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Seeking general principles in the design of defense systems against hydrogen peroxide

Dissertation presented to obtain the PhD degree in Bioengineering

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Alla mia famiglia senza la quale non sarei mai arrivato fino a qui.

A Pasquale, Maria e Valentina.

A human being should be able to change a diaper, plan an invasion, butcher a hog, conn a ship, design a building, write a sonnet, balance accounts, build a wall, set a bone, comfort the dying, take orders, give orders, cooperate, act alone, solve equations, analyse a new problem, pitch manure, program a computer, cook a tasty meal, fight efficiently, die gallantly. **Specialization is for insects**.

- Robert A. Heinlein

Declaration-Declaração

I declare that this dissertation is a result of my own research carried out between September 2011 and September 2016. The project was conceived and partially developed at the Computational and System Biology Group of Dr. Armindo Salvador, Centre for Neuroscience and Cell Biology-University of Coimbra, Portugal. Chapter 2 is an adapted version of a manuscript currently in preparation, authored by Selvaggio G., Oliveira V., Coelho P. M. B. M. and Salvador A entitled "*Design principles for thiol redox signaling: mapping the phenotypic repertoire of the cytoplasmic 2-Cys peroxiredoxin – thioredoxin system".*

Chapter 3 was carried out at the Synthetic Biology Group under the supervision of prof. Timothy K. Lu, Synthetic Biology Centre-MIT, Boston USA and has been published as Rubens J. R., Selvaggio G., Lu T. K. "Synthetic mixed-signal computation in living cells." Nat. Commun. (2016).

In vivo zebrafish experiments in Chapter 4 were performed at the Telomeres and Genome Stability Lab of Dr. Miguel Ferreira, Instituto Gulbenkian de Ciência in Oeiras, Portugal.

Declaro que esta dissertação é o resultato do meu próprio trabalho desenvolvido entre Setembro de 2011 e Setembro de 2016. O projecto foi concebido e parcialmente desenvolvido no Computational and System Biology Group do Dr. Armindo Salvador, Centro de Neurociências e Biologia Celular - Universidade de Coimbra, Portugal. O Capítulo 2 é uma versão adaptada do manuscrito em preparação, da autoria de Selvaggio G., Oliveira V., Coelho P. M. B. M. and Salvador A e titulado "*Design principles for thiol redox signaling: mapping the phenotypic repertoire of the cytoplasmic 2-Cys peroxiredoxin – thioredoxin system".*

O trabalho do Capítulo 3 foi realizado no Synthetic Biology Group do prof. Timothy K. Lu, Synthetic Biology Centre-MIT, Boston USA e foi publicado como Rubens J. R., Selvaggio G., Lu T. K. "Synthetic mixed-signal computation in living cells." Nat. Commun. (2016).

Experiências *in vivo* com peixe-zebra do Capítulo 4 foram realizadas no Telomeres and Genome Stability Lab do Dr. Miguel Ferreira, Instituto Gulbenkian de Ciência em Oeiras, Portugal.

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I have to thank many people at the end of this journey some of which became family to me, they all help to "matar a saudade" and to retrieve happiness in moments in which was hard to find light. It would take another thesis to thank you all for what you have done so I will try to be brief…and this never ended good.

I was a lucky person from the very beginning when I arrived in Portugal here I meet people like Enrico, Mariantonietta e Tina they made me call Lisbon home and feel happy in this place, I would have not made it through the first year without they invaluable friendship. I've to thank also David for granting me a nick name that will haunt me for long "Fraquinho", and Giulia made me smile in this last year. Although I was a "scary" person, and moreover an engineer, they became amazing friends.

In Coimbra I meet two of the three idiots Rui and Alessandro which made me pass beautiful days between cigarettes and coffee, together with David which has been not only of support to me in countless occasion but a trigger for me to get out some guts and make moves. I own to him a big share of my happiness. Pedro joined us after a while but he has been a pillar in helping and sustaining me in the period in the US and in all the trouble I got to get there, he was the one being there when rushed down to FC&T to yell a bit.

These people made me call Coimbra home. I like to imagine us at the math bar in the morning, with Ale having the n+1 coffee of the day, Rui arriving late and me David and Pedro ending up talking about some weird double meaning until we reach that limit that is time to go away.

In Boston was a tough period and although the first memory are mice related I had the opportunity to meet people like Mario, Carolina, Jaide and Sara. They have been special friends and one of the funniest birthday I had, eating at a Portuguese restaurant in Boston. It has been a bless to meet them in such a hard time.

In Oeiras, I found another major part of this big family that helped me moving forward. It was a very hard time but thanks to the friendship and the support of Tânia and Joana I managed through it. Our brakes with cigarettes and coffee, even if these last were not too good for my stress brought light to those days. I was again happy to go to work because I was going to meet such amazing people as you.

The "Telometros" lab has been a place where to be again amongst friends, Margarida and Ana the ninja shared with me desk and bench, and "nicely" reminded me that "I was supposed to write" although they started one year before the defense to say it. Mounir has been a very good friend to me despite him being half French. And then all the people of the lab: Pam, Inés, José, Edi, Gonçalo, Kirsten, Iris, Vanda, Akila, Rita and Raquel. You all have been so nice and kind to me that I will never forget this past year.

To all of you my friends of the past 5 years I'm bad with words and I think I don't have and know enough in English to say to you, with my bad accent, how much I love you, how much you represent to me and what is the happiness you brought to my life. The only thing I can do is that to all of you one by one I will hug you. Because is the only way in which this idiot can express feelings.

Last but not least, I've to thank Armindo, he welcomed me in his lab after few hours of discussions about science. I had started a PhD with the objective of learning how to do science, and under his mentorship I matured from the raw researcher I was. We had very interesting discussions and exchanged so many crazy ideas along these 5 years and we managed to carry at the end this project together.

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Abstract

Reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), are now known to play critical roles in signal transduction and in coordinating key cellular processes. However, these species can also covalently damage macromolecules and originate other even more deleterious compounds.

At the core of this twine between signaling and defense lays the Peroxiredoxin Thioredoxin Thioredoxin Reductase (PTTR) system. Experimental studies of the PTTRS highlighted many commonalities among different types of cells and organisms, but also intriguing differences in cells' responses to hydrogen peroxide.

The current work aims to study the PTTR system and its characteristics. Using a minimal mathematical model, we seek to uncover the general principles of how organisms exploit the properties of ROS for regulation of other protein while avoiding their deleterious effects.

These principles, in the form of relationships among rate constants and species concentrations, are thoroughly supported by experimental observations in a variety of organisms and allow to correlate proteins abundance patterns with the modes of response.

Depending on the relative abundances of peroxiredoxins, sulfiredoxin, thioredoxin, thioredoxin reductase and alternative H_2O_2-consuming proteins, the system is capable of distinct responses to changing hydrogen peroxide supplies, including proportional, ultrasensitive, and hysteretic (toggle switch) ones.

The complete characterization of the system however requires the definitions of the operative conditions in which the organism lives. A major and so far not univocally defined value is the maximum attained hydrogen peroxide concentration in vivo. To address this problem were developed a series of sensor with different thresholds and capable of memory functions. The peroxide classifier was then used in an inflammation animal model to measure the maximum attained concentrations.

The mathematical model developed in this system and the studies of the general principles underlying the PTTR system together with the experimental application of the H_2O_2 classifier could be used in clinical research or drug development.

Keywords: synthetic biology, redox signaling, peroxiredoxin, free radicals, system biology

Resumo

Várias espécies reactivas de oxigénio (ROS), tal como o peróxido de hidrogénio (H_2O_2), foram recentemente identificados como modeladores de sinalização e coordenação de importantes processos celulares. No entanto, estas partículas podem causar danos oxidativos em determinadas macromoléculas ou até originar outros metabolitos ainda mais reactivos.

Central a todo este processo de equilíbrio entre sinalização e dano oxidativo, encontra-se o importante sistema de defesa redox Peroxiredoxina Tioredoxina Tioredoxina-Reductase (PTTR). Várias evidências experimentais envolvendo o sistema PTTR, apontam para um grande nível de conservação entre diferentes tipos de células e organismos, mas no entanto é também evidente alguma disparidade em termos de resposta ao stress induzido por H_2O_2.

Este trabalho foi desenvolvido visando estudar o sistema PTTR. Através de um modelo matemático minimalista, procurámos descobrir os princípios base de como os organismos utilizam os ROS na regulação de outras proteínas de maneira a evitar os seus efeitos nefastos.

Os pricípios base foram desenvolvidos sob a forma de relações entre as constantes de reacção e a concentração das espécies. Princípios esses que foram solidamente apoiados por observações experimentais em diferentes organismos, tornando possível uma correlação clara entre o padrão de expressão das proteínas e a forma como o organismo responde ao stress.

Dependendo das concentrações relativas de peroxiredoxinas, sulfiredoxina, tioredoxina, tioredoxina-reductase e outras proteínas que alternativamente consomem o H_2O_2, o sistema é capaz de responder de forma diferente a mudanças de H_2O_2, incluíndo alterações proporcionais, ultra-sensíveis e histeresicas.

No entanto, a caracterização do sistema requer o conhecimento das condições oxidativas nas quais o organismo se desenvolve. Para isso, desenvolvemos uma série de sensores de H_2O_2 capazes de detectar diferentes níveis de exposição e capazes de memorizar esse mesmo contacto. O sensor foi posteriormente validado num modelo animal de inflamação de maneira a determinar os níveis máximos de exposição *in vivo*.

Neste trabalho desenvolveu-se um modelo matemático que em combinação com o sensor, podem vir a ser utilizados na prática clínica ou em ensaios toxicológicos.

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List of Abbreviations

List of abbreviations in alphabetical order.

Abbreviation	*Complete name*
ADC	Analog to digital converter
AhpC	Alkyl hydroperoxide reductase
Akt	Protein kinase B
ASK-1	apoptosis signaling kinase-1
aTc	anhydrotetracycline
ATP	Adenosine triphosphate
BAC	Bacterial artificial chromosome
BFP	Blue fluorescent protein
BOS	Basal oxidative stress
Cat	Catalase
CC	Copy control
Cys	Cysteine
DAC	Digital to analog converter
E.coli	Escherichia coli
EGF	Epidermal growth factor
FF	Fully folded
GFP	Green fluorescent protein
GMA	Generalized Mass Action
GPx	Glutathione peroxidase
GR	Glutathione Reductase
Grx	Glutaredoxin
GSH	Glutathione
H2DCF	2′,7′-dichlorodihydrofluorescein
H_2O_2	Hydrogen peroxide
HCP	High-copy plasmid
$HO^{\bullet}$	Hydroxyl radical
HOS	High oxidative stress
hpf	Hours post fertilization
hPrxI	Human Peroxiredoxin I
hPrxII	Human Peroxiredoxin II
hPrxVI	Human Peroxiredoxin VI

hpw	Hours post wounding
IL	Interleukin
IOS	Intermediate oxidative stress
LB medium	Luria-Bertani medium
LCP	Low-copy plasmid
LOS	Low oxidative stress
LU	Locally unfolded
MAPK/ERK	mitogen-activated protein kinases, originally called extracellular signal-regulated kinases
MCP	Medium-copy plasmid
Met	Methionine
NADPH	Nicotinamide adenine dinucleotide phosphate
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NOX	NADPH-oxidase
$O_2^{-}\cdot$	Superoxide anion
ODE	Ordinary differential equations
Orp1	Oxidant receptor protein
OS	Oxidative stress
PDGF	Platelet-derived growth factor
PI3K	phosphatidylinositol 3-kinase
PIP	PI 3,4,5-trisphosphate
PPP	Pentose Phosphate Pathway
Prx	Peroxiredoxin
PTEN	Phosphatase and tensin homolog
PTP-1B	Protein-tyrosine phosphatase 1B
PTTRS	Peroxiredoxin-Thioredoxin-Thioredoxin Reductase System
RBS	Ribosome binding site
RFP	Red fluorescent protein
ROS	Reactive oxygen species
S. pombe	Schizosaccharomyces pombe
SOD	Superoxide dismutase
Srx	Sulfiredoxin
STAT3	Signal transducer and activator of transcription 3
TNFα	Tumor necrosis factor α
Trx	Thioredoxin

TrxR	Thioredoxin reductase
TSAI	Thiol-specific antioxidant protein 1
TSAII	Thiol-specific antioxidant protein 2
YFP	Yellow fluorescent protein

Chapter 1 | General Introduction

Oxidative stress: a changing paradigm

The definition of oxidative stress (OS) has been changing over the years, due to a shift in the paradigm of the processes that involve some reactive oxygen species (ROS): from deleterious to necessary in the normal functions of the organism.

A comprehensive definition of this phenomenon (given by H. Sies and D. Jones) reported OS as**:** **"an imbalance between oxidants and anti-oxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage"** [1,2]. This definition highlights the importance of sensing oxidation sources, transmitting information carried by them, and activating proper responses. Such responses may eventually prevent the propagation of damage to key cellular components such as proteins, nucleic acids and lipidic membranes.

The OS sources can be endogenous (ensuing from mitochondrial respiration, protein folding autoxidation, etc.) or external to the organism. The latter may be originated by different factors (Table 1.1).

Table 1.1| Some known sources of oxidative stress.

Condition	Proposed Source	Likely reactive species produced
Hyperoxia; hypoxia; ischemia; reperfusion	Mitochondria; NADPH oxidases; xanthine oxidase; nitric oxide synthases	Superoxide, hydrogen peroxide, nitric oxide, peroxynitrite
Inflammation	Phagocyte NADPH oxidase; myeloperoxidase; inducible nitric oxide synthase	HOCl, chloramines, HOBr, bromamines, HOSCN, oxyradicals, nitrogen dioxide, carbonate radical, nitric oxide, peroxynitrite
Activation of receptors to agonists (e.g. Fas, TNF-α, angiotensin II)	NADPH oxidases; mitochondria; nitric oxide synthases	Superoxide, hydrogen peroxide, nitric oxide
Xenobiotic metabolism	Peroxidases; flavoprotein reductases; autoxidation	Oxyradicals, superoxide, hydrogen peroxide

Table 1.1 from ref. [3]

Deregulation of redox signaling pathways, with consequent OS, has been shown to be involved into the development of pathologies such as cardiovascular disease[4], inflammatory bowel disease [5,6], atherosclerosis, diabetes and metabolic disease [7,8] and neurodegenerative disease (e.g. Parkinson [9]).

OS play also a major role in tumor incidence and progression, where the antioxidant defenses are necessary for the initiation and the survival of the cancer [10,11]. Furthermore, in higher organisms the aging phenomenon is associated with an increase in the basal level of oxidants, called inflammaging [12].

It is thus important to analyze the signaling/defense system to better understand how to exploit the underlying mechanism of redox regulation for developing new drugs that target the diseases listed above.

Reactive oxygen species: why hydrogen peroxide?

ROS are a heterogeneous family containing both highly reactive oxygen radicals (e.g., ($O_2^{\bullet-}$) hydroxyl radical ($HO^{\bullet}$)) and less reactive non-radical oxidants (e.g., hydrogen peroxide H_2O_2, singlet oxygen ($^1O_2$)).

In the former group we find radicals like $HO^{\bullet}$, which is a very strong and indiscriminate oxidant. It can react with many amino acid side chains with diffusion-limited rate constants. The short lived nature of this ROS (estimations report 10^{-9} s at 37ºC [13]) spatially limit its interactions to the region where is generated.

$HO^{\bullet}$ radicals are generated by the reaction of transition metal ions (e.g. ferrous, cuprous) with H_2O_2 or via the iron catalyzed reaction between $O_2^{\bullet-}$ and H_2O_2.

$$O_2^{\bullet-} + H_2O_2 \rightarrow HO^{\bullet} + OH^- + O_2 \quad (1.1)$$

$$H_2O_2 + Fe^{2+} \rightarrow HO^- + HO^{\bullet} + Fe^{2+} \quad (1.2)$$

This can induce extensive damage especially in cells carrying heme groups[14].

$O_2^{\bullet-}$ is in general moderately reactive with biological macromolecules, but it can generates very reactive radicals such as $HO^{\bullet}$ and peroxinitrite [15]. Additionally, it is very reactive with iron-sulfur clusters of dehydratases, inactivating these enzymes and releasing Fe^{2+} in the process [16,17]. $O_2^{\bullet-}$ also reacts with reduced glutathione leading to the formation of sulfinyl and thiyl radicals to then regenerating itself in a self-sustaining cycle[18]. Furthermore, it promotes DNA damage [17]

$O_2^{\bullet-}$ is the primary product of NADPH oxidases [19] (NOX) and a byproduct of the mitochondrial respiration [20]. Because of its charged nature at physiological pH it can only permeate cell membranes through anion-channels [21]. $O_2^{\bullet-}$ is removed mainly by dismutation to O_2 and H_2O_2. in a reaction catalyzed by superoxide dismutase (SOD), with a catalytic rate constant of approximately 10^9 $M^{-1}s^{-1}$ [22,23].

Nearly all oxygen tolerant organisms contain SOD isoforms [24] that tightly control the intracellular concentration of $O_2^{\bullet-}$.In addiction to this, the $O_2^{\bullet-}$ limited membrane permeability describe a molecule with considerable limitation as signaling compound.

On the other hand, H_2O_2 possess crucial characteristics to behave as a redox messenger.

H_2O_2 is generated intracellularly, and extracellularly. Intracellular sources are mainly the dismutation of $O_2^{\bullet-}$ by SOD, in the mitochondria and in the cytoplasm or proteins autoxidation. Exogenous sources are instead related to the activation of NOX by cytokines and growth factor (e.g. PDGF, p53, thyrotropin, EGF, insulin, TNF-α) [19,25–29], presence of chemicals and immune (e.g. wound, inflammation) [30,31] or competitive responses (e.g. lactic-acid bacteria suppress competition by excreting H_2O_2)[32].

H_2O_2 is a small uncharged compound that can diffuse through the cell membrane either via passive diffusion or aquaporin channels [33], due to the high similarity with the water dipole. As a matter of fact organism have developed strategies to control this passive diffusion and to regulate the composition of the membrane[34] to make it harder to cross in response to sustained oxidative insults.

H_2O_2 and $O_2^{\cdot-}$ have a major role in redox biology, but considering the rapid conversion of the latter in H_2O_2 and the characteristic that this last has. We will focus on H_2O_2 as the principal ROS member, for signaling purposes.

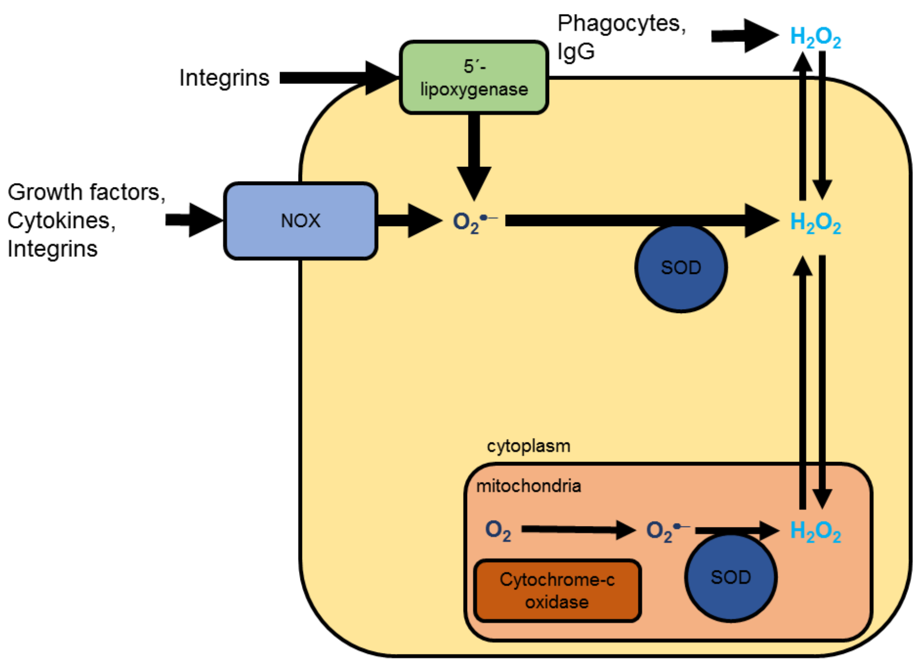


Figure 1.1| Different sources of hydrogen peroxide in eukaryotic cells. *Hydrogen peroxide can be produced extracellularly, for example by the immunoglobulin G-catalyzed oxidation of water, by receptor/ligand interactions, and by phagocytic immune cells.* $O_2^{\cdot-}$*, is produced by the partial reduction of the oxygen by cytochrome c oxidase in the mitochondria, by membrane associated NADPH oxidase, or by 5'-lipoxygenase in the cytoplasm, it is then rapidly converted to* H_2O_2 *by the action of cytoplasmic and mitochondrial SOD. Growth factor, cytokines and integrins stimulate the activation of NADPH oxidase and/or 5'-lipoxygenase. Figure and legend adapted from ref [35]*

Hydrogen peroxide targets: Cysteines

Cysteines (Cys) are the most nucleophilic amino acids in proteins, showing (together with methionine) a predisposition to oxidative modifications [36,37]. As a matter of fact, different enzymes families use these amino acids transformations as regulation (e.g. proteases, peroxidases, oxidoreductase [38]). The reactivity of a Cys thiol group, with H_2O_2 is dependent on the surrounding microenvironment and its acidity (pK_a). A free Cys in the cytoplasm has a pK_a between 8 and 9, which leaves the thiol group protonated and mostly non-reactive at physiological pH [39]. In some enzymes, however, the local aminoacidic arrangement can substantially modify the acidity to result in a pK_a as low as 4 to 5, deprotonating the thiols (R-S^-)[40]. However, the enhanced reactivity is not only correlated with the deprotonation but also depends on the stabilization of the thiolate , that increase its nucleophilicity [40].

Table 1.2| Acidity and Hydrogen Peroxide reactivity of several protein thiolates.

R-SH	pK_a	k_{RS^-} with H_2O_2 ($M^{-1}s^{-1}$)
Cysteine	8.3	26
Papain	3.4	62
PTP 1B	4.7	9
hPrxV	5.2	3.0 x 10^5
hPrxII	5.3	10^8

Table 1.2 adapted from ref [40]

Cys have several different post translational modification (Figure 1.2) depending on the oxygen degree of oxidation. The thiolate anion (R-S^-), the reactive form of Cys, may upon interaction with H_2O_2 generate the following oxidative modifications: sulfenic acid (R-SO^-), sulfinic acid (R-SO_2^-), sulfonic acid (R-SO_3^-).

$$H_2O_2 + R\text{-}S^- \rightarrow R\text{-}SO^- + H_2O \quad (1.3)$$

$$H_2O_2 + R\text{-}SO^- \rightarrow R\text{-}SO_2^- + H_2O \quad (1.4)$$

$$H_2O_2 + R\text{-}SO_2^- \rightarrow R\text{-}SO_3^- + H_2O \quad (1.5)$$

Protein Cys sulfenates can condense with protein thiols or low-molecular-weight thiols such as glutathione (glutathionylation) forming disulfides (henceforth denoted by R-SS-R or R-SSG, respectively):

$$R\text{-}S^- + H^+ + R'\text{-}SO^- \rightarrow R\text{-}S\text{-}S\text{-}R' + H_2O \quad (1.6)$$

$$GSH + R\text{-}SO^- \rightarrow R\text{-}SSG + H_2O \quad (1.7)$$

Thiol-disulfide oxidoreductase such as thioredoxin [41] or glutaredoxin [42] are able to resolve exposed disulfide bonds at the expense of reducing equivalents. However, it is possible that some cross-links are sterically hindered and thus irreversible.

Glutathionylation modifications are only resolvable through the glutaredoxins pathway [42,43].

Although R-SO_2^- of typical 2-Cys peroxiredoxins can be reduced at the expense of ATP and reducing equivalents under catalysis by sulfiredoxin [44–46], there are no evidence that R-SO_2^- from other proteins and R-SO_3^- in general can be rescued at biologically relevant rates.

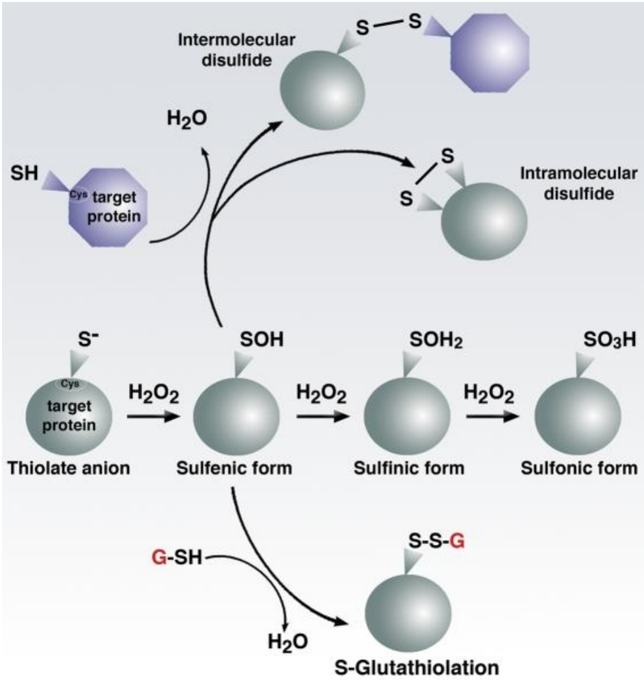


Figure 1.2| Cysteine biochemistry allows for redox-dependent signaling. *Specific reactive cysteine (Cys) residues within target proteins can be modified by oxidative stress. Thiolate form can be progressively oxidized by reacting with H_2O_2 in: sulfenic, sulfinic, sulfonic form. The sulfenic form (SO^-) is highly reactive and can condensate with another thiol forming an intra- or inter molecular disulfide or with a glutathione molecule. Higher states of oxidation generally, but not always lead to irreversible modifications. Figure from ref. [47]*

These reactions that lead to irreversible or non-repairable modifications are thus of particular concern because they may generate protein aggregates or misfolding leading to toxic effect for the organism. It is thus fundamental for an organism to maintain a tight control on the peroxide concentrations. As a matter of fact H_2O_2 has been reported to be a possible mutagenic source by damaging DNA already with sub-micromolar intracellular concentrations [48].

Hydrogen peroxide concentrations *in vivo*

Although high levels of H_2O_2 and other ROS generate cellular damage, it is becoming clear that low levels of H_2O_2 participate in cellular signaling to maintain homeostasis[49]. Despite the importance of H_2O_2 to cellular activities, the molecular mechanisms of its production, accumulation, function, and scavenging remain insufficiently understood. This is due to a large extent to the lack of knowledge of the actual concentrations and fluxes of H_2O_2 *in vivo*, and to persistent uncertainties about the roles of distinct antioxidant defenses in physiological context.

A wide range of peroxide concentration has been used in experiments to study the system (from µM to mM) with different and sometime subjective classifications. Lushchak [50] tried to give the following formal semi-quantitative definition of OS based on the observable phenotype produced (Figure 1.3).

Under basal oxidative stress (BOS) there are no observable outcomes. This state represents the normal functioning of the cell. An increase in the oxidative load will shift the organism to low-intensity oxidative stress (LOS), characterized by oxidation of the most reactive cellular components and induction of the redox-dependent response. Intermediate-intensity oxidative stress (IOS) is high enough that the up-regulation of the response is counterbalanced by its inactivation (e.g. substrate inactivation of antioxidant and associated enzymes that were upregulated in the LOS). This will generate an apparent negative response of the ROS- induced functions to increasing concentrations of the oxidant and an even higher oxidation of the available targets. Finally, in the high-intensity oxidative stress (HOS)virtually all available potential substrates are oxidized [50].

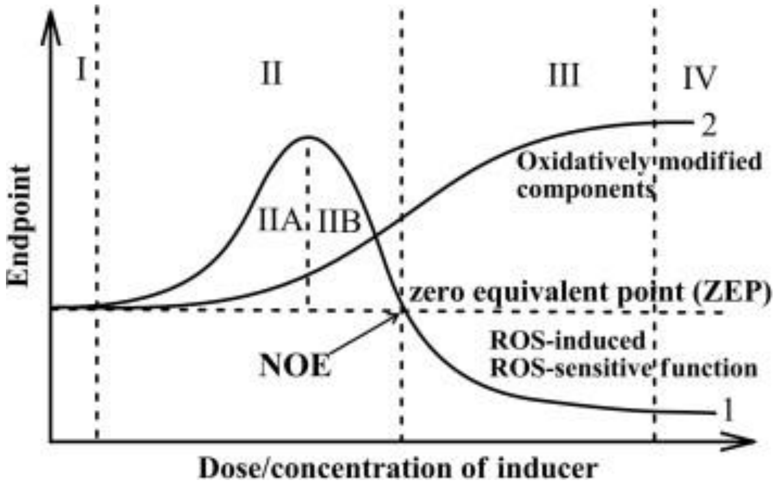


Figure 1.3| Schematic classification of the oxidative stress based on intensity. *This figure shows the behavior of the redox couples (**curve 2**) and of the ROS dependent response (**curve 1**) across different intensity of OS. I – basal oxidative stress zone (BOS); II – low intensity oxidative stress (LOS); III – intermediate intensity oxidative stress (IOS); and IV – high intensity oxidative stress (HOS). Figure from ref. [50]*

A sensitive and precise determination of H_2O_2 levels *in vivo* is essential to examining the role of H_2O_2 in physiological or pathological processes[51]. The acquisition of such knowledge has been delayed due to the difficulty of quantifying and tracking the small, diffusible and fast cleared H_2O_2 molecules in living cells. Various methods are nowadays available for the measurement of the H_2O_2 concentration.

A category of methods sensitive enough to determine physiological H_2O_2 concentrations is based on dihydro compounds such as 2′,7′-dichlorodihydrofluorescein (H2DCF) that fluoresce upon oxidation. They are widely used because of their sensitivity and simplicity, but these probes lack specificity, reacting with a variety of ROS including nitric oxide, peroxynitrite, and hypochloride in addition to H_2O_2 [52].

Another example are deprotection reaction-based probes that fluoresce upon H_2O_2-specific removal of a boronate group, rather than on nonspecific oxidation [53–55]. Intracellular H_2O_2 production can be also visualized by highly H_2O_2-specific, genetically encoded, and reversible fluorescent constructs, whose characteristics enable *in vivo* real-time dynamic H_2O_2 determinations.

Two main genetically encoded sensors are currently available. One is HyPer [56,57] and the other is Orp1-redox-sensitive green fluorescent protein 2 (roGFP2) [51]. In the latter, the reaction between Orp1 peroxidase and H_2O_2 results in a disulfide that changes the roGFP2 β-barrel structure, leading to changes in the spectrum of the fluorescent protein. Analogously, Hyper consists of a circularly permuted yellow fluorescent protein inserted into the regulatory domain of the prokaryotic H_2O_2-sensing protein, OxyR [58–60]. The oxidation of purified OxyR by H_2O_2 is very rapid (10^5 $M^{-1}s^{-1}$), with 100 nM of H_2O_2 sufficient to create a disulfide bond with a half-life of 30 s

[59]. The disulfide formation leads to conformational changes and hence to a change in the YFP excitation spectrum (400nm/500nm excitation and 516nm emission). The readout in both cases (roGFP2 and Hyper) is the ratio between the emission of light by the protein when excited with one or the other wavelength. In vitro assays for Hyper reported a ratio between 1.5-3.3 respectively to 25-250 nM of H_2O_2 [56]. A calibration curve, performed by FACS, over Hyper-expressing COS-7 cells exposed to various H_2O_2 concentrations showed a minimum external concentration of 5 µM to activate the sensor and a saturation at around 20 µM

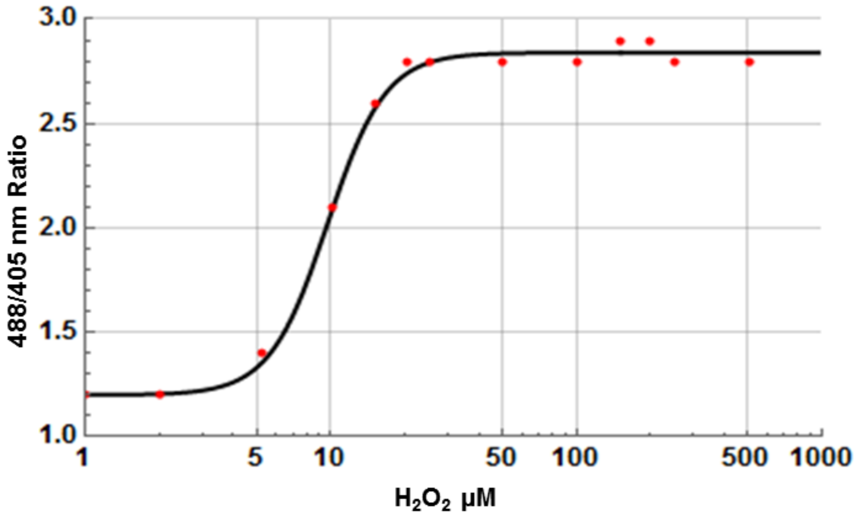


Figure 1.4| Hyper ratio in COS-7 cells exposed to different concentration of H_2O_2. *The ratio between the fluorescence excited by 488 nm and 405 nm lasers as a function of H_2O_2 concentration. In red are reported the experimental points in black the fitting curve (* $y = D - \frac{A-D}{1+\frac{x}{C}}; A = 4.5, B = 3.7, C = 9.7, D = 2.8$ *). Figure and legend adapted from ref. [56].*

The development of these intracellular redox sensors evolved over the past years improving their performances [57] and eventually being used in animal model. The study of the inflammation-like response in zebrafish performed by Niethammer *et al.* [61] showed the importance of H_2O_2 in the wound healing process and the establishment of a gradient in the tissue (due to NADPH-oxidases) that would function as chemotaxis signal for the immune system cells[30,57,62]. Both these work from Niethammer *et al.* and Pase *et al.* (respectively ref. [61] and ref. [30]) estimated, on the calibration curve previously done (ref. [56] and Figure 1.4), a concentration that ranged from 5-50 µM having the highest value of the gradient at the wounding site. This measurement unfortunately lacks a proper calibration. The cells examined are from various tissue and heterogeneous in antioxidant defense expressions and redox state. Furthermore, the calibration curve was calculated on human COS-7 cell. It spans over the entire linear region of the sensor possibly entering the saturation region. The Hyper mRNA injected can be degraded at different rates in different cells giving varying Hyper expressions.

Other estimations made on the data available in literature calculate the H_2O_2 concentration in the human plasma being between 1-5 µM in normal condition and increasing up to 30-50 µM in chronic inflammation conditions[63]. However, other *in vivo* studies ref. [64] reported that in healthy cells the H_2O_2 concentration rarely exceeds 1-15 µM.

The lack of consensus about the H_2O_2 concentration, together with the wide dynamic range covered by various experimental set-up (from µM-mM of external H_2O_2) make of the physiological oxidative load a puzzle that has still to be properly addressed.

Antioxidant defenses

Organisms have developed a variety of antioxidant defenses to counterbalance and control the ROS compounds. There are two major categories of defense: enzymatic (e.g. peroxidase, catalase, superoxide dismutase etc.) and non-enzymatic (e.g. vitamin C, A) [65]. In the following paragraphs we will focus on the Peroxiredoxin-Thioredoxin-Thioredoxin Reductase system (PTTRS), which is one of the main subjects of interest in this thesis

Peroxiredoxins

Peroxiredoxins (Prx) are a Cys-based class of scavengers for H_2O_2, which protect the cells from oxidative insults and prevent damage to cellular key components. Discovered to be ubiquitously present in several organisms, from archaea to humans [66], these proteins show abundances, structures similarities and properties that are conserved even amongst kingdoms [67] (Figure 1.5). Their catalytic cycle involves the oxidation of a peroxidatic Cys thiolate (C_P-S^-), located in a universally conserved PXXXTXXC motif, to sulfenic acid (C_P-SO^-). This sulfenic acid eventually reacts with a resolving cysteine (C_R) forming an inter- or intra- molecular disulfide that will be reduced restoring the thiolate.

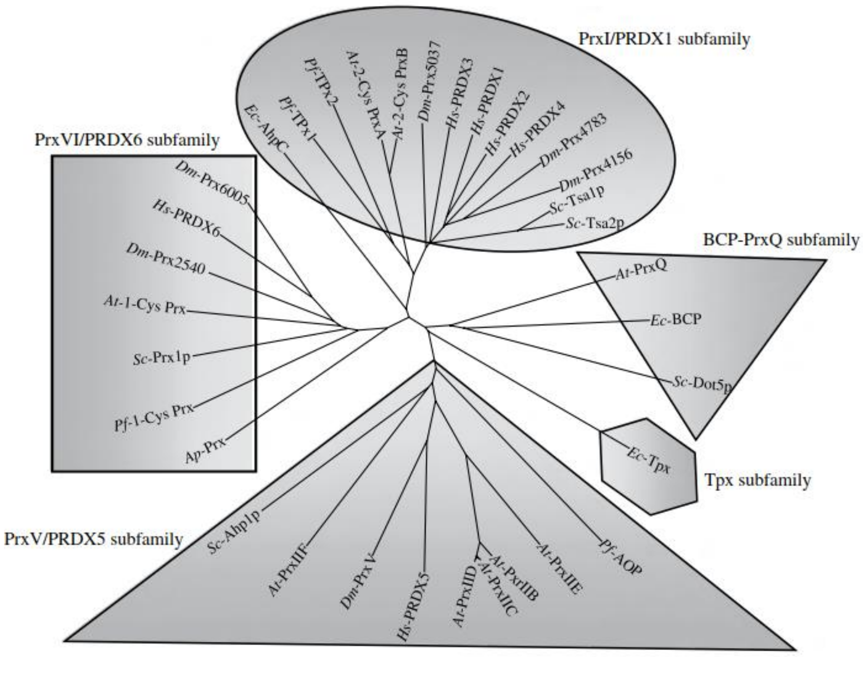


Figure 1.5| Phylogenetic tree of the peroxiredoxin family. *Protein alignment was performed with clustalX 1.81 program. Tree drawing was achieved with the neighbor-joining method. The unrooted tree was drawn with Treeview, and has been divided into five cluster (subfamilies) represented by the different shapes. Ec: Escherichia coli; Ap: Aeropyrum pernix; Sc: Saccharomyces cerevisiae; Pf: Plasmodium falciparum; At: Arabidopsis thaliana; Dm: Drosophila melanogaster; Hs: Homo sapiens. GenBank™ accession numbers of the peptide sequences are as follows: Ec-AhpC (NP_415138); Ec-Tpx (NP_415840); Ec-BCP (NP_416975); Ap-Prx (NP_148509); Sc-Tsa1p (NP_013684); Sc-Tsa2p (NP_010741); Sc-Prx1p (NP_009489); Sc-Dot5p (NP_012255); Sc-Ahp1p (NP_013210); Pf-TPx1 (AAF67110); Pf-TPx2 (AAK20024); Pf-1-Cys-Prx (AAG14353); Pf-AOP (1XIYA); At-PrxIIB (NP_176773); At-PrxIIC (NP_176772); At-PrxIID (NP_564763); At-PrxIIE (NP_190864); At-PrxIIF (NP_566268); At-2-Cys PrxA (NP_187769); At-2-Cys PrxB (NP_568166); At-1-Cys Prx (NP_175247); At-PrxQ (NP_189235); Dm-Prx4156 (NM_080263); Dm-Prx4783 (NM_167359); Dm-*

Prx5037 (NM_079663); Dm-PrxV (NM_176513); Dm-Prx6005 (NM_078739); Dm-Prx2540 (NM_165769); Hs-PRDX1 (NM_002574); Hs-PRDX2 (NM_005809); Hs-PRDX3 (NM_006793); Hs-PRDX4 (NM_006406); Hs-PRDX5 (NM_012094); Hs-PRDX6 (NM_004905). Figure and legend from ref [68].

Prx are divided into six different families depending on the mechanism of resolution of the sulfenic acid and their oligomeric state.

Table 1.3| Summary of Prx Subfamily phylogenetic distribution and structures.

Subfamily	Phylogenetic distribution	Structural distinctions relative to Prx core fold	Oligomeric states and interfaces
Prx1/AhpC	Archaea, bacteria, plants and other eukaryotes	Extended C terminus	B-type dimers, $(\alpha_2)_5$ decameres (and rare $(\alpha_2)_6$ dodecamers) through A interface
Prx6	Archaea, bacteria, plants, and other eukaryotes	Long, extended C terminus	B-type dimers, some $(\alpha_2)5$ decamers through A interface
AhpE	Bacteria	Extended loop at N terminus	A-type dimers
PrxQ	Archaea, bacteria, plants and fungi	Extended helix α5	Monomers and A-type dimers
Tpx	Bacteria	N-terminal hairpin	A-type dimers
Prx5	Bacteria, plants, and other eukaryotes	Pi helix insertion in β; ~20% fused with Grx domain	A-type dimers

Table 1.3 adapted from ref [69]

Prx1/AhpC and Prx6 subfamilies have the widest biological distribution (Table 1.3, Figure 1.5). The former are very abundant in cells, as illustrated by the following examples. They compose ~0.1%-1% of the soluble protein in rat and human cells [70], with peroxiredoxin II (hPrxII) being the third most abundant protein in human erythrocytes [71]. Thioredoxin peroxidase I (TSA1) constitutes the ~0.2-0.7% of the total soluble protein in *S. cerevisiae* [72]. Alkyl hydroperoxide reductase (AhpC) accounts for 0.4% of the proteome of *Escherichia coli* according to data from [73,74], and it is annotated amongst the ten most expressed proteins in this organism [75–77]. In eukaryotic cells, Prx1/AhpC peroxiredoxins are mainly located in the nucleus and cytoplasm.

The conserved fold at the active site of these proteins grant them high catalytic rates for the reduction of H_2O_2: hPrxII shows a second order rate constant of 10^8 $M^{-1}s^{-1}$ [78] , TSA1 of $2.2x10^7$ $M^{-1}s^{-1}$ [79] , AhpC of $4x10^7$ $M^{-1}s^{-1}$ [80].

The high abundances together with the high catalytic rates of ~10^6-10^8 $M^{-1}s^{-1}$ for H_2O_2 reduction may account, in the absence of inhibiting factors[81], for the consumption of more than the 90% of the cytosolic H_2O_2 under physiological conditions.

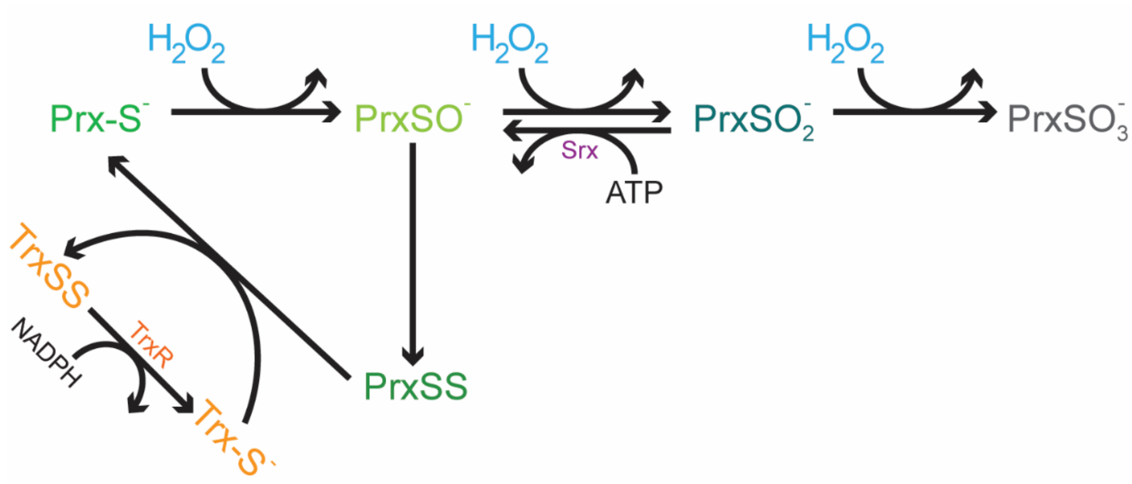


Figure 1.6| 2-Cys Peroxiredoxins catalytic cycle. *H_2O_2 oxidizes the peroxidatic cysteine to a sulfenic acid (Prx-SO$^-$), which then condenses with the resolving cysteine from an adjacent monomer to form a disulfide (PrxSS). This step requires first a local unfolding at the active sites that rearranges the peroxidatic and resolving cysteines regions. The cycle is then closed by the reduction of PrxSS often carried out by thioredoxin (Trx), at the expenditures of a reducing equivalent (NADPH) supplied through thioredoxin reductase (TrxR) eventually returning Prx to its fully folded structure.*

Peroxiredoxins of the Prx1/AhpC subfamily, commonly referred as typical 2-Cys peroxiredoxins, are pentamers of dimers. The subunits in each dimer are arranged in an antiparallel fashion with the C_P of one dimer facing the C_R of the other. These peroxiredoxins reduce H_2O_2 through a three-step cycle (Figure 1.6) that requires the formation of an inter-subunit disulfide bond, and that is maintained by the supply of reducing equivalents through the thioredoxin system.

Eukaryotic 2-Cys peroxiredoxins of the Prx1/AhpC subfamily, such as human peroxiredoxins I (hPrxI) and II (hPrxII), are susceptible to inactivation by their own substrates due to the conversion of their peroxidatic cysteines to sulfinic (Prx-SO_2^-) and sulfonic (Prx-SO_3^-) acids. This phenomenon, called hyperoxidation, is facilitated by two phylogenetically conserved structural motifs. Namely, a GGLG and a C-terminal extension with a YF, perturbing the unfolding-folding equilibrium of the active sites and thereby making them more prone to hyperoxidation[82]. The change in oxidative state also translates into a rearrangement of the quaternary structure of the Prx, this is usually associated with a change in function from scavenger to holdase/chaperone[83–85]. Prokaryotic peroxiredoxins are typically more resistant to hyperoxidation, with few exceptions[84,86]. They require H_2O_2 concentrations in the mM range to be inactivated [82] while eukaryotic ones, as hPrxII, are completely inactivated with 40 µM of H_2O_2 under similar conditions [87]. Prx-SO_2^- can be slowly reduced to the sulfenic form Prx-SO$^-$ at the expense of ATP and reducing equivalents under catalysis by Sulfiredoxin (Srx) [88].

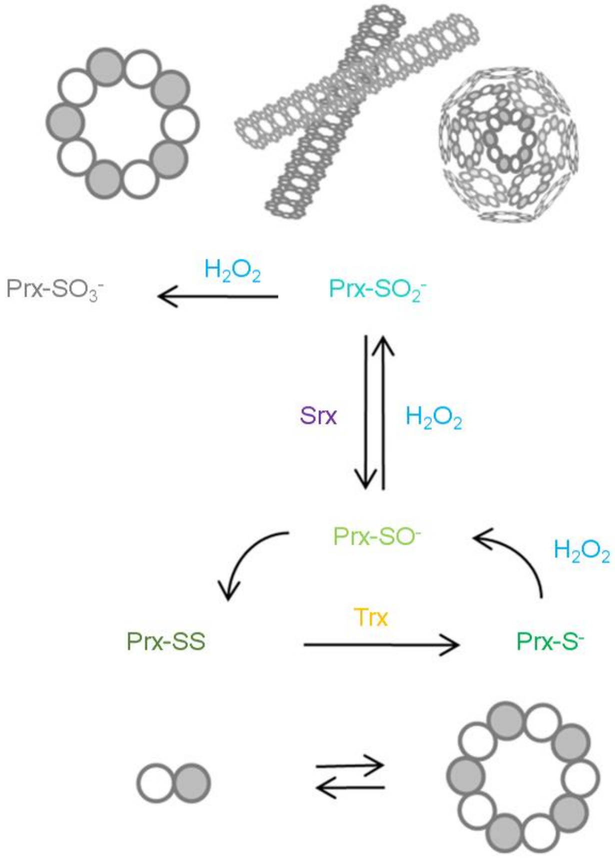


Figure 1.7| Typical 2-Cys peroxiredoxin quaternary structures. *During the reduction process, the Prx molecules alternate between dimeric and decameric states. The reduced, decameric form of the protein is the most reactive with H_2O_2. As the level of H_2O_2 increases, eukaryotic Prxs can react with a second H_2O_2 molecule to form the sulfinic acid form (Prx-SO_2^-) and, as a result, are inactivated. This hyperoxidation stabilizes the decameric state of the Prx molecule and can lead to the formation of filamentous and spherical, high molecular weight species, these play chaperon or holdase roles in the organism. Figure and legend adapted from ref [89]*

Srx is a very inefficient enzyme[45,90,91], it is widespread among eukaryotes, but exceptions exist where is not yet clear which enzymes recovers the sulfynilated form [92]. In several organisms hyperoxidation occurs not only upon extreme insults but also under physiological conditions[93].

The wide phylogenetic conservation of the above-mentioned structural motives suggests that sulfinylation confers properties that are favored by natural selection

Another possible evolutionary driving force that lead to this inactivation mechanism, is the so called "floodgate hypothesis". It proposes that a local inactivation of Prx allows H_2O_2 to increase locally thus propagating the signal to other target proteins that otherwise would be easily outcompeted by Prx.[82]

Moreover, Day et al.[94,95] showed that in *S. pombe* the survival to mM concentration of H_2O_2 was diminished when Prx was not inactivated anymore, demonstrating the importance of this form.

The Prx6 family, comprehend the 1-Cys peroxiredoxins (e.g. Human PrxVI). they are mostly cytosolic located, and their catalytic cycle requires GSH to be competed. In particular upon reaction with H_2O_2 the active site thiolate is oxidized to a sulfonate, whose reduction is dependent on glutathionylation by GSH-loaded glutathione S-transferase π [96,97]. hPrxVI may play an important role in H_2O_2 metabolism since from proteomics data we found its concentrations to be similar to those of hPrxI and hPrxII.

Thioredoxin and Thioredoxin Reductase

Thioredoxin (Trx), is a major disulfide reductase enzyme, widely distributed in all living organisms from bacteria to mammals (Figure 1.10)[98]. Trx is characterized by a conserved active domain Cys-Gly-Pro-Cys, which can reduce exposed disulfide substrate generating oxidized Trx [41]. The reducing equivalents necessary to support this reaction are provided by the FAD-containing enzyme thioredoxin reductase (TrxR).

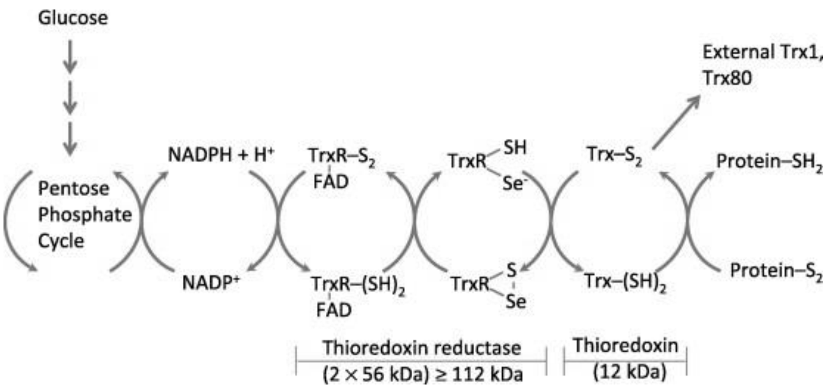


Figure 1.8| Redox reactions catalyzed by a mammalian Trx system comprising thioredoxin reductase (TrxR), thioredoxin (Trx) and NADPH. *The electron source of the Trx system is NADPH, which is largely produced from the pentose phosphate pathway. The oxidized thioredoxin (Trx-SS) is reduced by NADPH and the selenoenzyme TrxR. Electrons are transferred from NADPH to FAD, then to the N-terminal redox active disulfide in one subunit of TrxR, and finally to the C-terminal active site Gly–Cys–Sec–Gly of the other subunit*[99]*. Reduced thioredoxin (Trx-$(SH)_2$) catalyzes disulfide bond reduction in many proteins. Figure and legend from ref.* [100]

The reduction of Trx is carried out by a selenoenzyme TrxR. Two types TrxRs have been characterized, both belong to the flavoprotein family and both function as homodimers. The monomers possess a FAD prosthetic group, a NADPH-binding site and an active site [101]. However, the two groups are different in amino acid sequences and catalytic mechanisms [101,102], showing only a ~20% sequence identity [103].

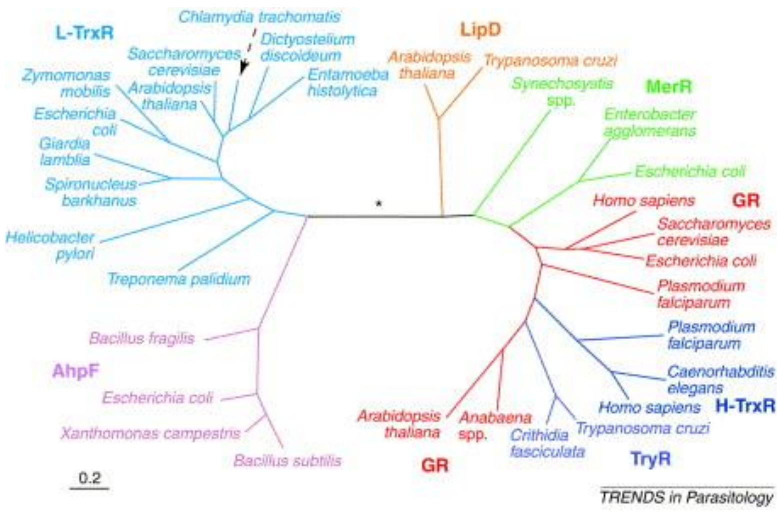


Figure 1.9| Phylogenetic relationships between high molecular weight thioredoxin reductase (H-TrxR), low molecular weight Thioredoxin reductase (L-TrxR). *In the figure the phylogenetic tree is also complemented by the enzymes which are closer to the L-TrxR and H-TrxR as: glutathione reductase (GR),*

mercuric reductase (MerR), lipoamidedehydrogenase (LipD), alkylhydroperoxide reductase F52A (AhpF). There is a large sequence divergence between the two TrxRs group and a complex gene history for all six enzymes. The tree was derived using maximum likelihood methods: 180 aminoacids aligned between different enzymes and used in phylogenetic inference. Scale bar represents inferred number of changes per site. Figure and legend from ref. [103].

The first TrxR type, is characterized by a high molecular weight (~55 kDa, H-TrxR), can be found mostly in higher organisms such as *Homo sapiens*, *C. elegans* and *Drosophila melanogaster* but also in the malaria parasite *P. falciparum* [104–107]. Mammalian TrxRs consist of two dimers arranged in an antiparallel fashion [99], they use one reducing equivalent from NADPH per molecule of Trx in a ping-pong type of reaction [102]. Bacteria, archaea, fungi and plants commonly possess another TrxR type with a low molecular weight (~35 kDa, L-TrxR) [108].

The thioredoxin system (Trx, TrxR and NADPH) can provide electrons to a large range of enzymes and was originally found to play a critical role in DNA repair and replication by being the reducing substrate of ribonucleotide reductase (RNR), together with Grx. [109].

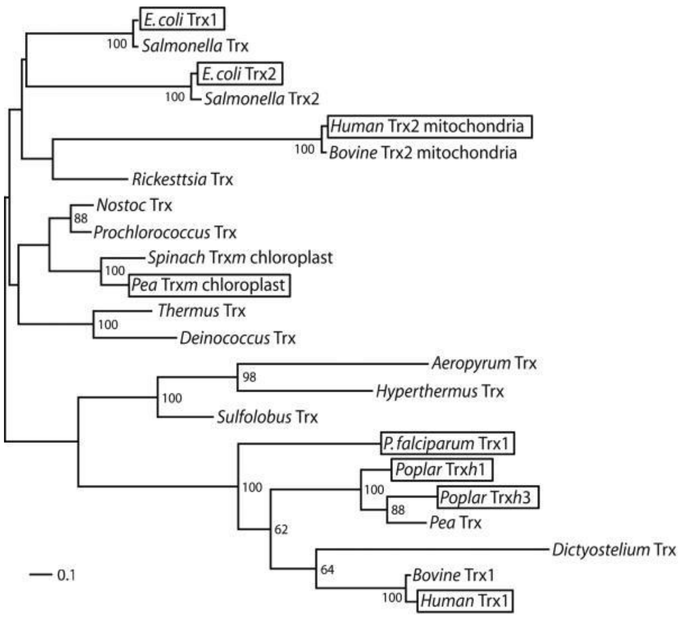


Figure 1.10| Phylogeny of Thioredoxin homologs from representative species of the three domains of life. *Branch lengths were estimated using maximum likelihood with rete variation modeled according to a gamma distribution. Scale bar represents amino acid replacements per site per unit evolutionary time. Posterior probabilities are shown at nodes of the phylogeny when greater than 50%. The lack of strong node supports deep in the phylogeny results from the ambiguous placement of mitochondrial sequences, possibly due to long branch attraction effects with nonbacterial sequences. Figure and legend from ref.* [98]

Eukarya possess two homologue thioredoxin systems, one cytoplasmatic and the other mitochondrial [98]. In addition to the catalytic active site thiols, mammalian cytoplasmic Trx possess three conserved thiols[110]. Two of these are closely located and can form an intramolecular disulfide; the remaining is located at the surface of the protein and can be either glutathionylated[111] or S-nitrosyilated[112] upon oxidative insults. The disulfide cannot be reduced by TrxR and delays the reduction of the Trx main active site by the same enzyme[110]. The presence of these other thiols groups has been postulated to be a further layer of control on Trx activity. Perer-Jimenez *et al.* [98] analysed the kinetics of 8 phylogenetically different Trxs. They share a common Michaelis-Menten mechanism in which the substrate disulfide first reorient and align with the catalytic Trx thiols and then react with a S_N2 mechanism. Interestingly the rate constant for this mechanism are of the same order of magnitude ~10^5 $M^{-1}s^{-1}$ (Table 1.4) and similar even between phylogenetic distant Trx like the human or the *E. coli* one (Figure 1.10).

Table 1.4| kinetic parameters for Thioredoxin from different organisms

Trx	Disulfide substrate	k ($M^{-1}s^{-1}$)	Temp (Cº)	pH	Ref.
E.coli **Trx1**	Insulin	10^5	25	7	[113]
E.coli **Trx1**	$(I27_{G32C-A75C})_8$	$2.5x10^5$	25	7.2	[98]
E.coli **Trx2**	$(I27_{G32C-A75C})_8$	$1.8x10^5$	25	7.2	[98]
Human Trx2	$(I27_{G32C-A75C})_8$	$6.5x10^5$	25	7.2	[98]
Human Trx1	$(I27_{G32C-A75C})_8$	$5.2x10^5$	25	7.2	[98]
Human Trx1	hPrxII	$2.1x10^5$	25	7.4	[78]
Pea Trxm	$(I27_{G32C-A75C})_8$	$2.8x10^5$	25	7.2	[98]
P. falciparum **Trx1**	$(I27_{G32C-A75C})_8$	$4.3x10^5$	25	7.2	[98]
Poplar Trx *h*3	$(I27_{G32C-A75C})_8$	$1.2x10^5$	25	7.2	[98]
Poplar Trx *h*1	$(I27_{G32C-A75C})_8$	$2.2x10^5$	25	7.2	[98]

$(I27_{G32C-A75C})_8$ used as a substrate is a polyprotein composed of eight domains of the 27th module of human cardiac titin in which each module contains an engineered disulfide bond between the 32nd and 75th positions [114]. *Table and legend adapted from ref.* [98,114,115].

The Trx reducing mechanism thus seems to be the outcome of an evolutionary pressure to develop an enzymatic process to reduce disulfide with rates constant that would have been not achievable with simple chemical reagents.

Sulfiredoxins

Sulfiredoxin (Srx) is the enzyme responsible for the reduction of Prx-SO_2^-. It is conserved majorly amongst eukaryotes (Figure 1.11), in agreement with observations that prokaryotic Prx are less sensible to hyperoxidation. Studies with the yeast Srx showed that the reduction requires also ATP hydrolysis, Mg^{2+}, and a thiol as an electron donor [44].

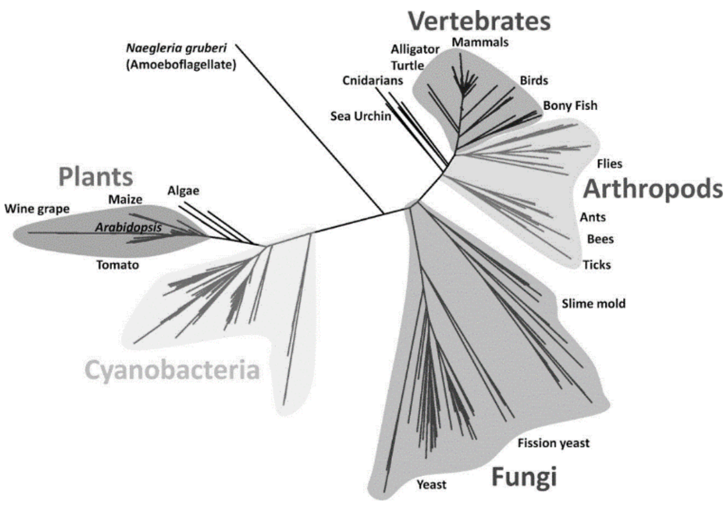


Figure 1.11| Relatedness tree for Sulfiredoxin sequences. *An unrooted phylogenetic tree of 335 Srx sequences is shown. Select organisms or groups of organisms are noted. Sequences were retrieved from the*

non-redundant protein database by BLAST on January 31, 2014, with an expect threshold of 100 using the human Srx1 sequence, and additional searches using distantly related Srx sequences did not identify further homologues. Sequences were aligned with MUSCLE, and evolutionary distances were calculated using PhyML. Figure and legend from ref [92]

The limiting step in this reaction is the formation of a thiosulfinate intermediate[45,89–91] (Srx-Prx) which existence has been confirmed for yeast [90] and human [116]. The resolution of this complex may generate an intramolecular disulfide bond Srx-SS, that is then recovered by Trx or use alternative pathways through GSH[117] (Figure 1.12).

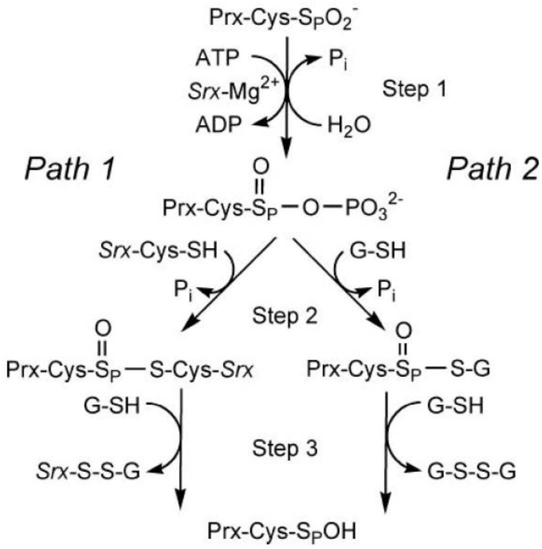


Figure 1.12| Comparison of the proposed sulfinic acid reduction mechanism of Sulfiredoxin. *Path 1 represents the mechanism originally proposed by Biteau et al. [44]. Path 2 incorporates modifications to the reaction pathway as suggested by Jeong et al. [117]. Step 1 involves the formation of the sulfinic acid phosphoryl ester intermediate. In Step 2 of the reaction, the addition of a thiol group leads to the formation of different thiosulfinate intermediates. This intermediate is subsequently resolved by GSH in Step 3. The resulting sulfenic acid form of Prx could then go on to react with Srx, GSH, and the resolving Cys of the adjacent Prx molecule to form Prx-S_P–S-Srx, Prx-S_P–S-G, Prx-S_P–S_R-Prx species. Figure and legend from ref [118].*

The reactivation is concentration dependent from Srx and limited by the Srx-Prx complex formation [45,91,116]. The k_{cat} values for the rat, human, and *Arabidopsis thaliana* Srx range from 0.1 to 1.8 min^{-1} [45,119–121].

The range of specific activities, 7–13 nmol min^{-1} mg^{-1} of protein (with hSrx ~10 nmol $min^{-1}mg^{-1}$), indicates that the Srx proteins are highly inefficient enzymes[121].

Alternative defense mechanism

Beyond Prx other enzymatic defenses have been developed by the organism to counterbalance H_2O_2 increasing concentrations.

Glutathione peroxidase (GPx) catalyzes the reduction of H_2O_2 and organic peroxides by GSH. The reaction is coupled with NADPH oxidation, via the reduction of GSSG catalyzed by glutathione reductase (GR). GPx has a homotetramer arrangement, in which every subunit contains a selenocystein that gets oxidized to selenenic acid. This reacts consecutively with two GSH molecules, the first forming a mixed selenenylsulfide, and the second reacting with this to produce reduced GPx and GSSG [122,123].

Catalase (Cat) is an enzyme typically located in peroxisomes that behaves as a dismutase at H_2O_2 concentrations above nanomolar and as a peroxidase at lower concentrations [124]. It is present in almost all aerobic organisms and many anaerobic ones. The catalase cycle takes place in two steps: the first H_2O_2 molecule oxidizes the heme to an oxyferryl species (compound I), the second H_2O_2 molecule is used as a reductant of compound I to regenerate catalase and release water and oxygen[125].

Hansen *et al*. [126] showed that the concentration of oxidizable protein thiols in human cell lines is in the order of 10 mM, which is comparable or higher than GSH concentrations. However, only a small fraction of these thiols are very reactive[37], and none of these is sufficiently abundant to contribute significantly for the H_2O_2 clearance capacity of the cells.

Hydrogen peroxide signal processing

The antioxidant enzymes (e.g. catalase, glutathione peroxidase, and the peroxiredoxins) maintain endogenous cellular concentrations of H_2O_2 in the sub-micromolar range, and they show redox-sensitive transcription in order to increase their activities in response to oxidative insults [58,127]. Thiolate groups are expected to react with H_2O_2 at rate constants in a range of 18-26 $M^{-1}s^{-1}$ at 37ºC [128]. Other than those in the active centers of peroxidases and peroxiredoxins, few protein thiols characterized to date have H_2O_2 reactivities above 100 $M^{-1}s^{-1}$ [129,130] (see Table 1.2), and none of these is sufficiently abundant to compete with the H_2O_2 clearance capacity of the cells.

Given the tight control of the H_2O_2 concentration and the low reactivities and expression of the possible signaling targets it is unlikely that a direct oxidation of these would be the main mechanism of signal transmission. A possible solution to this conundrum, the “flood-gate hypothesis”, was proposed by Wood *et al.* [82]. In this hypothesis, Prxs act as a peroxide floodgate, controlling the oxidants concentration and protecting the most reactive thiols. The inactivation of Prx by hyperoxidation caused by a local spike in the H_2O_2 concentration would allow H_2O_2 to temporarily accumulate and trigger the signaling cascade. This hypothesis relies on a high degree of coordination between NOX and antioxidant defense for the propagation of the signal, and although this may be possible in IOS conditions it seems unlikely under BOS/LOS and adds a potentially slow tier in the signaling cascade, delaying the sensing process.

An alternative, but not exclusive, explanation of the peroxide signal propagation is the “redox-relay”. In this scenario highly reactive proteins as Prxs or GPxs mediate the transduction by sensing and oxidizing specific protein thiols.

Here we focus on the capacity of the PTTRS to behave as a readout of the redox signals transferring disulfide moiety to the target proteins avoiding direct interaction, and possible irreversible damage

The Peroxiredoxin/Thioredoxin/Thioredoxin Reductase System as hydrogen peroxide sensor.

The conserved characteristics, even amongst kingdoms, and the high level of expression make of the PTTRS a perfect candidate for mediating redox signals. In particular, it is becoming more evident the presence and interaction of this system in several redox relays.

Recently Sobotta *et al.* ref. [131], reported that in HEK293T cells hPrxII directly oxidize the signaling protein STAT3 [131].

STAT3 is a member of the STAT protein family. Proteins in this family translocate to the cell nucleus upon activation, and there they activate the STAT response (*e.g.* pro-oncogenic factor, interleukin-6 pathways and NF-*k*B)[132]. Specifically, STAT3 responds to ligands interferons, growth factors and Interleukin-6 and may be activated also via MAPK [132].

Another redox regulated protein, linked to inflammatory response and cancer is NF-kB. Reduced Trx react with NF-*k*B allowing it to translocate in the nucleus and induce the response (i.e. IL-6 thus possibly activating STAT3)[133]. Important are also the evidences of redox relay that are activated upon oxidative insults due to the oxidations through Trx[134,135].

The PTTR system components, in particular Prx and Trx, show also synergies in the regulation of oxidative stress defense in a redox relay fashion by regulating the Pap1[95,136,137] and Yap1[138,139] pathways respectively in *S.pombe* and *S. cerevisiae*.

García-Santamarina *et al.* ref. [135] showed that treatment of Schizosaccharomyces pombe (*S. pombe*) with 0.2 mM H_2O_2 (below the toxicity levels for this organism), induces a transient general oxidation of thiols and the consequent formation of disulfide in many proteins. These include enzymes involved in antioxidant functions, Trx substrates, proteins related to proteasome, ribosomal proteins and metabolic enzymes. The authors also found mixed disulfides of Trx1 with target proteins in extracts of H_2O_2-treated cells. Such mixed disulfides are intermediates in the normal oxidation/reduction of protein thiols/disulfides by Trx. Subsequent studies from the same author, ref. [134], showed that in *S. pombe* ΔTrxR deletants the accumulated oxidized form of Trx oxidizes a variety of thiol-containing proteins, and that these proteins are not oxidized in ΔTrxΔTrxR double mutant. Likewise, Baty *et al.* ref. [140] showed that treatment of Jurkat cells with H_2O_2 leads to a selective oxidation of the most reactive protein thiols. Altogether, these observations indicate that oxidative pulses lead to a quick oxidation of the Trx pool and in turn trigger a substantial oxidation of solvent-exposed protein thiols to disulfides.

PTTRS components also interact with different crucial proliferation/apoptosis regulatory factors.

Reduced Trx is able to form a complex with apoptosis signaling kinase-1 (Ask-1) inhibiting its activation[141]. Upon oxidative stress Trx oxidation by hPrxI scavenging activity has been shown to lead to the dissociation and activation of Ask-1[142,143] and eventually to cell death (Figure 1.13).

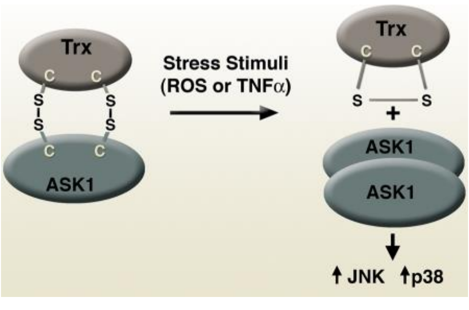


Figure 1.13| Trx-Ask1 interaction. *The interaction between Trx and ASK1 is redox dependent and in turn modulate the capacity of the transcription factor to activate effectors such as p38 MAPk and c-Jun-N-terminal kinase (JNK). Figure and legend adapted from ref. [47]*

Normally present as oligomers, Prxs can build higher-order complexes upon oxidative stress forming spherical aggregates or linear structure (Figure 1.7) which have been showed to have chaperone activities and are strictly linked with the hyperoxidation state of the reactive cysteine[83,89]. Day *et al.* ref. [94] showed hyperoxidation to be fundamental for survival of for S. pombe under extreme oxidative conditions. In these situation the deactivation of the Prx cycle would leave Trx able to cope with the organism redox unbalance. The chaperone activity of the sulfinic form of Prx would help in protecting the protein from aggregation and unfolding. Hyperoxidized form of hPrxI has been identified as regulatory for the c-ABI tyrosine kinase by inhibiting its activity, similarly with c-Myc or c-Jun [144].

Growth factor activation of the of the insulin pathway has be proposed by Kwon *et al.* ref.[145] to entail the local hyperoxidation of Prx. One of the fundamental transducer enzymes in the insulin signaling network, is phosphatidylinositol 3-kinase (PI3k). Upon stimulation of the cell with a growth factor this enzyme catalyzes the production of PI 3,4,5-trisphosphate (PIP3) which in turn activates Akt pathways. The formation of PIP3 is reversed by PTEN/PTP1B , which as other members of the PTP family is inactivated by H_2O_2 [146] (Figure 1.14).

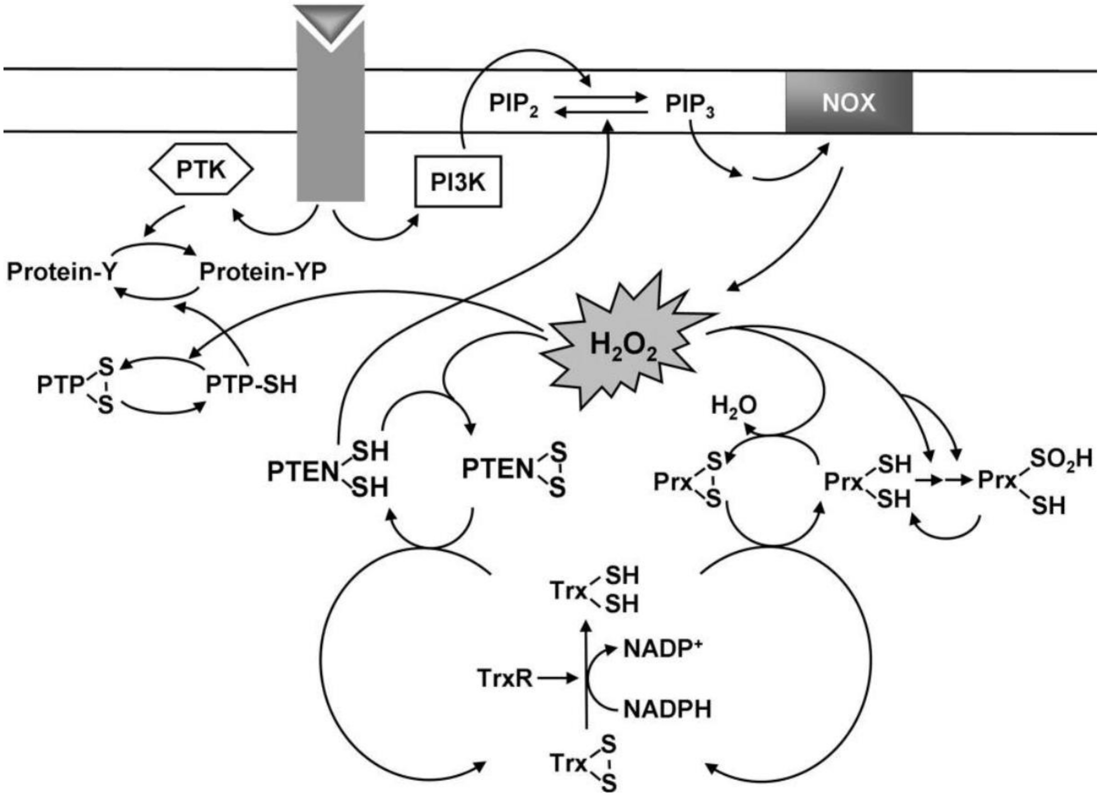


Figure 1.14| Model for the production, signaling role, and removal of H_2O_2 in growth factor-stimulated cells. *Stimulation of cells with a growth factor induces the activation of PI 3-kinase (PI3K), which catalyzes the conversion of PI(4,5)P_2 to PIP_3. PIP_3 activates the NADPH oxidase (NOX) complex, resulting in the production of H_2O_2. The H_2O_2 so generated likely mediates inactivation of cytosolic Prx molecules located nearby through a two-step oxidation of the active site Cys–SH to Cys–SO_2H. The inactivation of Prx in turn promotes local accumulation of H_2O_2. The results of the present study suggest that the accumulated H_2O_2 molecules inactivate PTEN by oxidizing the catalytic cysteine residue. This inactivation of PTEN increases the abundance of PIP_3 sufficiently to trigger downstream signaling events. The H_2O_2 signal is likely terminated by the reactivation of sulfinylated Prx and the consequent removal of H_2O_2. As the local concentration of H_2O_2 decreases, oxidized PTEN is reactivated by thioredoxin (Trx), which in turn receives reducing equivalents from NADPH by means of thioredoxin reductase (TrxR). Figure and legend ref.* [145]

The hyperoxidation of Prx allows local spikes in the H_2O_2 concentration and thus generate the oxidation of PTEN/PTP1B. Furthermore, recently it has been proved that hPrxI and PTEN/PTP1B physically interact in a redox dependent fashion [147] with the hyperoxidation of the former unbinding the complex PTEN-hPrxI and allowing for its activation.

The oxidized for of PTEN is then recovered by Trx [148]

But referring to Table 1.2 the reactivity of PTP1B is low [3] even when compared to the average thiolate rate constant, this would imply the maintenance of high intracellular concentration for prolonged time even if locally. It is thus till questionable a direct oxidation of PTEN by H_2O_2. The mediating role of PI3K in the overexpression of hPrxI, in response to LPS[149], could imply for an adaptation mechanism to the ligand presence by increasing the total Prx and limiting the H_2O_2 activity. This would in fact, in the longer period, increase the amount of reduced Prx that could bind PTEN limiting its activity and bringing back the system to a pre-stimulus situation.

Further downstream the PI3K pathways there are targets like Akt and the MAPk/ERK pathways [150], of which the latter has been proved to interact with hPrxI [151].

The concentration of the hyperoxidized form of Prx also undergoes a circadian oscillation in human erythrocytes [93,152].

It is thus evident the central role, even if through different complementary mechanism, of the PTTRS in transducing redox signals

Aim of the thesis

The present thesis combines theoretical, computational and experimental approaches with the aims of answering the following questions:

I. Considering the central role of the PTTRS in transducing the redox signaling and the different modes and mechanisms of response to OS. **Q1**: what qualitatively distinct types of stress responses are possible (e.g. proliferation vs apoptosis), and what conditions (*i.e.*, relative amounts and kinetic parameters of the PTTRS proteins) prompt each type of response? **Q2**: how do the PTTRS components transition with stress (e.g. proportional, ultrasensitivity etc.)? **Q3**: how the PTTRS components genes can be regulated to obtain perfect adaptation?
II. A central value in redox-biology is the concentration of H_2O_2 attained in physiological condition. Despite the several available methods for measuring it, there is still lack of a consensus about this value. **Q4**: what the maximum concentration of H_2O_2 attained *in vivo*?

The manuscript will follow a logic that drives the reader from the mathematical to the animal model exploring first the results of a theoretical approach and then showing the steps for obtaining the experimental values.

Chapter 2 | Design principles for thiol redox signaling: mapping the phenotypic repertoire of the cytoplasmic 2-Cys peroxiredoxin – thioredoxin system

This chapter is adapted from the current manuscript in preparation:

Selvaggio G., Oliveira V., Coelho P. M. B. M. and Salvador A
Design principles for thiol redox signaling: mapping the phenotypic repertoire of the cytoplasmic 2-Cys peroxiredoxin – thioredoxin system.

G.S. and A.S conceived the study. G.S., A.S., P.C. and V.O. performed analyses and collected data. G.S., A.S. and P.C discussed results and wrote the manuscript.

Abstract

Typical 2-Cys peroxiredoxins and thioredoxin are increasingly recognized to play a central role in antioxidant protection and redox signaling in the cytoplasm of eukaryotic cells. The molecular properties and cellular abundances of these proteins have been extensively characterized. Studies highlighted many commonalities among cells and organisms, but also intriguing differences in cells' responses to hydrogen peroxide. Relating these phenotypes to molecular properties and composition is crucial for understanding redox signaling and guiding potential therapeutic interventions, but non-trivial. Here we present a systematic analysis of this problem based on an idealized mathematical model that captures the features of the system that are common to most eukaryotic cells. The analysis identifies 12 regions of qualitatively distinct behavior in the protein composition space. This includes broad regions where effective antioxidant protection and reliable proportional signaling can coexist and regions where protection and/or signaling would be dysfunctional. Depending on the relative abundances of peroxiredoxins, sulfiredoxin, thioredoxin, thioredoxin reductase and alternative H_2O_2-consuming proteins, the system is capable of distinct responses to changing hydrogen peroxide supplies, including proportional, ultrasensitive, and hysteretic (toggle switch) ones. The model correctly predicts the distinct responses of human erythrocytes and Jurkat T cells to hydrogen peroxide based on these cells' composition. We predict that in cells that have abundant capacity for peroxiredoxin reduction and a limited peroxiredoxin-independent hydrogen peroxide clearance capacity the peroxiredoxin-thioredoxin system shows bistability and hysteresis at high hydrogen peroxide supply rates. Using proteomic data for multiple human cell lines, we show that their composition is commensurate with this phenotype under stress. Finally, we derive a set of design principles for effective redox signaling and antioxidant protection and examine the functional consequences of the distinct properties of human PrxI and PrxII, of modulations of the reactivity of these peroxiredoxins, and of gene expression changes.

Introduction

Peroxiredoxins (Prx) are a class of ubiquitously expressed proteins, from archaea to humans [66,67]. They show abundances, structural similarities and properties that are conserved even amongst kingdoms [153]. The Prx1/AhpC subfamily, commonly known as 2-Cys Prx [154,155] are highly express [70–77,155], localized both in nucleus and cytoplasm show rate constant for reaction with H_2O_2 that span from 10^6 to 10^8 $M^{-1}s^{-1}$ [78–80]. Their characteristics and location gives them key features to control cellular H_2O_2 concentrations and are proposed to modulate peroxide signaling [69]. These peroxiredoxins, which include human peroxiredoxin I and II (PrxI, PrxII), reduce H_2O_2 through the following three-step cycle (Figure 2.1).H_2O_2 oxidizes a thiolate (peroxidatic cysteine) in the active site to a sulfenic acid (Prx-SO^-), which then condenses with a Cys thiol (resolving cysteine) from an adjacent monomer to form a disulfide (Prx-SS). The rate of this step is limited by a conformational change (local unfolding, LU) that is required to bring the sulfenate and the resolving cysteine into close proximity. The cycle is then closed by the reduction of Prx-SS often carried out by Thioredoxin (Trx), returning Prx to its fully folded (FF) structure. The reducing equivalents to restore Trx to its reduced form and the thermodynamic driving force for the cycle come from NADPH oxidation, under Thioredoxin Reductase (TrxR)

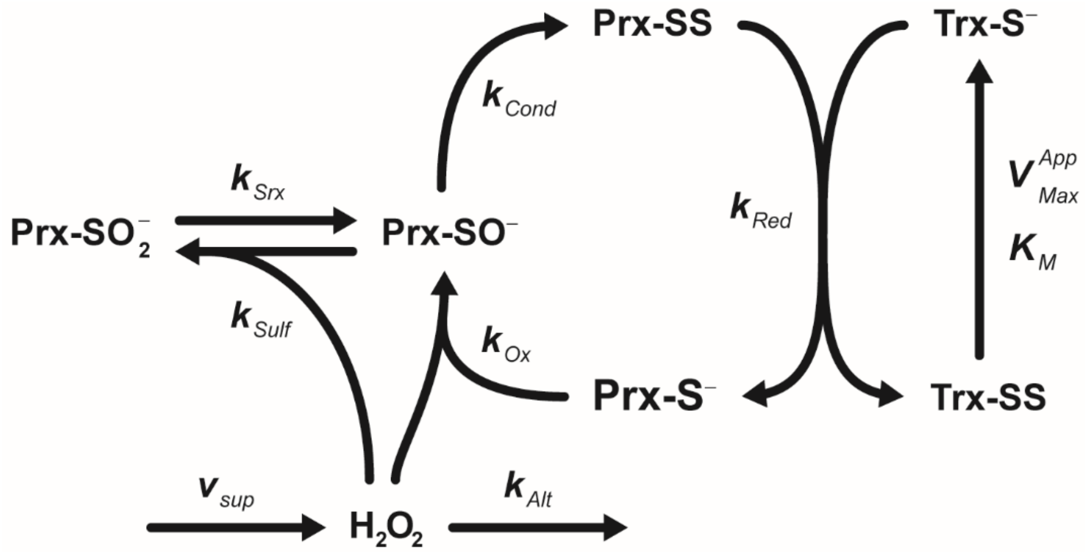


Figure 2.1| The peroxiredoxin / thioredoxin / thioredoxin reductase system (PTTRS) model. *this schematic representation of the real model aggregates the alternative sinks of H_2O_2 in a unique pseudo-first order consumption reaction, and assume a one substrate Michaelis Menten reaction for Thioredoxin Reductase activity, thus considering saturating concentrations of NADPH for the enzyme.*

Eukaryotic Prxs of the Prx1/AhpC subfamily are susceptible to inactivation by their own substrates due to the conversion of the sulfonate intermediate to sulfinate (Prx-SO_2^-) and sulfonate (Prx-SO_3^-) [87,156]. This phenomenon, called "hyperoxidation", is facilitated by phylogenetically conserved structural features that delay the local unfolding step, thereby promoting the accumulation of the sulfonate and its reaction with H_2O_2 [82]. PrxSO_2^- can be slowly reduced to the sulfenic form (Prx-SO^-) at the expense of ATP and reducing equivalents under catalysis by Sulfiredoxin (Srx) [88]. In several organisms hyperoxidation occurs not only upon extreme insults but also under physiological conditions [93].

The absence in Eukaryotic cells of a specific H_2O_2 sensors like OxyR [58] in bacteria, drive the research of possible transducer. The Peroxiredoxin/Thioredoxin/Thioredoxin Reductase system (PRTTRS) has emerged to play a crucial role in peroxide signal transmission. The species of the PTTRS show different mechanism of conveying the signal to the target proteins.

It has been shown that, in HEK293T cells, hPrxII directly oxidize and activates (redox relay) the signaling protein STAT3 [131] which is involved into cell growth and inflammatory response (*e.g.* pro-oncogenic factor, interleukin-6 pathways and NF-*k*B)[132]. Another redox relay regulation of a protein, linked to inflammatory response and cancer, is NF-kB. Reduced Trx react with NF-*k*B allowing it to translocate in the nucleus and induce the response (i.e. IL-6 thus possibly activating STAT3)[133]. It has been showed that hPrxI is able to inhibit NF-*k*B translocation into the nucleus[157]. Also in *S. pombe* and *S. cerevisiae*. the PTTR system components, in particular Prx and Trx, show regulation of oxidative stress defense in a redox relay fashion by regulating the Pap1[95,136,137] and Yap1[138,139] pathways. García-Santamarina *et al.* ref. [135] showed that treatment of Schizosaccharomyces pombe (*S. pombe*) with 0.2 mM H_2O_2, induces a transient general oxidation of thiols and the consequent formation of disulfide in many proteins. A similar results was obtained by Pillay *et al.* [158] that described, using a kinetic model of the Trx system in *E.coli* an ultrasensitivity response in the redox couples of the PTTRS based on the TrxR contribution.

Reduced Trx is also able to form a complex with Ask-1 inhibiting its activation[141], although Trx oxidation and hPrxI scavenging reactions lead to Ask-1 activation [142] and eventually to cell death. In the "floodgate hypothesis", Wood *et al.* [82] postulated that the hyperoxidation of Prx leads to a spike in the H_2O_2 concentration, that is then able to oxidize target proteins and propagate the signal. This mechanism however implies, if not mediated by other enzymes, long period for the response since the average protein thiols react with H_2O_2 with a rate constant of 18-26 $M^{-1}s^{-1}$ at 37ºC [128] In agreement with this hypothesis, hPrxI interact with PTEN[159], by forming a complex that is release upon hyperoxidation of the scavenger PTEN is then oxidized [146] and inhibits proliferation, this action is then rescued through Trx [148].

Whereas local inactivation of Prx requires coordination with NADPH-oxidase, a global hyperoxidation and thus a progressive increase of internal H_2O_2 has been observed in *S. pombe by Tomalin et al.* ref.[160]. This bi-phasic behavior has been associated with a progressive saturation of the internal antioxidant defense until a critical point where hyperoxidation becomes dominant. Being the main properties and the structure of the PTTR system conserved, we would expect that there are general principles connecting design to function and thus allowing us to make prediction on also the dynamic properties.

Intriguingly, whereas in some cell types exposure to H_2O_2 leads Prx to accumulate in the hyperoxidized form with limited Prx-driven thioredoxin oxidation (Phenotype S), in other cell types the same treatment leads both Prx and Trx to accumulate in the disulfide form (Phenotype D) [161]. This occurs even in organisms sharing the same genetic background and despite Phenotype S cell often carrying a lower proportion of the more hyperoxidation susceptible PrxII relative to PrxI (e.g. Erythrocytes and Jurkat cells [161]). It is so important to understand the system to answer to the following questions: **Q1**: what qualitatively distinct types of stress responses are possible, and what conditions (*i.e.*, relative amounts and kinetic parameters of the PTTRS proteins) prompt each type of response?

Q2: how do the PTTRS components transition with stress (e.g. proportional, ultrasensitivity etc.)?

Antioxidant defenses are upregulated in response to oxidative stress, this homeostatic regulation can allow the system to adapt to an increased basal H_2O_2 concentration restoring the previous redox state of the organism. **Q3**: what changes in PTTRS gene expression can return the system to a good region?

In order to address these questions we used the design space approach[162–164], a mathematical framework that allowed us, starting from a simple model, to study the different phenotypes that the PTTRS can produce and their characteristics.. Our results show that the system is cable of distinct responses to changing in H_2O_2 supplies, including proportional, ultrasensitive, and hysteretic ones, depending on the relative abundances of 2-Cys peroxiredoxins, Trx1, Srx, TrxR and alternative hydrogen peroxide sinks. The model correctly predicts the distinct responses of human erythrocytes and Jurkat T cells to hydrogen peroxide based on these cells' composition. The relative abundances of the above-mentioned proteins in all the 11 human cell lines examined is such as to avoid oxidation of Trx to the disulfide form at high hydrogen peroxide supply rates unless TrxR is inhibited or NADPH depleted. Further, they favor the occurrence of a hysteretic toggle-switch form a low hyperoxidation to a high hyperoxidation state under stress.

Finally, we derive a set of design principles for effective redox signaling and antioxidant protection and examine the functional consequences of the distinct properties of human PrxI and PrxII, of modulations of the reactivity of these peroxiredoxins, and of gene expression changes.

Model Formulation

We set up a minimal model that captures the basic features of the PTTRS common to most cells where it occurs (Figure 2.1). In this model the supply of H_2O_2 to the system (v_{sup}) includes both endogenous and exogenous sources.

The rate constant for H_2O_2 reduction by the thiolate form of Prx (k_{Ox}) is treated as an effective rate constant, which allows to consider the effect of an inhibition, such as recently postulated to happen in human erythrocytes [81]. H_2O_2 scavenging is divided between Prx-mediated and alternative sinks. The latter include the H_2O_2 efflux from the cytoplasm and the activities of catalase, peroxidases and 1-cys Prx. These were aggregated into a single flux and treated as a first-order.

The reduction of Prx-SO_2^- to Prx-SO^- is treated as a pseudo-first-order process. This process is catalyzed by Srx. Its rate-limiting step is the formation of a thiosulfinate intermediate [45,89–91,116] (Srx-Prx). The resolution of this complex may generate an intramolecular disulfide bond Srx-SS, that is then recovered by Trx or use alternative pathways through GSH [117]. The reactivation has been shown to be concentration dependent from Srx and limited by the Srx-Prx complex formation [45,91,116], based on this we approximate this reaction with a pseudo first order mechanism with rate dependent on the limiting step (Sulfiredoxin concentration and activity, Appendix A).

The reduction of Trx-SS was treated as a one-substrate Michaelis-Menten process. This process is catalyzed by TrxR and follows a ping-pong mechanism that uses NADPH as second substrate [165]. However, physiological concentrations of NADPH usually substantially exceed TrxR's apparent K_M for this substrate. For instance, human TrxR1 has a $K_{M,TrxR,NADPH}$ of 6 µM [166] and the apparent $K_M(\text{NADPH})$ will be substantially lower when the enzyme is far from saturation with Trx-SS. In turn, in absence of strong oxidative stress cytoplasmic NADPH concentrations are in the range 50-150 µM for *S. cerevisiae* and *E.coli* [167,168] and hRBCs contain ~40µM [169] but most of it is bound to proteins [170].

From the scheme in Figure 2.1 we derived the following system of algebraic differential equations:

$$\begin{cases} \frac{dH_2O_2}{dt} = v_{sup} - k_{Alt} \cdot H_2O_2 - k_{Ox} \cdot \text{Prx-S}^- \cdot H_2O_2 - k_{Sulf} \cdot \text{Prx-SO}^- \cdot H_2O_2 \\ \frac{d\text{Prx-SO}^-}{dt} = k_{Ox} \cdot \text{Prx-S}^- \cdot H_2O_2 + k_{Srx} \cdot \text{Prx-SO}_2^- - k_{Sulf} \cdot \text{Prx-SO}^- \cdot H_2O_2 - k_{Cond} \cdot \text{Prx-SO}^- \\ \frac{d\text{Prx-SO}_2^-}{dt} = k_{Sulf} \cdot \text{Prx-SO}^- \cdot H_2O_2 - k_{Srx} \cdot \text{Prx-SO}_2^- \\ \frac{d\text{Prx-SS}}{dt} = k_{Cond} \cdot \text{Prx-SO}^- - k_{Red} \cdot \text{Trx-S}^- \cdot \text{Prx-SS} \\ \frac{d\text{Trx-SS}}{dt} = k_{Red} \cdot \text{Trx-S}^- \cdot \text{Prx-SS} - V_{Max}^{App} \cdot \text{Trx-SS} \cdot X^{-1} \\ X = K_M + \text{Trx-SS} \\ \text{Prx}_T = \text{Prx-S}^- + \text{Prx-SS} + \text{Prx-SO}^- + \text{Prx-SO}_2^- \\ \text{Trx}_T = \text{Trx-S}^- + \text{Trx-SS} \end{cases} \quad (2.1)$$

Results

A phenotypic map of the PTTRS

We seek to map the properties of the system as function of kinetic parameters and protein concentrations. As a starting point, this requires analyzing the steady state solutions of the model described Equations (2.1). However, these solutions cannot be expressed in closed analytical form, and the large number of parameters prevents an effective numerical exploration. We therefore applied the system design space methodology [162,164] to obtain an intelligible approximate description. This methodology subdivides the parameters space into a set of regions. The dynamics in each region is described by a distinct combination of alternatively dominant production and consumption fluxes for each dynamic concentration, and of alternatively dominant concentrations among the forms included in each moiety-conservation cycle. Whenever a region contains a steady state solution, this is guaranteed to be unique and analytically described by a simple power law of the parameters. By the construction of the approximation, these regions represent qualitatively distinct behaviors of the system, and are accordingly denoted by "*phenotypic regions*". The parameters space partitioned into the phenotypic regions set of regions is called the system's "*design space*".

The construction of the design space for the PTTRS model is explained in the Appendix A. This design space contains 13 regions with positive steady state solutions. Not all of these are representative of the phenotypes of real cells, though. In order to select the biologically plausible regions, one has to consider the ranges of kinetic parameters and protein concentrations found in real cells. We consider the following three plausibility criteria cumulatively.

First, the maximum flux of reduction of the sulfinylated form of Peroxiredoxin is the lowest maximum flux of the system. Srx is an inefficient enzyme [45,88,90,91] and is much less abundant in cells than the other proteins considered in the model (Sulfiredoxin concentration and activity, Appendix A).

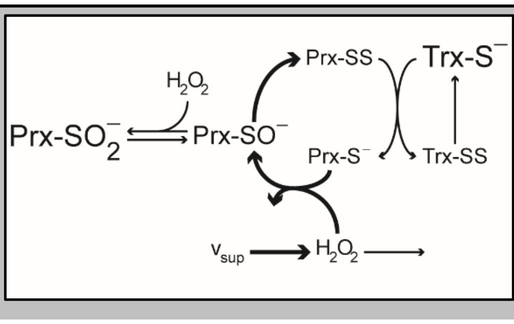
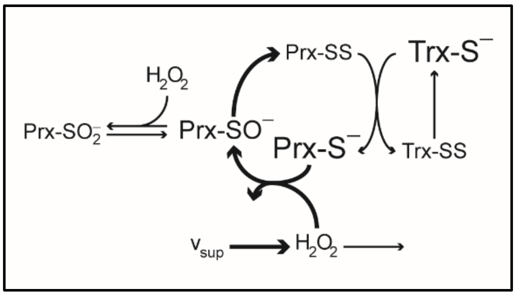
Second, the pseudo-first order-rate constant for H_2O_2 reduction by Prx-S^- strongly exceeds the rate constant for Prx-SO^- condensation. This follows from the high reactivity ($k_{Ox} \sim 10^6 - 10^8 M^{-1}s^{-1}$ [171])

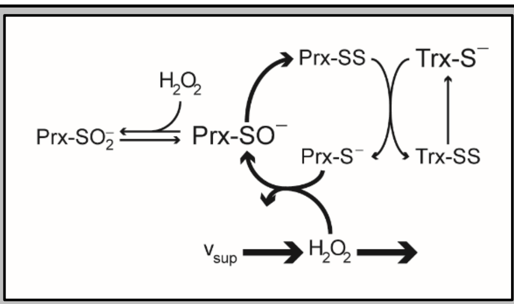
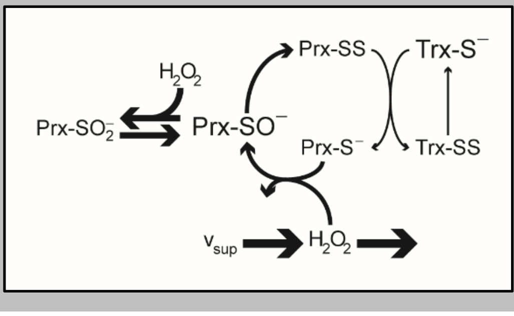
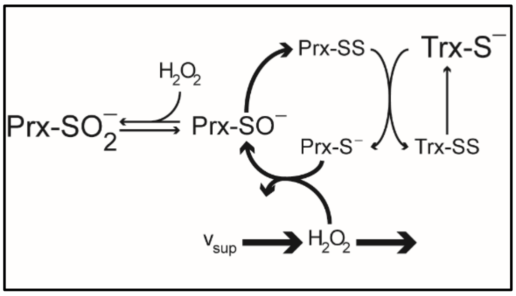
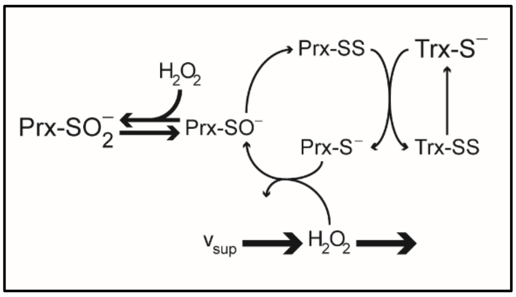
and abundance (tens to hundreds of μM, Supplementary Material) of typical 2-Cys peroxiredoxins in the cytoplasm. In turn, in eukaryotic typical 2-Cys peroxiredoxins the rate of condensation is limited by a local unfolding step that is required to bring the resolving cysteine into proximity with the sulfenate.

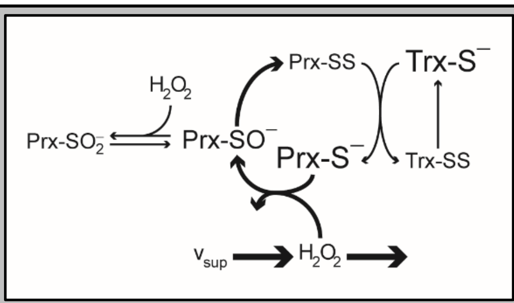
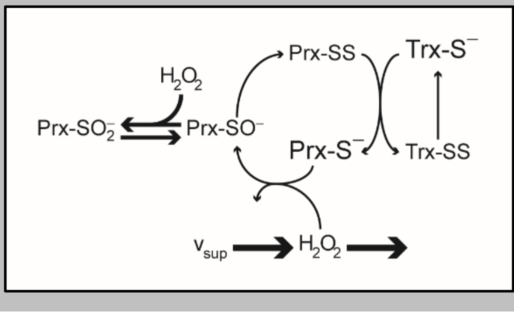
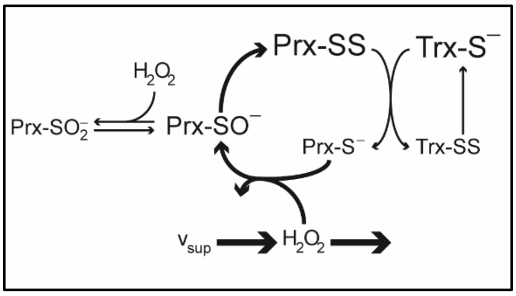
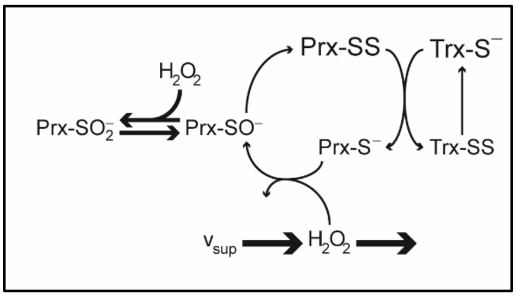
Third, Prx sulfinylation is the slowest among all (aggregated) H_2O_2-consuming processes in the model. The former process consumes H_2O_2 with a second order rate constant that has been measured for human PrxII as $1.2 \times 10^4\ M^{-1}s^{-1}$ [87].

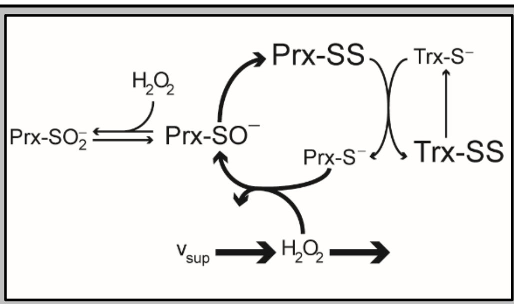
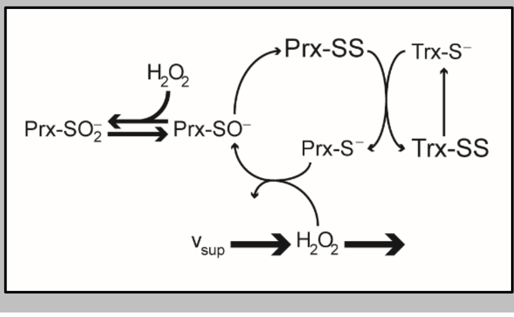
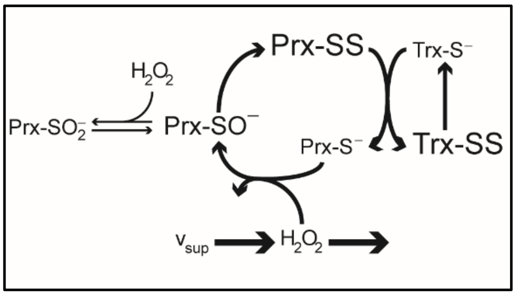
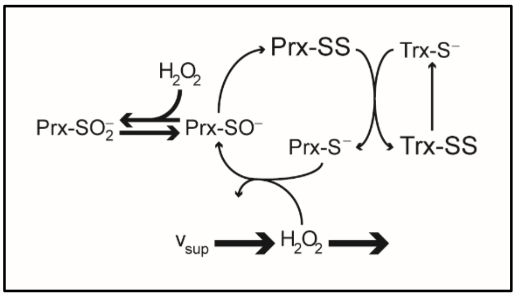
Only the eight phenotypic regions that we describe below (see Table 2 for properties) satisfy the three plausibility criteria above.

Table 2.1| Biologically relevant regimes represented by the dominant species in that region and the consequent scheme of the system. *The XYZW nomenclature of the regions describes the regions' properties as follows: X, oxidation state of Prx ("T": thiol," H": hyperoxidized," S": sulfenic," D": disulfide); Y, oxidation state of Trx ("T": thiol," D": disulfide); Z, major H_2O_2 scavenger ("P": Prx," A": alternative sinks); W saturation of TrxR enzyme with Trx-SS ("U": unsaturated," S": saturated). Relative symbol sizes and arrow widths reflect relative concentrations and relative fluxes, respectively. * Unstable steady state region.*

Region	Dominant Species	Phenotype
HTPU	$Prx\text{-}SO_2^-$ $Trx\text{-}S^-$	
TTPU	$Prx\text{-}S^-$ $Trx\text{-}S^-$	

STAU	Prx-SO$^-$ Trx-S$^-$	 
HTAU	Prx-SO$_2^-$ Trx-S$^-$	 

TTAU	Prx-S$^-$ Trx-S$^-$	 
DTAU	Prx-SS Trx-S$^-$	 

DDAU	Prx-SS Trx-SS	 
DDAS	Prx-SS Trx-SS	 

Phenotypic regions TTPU and TTAU are characterized by the thiol(ate) forms of Prx and Trx being the dominant ones and differ on whether most of the H_2O_2 is consumed by Prx (TTPU) or by alternative sinks (TTAU). These regions occur where cumulatively the H_2O_2 supply is low and the TrxR activity is not too low. In these regions, the concentrations of Prx-SO$^-$, Prx-SS and Trx-SS show a linear response to changes in v_{sup}.

Region HTAU is characterized by extensive Prx sulfinylation and low Trx oxidation. This region occurs where, cumulatively v_{sup} is very high and the TrxR activity is not too low.

Under some conditions, regions TTPU and HTAU overlap. When this occurs there is also a region (HTPU) of unstable steady states that coincides with the overlap between TTPU and HTAU. This feature reveals the possibility of bistability and hysteresis in this system. We discuss the conditions where this behavior occurs and its implications in a subsequent section.

Regions STAU and DTAU are characterized by Trx being predominantly in thiol form and the dominant Prx forms being the sulfenic or the disulfide ones, respectively. Both regions occur at intermediate H_2O_2 supplies and high TrxR activities, only under conditions where regions TTPU and HTAU do not overlap. In both STAU and DTAU, the concentrations of the sulfenic and disulfide forms of Prx and of the thiol and disulfide forms of Trx are all virtually independent of the H_2O_2 supply. The system is thus unable to function as a redox relay in these regimes (response saturation).

Finally, regions DDAU and DDAS are characterized by the dominance of the disulfide forms of both Prx and Trx and differ on whether TrxR is saturated (DDAS) or not (DDAU). They occur at intermediate H_2O_2 supplies and low TrxR activities. In most cells a strong oxidation of Trx leads to apoptosis [172], which is likely due to release of ASK1 from inhibition by Trx-SH [141].

The analysis of the design space permits the following generalizations. First, provided that cells have some TrxR activity, the system can always be driven to either TTPU or TTAU by making v_{sup} sufficiently low, and to HTAU by making v_{sup} sufficiently high. However, the latter v_{sup} values are not necessarily physiological. Second, the system can always be driven to regions DDAU and DDAS through a strong enough inhibition/under expression of TrxR or Trx, though DDAS becomes unreachable at low Trx expression.

Identifying the best region for signaling and protection

Given the potential role of the PTTRS in protecting the cytoplasm against excessive H_2O_2 concentrations and in redox signaling, we now investigate in what regions both functions can be effectively fulfilled.

Our analysis will first address a mode of signaling where the output is proportional to the input, by opposition to discrete-state (e.g. binary, digital) signaling. As input variables of biological relevance, we will consider the H_2O_2 supply (v_{sup}) and TrxR activity (V_{Max}). The former input is a natural choice, as cells generate H_2O_2 as an initial response to a variety of stimuli, such as mitogenic factors [28,173]. The choice of the latter input follows from the observation that TrxR is strongly inhibited by electrophilic substances such as 4-hydroxynonenal that are produced under oxidative stress [174]. Further, TrxR inhibition is being considered as an anti-cancer therapy [175]. We consider the following broad performance criteria:

(a) **Gains**: Prx-SO$^-$, Prx-SO$_2^-$, Prx-SS and Trx-SS from the evidence in literature are responsible to transduce the H_2O_2 signal. It is thus required a positive and high sensitivity to v_{sup};

(b) **Signal robustness**: the above mentioned species must transduce a signal independently from the changes in structural parameters (e.g. protein concentrations, kinetic rates). It is thus required that the steady state values of these species are minimally dependent from parameters other than v_{sup};

(c) **Signal Stability**: the system steady state must be stable. Biological systems are intrinsically noisy, there are continuous perturbations of the steady state: an unstable system would break the signaling chain diverging even for small variation of v_{sup}.

Table 2 summarizes the performance of the system within each of the eight plausible phenotypic regions (full description in Table A.4 Appendix A). Regions TTPU and TTAU show the best performances according to most of the criteria. Performances are more robust in TTPU than in TTAU, but the dynamic range for v_{sup} extends to higher values in TTAU (Appendix A) owing to the higher contribution of the alternative sinks for clearing H_2O_2.

In both TTPU and TTAU the settling time is characterized by $\frac{k_{Cond}}{k_{Srx}}$ (see Appendix A, Stability analysis). The higher this ratio the slower the system will reach the new equilibrium. The rise time instead has a strong correlation with $\frac{k_{Cond}}{k_{Ox} \cdot Prx_T}$ for TTPU, and $\frac{k_{Cond}}{k_{Alt}}$ for TTAU (Appendix A, Stability analysis). Therefore, within these regions the establishment of a new equilibrium is limited by the Srx activity and thus very slow, but the rise time depends on the dominant scavenging activity and can be shortened by increasing its expression.

Table 2.2| Evaluation of the local performance in all biologically relevant Regions. *The criteria were listed under the sub-section "Performance criteria" of the Methods. The symbols: {"++","+","-","--"} indicates the degree of compliancy to the criteria, respectively from good to worse (the values are reported in the supplementary material).*

Criteria\Regions	HTPU	TTPU	STAU	HTAU	TTAU	DTAU	DDAU	DDAS
Sensitivity of Prx-SO- to v_{sup}	+	+	--	--	+	--	--	--
Robust Prx-SO$^-$	++	++	++	-	-	-	-	+
Sensitivity of Prx-SO$_2^-$ to v_{sup}	--	++	+	--	++	+	+	+
Robust Prx-SO$_2^-$	++	-	-	++	--	--	--	-
Sensitivity of Prx-SS to v_{sup}	+	+	--	--	+	--	--	--
Robust Prx-SS	+	+	-	--	-	++	++	++
Sensitivity of Trx-SS to v_{sup}	+	+	--	--	+	--	--	--
Robust Trx-SS	+	+	-	--	-	-	++	++
Stability	--	++	++	++	++	++	++	++
Overall	-	++	--	--	+	--	--	--

The system will always be in the optimal region (TTAU or TTPU) as long as the following condition is satisfied:

$$v_{\text{sup}} < \frac{\text{Min}\left(k_{Cond}\cdot \text{Prx}_\text{T}, \sqrt{\frac{k_{Cond}\cdot k_{Ox}\cdot k_{Srx}}{k_{Sulf}}}\cdot \text{Prx}_\text{T}, k_{Red}\cdot \text{Trx}_\text{T}\cdot \text{Prx}_\text{T}, V_{Max}^{App}, \frac{V_{Max}^{App}\cdot \text{Trx}_\text{T}}{\text{K}_\text{M}}\right)}{\text{Min}\left(1, \frac{k_{Ox}\cdot \text{Prx}_\text{T}}{k_{Alt}}\right)} \tag{2.2}$$

Defining for all organism the possible dynamic range of the peroxide signal that is optimally sensed by the system.

Responses to stress

Strong increases in H_2O_2 supply and/or inhibitions of TrxR eventually drive the system from the good-performance TTAU/TTPU regions into any of the sub-optimal regions. These excursions across the boundaries of the good regions represent stress responses. Below we address the following two questions about these stress responses. **Q1**: what qualitatively distinct types of stress responses are possible, and what conditions (*i.e.*, relative amounts and kinetic parameters of the PTTRS proteins) prompt each type of response? **Q2**: how does the system transition to stress? In order to do so systematically, we analyzed the topologically distinct ways how the 8 plausible phenotypic regions can be arranged over the plane defined by the scaled parameters $\phi = \frac{v_{\text{sup}}}{k_{Cond}\cdot Prx_T}$ and $\sigma = \frac{V_{Max}}{k_{Cond}\cdot Prx_T}$. Each of these arrangements is determined by the abundances and the kinetic parameters of the PTTRS and can be viewed as a distinct response hypersurface. Trajectories over a response hypersurface define response phenotypes.

There are 1152 qualitatively distinct response hypersurfaces in the (σ, ϕ) plane that satisfy the three biological plausibility criteria (Appendix A). Their analysis reveals only 12 possible ways of arranging the regimes under biological relevant conditions (Figure 2.2). These 12 topologies reflect all the possible stress responses.

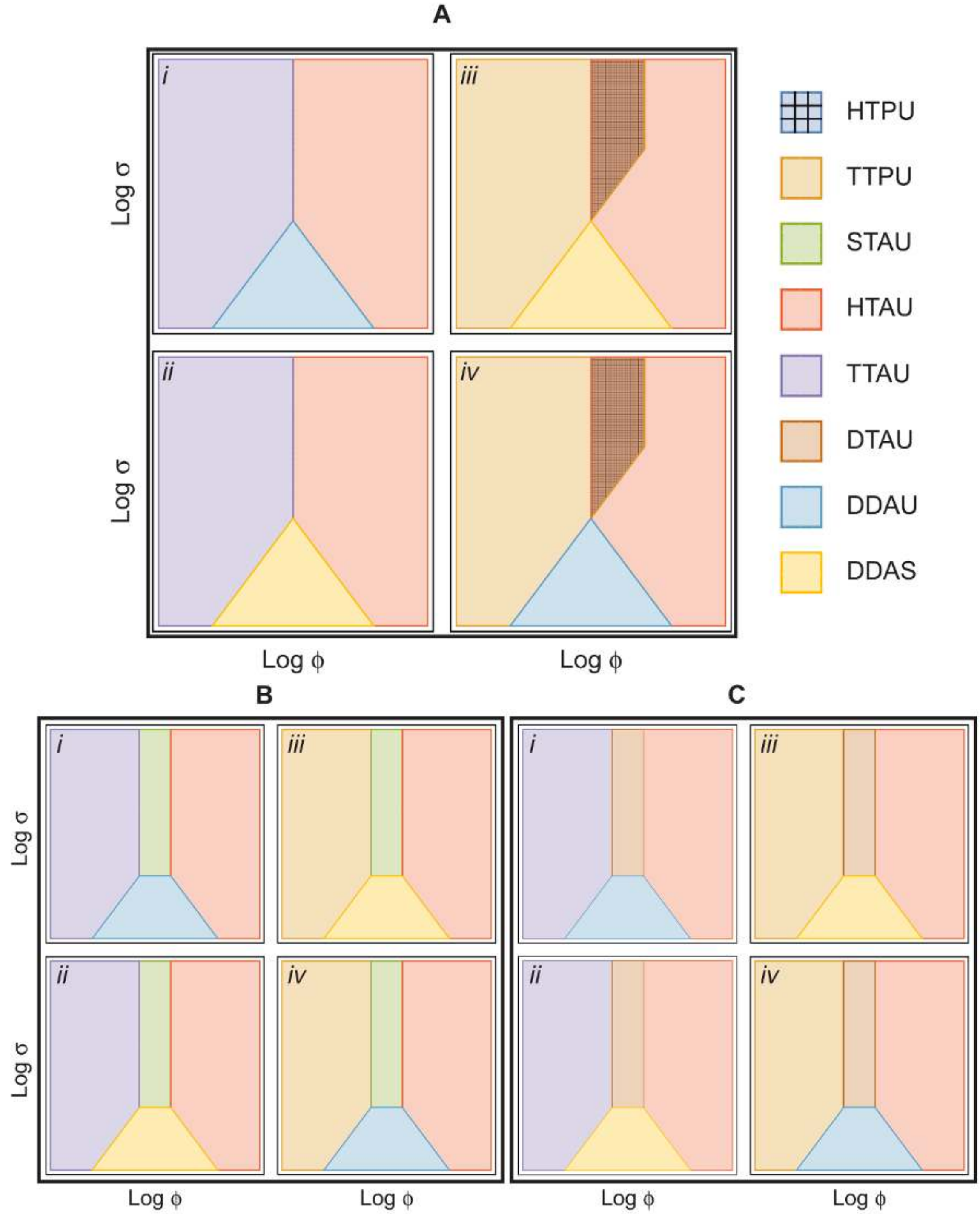


Figure 2.2| Allowed topologies of the various regions of distinct behavior of the PTTRS in the parameters space for biologically plausible conditions.

An analysis of the macroscopic phenotypes (dominant species) can be used, by pairing the regimes characteristics in Table 2.2 with the topology in Figure 2.2, to define super-families (A, B, C) of topologies which share identical modes of response along the two principal dimensions (σ,ϕ) .

It is possible to discriminate if an organism belongs to one of these by knowing the maximum flux of reduction of Prx ($k_{\text{Red}} \cdot \text{Prx}_\text{T} \cdot \text{Trx}_\text{T}$), the maximum flux of condensation of Prx ($k_{Cond} \cdot \text{Prx}_\text{T}$) and the relative activities between Srx, alternative sinks and Prx hyperoxidation ($\frac{k_{Srx} \cdot k_{Alt}}{k_{Sulf}}$).

Table 2.3| Topology Superfamilies definitions.

Super family	Conditions
A	$k_{\text{Red}} \cdot \text{Prx}_\text{T} \cdot \text{Trx}_\text{T} > \sqrt{k_{Cond} \cdot \text{Prx}_\text{T} \cdot \frac{k_{Srx} \cdot \text{Max}[k_{Alt}, k_{Ox} \cdot \text{Prx}_\text{T}]}{k_{Sulf}}} \wedge k_{Cond} \cdot \text{Prx}_\text{T} > \frac{k_{Srx} \cdot k_{Alt}}{k_{Sulf}}$
B	$\text{k}_{Cond} \cdot \text{Prx}_\text{T} < \text{Min}[k_{\text{Red}} \cdot \text{Trx}_\text{T} \cdot \text{Prx}_\text{T}, \frac{k_{Srx} \cdot k_{Alt}}{k_{Sulf}}]$
C	$k_{\text{Red}} \cdot \text{Prx}_\text{T} \cdot \text{Trx}_\text{T} < \text{Min}[k_{Cond} \cdot \text{Prx}_\text{T}, \sqrt{k_{Cond} \cdot \text{Prx}_\text{T} \cdot \frac{k_{Alt} \cdot k_{Srx}}{k_{Sulf}}}]$

Within a super-family we can identify 4 possible variations. If Prx scavenge the majority of H_2O_2 from the organism (condition *iii-iv*), then the basal regime will be TTPU vice versa TTAU (condition *i-ii*). If TrxR can be saturated by Trx-SS (condition *i-iii*), then for low TrxR activity the operating regime will be DDAS vice versa (condition *ii-iv*) will be DDAU. Although the 4 conditions (*i, ii, iii, iv*) share the same macroscopic phenotype there is a change in performances depending on the regimes involved, in particular at low-moderate v_{sup} (*e.g.* TTPU or TTAU). Moreover, conditions *iii-iv* show an ultrasensitivity transition in the internal H_2O_2 concentration when leaving regime TTPU. Instead TTAU shows a smooth transition with progressively increasing concentrations.

Differences amongst super-families arise in the behavior at moderate oxidative loads and for high activity of TrxR. The superfamily B and C here show a regime that has lost capacity to transduce oxidative signals through the PTTR system and that shows a fully reduced Trx and a Prx predominantly in the sulfenic (STAU) or disulfide form (DTAU), respectively for the superfamily B and C. At high oxidative loads, all the topologies move to regime HTAU which shows an accumulation of the hyperoxidized form of Prx. Moreover, in HTAU there is an inversion in the sensitivities of the system, this will generate a negative response to increasing concentrations of H_2O_2.

The presence of STAU and DTAU defines a region of saturation of the transducer that is no longer able to propagate further increment in the v_{sup}. Interestingly the superfamily A doesn't possess this "buffering" regimes but switch from TTPU or TTAU to HTAU. This may happen in different way within the superfamily: in the topologies A-*i* and A-*ii* this is a simple threshold-type switch, while in the topologies A-*iii* and A-*iv* there is a region of overlap that create a bistability with hysteretic behavior.

Dichotomy Threshold

What emerges from Figure 2.2 is that all the topologies can show Phenotype-S or D depending on a σ_{crit} value, directly related to the TrxR activity.

The analysis of the regimes DDAU and DDAS border, produces the following expression for σ_{crit} :

This constraint can be expressed in kinetic terms as:

$$V_{Max,TrxR}^{App} = \frac{\text{Min}\left(k_{Cond} \cdot \text{Prx}_T, k_{Red} \cdot \text{Trx}_T \cdot \text{Prx}_T, \sqrt{\frac{k_{Srx} \cdot k_{Cond} \cdot k_{Ox}}{k_{Sulf}}} \cdot \text{Prx}_T \right)}{\text{Max}\left(1, \frac{\text{Trx}_T}{K_{M,TrxSS}} \right)} \tag{2.3}$$

The tolerance of a phenotype is defined by the ratio between the actual TrxR activity and this critical value. Interestingly a prolonged oxidative insult, will progressively deplete the NADPH pool of the organism desaturating TrxR and thus decreasing $V_{Max,TrxR}^{App}$ this means that an insufficient supply of reducing equivalents combined with extreme and sustained oxidative scenarios may lead to a change in the phenotype expressed.

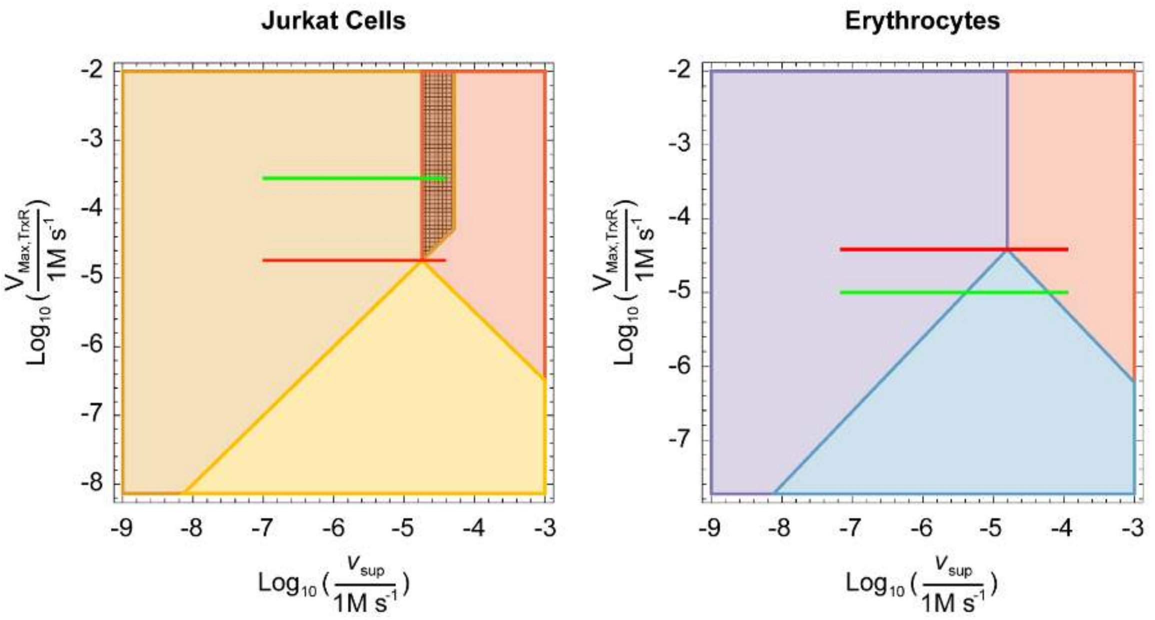


Figure 2.3| Design space of the PTTRS for human Jurkat T cells and erythrocytes. *The red lines indicate the critical value above which Phenotype S holds; the green lines indicate the physiological operating range for each cell type. The lines span over a dynamic range of external concentration that goes from 1nM-10µM of H_2O_2. The color code for the regions is the same as in the Figure 2.2.*

In Figure 2.3 are represented the design spaces of two biological instances: Human RBC and Jurkat T Cells. These two cell type represent an example of the dichotomy. hRBC possess a very high concentration (third most abundant protein) of hPrxII, which is prone to hyperoxidation. Jurkat cells on the other hand have more hPrxI than hPrxII, being the former more resistant to sulfinylation, but globally these cells have a lower Prx content when compared to RBC. Given these premises it is surprising to observe an S-phenotype in the Jurkat cells while a D-phenotype in the hRBC.

The red lines indicate the critical value $V_{Max,TrxR}^{App}$ above which Phenotype S holds; the green lines indicate the physiological operating range for each cell type. Both Jurkat T cells and erythrocytes operate robustly above and below the threshold, respectively. Further, the operating range for Jurkat T cells extends into the multi-stability region.

Bistability Region

The bistable region between TTPU and HTAU translates into a hysteretic dynamic, because upon intensive oxidative insults above a certain v_{sup} peroxiredoxin will accumulate from reduced to its hyperoxidized form. The reverse phenomenon, after the progressive removal of the stress, will instead occur at a lower v_{sup}. Therefore, in the overlapping region, for the same H_2O_2 supply Prx can be either very or moderately hyperoxidized, and H_2O_{2i} have either µM or nM concentrations, depending on whether cells had been previously exposed to high or low H_2O_2, respectively.

The bistability region exists only for the topology superfamily A, conditions *iii-iv*. As a matter of fact, the unstable regime HTPU, whose existence defines the bistable behavior has as necessary and sufficient requirements the ones that defines the superfamily A (Table 2.3), in particular conditions *iii-iv* (Prxs major H_2O_2 scavenger).

The bistability region is present when the TrxR activity is greater than the threshold defined in Equation (2.3) and coincide with HTPU regimes borders:

$$\mathrm{Min}\left(k_{Cond}\cdot \mathrm{Prx_T}, k_{\mathrm{Red}}\cdot \mathrm{Prx_T}\cdot \mathrm{Trx_T}, \sqrt{k_{Cond}\cdot \mathrm{Prx_T}\frac{k_{Ox}\cdot k_{Srx}}{k_{Sulf}}}\right) > v_{\mathrm{sup}} > \sqrt{k_{Cond}\cdot \mathrm{Prx_T}\frac{k_{Alt}\cdot k_{Srx}}{k_{Sulf}}} \qquad (2.4)$$

Strikingly as can be also seen in the Appendix A, the bistability region is largely present in nature[160]. In particular cancer cell lines present an A-*iii* topology and show a S-phenotype; but it may or may not be reached because of the intensity of external stimuli or of the TrxR activity which is usually upregulate in cancer[176].

Adaptation

Biological systems show the capacity, through feed-back control, to adapt themselves to a persistent signal by returning to a pre-stimulus internal condition (perfect adaptation). How should the adaptive gene expression program respond to a sustained oxidative load in order to restore the PTTRS to the region of best performance? The question is motivated by the observation that preconditioning of organism (hormesis) lead to a general over-expression of the defense and to a higher resistance. Also, high H_2O_2 levels may trigger the ARE-mediated response, which includes the induction of most of the proteins considered in the model.

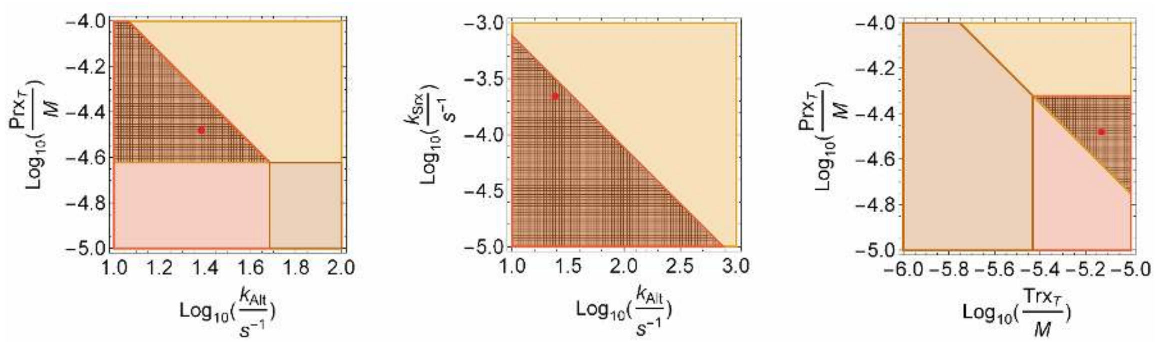


Figure 2.4| Gene regulation of the PTTRS components to induce adaptation. *Design space of Jurkat T cells for external H_2O_2 concentration of 10µM and k_{Alt}=24.2 (including PrxVI activity). The red dot identifies the operational point of the cell line. The color code of the regions is the same as in Figure 2.2.*

Figure 2.4 highlights possible control solutions that can lead to perfect adaptation. The overexpression of Prxs, peroxidases, catalase or Srx can by itself return the operating point to the good performance TTPU region. However, neither a decrease nor an increase in Trx abundance can have that effect.

Discussion

Peroxiredoxins are abundantly expressed in several organisms, they possess a high catalytic rate, which requires a very specific amino acidic arrangement of the active site. The substrate inactivation, to which the Prx1/AhpC-subfamily are sensitive, is detrimental to the scavenging capacity of this enzyme. This would have implied the loss of these defenses gene from the genomes instead they are widely expressed and conserved due to a selective pressure.

Various oxidized forms of the Prx and Trx can specifically oxidize other proteins, thereby regulating their activities [95,131,177] in a redox-dependent manner. The PTTRS can thus participate not only in defense against peroxides but also as a peroxide signal transducer. The study we perform on the possible modes of response of this system to hydrogen peroxide supply highlights two regions (TTAU and TTPU) where the orchestration of protein concentrations and kinetic parameters grant best performances for proportional (*i.e.* analogic) signaling. In these regions, the fractions of Prx in sulfenic and disulfide forms and of Trx in disulfide form — which are hypothesized to relay oxidizing equivalents to redox signaling targets — respond linearly to increasing v_{sup}, and signaling is robust to fluctuations in most parameters and total protein concentrations. The approximate dynamic range for proportional signaling by the PTTRS is given by Equation (2.2).Remarkably, the operating range for all the twelve cell types that we tested lies in these regions for physiological values of v_{sup}. In particular, the upper bounds of the physiological (non-stress) range of v_{sup} lie within the dynamic range defined above. These observations indicate that the PTTRS not only can work as part of a redox relay as proposed before [134,135] but is designed by natural selection to ensure reliable analogic signal transduction. .

Our analysis also highlights that the PTTSR can exhibit several qualitatively distinct stress responses to high v_{sup} or diminished TrxR activity, and maps these stress phenotypes to protein composition. The TrxR activity threshold defined by Equation (2.3) allows to discriminate between cells that will show Phenotype-S or Phenotype-D.

Factors like: Yap1[138], Pap1[136], Ask-1 [141,143] are activated by accumulation of Trx in disulphide form. The two regions DDAU and DDAS, which cells showing phenotype D enter at high oxidative loads, can thus trigger apoptosis. The latter does not happen in erythrocytes, which lack a ASK-1-mediated apoptosis pathway, but would be a fatal problem for other cells. Remarkably, among the 12 human cell types we analyzed, erythrocytes are the only ones predicted to exhibit Phenotype D. All the proliferative cell lines are predicted to show Phenotype S (Figure A.6).

Phenotype-S instead presents an accumulation of Prx in its sulfinic form and ultimately preserves the Trx pool reduced, this has been showed by Day *et al.*[94] to be fundamental for survival of for *S. pombe* under extreme oxidative conditions, arising the possibility that a deactivation of the Prx cycle would leave Trx able to cope with the organism redox equilibrium. In particular, it has been shown that reduced Trx inhibits ASK-1 by forming a complex with the protein, thus preventing apoptosis initiation. Furthermore, the chaperone activity of the sulfinic form of Prx helps in protecting the protein from aggregation and unfolding.

The modes of response of the PTTRS may change depending on the relative concentrations of these proteins at intermediate oxidative loads. There are three super-families of response: B and C present a regime that has lost capacity to transduce oxidative signals and that shows a fully reduced Trx and a Prx predominantly in the sulfenic (STAU) or disulfide form (DTAU), respectively for the superfamily B and C. This regime is located between the low oxidative stress operating region (TTAU or TTPU) and the high oxidative load region HTAU. It is reachable only for TrxR activities that are above the threshold defined by Equation (2.3). While superfamily A lacks this unresponsive regimes.

In the work of Tomalin *et al.* ref. [160] *S. pombe* and HEK293 cells show a drastic increase in the intracellular concentration of H_2O_2 paired with a stiff increase off the hyperoxidized form of the Prx isoform present in the organism. Previous work showed that a treatment with 0.2mM cause the majority of Trx to become rapidly oxidized [95,178]. These two characteristics identify *S. pombe* as belonging to superfamily A. In Figure A.6 our analysis predicts for HEK293 cells a phenotype A (in agreement with the results of Tomalin *et al.*) but considering the relative proximity of the operating point (green line) to the threshold it is possible that for prolonged insults the TrxR activity is lowered by a progressive exhaustion of the NADPH pool this could eventually lead to cross the disulfide regimes. The proteomic data however, present quantitative estimations issues, in particular related to the correct representation of the proteome. A possible solution would be to design specific experiments in order to address the bistability and oxidation state of Trx and Prx together.

The Phenotype-S, in the case A-*iii* and A-*iv* of Figure 2, with its bistability region perfectly implements in vivo yet another example of a Schmidt Trigger, were the system is able to sense an input in a noisy environment activating the downstream cascade of events only after a threshold is trespassed and deactivating it when the input drops below a lower one. This bistable window within the two thresholds identify the amplitude of the noise that is eventually tolerated by the system, thus its robustness to the signal variation. This structure usually accounts for important decision-making phenomenon (e.g. neuron action-potential), which activation implies a very high cost for the organism: here we hypothesize that this correspond to the drastic increase in cytosolic H_2O_2

concentration. Below Th_{high} the propagation of the redox signal is assigned to peroxiredoxins that due to their abundance and catalytic rates are able to rapidly consume the peroxide and activate the cascades with a higher specificity than the ROS. The progressive inactivation of the scavenging peroxiredoxins activity with a drastic accumulation of the sulfinic form, once v_{sup} trespass Th_{high}, allows H_2O_2 to actively propagate its signal while activating at the same time the protecting holdase activity of peroxiredoxin. The role in proliferation pathways, as the control of PTEN and AKT activity, could explain why a deregulation of the PTTR system is involved in several type of cancer in human[159,179–181].

Material and Methods

Parameters for human erythrocytes were estimated as described in [81], those for Jurkat T, A549, GAMG, HEK293, Hela, HepG2, K562, LnCap, MCF7, RKO, U2OS were estimated from the literature[79,182–186] as described in Appendix A

The design space analysis, determination of the steady state properties and numerical calculations were performed in Mathematica 10.3 [187].

Chapter 3 | Hydrogen peroxide concentrations classifier

This chapter is based on the following publication:

Rubens J. R., Selvaggio G., Lu T. K. *Synthetic mixed-signal computation in living cells.* Nat. Commun. 7:11658 doi: 10.1038/ncomms11658 (2016).

J.R.R. and T.K.L. conceived the study. J.R.R. and G.S. performed experiments and collected data. All authors analyzed the data, discussed results, and wrote the manuscript. In particular, GS built and characterized the three H_2O_2 sensors and the band pass showed in Figure 3.2 and Figure 3.3.

The authors have filed patents based on this work.:

ANALOG TO DIGITAL COMPUTATIONS IN BIOLOGICAL SYSTEMS,

International Publication Number **WO 2016/106319**

PROBIOTIC ORGANISMS FOR DIAGNOSIS, MONITORING, AND TREATMENT OF INFLAMMATORY BOWEL DISEASE,

International Publication Number **WO 2016/106343**

Abstract

Living cells implement complex computations upon the continuous environmental signals that they encounter. These computations involve both analog and digital-like processing of signals to give rise to complex developmental programs, context-dependent behaviors, and homeostatic activities. In contrast to natural biological systems, synthetic biological systems have largely focused on either digital or analog computation separately. Here, we integrate analog and digital computation to implement complex hybrid synthetic genetic programs in living cells. In particular, we present a framework for building comparator gene circuits to digitize analog inputs based on different thresholds. We then demonstrate that comparators can be predictably composed together to build bandpass filters, ternary logic systems, and multi-level analog-to-digital converters (ADCs). Additionally, we interface these analog-to-digital circuits with other digital gene circuits to enable concentration-dependent logic.

The sensors developed in this work are able, when combined to work as a hydrogen peroxide classifier, by opportunely color coding different concentrations of the molecule. The integration of analog and digital element allows to sense and memorize external stimuli.

We expect that this hybrid computational paradigm will enable new industrial, diagnostic, and therapeutic applications with engineered cells.

Introduction

Analog and digital computation each have distinct advantages for cellular computing[188]. Digital computation in synthetic [189–195] and natural biological systems is useful for signal integration given its relative robustness to noise [196] and is exemplified by decision-making circuits, such as those in developmental programs that lead cells into differentiated states [197]. Analog computation is useful for signal processing in synthetic [198–200] or natural biological systems when the output needs to be dependent on graded information or continuous functions of the inputs, such as the sum or ratio of energy sources [201,202]. However, analog signal integration is susceptible to noise, making it challenging to design robust synthetic genetic programs [203]. Here, we combine the benefits of analog signal processing with digital signal integration to create artificial mixed-signal gene networks that carry out new hybrid functions in living cells.

Our approach is to process signals from front-end analog sensors with composable input-discretization devices that are analogous to electronic comparators. The outputs of these devices can then be processed in a digital fashion with downstream circuits. This strategy of explicitly digitizing analog signals followed by digital computing stages is conceptually different than other mixed-signal computing approaches, such as fuzzy logic, neural networks, and hybrid automata, in which analog and digital processing are intricately coupled. However, the components developed here may be useful for future gene circuits implementing the latter form of hybrid computing. Electronic comparators compare analog voltages between two terminals (V_+ and V_-) and output a digital OFF or ON signal (or "LO" or "HI") if $V_+ < V_-$ or $V_+ > V_-$, respectively[204]. Rather than voltage, our genetic comparators take the concentration of an activated transcription factor as their input. The transcription factor acts a front-end sensor for continuous information (e.g., the concentration of a small molecule), and should ideally operate over a wide input dynamic range to enable multiple genetic comparators with different thresholds to discretize the same input into multiple distinct outputs. In contrast to previously developed thresholding circuits that modulate continuous levels of gene expression in response to molecular concentration and could be used as comparators [205–209], our comparators convert molecular concentration into digital gene expression. This enabled us to create higher-order mixed-signal circuits that also take on digital gene expression states, such as 2-bit analog to digital converters and ternary logic circuits, in contrast to previous mixed-signal circuits, such as filters that are essentially 1-bit analog-to-digital converters [210–213].

Results

Genetic Comparators Digitize Analog Gene Expression

We first created an analog sensor for the reactive oxygen species hydrogen peroxide (H_2O_2). H_2O_2 plays intricate biological roles across all kingdoms of life, and its regulation is linked to human health and disease[214]. H_2O_2 oxidizes and activates the *E. coli* transcription factor OxyR [58,59,215,216]. We constitutively expressed OxyR to set a minimum concentration of OxyR in the cell, since genomically expressed *oxyR* is auto-negatively regulated, and we placed *gfp* under the control of the OxyR-regulated oxyS promoter (oxySp) on the same low copy plasmid (LCP) (Figure B.1). We found that GFP expression was continuously increased by H_2O_2 over more than two orders of magnitude of concentration, indicating that OxyR is a wide-dynamic-range analog sensor for H_2O_2 in this context.

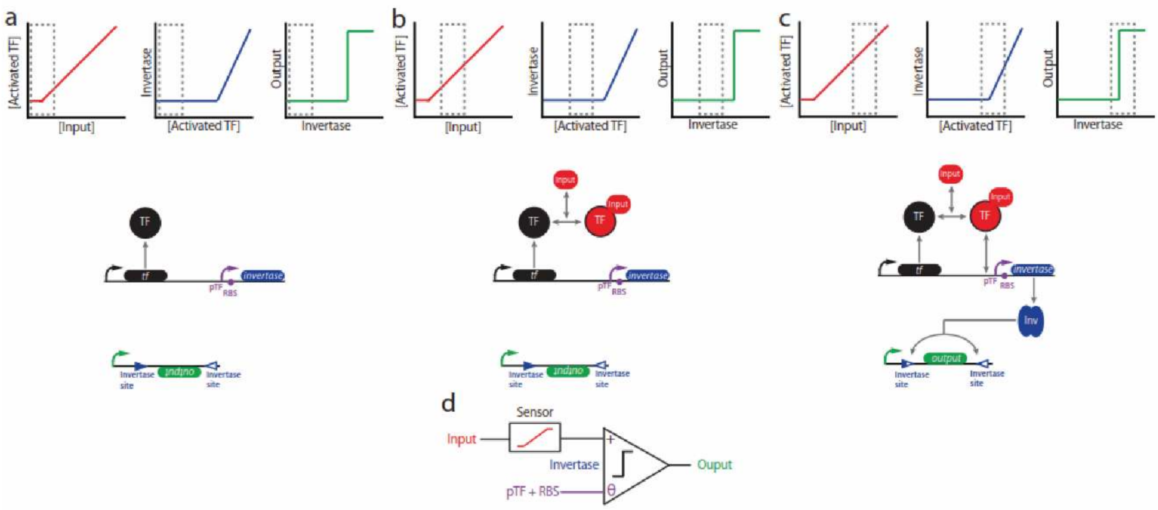


Figure 3.1| Comparator overview. *(a). At low input concentrations, the transcription factor gene (tf) is constitutively expressed, but the TF is not activated to a significant level. Consequently, the invertase gene is not expressed. (b). At medium input concentrations, the TF is activated (red TF bound to Input), but it is below the concentration needed for significant expression of the invertase gene. (c). At high input concentrations, the concentration of activated TF is sufficient to activate expression of the invertase from a specific promoter (pTF). The Invertase (Inv) binds to the invertase sites (triangles) and inverts the DNA between the sites. This results in the expression of the output gene by the upstream promoter (green arrow), leading to output expression. (d). A genetic comparator abstraction. It is composed of the threshold module (purple), the digitization module (blue) and the output module (green). An input activates a sensor (such as a transcription factor), and this transcription factor activates the expression of an invertase at an input threshold θ defined by the affinity of the invertase promoter for the activated transcription factor and by the translation strength of the invertase as defined by its RBS. When the invertase is expressed, the output is switched ON.*

We then created genetic comparators (Figure 3.1), which can be conceptualized as composed of three components. The first component is the threshold module. It includes a promoter, which is regulated by the transcription factor, and a ribosome binding site (RBS) that together set the expression level of the downstream recombinase gene and determine the threshold for comparator activation. This is in contrast to electronic comparators, where a second input can dynamically set the threshold (e.g., V_-). The second module is the digitization module, which is composed of a recombinase whose expression is controlled by the threshold module. The recombinase digitizes the input value by inverting the orientation of a targeted DNA segment maintained at a very low copy number. The third module is the DNA that is inverted by the recombinase, which can contain a gene or gene-regulatory elements, such as a transcriptional promoters or terminators, to alter expression of the desired output(s).

The digitization aspect of the comparator relies on recombinases, and thus we explored how the number of sites targeted by recombinases affects signal digitization into two distinct gene expression states within individual cells. The serine integrases (recombinases) we used flip, excise, or integrate DNA depending on the orientation of *attB* and *attP* recombinase-recognition sites, and their activity is unidirectional unless co-factors are present[217]. Recombinases have been used to build digital counters[218], integrate logic and memory[219], and amplify input-output transfer functions[220]. To discretize H_2O_2 input levels, we placed the Bxb1 recombinase under the control of the oxySp promoter on a LCP. In order to keep the basal level of *bxb1* minimal such that there is little recombinase activity in the cell in the un-induced state, we added a ClpXP-mediated degradation tag to the 3' end of the *bxb1* coding sequence[221] (Figure B.2-a). We tested two options as reporters for recombinase activity: a medium copy plasmid (MCP, maintained at 20-30 copies per cell[222]) and a bacterial artificial chromosome (BAC, maintained at 1-2 copies per cell[223]), each of which contained a constitutive promoter upstream of an inverted *gfp* gene flanked by oppositely oriented *attB* and *attP* sites.

We induced *bxb1* expression at different concentrations of H_2O_2 and measured GFP expression via flow cytometry (Figure B.2-b,d). We set a threshold for calling cells GFP "ON" or "OFF" and used this threshold to calculate the percent of cells that were ON (%ON) at each concentration of H_2O_2 (Data Processing and Calculations appendix). The %ON vs. H_2O_2 concentration data was fit to a sigmoidal function to generate input-output transfer functions (Data Processing and Calculations appendix). The MCP and BAC reporters had similar transfer functions, although cells using the MCP reporter had a higher percent of cells ON at the basal H_2O_2 concentration (Figure B.2-b). However, GFP expression in cells with the MCP reporter exhibited a multi-modal distribution especially at intermediate concentrations of H_2O_2, which suggests partial plasmid flipping and thus mixed GFP expression levels in different cells (Figure B.2-d). This effect was further demonstrated by increases in the geometric mean of GFP levels with increasing H_2O_2 in the ON population (Figure B.2-f). In contrast, cells with the BAC reporter only exhibited a bi-modal distribution (Figure B.2-c), and the geometric mean of the ON population only marginally increased with H_2O_2 concentration (Figure B.2-e). Thus, we concluded that the BAC reporter converts the input concentration of H_2O_2 into digital OFF and ON gene expression states within individual cells better than the MCP reporter.

We further sought to demonstrate that our analog-to-digital comparator circuits could be used to drive downstream circuits in a trans-acting fashion. To construct a cascade, we replaced *gfp* in the BAC expression operon with *tetR* and placed *gfp* under the control of the TetR-regulated promoter pLtetO on a MCP (Figure B.3). In the absence of H_2O_2, the majority of cells expressed *gfp* and were in the ON state. In the presence of H_2O_2, *gfp* expression from pLtetO was efficiently repressed and the majority of cells were switched into an OFF state. These results demonstrate that recombinase circuits can be used together with trans-acting regulation to assemble functional cascades. We also developed a method to simplify the quantification of OFF versus ON since fluorescent gene expression levels from the BAC are low and can result in overlapping OFF and ON gene expression distributions in flow cytometry. This method amplifies the copy number of the reporter from low to high but preserves the bi-modal nature of the OFF and ON populations, thus confirming the digital flipping of the BAC (Figure B.4).

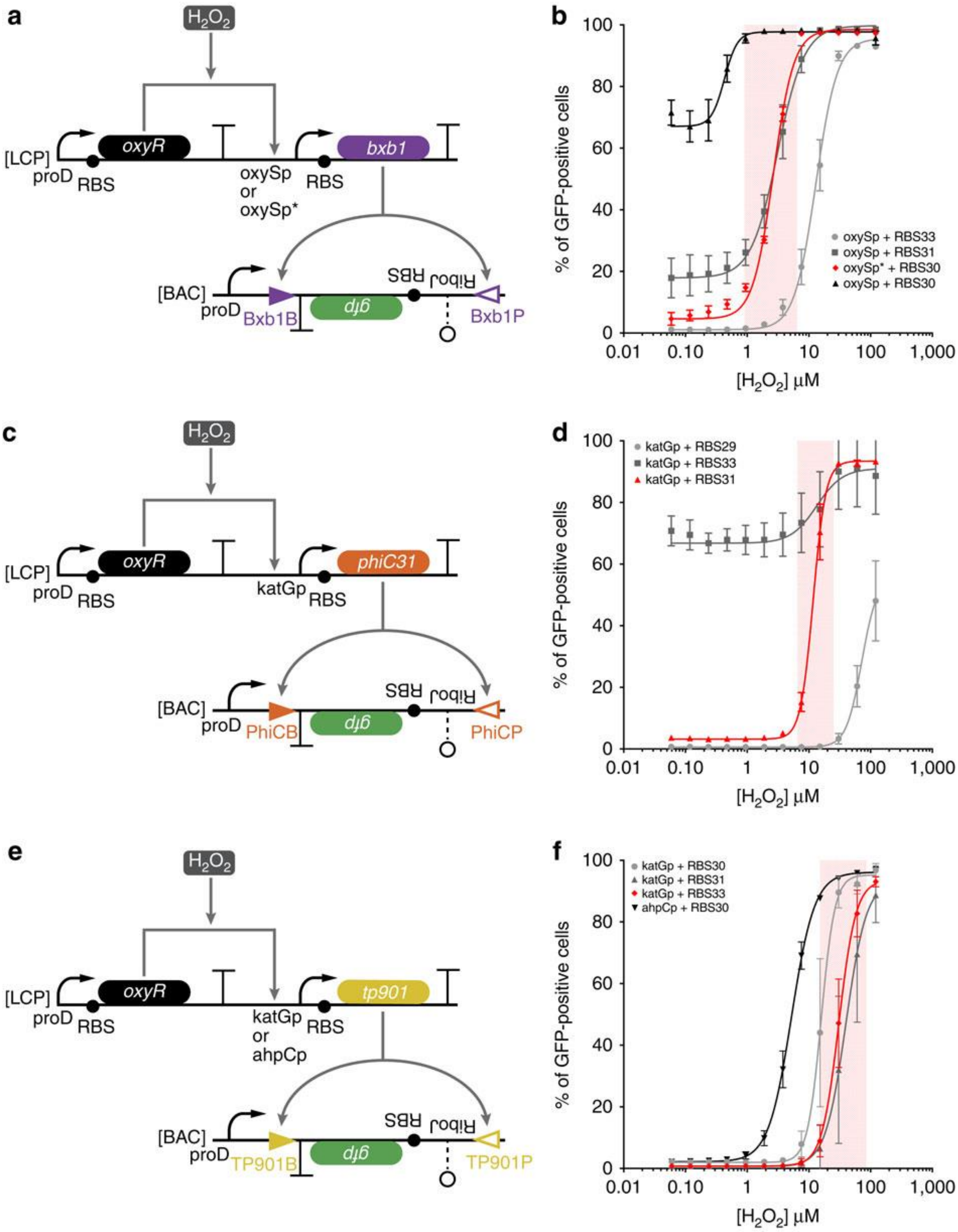


Figure 3.2| Genetic comparators with different activation thresholds. *(a). The low-threshold H_2O_2 comparator circuit. OxyR is constitutively expressed from a low-copy plasmid (LCP) and activates transcription of bxb1 recombinase from either the oxySp or oxySp* promoter on the same LCP in response to H_2O_2. Bxb1 translation is altered by the strength of the ribosome binding site (RBS). Bxb1 inverts the gfp expression cassette located between inversely oriented attB and attP sites (triangles) on a bacterial artificial chromosome (BAC), thus turning on GFP expression. The gfp cassette has a ribozyme sequence for cleaving the 5' untranslated region of an mRNA transcript (RiboJ)* [224]*, a computationally designed RBS* [225]*, the gfp coding sequence, and a transcriptional terminator. (b). The percent of GFP positive cells at different H_2O_2 concentrations as measured by flow cytometry. Different combinations of oxySp and oxySp* promoters and RBSs exhibit different H_2O_2 thresholds and basal levels for GFP activation. The oxySp* and RBS30*

combination (red diamonds) had the lowest threshold and a narrow transition band (shaded region). (c). The medium-threshold H_2O_2 comparator circuit. The same as Figure 3.2-a, except with the katGp promoter instead of the oxySp or oxySp promoters, and phiC31 recombinase and att inversion sites instead of bxb1 recombinase and att inversion sites. (d). Different combinations of the katGp promoter and RBSs had different H_2O_2 thresholds and basal levels for GFP activation. The katGp and RBS31 combination (red triangles) had a medium H_2O_2 threshold and narrow transition band (shaded region). (e). The high-threshold H_2O_2 comparator circuit. The same as Figure 3.2-a, except with either the katGp promoter or ahpCp promoter instead of the oxySp or oxySp* promoters, and tp901 recombinase and att inversion sites instead of bxb1 recombinase and att inversion sites. (f). Different combinations of katGp and ahpCp promoters and RBSs exhibited different H_2O_2 thresholds for GFP activation. The katGp and RBS33 combination (red diamonds) had the highest threshold and a narrow transition band (shaded region). Lines are sigmoidal fits to the data (Data Processing and Calculations appendix). The errors (standard deviation) are derived from flow cytometry experiments of three biological replicates, each of which involved n > 30,000 gated events.*

The threshold module of the comparator can be used to shift the discretization threshold. We created comparators with different thresholds and transition bands (e.g., the input dynamic range) by assembling combinations of promoters with different transcription-factor affinities, ribosome binding sites, and recombinases (Figure 3.2). We defined the transition band as the range of H_2O_2 concentrations across which the percent of cells expressing the output fluorophore is between 10% and 90% as interpolated from the transfer function (though on a single cell level, gene expression is binary), and we calculated the "relative input range" of the transition band to define its width (Data Processing and Calculations appendix). A narrow relative input range is indicative of low variability across the cell population around the input threshold for state switching, which is important for robustness to noise[226].

The low-threshold comparator used the Bxb1 recombinase and the oxySp promoter, which is activated at low H_2O_2 concentrations. We screened different RBSs in this construct and found that none of these circuits turned ON below 1 µM H_2O_2 without also exhibiting a high basal level of recombinase activity (Figure 3.2-a). To address this issue and reduce basal bxb1 expression, we used a strong RBS (RBS30) and randomly mutated the -10 region of the oxySp promoter to create a low-threshold comparator that had a transition band between 0.91-6.44 µM H_2O_2, giving it a relative input range of 7.10 (Figure 3.2-b,

Figure 3.1-a). To create a medium-threshold comparator, we tested different RBSs controlling phiC31 recombinase translation from the katGp promoter (Figure 3.2-c). A circuit with RBS31 had a transition band of 6.50-25.13 µM, which is a relative input range of 3.87 (Figure 3.2-d,

Figure 3.1-b). To create a high-threshold comparator, we used *tp901* recombinase and screened different RBS and promoter combinations (Figure 3.2-e). We first tried the ahpCp promoter, but found that this promoter-recombinase combination had an intermediate activation threshold. We instead turned to the katGp promoter and tested different RBSs. Using RBS33 yielded a circuit with improved behavior, with a transition band of 15.19-85.49 µM H_2O_2 and relative input range of 5.63 (Figure 3.2-f**,** Figure 3.1-c).

Complex Signal Processing Circuits Composed of Genetic Comparators

Comparators with different thresholds can be composed together to build more complex signal-processing circuits in living cells (Figure 3.3 and Figure 3.4). For example, circuits that turn gene expression ON with increasing input concentrations (as in Figure 3.2) can be considered high-pass circuits (since they allow high-concentration inputs to "pass" or be outputted). Next, to create low-pass circuits (which only allow low-concentration inputs to "pass"), we built a gene expression cassette that was ON in the basal state and used an inducible recombinase circuit to turn the output gene OFF by inverting the upstream promoter. Then, to create bandpass filters (Figure 3.3), we combined a low-threshold high-pass circuit with either a medium- or high-threshold low-pass circuit (Figure 3.3-a,c), thus implementing the logic in Figure 3.3-e.

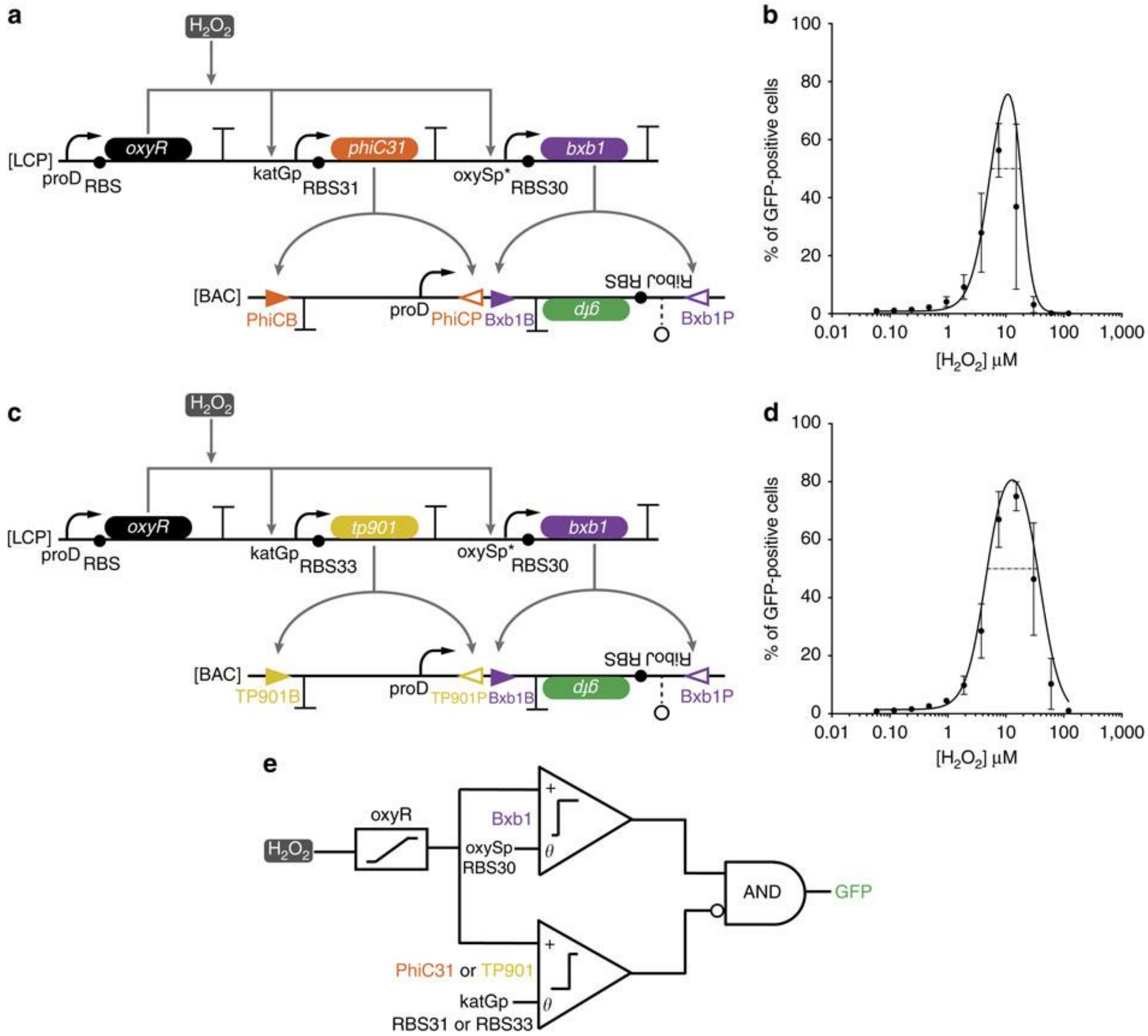


Figure 3.3| Bandpass filters assembled from low-pass and high-pass filters. *(a). The low-threshold and medium-threshold bandpass filter circuit. OxyR is constitutively expressed and activates transcription of bxb1 and phiC31 in response to H_2O_2. Bxb1 inverts the gfp cassette to enable expression from the upright proD promoter, while PhiC31 inverts the proD promoter to turn off GFP production. (b). The percent of GFP positive cells at different H_2O_2 concentrations as measured by flow cytometry for the circuit shown in Figure 3.3-a (black circles). The transfer functions of the comparators composing the bandpass were characterized to generate the predicted bandpass transfer function (black line), $R^2 = 0.75$ (Figure B.6). The dashed black line demarcates the 50% ON relative input range. (c). The low-threshold and high-threshold bandpass filter circuit. Same as Figure 3.3-a, except RBS33 and tp901 replace RBS31 and phiC31, respectively. (d). Same as Figure 3.3-b, but for the circuit shown in Figure 3.3-c. $R^2 = 0.95$. The transfer functions of the comparators are shown in Figure B.7. (e). Abstraction of bandpass genetic circuits. H_2O_2 activates OxyR in an analog fashion. Activated OxyR activates expression of bxb1 and either phiC31 or tp901 depending on the circuit used (Figure 3.3-a or Figure 3.3-c, respectively). The activation threshold is set by the promoters and RBS controlling recombinase expression. The expression of GFP is dependent upon bxb1 expression AND (NOT) phiC31 or tp901 expression. The errors (standard deviation) are derived from flow cytometry experiments of three biological replicates, each of which involved n > 30,000 gated events.*

The bandpass circuits switched GFP expression ON at low concentrations of H_2O_2 and switched GFP OFF at either medium or high concentrations of H_2O_2, depending on the threshold of the low-pass circuit (Figure 3.3-b,d, Figure B.6 and Figure B.7). The transfer function of each bandpass circuit could be predicted from straightforward addition of the transfer function of the high-pass circuit with the transfer function of the low-pass circuit that composed it (Data Processing and Calculations appendix). To determine the transfer functions of the high-pass and low-pass circuits, we measured GFP activation by the comparators using the same reporters for each recombinase as in Figure 3.2 (Figure B.6 and Figure B.7). We defined the bandwidth of a bandpass filter as the relative input range over which the circuit switched from 50% ON to 50% OFF. The bandpass circuit composed of the low-threshold high-pass and medium-threshold low-pass had a relative input range of 3.16; the bandpass circuit composed of the low-threshold high-pass and high-threshold low-pass had a wider relative input range of 7.34. This circuit architecture can be adapted to create band-stop filters by making the low-threshold circuit a low-pass and making the high-threshold circuit a high-pass.

Higher-order signal-processing circuits can be designed to convert a single analog input into multiple distinct outputs. For instance, we built analog-to-digital converters[204] that convert input H_2O_2 into the expression of multiple genes (Figure 3.4). For example, we built a circuit that can be used to output a pair of signals that encode the information of a ternary output. The circuit measures input H_2O_2 concentration and converts it into three gene expression states that represent a confirmed low concentration ("-1"), an intermediate concentration ("0"), or a confirmed high concentration ("+1"). To construct this circuit (Figure 3.4-a,b), we altered the bandpass circuit in Figure 3.3-a such that *gfp* was initially expressed by the proD promoter but would be shut off by Bxb1 production. We then added a copy of *rfp* that could be activated by inversion of the promoter by PhiC1 production. We defined the "-1" state as when >90% of cells were GFP positive and the "1" state as when >90% of cells were RFP positive. This resulted in three distinct gene expression states within the cells that were toggled at different H_2O_2 concentrations (Figure 3.4-c, Figure B.8). In future work, the *rfp* and *gfp* outputs could be replaced by other genetic regulators that feed into downstream computing circuits. These types of circuits could be extended to implement ternary logic, to report inequalities (such as <, =, >), or to encode distinct outputs at low or high input levels to actuate downstream circuits.

We also built a circuit where multiple comparators with different thresholds were each used to drive expression of a different fluorophore, thus implementing an ADC (Figure 3.4-d,e). This circuit classified H_2O_2 concentrations into one of four gene expression states in each cell ([*gfp, rfp, bfp*] = 000, 100, 110, 111) due to successive Bxb1, PhiC31, and TP901 expression with increasing H_2O_2, thereby encoding 2 bits of information (Figure 3.4-f, Figure B.9). The relative input ranges of the threshold circuits (horizontal lines in Figure 3.4-f) were 7.79, 5.08, and 6.42 for *gfp*, *rfp*, and *bfp* expression respectively, demonstrating that the ADC operates similarly in each concentration range. The resolution of an electronic analog-to-digital converter is a measure of the number of output discrete values encoded across a continuous input voltage range[227]. We created an analogous figure of merit for genetic analog-to-digital converters, where we measure the number of bits encoded across the ADC relative input range (Data Processing and Calculations Appendix B). We calculated this relative resolution (*RQ)* for our ADC to be 3.84. Adding XOR and buffer gates downstream of the current GFP, RFP, and BFP outputs should implement a canonical 2-bit ADC that generates a binary 2-bit output.

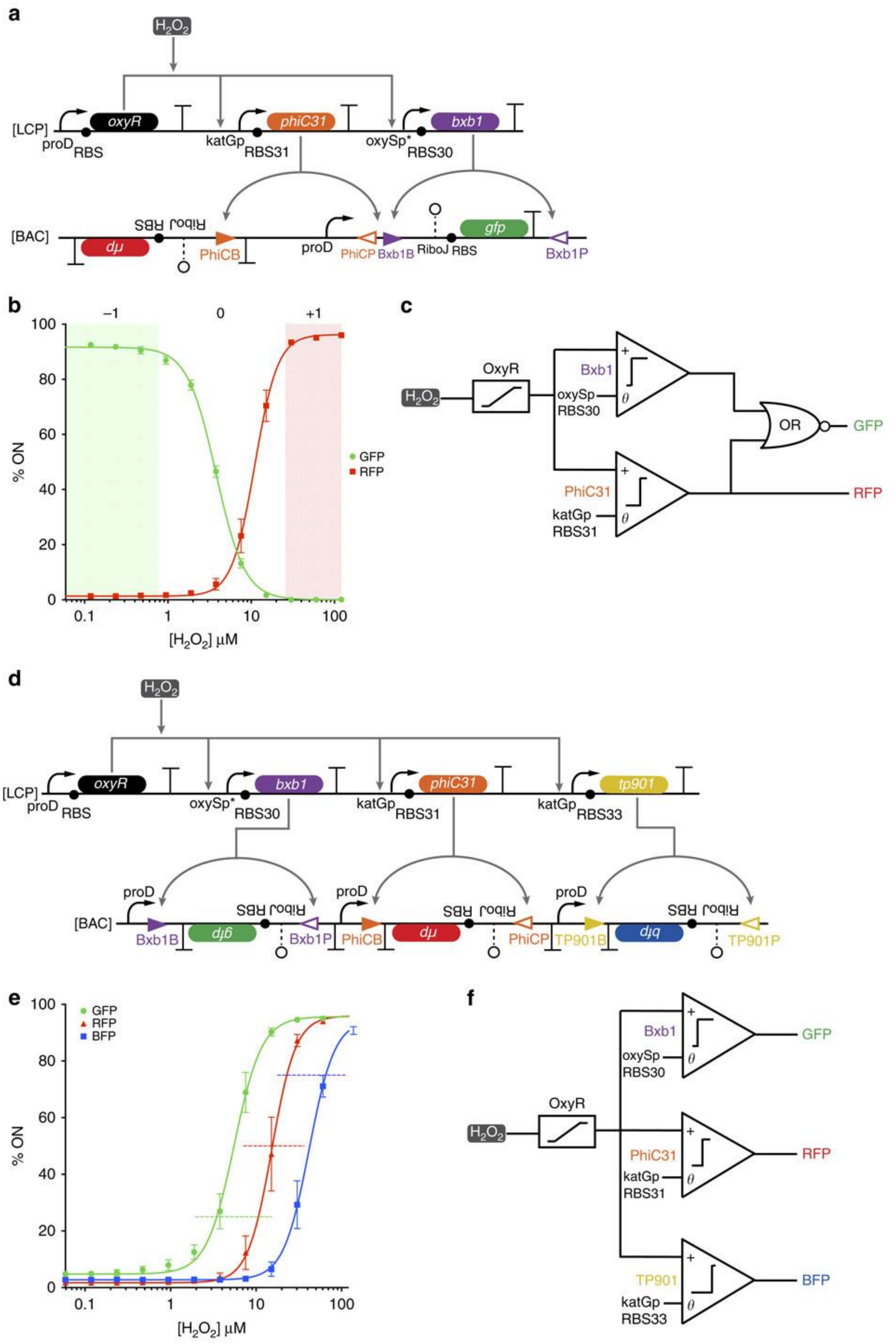


Figure 3.4| Multi-bit analog to digital converters. *(a). Ternary (three-state) logic gene circuit. OxyR is constitutively expressed and activates transcription of bxb1 and phiC31 in response to increasing concentrations of* H_2O_2*. Bxb1 unpairs the gfp cassette from the proD promoter, and PhiC31 unpairs the proD promoter from the gfp cassette and pairs it with the rfp cassette. (b). The percent of cells expressing GFP (green circle) and the percent of cells expressing RFP (red square) were fit to sigmoidal functions (solid lines). The "-1" state (shaded green) is defined as >90% cells being GFP positive. The "+1" (shaded red) is defined*

as >90% of cells being RFP positive. The "0" state is when neither -1 or +1 conditions are met. (c). Abstraction of ternary logic genetic circuit. H2O2 activates OxyR, which then activates expression of bxb1 and phiC31 depending upon the thresholds set by the promoters and RBS of their respective circuits. GFP expression is repressed by bxb1 OR phiC31 activation, whereas RFP activation is dependent upon phiC31 activation. (d). 2-bit analog-to-digital converter. OxyR is constitutively produced and activates transcription of bxb1, phiC31, and tp901 in response to increasing thresholds of H2O2. Bxb1, PhiC31, and TP901 invert gfp, rfp, and bfp, respectively, to enable expression from three different upstream proD promoters. (e). The percent of cells expressing GFP (green circle), RFP (red triangle), or BFP (blue square) were fit to sigmoidal functions (solid lines). The transition band for each circuit is demarcated by a horizontal dashed line of the same color. Each transfer function had a similar relative input range. (f). Abstraction of 2-bit analog-to-digital converter. H2O2 activates OxyR, which then activates expression of bxb1, phiC31, tp901 depending upon the thresholds set by the promoters and RBS of their respective circuits. Bxb1, PhiC31, and TP901 then activate gfp, rfp, and bfp expression, respectively. The errors (standard deviation) are derived from flow cytometry experiments of three biological replicates, each of which involved n > 30,000 gated events.

A Mixed-Signal Processing Gene Circuit

Analog-to-digital circuits can be further interfaced with digital circuits to form mixed-signal processing circuits (Figure 3.5). We built a variant of the bandpass circuit where the low-threshold comparator and medium-threshold comparator circuits both flip the directionality of *gfp.* This resulted in an analog-to-digital circuit where only intermediate H_2O_2 levels enable GFP production, which is analogous to an XOR gate on H_2O_2 concentrations digitized using two different thresholds (Figure 3.5-a,b). In addition, we placed *tp901* under control of the TetR-repressed pLtetO promoter and constitutively expressed *tetR*, thereby making *tp901* digitally inducible by anhydrotetracycline (aTc) [222]. We then used *tp901* to control the direction of the promoter driving transcription of *gfp*. We assayed GFP levels at different H_2O_2 concentrations in the presence and absence of aTc and found a majority of GFP-positive cells only at intermediate concentrations of H_2O_2 and when aTc was absent (Figure 3.5-b), thus implementing the concentration-dependent logic shown in Figure 3.5-c. Concentration-dependent logic could allow cells to carry out distinct activities at intermediate input levels, as opposed to extreme ones, and to encode a greater density of information into biological signals.

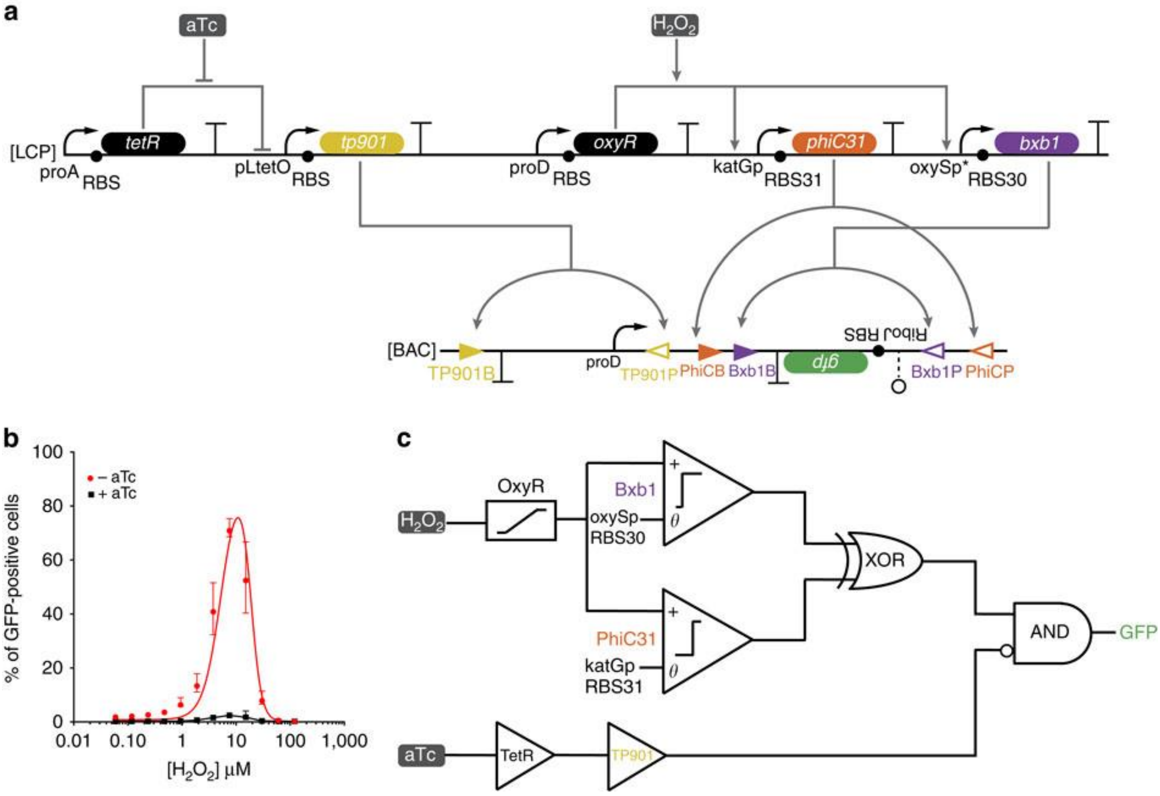


Figure 3.5| Mixed-signal computation and concentration-dependent logic. *(a). Mixed-signal gene circuit. OxyR is constitutively produced and activates transcription of bxb1 and phiC31 at two different thresholds of H_2O_2. Both Bxb1 and PhiC31 can invert a gfp expression cassette. Bxb1-based flipping occurs at a lower H_2O_2 concentration than PhiC31-based flipping such that gfp is only in an upright orientation over an intermediate range of H_2O_2. Furthermore, TetR is constitutively produced and represses the pLtetO promoter; this repression is relieved by the presence of aTc. TP901 is expressed from the pLtetO promoter and inverts the proD promoter such that it cannot drive expression from an upright gfp cassette. The resulting circuit implements concentration-dependent logic with an output (GFP) that is ON only if an intermediate level of the input H_2O_2 is present and aTc is not present. (b). The percent of cells expressing GFP at different concentrations of H_2O_2 in the presence (black square) and absence (red circle) of aTc. When aTc is absent, the circuit implements a bandpass response to H_2O_2, where the data is well-fit by the same transfer function (red line) as the black line in Figure 3.3-b, $R^2 = 0.94$. When aTc is present, the circuit is OFF. The black line is a straight line between each data point. (c). Abstraction of the mixed-signal gene circuit. H_2O_2 activates OxyR, which then activates expression of bxb1 and phiC31 depending upon the thresholds set by the promoters and RBS of their respective circuits. aTc activates expression of tp901 via inactivation of TetR. GFP is expressed when either Bxb1 or PhiC31 are present AND NOT when TP901 is activated. The errors (standard deviation) are derived from flow cytometry experiments of three biological replicates, each of which involved n > 30,000 gated events.*

Discussion

We have shown that cells can be engineered to implement synthetic computations that convert continuous information into discrete information. These computations rely on gene circuits that threshold and discretize signals from sensors, analogous to comparators in electronics. Our basic comparator design should be adaptable to other cellular contexts and for sensing inputs besides chemical concentration, such as light [200] or contact [197]. There are other known ways to implement thresholding circuits [205–208] and to dynamically alter thresholds [209], suggesting that it would be possible to implement a negative input terminal analogous to that in electronic comparators, rather than the fixed threshold that we implemented here.

Our comparators can be composed together to build multi-threshold analog-to-digital converters. In contrast to previously described genetic bandpass-filters [210–213], our bandpass filters convert continuous information into distinct gene expression states instead of altering continuous gene expression. Furthermore, the outputs from our analog-to-digital converters can be integrated with other digital circuits (Figure 3.5). Alternatively, multiple analog signals could be integrated at the front end to calculate complex analog functions[198] before feeding the output(s) into downstream analog-to-digital converters. We have engineered the outputs of our circuits to be Boolean (Figure 3.3, Figure 3.5), ternary (Figure 3.4-a,c), or multi-state digital (Figure 3.4-d,f). It may be possible to further increase ADC resolution by increasing the number of comparators across the same range of H_2O_2 or by adding comparators that can respond to lower or higher concentrations of H_2O_2.

There are a number of potential challenges involved in scaling mixed-signal gene circuits. First, it is important that comparators do not substantially affect cell growth. We found that the number of plasmids on which comparator circuits are encoded impacted cell growth more than the number of comparator circuits (Figure B.12 and Figure B.13). Thus, to scale mixed-signal computation it will be important to decrease the number of episomal DNA constructs, for example by moving comparators to the chromosome. Furthermore, to increase ADC resolution, comparators will need to have sharper thresholds at the population-level (i.e., more consistency in the behavior of each cell around the threshold point). We surmise that this may be possible by implementing negative feedback in the analog sensor circuit, which can reduce population-level heterogeneity[228]. Screening large promoter / RBS / recombinase libraries could enable the identification of circuits that implement various thresholds upon a given analog input. Utilizing novel orthogonal recombinases could aid in the scaling of mixed-signal gene circuits[229]. For certain analog inputs, it may also be necessary to implement a graded positive-feedback[198] or negative-feedback loop[230] to enable wide input dynamic range activation of the sensor transcription factor.

ADCs are the complement of digital-to-analog converters (DACs): ADCs convert an analog input signal into discrete output signals, whereas DACs convert discrete input signals into analog output signals (Figure B.11-a,c). For example, DACs that we previously implemented in living cells accepted two digital inputs and produced four different gene expression levels as outputs depending on the specific combination of inputs (Figure B.11-d)[219]. Here, we built ADCs that translate a single analog input in the form of inducer concentration to multiple discrete outputs, represented by triggering the expression of different genes (Figure B.11-e).

These mixed-signal circuits constitute a first step towards advanced analog-digital hybrid computational approaches. For instance, to implement an artificial neural network circuit, multiple analog inputs could be fed into the promoter controlling recombinase expression, and the weights of the analog inputs could be tuned via their binding affinity to the promoter. Linking these artificial circuits together could allow the creation of artificial neuronal networks in living cells. The comparators could also be used in a hybrid automaton if they were integrated with state machines, wherein the state switches based upon analog thresholds. Additionally, the ternary logic circuit (Figure 3a) could be used to implement fuzzy logic by converting the “0” state into the expression of a third gene.

We envision that mixed-signal processing will enable a wide range of industrial [231], diagnostic, and therapeutic engineered cell applications [232,233]. For example, cells could be designed to produce quorum-sensing signals that trigger multiple distinct production pathways as the quorum-sensing molecules accumulate in a bioreactor. The first phase could be focused on biomass accumulation, the second phase dedicated to secreting the desired product, such as a biologic protein drug fused to a secretion tag, and the third committed to secreting product-modifying enzymes, such as a protease to separate the secretion tag from the active drug. Such behavior could be programmed with an ADC that senses the concentration of an accumulating quorum-sensing molecule as an input and triggers successive circuits with higher concentrations, similar to the system shown in Figure 3.4-d,e. As a first step towards such industrial applications, we scaled up the operational-volume of the ADC circuit by 100x and found the circuit functioned, albeit with shifted thresholds (Figure B.14).

In addition, cells could be designed to detect continuous quantities of multiple biomarkers, integrate these signals to diagnose disease conditions, and produce reporter output(s) for non-invasive biosensing applications. For instance, probiotic or commensal[234] bacteria could be engineered to sense the concentration of multiple biomarkers for inflammatory bowel disease (e.g., reactive oxygen species, nitric oxide, blood), discretize the magnitude of each of these analog signals using ADCs with a range of thresholds, integrate the resulting information with Boolean logic (e.g., a multi-input AND gate) to decide whether a disease flare-up is occurring and how severe it is, and produce discrete reporters that can be detected outside of the body. Reporting on disease states and severity with digitized outputs (e.g., different fluorescent or colorimetric reporters) could be more robust than analog outputs (e.g., a single fluorescent reporter expressed at different levels) since the latter is more susceptible to noise. Our analog-to-digital converters could also be used as peak detectors due to the inherent memory feature of recombinase-based switches. For instance, probiotic bacteria could be engineered to remember the maximum concentration of a biomarker that they detected while passing through the intestine. Similar circuits could be used to create environmental sensors that sense and record maximum pollutant levels [235].

Mixed-signal circuits could also be useful for engineering cell therapies whose therapeutic outputs are regulated by quantitative levels of disease biomarkers. For example, mammalian gene circuits could be designed such that blood glucose levels below the normal region (“-1” in a ternary logic system) would switch on glucagon secretion, blood glucose levels in the desired region (“0” in a ternary logic system) would result in no hormone secretion, and blood glucose levels above the normal region (“1” in in a ternary logic system) would trigger insulin secretion. The ability to trigger distinct outputs in response to different conditions could enable new “homeostatic” therapies. Such applications would benefit from resettable mixed-signal circuits, which could be implemented using transcriptional regulators, rather than the permanent-memory mixed-signal circuits described here.

In summary, mixed-signal gene circuits merge analog and digital signal processing to enable both continuous information sensing and robust multi-signal integration and computing in living cells. Ultimately, we expect that this hybrid analog-digital computational paradigm will allow synthetic biological systems to begin to approach the nuanced complexities found in natural biological systems [201,202,205,236–245].

Material and Methods

Strains and plasmids

All plasmids were constructed using PCR and Gibson assembly starting from DNA sources as referenced in Table B.3 or from gBlocks manufactured by IDT. All plasmids were constructed with standard cloning procedures. *Escherichia coli* EPI300 (*F⁻ mcrA Δ(mrr-hsdRMS-mcrBC) Φ80dlacZΔM15 ΔlacX74 recA1 endA1 araD139 Δ(ara, leu)7697 galU galK λ⁻ rpsL (Str^R) nupG trfA dhfr*) was used for all experiments. Parts and plasmids used in this study are detailed in Plasmids appendix, Table B.1, Table B.3 and Table B.2. Plasmid sequences and plasmid DNA can be obtained at Addgene under ID numbers 78211–78229.

Circuit characterization

Plasmids were transformed into chemically competent E. coli EPI300, plated on LB medium with appropriate antibiotics and grown overnight at 37ºC. Antibiotic concentrations were carbenicillin (50 mg mL^{-1}), kanamycin (30 mg mL^{-1}) and chloramphenicol (25 mg mL^{-1}). The next day, single colonies were inoculated into Teknova Hi-Def Azure Media with appropriate antibiotics and 0.2% glucose, and incubated shaking aerobically for 16–18 h at 37ºC. Cultures were then diluted 2,500x into fresh Hi-Def Azure Media with appropriate antibiotics and 0.2% glucose, and shaken for 20 min aerobically at 37ºC. After 20 min, 200 µL of culture was transferred to a 96-well plate, and H_2O_2 (Sigma–Aldrich H1009-100ML) was added at appropriate concentration via serial dilution. For the experiment in Figure 3.5, aTc (Cayman Chemical 10009542) was added to a final concentration of 75 ng mL^{-1}. Plates were incubated aerobically with shaking for 20 h at 30ºC for all experiments except those in Figure B.1, in which plates were incubated for 3 h. After incubation, the optical densities of cultures were measured at 600 nm in a plate reader. For experiments in Figure B.2-Figure B.4, cells were then assayed on the flow cytometer. For all other experiments (Figure 2.1-Figure 2.4; Figure B.5-Figure B.10), cells were washed with PBS, diluted 8x into fresh Hi-Def Azure Media with appropriate antibiotics, 0.4% glycerol and 1x Copy Control Induction Solution (Epicentre), and incubated shaking aerobically for a further 10 h at 30ºC. After this incubation, the optical densities of cultures were measured at 600 nm in a plate reader. For all flow cytometer experiments, cells were diluted into ice-cold 1x PBS to an optical density at 600 nm of <0.02 and assayed on a BD LSR-Fortessa using the high-throughput sampler. At least 30,000 gated events were recorded. GFP expression was measured via the fluorescein isothiocyanate channel, RFP expression was measured via the TexasRed channel and BFP expression was measured via the Pacific Blue channel. FCS files were exported and processed in FlowJo software. Events were gated for live *E.coli* via forward scatter area and side scatter area, and then analyzed as in Data Processing and Calculations (Appendix B). The y axis on the flow cytometry histograms is normalized to the mode for each sample. At least three biological replicates were conducted for each experiment.

Chapter 4 | Measuring hydrogen peroxide concentrations *in vivo*

This chapter is based on the ongoing work:

Measuring maximal hydrogen peroxide concentrations attained in an inflammation animal model

Selvaggio G., Ferreira T., Ferreira M. and Salvador A.

G.S. and A.S conceived the study. G.S., T.F., performed analyses and collected data. All the authors discussed results and wrote the manuscript.

Abstract

Despite the importance of H_2O_2 to cellular activities, the molecular mechanisms of its production, accumulation, function, and scavenging remain insufficiently understood. This is due to a large extent to the lack of knowledge of the actual concentrations and fluxes of H_2O_2 *in vivo*.

Here we present a quantitative measurement of the extracellular H_2O_2 concentrations attained in a wound healing model in zebrafish. To accomplish it we used one of the genetically engineered bacterial sensors from the previous chapter. This sensor is able to measure and memorize extracellular H_2O_2 ranges encoding them into different fluorescent proteins. As previously reported inflammation by wounding generates a gradient of H_2O_2 concentration starting from the wounding site. Our sensor was able to map also in space the different peroxide concentrations.

Introduction

The lack of consensus about the H_2O_2 concentrations *in vivo*, together with the wide dynamic range covered by various experimental setups (from µM-mM of external H_2O_2) raise questions over the physiological plausible oxidative loads and their implications.

Estimations made on the data available in literature calculate the H_2O_2 concentration in the human plasma being between 1-5 µM in normal condition and increasing up to 30-50 µM in chronic inflammation conditions [63]. However, other *in vivo* studies ref. [64] reported that in healthy cells the H_2O_2 concentration rarely exceeds 1-15 µM. Benfeitas *et al.* ref. [81] reported, based on an analysis of the literature, that in absence of inflammation or infection the H_2O_2 concentrations are in the nM range.

All these estimates are based on very indirect evidence.

An absolute measurement of H_2O_2 concentrations in a living organism would allow to understand if the responses to H_2O_2 stimulation observed *in vitro* reflect organism characteristics or are artifacts induced by unphysiological treatments.

The development of genetically encoded sensors (e.g. HyPer [56,57], roGFP2 [51]) eventually allowed the study of the oxidative response in animal models [61,246] and cell culture with a higher degree of specificity compared to past chemical compounds like dichlorofluoresceins.

Hyper in particular uses an OxyR active site to sense H_2O_2, by inducing a conformational change in the attached YFP modifying the excitation spectrum of the protein.

Niethammer et al. [61] showed the importance of H_2O_2 in the wound healing process in zebrafish and the establishment of a H_2O_2 concentration gradient in the tissue (due to NADPH-oxidases) that functions as chemotaxis signal for the immune system cells [30,57,62]. The gradient was abolished once the oxidases were knocked-out.

Niethammer *et al.* [61] and then Pase *et al.* ref. [30] estimated, based on previous calibration (ref. [56] and Figure 1.4), concentrations that ranged from 0.5-50 µM having the highest value of the gradient at the wounding site. However, the fact that the signal in their experimental setup reflects a balance between probe oxidation by H_2O_2 and probe reduction by cellular reductants whose concentrations may change from cell to cell and over time raises questions about the reliability of these values. Here we measured H_2O_2 concentration in an inflammation response to fin-clip in zebrafish. To accomplish this, we will build on the work of Niethammer *et al.* and of Pase *et al.*, who observed H_2O_2 gradients in response to cuts in the zebrafish fin. We injected this model with bacteria that carried an H_2O_2 classifier (described in Chapter 3) that encodes different concentrations in different fluorescence colors by expressing different fluorescent proteins (Table 4.1).

Table 4.1| Sensor activation thresholds.

	Low	*Medium*	*High*
H_2O_2 *Threshold*	5.5 µM	15.3 µM	41.6 µM

The values reported in Table 4.1, are based on Figure 3.4 and represent the theoretical thresholds of the sigmoid like shape of the sensors activation.

Results

The injection of the bacteria carrying the sensor was performed near the wounding region (Figure 4.1-a), the confocal images were processed using ImageJ 1.51f[247] as described in the Material and Methods section. Comparing our method to the previous results obtained with Hyper, we tried to reproduce the establishment of a gradient along the direction perpendicular to the wound. We classified each bacterium with the corresponding highest sensor expressed; this eventually should lead to a three mono-modal probability distribution of the sensor state depending from the distance (Figure 4.1-b).

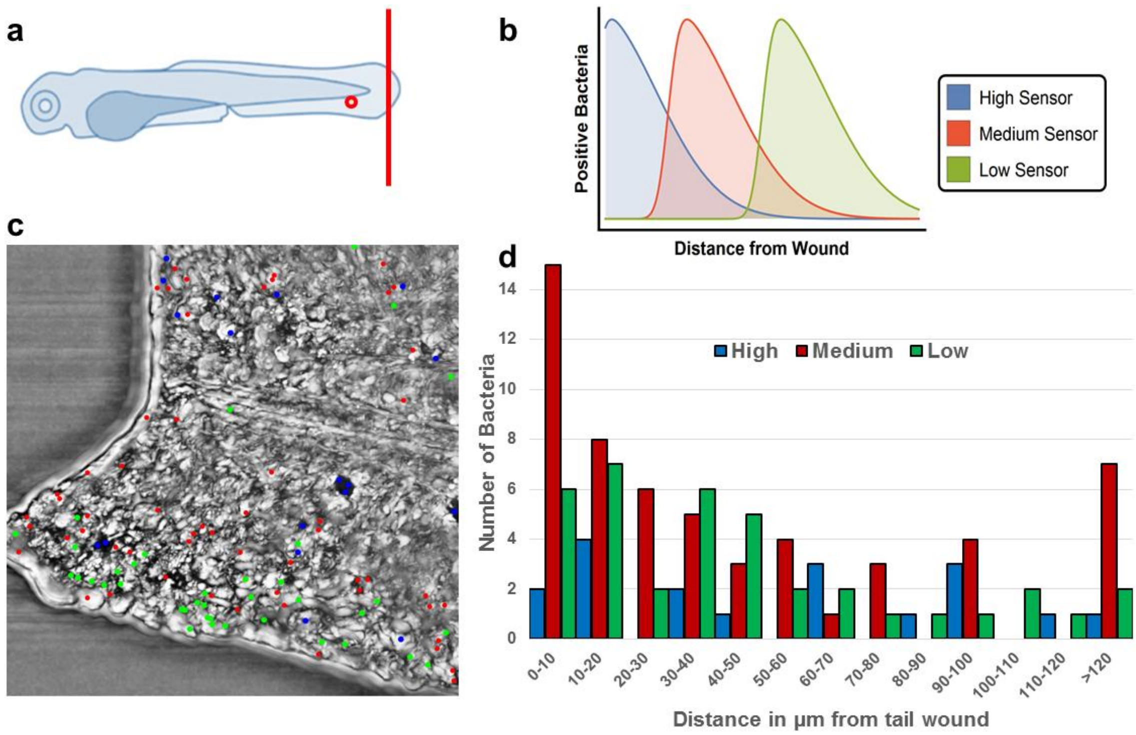


Figure 4.1| Measuring hydrogen peroxide gradients in zebrafish. *(a) a schematic representation of the fin clip and injection sites. (b) ideal representation of the probability of activation of the sensors based on the distance from the wounding site. (c) pseudo image representing the pattern of activation in a fin-clip experiment*

(color code as in panel b). It is possible to notice the degree of activation of the high sensor in the injection site close to the notochord. (d) histogram representing the number of activated bacteria per sensor and their distance from the tail wound.

It was not possible to univocally detect the gradient with our sensor, mainly due to the fact that the injection point creates a small wound with a local inflammation. But it emerged clear that the highest sensor (threshold 41.65 µM of H_2O_2) was activated only at the wounding site (either injection of fin clip).

The pattern of sensors activation (Figure 4.1-d) shows a progressive decrease in the medium and high sensors activation that correlates with an increase in the lowest one, this is eventually halted by the presence of the wound caused by the injection site. In Figure 4.2 it is possible to appreciate this activation and how the medium sensor reach the peak of its activation in between the two wounding sites.

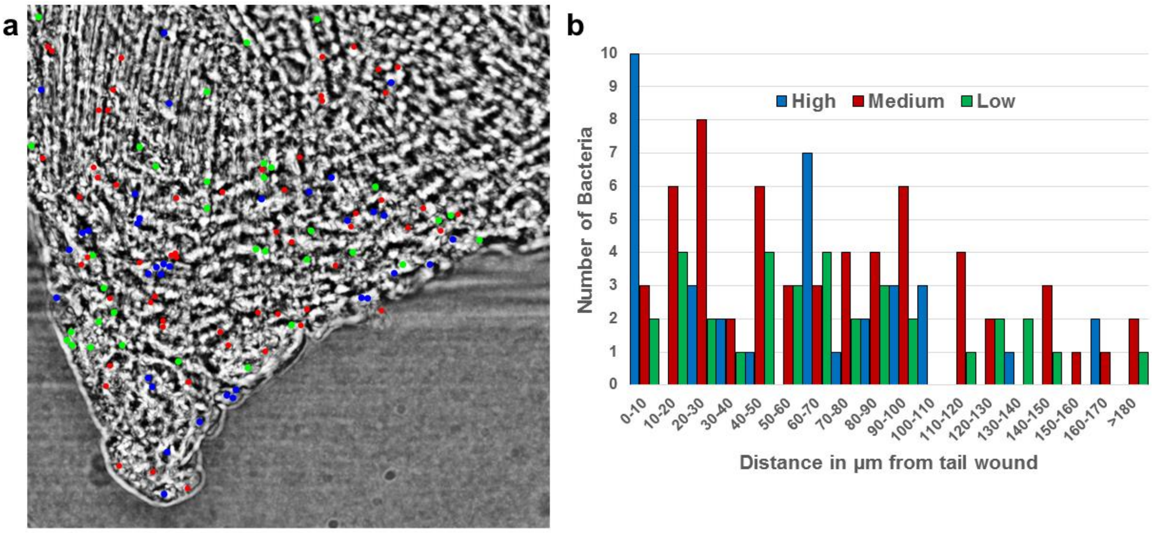


Figure 4.2| Sensors activation between two wounds *(a) a pseudo image representing the pattern of activation in a fin-clip experiment, on the left of the fin it is possible to identify the injection site. (b) histogram of the distances from the wound, it shows two peaks of activation in correspondence of the two wounds (injection and fin-clip) and a bell shape activation in between the two injuries.*

Discussion

We performed the experiment described above with the aim of measuring the highest extracellular H_2O_2 concentration attained *in vivo* upon wounding induced inflammation. The measurement performed by Niethammer *et al.* [61] unfortunately presents some incongruences. They estimated a range for the peroxide gradient of 0.5-50 µM. The lowest value sensed by cells expressing Hyper, accordingly to Belousov *et al.* ref.[56] is 5µM thus raising the lower limit of the gradient of 1 order of magnitude. The calibration curve was calculated on human COS-7 cell while the cells examined are from various tissue and heterogeneous in antioxidant defense expressions and redox state. The measure performed by Niethammer *et al.* spans over the entire linear region of the sensor entering the saturation region (~20 µM). The Hyper mRNA injected can be degraded at different rates in different cells giving varying Hyper expressions.

In turn, the usage of the same cell type (*E.coli* EPI300), carrying the sensor in the present experiments minimizes the variability associated to species and cell-type differences. The necessity of inducing another wounding site highlighted the impossibility to reproduce the gradient experiment, but allowed us to estimate the maximum concentration reached *in vivo* to exceed 40µM only at the wound border

, otherwise being comprised between 15 and 40 µM (medium and high sensor thresholds Appendix B). The characterization of the strain used in the experiments allows for an estimation of the upper limit of the H_2O_2 concentration. As a matter of fact, the *E.coli* cells used are unable to tolerate concentration higher than 120µM of H_2O_2, this is of course an overestimation of the upper bound but allows us to further limit the possible *in vivo* dynamic range of H_2O_2 concentrations.

Although coming from a model organism this measure allows us to better evaluate existent literature results on peroxide signaling. The redox relay between STAT3 and hPrxII observed by Sobotta *et al.* ref. [131], was in high oxidative conditions 100µM H_2O_2. This implies based on our measurement that this could only happen in the very proximity of the inflammation site where the wound healing process would require first a rapid proliferation and then apoptosis[248]; both these mechanism have in STAT3 a principal actor. Furthermore, the study from Tomalin *et al.* ref. [160] observed the hyperoxidation with consequent increase of intracellular H_2O_2 in HEK293 cell when the external concentration was ~40µM. Hyperoxidized Prx is not able anymore to form complex with PTEN which in turn is an inhibitor of proliferation. The accumulation of the hyperoxidized form of Prx can then lead to the inactivation of mitotic pathways due to PTEN oxidation. Instead the lower H_2O_2 concentration attained a few microns from the wounding site are not sufficient to generate the sulfinylation trigger and thus still capable of proliferate.

Material and Methods

Strains and bacteria preparation

Escherichia coli EPI300 (*F⁻ mcrA Δ(mrr-hsdRMS-mcrBC) Φ80dlacZΔM15 ΔlacX74 recA1 endA1 araD139 Δ(ara, leu)7697 galU galK λ⁻ rpsL (Str^R) nupG trfA dhfr*) was used for all experiments.

We freshly transform plasmid DNA of the circuit described in Chapter 3 into competent cells and plate them on the appropriate antibiotics plate. A single negative (non fluorescent) colony was screened from the plate, using an epi-fluorescent stereoscope (Zeiss Stereo Lumar.V12), and grow overnight in 5mL of LB supplemented with antibiotics at 37º. We pellet the culture by centrifugation at 4000g for 15 min and we then resuspendend the pellet in 100 µL of PBS 1x plus antibiotics. We used a 27G syringe to aspirate and eject the suspension in order to separate the bacterial clumps.

Zebrafish Husbandry

Experiments were performed using the Casper zebrafish strain (ZIRC ZL1714)[249]. Recently spawned eggs were collected in a Petri dish filled with embryonic medium (E3) and incubated at 28 ºC. Between 48 and 72 hours post fertilization (hpf) the larvae were dechorionated, and again incubated at 28ºC.

Injections

Injection bedding was prepared by dissolving 1% agarose in E3 and covering the bottom of a Petri dish. 72 hpf larvae were anesthetize by incubation in Tricaine (MS-222, 0.6 mM in E3) supplemented with antibiotics. Antibiotic concentrations were ampicillin (50 mg mL^{-1}), kanamycin (30 mg mL^{-1}) and chloramphenicol (25 mg mL^{-1}). Larvae were injected with a bacterial suspension mixed with phenol red to properly identify the site of injection. An average of 100-200 bacteria cell per larvae were injected (1-2 nL of injection volume)[250,251]. To control the procedure, we used a stereoscopic dissecting microscope (Olympus SZX10); a pneumatic picopump (PV820, World Precision Instruments) and a micromanipulator with pulled microcapillar pipettes)

Fin-fold amputation

The injected zebrafish larvae were staged and using a scalpel we made a full incision, clipping the fin fold distal to the notochord[30,61,252]. The larvae were then transfer into fresh E3, supplemented with antibiotics, to allow to recovery from anesthesia and then incubated at 30ºC for 5h.

Image acquisition

5 hours post wounding (hpw), living zebrafish were staged in E3 1% low melting point agarose, and immerse in E3 supplemented with Tricaine. Confocal Z-stacks were acquired on a Leica SP5 confocal, using a 20x 0.70NA dry objective, using HyD and PMT detectors in Standard Mode

Imaging

Z-stack were analyzed using ImageJ 1.51f[247]. the image was first equalized in order to use the entire dynamic range of the system. Then a convoluted background subtraction and a pseudo flat correction was applied to the stack (Plugin Biovoxxel). In order to extract the fluorescent bacteria a Laplacian of Gaussian with a 3σ square kernel was applied to each plane individually. Bacteria where then counted, on the STD-projection of the z-stack. using the cell counter plugin of ImageJ.

Distance analysis

Distance was calculated from an ideal line that cross the fish along the wounding site. The formula used was:

$$d(P_1, P_2, (x_0, y_0)) = \frac{\left|(y_2 - y_1) \cdot x_0 - (x_2 - x_1) \cdot y_0 + x_2 \cdot y_1 - y_2 \cdot x_1\right|}{\sqrt{(y_2 - y_1)^2 + (x_2 - x_1)^2}}$$

Histograms and distances were calculated using MS-Excel.

Chapter 5 | General discussion and future perspectives

Typical 2-Cys peroxiredoxins and thioredoxin are increasingly recognized to play a central role in antioxidant protection and redox signaling in the cytoplasm of eukaryotic cells. The molecular properties and cellular abundances of these proteins have been extensively characterized. Studies highlighted many commonalities among cells and organisms, but also intriguing differences in cells' responses to hydrogen peroxide.

The study performed in Chapter 2 allowed us to map all the possible phenotypic response that the PTTR system could show to a variation in H_2O_2 supply.

In all biological instances tested (Figure A.6) the design of the system is robust and locates the basal operative point in the region where the best signaling performances are granted. The uncertainties related with the proteomic source, of the data used for obtaining the design space, may lead to changes in the superfamily (response to moderate OS) but the behavior at LOS is consistent and conserved. Furthermore, the model correctly predicts the distinct responses of human erythrocytes and Jurkat T cells to hydrogen peroxide based on these cells' composition.

The importance of understanding the possible phenotypes of redox-signaling expressed by different organisms is to guide drug development and target specific pathways.

As a matter of fact, deregulation of redox signaling pathways, with or without consequent OS, is involved into the development of pathologies such as cardiovascular disease[4], inflammatory bowel disease [5,6], atherosclerosis, diabetes and metabolic disease [7,8] and neurodegenerative disease (e.g. Parkinson [9]). Redox signaling and OS play also a major role in tumor incidence and progression, where the antioxidant defenses are necessary for the initiation and the survival of the cancer [10,11]. Furthermore, in higher organisms the aging phenomenon is associated with an increase in the basal level of oxidants, called inflammaging [12].

The difficulty in developing drugs that target the component of the PTTRS is their high degree of conservation even amongst different organism, it is thus important to develop a holistic approach to the system that allows for a modification of the unwanted phenotype. As example, since the pathogens infectivity and viability is strictly related with Prx activity, it has been hypothesized as possible approach to drive the system into hyperoxidation: due to the lack of Srx in prokaryotes[92].

Important in cancer therapies is also the increased resistance that some cell show to chemotherapy compounds. MCF7 cells, breast cancer, show Adriamycin (doxorubicin) resistance when there is an overexpression of the Hexose monophosphate shunt[176]. The first product of this pathway is NADPH. An increased amount of reducing equivalent does increase the antioxidant capacity of the cell and thus limit the cytotoxicity of Adriamycin related to OS. If we consider Figure A.6 for MCF7 we could hypothesize of induce cell death by decreasing TrxR activity (thus activating the ASK-1 pathway). Or we could limit the effect of the NADPH overproduction by inducing a change in the superfamily from A to C. This will increase the amount of reducing equivalent used by the PTTRS limiting other enzymes and eventually saturating Trx and halting cell replication (by removing energy supply to RNR).

The classifier developed in Chapter 3 and tested *in vivo* in Chapter 4 opens for a new set of tool created by synthetic biology to measure and integrate biological variable. In particular, this kind of system could be used to monitor disease like inflammatory bowels (IBD) and counteract by releasing on command anti-inflammatory compounds upon chronic inflammation. In research it would be possible to use them for drug screening measuring H_2O_2 concentration in the guts of an animal model. It is possible to treat zebrafish with Dextran Sulfate Sodium (DSS) which is capable to induce a colitis-like status in the animal[253]. This combined with the transparency of the fish and a cost effective scalability of the screening would allow for a higher parallelization of the analysis. Measuring H_2O_2 concentration in response to a systemic inflammation by injection in the blood stream (or other systemic compartments) can also help in the studies of the immune response to pathogen infections [254,255].

Appendix A | Design principles for thiol redox signaling: mapping the phenotypic repertoire of the cytoplasmic 2-Cys peroxiredoxin – thioredoxin system supplementary information

The systems design space methodology for characterizing the phenotypic repertoire of biochemical circuits

The analysis of the dynamic properties of the PTTRS is based on the systems design space methodology [162,164,256–259], with modifications relative to the published techniques. The modifications to be described below aim to improve the handling of cycles and moiety conservation relationships.

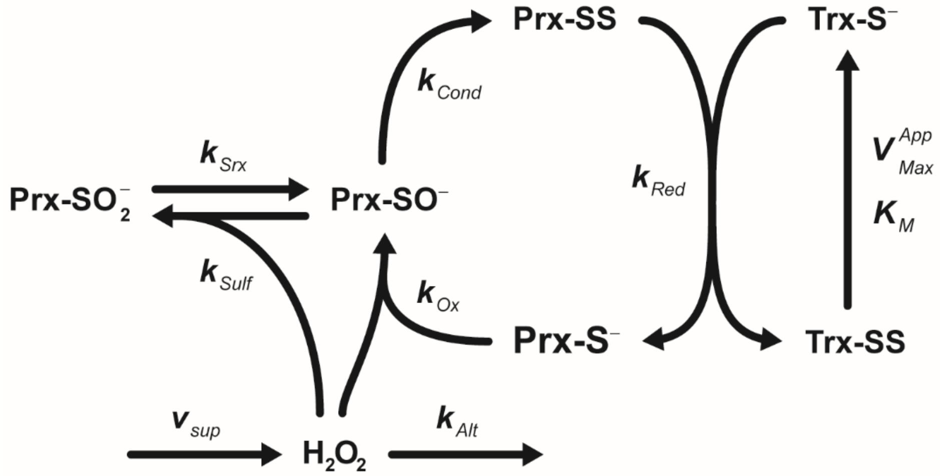


Figure A.1| PTTRS model.

The model in Figure A.1 translates in to the following system of ordinary differential equations:

$$\begin{aligned}
\frac{d\mathrm{H_2O_2}}{dt} &= v_{\mathrm{sup}} - k_{Alt}[\mathrm{H_2O_2}] - k_{Ox}[\mathrm{PrxS^-}][\mathrm{H_2O_2}] - k_{Sulf}[\mathrm{PrxSO^-}][\mathrm{H_2O_2}] \\
\frac{d\mathrm{PrxS^-}}{dt} &= k_{Red}[\mathrm{TrxS^-}][\mathrm{PrxSS}] - k_{Ox}[\mathrm{PrxS^-}][\mathrm{H_2O_2}] \\
\frac{d\mathrm{PrxSO^-}}{dt} &= k_{Ox}\cdot\mathrm{PrxS^-}\cdot\mathrm{H_2O_2} + k_{Srx}\cdot\mathrm{PrxSO_2^-} - k_{Sulf}\cdot\mathrm{PrxSO^-}\cdot\mathrm{H_2O_2} - k_{Cond}\cdot\mathrm{PrxSO^-} \\
\frac{d\mathrm{PrxSO_2^-}}{dt} &= k_{Sulf}\cdot\mathrm{PrxSO^-}\cdot\mathrm{H_2O_2} - k_{Srx}\cdot\mathrm{PrxSO_2^-} \\
\frac{d\mathrm{PrxSS}}{dt} &= k_{Cond}\cdot\mathrm{PrxSO^-} - k_{Red}\cdot\mathrm{TrxS^-}\cdot\mathrm{PrxSS} \\
\frac{d\mathrm{TrxS^-}}{dt} &= \frac{V_{Max}^{App}\cdot\mathrm{TrxSS}}{K_M+\mathrm{TrxSS}} - k_{Red}\mathrm{TrxS^-}\cdot\mathrm{PrxSS} \\
\frac{d\mathrm{TrxSS}}{dt} &= k_{Red}\cdot\mathrm{TrxS^-}\cdot\mathrm{PrxSS} - \frac{V_{Max}^{App}\cdot\mathrm{TrxSS}}{K_M+\mathrm{TrxSS}}
\end{aligned} \tag{A.1}$$

In order to apply the system design space methodology, we must recast this system to a canonical form, called a Generalized Mass Action (GMA) system, such that each term in the right hand side of the equations becomes a product of power laws. In the present case, this can be straightforwardly accomplished by defining a new ancillary variable $X = K_M + \mathrm{TrxSS}$. Further, we note that $\frac{d\mathrm{PrxS^-}}{dt} + \frac{d\mathrm{PrxSO^-}}{dt} + \frac{d\mathrm{PrxSO_2^-}}{dt} + \frac{d\mathrm{PrxSS}}{dt} = \frac{d(\mathrm{PrxS^-} + \mathrm{PrxSO^-} + \mathrm{PrxSO_2^-} + \mathrm{PrxSS})}{dt} = 0$ This shows that $\text{PrxS}^- + \text{PrxSO}^- + \text{PrxSO}_2^- + \text{PrxSS} = \text{Prx}_T$ is a conserved quantity, corresponding to the total concentration of peroxiredoxin.

Likewise, $\frac{d\text{TrxS}^-}{dt} + \frac{d\text{TrxSS}}{dt} = \frac{d(\text{TrxS}^- + \text{TrxSS})}{dt} = 0$, showing that $\text{TrxS}^- + \text{TrxSS} = \text{Trx}_T$ is also a conserved quantity, corresponding to the total concentration of thioredoxin. We can simplify the ODE system (A.1) by replacing two of the differential equations by these conservation relationships. Upon recasting and simplification, the equations are transformed to the equivalent form:

$$
\begin{aligned}
\frac{d\text{H}_2\text{O}_2}{dt} &= v_{\text{sup}} - k_{Alt} \cdot \text{H}_2\text{O}_2 - k_{Ox} \cdot \text{PrxS}^- \cdot \text{H}_2\text{O}_2 - k_{Sulf} \cdot \text{PrxSO}^- \cdot \text{H}_2\text{O}_2 \\
\frac{d\text{PrxSO}^-}{dt} &= k_{Ox} \cdot \text{PrxS}^- \cdot \text{H}_2\text{O}_2 + k_{Srx} \cdot \text{PrxSO}_2^- - k_{Sulf} \cdot \text{PrxSO}^- \cdot \text{H}_2\text{O}_2 - k_{Cond} \cdot \text{PrxSO}^- \\
\frac{d\text{PrxSO}_2^-}{dt} &= k_{Sulf} \cdot \text{PrxSO}^- \cdot \text{H}_2\text{O}_2 - k_{Srx} \cdot \text{PrxSO}_2^- \\
\frac{d\text{PrxSS}}{dt} &= k_{Cond} \cdot \text{PrxSO}^- - k_{Red} \cdot \text{TrxS}^- \cdot \text{PrxSS} \\
\frac{d\text{TrxSS}}{dt} &= k_{Red} \cdot \text{TrxS}^- \cdot \text{PrxSS} - V_{Max}^{App} \cdot \text{TrxSS} \cdot X^{-1} \\
0 &= K_M + \text{TrxSS} - X \\
0 &= \text{PrxS}^- + \text{PrxSO}^- + \text{PrxSO}_2^- + \text{PrxSS} - \text{Prx}_T \\
0 &= \text{TrxS}^- + \text{TrxSS} - \text{Trx}_T
\end{aligned}
\qquad (A.2)
$$

Although not necessary for application of the system design space methodology, one can reduce the dimensionality of the parameters space by scaling all parameters and variables. We used the scaling in Table A.1, which makes all variables and parameters dimensionless.

Table A.1: Dimensionless Groups

Symbol	Expression	Biological meaning
ϕ	$\frac{v_{\text{sup}}}{k_{Cond} \cdot \text{Prx}_T}$	**Scaled H_2O_2 supply**
α	$\frac{k_{Ox} \cdot \text{Prx}_T}{k_{Cond} \cdot (1 + K_i)}$	Scaled max reduction of H_2O_2 by Prx
β	$\frac{k_{Alt}}{k_{Cond}}$	Scaled max rate constant for alternative scavengers
ρ	$\frac{k_{Red} \cdot \text{Trx}_T}{k_{Cond}}$	Scaled max reduction of Prx by Trx
σ	$\frac{V_{Max}^{App}}{k_{Cond} \cdot \text{Prx}_T}$	**Scaled V_{Max}^{app} of TrxR**
χ	$\frac{K_{M,TrxSS}}{\text{Trx}_T}$	Scaled $K_{M,TSS}$ of TrxR
η	$\frac{k_{Srx}}{k_{Cond}}$	Scaled max reduction of hyperoxidized Prx by Srx

ψ	$\frac{k_{Sulf} \cdot \mathrm{Prx_T}}{k_{Cond}}$	Scaled max sulfinylation of Prx
μ	$\frac{\mathrm{Trx_T}}{\mathrm{Prx_T}}$	Ratio between Trx and Prx concentrations
τ	$t \cdot k_{Cond}$	Scaled time
h	$\frac{\mathrm{H_2}O_2}{\mathrm{Prx_T}}$	Dimensionless H_2O_2 concentration
x	$\frac{\mathrm{PrxS^-}}{\mathrm{Prx_T}}$	Normalized Prx-S^- concentrations
y	$\frac{\mathrm{PrxSO^-}}{\mathrm{Prx_T}}$	Normalized Prx-SO^- concentrations
w	$\frac{\mathrm{PrxSO_2^-}}{\mathrm{Prx_T}}$	Normalized Prx- concentrations
z	$\frac{\mathrm{PrxSS}}{\mathrm{Prx_T}}$	Normalized Prx-SS concentrations
u	$\frac{X}{\mathrm{Trx_T}}$	Normalized ancillary variable X
r	$\frac{\mathrm{TrxS^-}}{\mathrm{Trx_T}}$	Ratio between Trx-S^- concentrations
s	$\frac{\mathrm{TrxSS}}{\mathrm{Trx_T}}$	Normalized Trx-SS concentrations

Scaled variables x, y, w and z represent the fractions of the peroxiredoxin pool in each form, and scaled variables r, s represent the fractions of the thioredoxin pool in each form. Upon this scaling, equations (A.2) become:

$$
\begin{aligned}
\frac{dh}{d\tau} &= \phi - (\alpha xh + \beta h + \psi yh) \\
\frac{dy}{d\tau} &= (\alpha xh + \eta w) - (y + \psi yh) \\
\frac{dw}{d\tau} &= \psi yh - \eta w \\
\frac{dz}{d\tau} &= y - \rho rz \\
\mu \frac{ds}{d\tau} &= \rho rz - \sigma su^{-1} \\
0 &= (\chi + s) - u \\
0 &= (x + y + w + z) - 1 \\
0 &= (r + s) - 1
\end{aligned}
\tag{A.3}
$$

The parameters space is thereby reduced from 11 $(v_{\sup}, k_{Alt}, k_{Ox}, k_{Cond}, k_{Sulf}, k_{Red}, k_{Srx}, K_M, V_{Max}^{App}, \mathrm{Prx_T}, \mathrm{Trx_T})$ to 9 dimensions, of which one is immaterial for steady state analysis.

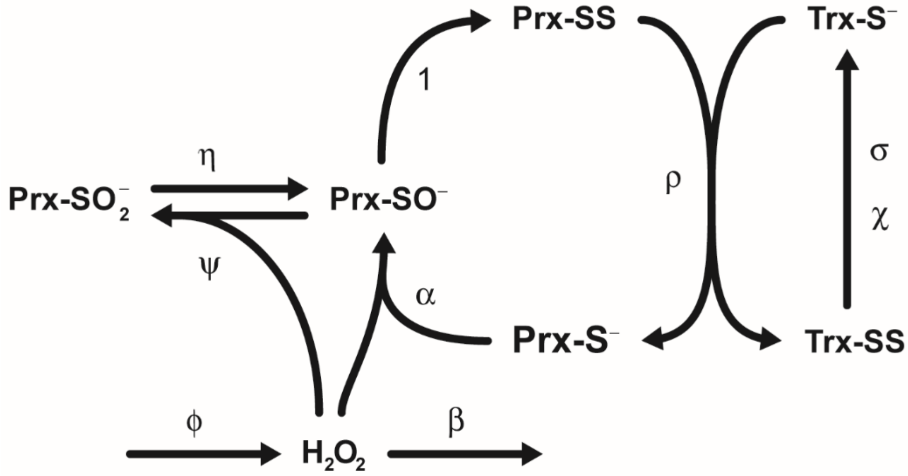


Figure A.2| Dimensionless PTTRS model.

The parentheses in equations (A.3) highlight that the right hand parts of these equations are differences between two positive-coefficient linear combinations of non-negative terms. Under most conditions the value of each of these linear combinations is dominated by one of its terms. Henceforth we will denote by *dominant positive term* and *dominant negative term* the dominant terms in the positive and negative linear combinations (respectively) in an equation. For instance, if $\alpha = 20, x = 0.9, h = 0.05, \eta = 0.001, w = 0.01, y = 0.05, \psi = 0.1$, then αxh is the positive dominant term and y is the negative dominant term for the second equation in (A.3).

We will denote by *dominant subsystem* any subsystem of (A.3) that retains only the dominant terms of the whole system. For instance, in the case where the second consumption term for h and all the first terms in all other linear combinations are the dominant ones we obtain the dominant subsystem:

$$\begin{aligned}
\frac{dh}{d\tau} &= \phi - \beta h \\
\frac{dy}{d\tau} &= \alpha xh - y \\
\frac{dw}{d\tau} &= \psi yh - \eta w \\
\frac{dz}{d\tau} &= y - \rho rz \\
\mu \frac{ds}{d\tau} &= \rho rz - \sigma su^{-1} \\
0 &= \chi - u \\
0 &= x - 1 \\
0 &= r - 1
\end{aligned} \quad . \qquad \text{(A.4)}$$

Each system can generate $S = \prod_{i=1}^{e} P_i \cdot N_i$ dominant subsystems, where e stands for the number of equations, and P_i, N_i stand for the number of positive and negative terms (respectively) in equation *i*. For instance, the present system can generate $S = (1{\times}3)(2{\times}2)(1{\times}1)(1{\times}1)(1{\times}1)(2{\times}1)$ $(4{\times}1)(2{\times}1)$ = 192 dominant subsystems.

Importantly, all dominant subsystems share a canonical nonlinear form such that the right hand side of the differential equations is a difference between products of power laws. Systems exhibiting this canonical form are known as S systems [260–262] and have many desirable mathematical properties [263]. Of interest in the present context, closed form analytical steady state solutions can be straightforwardly obtained upon a logarithmic transformation of all variables and parameters. For instance, for dominant subsystem (A.4), defining $a^* = \log(a)$, we obtain:

$$\begin{aligned}
&\phi^* = \beta^* + h^* \\
&\alpha^* + x^* + h^* = y^* \\
&\psi^* + y^* + h^* = \eta^* + w^* \\
&y^* = \rho^* + r^* + z^* \\
&\rho^* + r^* + z^* = \sigma^* + s^* - u^* \\
&\chi^* = u^* \\
&x^* = 0 \\
&r^* = 0
\end{aligned} \tag{A.5}$$

which yields the solution:

$$\begin{aligned}
&h^* = \phi^* - \beta^* \\
&x^* = 0 \\
&y^* = \alpha^* + \phi^* - \beta^* \\
&w^* = \psi^* + 2\phi^* - \eta^* - \alpha^* \\
&z^* = \alpha^* + \phi^* - \beta^* - \rho^* \\
&r^* = 0 \\
&s^* = \alpha^* + \chi^* + \phi^* - \beta^* - \sigma^*
\end{aligned} \tag{A.6}$$

Each dominant subsystem approximates the behavior of the system in the region where the respective *dominance conditions* are valid. These conditions are the inequalities that define where each dominant term is higher than every other one in the respective positive or negative linear combination. For instance, the dominant subsystem (A.4) holds where the following set of dominance conditions is valid:

$$\begin{aligned}
&\beta h > \alpha x h \wedge \beta h > \psi y h \wedge \\
&\alpha x h > \eta w \wedge y > \psi y h \wedge \\
&\chi > s \wedge \\
&x > y \wedge x > w \wedge x > z \wedge \\
&r > s
\end{aligned} \tag{A.7}$$

The dominance conditions define a *dominance region* in the phase space, which depends on parameters. A dominant subsystem may or may not be able to reach a steady state within its dominance region. In order to define the region of the parameters space where a dominant subsystem is able to attain a steady state within its dominance region we replace its steady state solution into the dominance conditions. Again, we can transform these nonlinear inequalities to linear ones by applying the logarithmic transformation. The replaced inequalities thus become:

$$\begin{aligned}
&\beta^* - \alpha^* - \phi^* > 0 \\
&\beta^* + \rho^* - \alpha^* - \phi^* > 0 \\
&\beta^* + \sigma^* - \alpha^* - \chi^* - \phi^* > 0 \\
&2\beta^* + \eta^* - \alpha^* - \psi^* - 2\phi^* > 0 \\
&\beta^* - \alpha^* > 0 \\
&\beta^* - \psi^* - \phi^* > 0 \\
&\beta^* + \sigma^* - \alpha^* - \phi^* > 0
\end{aligned} \tag{A.8}$$

We will call these the *boundary conditions* for the dominant subsystem, and we will call the dominant subsystem *valid* if its boundary conditions are feasible.

The boundary conditions for all the valid dominant subsystems pave the parameters space into up to S discrete regions whose topology and geometry is determined by the system's interaction structure (*design*). We call this partitioned space the *system design space*.

Some dominant subsystems can be sub-determinate. This happens in systems where a fast (quasi-equilibrium) subsystem establishes under some conditions. These cases require special consideration, and can be handled in a more expedite way through the matrix formulation presented below.

System (A.3) can be represented in matrix form as:

$$\dot{\mathbf{X}} = \mathbf{T}.\mathbf{f}\ , \tag{A.9}$$

where $\dot{\mathbf{X}}$ is the vector of time derivatives (possibly 0 for constant quantities), $\mathbf{T}$ is the $E \times T$, with T the number of different terms, is *term coefficients matrix,* and f is the *terms vector*. Here,

$$\mathbf{T} = \begin{bmatrix}
1 & -1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & -1 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & -1 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 & 0 & -1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 1 & -1
\end{bmatrix}, \tag{A.10}$$

$$\mathbf{f} = \begin{bmatrix} \phi \\ \alpha xh \\ \beta h \\ \psi yh \\ \eta w \\ y \\ \rho rz \\ \sigma su^{-1} \\ \chi \\ s \\ u \\ x \\ w \\ z \\ r \\ 1 \end{bmatrix} \qquad \text{(A.11)}$$

The upper left 5×8 submatrix of $\mathbf{T}$ is the reduced stoichiometric matrix of the system, and the remaining rows account for the ancillary variable and for the conservation relationships. Term coefficients matrices for dominant subsystems are obtained by selecting from each row in $\mathbf{T}$ one positive and one negative element and setting all other elements to 0. We identify each dominant subsystem by a signature in the form $(p_1, n_1, p_2, n_2, \ldots, p_e, n_e)$ where p_i and n_i are the indexes of the selected positive and negative elements in the i^{th} row of $\mathbf{T}$. Thus,

$$\mathbf{T}_{(1,3,2,6,4,5,6,7,8,7,9,11,12,16,15,16)} = \begin{bmatrix} 1 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 \end{bmatrix}$$

(A.12)

is the term coefficients matrix for the dominant subsystem (A.4). The rows of this matrix are linearly independent, and therefore this dominant subsystem has a unique steady state solution as seen above. However, this is not the case for, say, the dominant subsystem (1,2,5,4,4,5,6,7,8,7,9,11, 12,16,15,16):

$$\mathbf{T}_{(1,3,5,4,4,5,6,7,8,7,9,11,12,16,15,16)} = \begin{bmatrix} 1 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 \end{bmatrix},$$

(A.13)

Here, the second and third rows, corresponding to the differential equations for y and w, are linearly dependent. This sub-determinate dominant subsystem thus does not permit the simultaneous determination of both y and w, but yields instead an algebraic relationship among these variables:

$$\frac{w}{y} = \frac{\psi}{\eta} h \ . \tag{A.14}$$

This translates the fact that under the conditions where this dominant subsystem holds a quasi-equilibrium establishes between PrxSO$^-$ and PrxSO$_2^-$ owing to rapid recycling between the sulfinylation and the sulfiredoxin-catalyzed reduction of the sulfinic acid. (Physiologically implausible but possible.) PrxSO$^-$ and PrxSO$_2^-$ thus form an aggregated pool whose total concentration moves in a slower time scale and is determined by subdominant processes in the system. The subdominant terms that can potentially determine the concentration of the aggregated pool are those that do not cancel out upon addition of the differential equations for y and w, which expresses $\frac{d(w+y)}{dt}$. That is, those terms corresponding to non-null elements in the sum of the second and third rows of $\mathbf{T}$. By replacing one of the linearly dependent rows in $T_{(1,3,5,4,4,5,6,7,8,7,9,11,6,16,10,16)}$ by this sum one obtains a full-rank matrix:

$$\mathbf{T}_{(1,3,(5,2),(4,6),4,5,6,7,8,7,9,11,12,16,15,16)} = \begin{bmatrix} 1 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 \end{bmatrix}$$

(A.15)

corresponding to a fully determinate dominant subsystem. [In this notation, the indexes in (…,(…,i),(…,j),…) express the selected subdominant terms. We will call a system generated in this way by choosing a set of subdominant terms a *subdominant subsystem*.] Note that $\mathbf{T}_{(1,3,(5,2),(4,6),4,5,6,7,8,7,9,11,12,16,15,16)} = \mathbf{T}_{(1,3,2,6,4,5,6,7,8,7,9,11,12,16,15,16)}$, and therefore the dominant subsystem

(1,3,5,4,4,5,6,7,8,7,9,11,12,16,15,16) has the same steady state as the dominant subsystem (1,3,2,6,4,5,6,7,8,7,9,11,12,16,15,16). However, it holds in its own dominance region:

$$\begin{array}{l} \beta h > \alpha xh \wedge \beta h > \psi yh \wedge \\ \eta w > \alpha xh \wedge \psi yh > y \wedge \\ \chi > s \wedge \\ x > y \wedge x > w \wedge x > z \wedge \\ r > s \end{array}, \tag{A.16}$$

with ensuing boundary conditions

$$\begin{array}{l} \beta^* - \alpha^* - \phi^* > 0 \\ \beta^* + \rho^* - \alpha^* - \phi^* > 0 \\ \beta^* + \sigma^* - \alpha^* - \chi^* - \phi^* > 0 \\ 2\beta^* + \eta^* - \alpha^* - \psi^* - 2\phi^* > 0 \\ 2\beta^* - \alpha^* - \psi^* - \phi^* > 0 \\ \psi^* + \phi^* - \beta^* > 0 \\ \beta^* + \sigma^* - \alpha^* - \phi^* > 0 \end{array}. \tag{A.17}$$

For purposes of steady state analysis one may thus merge boundary conditions (A.8) and (A.17) into a single region.

The present example illustrates a relatively straightforward case of sub-determined dominant subsystem. However, there may be multiple quasi-equilibrium subsystems, the corresponding slow aggregated variables may not be straightforwardly identifiable, subdominant systems may have multiple subdominant subsystems, and some of the latter may be sub-determinate.

Design space analysis of the PTTRS model

After applying the above described approach to the 192 phenotypic regions of the PTTRS model (Equations (A.3)), only 13 regions had a steady state solution in agreement with the dominant conditions that defined them (self-consistency), and are reported in Table A.2.

Table A.2| Phenotypic regions inequalities.

Regions	Inequalities
HTPU	$\text{Max}[\eta^*, \frac{\beta^* + \eta^* - \psi^*}{2}] < \phi^* < \text{Min}[0, \rho^*, \sigma^*, \sigma^* - \chi^*, \frac{\alpha^* + \eta^* - \psi^*}{2}]$
TTPU	$\phi^* < \text{Min}[0, \rho^*, \sigma^*, \sigma^* - \chi^*, \frac{\alpha^* + \eta^* - \psi^*}{2}, \alpha^* - \psi^*] \wedge \alpha^* > \beta^*$
STAU	$\sigma^* > \text{Max}[0, \chi^*] \wedge \rho^* > 0 \wedge \beta^* - \alpha^* < \phi^* < \beta^* + \eta^* - \psi^* \wedge \left(\left(0 < \phi^* < \beta^* - \psi^*\right) \vee \left(\beta^* > \psi^* \wedge \phi^* > \beta^* - \psi^*\right) \right)$
HTAU	$\phi^* > \beta^* + \eta^* - \psi^* - \text{Min}[\sigma^*, \sigma^* - \chi^*, \rho^*, \frac{\alpha^* + \eta^* - \psi^*}{2}] \wedge \left(\left(\frac{\beta^* + \eta^* - \psi^*}{2} < \phi^* < \beta^* - \psi^* \right) \vee \left(\phi^* > \text{Max}[\eta^*, \beta^* - \psi^*] \right) \right)$

TTAU	$\phi^* < \text{Min}[0,\rho^*,\sigma^*,\sigma^*-\chi^*,\frac{\alpha^*+\eta^*-\psi^*}{2}]-(\beta^*-\alpha^*) \wedge ((\beta^*>\alpha^* \wedge \phi^* < \beta^*-\psi^*) \vee (\phi^* < (\beta^*-\alpha^*)+(\beta^*-\psi^*) \wedge \phi^* > \beta^*-\psi^*))$
DTAU	$(\beta^*-\alpha^*)-\rho^* < \phi^* < (\beta^*+\eta^*-\psi^*)-\rho^* \wedge \rho^*>0 \wedge \sigma^* > \text{Max}[0,\chi^*]-\rho^* \wedge ((\beta^*-\psi^*>\rho^* \wedge \phi^*>\beta^*-\psi^*) \vee (\rho^* < \phi < \beta^*-\psi^*))$
DDAU	$\sigma^* < \chi^* + \text{Min}[0,\rho^*] \wedge \chi^*>0 \wedge \beta^*-\alpha^*+\sigma^*-\chi^* < \phi^* < \beta^*+\eta^*-\psi^*-(\sigma^*-\chi^*) \wedge ((\sigma^*-\chi^*<\phi^*<\beta^*-\psi^*) \vee (\sigma^*-\chi^*<\beta^*-\psi^* \wedge \phi^*>\beta^*-\psi^*))$
STPU††	$\text{Max}[0,\psi^*-\alpha^*] < \phi^* < \eta^* \wedge \psi^* > \beta^* \wedge \rho^* > 0 \wedge \sigma^* > \text{Max}[0,\chi^*]$
TTPU††	$2\cdot\beta^*-\alpha^*-\psi^* < \phi^* < 2\cdot\text{Min}[0,\sigma^*,\sigma^*-\chi^*,\rho^*,\frac{\alpha^*+\eta^*-\psi^*}{2}]+(\psi^*-\alpha^*)$
DTPU††	$\sigma^* > \text{Max}[0,\chi^*]+\rho^* \wedge \rho^* > \text{Max}[0,\beta^*-\psi^*] \wedge Max[\rho^*,2\cdot\rho^*+\psi^*-\alpha^*] < \phi^* < \eta^*$
DDPU††	$\chi^*>0 \wedge \sigma^* < \chi^*+\text{Min}[0,\rho^*,\psi^*-\beta^*] \wedge \text{Max}[\sigma^*-\chi^*+\psi^*-\alpha^*,0]+\sigma^*-\chi^* < \phi^* < \eta^*$
DDAS	$\chi^*<0 \wedge \sigma^* < \text{Min}[0,\rho^*] \wedge \phi^* < \text{Min}[\beta^*-\alpha^*,\beta^*-\psi^*+\eta^*]-\sigma^* \wedge \left(\left(\sigma^*<\psi^*-\beta^* \wedge \phi^*>\beta^*-\psi^*\right) \vee \left(\phi^*<\beta^*-\psi^* \wedge \phi^*>\sigma^*\right)\right)$
DDPS††	$\chi^*<0 \wedge \beta^*-\psi^* < \sigma^* < \text{Min}[0,\rho^*] \wedge \text{Max}[0,\psi^*-\alpha^*+\sigma^*]+\sigma^* < \phi^* < \eta^*$

†† Peroxiredoxin hyperoxidation reaction is higher than either peroxiredoxin and alternative sinks scavenging

Not all of these are representative of the phenotypes of real cells, though. In order to select the biologically plausible regions, one has to consider the ranges of kinetic parameters and protein concentrations found in real cells. We consider the following three plausibility criteria cumulatively.

First, the maximum flux of reduction of the sulfinylated form of Peroxiredoxin is the lowest maximum flux of the system. Srx is an inefficient enzyme [45,88,90,91] and is much less abundant in cells than the other proteins considered in the model Table A.6.

In dimensionless term this is expressed as the following inequality:

$$\eta^* < \text{Min}[\alpha^*,\beta^*,\psi^*,\rho^*,\sigma^*,\sigma^*-\chi^*,0]$$

Second, the pseudo-first order-rate constant for Prx-S$^-$ oxidation by H_2O_2 strongly exceeds the rate constant for Prx-SO$^-$ condensation. This follows from the high reactivity ($k_{Ox} \sim 10^6 - 10^8 M^{-1}s^{-1}$ [171]) and abundance (tens to hundreds of μM, Table A.6) of typical 2-Cys peroxiredoxins in the cytoplasm. In turn, in eukaryotic typical 2-Cys peroxiredoxins the rate of condensation is limited by a local unfolding step that is required to bring the resolving cysteine into proximity with the sulfenate.

In dimensionless term this is expressed as the following inequality:

$$\alpha^* > \psi^* \wedge \alpha^* > 0$$

Third, Prx sulfinylation is the slowest among all (aggregated) H_2O_2-consuming processes in the model. The former process consumes H_2O_2 with a second order rate constant that has been measured for human PrxII as $1.2\times10^4\ M^{-1}s^{-1}$ [87].

$$\psi^* < \beta^*$$

Only the eight phenotypic regions that satisfy the three plausibility criteria above and their steady states are reported in Table A.3.

Table A.3| Biologically plausible phenotypic region steady state.

Regions	Variables						
	h	x	y	z	w	r	s
HTPU	$\frac{\eta}{\phi\cdot\psi}$	$\frac{\phi^2\cdot\psi}{\alpha\cdot\eta}$	ϕ	$\frac{\phi}{\rho}$	1	1	$\frac{\phi\cdot\chi}{\sigma}$
TTPU	$\frac{\phi}{\alpha}$	1	ϕ	$\frac{\phi}{\rho}$	$\frac{\phi^2\cdot\psi}{\alpha\cdot\eta}$	1	$\frac{\phi\cdot\chi}{\sigma}$
STAU	$\frac{\phi}{\beta}$	$\frac{\beta}{\alpha\cdot\phi}$	1	$\frac{1}{\rho}$	$\frac{\phi\cdot\psi}{\beta\cdot\eta}$	1	$\frac{\chi}{\sigma}$
HTAU	$\frac{\phi}{\beta}$	$\frac{\beta^2\cdot\eta}{\alpha\cdot\phi^2\cdot\psi}$	$\frac{\beta\cdot\eta}{\phi\cdot\psi}$	$\frac{\beta\cdot\eta}{\rho\cdot\phi\cdot\psi}$	1	1	$\frac{\beta\cdot\eta\cdot\chi}{\sigma\cdot\phi\cdot\psi}$
TTAU	$\frac{\phi}{\beta}$	1	$\frac{\alpha\cdot\phi}{\beta}$	$\frac{\alpha\cdot\phi}{\beta\cdot\rho}$	$\frac{\alpha\cdot\phi^2\cdot\psi}{\beta^2\cdot\eta}$	1	$\frac{\alpha\cdot\phi\cdot\chi}{\beta\cdot\sigma}$
DTAU	$\frac{\phi}{\beta}$	$\frac{\beta\cdot\rho}{\alpha\cdot\phi}$	ρ	1	$\frac{\rho\cdot\phi\cdot\psi}{\beta\cdot\eta}$	1	$\frac{\rho\cdot\chi}{\sigma}$
DDAU	$\frac{\phi}{\beta}$	$\frac{\beta\cdot\sigma}{\alpha\cdot\phi\cdot\chi}$	$\frac{\sigma}{\chi}$	1	$\frac{\sigma\cdot\phi\cdot\psi}{\beta\cdot\eta\cdot\chi}$	$\frac{\sigma}{\rho\cdot\chi}$	1
DDAS	$\frac{\phi}{\beta}$	$\frac{\beta\cdot\sigma}{\alpha\cdot\phi}$	σ	1	$\frac{\sigma\cdot\phi\cdot\psi}{\beta\cdot\eta}$	$\frac{\sigma}{\rho}$	1

Performance criteria

The local performance of a system can be characterized by how its steady-state responds to changes in the independent state variables (Logarithmic Gain) and parameters (Parameter Sensitivities). However, the study of the system's steady-state behavior will not give us insight into its dynamical properties. The study of the local dynamics will require examining the properties of the system's differential equations.

Logarithmic Gain

A logarithmic gain can be defined as

$$L(y,x) = \frac{\partial Log(y)}{\partial Log(x)}$$

Where: $L(y,x)$ represents the percent change in the dependent variable, y resulting from an infinitesimal change in the independent variable x while all other independent variables are held constant. A positive Logarithmic Gain implies direct proportionality between independent and dependent variable vice-versa if negative. Furthermore, if $L(y,x)$ is greater than 1 it implies an amplification of the signal. Similarly, to what defined above for variable is possible to calculate logarithmic gains for fluxes.

Sensitivities

The sensitivity can be defined as:

$$S(y,k)=\frac{\partial Log(y)}{\partial Log(k)}$$

where $S(y,k)$ represents the percent change in the dependent variable, y resulting from an infinitesimal change in the constant k while all other kinetic parameters are held constant.

Robustness

The robustness can be defined as:

$$I(y)_{p-k}=\sum_{i=1}^{\#p}\left|S(y,p_i)\right|$$

Where $I(y)_{p-k}$ represent the sum, in absolute value of all the sensitivities of the dependent variable y to all the kinetic parameters p except the one desired k. This measure account for the degree of cross talk and unwanted variation of the variable y.

Using the values in Table A.3 to calculate the performance criteria defined above, we obtain:

Table A.4| Performance criteria evaluated for the biological plausible regions

Criteria	Regions							
	HTPU	TTPU	STAU	HTAU	TTAU	DTAU	DDAU	DDAS
$S[x,v_{sup}]$	2	0	−1	−2	0	−1	−1	−1
$S[y,v_{sup}]$	1	1	0	−1	1	0	0	0
$S[z,v_{sup}]$	1	1	0	−1	1	0	0	0
$S[w,v_{sup}]$	0	2	1	0	2	1	1	1
$S[r,v_{sup}]$	0	0	0	0	0	0	0	0
$S[s,v_{sup}]$	1	1	0	−1	1	0	0	0
$I[x]_{p-vsup}$	$5+\frac{K_i}{1+K_i}$	1	$4+\frac{K_i}{1+K_i}$	$7+\frac{K_i}{1+K_i}$	1	$5+\frac{K_i}{1+K_i}$	$5+\frac{K_i}{1+K_i}$	$3+\frac{K_i}{1+K_i}$
$I[y]_{p-vsup}$	1	1	1	4	$4+\frac{K_i}{1+K_i}$	4	4	2

I[z]p-vsup	2	2	4	7	$5+\frac{K_i}{1+K_i}$	1	1	1
I[w]p-vsup	1	$5+\frac{K_i}{1+K_i}$	4	1	$7+\frac{K_i}{1+K_i}$	7	7	5
I[r]p-vsup	1	1	1	1	1	1	5	3
I[s]p-vsup	2	2	4	7	$5+\frac{K_i}{1+K_i}$	5	1	1
VTrxR								
S[VTrxR,vsup]	1	1	0	−1	1	0	0	0
I[VTrxR]p-vsup	0	0	2	5	$3+\frac{K_i}{1+K_i}$	3	3	1

Stability analysis

The S-system that described the PTTRS in each region were linearized in the respective equilibrium point. Where possible the eigenvalues were analytically obtained (TTPU, TTAU). Otherwise the Routh criteria were applied to determine the presence of positive eigenvalues.

Region HTPU

The S-system that describe this region assumes that r~1 and w ~1, this implied that the dynamics of the system will be dominated, for small perturbation, from the other variable. The ODE of the region thus becomes:

$$\begin{cases} \frac{dh}{d\tau} = \phi - h \cdot x \cdot \alpha \\ \frac{dx}{d\tau} = r \cdot z \cdot \rho + w \cdot \eta - h \cdot x \cdot \alpha - h \cdot y \cdot \psi \\ \frac{dy}{d\tau} = h \cdot x \cdot \alpha - y \\ \frac{dz}{d\tau} = y - r \cdot z \cdot \rho \\ \frac{ds}{d\tau} = -\frac{s \cdot \sigma}{\mu \cdot \chi} + \frac{r \cdot z \cdot \rho}{\mu} \end{cases}$$

To linearize the system, we calculate the Jacobian:

$$\begin{pmatrix} -x \cdot \alpha & -h \cdot \alpha & 0 & 0 & 0 \\ -x \cdot \alpha - y \cdot \psi & -h \cdot \alpha & -h \cdot \psi & r \cdot \rho & 0 \\ x \cdot \alpha & h \cdot \alpha & -1 & 0 & 0 \\ 0 & 0 & 1 & -r \cdot \rho & 0 \\ 0 & 0 & 0 & \frac{r \cdot \rho}{\mu} & -\frac{\sigma}{\mu \cdot \chi} \end{pmatrix}$$

Calculating the eigenvalues (with Eigenvalues instruction from Mathematica v10.3[187]) of the Jacobian in the equilibrium point we obtain:

$$\lambda_1 = -\frac{\sigma}{\mu \cdot \chi}$$

And a 4th grade polynomial that cannot be resolved:

$$\begin{aligned}
&-\alpha \cdot \eta^5 \cdot \mu^4 \cdot \rho \cdot \phi^4 \cdot \chi^4 \cdot \psi^4 \\
&+\left(\alpha \cdot \eta^5 \cdot \mu^3 \cdot \rho \cdot \phi \cdot \chi^3 \cdot \psi^2 - \alpha \cdot \eta^4 \cdot \mu^3 \cdot \phi^3 \cdot \chi^3 \cdot \psi^3 - \alpha \cdot \eta^4 \cdot \mu^3 \cdot \rho \cdot \phi^3 \cdot \chi^3 \cdot \psi^3 + \eta^2 \cdot \mu^3 \cdot \rho \cdot \phi^5 \cdot \chi^3 \cdot \psi^4\right) \cdot \lambda \\
&+\eta \cdot \mu^2 \cdot \chi^2 \cdot \psi \left(\phi^2 \cdot \psi \left(\eta \cdot \rho + (1+\rho)\phi^2 \cdot \psi\right) + \alpha \cdot \eta^2 \left(\eta + \phi(1+\rho - \phi \cdot \psi)\right)\right) \cdot \lambda^2 \\
&+\left(\alpha \cdot \eta^2 \cdot \mu \cdot \chi + \eta \cdot \mu \cdot \phi \cdot \chi \cdot \psi + \eta \cdot \mu \cdot \rho \cdot \phi \cdot \chi \cdot \psi + \mu \cdot \phi^3 \cdot \chi \cdot \psi^2\right) \cdot \lambda^3 \\
&+\lambda^4
\end{aligned}$$

It is possible to apply the Routh criteria to this polynomial to calculate the presence of positive eigenvalues:

$$a \cdot \lambda^4 + b \cdot \lambda^3 + c \cdot \lambda^2 + d \cdot \lambda^1 + e \cdot \lambda^0;$$

λ^4	a	c	e	0
λ^3	b	d	0	0
λ^2	$\frac{b \cdot c - a \cdot d}{b}$	e	0	0
λ^1	$\frac{\frac{b \cdot c - a \cdot d}{b} \cdot d - b \cdot e}{\frac{b \cdot c - a \cdot d}{b}}$	0	0	0
λ^0	e	0	0	0

The analysis reveals one change of sign so the regime has one positive eigenvalue making it unstable.

Region TTPU

The S-system that describe this region assumes that r~1 and x ~1, this implied that the dynamics of the system will be dominated, for small perturbation, from the other variable. The ODE of the region thus becomes:

$$\begin{cases}
\frac{dh}{d\tau} = \phi - h \cdot x \cdot \alpha \\
\frac{dw}{d\tau} = -w \cdot \eta + h \cdot y \cdot \psi \\
\frac{dy}{d\tau} = h \cdot x \cdot \alpha - y \\
\frac{dz}{d\tau} = y - r \cdot z \cdot \rho \\
\frac{ds}{d\tau} = -\frac{s \cdot \sigma}{\mu \cdot \chi} + \frac{r \cdot z \cdot \rho}{\mu}
\end{cases}$$

To linearize the system, we calculate the Jacobian:

$$\begin{pmatrix} -x\cdot\alpha & 0 & 0 & 0 & 0 \\ y\cdot\psi & -\eta & h\cdot\psi & 0 & 0 \\ x\cdot\alpha & 0 & -1 & 0 & 0 \\ 0 & 0 & 1 & -r\cdot\rho & 0 \\ 0 & 0 & 0 & \frac{r\cdot\rho}{\mu} & -\frac{\sigma}{\mu\cdot\chi} \end{pmatrix}$$

Calculating the eigenvalues (with Eigenvalues instruction from Mathematica v10.3[187]) of the Jacobian in the equilibrium point we obtain:

$$\lambda_1 = -\frac{\sigma}{\mu\cdot\chi};\lambda_2 = -1;\lambda_3 = -\alpha;\lambda_4 = -\eta;\lambda_5 = -\rho$$

Being all the dimensionless group positive, the eigenvalues are all negative and thus the system is stable

Region STAU

The S-system that describe this region assumes that r~1 and y ~1, this implied that the dynamics of the system will be dominated, for small perturbation, from the other variable. The ODE of the region thus becomes:

$$\begin{cases} \frac{dh}{d\tau} = \phi - h\cdot\beta \\ \frac{dw}{d\tau} = h\cdot y\cdot\psi - w\cdot\eta \\ \frac{dx}{d\tau} = r\cdot z\cdot\rho + w\cdot\eta - h\cdot x\cdot\alpha - h\cdot y\cdot\psi \\ \frac{dz}{d\tau} = y - r\cdot z\cdot\rho \\ \frac{ds}{d\tau} = -\frac{s\cdot\sigma}{\mu\cdot\chi} + \frac{r\cdot z\cdot\rho}{\mu} \end{cases}$$

To linearize the system, we calculate the Jacobian:

$$\begin{pmatrix} -\beta & 0 & 0 & 0 & 0 \\ y\cdot\psi & -\eta & 0 & 0 & 0 \\ -x\cdot\alpha - y\cdot\psi & \eta & -h\cdot\alpha & r\cdot\rho & 0 \\ 0 & 0 & 0 & -r\cdot\rho & 0 \\ 0 & 0 & 0 & \frac{r\cdot\rho}{\mu} & -\frac{\sigma}{\mu\cdot\chi} \end{pmatrix}$$

Calculating the eigenvalues (with Eigenvalues instruction from Mathematica v10.3[187]) of the Jacobian in the equilibrium point we obtain:

$$\lambda_1 = -\frac{\sigma}{\mu\cdot\chi};\lambda_2 = -\beta;\lambda_3 = -\frac{\alpha\cdot\phi}{\beta};\lambda_4 = -\eta;\lambda_5 = -\rho$$

Being all the dimensionless group positive, the eigenvalues are all negative and thus the system is stable

Region HTAU

The S-system that describe this region assumes that r~1 and w ~1, this implied that the dynamics of the system will be dominated, for small perturbation, from the other variable. The ODE of the region thus becomes:

$$\begin{cases} \frac{dh}{d\tau} = \phi - h \cdot \beta \\ \frac{dx}{d\tau} = r \cdot z \cdot \rho + w \cdot \eta - h \cdot x \cdot \alpha - h \cdot y \cdot \psi \\ \frac{dy}{d\tau} = h \cdot x \cdot \alpha - y \\ \frac{dz}{d\tau} = y - r \cdot z \cdot \rho \\ \frac{ds}{d\tau} = -\frac{s \cdot \sigma}{\mu \cdot \chi} + \frac{r \cdot z \cdot \rho}{\mu} \end{cases}$$

To linearize the system, we calculate the Jacobian:

$$\begin{pmatrix} -\beta & 0 & 0 & 0 & 0 \\ -x \cdot \alpha - y \cdot \psi & -h \cdot \alpha & -h \cdot \psi & r \cdot \rho & 0 \\ x \cdot \alpha & h \cdot \alpha & -1 & 0 & 0 \\ 0 & 0 & 1 & -r \cdot \rho & 0 \\ 0 & 0 & 0 & \frac{r \cdot \rho}{\mu} & -\frac{\sigma}{\mu \cdot \chi} \end{pmatrix}$$

Calculating the eigenvalues (with Eigenvalues instruction from Mathematica v10.3[187]) of the Jacobian in the equilibrium point we obtain:

$$\lambda_1 = -\frac{\sigma}{\mu \cdot \chi}; \lambda_2 = -\beta$$

And a 3rd order polynomial that cannot be solved.

$$\begin{aligned} &\alpha \cdot \beta \cdot \mu^3 \cdot \rho \cdot \phi^8 \cdot \chi^3 \cdot \psi^4 \\ &+\left(\beta^2 \cdot \mu^2 \cdot \rho \cdot \phi^4 \cdot \chi^2 \cdot \psi^2 + \alpha \cdot \beta \cdot \mu^2 \cdot \phi^5 \cdot \chi^2 \cdot \psi^2 + \alpha \cdot \beta \cdot \mu^2 \cdot \rho \cdot \phi^5 \cdot \chi^2 \cdot \psi^2 + \alpha \cdot \mu^2 \cdot \phi^6 \cdot \chi^2 \cdot \psi^3\right) \cdot \lambda \\ &+\left(\beta \cdot \mu \cdot \phi^2 \cdot \chi \cdot \psi + \beta \cdot \mu \cdot \rho \cdot \phi^2 \cdot \chi \cdot \psi + \alpha \cdot \mu \cdot \phi^3 \cdot \chi \cdot \psi\right) \cdot \lambda^2 \\ &+\lambda^3 \end{aligned}$$

Applying the Routh criteria as described previously we found no change in sign meaning that the system is stable.

Region TTAU

The S-system that describe this region assumes that r~1 and x ~1, this implied that the dynamics of the system will be dominated, for small perturbation, from the other variable. The ODE of the region thus becomes:

$$\begin{cases} \frac{dh}{d\tau} = \phi - h \cdot \beta \\ \frac{dw}{d\tau} = -w \cdot \eta + h \cdot y \cdot \psi \\ \frac{dy}{d\tau} = h \cdot x \cdot \alpha - y \\ \frac{dz}{d\tau} = y - r \cdot z \cdot \rho \\ \frac{ds}{d\tau} = -\frac{s \cdot \sigma}{\mu \cdot \chi} + \frac{r \cdot z \cdot \rho}{\mu} \end{cases}$$

To linearize the system, we calculate the Jacobian:

$$\begin{pmatrix} -\beta & 0 & 0 & 0 & 0 \\ y \cdot \psi & -\eta & h \cdot \psi & 0 & 0 \\ x \cdot \alpha & 0 & -1 & 0 & 0 \\ 0 & 0 & 1 & -r \cdot \rho & 0 \\ 0 & 0 & 0 & \frac{r \cdot \rho}{\mu} & -\frac{\sigma}{\mu \cdot \chi} \end{pmatrix}$$

Calculating the eigenvalues (with Eigenvalues instruction from Mathematica v10.3[187]) of the Jacobian in the equilibrium point we obtain:

$$\lambda_1 = -\frac{\sigma}{\mu \cdot \chi}; \lambda_2 = -1; \lambda_3 = -\beta; \lambda_4 = -\eta; \lambda_5 = -\rho$$

Being all the dimensionless group positive, the eigenvalues are all negative and thus the system is stable

Region DTAU

The S-system that describe this region assumes that r~1 and z ~1, this implied that the dynamics of the system will be dominated, for small perturbation, from the other variable. The ODE of the region thus becomes:

$$\begin{cases} \frac{dh}{d\tau} = \phi - h \cdot \beta \\ \frac{dx}{d\tau} = r \cdot z \cdot \rho + w \cdot \eta - h \cdot x \cdot \alpha - h \cdot y \cdot \psi \\ \frac{dy}{d\tau} = h \cdot x \cdot \alpha - y \\ \frac{dz}{d\tau} = y - r \cdot z \cdot \rho \\ \frac{ds}{d\tau} = -\frac{s \cdot \sigma}{\mu \cdot \chi} + \frac{r \cdot z \cdot \rho}{\mu} \end{cases}$$

To linearize the system, we calculate the Jacobian:

$$\begin{pmatrix} -\beta & 0 & 0 & 0 & 0 \\ -x\cdot\alpha - y\cdot\psi & -h\cdot\alpha & -h\cdot\psi & r\cdot\rho & 0 \\ x\cdot\alpha & h\cdot\alpha & -1 & 0 & 0 \\ 0 & 0 & 1 & -r\cdot\rho & 0 \\ 0 & 0 & 0 & \frac{r\cdot\rho}{\mu} & -\frac{\sigma}{\mu\cdot\chi} \end{pmatrix}$$

Calculating the eigenvalues (with Eigenvalues instruction from Mathematica v10.3[187]) of the Jacobian in the equilibrium point we obtain:

$$\lambda_1 = -\frac{\sigma}{\mu\cdot\chi}; \lambda_2 = -\beta$$

And a 3rd order polynomial that cannot be resolved.

$$\begin{aligned} &\alpha\cdot\beta\cdot\mu^3\cdot\rho\cdot\phi^5\cdot\chi^3\cdot\psi \\ &+\left(\beta^2\cdot\mu^2\cdot\rho\cdot\phi^2\cdot\chi^2 + \alpha\cdot\beta\cdot\mu^2\cdot\phi^3\cdot\chi^2 + \alpha\cdot\beta\cdot\mu^2\cdot\rho\cdot\phi^3\cdot\chi^2 + \alpha\cdot\mu^2\cdot\phi^4\cdot\chi^2\cdot\psi\right)\cdot\lambda \\ &+\left(\beta\cdot\mu\cdot\phi\cdot\chi + \beta\cdot\mu\cdot\rho\cdot\phi\cdot\chi + \alpha\cdot\mu\cdot\phi^2\cdot\chi\right)\cdot\lambda^2 \\ &+\lambda^3 \end{aligned}$$

Applying the Routh criteria as described previously we found no change in sign meaning that the system is stable.

Region DDAU

The S-system that describe this region assumes that s~1 and z ~1, this implied that the dynamics of the system will be dominated, for small perturbation, from the other variable. The ODE of the region thus becomes:

$$\begin{cases} \frac{dh}{d\tau} = \phi - h\cdot\beta \\ \frac{dw}{d\tau} = -w\cdot\eta + h\cdot y\cdot\psi \\ \frac{dy}{d\tau} = h\cdot x\cdot\alpha - y \\ \frac{dx}{d\tau} = -h\cdot x\cdot\alpha + w\cdot\eta + r\cdot z\cdot\rho - h\cdot y\cdot\psi \\ \frac{dr}{d\tau} = +\frac{s\cdot\sigma}{\mu\cdot\chi} - \frac{r\cdot z\cdot\rho}{\mu} \end{cases}$$

To linearize the system, we calculate the Jacobian:

$$\begin{pmatrix} -\beta & 0 & 0 & 0 & 0 \\ \psi\cdot y & -\eta & h\cdot\psi & 0 & 0 \\ x\cdot\alpha & 0 & -1 & h\cdot\alpha & 0 \\ -x\cdot\alpha - y\cdot\psi & \eta & -h\cdot\psi & -h\cdot\alpha & z\cdot\rho \\ 0 & 0 & 0 & 0 & -\frac{z\cdot\rho}{\mu} \end{pmatrix}$$

Calculating the eigenvalues (with Eigenvalues instruction from Mathematica v10.3[187]) of the Jacobian in the equilibrium point we obtain:

$$\lambda_1 = -\frac{\rho}{\mu}; \lambda_2 = -\beta$$

And a 3rd order polynomial that cannot be resolved

$$\begin{aligned}
&+\alpha \cdot \beta^2 \cdot \eta \cdot \mu^3 \cdot \phi^4 \cdot \chi^3 \\
&+\left(\beta^2 \cdot \eta \cdot \mu^2 \cdot \phi^2 \cdot \chi^2 + \alpha \cdot \beta \cdot \mu^2 \cdot \phi^3 \cdot \chi^2 + \alpha \cdot \beta \cdot \eta \cdot \mu^2 \cdot \phi^3 \cdot \chi^2 + \alpha \cdot \mu^2 \cdot \phi^4 \cdot \chi^2 \cdot \psi\right) \cdot \lambda \\
&+\left(\beta \cdot \mu \cdot \phi \cdot \chi + \beta \cdot \eta \cdot \mu \cdot \phi \cdot \chi + \alpha \cdot \mu \cdot \phi^2 \cdot \chi\right) \cdot \lambda^2 \\
&+\lambda^3
\end{aligned}$$

Applying the Routh criteria as described previously we found no change in sign meaning that the system is stable.

Region DDAS

The S-system that describe this region assumes that s~1 and z ~1, this implied that the dynamics of the system will be dominated, for small perturbation, from the other variable. The ODE of the region thus becomes:

$$\begin{cases}
\frac{dh}{d\tau} = \phi - h \cdot \beta \\
\frac{dw}{d\tau} = -w \cdot \eta + h \cdot y \cdot \psi \\
\frac{dy}{d\tau} = h \cdot x \cdot \alpha - y \\
\frac{dx}{d\tau} = -h \cdot x \cdot \alpha + w \cdot \eta + r \cdot z \cdot \rho - h \cdot y \cdot \psi \\
\frac{dr}{d\tau} = +\frac{s \cdot \sigma}{\mu \cdot \chi} - \frac{r \cdot z \cdot \rho}{\mu}
\end{cases}$$

To linearize the system, we calculate the Jacobian:

$$\begin{pmatrix}
-\beta & 0 & 0 & 0 & 0 \\
\psi \cdot y & -\eta & h \cdot \psi & 0 & 0 \\
x \cdot \alpha & 0 & -1 & h \cdot \alpha & 0 \\
-x \cdot \alpha - y \cdot \psi & \eta & -h \cdot \psi & -h \cdot \alpha & z \cdot \rho \\
0 & 0 & 0 & 0 & -\frac{z \cdot \rho}{\mu}
\end{pmatrix}$$

Calculating the eigenvalues (with Eigenvalues instruction from Mathematica v10.3[187]) of the Jacobian in the equilibrium point we obtain:

$$\lambda_1 = -\frac{\rho}{\mu}; \lambda_2 = -\beta$$

And a 3rd order polynomial that cannot be resolved

$$\alpha \cdot \beta^2 \cdot \eta \cdot \mu^3 \cdot \phi^4$$
$$+\left(\beta^2 \cdot \eta \cdot \mu^2 \cdot \phi^2 + \alpha \cdot \beta \cdot \mu^2 \cdot \phi^3 + \alpha \cdot \beta \cdot \eta \cdot \mu^2 \cdot \phi^3 + \alpha \cdot \mu^2 \cdot \phi^4 \cdot \psi\right) \cdot \lambda$$
$$+\left(\beta \cdot \mu \cdot \phi + \beta \cdot \eta \cdot \mu \cdot \phi + \alpha \cdot \mu \cdot \phi^2\right) \cdot \lambda^2$$
$$+\lambda^3$$

Applying the Routh criteria as described previously we found no change in sign meaning that the system is stable.

PTTRS Topologies

The determination of the best performing regime (TTAU, TTPU) allowed to identify the ideal optimal location of the operating point for the PTTRS. However, an increase in H_2O_2 supply or changes in other parameters may induce a transition to a suboptimal regime. In order to map all the possible regimes arrangement into the design space we reduced the regimes inequalities to minimal expressions, applying the above defined biological constrains

Table A.5| Minimized physiologically plausible regimes inequalities.

Regions	Inequalities
HTPU	$\frac{\beta^* + \eta^* - \psi^*}{2} < \phi^* < \text{Min}\left[0, \rho^*, \sigma^*, \sigma^* - \chi^*, \frac{\alpha^* + \eta^* - \psi^*}{2}\right]$
TTPU	$\phi^* < \text{Min}\left[0, \rho^*, \sigma^*, \sigma^* - \chi^*, \frac{\alpha^* + \eta^* - \psi^*}{2}\right] \wedge \alpha^* > \beta^*$
STAU	$\beta^* + \eta^* - \psi^* > \phi^* > \text{Max}\left[0, -\alpha^* + \beta^*\right] \wedge \sigma^* > \text{Max}\left[0, \chi^*\right] \wedge \rho^* > 0$
HTAU	$\beta^* + \eta^* > \phi^* > \beta^* + \eta^* - \psi^* + \text{Max}\left[0, -\rho^*, -\sigma^*, -\sigma^* + \chi^*, \frac{-\alpha^* - \eta^* + \psi^*}{2}, \frac{-\beta^* - \eta^* + \psi^*}{2}\right]$ $\vee\, \phi^* > \beta^* + \eta^* - \psi^* + \text{Max}\left[-\eta^*, \frac{\alpha^* + \eta^* - \psi^*}{2}\right]$
TTAU	$\text{Min}\left[0, \rho^*, \sigma^*, \sigma^* - \chi^*, \frac{\alpha^* + \eta^* - \psi^*}{2}\right] > \alpha^* - \beta^* + \phi^* \wedge \alpha^* < \beta^*$
DTAU	$\beta^* + \eta^* - \psi^* - \rho^* > \phi^* > \rho^* + \text{Max}\left[0, -\alpha^* + \beta^*\right] \wedge \sigma^* > \rho^* + \text{Max}\left[0, \chi^*\right] \wedge \rho^* < 0$
DDAU	$\beta^* + \eta^* + \chi^* - \psi^* - \sigma^* > \phi^* > \sigma^* - \chi^* + \text{Max}\left[0, -\alpha^* + \beta^*\right]$ $\wedge \sigma^* < \chi^* + \text{Min}\left[0, \rho^*\right] \wedge \chi^* > 0$
DDAS	$\beta^* + \eta^* - \psi^* - \sigma^* > \phi^* > \sigma^* + \text{Max}\left[0, -\alpha^* + \beta^*\right] \wedge \sigma^* < \text{Min}\left[0, \rho^*\right] \wedge \chi^* < 0$

The inequalities can be fully resolved in the (σ, ϕ) plane by establishing the relation between the following expressions:

$$\{\rho^*, \chi^*, 0, \alpha^* - \beta^*, \alpha^* - \psi^* + \eta^*, \beta^* - \psi^* + \eta^*, \frac{\alpha^* - \beta^*}{2}, \frac{\beta^* - \psi^* + \eta^*}{2}, \frac{\alpha^* - \psi^* + \eta^*}{2}, \eta^*\}$$

The permutation of the elements of this vector will define a sector of the design space where the topology will be defined by the relations amongst the groups

i.e.

$$\{\eta^* < \rho^* < \chi^* < 0 < \alpha^* - \beta^* < \frac{\alpha^* - \beta^*}{2} < \beta^* - \psi^* + \eta^* < \frac{\beta^* - \psi^* + \eta^*}{2} < \alpha^* - \psi^* + \eta^* < \frac{\alpha^* - \psi^* + \eta^*}{2}\}$$

This will generate 362880 possible sectors of the space, of these sectors only 1152 will be biologically relevant (once applied the biological constrains defined above).

After analyzing the possible arrangements, we found that only 12 possible topologies were allowed. An analysis of the macroscopic phenotypes (dominant species) can be used, by pairing the regimes characteristics in Table 2.2 with the topology in Figure 2.2, to define super-families (A, B, C) of topologies which share identical modes of response. Within a super-family we can identify 4 possible variations. If Prx scavenge the majority of H_2O_2 from the organism (condition *iii-iv*), then the basal regime will be TTPU vice versa TTAU (condition *i-ii*). If TrxR can be saturated by Trx-SS (condition *i-iii*), then for low TrxR activity the operating regime will be DDAS vice versa (condition *ii-iv*) will be DDAU.

Topology A-i

This topology is characterized by having the alternative sink scavenging the majority of H_2O_2 under basal oxidative stress and TrxR cannot be saturated by Trx-SS. It is possible in 20 sectors of the design space out of the 1152.

The topology minimal requirements can be extracted by analyzing the 20 permutation that describe the sectors. In particular, by assigning a defined position to each permutation term it is possible to calculate how many times it appears lower or greater than each other. This creates a correlation matrix like in which the maxima identify the conserved relation amongst all 20 relations belonging to the A-*i* topology (positive maximum indicates column element greater than the row, negative vice versa).

	ρ^*	χ^*	0	$\alpha^* - \beta^*$	$\alpha^* - \psi^* + \eta^*$	$\beta^* - \psi^* + \eta^*$	$\frac{\alpha^* - \beta^*}{2}$	$\frac{\alpha^* - \psi^* + \eta^*}{2}$	$\frac{\beta^* - \psi^* + \eta^*}{2}$
ρ^*	0	–12	–4	18	20	18	10	20	10
χ^*	0	0	20	20	20	20	20	20	20
0	0	0	0	20	20	20	20	20	20
$\alpha^* - \beta^*$	0	0	0	0	20	0	–20	0	–10
$\alpha^* - \psi^* + \eta^*$	0	0	0	0	0	–20	–20	–20	–20
$\beta^* - \psi^* + \eta^*$	0	0	0	0	0	0	–10	0	–20
$\frac{\alpha^* - \beta^*}{2}$	0	0	0	0	0	0	0	20	0
$\frac{\alpha^* - \psi^* + \eta^*}{2}$	0	0	0	0	0	0	0	0	–20
$\frac{\beta^* - \psi^* + \eta^*}{2}$	0	0	0	0	0	0	0	0	0

The analysis of this matrix yield the following topology definition:

$$\alpha^* < \beta^* \wedge \frac{\alpha^* + \eta^* - \psi^*}{2} < \rho^* \wedge \beta^* + \eta^* - \psi^* < 0 \wedge \chi^* > 0$$

Topology A-ii

This topology is characterized by having the alternative sink scavenging the majority of H_2O_2 under basal oxidative stress and TrxR can be saturated by Trx-SS. It is possible in 124 sectors of the design space out of the 1152. As previously described it is possible to define a correlation matrix:

	ρ^*	χ^*	0	$\alpha^* - \beta^*$	$\alpha^* - \psi^* + \eta^*$	$\beta^* - \psi^* + \eta^*$	$\frac{\alpha^* - \beta^*}{2}$	$\frac{\alpha^* - \psi^* + \eta^*}{2}$	$\frac{\beta^* - \psi^* + \eta^*}{2}$
ρ^*	0	76	–68	108	124	108	44	124	44
χ^*	0	0	–124	18	92	18	–54	28	–54
0	0	0	0	124	124	124	124	124	124
$\alpha^* - \beta^*$	0	0	0	0	124	0	–124	0	–62
$\alpha^* - \psi^* + \eta^*$	0	0	0	0	0	–124	–124	–124	–124
$\beta^* - \psi^* + \eta^*$	0	0	0	0	0	0	–62	0	–124
$\frac{\alpha^* - \beta^*}{2}$	0	0	0	0	0	0	0	124	0
$\frac{\alpha^* - \psi^* + \eta^*}{2}$	0	0	0	0	0	0	0	0	–124
$\frac{\beta^* - \psi^* + \eta^*}{2}$	0	0	0	0	0	0	0	0	0

The analysis of this matrix yield the following topology definition:

$$\alpha^* < \beta^* \wedge \chi^* < 0 \wedge \frac{\alpha^* + \eta^* - \psi^*}{2} < \rho^* \wedge \beta^* + \eta^* - \psi^* < 0$$

Topology A-iii

This topology is characterized by having the Prxs scavenging the majority of H_2O_2 under basal oxidative stress and TrxR can be saturated by Trx-SS. It is possible in 98 sectors of the design space out of the 1152. As previously described it is possible to define a correlation matrix:

	ρ^*	χ^*	0	$\alpha^* - \beta^*$	$\alpha^* - \psi^* + \eta^*$	$\beta^* - \psi^* + \eta^*$	$\frac{\alpha^* - \beta^*}{2}$	$\frac{\alpha^* - \psi^* + \eta^*}{2}$	$\frac{\beta^* - \psi^* + \eta^*}{2}$
ρ^*	0	76	22	–66	40	98	–28	34	98
χ^*	0	0	–98	–98	–36	52	–98	–72	–4
0	0	0	0	–98	22	98	–98	22	98
$\alpha^* - \beta^*$	0	0	0	0	98	98	98	98	98
$\alpha^* - \psi^* + \eta^*$	0	0	0	0	0	98	–60	–22	44
$\beta^* - \psi^* + \eta^*$	0	0	0	0	0	0	–98	–98	–98
$\frac{\alpha^* - \beta^*}{2}$	0	0	0	0	0	0	0	98	98
$\frac{\alpha^* - \psi^* + \eta^*}{2}$	0	0	0	0	0	0	0	0	98
$\frac{\beta^* - \psi^* + \eta^*}{2}$	0	0	0	0	0	0	0	0	0

The analysis of this matrix yield the following topology definition:

$$\beta^* < \alpha^* \wedge \chi^* < 0 \wedge \beta^* + \eta^* - \psi^* < \wedge \frac{\beta^* + \eta^* - \psi^*}{2} < \rho^*$$

Topology A-iv

L2L1L4L7

This topology is characterized by having the Prxs scavenging the majority of H_2O_2 under basal oxidative stress and TrxR cannot be saturated by Trx-SS. It is possible in 109 sectors of the design space out of the 1152. As previously described it is possible to define a correlation matrix:

	ρ^*	χ^*	0	$\alpha^* - \beta^*$	$\alpha^* - \psi^* + \eta^*$	$\beta^* - \psi^* + \eta^*$	$\frac{\alpha^* - \beta^*}{2}$	$\frac{\alpha^* - \psi^* + \eta^*}{2}$	$\frac{\beta^* - \psi^* + \eta^*}{2}$
ρ^*	0	−25	59	−69	23	109	−17	47	109
χ^*	0	0	109	−55	39	109	13	81	109
0	0	0	0	−109	−31	109	−109	−31	109
$\alpha^* - \beta^*$	0	0	0	0	109	109	109	109	109
$\alpha^* - \psi^* + \eta^*$	0	0	0	0	0	109	−39	31	73
$\beta^* - \psi^* + \eta^*$	0	0	0	0	0	0	−109	−109	−109
$\frac{\alpha^* - \beta^*}{2}$	0	0	0	0	0	0	0	109	109
$\frac{\alpha^* - \psi^* + \eta^*}{2}$	0	0	0	0	0	0	0	0	109
$\frac{\beta^* - \psi^* + \eta^*}{2}$	0	0	0	0	0	0	0	0	0

The analysis of this matrix yield the following topology definition:

$$\beta^* < \alpha^* \wedge \beta^* + \eta^* - \psi^* < 0 \wedge \frac{\beta^* + \eta^* - \psi^*}{2} < \rho^* \wedge 0 < \chi^*$$

Topology B-i

This topology is characterized by having the alternative sink scavenging the majority of H_2O_2 under basal oxidative stress and TrxR cannot be saturated by Trx-SS. It also presents a saturation response regime, where the signaling pathways of the PTTRS are unresponsive to change in H_2O_2. It is possible in 84 sectors of the design space out of the 1152. As previously described it is possible to define a correlation matrix:

	ρ^*	χ^*	0	$\alpha^*-\beta^*$	$\alpha^*-\psi^*+\eta^*$	$\beta^*-\psi^*+\eta^*$	$\frac{\alpha^*-\beta^*}{2}$	$\frac{\alpha^*-\psi^*+\eta^*}{2}$	$\frac{\beta^*-\psi^*+\eta^*}{2}$
ρ^*	0	0	84	84	24	–44	84	60	8
χ^*	0	0	84	84	24	–44	84	60	8
0	0	0	0	84	–36	–84	84	–36	–84
$\alpha^*-\beta^*$	0	0	0	0	–84	–84	–84	–84	–84
$\alpha^*-\psi^*+\eta^*$	0	0	0	0	0	–84	60	36	–24
$\beta^*-\psi^*+\eta^*$	0	0	0	0	0	0	84	84	84
$\frac{\alpha^*-\beta^*}{2}$	0	0	0	0	0	0	0	–84	–84
$\frac{\alpha^*-\psi^*+\eta^*}{2}$	0	0	0	0	0	0	0	0	–84
$\frac{\beta^*-\psi^*+\eta^*}{2}$	0	0	0	0	0	0	0	0	0

The analysis of this matrix yield the following topology definition:

$$\alpha^* < \beta^* \wedge 0 < \beta^* + \eta^* - \psi^* \wedge 0 < \chi^* \wedge 0 < \rho^*$$

Topology B-ii

This topology is characterized by having the alternative sink scavenging the majority of H_2O_2 under basal oxidative stress and TrxR can be saturated by Trx-SS. It also presents a saturation response regime, where the signaling pathways of the PTTRS are unresponsive to change in H_2O_2. It is possible in 60 sectors of the design space out of the 1152. As previously described it is possible to define a correlation matrix:

	ρ^*	χ^*	0	$\alpha^*-\beta^*$	$\alpha^*-\psi^*+\eta^*$	$\beta^*-\psi^*+\eta^*$	$\frac{\alpha^*-\beta^*}{2}$	$\frac{\alpha^*-\psi^*+\eta^*}{2}$	$\frac{\beta^*-\psi^*+\eta^*}{2}$
ρ^*	0	60	60	60	30	–28	60	48	10
χ^*	0	0	–60	28	–30	–60	–10	–48	–60
0	0	0	0	60	0	–60	60	0	–60
$\alpha^*-\beta^*$	0	0	0	0	–60	–60	–60	–60	–60
$\alpha^*-\psi^*+\eta^*$	0	0	0	0	0	–60	30	0	–30
$\beta^*-\psi^*+\eta^*$	0	0	0	0	0	0	60	60	60
$\frac{\alpha^*-\beta^*}{2}$	0	0	0	0	0	0	0	–60	–60
$\frac{\alpha^*-\psi^*+\eta^*}{2}$	0	0	0	0	0	0	0	0	–60
$\frac{\beta^*-\psi^*+\eta^*}{2}$	0	0	0	0	0	0	0	0	0

The analysis of this matrix yield the following topology definition:

$$\chi^* < 0 \wedge 0 < \rho^* \wedge \alpha^* < \beta^* \wedge 0 < \beta^* + \eta^* - \psi^*$$

Topology B-iii

This topology is characterized by having the Prxs scavenging the majority of H_2O_2 under basal oxidative stress and TrxR can be saturated by Trx-SS. It also presents a saturation response regime, where the signaling pathways of the PTTRS are unresponsive to change in H_2O_2. It is possible in 28 sectors of the design space out of the 1152. As previously described it is possible to define a correlation matrix:

	ρ^*	χ^*	0	$\alpha^*-\beta^*$	$\alpha^*-\psi^*+\eta^*$	$\beta^*-\psi^*+\eta^*$	$\frac{\alpha^*-\beta^*}{2}$	$\frac{\alpha^*-\psi^*+\eta^*}{2}$	$\frac{\beta^*-\psi^*+\eta^*}{2}$
ρ^*	0	28	28	–2	–20	–2	14	–4	14
χ^*	0	0	–28	–28	–28	–28	–28	–28	–28
0	0	0	0	–28	–28	–28	–28	–28	–28
$\alpha^*-\beta^*$	0	0	0	0	–28	0	28	0	14
$\alpha^*-\psi^*+\eta^*$	0	0	0	0	0	28	28	28	28
$\beta^*-\psi^*+\eta^*$	0	0	0	0	0	0	14	0	28
$\frac{\alpha^*-\beta^*}{2}$	0	0	0	0	0	0	0	–28	0
$\frac{\alpha^*-\psi^*+\eta^*}{2}$	0	0	0	0	0	0	0	0	28
$\frac{\beta^*-\psi^*+\eta^*}{2}$	0	0	0	0	0	0	0	0	0

The analysis of this matrix yield the following topology definition:

$\chi^* < 0 \wedge 0 < \rho^* \wedge 0 < \beta^* + \eta^* - \psi^* \wedge \beta^* < \alpha^*$

Topology B-iv

This topology is characterized by having the Prxs scavenging the majority of H_2O_2 under basal oxidative stress and TrxR cannot be saturated by Trx-SS. It also presents a saturation response regime, where the signaling pathways of the PTTRS are unresponsive to change in H_2O_2. It is possible in 224 sectors of the design space out of the 1152. As previously described it is possible to define a correlation matrix:

	ρ^*	χ^*	0	$\alpha^*-\beta^*$	$\alpha^*-\psi^*+\eta^*$	$\beta^*-\psi^*+\eta^*$	$\frac{\alpha^*-\beta^*}{2}$	$\frac{\alpha^*-\psi^*+\eta^*}{2}$	$\frac{\beta^*-\psi^*+\eta^*}{2}$
ρ^*	0	0	224	–16	–160	–16	112	–32	112
χ^*	0	0	224	–16	–160	–16	112	–32	112
0	0	0	0	–224	–224	–224	–224	–224	–224
$\alpha^*-\beta^*$	0	0	0	0	–224	0	224	0	112
$\alpha^*-\psi^*+\eta^*$	0	0	0	0	0	224	224	224	224
$\beta^*-\psi^*+\eta^*$	0	0	0	0	0	0	112	0	224
$\frac{\alpha^*-\beta^*}{2}$	0	0	0	0	0	0	0	–224	0
$\frac{\alpha^*-\psi^*+\eta^*}{2}$	0	0	0	0	0	0	0	0	224
$\frac{\beta^*-\psi^*+\eta^*}{2}$	0	0	0	0	0	0	0	0	0

The analysis of this matrix yield the following topology definition:

$\beta^* < \alpha^* \wedge 0 < \chi^* \wedge 0 < \beta^* + \eta^* - \psi^* \wedge 0 < \rho^*$

Topology C-i

This topology is characterized by having the alternative sink scavenging the majority of H_2O_2 under basal oxidative stress and TrxR cannot be saturated by Trx-SS. It also presents a saturation response regime, where the signaling pathways of the PTTRS are unresponsive to change in H_2O_2. It is possible in 76 sectors of the design space out of the 1152. As previously described it is possible to define a correlation matrix:

	ρ^*	χ^*	0	$\alpha^*-\beta^*$	$\alpha^*-\psi^*+\eta^*$	$\beta^*-\psi^*+\eta^*$	$\frac{\alpha^*-\beta^*}{2}$	$\frac{\alpha^*-\psi^*+\eta^*}{2}$	$\frac{\beta^*-\psi^*+\eta^*}{2}$
ρ^*	0	–76	–76	20	–22	–68	–26	–56	–76
χ^*	0	0	76	76	46	–12	76	64	26
0	0	0	0	76	16	–44	76	16	–44
$\alpha^*-\beta^*$	0	0	0	0	–44	–60	–76	–60	–68
$\alpha^*-\psi^*+\eta^*$	0	0	0	0	0	–76	14	–16	–46
$\beta^*-\psi^*+\eta^*$	0	0	0	0	0	0	52	60	44
$\frac{\alpha^*-\beta^*}{2}$	0	0	0	0	0	0	0	–44	–60
$\frac{\alpha^*-\psi^*+\eta^*}{2}$	0	0	0	0	0	0	0	0	–76
$\frac{\beta^*-\psi^*+\eta^*}{2}$	0	0	0	0	0	0	0	0	0

The analysis of this matrix yield the following topology definition:

$$\rho^* < 0 \wedge 0 < \chi^* \wedge \alpha^* < \beta^* \wedge \rho^* < \frac{\beta^*+\eta^*-\psi^*}{2}$$

Topology C-ii

This topology is characterized by having the alternative sinks scavenging the majority of H_2O_2 under basal oxidative stress and TrxR can be saturated by Trx-SS. It also presents a saturation response regime, where the signaling pathways of the PTTRS are unresponsive to change in H_2O_2. It is possible in 212 sectors of the design space out of the 1152. As previously described it is possible to define a correlation matrix:

	ρ^*	χ^*	0	$\alpha^*-\beta^*$	$\alpha^*-\psi^*+\eta^*$	$\beta^*-\psi^*+\eta^*$	$\frac{\alpha^*-\beta^*}{2}$	$\frac{\alpha^*-\psi^*+\eta^*}{2}$	$\frac{\beta^*-\psi^*+\eta^*}{2}$
ρ^*	0	–48	–212	20	40	–148	–136	–124	–212
χ^*	0	0	–212	44	64	–84	–80	–52	–156
0	0	0	0	212	164	44	212	164	44
$\alpha^*-\beta^*$	0	0	0	0	44	–84	–212	–84	–148
$\alpha^*-\psi^*+\eta^*$	0	0	0	0	0	–212	–104	–164	–188
$\beta^*-\psi^*+\eta^*$	0	0	0	0	0	0	20	84	–44
$\frac{\alpha^*-\beta^*}{2}$	0	0	0	0	0	0	0	44	–84
$\frac{\alpha^*-\psi^*+\eta^*}{2}$	0	0	0	0	0	0	0	0	–212
$\frac{\beta^*-\psi^*+\eta^*}{2}$	0	0	0	0	0	0	0	0	0

The analysis of this matrix yield the following topology definition:

$$\alpha^* < \beta^* \wedge \chi^* < 0 \wedge \rho^* < 0 \wedge \rho^* < \frac{\beta^* + \eta^* - \psi^*}{2}$$

Topology C-iii

This topology is characterized by having the Prxs scavenging the majority of H_2O_2 under basal oxidative stress and TrxR can be saturated by Trx-SS. It also presents a saturation response regime, where the signaling pathways of the PTTRS are unresponsive to change in H_2O_2. It is possible in 54 sectors of the design space out of the 1152. As previously described it is possible to define a correlation matrix:

	ρ^*	χ^*	0	$\alpha^*-\beta^*$	$\alpha^*-\psi^*+\eta^*$	$\beta^*-\psi^*+\eta^*$	$\frac{\alpha^*-\beta^*}{2}$	$\frac{\alpha^*-\psi^*+\eta^*}{2}$	$\frac{\beta^*-\psi^*+\eta^*}{2}$
ρ^*	0	–16	–54	–54	–42	–2	–54	–54	–54
χ^*	0	0	–54	–54	–26	12	–54	–44	–22
0	0	0	0	–54	6	38	–54	6	38
$\alpha^*-\beta^*$	0	0	0	0	38	46	54	46	50
$\alpha^*-\psi^*+\eta^*$	0	0	0	0	0	54	–22	–6	18
$\beta^*-\psi^*+\eta^*$	0	0	0	0	0	0	–42	–46	–38
$\frac{\alpha^*-\beta^*}{2}$	0	0	0	0	0	0	0	38	46
$\frac{\alpha^*-\psi^*+\eta^*}{2}$	0	0	0	0	0	0	0	0	54
$\frac{\beta^*-\psi^*+\eta^*}{2}$	0	0	0	0	0	0	0	0	0

The analysis of this matrix yield the following topology definition:

$$\chi^* < 0 \wedge \rho^* < 0 \wedge \rho^* < \frac{\beta^* + \eta^* - \psi^*}{2} \wedge \beta^* < \alpha^*$$

Topology C-iv

This topology is characterized by having the alternative sink scavenging the majority of H_2O_2 under basal oxidative stress and TrxR cannot be saturated by Trx-SS. It also presents a saturation response regime, where the signaling pathways of the PTTRS are unresponsive to change in H_2O_2 It is possible in 63 sectors of the design space out of the 1152. As previously described it is possible to define a correlation matrix:

	ρ^*	χ^*	0	$\alpha^*-\beta^*$	$\alpha^*-\psi^*+\eta^*$	$\beta^*-\psi^*+\eta^*$	$\frac{\alpha^*-\beta^*}{2}$	$\frac{\alpha^*-\psi^*+\eta^*}{2}$	$\frac{\beta^*-\psi^*+\eta^*}{2}$
ρ^*	0	–63	–63	–63	–57	–25	–63	–63	–63
χ^*	0	0	63	–19	–5	33	19	23	49
0	0	0	0	–63	–33	7	–63	–33	7
$\alpha^*-\beta^*$	0	0	0	0	7	35	63	35	49
$\alpha^*-\psi^*+\eta^*$	0	0	0	0	0	63	13	33	45
$\beta^*-\psi^*+\eta^*$	0	0	0	0	0	0	–21	–35	–7
$\frac{\alpha^*-\beta^*}{2}$	0	0	0	0	0	0	0	7	35
$\frac{\alpha^*-\psi^*+\eta^*}{2}$	0	0	0	0	0	0	0	0	63
$\frac{\beta^*-\psi^*+\eta^*}{2}$	0	0	0	0	0	0	0	0	0

The analysis of this matrix yield the following topology definition:

$$\rho^* < 0 \wedge 0 < \chi^* \wedge \rho^* < \frac{\beta^*+\eta^*-\psi^*}{2} \wedge \beta^* < \alpha^*$$

Dichotomy threshold Calculation

As showed in Figure 2.2 in all the topologies there is a critical value for σ , that allows to discriminate between a response that lead to an accumulation of the Prx-SS and Trx-SS (D-Phenotype) and the counterpart in which there is accumulation of Prx-SO_2^- and Trx-SH (S-Phenotype). The value σ_{crit} is defined by the borders of the two regions DDAU and DDAS (Table A.5), properly re-arranging the inequalities we obtain:

$$\sigma^*_{crit} - \text{Max}[0,\chi^*] = \text{Min}[\text{Min}[0,\rho^*],\frac{\beta^*-\psi^*+\eta^*}{2}\text{-Max}[0,\frac{\alpha^*-\beta^*}{2}]];$$

$$\sigma^*_{crit} - \text{Max}[0,\chi^*] = \text{Min}[\text{Min}[0,\rho^*],\text{-Max}[-\frac{\beta^*-\psi^*+\eta^*}{2},-\frac{\alpha^*-\psi^*+\eta^*}{2}]];$$

$$\sigma^*_{crit} - \text{Max}[0,\chi^*] = \text{Min}[\text{Min}[0,\rho^*],\text{Min}[\frac{\beta^*-\psi^*+\eta^*}{2},\frac{\alpha^*-\psi^*+\eta^*}{2}]];$$

$$\sigma^*_{crit} = \text{Min}[0,\rho^*,\frac{\beta^*-\psi^*+\eta^*}{2},\frac{\alpha^*-\psi^*+\eta^*}{2}]+\text{Max}[0,\chi^*] \quad \text{(A.18)}$$

Parameters Estimations

Estimation of protein concentrations from proteomic datasets

Where more reliable determinations were lacking, we estimated protein concentrations based on the proteomic iBAQ dataset from Geiger *et al.* [183] as reported in the Proteomaps database (http://www.proteomaps.net/) [185]. The estimates follow the method outlined by Milo *et al.* [264]. They are based on the observation that most mammalian cells have a mean protein density of C_p= 0.2 g/mL cell volume [265,266]. For instance, Jurkat T cells contain 0.14 mg protein/10^6 cells [267], which translates into C_p= 0.21 g/mL, considering a mean Jurkat T cell volume of $6.6\pm0.46\times10^{-13}$ dm^3 [268]. Then, considering that an average human protein contains 375 aminoacyl residues ($\overline{Laa}$ below) [269], and a mean molecular weight of 110Da per aminoacid we obtain the following average concentration of total protein in a human cell:

$$C_{tot}^{Organism}[\mathrm{M}] = \frac{C_p[\mathrm{g/mL}]}{110[\mathrm{Da}]\times 375[\mathrm{aa}]} = 4.9\mathrm{mM} \qquad \text{(A.19)}$$

Knowing the mass fraction (φ_{Prot} , expressed as “size weighted abundance” in the Proteomaps database) and its primary sequence length (Laa_{Prot}) one can then calculate its concentration by applying the following formula:

$$C_{\mathrm{Prot}}^{Organism} = \frac{\varphi_{\mathrm{Prot}}\cdot C_{tot}^{Organism}}{\dfrac{Laa_{\mathrm{Prot}}}{\overline{Laa}}} \qquad \text{(A.20)}$$

Jurkat T Cells

Peroxiredoxins concentration and rate constants

We consider Prx total concentration as the sum of the concentration of PrxI (Prdx1, 199aa, 22.11 kDa) and PrxII (Prxd2, 196aa, 21.892 kDa) the two main 2-cys cytoplasmic peroxiredoxin.

Rhee *et al.* [270] determined the PrxI and PrxII contents in Jurkat T cells as $R_{\mathrm{PrxI/T}}$ = 2.7 µg/mg of total soluble protein, and $R_{\mathrm{PrxII/T}}$ = 1 µg/mg of soluble protein. Considering an average cell volume of 6.6×10^{-13} dm^3 [268], an average protein content of 200 g/dm^3 [264] and the molecular weights of PrxI (22,110 Da) and PrxII (21,892 Da) we obtain, the following concentrations:

$$PrxI = \frac{C_{tot}^{Jurkat}\cdot R_{\mathrm{PrxI/T}}}{MW} = \frac{200(\mathrm{g/dm^3})\times 2.7\times10^{-3}(\mathrm{g\ PrxII/g})}{2.21\times10^4(\mathrm{g/mol})} = 24.\mathrm{\mu M}$$

$$PrxII = \frac{C_{tot}^{Jurkat}\cdot R_{\mathrm{PrxII/T}}}{MW} = \frac{200(\mathrm{g/dm^3})\times 10^{-3}(\mathrm{g\ PrxII/g})}{2.19\times10^4(\mathrm{g/mol})} = 9.1\mathrm{\mu M}$$

The rate constants for the oxidation of PrxII-S^- to PrxII-SO^- and of PrxII-SO^- to PrxII-SO_2^- , as well as the rate constant for conversion of PrxII-SO^- to PrxII-SS, were determined experimentally as

$k_{Ox} = 10^8 \mathrm{M^{-1}s^{-1}}$ [78], $k_{Sulf} = 1.2\times10^4 \mathrm{M^{-1}s^{-1}}$, $k_{Cond} = 1.7\mathrm{s^{-1}}$ [87], respectively. The rate constant for PrxII-SS reduction was determined as $k_{\mathrm{Red}} = 2.1\times10^5 \mathrm{M^{-1}s^{-1}}$ [78].

The kinetic properties of PrxI are less well characterized, but considering the strong homology between PrxI and PrxII we assumed that these proteins have the same value of k_{Ox}. In turn, PrxI is known to be more resistant to hyperoxidation than PrxII [271], which may be due to a higher value of k_{Sulf} and/or a lower value of k_{Cond}. Because the lower sensitivity of PrxIII to sulfinylation is entirely due to a lower value of k_{Cond}, the value of k_{Sulf} being virtual identical to that for PrxII [87], we assumed that the same holds for PrxI. We estimated the value of k_{Cond} by fitting the first 110 s of the time course of NADPH consumption reported in Figure 6A of ref. [271], after subtracting the basal NADPH consumption rate as computed from the late phase (t > 120 s) of the curve, to the following kinetic model of the experiment:

$$\frac{d\mathrm{H_2O_2}}{dt} = k_{Ox}\cdot \mathrm{Prx\text{-}S^-}\cdot \mathrm{H_2O_2} - k_{Sulf}\cdot \mathrm{Prx\text{-}SO^-}\cdot \mathrm{H_2O_2}$$

$$\frac{d\mathrm{Prx\text{-}S^-}}{dt} = k_{Red}\cdot \mathrm{Trx\text{-}S^-}\cdot \mathrm{Prx\text{-}SS} - k_{Ox}\cdot \mathrm{Prx\text{-}S^-}\cdot \mathrm{H_2O_2}$$

$$\frac{d\mathrm{Prx\text{-}SO^-}}{dt} = k_{Ox}\cdot \mathrm{Prx\text{-}S^-}\cdot \mathrm{H_2O_2} + k_{Srx}\cdot \mathrm{Prx\text{-}SO_2^-} - k_{Sulf}\cdot \mathrm{Prx\text{-}SO^-}\cdot \mathrm{H_2O_2} - k_{Cond}\cdot \mathrm{Prx\text{-}SO^-}$$

$$\frac{d\mathrm{Prx\text{-}SO_2^-}}{dt} = k_{Sulf}\cdot \mathrm{Prx\text{-}SO^-}\cdot \mathrm{H_2O_2}$$

$$\frac{d\mathrm{Prx\text{-}SS}}{dt} = k_{Cond}\cdot \mathrm{Prx\text{-}SO^-} - k_{Red}\cdot \mathrm{Trx\text{-}S^-}\cdot \mathrm{Prx\text{-}SS}$$

$$\frac{dNADPH}{dt} = -k_{Red}\cdot \mathrm{Trx\text{-}S^-}\cdot \mathrm{Prx\text{-}SS}$$

The rate constant for the reactivity towards H_2O_2, as reported in literature, was set to $k_{Ox} = 10^8 \mathrm{M^{-1}s^{-1}}$ for PrxII [78]. The condensation and sulfinylation rates constant have been measured[87] for PrxII as $k_{Cond} = 1.7\mathrm{s^{-1}}$ and $k_{Sulf} = 1.2\cdot10^4\mathrm{M^{-1}s^{-1}}$, respectively. Due to the high homology between the two peroxiredoxins I and II we consider them having the same rate constant for sulfinylation and reduction of H_2O_2. We rather adduce the difference in hyperoxidation sensibility to differences in the condensation step as highlighted by the following simulations.

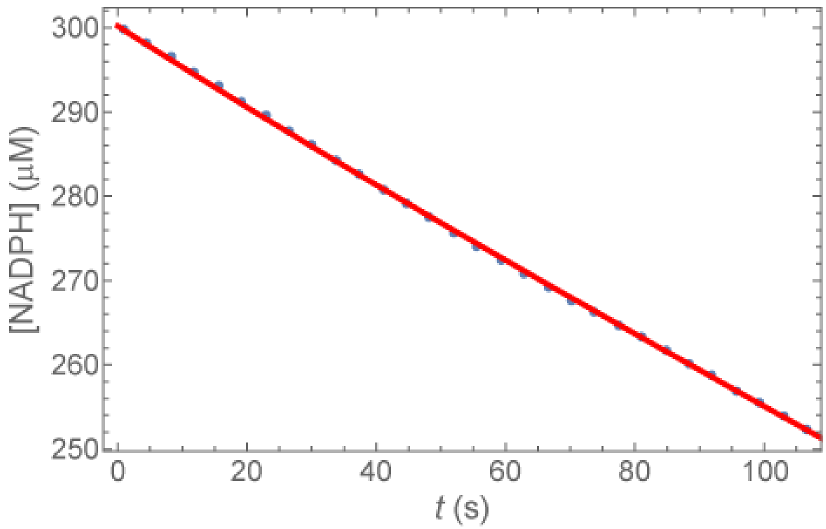


Figure A.3| Fit to the time course of NADPH consumption reported in Figure 6A of ref. [24]. *Dots, sampled points; red line, fitted curve for* $k_{Cond} = 88.4\,s^{-1}$, $k_{Red} = 1.1\times10^{5}\,M^{-1}s^{-1}$.

The parameters k_{Ox} and k_{Sulf} were fixed at the values indicated above, whereas k_{Cond} and k_{Red} were left as adjustable parameters. The fit was made using *Mathematica*™ v 14.0 FindFit function with default settings. An excellent fit was obtained for $k_{Cond} = 88.4\,s^{-1}$ and $k_{Red} = 1.1\times10^{5}\,M^{-1}s^{-1}$ (Figure A.3). The value of k_{Red} is comparable to that reported for PrxII [78]. For this reason and because we could not determine the origin of the Trx used in the experiments in ref. [271] we assumed that the value of k_{Red} for PrxI is the same as for PrxII.

For simplicity, in the design space analysis we considered a single 2-Cys peroxiredoxin with a k_{Cond} that is a concentration-weighted average of the values for PrxI and PrxII:

$$k^{*}_{Cond} = \frac{k^{PrxI}_{Cond}\cdot \text{PrxI} + k^{PrxII}_{Cond}\cdot \text{PrxII}}{\text{PrxI}+\text{PrxII}} = \frac{88.4\cdot 24.4 + 1.7\cdot 9}{24.4+9} \cong 65\text{s}^{-1}$$

Thioredoxin concentration and rate constant

The concentration of Trx1 (TXN, 105aa) was estimated using the method described above:

$$Trx1 = \frac{\varphi_{Trx}\cdot C^{Jurkat}_{tot}}{\frac{Laa_{Trx}}{\overline{Laa}}} = \frac{4.3\cdot 10^{-4}\times 4.85\cdot 10^{-3}(\text{M})}{\frac{105}{375}} \cong 7.4\mu\text{M}$$

while the rate constant for the reduction of the Prx was set to $k_{Red} = 2.1\times10^{5}\text{M}^{-1}\text{s}^{-1}$, as previously reported [78].

Thioredoxin Reductase concentration and activity

Low et al. [272] determined the activity of Trx reductase in Jurkat T cells for 5-(3-Carboxy-4-nitrophenyl) disulfanyl-2-nitrobenzoic acid (DTNB) as substrate as 1.63±0.35 nmol/10^6 cells/min, at 37 °C, pH 7.4. We estimated [81] that the activity with human Trx1 as substrate is 1.3-fold higher.

Therefore, considering a mean Jurkat T cell volume of 6.6×10^{-13} dm^3 and a cytoplasmic fraction of 0.3 [268] one can estimate:

$$V_{Max,TrxR} = 1.3\frac{1.6\times10^{-9}(\text{mol})}{0.3\times6.6\times10^{-9}(\text{dm}^3)\times60(\text{s/min})} = 0.18\,\text{mMs}^{-1}\ .$$

This is the value we will use as reference in our modelling.

A partly independent estimate follows from the mass fraction of thioredoxin reductase (TxnRd1, Laa= 649) ($\varphi_{\text{TrxR}} = 4.04\times10^{-4}$) obtained from the proteomic iBAQ dataset from Geiger *et al.* [183] and as reported in the Proteomaps database [185]. Applying the method described in section Estimation of protein concentrations from proteomic datasets (Appendix A), this corresponds to the following concentration:

$$TrxR = \frac{\varphi_{\text{TrxR}}\cdot C_{tot}^{Jurkat}}{\frac{Laa_{\text{TrxR}}}{\overline{Laa}}} = \frac{4.0\times10^{-4}\times4.85\times10^{-3}(\text{M})}{\frac{649}{375}} = 1.1\,\mu\text{M}$$

Considering the $k_{cat} = 76.3\,\text{s}^{-1}$ estimated in ref. [81] this concentration yields $V_{Max,TrxR} = 0.084$ mMs^{-1}, in reasonable agreement with the previous estimate.

TrxR follows a ping-pong catalytic mechanism [102][273] whose kinetics can be described by:

$$v = \frac{V_{Max,TrxR}}{1+\frac{K_{M,TrxR,NADPH}}{NADPH}+\frac{K_{M,TrxR,TrxSS}}{TrxSS}}$$

We considered $K_{M,TrxR,TrxSS} = 1.8\,\mu$M [274]. The low $K_{M,TrxR,NADPH} = 6.0\,\mu$M [275] implies that except under strong and prolonged oxidative stress NADPH concentrations can be considered saturating. This should be especially true for tumor cell lines, which tend to over-express the pentose phosphates pathway [276] and thus have a large capacity to reduce $NADP^+$ to NADPH. Therefore, we assume that TrxR is saturated with NADPH and approximate its kinetics as

$$v = \frac{V_{Max,TrxR}\cdot TrxSS}{TrxSS + K_{M,TrxR,TrxSS}}$$

H_2O_2 Permeability

Antunes and Cadenas [277] determined the permeability of the Jurkat T cell membrane as $\kappa_p = 2\times10^{-5}\text{dm}\,\text{s}^{-1}$. Considering a mean Jurkat T cell volume $V =$ 6.6×10^{-13} dm^3, a surface area $S = 3.7\pm1.7\times10^{-8}$ dm^2 and a cytoplasmic fraction of 0.3 [268] one obtains a first order rate constant for H2O2 influx from the extracellular medium into the cytoplasm of:

$$k_{\text{inf}} = \frac{\kappa_p\cdot S}{V} = \frac{2\times10^{-5}(\text{dm}\,\text{s}^{-1})\times3.7\times10^{-8}(\text{dm}^2)}{0.3\times6.6\times10^{-13}(\text{dm}^3)} = 3.7\,\text{s}^{-1}$$

Alternative H_2O_2 sinks

The capacity of Jurkat T cells to clear cytoplasmic H_2O_2 through processes other than reduction by PrxI and PrxII is arguably the most uncertain parameter in the model. One has to consider at least the five processes that will be discussed below.

Reduction by glutathione peroxidase

At low H_2O_2 supply rates the kinetics of glutathione peroxidase 1 is well approximated by a simple mass action rate expression [277]. Antunes and Cadenas [277] determined the pseudo-first-order rate constant for this process as 4.1 s^{-1}.

Reduction by peroxiredoxin VI

Recent proteomic studies [183] point to a substantial concentration of the 1-Cys peroxiredoxin PrxVI in Jurkat T cells. Using the estimation method described in section Estimation of protein concentrations from proteomic datasets (Appendix A) we obtain:

$$PrxVI = \frac{\varphi_{\mathrm{PrxVI}} \cdot C_{tot}^{Jurkat}}{\frac{Laa_{\mathrm{PrxVI}}}{\overline{Laa}}} = \frac{6.4\times10^{-4}\times4.85\times10^{-3}(\mathrm{M})}{\frac{224}{375}} = 5.2\,\mu\mathrm{M}$$

Considering a rate constant for H_2O_2 reduction of $k_{\mathrm{Ox,PrxVI}} =$ 3×10^6 $M^{-1}s^{-1}$, this translates into a pseudo-first-order rate constant of $k_{\mathrm{Pr}\,xVI} = 3.0\times10^{6}(\mathrm{M^{-1}s^{-1}})\times5.2\times10^{-6}(\mathrm{M}) = 16.\ \mathrm{s^{-1}}$ when all the protein is in thiolate form.

Upon reaction with H_2O_2 the active site thiolate is oxidized to a sulfenate whose reduction is dependent on glutathionylation by GSH-loaded glutathione S-transferase π [96,97], which is also abundant in Jurkat T cells [185]. At high H2O2 concentrations the rate-limiting step in the catalytic cycle may be the reduction of the glutathionylated PrxVI molecule by another GSH molecule.[96]

Reduction by other thiol proteins

Hansen *et al.* [126] showed that the concentration of oxidizable protein thiols in human cell lines is in the order of 10 mM, which is comparable or higher than GSH concentrations. However, only a small fraction of these thiols are very reactive.[37] Most protein thiols are expected to react with H2O2 at rate constants ~1 $M^{-1}s^{-1}$ or lower, similar to GSH ($k =$ 0.87 $M^{-1}s^{-1}$ [128]). Other than those in the active centers of peroxidases and peroxiredoxins, few protein thiols characterized to date have H2O2 reactivities in excess of 100 $M^{-1}s^{-1}$ [129][130], and none of these is sufficiently abundant to contribute significantly for the H2O2 clearance capacity of the cells.

However, a quantitative analysis based on a mathematical model for H2O2 metabolism in Jurkat T cells [278] suggested that these cells contain an abundant pool (1 mM) of quite reactive (5×10^5 $M^{-1}s^{-1}$) protein thiols. More recently, a thorough analysis of the redox response of the 2-Cys peroxiredoxin Tpx1 from the fission yeast *Schizosaccharomyces pombe* to high concentrations of ectopic H_2O_2 also postulated the existence of a large (~13 mM) pool of moderately H2O2-reactive (5×10^2 $M^{-1}s^{-1}$) protein thiols. [160] This would correspond to a pseudo-first-order rate constant of ~6.5 s^{-1} for H2O2 consumption.

None of these works identified the thiol proteins that might be oxidized at such rates. And in both cases reactivities and pool sizes were estimated quite indirectly by fitting a kinetic model to experimentally determined time courses. Such estimates are very sensitive to the considerable uncertainties in both data and models. For instance, estimations in ref. [278] were based on experimental determinations of the redox potential of GSH that did not account for subcellular distribution of GSSG, which is now known to be concentrated in lysosomes and present at much lower concentrations in the cytoplasm [279]. The contribution of the thiol proteome for H2O2 clearance thus remains poorly defined. We therefore neglected it.

Dismutation by catalase

In Jurkat T cells, as in most human cells, all catalase is contained within peroxisomes. As consequence, the consumption of cytoplasmic H2O2 by catalase is rate limited by the permeation of the peroxisomal membrane [277]. Taking this fact into account, Antunes and Cadenas [277] estimated the contribution of catalase for the clearance of cytoplasmic H_2O_2 as $k_{Cat} =$ 0.4 s^{-1}.

Efflux

Because the plasma membrane is relatively permeable, part of the H2O2 can leave the cell. The rate constant for this process is $k_{efl} = k_{\inf} =$ 3.7 s^{-1} as determined above.

Unlike all the other H_2O_2 clearance processes discussed above, catalase and the efflux are virtually non-saturable.[125,280] Therefore, at very high H_2O_2 supply rates able to saturate all other processes, cytoplasmic H_2O_2 will nearly equilibrate with the extracellular environment, because $k_{efl} \gg k_{Cat}$.

Altogether, the H_2O_2 clearance capacity through processes other than reduction by the typical 2-Cys peroxiredoxins adds up to:

$$k_{Alt} = (4.1 + 16. + 0.4 + 3.7)\,\text{s}^{-1} = 24.\ \text{s}^{-1}$$

at low oxidative loads, and to

$$k_{Alt} = (0.40 + 3.7)\,\text{s}^{-1} = 4.1\ \text{s}^{-1}$$

under strong enough oxidative loads to deplete GSH.

Sulfiredoxin concentration and activity

The reduction of $PrxSO_2^-$ to $PrxSO^-$ requires ATP and Trx1SH and is catalyzed by sulfiredoxin. The rate-limiting step in this process is the formation of a thiosulfinate intermediate [45,89–91] (Srx-Prx) whose existence for the human enzyme has been confirmed [116]. The resolution of this complex generates an intramolecular disulfide bond SrxSS, that is then reduced by Trx1SH.[117] Human Srx has a catalytic constant of 3.0×10^{-3} s^{-1} for PrxI-SO_2^-, K_M(Trx1SH) = 1.2 µM and K_M(ATP) = 30 µM [121]. Therefore, the enzyme is normally saturated with these substrates. However, the MIchaelis constant for $PrxSO_2^-$ has not been characterized, which prevents a detailed modeling of its kinetics. On the other hand, we were able to estimate a pseudo-first-order rate constant for PrxI-

SO2- reduction in A549 cells previously exposed to 250 µM H_2O_2 from the immunoblot images for "Control RNA","α-PrxSO2" panel in Figure 8 from ref. [121].

Figure A.4| Immunoblot image hPrxI-SO₂.

Densitometry analysis of the image reveals a mono-exponential decay of the concentration of PrxI-SO2-, which is well fitted (R^2=0.996) by a $k_{Alt}^{A549} = 4.45\times10^{-3}\ \mathrm{s}^{-1}$ (Figure A.5| Sulfiredoxin activity estimation from fit of ref. [121]).

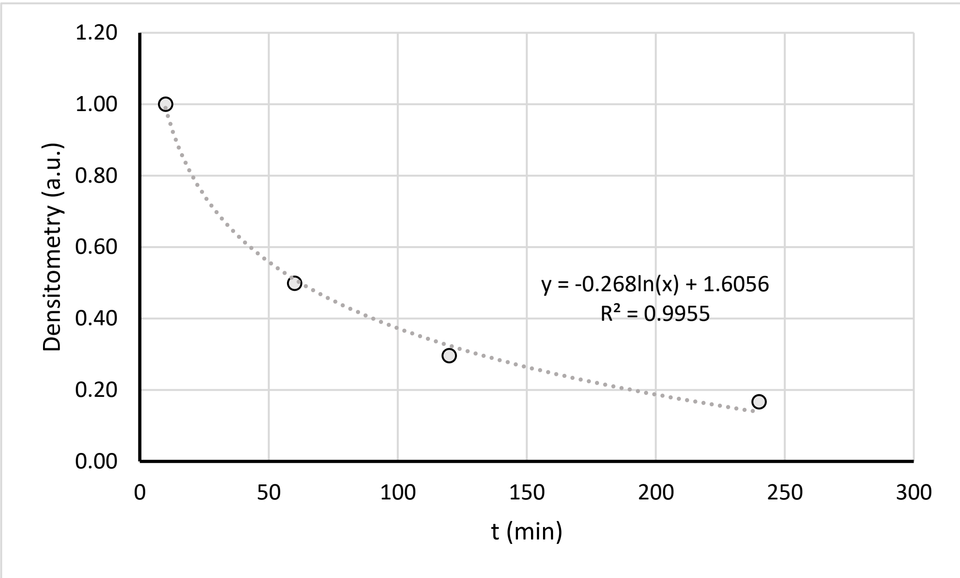


Figure A.5| Sulfiredoxin activity estimation from fit of ref. [121]

From the data in the Proteomaps database, using the method described in Section Estimation of protein concentrations from proteomic datasets (Appendix A), we estimated the concentration of Srx in A549 and in Jurkat T cells as:

$$Srx(\mathrm{A549}) = \frac{\varphi_{\mathrm{Srx}}^{\mathrm{A549}} \cdot C_{tot}^{A549}}{\frac{Laa_{\mathrm{Srx}}}{\overline{Laa}}} = \frac{\ldots\times10^{-6}\times\ldots\times10^{-3}(\mathrm{M})}{\frac{145}{375}} = 0.50\,\mu\mathrm{M}$$

$$Srx(\mathrm{Jurkat}) = \frac{1.9\cdot10^{-6}\cdot4.85\cdot10^{-3}(\mathrm{M})}{\frac{145}{375}} = 0.02\,\mu\mathrm{M}.$$

Therefore, assuming that the pseudo-first-order rate constant is proportional to the concentration of Srx in each cell, we obtained:

$$k_{\mathrm{Srx}} = \frac{0.02\,\mu\mathrm{M}}{0.5\,\mu\mathrm{M}} 4.45\times10^{-3}\ \mathrm{s}^{-1} = 1.8\times10^{-4}\ \mathrm{s}^{-1}\ .$$

Other cells

The concentrations value of the other cell type considered in the work, were calculated with the mass fraction method using the data generated by Geiger et al 2012[183] and which values are resumed in

Alternative sinks and Catalase activity

Where in literature were missing data about H_2O_2 consumption by alternative sinks, catalase activity and efflux were used as an approximation of the cell capacity to scavenge H_2O_2.

Catalases are able to dismutase H_2O_2 at a rate that shows no saturation and is directly proportional to their concentration[125,280]. It is possible to calculate the ratio k_{cat}/K_M as a proxy of their activity, together with the level of expression estimates the pseudo-first order rate constant.

For human catalase the ratio has been reported to be

$$k_{Catalase} = \frac{k_{cat}}{K_M} = 7.34 \mu M^{-1} s^{-1}$$ [125,281]

Catalases are mostly confined into peroxisomes thus intertwining another layer that H_2O_2 has to cross [277]. Estimations peroxisomes permeability to H_2O_2 gave, for rat liver cells, $P^{peroxisomes} = 3 \cdot 10^{-3} cms^{-1}$ [282–284]. An average of two peroxisomes per cells has been reported in literature for Jurkat T cells[285], the diameter for these organelles in Eukarya ranges from 0.1 to 1 µm[286]

Assuming a perfect sphere shape, we can obtain:

$$k_{\inf}^{per} = \frac{P^{peroxisomes} \cdot S}{A} = \frac{P^{peroxisomes} \cdot 3}{r} \cdot 2 = \frac{3 \cdot 10^{-5} \cdot 3 \cdot 2}{1 \cdot 10^{-6}} = 1.8 s^{-1}$$

The H_2O_2 concentration in the peroxisome membrane can be described by the following differential equation:

$$\frac{dH_2O_{2per}}{dt} = k_p \cdot H_2O_{2cyt} - k_p \cdot H_2O_{2per} - k_{Catalase} \cdot Catalase \cdot H_2O_{2per}$$

At steady state the permeation through the membrane of the peroxisomes and the consumption of catalase reach an equilibrium according to the following law:

$$0 = k_p \cdot H_2O_{2cyt} - k_p \cdot H_2O_{2per} - k_{Catalase} \cdot Catalase \cdot H_2O_{2per}$$

From this is it possible to calculate the relative contribution of Catalase to the Alternative Sinks pool:

$$k_{Cat}^{*} = \frac{k_p \cdot k_{Cat} \cdot Catalase}{k_p + k_{Cat} \cdot Catalase}$$

This has then been added to the efflux constant to obtain the alternative sinks rate for the other cells.

PTTRS parameters other cell line

Parameters for human erythrocytes were estimated as described in [81], those for Jurkat T, A549, GAMG, HEK293, Hela, HepG2, K562, LnCap, MCF7, RKO, U2OS were estimated from the literature[79,182–186] as reported above concentrations were derived from Geiger *et al.* 2012 [183].

Table A.6| Cell lines kinetic parameters.

	Cell lines											
	A549	GAMG	HEK293	HeLa	HepG2	Jurkat	K562	LnCap	MCF7	RKO	U2OS	hRBC
hPrxI [μM]	23.2	24.6	33.4	27.4	30.1	24	25	19.4	19.3	20.5	25.3	nd
hPrxII [μM]	1	1.9	9.8	8.2	10.6	9.1	10.2	14	10.7	9.8	3.9	570
hPrxVI [μM]	5.5	4.8	13.6	14.3	15.8	5.2	19	12.9	5.5	15	9.4	nd
GPxI [μM]	0.1	1.3	1.4	0.5	1.1	0.3	0	2.2	1.1	0.4	0.5	nd
Cat [μM]	0.7	1.1	0.3	1	2	0.9	1.4	3.8	0.6	0.3	0.6	nd
Srx [μM]	0.5	0.2	0	0.1	0.2	0	0	0.1	0.2	0.5	0.2	nd
Trx [μM]	14.6	15.4	14	12.9	11.8	7.4	9.6	6.5	7.4	23.8	6.3	0.56
TrxR [μM]	3.9	2.6	0.8	1.7	0.7	1.1	0.6	1.6	1	1.2	1.5	nd
V_{Max} [$M^{-1}s^{-1}$]	$2.8\cdot10^{-4}$	$1.9\cdot10^{-4}$	$5.6\cdot10^{-5}$	$1.2\cdot10^{-4}$	$4.9\cdot10^{-5}$	$1.8\cdot10^{-4}$	$4.5\cdot10^{-5}$	$1.2\cdot10^{-4}$	$7.2\cdot10^{-5}$	$9.1\cdot10^{-5}$	$1.1\cdot10^{-4}$	10^{-5}
k_{Cat} [s^{-1}]	1.3	1.5	1.1	1.5	1.6	0.4	1.5	1.7	1.3	1	1.3	218
k_{Srx} [s^{-1}]	$4.5\cdot10^{-3}$	$1.8\cdot10^{-3}$	$2.5\cdot10^{-4}$	$1.1\cdot10^{-3}$	$2.2\cdot10^{-3}$	$2.2\cdot10^{-4}$	$2.4\cdot10^{-4}$	$7.3\cdot10^{-4}$	$2.2\cdot10^{-3}$	$4.6\cdot10^{-3}$	$1.7\cdot10^{-3}$	10^{-5}
k_{Cond} [s^{-1}]	84.8	82.2	68.8	68.4	65.8	65	63.3	52.1	57.3	60.4	76.8	1.7
k_{Sulf} [$M^{-1}s^{-1}$]	$1.2\cdot10^{4}$	$1.2\cdot10^{4}$	$1.2\cdot10^{4}$	$1.2\cdot10^{4}$	$1.2\cdot10^{4}$	$1.2\cdot10^{4}$	$1.2\cdot10^{4}$	$1.2\cdot10^{4}$	$1.2\cdot10^{4}$	$1.2\cdot10^{4}$	$1.2\cdot10^{4}$	$1.2\cdot10^{4}$
k_{Red} [$M^{-1}s^{-1}$]	$2.1\cdot10^{5}$	$2.1\cdot10^{5}$	$2.1\cdot10^{5}$	$2.1\cdot10^{5}$	$2.1\cdot10^{5}$	$2.1\cdot10^{5}$	$2.1\cdot10^{5}$	$2.1\cdot10^{5}$	$2.1\cdot10^{5}$	$2.1\cdot10^{5}$	$2.1\cdot10^{5}$	$2.1\cdot10^{5}$
k_{Inf} [s^{-1}]	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	10.9
k_{Ox} [$M^{-1}s^{-1}$]	10^{8}	10^{8}	10^{8}	10^{8}	10^{8}	10^{8}	10^{8}	10^{8}	10^{8}	10^{8}	10^{8}	10^{8}
k_{Alt} [s^{-1}]	5	5.2	4.8	5.2	5.3	8.2	5.2	5.4	5	4.7	5	228.9

Design space PTTRS other cell lines

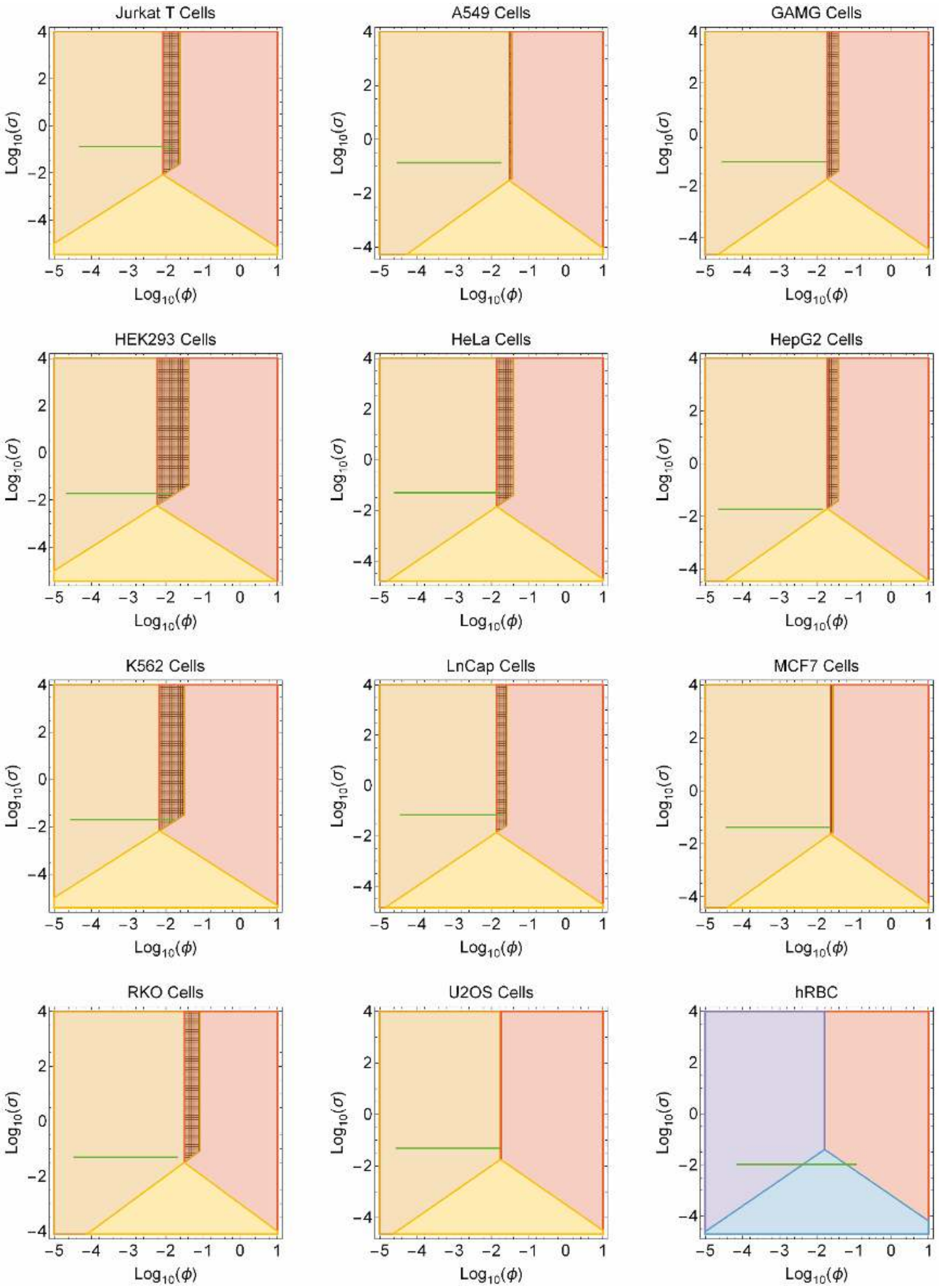


Figure A.6| Design space of the PTTRS for other cell lines.

Appendix B | Hydrogen peroxide concentrations classifier supplementary information

Supplementary figures

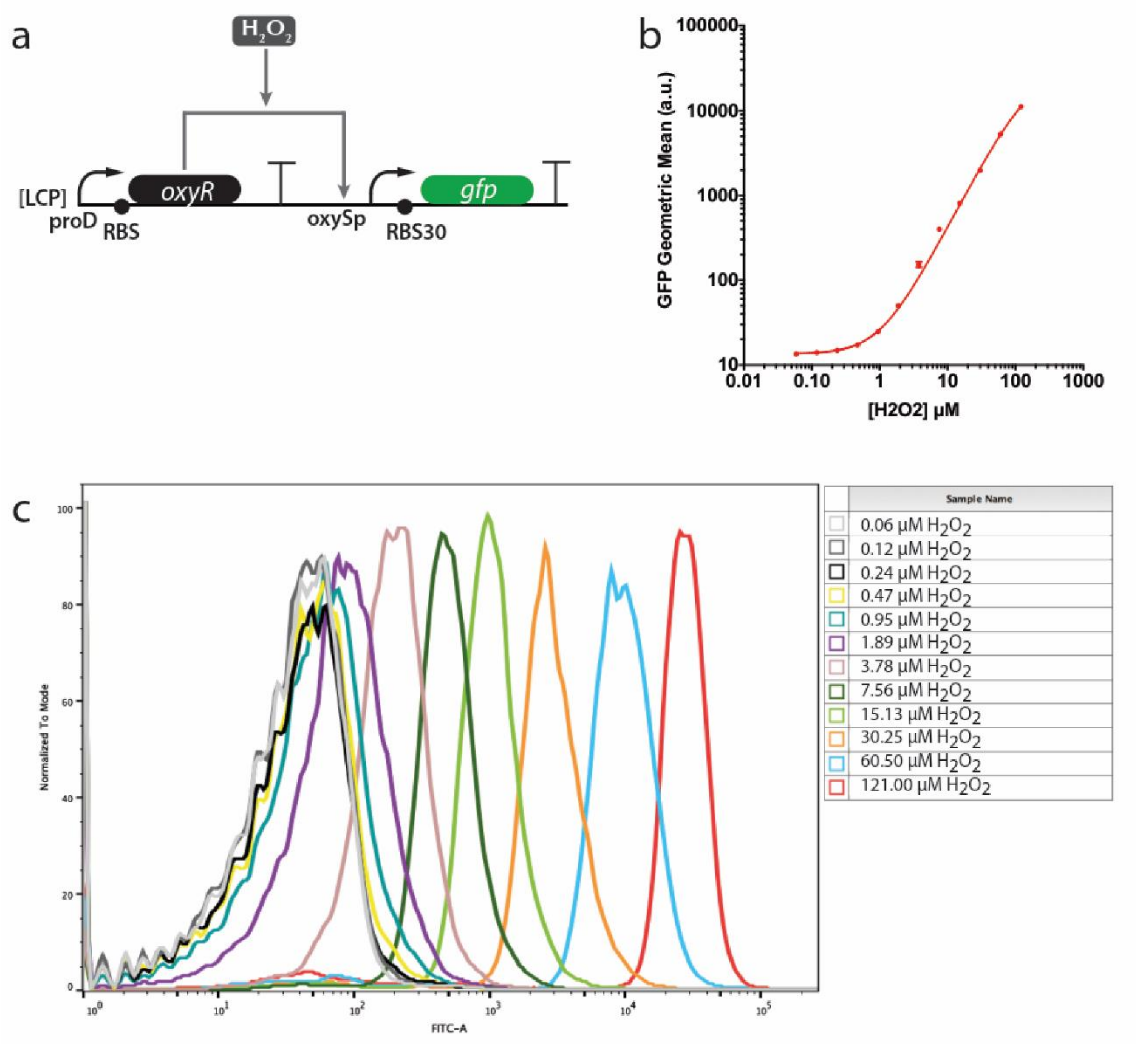
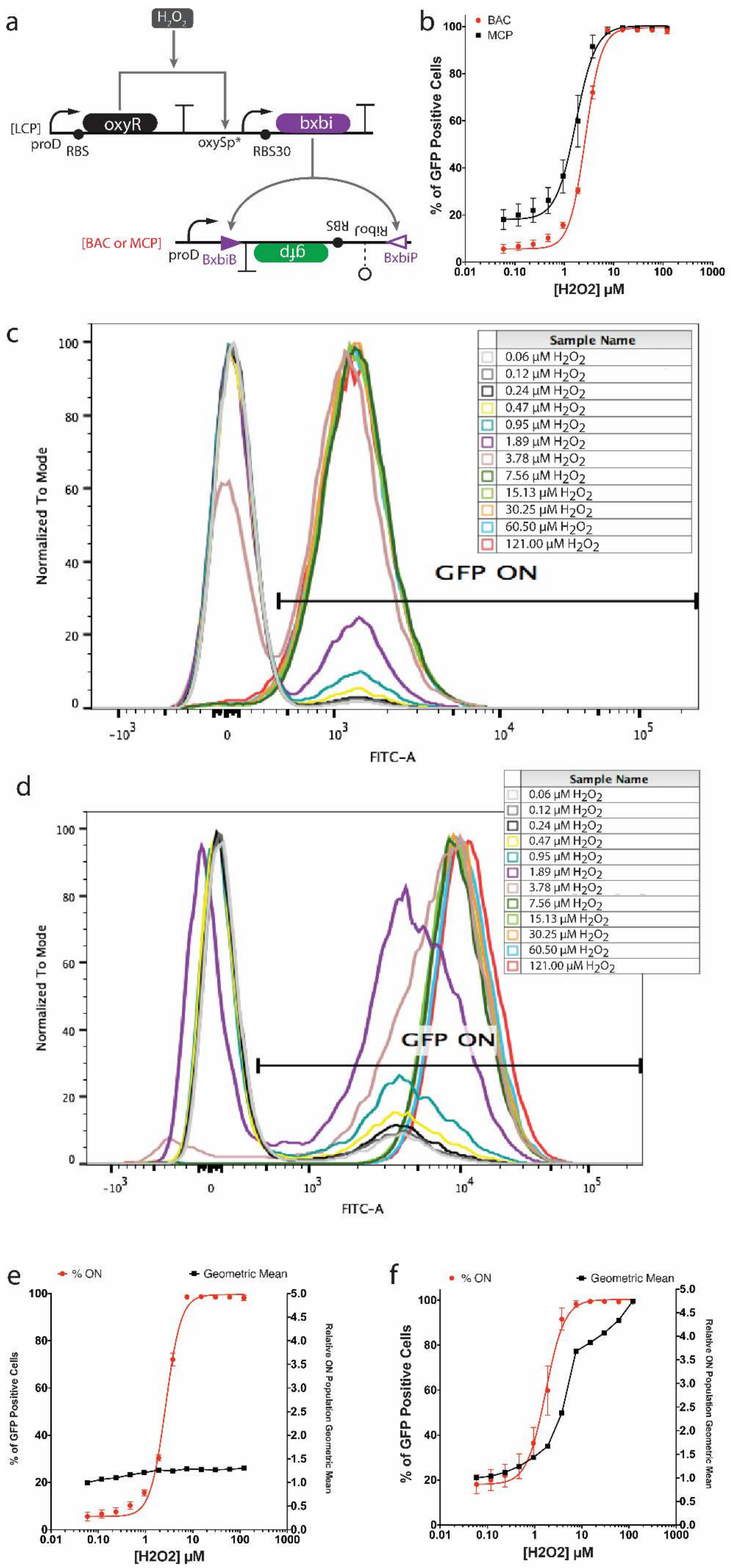


Figure B.1| Analog H_2O_2–sensor. *(a). OxyR is constitutively expressed from a low-copy plasmid (LCP) and activates transcription of gfp from the oxySp promoter on the same LCP in response to H_2O_2. (b). The geometric mean of GFP expression at different concentrations of H_2O_2 was measured three hours after induction. The line is a Hill function fit to the data. The errors (standard error of the mean) are derived from flow cytometry experiments of three biological replicates, each of which involved n>30,000 gated events. (c). Representative flow cytometry histograms for the analog circuit shown at in Figure B.1-a at different H_2O_2 concentrations. GFP is measured with FITC. GFP expression is continuously activated with increasing H_2O_2 over at least two orders of magnitude of the input.*


a
H_2O_2
[LCP]
proD
RBS
oxyR
oxySp*
RBS30
bxbi
[BAC or MCP]
proD
BxbiB
gfp
RBS
RiboJ
BxbiP
b
BAC
MCP
% of GFP Positive Cells
[H2O2] µM
c
Normalized To Mode
FITC-A
GFP ON
Sample Name
0.06 µM H_2O_2
0.12 µM H_2O_2
0.24 µM H_2O_2
0.47 µM H_2O_2
0.95 µM H_2O_2
1.89 µM H_2O_2
3.78 µM H_2O_2
7.56 µM H_2O_2
15.13 µM H_2O_2
30.25 µM H_2O_2
60.50 µM H_2O_2
121.00 µM H_2O_2
d
Normalized To Mode
FITC-A
GFP ON
e
% ON
Geometric Mean
% of GFP Positive Cells
Relative ON Population Geometric Mean
[H2O2] µM
f
% ON
Geometric Mean
% of GFP Positive Cells
Relative ON Population Geometric Mean
[H2O2] µM

g

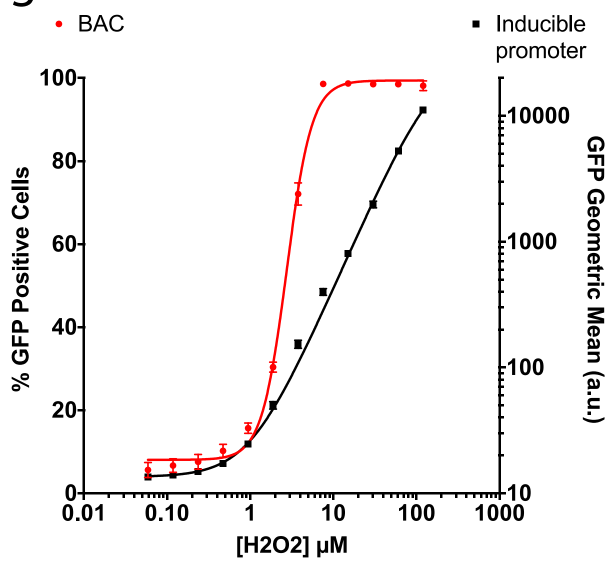


Figure B.2| Digitization of an analog input by inverting target DNA on a medium-copy plasmid (MCP) versus a bacterial artificial chromosome (BAC). *(a). OxyR is constitutively expressed from a LCP and activates transcription of bxb1 from the oxySp* promoter on the same LCP in response to* H_2O_2*. Bxb1 inverts the gfp expression construct on a BAC or MCP, turning on gfp expression by pairing it with an upstream proD promoter. (b). The percent of GFP positive cells at different* H_2O_2 *concentrations as measured by flow cytometry. The BAC (red circles) and MCP (black squares) have similar transfer functions. However, the MCP exhibits a higher basal level of cells that are GFP positive. The errors (standard deviation) are derived from flow cytometry experiments of three biological replicates, each of which involved n > 30,000 gated events. (c). Representative flow cytometry histograms for the BAC circuit shown in Figure B.2-a at different* H_2O_2 *concentrations. GFP is measured with FITC. The GFP-positive cells maintain a consistent level of GFP fluorescence even with increased* H_2O_2*, indicating a homogeneous population. (d). Representative flow cytometry histograms for the MCP circuit shown in Figure B.2-a at different* H_2O_2 *concentrations. The GFP-positive cells demonstrate increasing levels of GFP fluorescence with increased* H_2O_2*, indicating that there are multiple heterogeneous subpopulations. (e). The % of GFP positive cells vs. concentration of* H_2O_2 *(red circles) for the BAC circuit from Figure B.2-a is fit to a transfer function and plotted on the left y-axis. The geometric mean of the GFP positive cells in Figure B.2-c relative to the minimum geometric mean of the GFP positive cells in the same experiment vs. concentration of* H_2O_2 *(black squares) is plotted on the right y-axis and adjacent points are directly connected by straight lines (black line). The geometric mean does not considerably increase with* H_2O_2*, indicating that GFP positive cells in Figure B.2-c constitute one population even at different levels of the input. (f). The % of GFP positive cells vs. concentration of* H_2O_2 *(red circles) for the MCP circuit from Figure B.2-a is fit to a transfer function and plotted on the left y-axis. The geometric mean of the GFP positive cells in Figure B.2-d relative to the minimum geometric mean of the GFP positive cells in the same experiment vs. concentration of* H_2O_2 *(black squares) is plotted on the right y-axis and adjacent points are directly connected by straight lines (black line). The geometric mean increases considerably with* H_2O_2*, indicating that GFP positive cells in Figure B.2-d take on multiple populations with different* H_2O_2 *levels. (g). Digitization of the input by the comparator circuit. The percent of GFP positive cells at different* H_2O_2 *concentrations as measured by flow cytometry for the BAC comparator circuit (red circles) is plotted on the left axis (same data as black squares in Figure B.2-b). For comparison, we have also plotted the geometric mean of GFP expression at different concentrations of* H_2O_2 *(black squares) on the right axis (same data as red circles in Figure B.1-b). The five-highest tested concentrations of* H_2O_2 *continuously increase GFP expression from the inducible promoter but do not increase the percent of GFP positive cells from a comparator.*

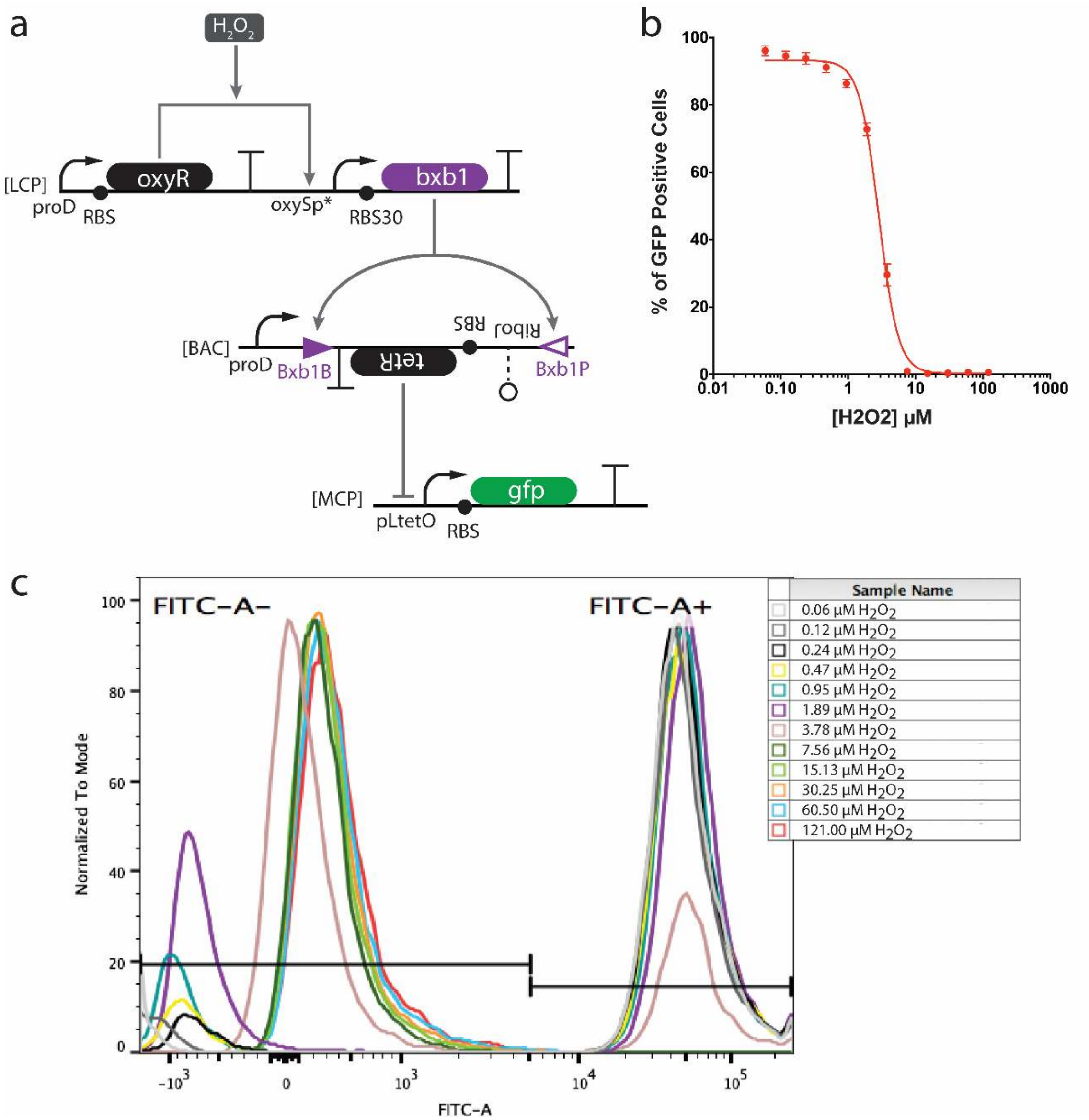
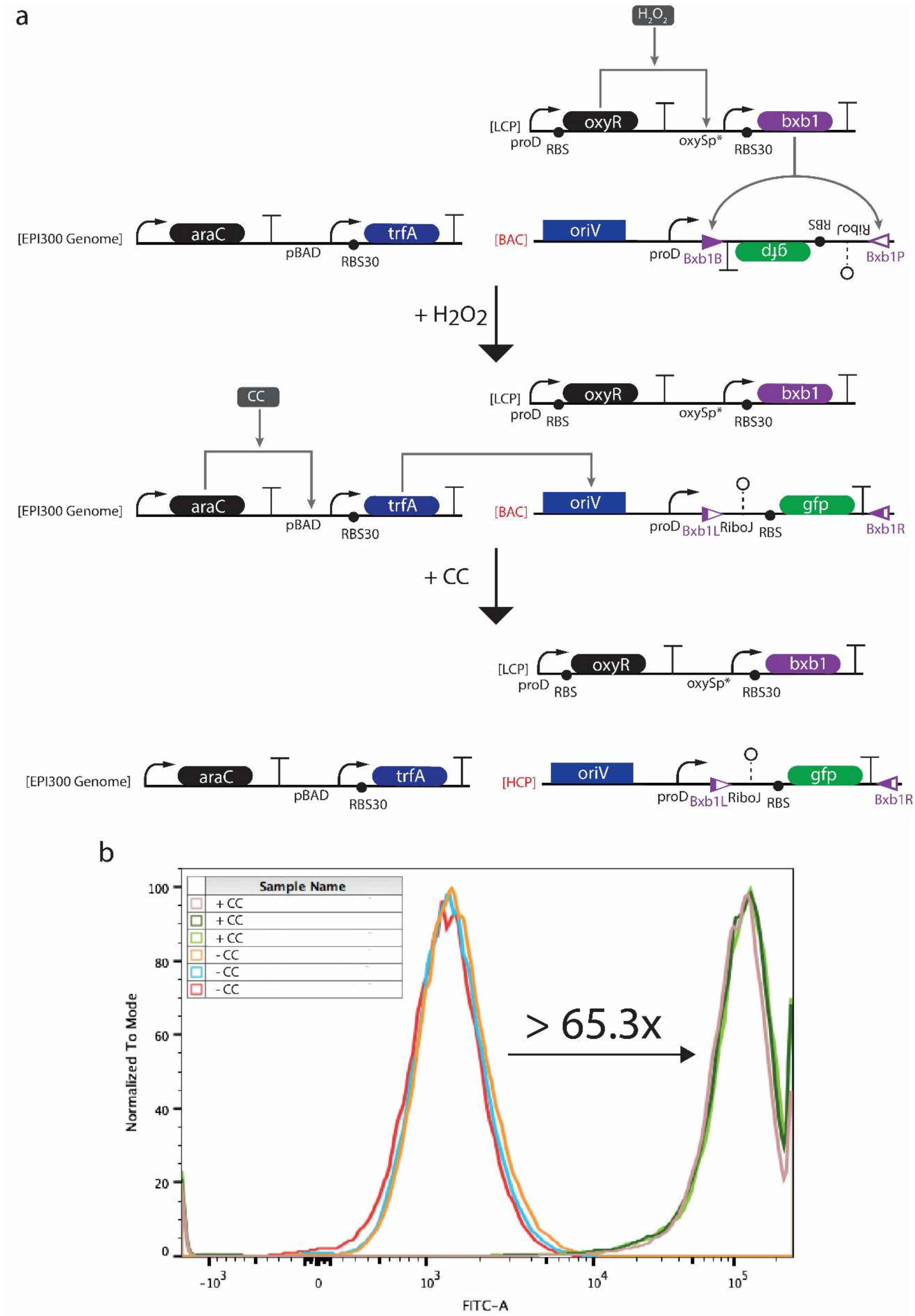


Figure B.3| Feedforward cascade involving a recombinase-invertible trans-acting transcriptional element on a BAC. *(a). OxyR is constitutively expressed from a LCP and activates transcription of bxb1 from the oxySp* promoter on the same LCP in response to H_2O_2. Bxb1 inverts the tetR expression cassette on a BAC, turning on TetR expression by pairing it with the proD promoter. TetR represses gfp expression from pLtetO on a MCP. (b). The percent of GFP positive cells at different H_2O_2 concentrations as measured by flow cytometry. The transfer function has a narrow switching range. The errors (standard deviation) are derived from flow cytometry experiments of three biological replicates, each of which involved n>30,000 gated events. (c). Representative flow cytometry histograms for the circuit shown at in Figure B.3-a at different H_2O_2 concentrations. GFP is measured with FITC. The GFP-positive cells fall into one population.*


a
H2O2
[LCP]
proD
RBS
oxyR
oxySp*
RBS30
bxb1
[EPI300 Genome]
araC
pBAD
trfA
RBS30
[BAC]
oriV
proD
Bxb1B
gfp
RBS
RiboJ
Bxb1P
+ H2O2
CC
Bxb1L
Bxb1R
+ CC
[HCP]
b
Sample Name
+ CC
+ CC
+ CC
- CC
- CC
- CC
> 65.3x
Normalized To Mode
FITC-A

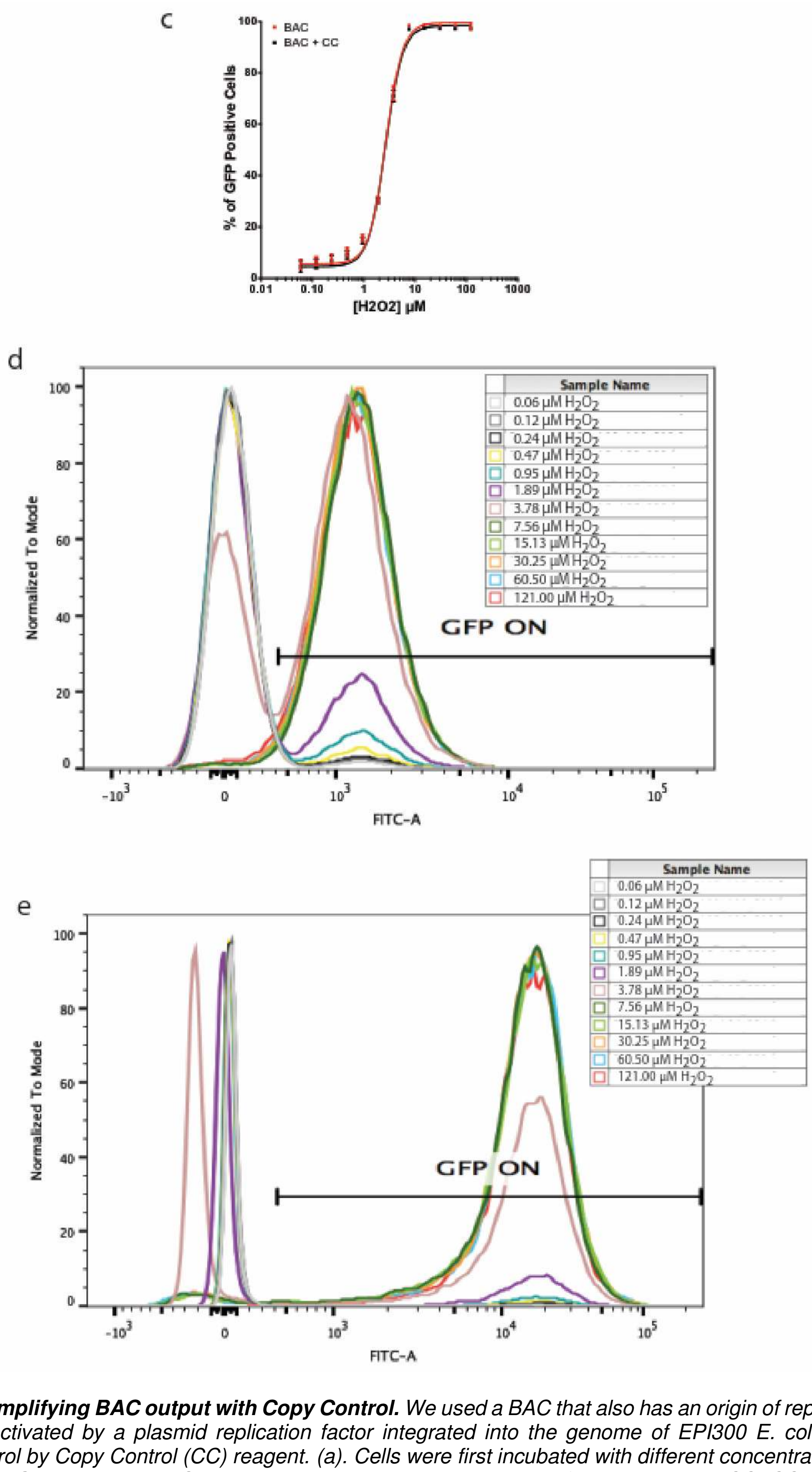


Figure B.4| Amplifying BAC output with Copy Control. *We used a BAC that also has an origin of replication that can be activated by a plasmid replication factor integrated into the genome of EPI300 E. coli under inducible control by Copy Control (CC) reagent. (a). Cells were first incubated with different concentrations of* H_2O_2 *to induce GFP expression. Cells were then washed and diluted into fresh media with CC. CC induces trfA expression from the pBAD promoter via activation of AraC, which are both expressed from the EPI300 chromosome. TrfA amplifies the BAC from 1-2 copies per cell to a high copy plasmid (HCP) at* $\sim\mathbf{100}$ *copies per cell. (b). Flow cytometry histograms for GFP expression from the BAC with CC (purple, dark green, light green) and without CC (orange, blue, red) at 121 µM* H_2O_2*. CC amplifies GFP expression at least 63.5x as measured by the geometric means of the populations. (c). The transfer functions for the BAC with CC (black* *line, black squares) and without CC (red line, red circles) are nearly identical. The errors (standard deviation) are derived from flow cytometry experiments of three biological replicates, each of which involved n>30,000 gated events. (d). Representative flow cytometry histograms for the BAC at different concentrations of H_2O_2 without CC for the data in Figure B.4-c. (e). Representative flow cytometry histograms for the BAC at different concentrations of H_2O_2 with CC for the data in Figure B.4-c. Note that the experiments in figures Figure B.4-b,d were measured with the same FITC voltage on the flow cytometer, and Figure B.4-e was measured with a different, lower FITC voltage on the flow cytometer because GFP expression from the BAC+CC was greater than the measurable fluorescence at the higher FITC voltage (as can be seen in the +CC data in Figure B.4-b).*

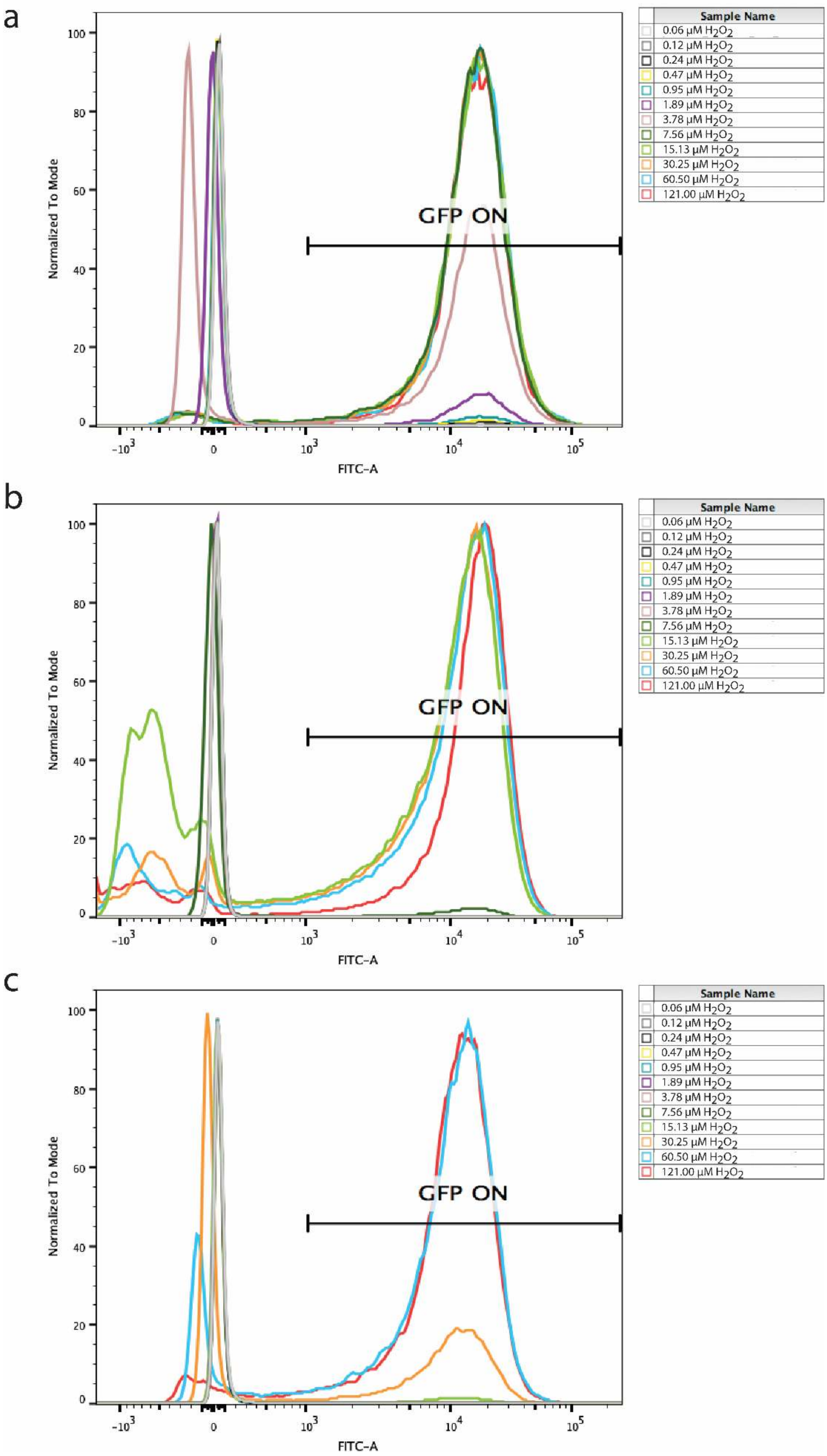
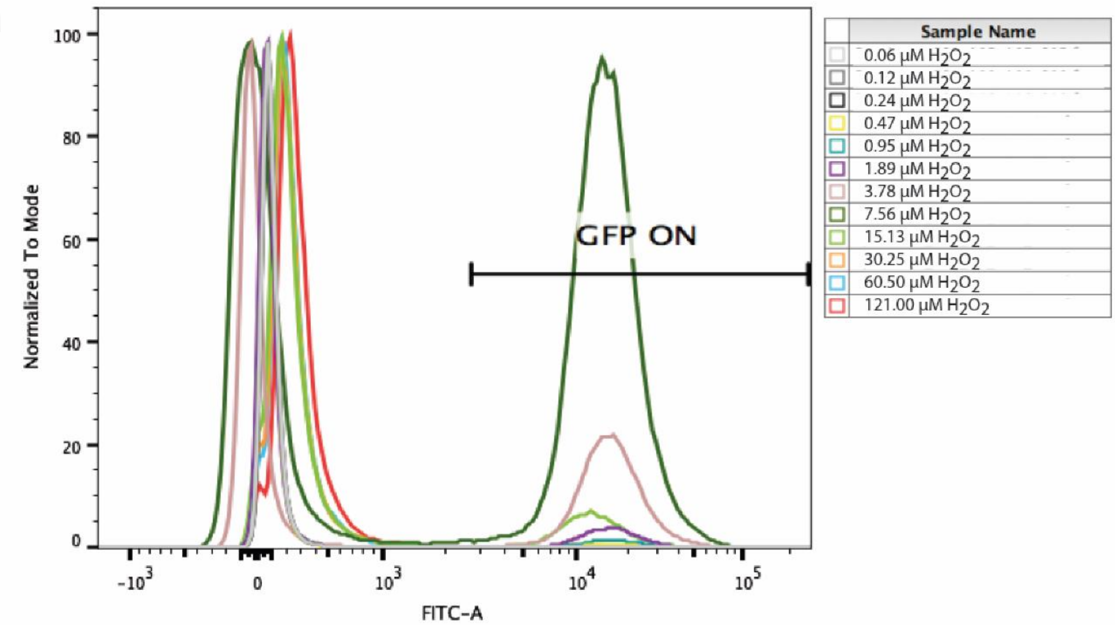


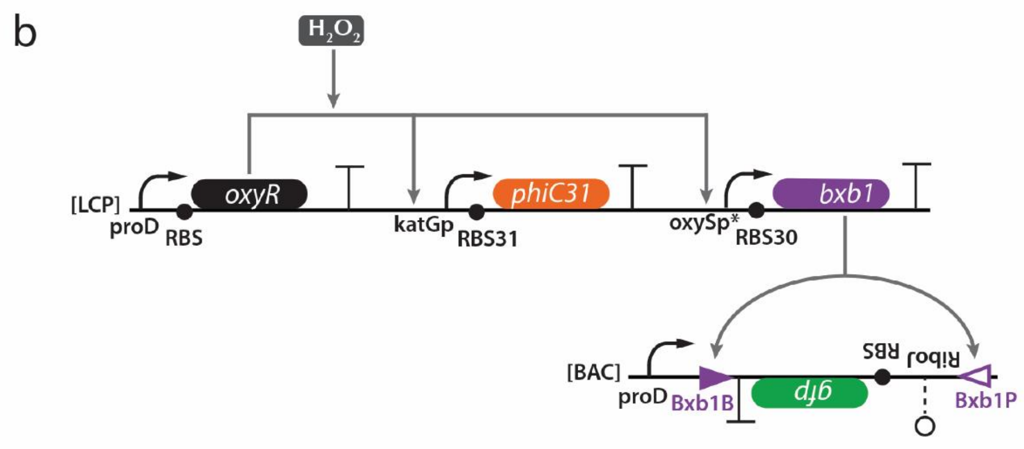
Figure B.5| Flow cytometry histograms for comparators with different activation thresholds. *(Figure 3.2). (a). Representative flow cytometry histograms for GFP expression for the low threshold circuit shown in Figure 3.2-a with oxySp* and RBS30, which correspond to the red diamonds and red line in Figure 3.2-b. (b). Representative flow cytometry histograms for GFP expression for the medium threshold circuit shown in Figure*

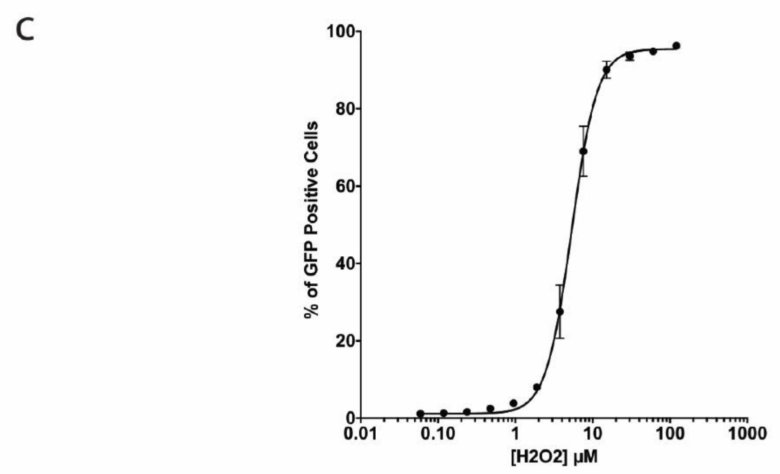
3.2-c with katGp and RBS31, which correspond to the red triangles and red line in Figure 3.2-d. (c). Representative flow cytometry histograms for GFP expression for the high threshold circuit shown in Figure 3.2-e with katGp and RBS33, which correspond to the red diamonds and red line in Figure 3.2-f.

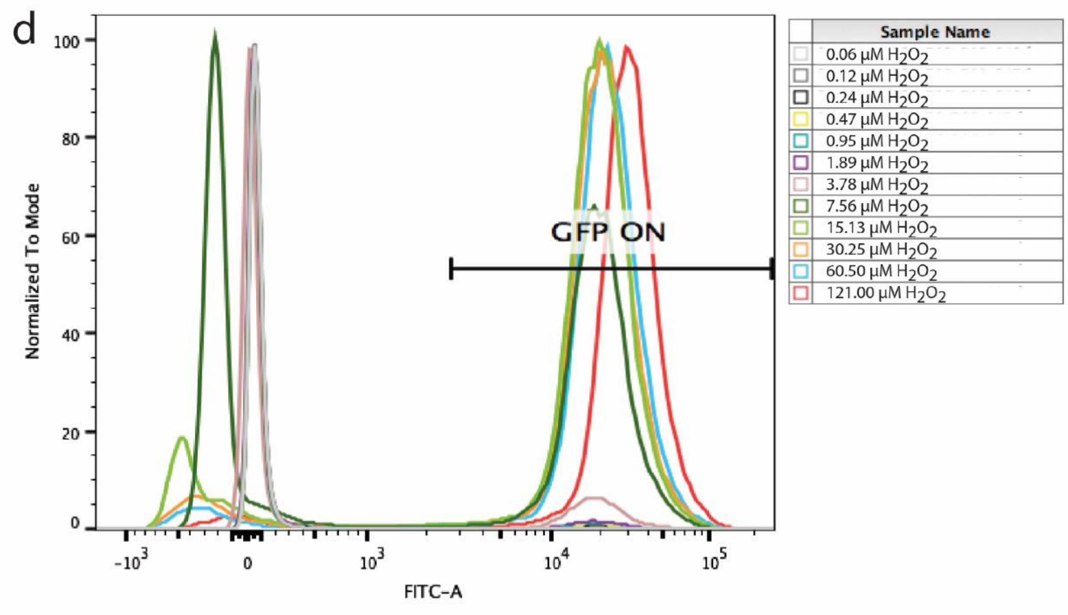
a



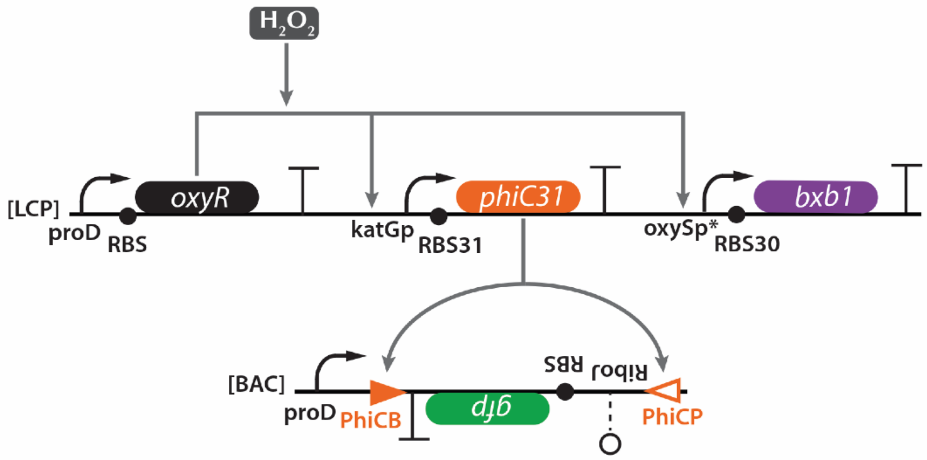
Normalized To Mode
100
80
60
40
20
0
GFP ON
-10^3
0
10^3
10^4
10^5
FITC-A
Sample Name
0.06 µM H2O2
0.12 µM H2O2
0.24 µM H2O2
0.47 µM H2O2
0.95 µM H2O2
1.89 µM H2O2
3.78 µM H2O2
7.56 µM H2O2
15.13 µM H2O2
30.25 µM H2O2
60.50 µM H2O2
121.00 µM H2O2


b
H2O2
[LCP]
proD
RBS
oxyR
katGp
RBS31
phiC31
oxySp*
RBS30
bxb1
[BAC]
proD
Bxb1B
gfp
RBS
RiboJ
Bxb1P

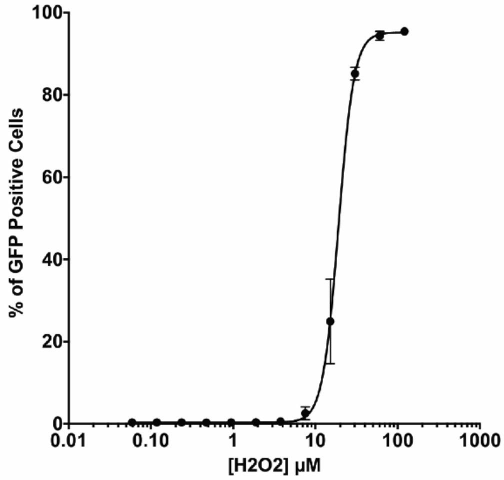

c
% of GFP Positive Cells
[H2O2] µM
0.01
0.10
1
10
100
1000


d
Normalized To Mode
FITC-A
GFP ON
Sample Name
0.06 µM H2O2
0.12 µM H2O2
0.24 µM H2O2
0.47 µM H2O2
0.95 µM H2O2
1.89 µM H2O2
3.78 µM H2O2
7.56 µM H2O2
15.13 µM H2O2
30.25 µM H2O2
60.50 µM H2O2
121.00 µM H2O2

e



f



g

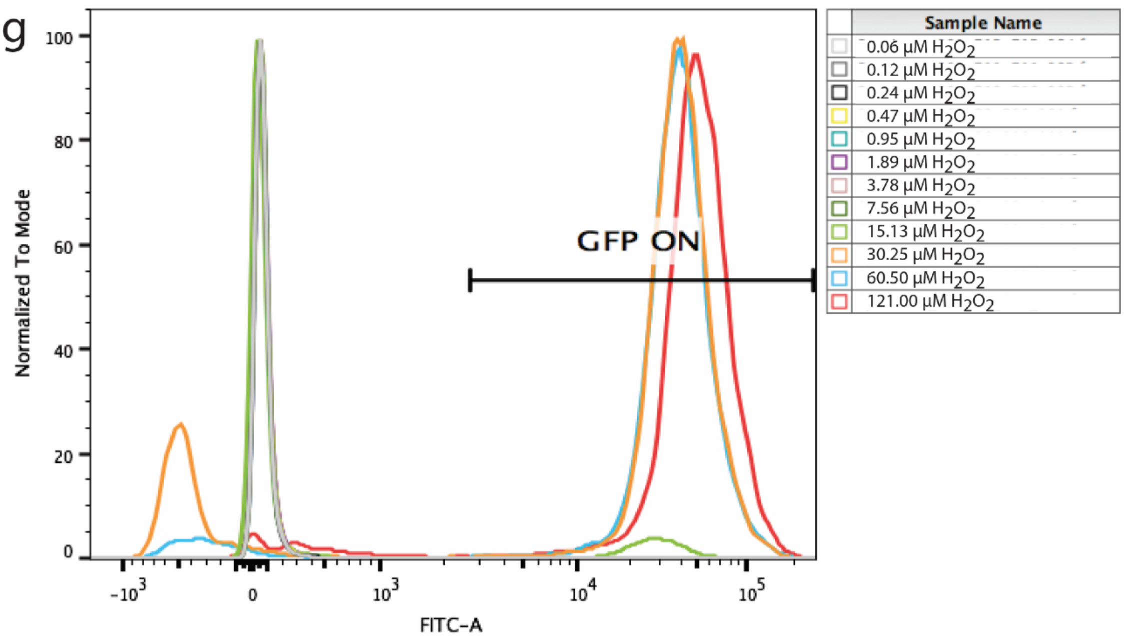
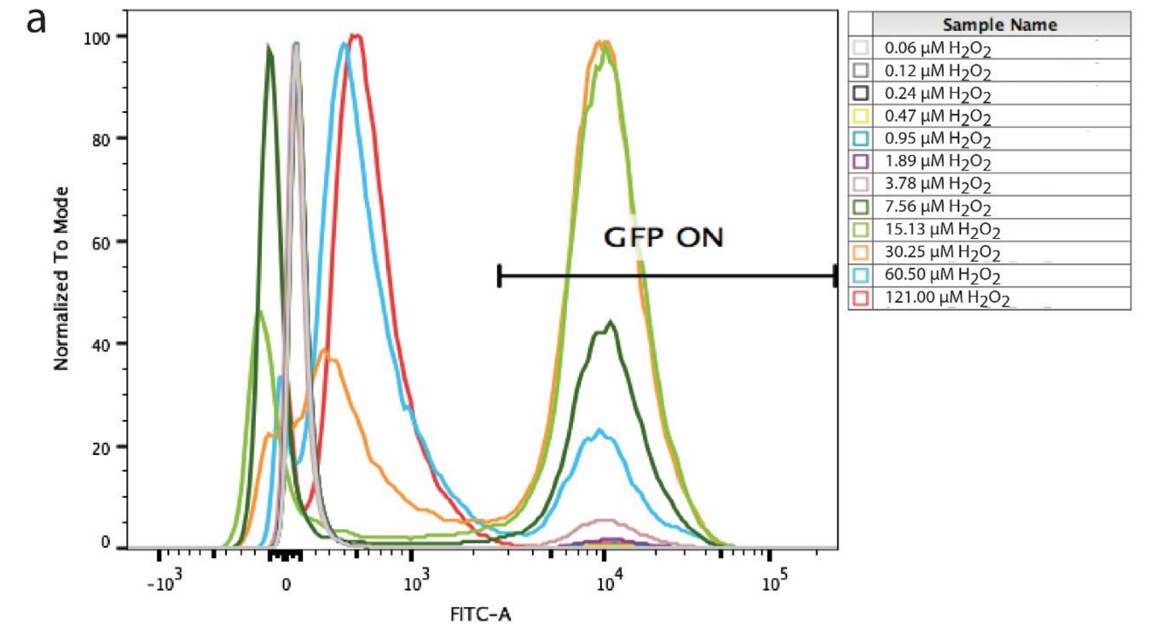
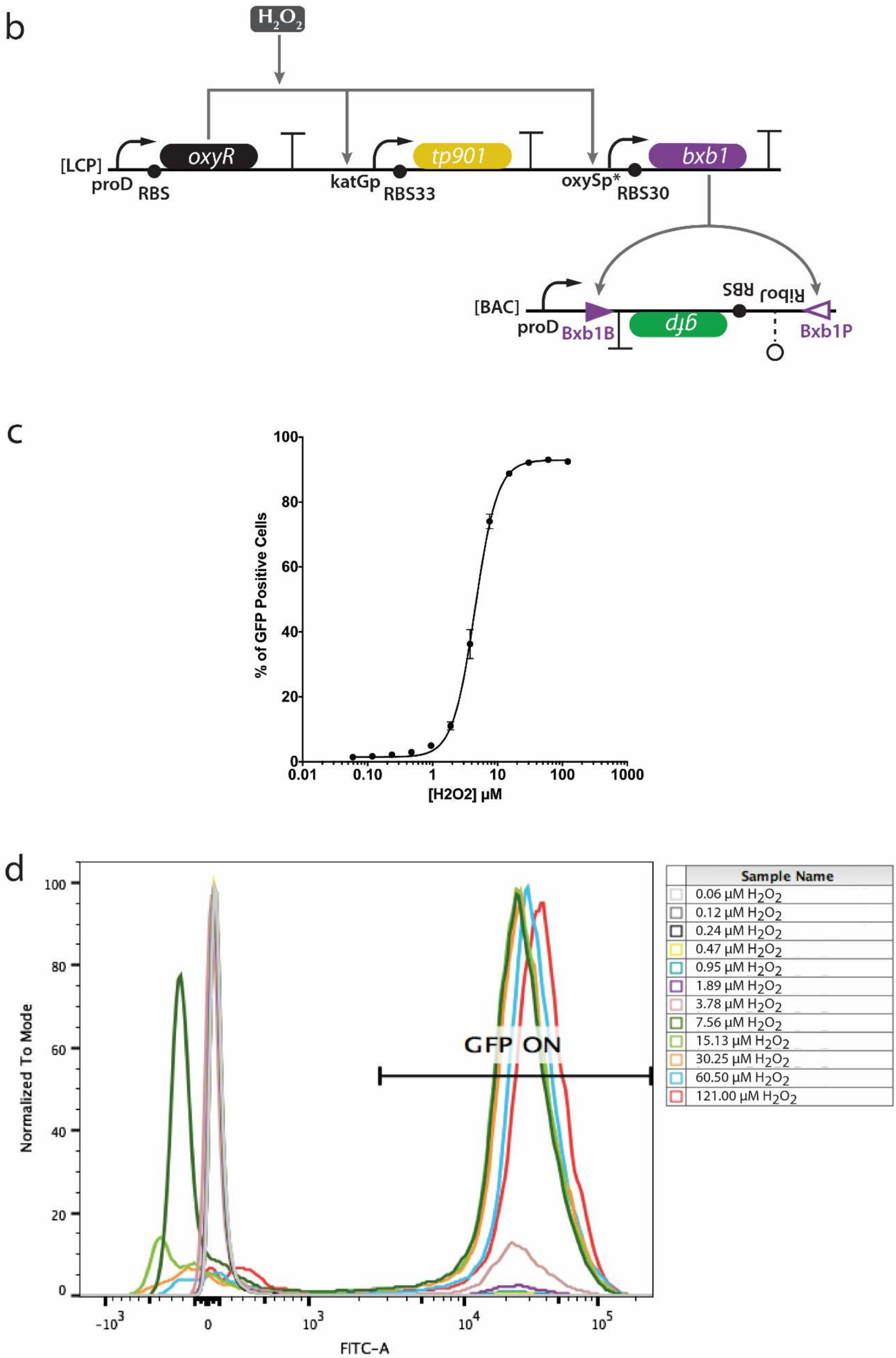


Figure B.6| A bandpass filter assembled from a low-threshold high-pass circuit and a medium-threshold low-pass circuit. *(Figure 3.3-a,b). (a). Representative flow cytometry histograms for GFP expression from the bandpass circuit shown in Figure 3.3-a,b. (b). The circuit used to characterize the transfer function of the low-threshold comparator that operates as a high-pass in the bandpass circuit in Figure 3.3-*

a,b. OxyR is constitutively expressed from a LCP and activates transcription of bxb1 from the oxySp promoter and phiC31 from the katGp promoter on the same LCP in response to H_2O_2. Bxb1 inverts the gfp expression cassette on a BAC, turning on GFP expression by pairing it with the proD promoter. (c). The transfer function of the low-threshold comparator that operates as a high-pass in the bandpass circuit in Figure 3.3-a,b. Black line is a sigmoidal fit to the data. This fit was used to generate the high-pass variables in the bandpass function (Data Processing and Calculation). The errors (standard deviation) are derived from flow cytometry experiments of three biological replicates, each of which involved n > 30,000 gated events. (d). Representative flow cytometry histograms for GFP expression for the data shown in Figure B.6-c. (e). The circuit used to characterize the transfer function of the medium-threshold comparator that operates as a low-pass transfer function in the bandpass circuit in Figure 3.3-a,b. Here, we characterized the comparator by turning on GFP expression, rather than turning it off as in Figure 3.3. OxyR is constitutively expressed from a LCP and activates transcription of bxb1 from the oxySp* promoter and phiC31 from the katGp promoter on the same LCP in response to H_2O_2. PhiC31 inverts the gfp cassette on a BAC, turning on GFP expression by pairing it with the proD promoter. (f). The transfer function of the medium-threshold comparator that operates as a low-pass in the bandpass circuit in Figure 3.3-a,b. This fit was used to generate the low-pass variables in the bandpass function (Data Processing and Calculations). The errors (standard deviation) are derived from flow cytometry experiments of three biological replicates, each of which involved n > 30,000 gated events. (g). Representative flow cytometry histograms for GFP expression for the data shown in Figure B.6-f.*




b
H2O2
[LCP]
proD
RBS
oxyR
katGp
RBS33
tp901
oxySp*
RBS30
bxb1
[BAC]
proD
Bxb1B
gfp
RiboJ RBS
Bxb1P
c
% of GFP Positive Cells
[H2O2] µM
d
Normalized To Mode
FITC-A
GFP ON
Sample Name
0.06 µM H2O2
0.12 µM H2O2
0.24 µM H2O2
0.47 µM H2O2
0.95 µM H2O2
1.89 µM H2O2
3.78 µM H2O2
7.56 µM H2O2
15.13 µM H2O2
30.25 µM H2O2
60.50 µM H2O2
121.00 µM H2O2

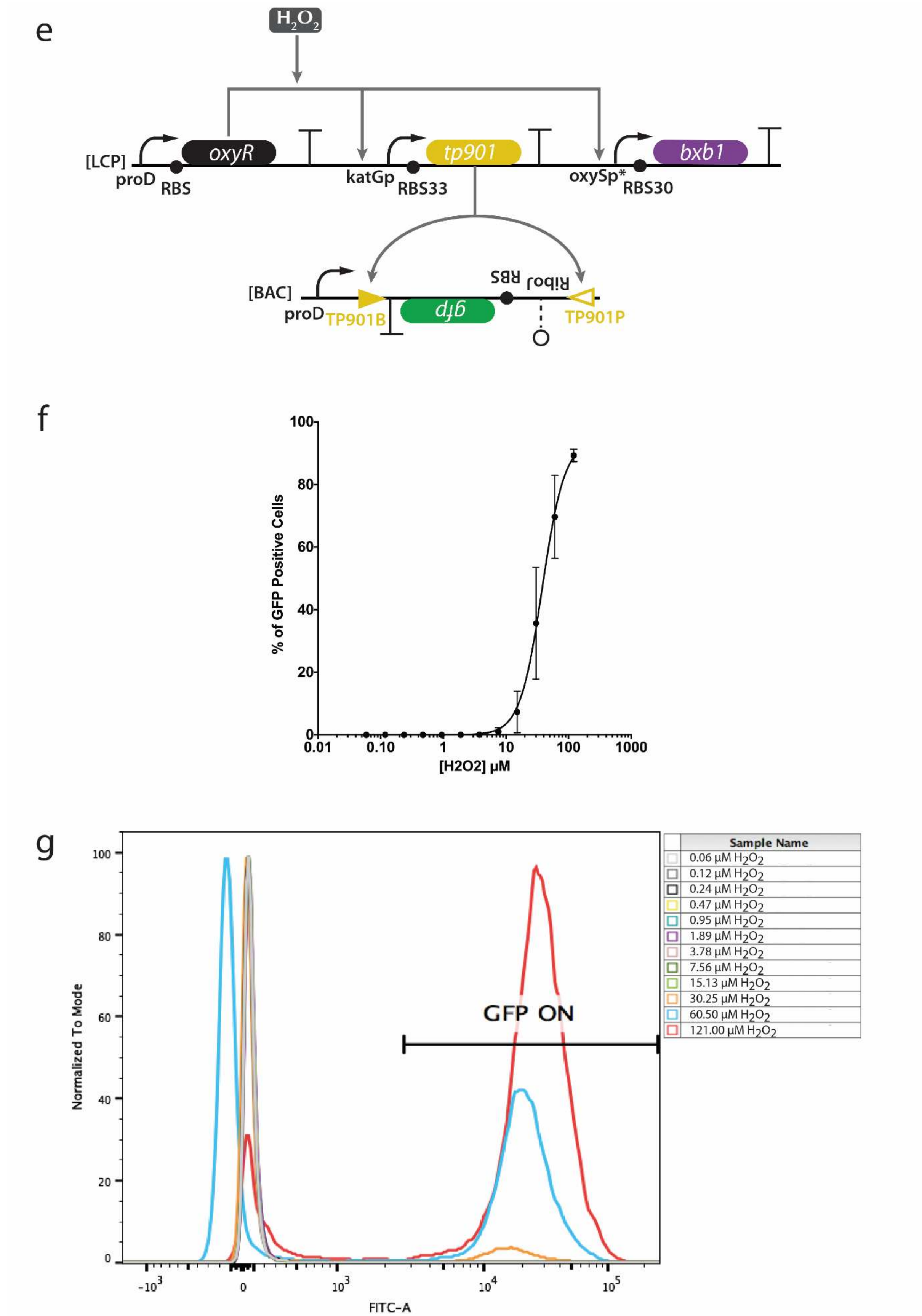
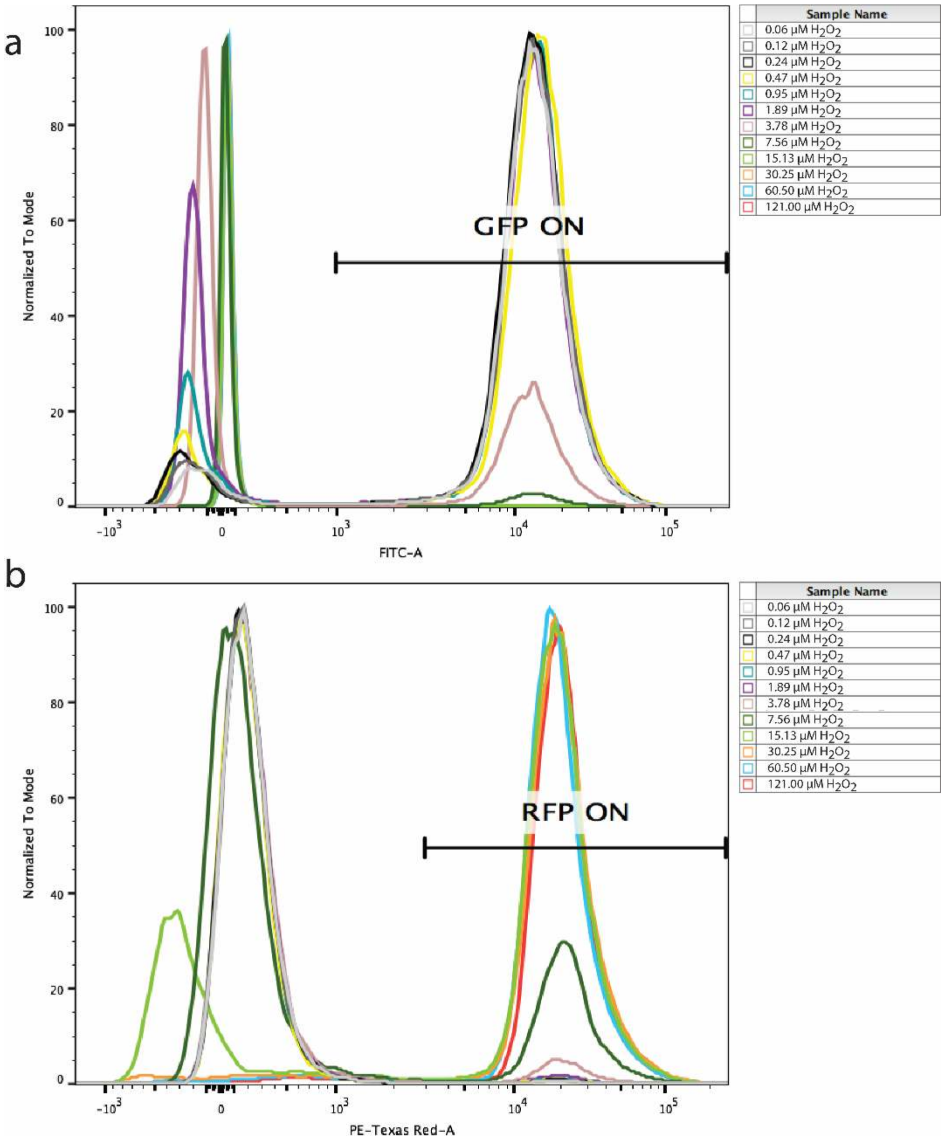
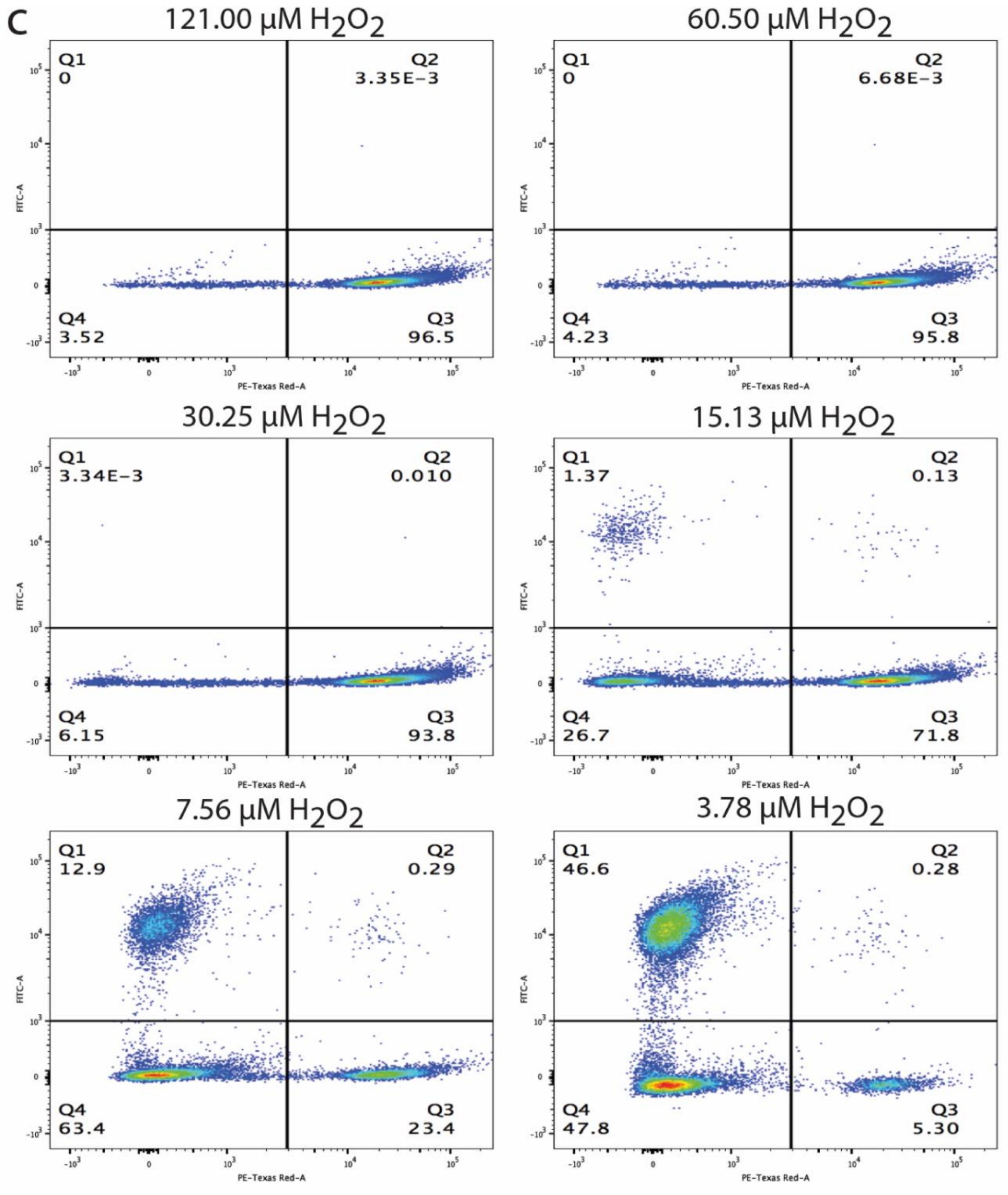


Figure B.7| A bandpass filter assembled from a low-threshold high-pass circuit and a high-threshold low-pass circuit. *Figure 3.3-c,d. (a). Representative flow cytometry histograms for GFP expression from the bandpass circuit shown in Figure 3.3-c,d. (b). The circuit used to characterize the transfer function of the low-threshold comparator that operates as a high-pass in the bandpass circuit in Figure 3.3-c,d. OxyR is* *constitutively expressed from a LCP and activates transcription of bxb1 from the oxySp* promoter and tp901 from the katGp promoter on the same LCP in response to H_2O_2. Bxb1 inverts the gfp cassette on a BAC, turning on GFP expression by pairing it with the proD promoter. (c). The transfer function of the low-threshold comparator that operates as a high-pass in the bandpass circuit in Figure 3.3-c,d. This fit was used to generate the high-pass variables in the bandpass function (Data Processing and Calculations). The errors (standard deviation) are derived from flow cytometry experiments of three biological replicates, each of which involved n > 30,000 gated events. (d). Representative flow cytometry histograms for GFP expression for the data shown in Figure B.7-c. (e). The circuit used to characterize the transfer function of the high-threshold comparator that operates as a low-pass transfer function in the bandpass circuit in Figure 3.3-c,d. Here, we characterized the comparator by turning on GFP expression, rather than turning it off as in Figure 3.3. OxyR is constitutively expressed from a LCP and activates transcription of bxb1 from the oxySp* promoter and tp901 from the katGp promoter on the same LCP in response to H_2O_2. TP901 inverts the gfp cassette on a BAC, turning on GFP expression by pairing it with the proD promoter. (f). The transfer function of the high-threshold comparator that operates as a low-pass in the bandpass circuit in Figure 3.3-c,d. This fit was used to generate the low-pass variables in the bandpass function (Data Processing and Calculations). (g). Representative flow cytometry histograms for GFP expression for the data shown in Figure B.7-f.*




C
121.00 µM H2O2
Q1 0
Q2 3.35E-3
Q4 3.52
Q3 96.5
60.50 µM H2O2
Q1 0
Q2 6.68E-3
Q4 4.23
Q3 95.8
30.25 µM H2O2
Q1 3.34E-3
Q2 0.010
Q4 6.15
Q3 93.8
15.13 µM H2O2
Q1 1.37
Q2 0.13
Q4 26.7
Q3 71.8
7.56 µM H2O2
Q1 12.9
Q2 0.29
Q4 63.4
Q3 23.4
3.78 µM H2O2
Q1 46.6
Q2 0.28
Q4 47.8
Q3 5.30
FITC-A
PE-Texas Red-A

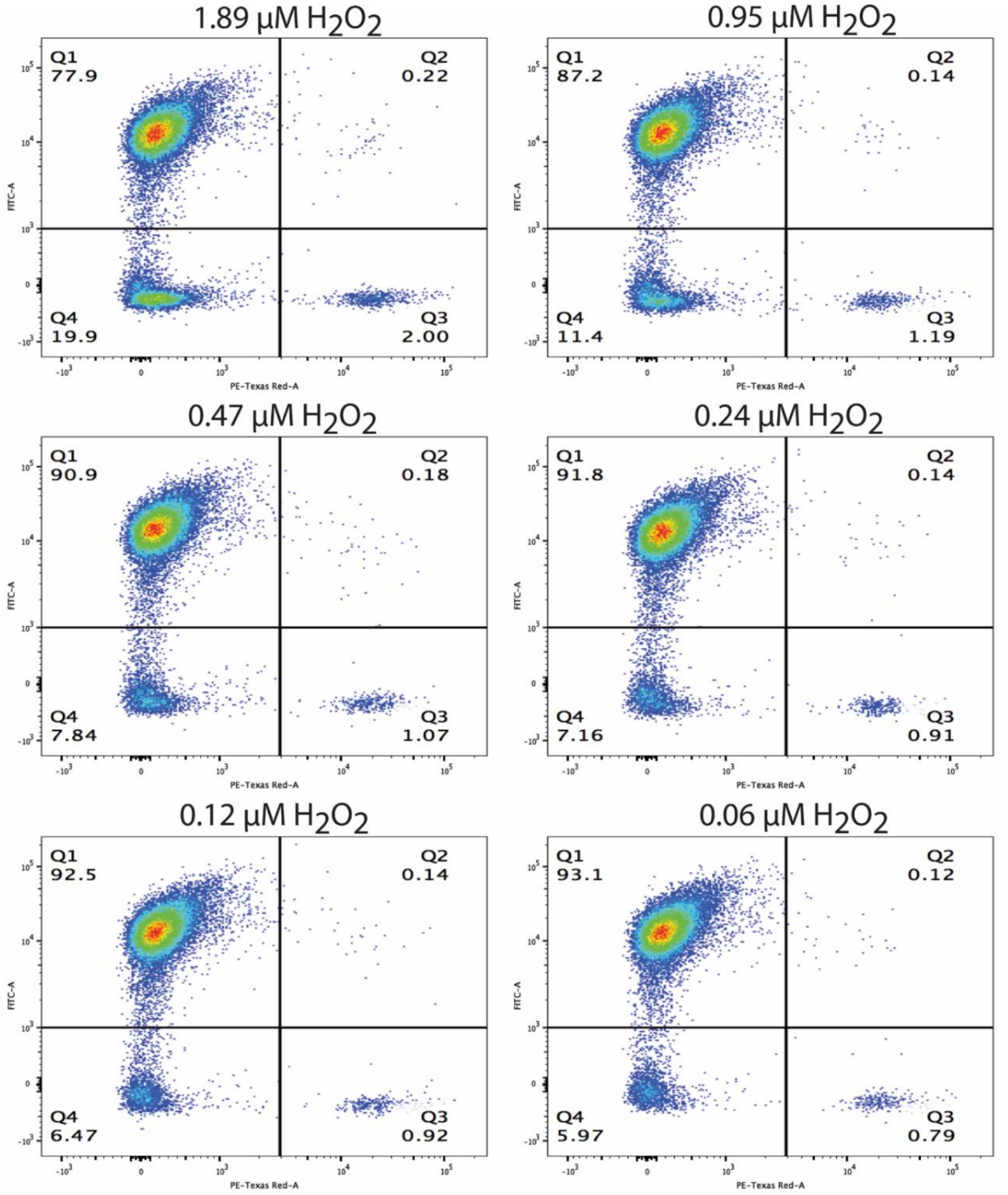


Figure B.8| Ternary Logic. *(Figure 3.4-a,b). (a). Representative flow cytometry histograms for GFP expression for the ternary logic circuit shown in Figure 3.4-a, and the data in Figure 3.4-b. (b). Representative flow cytometry histograms for RFP expression for the ternary logic circuit shown in Figure 3.4-a, and the data in Figure 3.4-b. (c). Representative flow cytometry plots for GFP and RFP expression for the ternary logic circuit shown in Figure 3.4-a, and the data in Figure 3.4-b.*

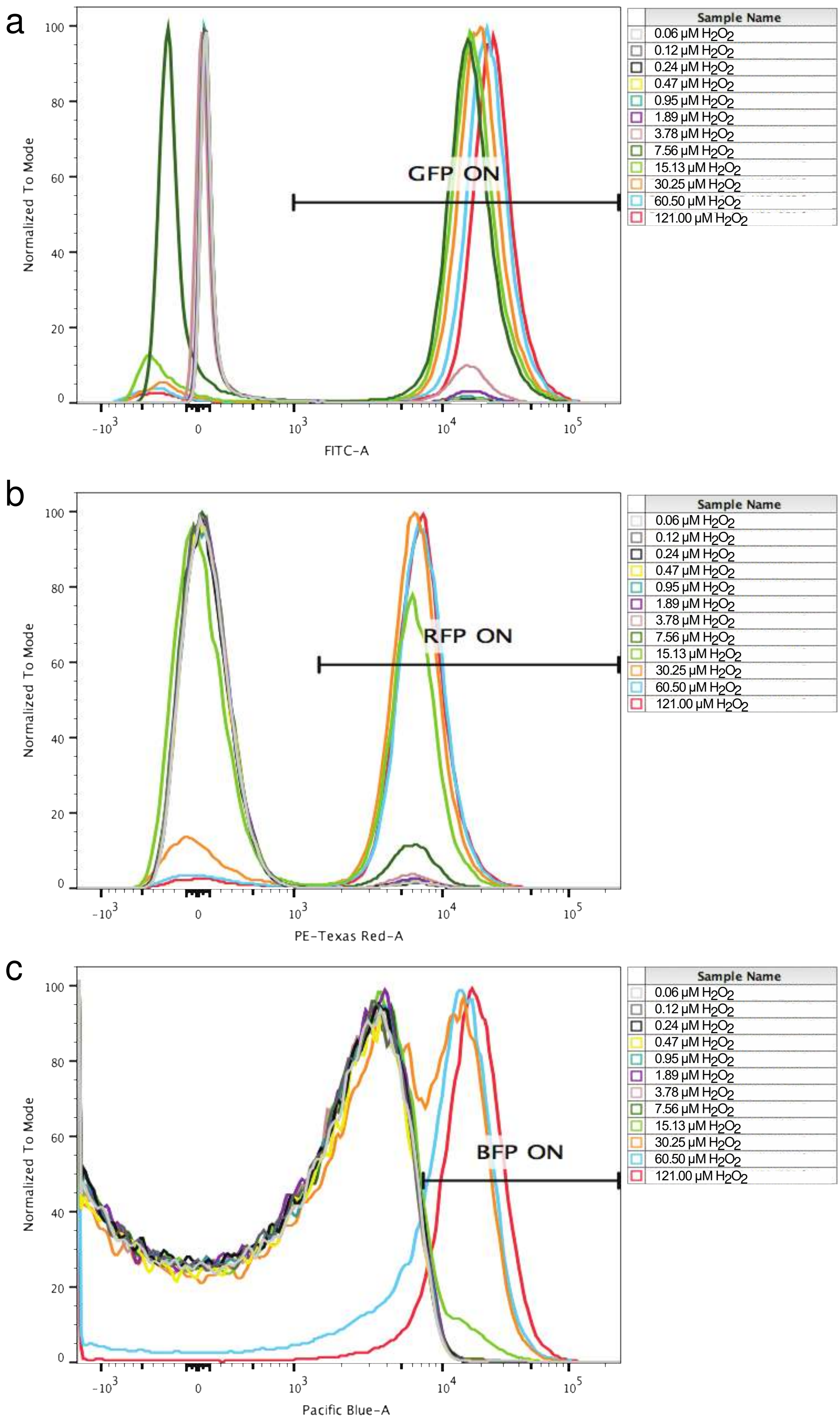


Figure B.9| 2-bit Analog-to-digital Converter. *(Figure 3.4-d,e). (a). Representative flow cytometry histograms for GFP expression from the analog-to-digital converter circuit shown in Figure 3.4-d, and the data in Figure 3.4-e. (b). Representative flow cytometry histograms for RFP expression from the analog-to-digital converter circuit shown in Figure 3.4-d, and the data in Figure 3.4-e. (c). Representative flow cytometry* *histograms for BFP expression from the analog-to-digital converter circuit shown in Figure 3.4-d, and the data in Figure 3.4-e.*

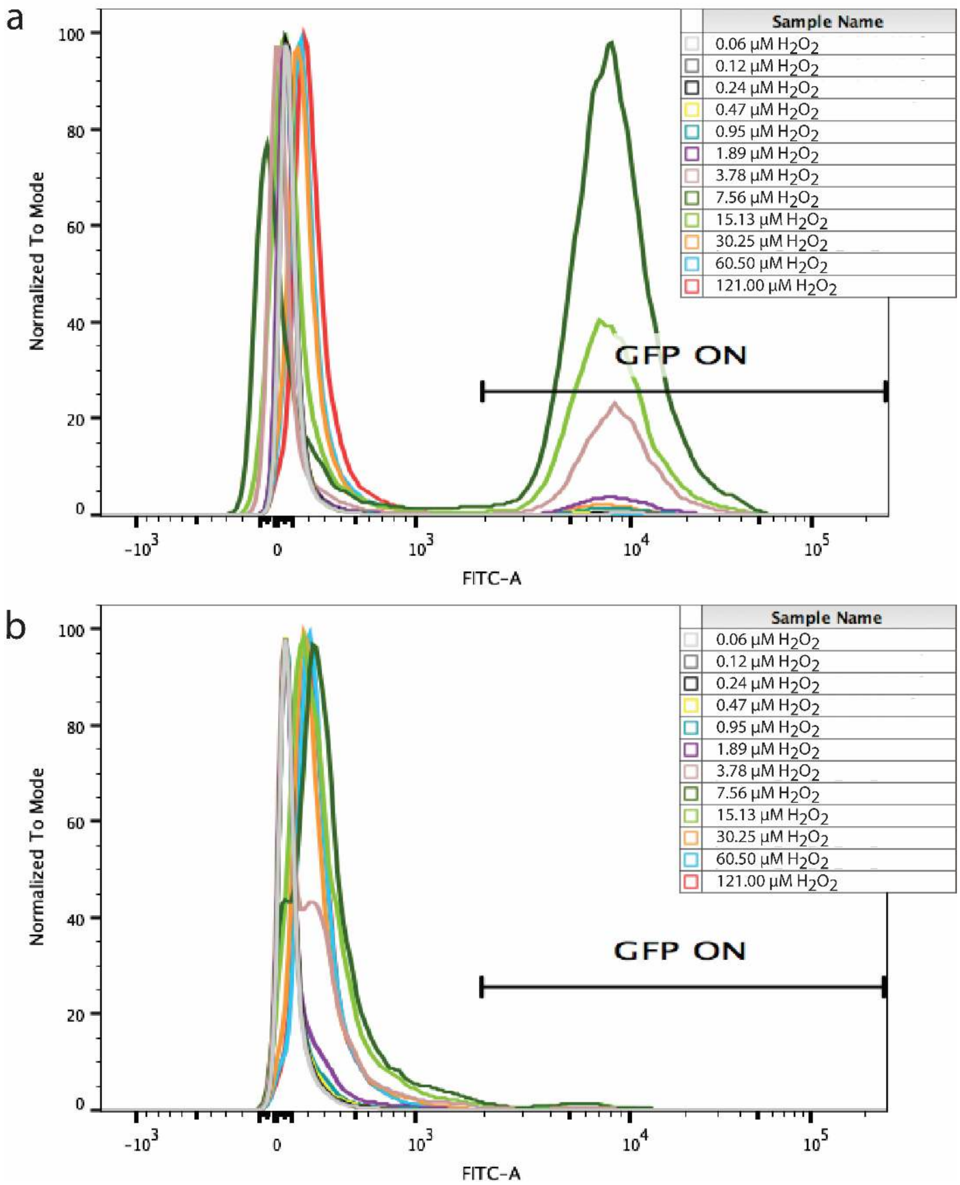


Figure B.10| Mixed-signal processing and concentration-dependent logic. *(Figure 3.5). (a). Representative flow cytometry histograms for GFP expression from the mixed-signal processing circuit shown in Figure 3.5-a, and the data in Figure 3.5-b, without aTc. (b). Representative flow cytometry histograms for GFP expression from the mixed- signal processing circuit shown in Figure 3.5-a, and the data in Figure 3.5-b, with aTc.*

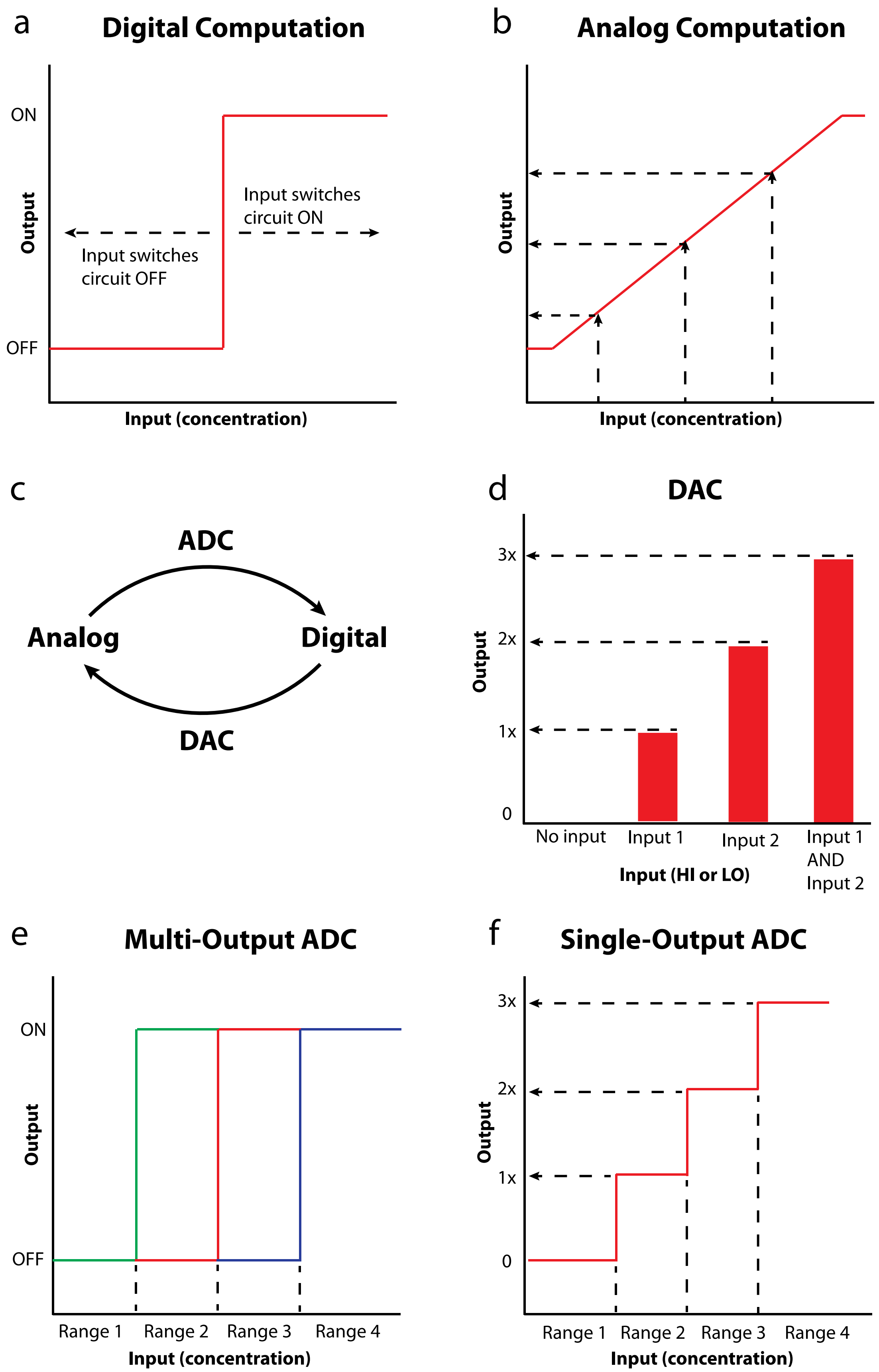
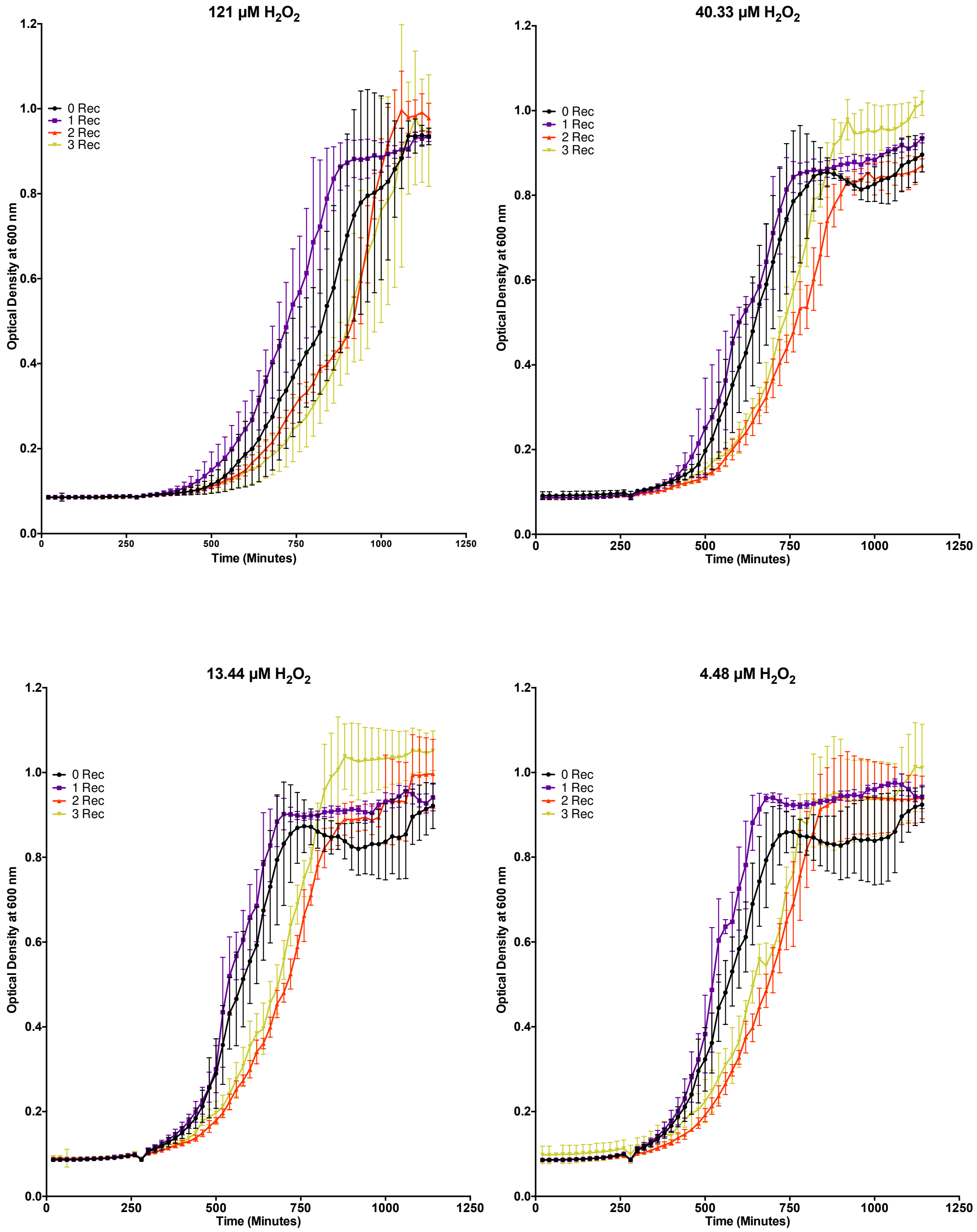


Figure B.11| Digital-to-analog converters and analog-to-digital converters are complementary systems that translate digital signals to analog signals, and vice versa. *(a). In the digital computation paradigm, signals are defined as OFF or ON and computing is based on Boolean logic. (b) In the analog computation* *paradigm, circuits convert continuous, analog inputs to continuous outputs according to mathematical relationships. (c) Analog information is converted to digital information with analog-to-digital converters (ADC). Digital information is converted to analog information with digital-to-analog converters (DAC). (d) A digital-to-analog converter that accepts various digital combinations of inputs and outputs quantized levels of a single output. (e) An analog-to-digital converter that accepts the continuous, analog concentration of an input and classifies discrete ranges of the input to different output molecules. (f) An analog-to-digital converter that accepts the continuous, analog concentration of an input and classifies discrete ranges of the input to discrete levels of a single output.*



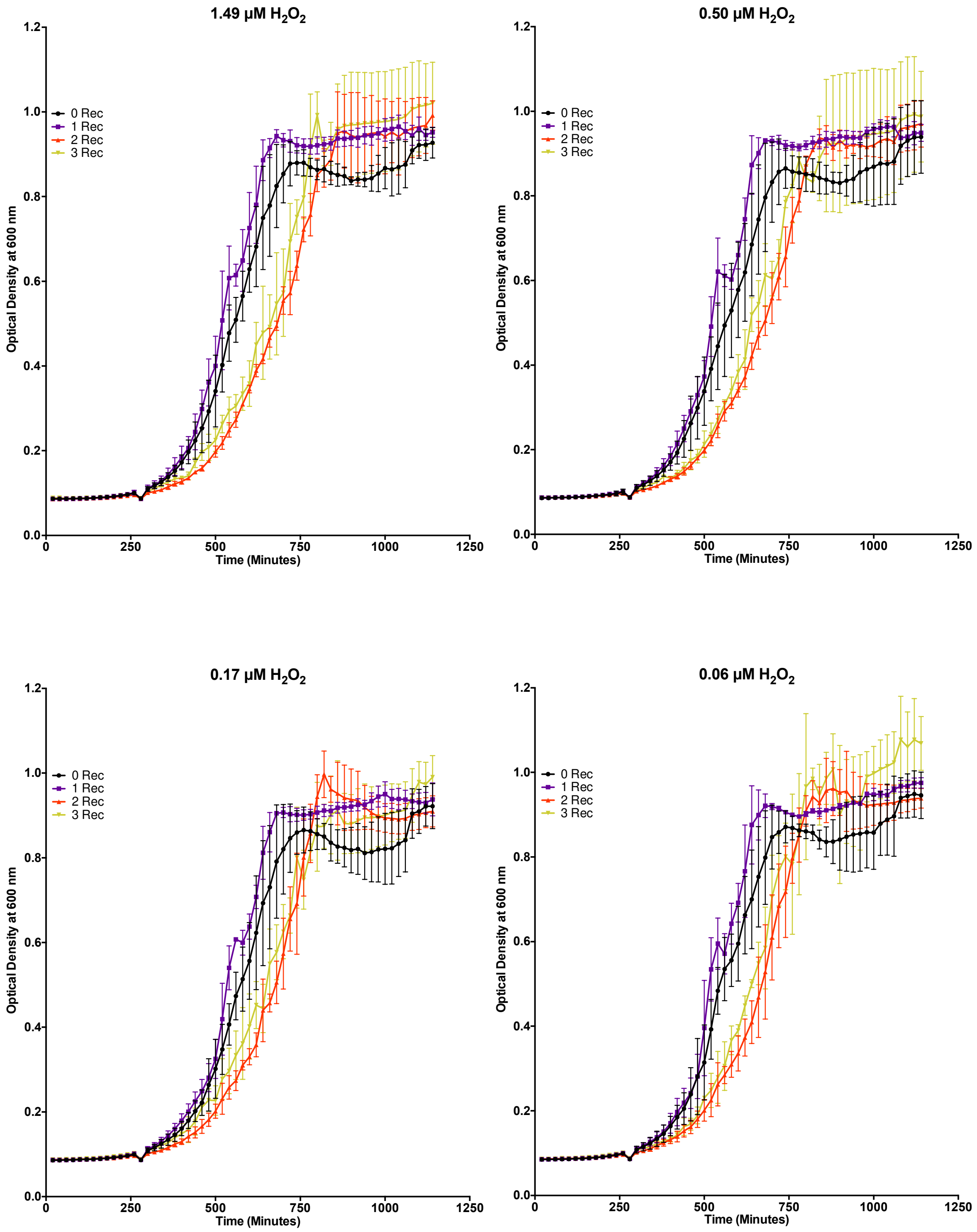
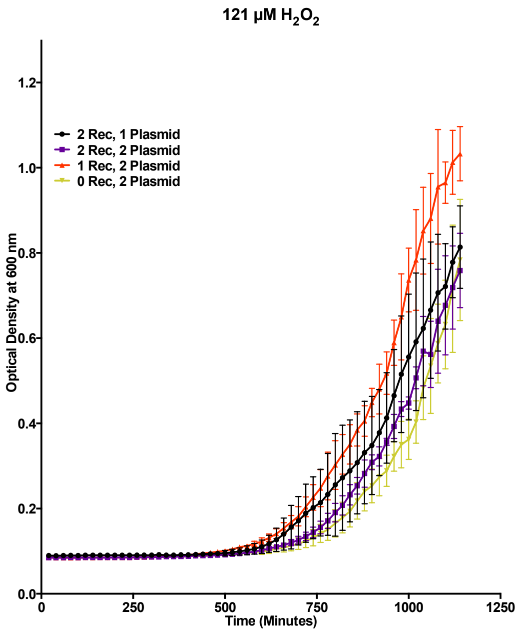
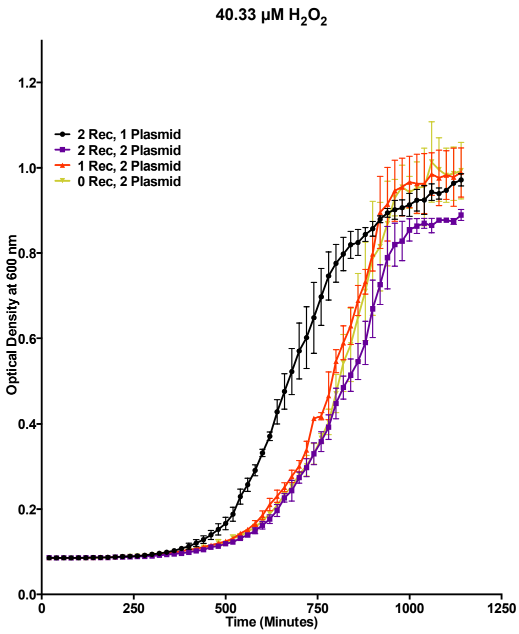
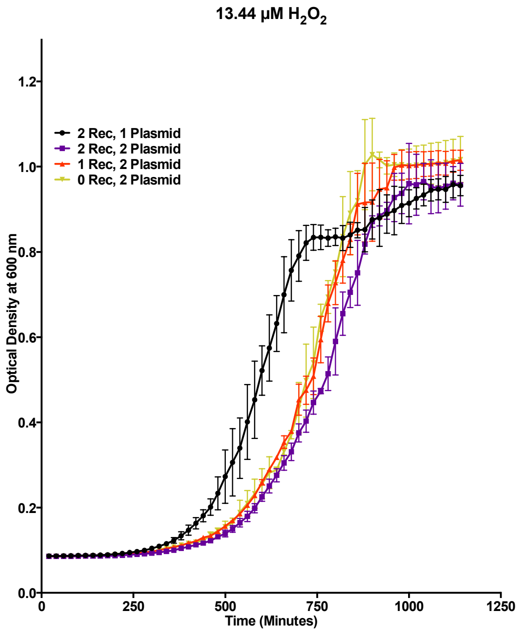
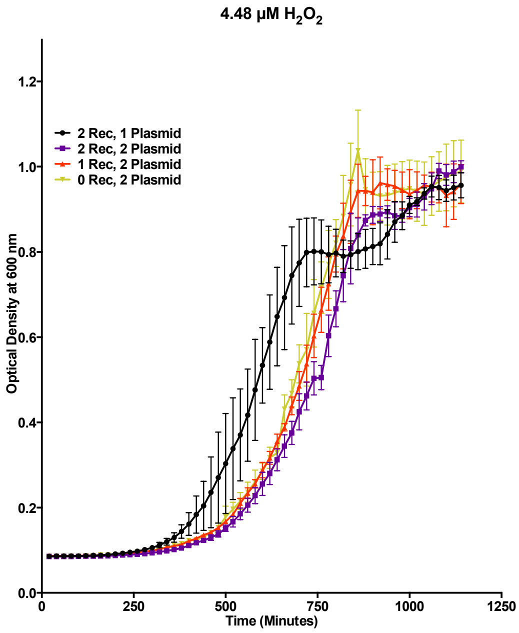


Figure B.12| Growth curves for cells containing 0, 1, 2, or 3 recombinases at different concentrations of H_2O_2. *Cells were induced with H_2O_2 and incubated in a plate reader shaking at 30 degrees for 20 hours. Optical density measurements at 600 nm were taken every 20 minutes. The mean and standard deviation are derived from three biological replicates, and the lines are direct connections between adjacent measurements. The cells did not contain reporter plasmids. The cells with 1 recombinase (Rec) contain the low-pass circuit encoded on 1 plasmid (pZS2oxySp*-RBS30-Bxbi-proD-oxyR). The cells with 2 recombinases contain the low-pass and medium-pass circuit on 2 plasmids (pZS2oxySp*-RBS30-Bxbi-proD-oxyR and pZS1katGp-RBS31-PhiC31-proD-oxyR). The cells with 3 recombinases contain the low-pass, medium-pass, and high-pass circuit on 2 plasmids (pZS1oxySp*-RBS30-bxbi-katGp-RBS31-PhiC31-proD-oxyR + pZS2katGp-RBS33-TP901-proD-oxyR).*


121 µM H2O2
2 Rec, 1 Plasmid
2 Rec, 2 Plasmid
1 Rec, 2 Plasmid
0 Rec, 2 Plasmid
Optical Density at 600 nm
Time (Minutes)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0
250
500
750
1000
1250


40.33 µM H2O2
2 Rec, 1 Plasmid
2 Rec, 2 Plasmid
1 Rec, 2 Plasmid
0 Rec, 2 Plasmid
Optical Density at 600 nm
Time (Minutes)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0
250
500
750
1000
1250


13.44 µM H2O2
2 Rec, 1 Plasmid
2 Rec, 2 Plasmid
1 Rec, 2 Plasmid
0 Rec, 2 Plasmid
Optical Density at 600 nm
Time (Minutes)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0
250
500
750
1000
1250


4.48 µM H2O2
2 Rec, 1 Plasmid
2 Rec, 2 Plasmid
1 Rec, 2 Plasmid
0 Rec, 2 Plasmid
Optical Density at 600 nm
Time (Minutes)
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0.2
0.4
0.6
0.8
1.0
1.2
0
250
500
750
1000
1250

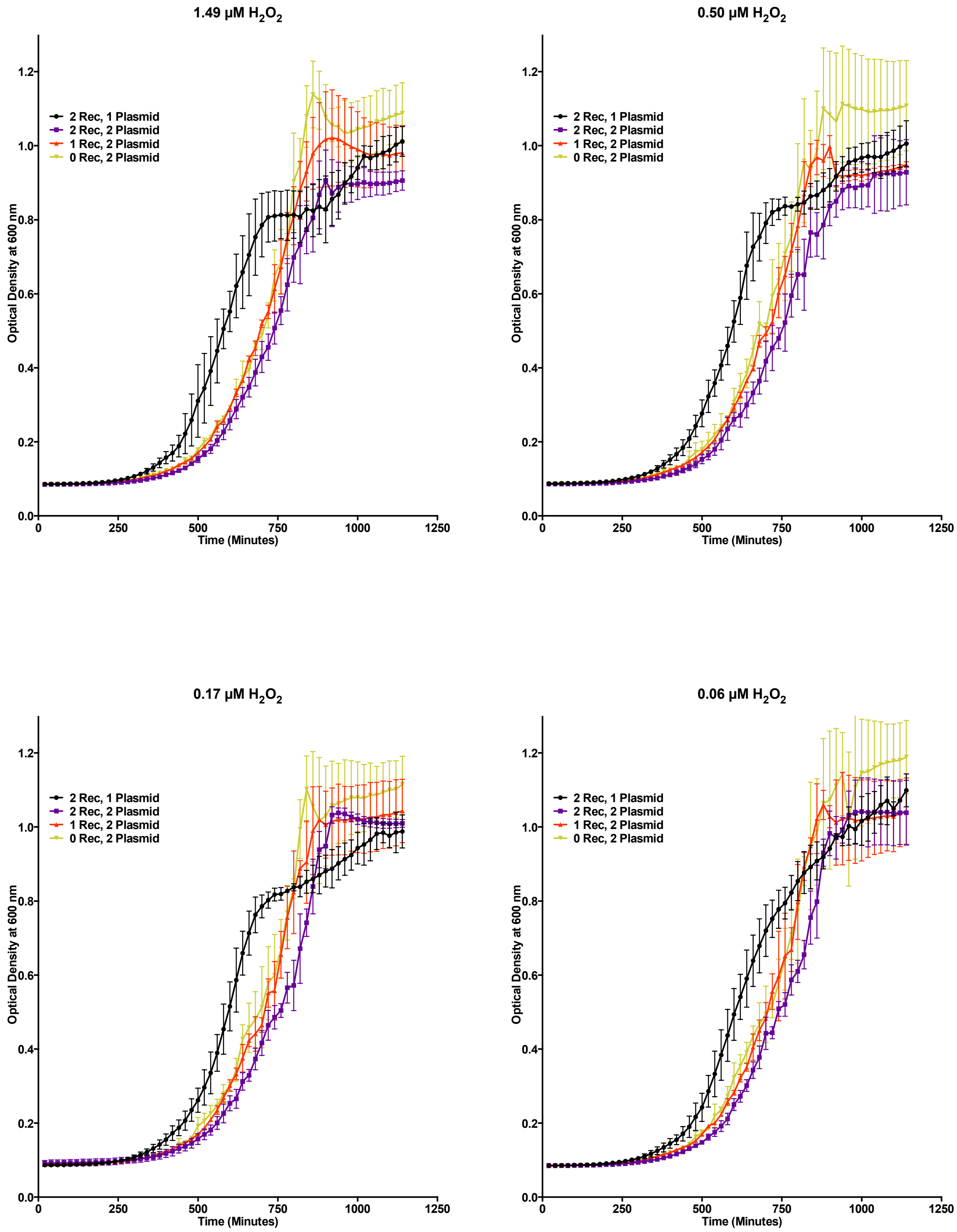


Figure B.13| Growth curves for cells containing 0, 1, or 2 recombinases on 2 plasmids or 2 recombinases on 1 plasmid at different concentrations of H_2O_2. *Cells were induced with H_2O_2 and incubated in a plate reader shaking at 30 degrees for 20 hours. Optical density measurements at 600 nm were taken every 20 minutes. The mean and standard deviation are derived from three biological replicates, and the lines are direct connections between adjacent measurements. The cells did not contain reporter plasmids. The cells with 2 recombinases (Rec) on 1 plasmid contain a plasmid encoding the low-threshold and medium-threshold circuits (plasmid pZS1oxySp*-RBS30-bxbi-katGp-RBS31-PhiC31-proD-oxyR). The cells with 2 recombinases on 2 plasmids contain the low-pass and medium-pass circuit (pZS2oxySp*-RBS30-Bxbi-proD-oxyR and pZS1katGp-RBS31-PhiC31-proD-oxyR). The cells with 1 recombinase and 2 plasmids contain the low-pass circuit (pZS2oxySp*-RBS30-Bxbi-proD-oxyR) and a second plasmid that does not encode a recombinase (pSC101 origin, carbenicillin resistance). The cells with 0 recombinase and 2 plasmids contain 2 plasmids that do not express recombinases (pSC101 origins, carbenicillin or kanamycin resistance).*

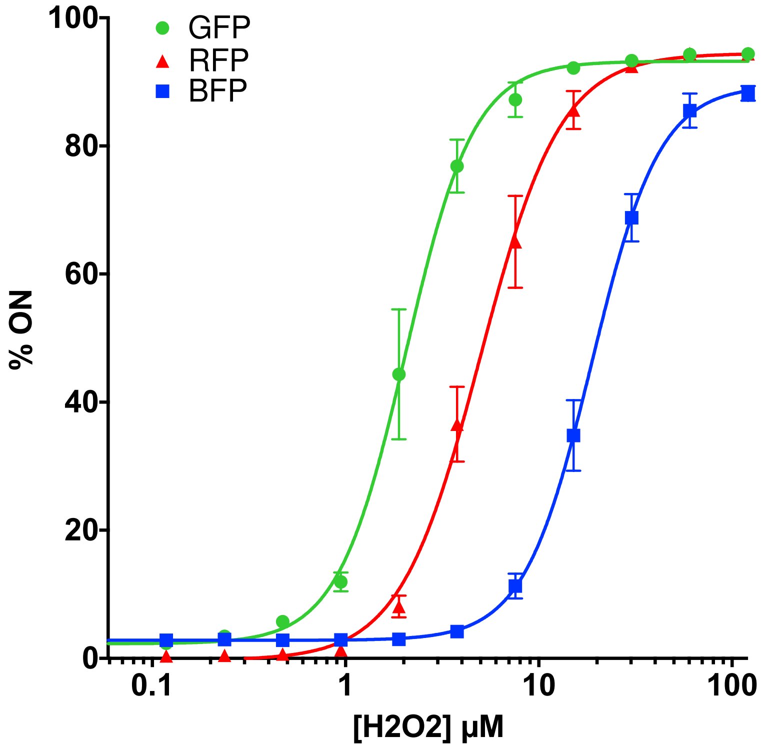


Figure B.14| Scale-up of the 2-bit ADC circuit. *Cells containing the 2-bit ADC circuit (Figure 3.4-d) were grown within flasks with different concentrations of H_2O_2 at a volume of 20 mL, which is a 100x greater volume than which was used to generate the data in Figure 3.4-e. The thresholds were shifted slightly to lower concentrations of H_2O_2 in higher volumes compared to Figure 3.4-e but still show good separation. The data is the mean and standard deviation of the percent of fluorophore-positive cells from flow cytometry experiments with three biological replicates.*

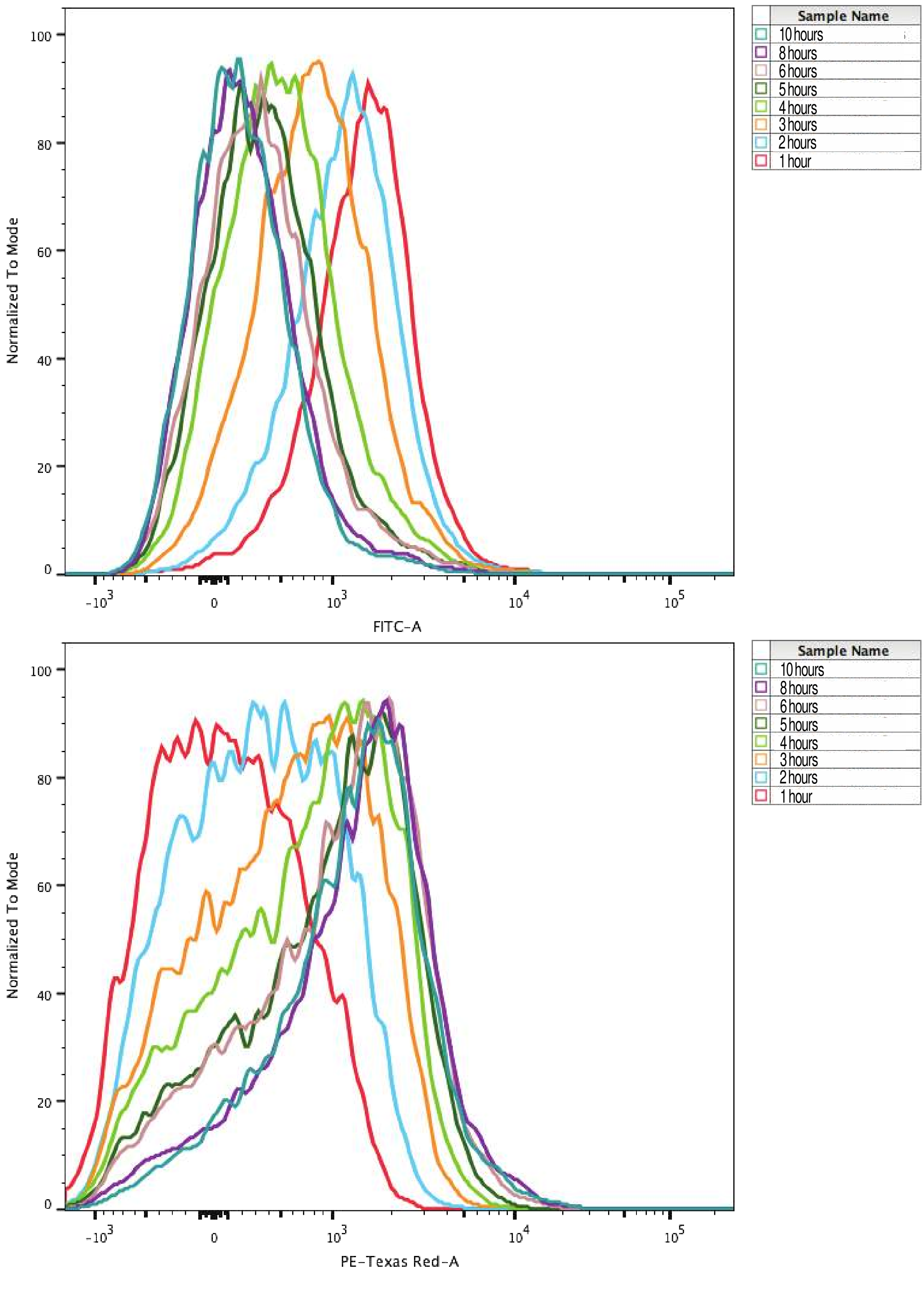
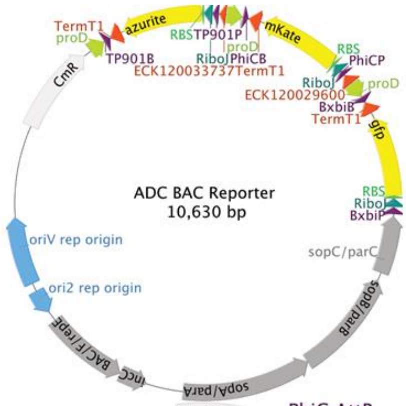
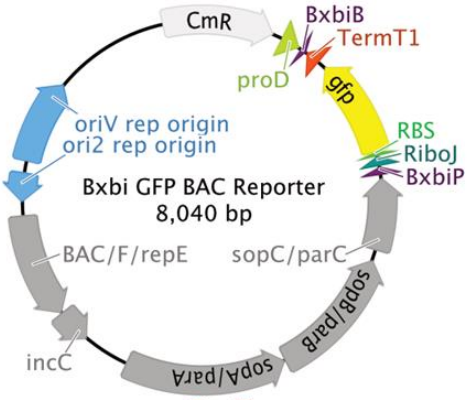
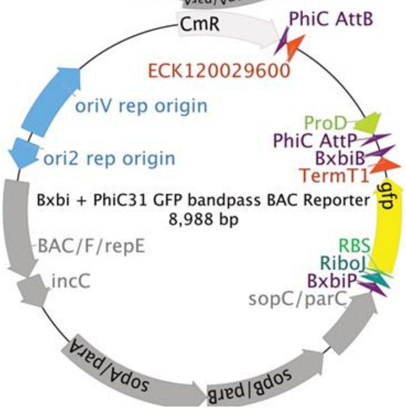


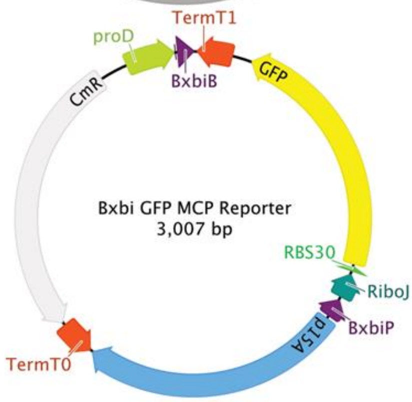
Figure B.15| Time-course experiment for the ternary logic circuit. *Representative flow cytometry histograms from three biological replicates for GFP expression (top) and RFP expression (bottom) from the ternary logic circuit shown in Figure 3.4-a at a fixed concentration of* H_2O_2 *(20.2 µM), which is expected to result in a RFP ON and GFP OFF state. The cells were induced in 50 mL of media in shaking flasks. At each indicated time point, samples were taken from batch culture and run on the flow cytometer. Because the cells were not induced with Copy Control, the separation between ON and OFF states is smaller than measured in Figure B.8. The cells likely induce recombination within the first 2 hours, as shown by the population-level changes in RFP and GFP expression, though it takes more time for the cells to reach steady-state gene expression by accumulating RFP and diluting GFP through cell division.*

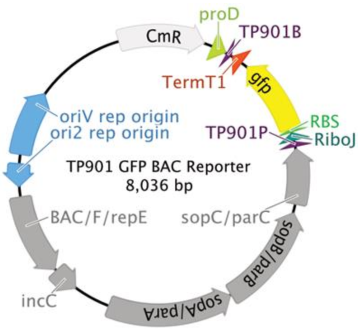
Plasmids and synthetic parts

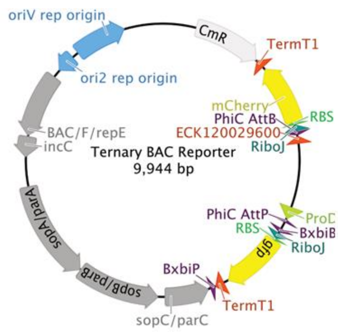


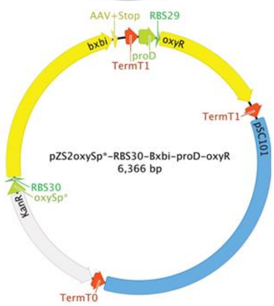


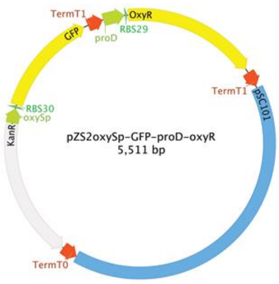


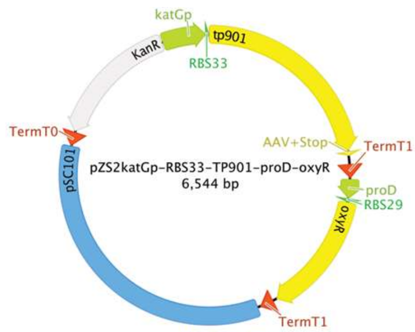








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tp901
KanR
RBS33
TermT0
AAV+Stop
TermT1
pZS2katGp-RBS33-TP901-proD-oxyR
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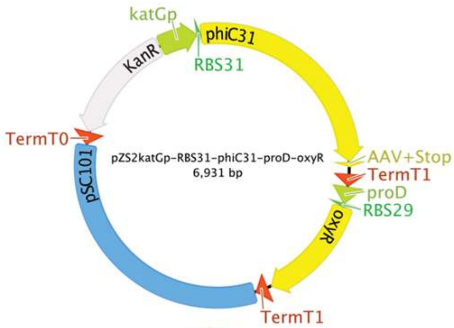
pSC101
proD
RBS29
oxyR
TermT1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KanR
RBS31
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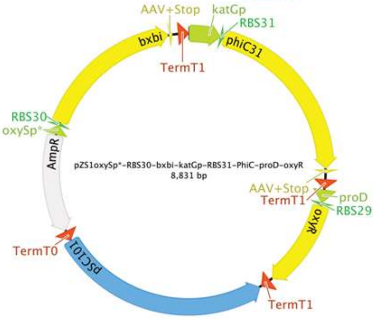
TermT1
proD
RBS29
oxyR
TermT1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RBS31
bxbi
phiC31
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RBS30
oxySp*
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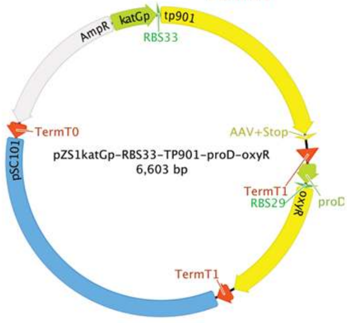
proD
RBS29
oxyR
TermT0
pSC101
TermT1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tp901
RBS33
TermT0
AAV+Stop
pSC101
pZS1katGp-RBS33-TP901-proD-oxyR
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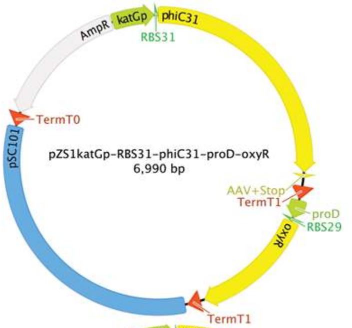
TermT1
RBS29
oxyR
proD
TermT1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phiC31
RBS31
TermT0
pSC101
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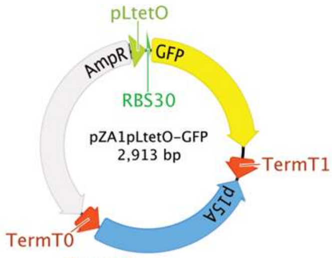
TermT1
proD
RBS29
oxyR
TermT1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RBS30
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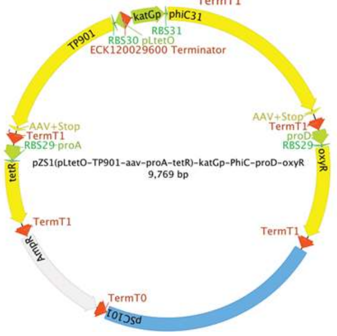
TermT1
P15A
TermT0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TP901
RBS31
RBS30
pLtetO
ECK120029600 Terminator
AAV+Stop
TermT1
RBS29
proA
AAV+Stop
TermT1
proD
RBS29
oxyR
tetR
pZS1(pLtetO-TP901-aav-proA-tetR)-katGp-PhiC-proD-oxyR
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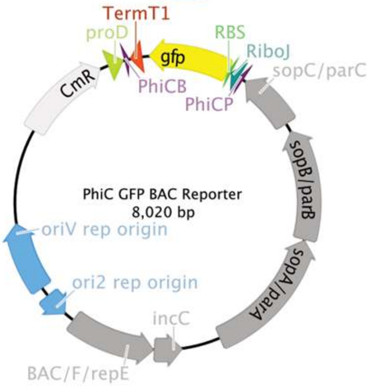
TermT1
TermT1
AmpR
TermT0
pSC101


TermT1
proD
RBS
RiboJ
gfp
sopC/parC
CmR
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PhiCP
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PhiC GFP BAC Reporter
8,020 bp
oriV rep origin
ori2 rep origin
sopA/parA
incC
BAC/F/repE

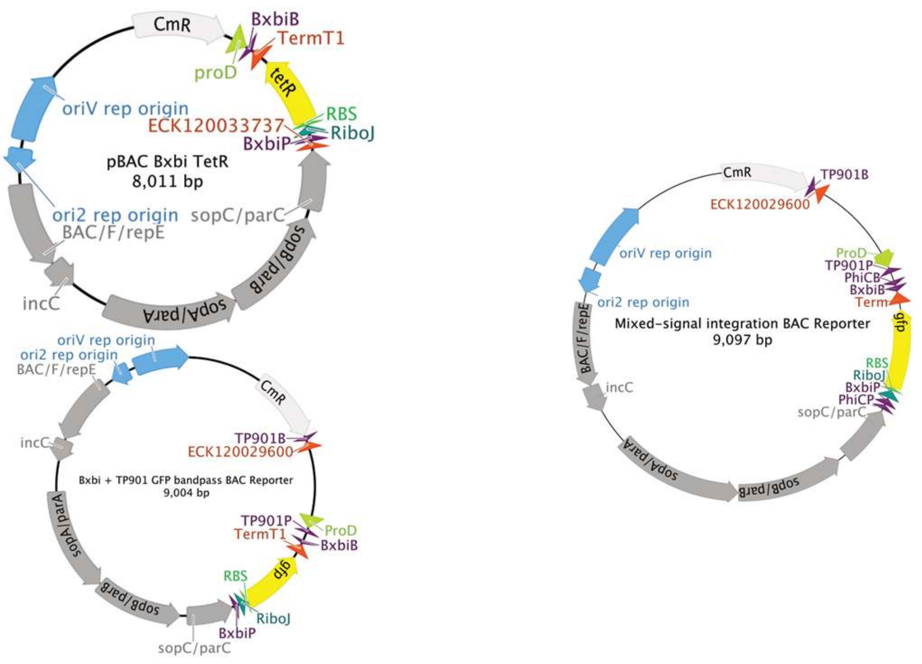

Table B.1| List of plasmids used in experiments

Figure	Plasmids
Figure 3.2-a	pZS2oxySp*-RBS30-Bxbi-proD-oxyR + Bxbi GFP BAC Reporter
Figure 3.2-c	pZS2katGp-RBS31-PhiC31-proD-oxyR + PhiC31 GFP BAC Reporter
Figure 3.2-e	pZS2katGp-RBS33-TP901-proD-oxyR + TP901 GFP BAC Reporter
Figure 3.3-a	pZS2oxySp*-RBS30-Bxbi-proD-oxyR + pZS1katGp-RBS31-PhiC31-proD-oxyR + Bxbi+PhiC31 GFP Bandpass BAC Reporter
Figure 3.3-c	pZS2oxySp*-RBS30-Bxbi-proD-oxyR + pZS1katGp-RBS33-TP901-proD-oxyR + Bxbi+TP901 GFP Bandpass BAC Reporter
Figure 3.4-a	pZS2oxySp*-RBS30-Bxbi-proD-oxyR + pZS1katGp-RBS31-PhiC31-proD-oxyR + Ternary BAC Reporter
Figure 3.4-d	pZS1oxySp*-RBS30-bxbi-katGp-RBS31-PhiC31-proD-oxyR + pZS2katGp-RBS33-TP901-proD-oxyR + ADC BAC Reporter
Figure 3.5-a	pZS2oxySp*-RBS30-Bxbi-proD-oxyR + pZS1(pLtetO-TP901-aav-proA-tetR)-katGp-PhiC31-proD-oxyR + Mixed-signal integration BAC Reporter
Figure B.1-a	pZS2oxySp-GFP-proD-oxyR
Figure B.2-a	pZS2oxySp*-RBS30-Bxbi-proD-oxyR + Bxbi GFP MCP Reporter
Figure B.3-a	pZS2oxySp*-RBS30-Bxbi-proD-oxyR + pBAC Bxbi TetR + pZA1pLtetO-GFP

Figure B.6-b	pZS2oxySp*-RBS30-Bxbi-proD-oxyR + pZS1katGp-RBS31-PhiC31-proD-oxyR + Bxbi GFP BAC Reporter
Figure B.6-e	pZS2oxySp*-RBS30-Bxbi-proD-oxyR + pZS1katGp-RBS31-PhiC31-proD-oxyR + PhiC31 GFP BAC Reporter
Figure B.7-b	pZS2oxySp*-RBS30-Bxbi-proD-oxyR + pZS1katGp-RBS33-TP901-proD-oxyR + Bxbi GFP BAC Reporter
Figure B.7-e	pZS2oxySp*-RBS30-Bxbi-proD-oxyR + pZS1katGp-RBS33-TP901-proD-oxyR + TP901 GFP BAC Reporter

Table B.2| Plasmid Sequences.

Plasmid Name	Sequence
pZS2oxySp*-RBS30-Bxbi-proD-oxyR	CACAGCTAACACCACGTCGTCCCTATCTGCTGCCCTAGGTCTATGAGTGGTTGCTGGATAA CTTTACGGGCATGCATAAGGCTCGTATAATATATTCAGGGAGACCACAACGGTTTCCCTCTA CAAATAATTTTGTTTAACTTTGAATTCTTCACACAGGAAACCGGTACCATGAATATTCGTGAT CTTGAGTACCTGGTGGCATTGGCTGAACACCGCCATTTTCGGCGTGCGGCAGATTCCTGCC ACGTTAGCCAGCCGACGCTTAGCGGGCAAATTCGTAAGCTGGAAGATGAGCTGGGCGTGA TGTTGCTGGAGCGGACCAGCCGTAAAGTGTTGTTCACCCAGGCGGGAATGCTGCTGGTGG ATCAGGCGCGTACCGTGCTGCGTGAGGTGAAAGTCCTTAAAGAGATGGCAAGCCAGCAGG GCGAGACGATGTCCGGACCGCTGCACATTGGTTTGATTCCCACAGTTGGACCGTACCTGCT ACCGCATATTATCCCTATGCTGCACCAGACCTTTCCAAAGCTGGAAATGTATCTGCATGAAG CACAGACCCACCAGTTACTGGCGCAACTGGACAGCGGCAAACTCGATTGCGTGATCCTCGC GCTGGTGAAAGAGAGCGAAGCATTCATTGAAGTGCCGTTGTTTGATGAGCCAATGTTGCTG GCTATCTATGAAGATCACCCGTGGGCGAACCGCGAATGCGTACCGATGGCCGATCTGGCA GGGGAAAAACTGCTGATGCTGGAAGATGGTCACTGTTTGCGCGATCAGGCAATGGGTTTCT GTTTTGAAGCCGGGGCGGATGAAGATACACACTTCCGCGCGACCAGCCTGGAAACTCTGC GCAACATGGTGGCGGCAGGTAGCGGGATCACTTTACTGCCAGCGCTGGCTGTGCCGCCGG AGCGCAAACGCGATGGGGTTGTTTATCTGCCGTGCATTAAGCCGGAACCACGCCGCACTAT TGGCCTGGTTTATCGTCCTGGCTCACCGCTGCGCAGCCGCTATGAGCAGCTGGCAGAGGC CATCCGCGCAAGAATGGATGGCCATTTCGATAAAGTTTTAAAACAGGCGGTTTAACCCGGG GGATCCCATGGTACGCGTGCTAGAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTG GGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGC CCTAGACCTAGGGCCTAGGGTACGGGTTTTGCTGCCCGCAAACGGGCTGTTCTGGTGTTGC TAGTTTGTTATCAGAATCGCAGATCCGGCTTCAGGTTTGCCGGCTGAAAGCGCTATTTCTTC CAGAATTGCCATGATTTTTTCCCCACGGGAGGCGTCACTGGCTCCCGTGTTGTCGGCAGCT TTGATTCGATAAGCAGCATCGCCTGTTTCAGGCTGTCTATGTGTGACTGTTGAGCTGTAACA AGTTGTCTCAGGTGTTCAATTTCATGTTCTAGTTGCTTTGTTTTACTGGTTTCACCTGTTCTAT TAGGTGTTACATGCTGTTCATCTGTTACATTGTCGATCTGTTCATGGTGAACAGCTTTAAATG CACCAAAAACTCGTAAAAGCTCTGATGTATCTATCTTTTTTACACCGTTTTCATCTGTGCATAT GGACAGTTTTCCCTTTGATATCTAACGGTGAACAGTTGTTCTACTTTTGTTTGTTAGTCTTGA TGCTTCACTGATAGATACAAGAGCCATAAGAACCTCAGATCCTTCCGTATTTAGCCAGTATG TTCTCTAGTGTGGTTCGTTGTTTTTGCGTGAGCCATGAGAACGAACCATTGAGATCATGCTT ACTTTGCATGTCACTCAAAAATTTTGCCTCAAAACTGGTGAGCTGAATTTTTGCAGTTAAAGC ATCGTGTAGTGTTTTTCTTAGTCCGTTACGTAGGTAGGAATCTGATGTAATGGTTGTTGGTAT TTTGTCACCATTCATTTTTATCTGGTTGTTCTCAAGTTCGGTTACGAGATCCATTTGTCTATCT

	AGTTCAACTTGGAAAATCAACGTATCAGTCGGGCGGCCTCGCTTATCAACCACCAATTTCAT ATTGCTGTAAGTGTTTAAATCTTTACTTATTGGTTTCAAAACCCATTGGTTAAGCCTTTTAAAC TCATGGTAGTTATTTTCAAGCATTAACATGAACTTAAATTCATCAAGGCTAATCTCTATATTTG CCTTGTGAGTTTTCTTTTGTGTTAGTTCTTTTAATAACCACTCATAAATCCTCATAGAGTATTT GTTTTCAAAAGACTTAACATGTTCCAGATTATATTTTATGAATTTTTTTAACTGGAAAAGATAA GGCAATATCTCTTCACTAAAAACTAATTCTAATTTTTCGCTTGAGAACTTGGCATAGTTTGTC CACTGGAAAATCTCAAAGCCTTTAACCAAAGGATTCCTGATTTCCACAGTTCTCGTCATCAG CTCTCTGGTTGCTTTAGCTAATACACCATAAGCATTTTCCCTACTGATGTTCATCATCTGAGC GTATTGGTTATAAGTGAACGATACCGTCCGTTCTTTCCTTGTAGGGTTTTCAATCGTGGGGT TGAGTAGTGCCACACAGCATAAAATTAGCTTGGTTTCATGCTCCGTTAAGTCATAGCGACTA ATCGCTAGTTCATTTGCTTTGAAAACAACTAATTCAGACATACATCTCAATTGGTCTAGGTGA TTTTAATCACTATACCAATTGAGATGGGCTAGTCAATGATAATTACTAGTCCTTTTCCTTTGAG TTGTGGGTATCTGTAAATTCTGCTAGACCTTTGCTGGAAAACTTGTAAATTCTGCTAGACCCT CTGTAAATTCCGCTAGACCTTTGTGTGTTTTTTTTGTTTATATTCAAGTGGTTATAATTTATAG AATAAAGAAAGAATAAAAAAAGATAAAAAGAATAGATCCCAGCCCTGTGTATAACTCACTACT TTAGTCAGTTCCGCAGTATTACAAAAGGATGTCGCAAACGCTGTTTGCTCCTCTACAAAACA GACCTTAAAACCCTAAAGGCTTAAGTAGCACCCTCGCAAGCTCGGGCAAATCGCTGAATATT CCTTTTGTCTCCGACCATCAGGCACCTGAGTCGCTGTCTTTTTCGTGACATTCAGTTCGCTG CGCTCACGGCTCTGGCAGTGAATGGGGGTAAATGGCACTACAGGCGCCTTTTATGGATTCA TGCAAGGAAACTACCCATAATACAAGAAAAGCCCGTCACGGGCTTCTCAGGGCGTTTTATG GCGGGTCTGCTATGTGGTGCTATCTGACTTTTTGCTGTTCAGCAGTTCCTGCCCTCTGATTT TCCAGTCTGACCACTTCGGATTATCCCGTGACAGGTCATTCAGACTGGCTAATGCACCCAGT AAGGCAGCGGTATCATCAACAGGCTTACCCGTCTTACTGTCCCTAGTGCTTGGATTCTCACC AATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTC ATTACTGGATCTATCAACAGGAGTCCAAGCGAGCTCTCGAACCCCAGAGTCCCGCTCAGAA GAACTCGTCAAGAAGGCGATAGAAGGCGATGCGCTGCGAATCGGGAGCGGCGATACCGTA AAGCACGAGGAAGCGGTCAGCCCATTCGCCGCCAAGCTCTTCAGCAATATCACGGGTAGC CAACGCTATGTCCTGATAGCGGTCCGCCACACCCAGCCGGCCACAGTCGATGAATCCAGAA AAGCGGCCATTTTCCACCATGATATTCGGCAAGCAGGCATCGCCATGGGTCACGACGAGAT CCTCGCCGTCGGGCATGCGCGCCTTGAGCCTGGCGAACAGTTCGGCTGGCGCGAGCCCC TGATGCTCTTCGTCCAGATCATCCTGATCGACAAGACCGGCTTCCATCCGAGTACGTGCTC GCTCGATGCGATGTTTCGCTTGGTGGTCGAATGGGCAGGTAGCCGGATCAAGCGTATGCA GCCGCCGCATTGCATCAGCCATGATGGATACTTTCTCGGCAGGAGCAAGGTGAGATGACAG GAGATCCTGCCCCGGCACTTCGCCCAATAGCAGCCAGTCCCTTCCCGCTTCAGTGACAACG TCGAGCACAGCTGCGCAAGGAACGCCCGTCGTGGCCAGCCACGATAGCCGCGCTGCCTCG TCCTGCAGTTCATTCAGGGCACCGGACAGGTCGGTCTTGACAAAAAGAACCGGGCGCCCCT GCGCTGACAGCCGGAACACGGCGGCATCAGAGCAGCCGATTGTCTGTTGTGCCCAGTCAT AGCCGAATAGCCTCTCCACCCAAGCGGCCGGAGAACCTGCGTGCAATCCATCTTGTTCAAT CATGCGAAACGATCCTCATCCTGTCTCTTGATCAGATCTTGATCCCCTGCGCCATCAGATCC TTGGCGGCAAGAAAGCCATCCAGTTTACTTTGCAGGGCTTCCCAACCTTACCAGAGGGCGC CCCAGCTGGCAATTCCGACGTCTTCATTATCCATCCTCCATCGCCACGATAGTTCATGGCGA TAGGTAGAATAGCAATGAACGATTATCCCTATCAAGCATTCTGACTGAGCATTGCTCACACG AATTCATTAAAGAGGAGAAAGGTACCATGAGAGCCCTGGTAGTCATCCGCCTGTCCCGCGT CACCGATGCTACGACTTCACCGGAGCGTCAGCTGGAGTCTTGCCAGCAGCTCTGCGCCCA GCGCGGCTGGGACGTCGTCGGGGTAGCGGAGGATCTGGACGTCTCCGGGGCGGTCGATC CGTTCGACCGGAAGCGCAGACCGAACCTGGCCCGGTGGCTAGCGTTCGAGGAGCAACCGT TCGACGTGATCGTGGCGTACCGGGTAGACCGGTTGACCCGATCGATCCGGCATCTGCAGC AGCTGGTCCACTGGGCCGAGGACCACAAGAAGCTGGTCGTCTCCGCGACCGAAGCGCACT TCGATACGACGACGCCGTTTGCGGCGGTCGTCATCGCGCTTATGGGAACGGTGGCGCAGA TGGAATTAGAAGCGATCAAAGAGCGGAACCGTTCGGCTGCGCATTTCAATATCCGCGCCGG

	GAAATACCGAGGATCCCTGCCGCCGTGGGGATACCTGCCTACGCGCGTGGACGGGGAGTG GCGGCTGGTGCCGGACCCTGTGCAGCGAGAGCGCATCCTCGAGGTGTATCACCGCGTCGT CGACAACCACGAGCCGCTGCACCTGGTGGCCCACGACCTGAACCGGCGTGGTGTCCTGTC GCCGAAGGACTACTTCGCGCAGCTGCAAGGCCGCGAGCCGCAGGGCCGGGAGTGGTCGG CTACCGCGCTGAAGCGATCGATGATCTCCGAGGCGATGCTCGGGTACGCGACTCTGAACG GTAAGACCGTCCGAGACGACGACGGAGCCCCGCTGGTGCGGGCTGAGCCGATCCTGACC CGTGAGCAGCTGGAGGCGCTGCGCGCCGAGCTCGTGAAGACCTCCCGGGCGAAGCCCGC GGTGTCTACCCCGTCGCTGCTGCTGCGGGTGTTGTTCTGTGCGGTGTGCGGGGAGCCCGC GTACAAGTTCGCCGGGGGAGGACGTAAGCACCCGCGCTACCGCTGCCGCTCGATGGGGTT CCCGAAGCACTGCGGGAACGGCACGGTGGCGATGGCCGAGTGGGACGCGTTCTGCGAGG AGCAGGTGCTGGATCTGCTCGGGGACGCGGAGCGTCTGGAGAAAGTCTGGGTAGCCGGCT CGGACTCCGCGGTCGAACTCGCGGAGGTGAACGCGGAGCTGGTGGACCTGACGTCGCTG ATCGGCTCCCCGGCCTACCGGGCCGGCTCTCCGCAGCGAGAAGCACTGGATGCCCGTATT GCGGCGCTGGCCGCGCGGCAAGAGGAGCTGGAGGGTCTAGAGGCTCGCCCGTCTGGCTG GGAGTGGCGCGAGACCGGGCAGCGGTTCGGGGACTGGTGGCGGGAGCAGGACACCGCG GCAAAGAACACCTGGCTTCGGTCGATGAACGTTCGGCTGACGTTCGACGTCCGCGGCGGG CTGACTCGCACGATCGACTTCGGGGATCTGCAGGAGTACGAGCAGCATCTCAGGCTCGGC AGCGTGGTCGAACGGCTACACACCGGGATGTCGAGGCCTGCAGCAAACGACGAAAACTAC GCTGCAGCAGTTTAGACACATGGCATGGATGAACTATACAAATAACCCGGGGGATCCCATG GTACGCGTGCTAGAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGT TTTATCTGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGCCCTAGACCTA G

pZS2katGp- RBS33- TP901-proD- oxyR	ATGACTAAGAAAGTAGCAATCTATACACGAGTATCCACTACTAACCAAGCAGAGGAAGGCTT CTCAATTGATGAGCAAATTGACCGTTTAACAAAATATGCTGAAGCAATGGGGTGGCAAGTAT CTGATACTTATACTGATGCTGGTTTTTCAGGGGCCAAACTTGAACGCCCAGCAATGCAAAGA TTAATCAACGATATCGAGAATAAAGCTTTTGATACAGTTCTTGTATATAAGCTAGACCGCCTT TCACGTAGTGTAAGAGATACTCTTTATCTTGTTAAGGATGTGTTCACAAAAAATAAAATAGAC TTTATCTCGCTTAATGAAAGTATTGATACTTCTTCTGCTATGGGTAGCTTGTTTCTCACTATTC TTTCTGCAATTAATGAGTTTGAAAGAGAGAATATAAAAGAACGCATGACTATGGGTAAACTAG GGCGAGCGAAATCTGGTAAGTCTATGATGTGGACTAAGACAGCTTTTGGGTATTACCACAAC AGAAAGACAGGTATATTAGAAATTGTTCCTTTACAAGCTACAATAGTTGAACAAATATTCACT GATTATTTATCAGGAATATCACTTACAAAATTAAGAGATAAACTCAATGAATCTGGACACATC GGTAAAGATATACCGTGGTCTTATCGTACCCTAAGACAAACACTTGATAATCCAGTTTACTGT GGTTATATCAAATTTAAGGACAGCCTATTTGAAGGTATGCACAAACCAATTATCCCTTATGAG ACTTATTTAAAAGTTCAAAAAGAGCTAGAAGAAAGACAACAGCAGACTTATGAAAGAAATAAC AACCCTAGACCTTTCCAAGCTAAATATATGCTGTCAGGGATGGCAAGGTGCGGTTACTGTG GAGCACCTTTAAAAATTGTTCTTGGCCACAAAAGAAAAGATGGAAGCCGCACTATGAAATAT CACTGTGCAAATAGATTTCCTCGAAAAACAAAAGGAATTACAGTATATAATGACAATAAAAAG TGTGATTCAGGAACTTATGATTTAAGTAATTTAGAAAATACTGTTATTGACAACCTGATTGGAT TTCAAGAAAATAATGACTCCTTATTGAAAATTATCAATGGCAACAACCAACCTATTCTTGATAC TTCGTCATTTAAAAAGCAAATTTCACAGATCGATAAAAAAATACAAAAGAACTCTGATTTGTAC CTAAATGATTTTATCACTATGGATGAGTTGAAAGATCGTACTGATTCCCTTCAGGCTGAGAAA AAGCTGCTTAAAGCTAAGATTAGCGAAAATAAATTTAATGACTCTACTGATGTTTTTGAGTTA GTTAAAACTCAGTTGGGCTCAATTCCGATTAATGAACTATCATATGATAATAAAAAGAAAATC GTCAACAACCTTGTATCAAAGGTTGATGTTACTGCTGATAATGTAGATATCATATTTAAATTC CAACTCGCTAGGCCTGCAGCAAACGACGAAAACTACGCTGCAGCAGTTTAGACACATGGCA TGGATGAACTATACAAATAACCCGGGGGATCCCATGGTACGCGTGCTAGAGGCATCAAATA AAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGC TCTCCTGAGTAGGACAAATCCGCCGCCCTAGACCTAGCACAGCTAACACCACGTCGTCCCT ATCTGCTGCCCTAGGTCTATGAGTGGTTGCTGGATAACTTTACGGGCATGCATAAGGCTCGT ATAATATATTCAGGGAGACCACAACGGTTTCCCTCTACAAATAATTTTGTTTAACTTTGAATTC TTCACACAGGAAACCGGTACCATGAATATTCGTGATCTTGAGTACCTGGTGGCATTGGCTGA ACACCGCCATTTTCGGCGTGCGGCAGATTCCTGCCACGTTAGCCAGCCGACGCTTAGCGG GCAAATTCGTAAGCTGGAAGATGAGCTGGGCGTGATGTTGCTGGAGCGGACCAGCCGTAA AGTGTTGTTCACCCAGGCGGGAATGCTGCTGGTGGATCAGGCGCGTACCGTGCTGCGTGA GGTGAAAGTCCTTAAAGAGATGGCAAGCCAGCAGGGCGAGACGATGTCCGGACCGCTGCA CATTGGTTTGATTCCCACAGTTGGACCGTACCTGCTACCGCATATTATCCCTATGCTGCACC AGACCTTTCCAAAGCTGGAAATGTATCTGCATGAAGCACAGACCCACCAGTTACTGGCGCA ACTGGACAGCGGCAAACTCGATTGCGTGATCCTCGCGCTGGTGAAAGAGAGCGAAGCATTC ATTGAAGTGCCGTTGTTTGATGAGCCAATGTTGCTGGCTATCTATGAAGATCACCCGTGGGC GAACCGCGAATGCGTACCGATGGCCGATCTGGCAGGGGAAAAACTGCTGATGCTGGAAGA TGGTCACTGTTTGCGCGATCAGGCAATGGGTTTCTGTTTTGAAGCCGGGGCGGATGAAGAT ACACACTTCCGCGCGACCAGCCTGGAAACTCTGCGCAACATGGTGGCGGCAGGTAGCGGG ATCACTTTACTGCCAGCGCTGGCTGTGCCGCCGGAGCGCAAACGCGATGGGGTTGTTTATC TGCCGTGCATTAAGCCGGAACCACGCCGCACTATTGGCCTGGTTTATCGTCCTGGCTCACC GCTGCGCAGCCGCTATGAGCAGCTGGCAGAGGCCATCCGCGCAAGAATGGATGGCCATTT CGATAAAGTTTTAAAACAGGCGGTTTAACCCGGGGGATCCCATGGTACGCGTGCTAGAGGC ATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCG GTGAACGCTCTCCTGAGTAGGACAAATCCGCCGCCCTAGACCTAGGGCCTAGGGTACGGG TTTTGCTGCCCGCAAACGGGCTGTTCTGGTGTTGCTAGTTTGTTATCAGAATCGCAGATCCG GCTTCAGGTTTGCCGGCTGAAAGCGCTATTTCTTCCAGAATTGCCATGATTTTTTCCCCACG GGAGGCGTCACTGGCTCCCGTGTTGTCGGCAGCTTTGATTCGATAAGCAGCATCGCCTGTT

	TCAGGCTGTCTATGTGTGACTGTTGAGCTGTAACAAGTTGTCTCAGGTGTTCAATTTCATGTT CTAGTTGCTTTGTTTTACTGGTTTCACCTGTTCTATTAGGTGTTACATGCTGTTCATCTGTTAC ATTGTCGATCTGTTCATGGTGAACAGCTTTAAATGCACCAAAAACTCGTAAAAGCTCTGATGT ATCTATCTTTTTTACACCGTTTTCATCTGTGCATATGGACAGTTTTCCCTTTGATATCTAACGG TGAACAGTTGTTCTACTTTTGTTTGTTAGTCTTGATGCTTCACTGATAGATACAAGAGCCATA AGAACCTCAGATCCTTCCGTATTTAGCCAGTATGTTCTCTAGTGTGGTTCGTTGTTTTTGCGT GAGCCATGAGAACGAACCATTGAGATCATGCTTACTTTGCATGTCACTCAAAAATTTTGCCT CAAAACTGGTGAGCTGAATTTTTGCAGTTAAAGCATCGTGTAGTGTTTTTCTTAGTCCGTTAC GTAGGTAGGAATCTGATGTAATGGTTGTTGGTATTTTGTCACCATTCATTTTTATCTGGTTGT TCTCAAGTTCGGTTACGAGATCCATTTGTCTATCTAGTTCAACTTGGAAAATCAACGTATCAG TCGGGCGGCCTCGCTTATCAACCACCAATTTCATATTGCTGTAAGTGTTTAAATCTTTACTTA TTGGTTTCAAAACCCATTGGTTAAGCCTTTTAAACTCATGGTAGTTATTTTCAAGCATTAACAT GAACTTAAATTCATCAAGGCTAATCTCTATATTTGCCTTGTGAGTTTTCTTTTGTGTTAGTTCT TTTAATAACCACTCATAAATCCTCATAGAGTATTTGTTTTCAAAAGACTTAACATGTTCCAGAT TATATTTTATGAATTTTTTTAACTGGAAAAGATAAGGCAATATCTCTTCACTAAAAACTAATTCT AATTTTTCGCTTGAGAACTTGGCATAGTTTGTCCACTGGAAAATCTCAAAGCCTTTAACCAAA GGATTCCTGATTTCCACAGTTCTCGTCATCAGCTCTCTGGTTGCTTTAGCTAATACACCATAA GCATTTTCCCTACTGATGTTCATCATCTGAGCGTATTGGTTATAAGTGAACGATACCGTCCGT TCTTTCCTTGTAGGGTTTTCAATCGTGGGGTTGAGTAGTGCCACACAGCATAAAATTAGCTT GGTTTCATGCTCCGTTAAGTCATAGCGACTAATCGCTAGTTCATTTGCTTTGAAAACAACTAA TTCAGACATACATCTCAATTGGTCTAGGTGATTTTAATCACTATACCAATTGAGATGGGCTAG TCAATGATAATTACTAGTCCTTTTCCTTTGAGTTGTGGGTATCTGTAAATTCTGCTAGACCTTT GCTGGAAAACTTGTAAATTCTGCTAGACCCTCTGTAAATTCCGCTAGACCTTTGTGTGTTTTT TTTGTTTATATTCAAGTGGTTATAATTTATAGAATAAAGAAAGAATAAAAAAAGATAAAAAGAA TAGATCCCAGCCCTGTGTATAACTCACTACTTTAGTCAGTTCCGCAGTATTACAAAAGGATGT CGCAAACGCTGTTTGCTCCTCTACAAAACAGACCTTAAAACCCTAAAGGCTTAAGTAGCACC CTCGCAAGCTCGGGCAAATCGCTGAATATTCCTTTTGTCTCCGACCATCAGGCACCTGAGTC GCTGTCTTTTTCGTGACATTCAGTTCGCTGCGCTCACGGCTCTGGCAGTGAATGGGGGTAA ATGGCACTACAGGCGCCTTTTATGGATTCATGCAAGGAAACTACCCATAATACAAGAAAAGC CCGTCACGGGCTTCTCAGGGCGTTTTATGGCGGGTCTGCTATGTGGTGCTATCTGACTTTTT GCTGTTCAGCAGTTCCTGCCCTCTGATTTTCCAGTCTGACCACTTCGGATTATCCCGTGACA GGTCATTCAGACTGGCTAATGCACCCAGTAAGGCAGCGGTATCATCAACAGGCTTACCCGT CTTACTGTCCCTAGTGCTTGGATTCTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTT CTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGATCTATCAACAGGAGTCCAAGCGA GCTCTCGAACCCCAGAGTCCCGCTCAGAAGAACTCGTCAAGAAGGCGATAGAAGGCGATG CGCTGCGAATCGGGAGCGGCGATACCGTAAAGCACGAGGAAGCGGTCAGCCCATTCGCCG CCAAGCTCTTCAGCAATATCACGGGTAGCCAACGCTATGTCCTGATAGCGGTCCGCCACAC CCAGCCGGCCACAGTCGATGAATCCAGAAAAGCGGCCATTTTCCACCATGATATTCGGCAA GCAGGCATCGCCATGGGTCACGACGAGATCCTCGCCGTCGGGCATGCGCGCCTTGAGCCT GGCGAACAGTTCGGCTGGCGCGAGCCCCTGATGCTCTTCGTCCAGATCATCCTGATCGACA AGACCGGCTTCCATCCGAGTACGTGCTCGCTCGATGCGATGTTTCGCTTGGTGGTCGAATG GGCAGGTAGCCGGATCAAGCGTATGCAGCCGCCGCATTGCATCAGCCATGATGGATACTTT CTCGGCAGGAGCAAGGTGAGATGACAGGAGATCCTGCCCCGGCACTTCGCCCAATAGCAG CCAGTCCCTTCCCGCTTCAGTGACAACGTCGAGCACAGCTGCGCAAGGAACGCCCGTCGT GGCCAGCCACGATAGCCGCGCTGCCTCGTCCTGCAGTTCATTCAGGGCACCGGACAGGTC GGTCTTGACAAAAAGAACCGGGCGCCCCTGCGCTGACAGCCGGAACACGGCGGCATCAGA GCAGCCGATTGTCTGTTGTGCCCAGTCATAGCCGAATAGCCTCTCCACCCAAGCGGCCGGA GAACCTGCGTGCAATCCATCTTGTTCAATCATGCGAAACGATCCTCATCCTGTCTCTTGATC AGATCTTGATCCCCTGCGCCATCAGATCCTTGGCGGCAAGAAAGCCATCCAGTTTACTTTGC AGGGCTTCCCAACCTTACCAGAGGGCGCCCCAGCTGGCAATTCCGACGTCTGTGGCTTTTA

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pZS2katGp-RBS31-phiC31-proD-oxyR	ATGACACAAGGGGTTGTGACCGGGGTGGACACGTACGCGGGTGCTTACGACCGTCAGTCG CGCGAGCGCGAGAATTCGAGCGCAGCAAGCCCAGCGACACAGCGTAGCGCCAACGAAGA CAAGGCGGCCGACCTTCAGCGCGAAGTCGAGCGCGACGGGGGCCGGTTCAGGTTCGTCG GGCATTTCAGCGAAGCGCCGGGCACGTCGGCGTTCGGGACGGCGGAGCGCCCGGAGTTC GAACGCATCCTGAACGAATGCCGCGCCGGGCGGCTCAACATGATCATTGTCTATGACGTGT CGCGCTTCTCGCGCCTGAAGGTCATGGACGCGATTCCGATTGTCTCGGAATTGCTCGCCCT GGGCGTGACGATTGTTTCCACTCAGGAAGGCGTCTTCCGGCAGGGAAACGTCATGGACCT GATTCACCTGATTATGCGGCTCGACGCGTCGCACAAAGAATCTTCGCTGAAGTCGGCGAAG ATTCTCGACACGAAGAACCTTCAGCGCGAATTGGGCGGGTACGTCGGCGGGAAGGCGCCT TACGGCTTCGAGCTTGTTTCGGAGACGAAGGAGATCACGCGCAACGGCCGAATGGTCAATG TCGTCATCAACAAGCTTGCGCACTCGACCACTCCCCTTACCGGACCCTTCGAGTTCGAGCC CGACGTAATCCGGTGGTGGTGGCGTGAGATCAAGACGCACAAACACCTTCCCTTCAAGCCG GGCAGTCAAGCCGCCATTCACCCGGGCAGCATCACGGGGCTTTGTAAGCGCATGGACGCT GACGCCGTGCCGACCCGGGGCGAGACGATTGGGAAGAAGACCGCTTCAAGCGCCTGGGA CCCGGCAACCGTTATGCGAATCCTTCGGGACCCGCGTATTGCGGGCTTCGCCGCTGAGGT GATCTACAAGAAGAAGCCGGACGGCACGCCGACCACGAAGATTGAGGGTTACCGCATTCA GCGCGACCCGATCACGCTCCGGCCGGTCGAGCTTGATTGCGGACCGATCATCGAGCCCGC TGAGTGGTATGAGCTTCAGGCGTGGTTGGACGGCAGGGGGCGCGGCAAGGGGCTTTCCC GGGGGCAAGCCATTCTGTCCGCCATGGACAAGCTGTACTGCGAGTGTGGCGCCGTCATGA CTTCGAAGCGCGGGGAAGAATCGATCAAGGACTCTTACCGCTGCCGTCGCCGGAAGGTGG TCGACCCGTCCGCACCTGGGCAGCACGAAGGCACGTGCAACGTCAGCATGGCGGCACTCG ACAAGTTCGTTGCGGAACGCATCTTCAACAAGATCAGGCACGCCGAAGGCGACGAAGAGAC GTTGGCGCTTCTGTGGGAAGCCGCCCGACGCTTCGGCAAGCTCACTGAGGCGCCTGAGAA GAGCGGCGAACGGGCGAACCTTGTTGCGGAGCGCGCCGACGCCCTGAACGCCCTTGAAG AGCTGTACGAAGACCGCGCGGCAGGCGCGTACGACGGACCCGTTGGCAGGAAGCACTTCC GGAAGCAACAGGCAGCGCTGACGCTCCGGCAGCAAGGGGCGGAAGAGCGGCTTGCCGAA CTTGAAGCCGCCGAAGCCCCGAAGCTTCCCCTTGACCAATGGTTCCCCGAAGACGCCGAC GCTGACCCGACCGGCCCTAAGTCGTGGTGGGGGCGCGCGTCAGTAGACGACAAGCGCGT GTTCGTCGGGCTCTTCGTAGACAAGATCGTTGTCACGAAGTCGACTACGGGCAGGGGGCA GGGAACGCCCATCGAGAAGCGCGCTTCGATCACGTGGGCGAAGCCGCCGACCGACGACG ACGAAGACGACGCCCAGGACGGCACGGAAGACGTAGCGGCGAGGCCTGCAGCAAACGAC GAAAACTACGCTGCAGCAGTTTAGACACATGGCATGGATGAACTATACAAATAACCCGGGG GATCCCATGGTACGCGTGCTAGAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGG GCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGCC CTAGACCTAGCACAGCTAACACCACGTCGTCCCTATCTGCTGCCCTAGGTCTATGAGTGGTT GCTGGATAACTTTACGGGCATGCATAAGGCTCGTATAATATATTCAGGGAGACCACAACGGT TTCCCTCTACAAATAATTTTGTTTAACTTTGAATTCTTCACACAGGAAACCGGTACCATGAATA TTCGTGATCTTGAGTACCTGGTGGCATTGGCTGAACACCGCCATTTTCGGCGTGCGGCAGA TTCCTGCCACGTTAGCCAGCCGACGCTTAGCGGGCAAATTCGTAAGCTGGAAGATGAGCTG GGCGTGATGTTGCTGGAGCGGACCAGCCGTAAAGTGTTGTTCACCCAGGCGGGAATGCTG CTGGTGGATCAGGCGCGTACCGTGCTGCGTGAGGTGAAAGTCCTTAAAGAGATGGCAAGC CAGCAGGGCGAGACGATGTCCGGACCGCTGCACATTGGTTTGATTCCCACAGTTGGACCGT ACCTGCTACCGCATATTATCCCTATGCTGCACCAGACCTTTCCAAAGCTGGAAATGTATCTG CATGAAGCACAGACCCACCAGTTACTGGCGCAACTGGACAGCGGCAAACTCGATTGCGTGA TCCTCGCGCTGGTGAAAGAGAGCGAAGCATTCATTGAAGTGCCGTTGTTTGATGAGCCAAT GTTGCTGGCTATCTATGAAGATCACCCGTGGGCGAACCGCGAATGCGTACCGATGGCCGAT CTGGCAGGGGAAAAACTGCTGATGCTGGAAGATGGTCACTGTTTGCGCGATCAGGCAATGG GTTTCTGTTTTGAAGCCGGGGCGGATGAAGATACACACTTCCGCGCGACCAGCCTGGAAAC TCTGCGCAACATGGTGGCGGCAGGTAGCGGGATCACTTTACTGCCAGCGCTGGCTGTGCC GCCGGAGCGCAAACGCGATGGGGTTGTTTATCTGCCGTGCATTAAGCCGGAACCACGCCG

	CACTATTGGCCTGGTTTATCGTCCTGGCTCACCGCTGCGCAGCCGCTATGAGCAGCTGGCA GAGGCCATCCGCGCAAGAATGGATGGCCATTTCGATAAAGTTTTAAAACAGGCGGTTTAAC CCGGGGGATCCCATGGTACGCGTGCTAGAGGCATCAAATAAAACGAAAGGCTCAGTCGAAA GACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCC GCCGCCCTAGACCTAGGGCCTAGGGTACGGGTTTTGCTGCCCGCAAACGGGCTGTTCTGG TGTTGCTAGTTTGTTATCAGAATCGCAGATCCGGCTTCAGGTTTGCCGGCTGAAAGCGCTAT TTCTTCCAGAATTGCCATGATTTTTTCCCCACGGGAGGCGTCACTGGCTCCCGTGTTGTCGG CAGCTTTGATTCGATAAGCAGCATCGCCTGTTTCAGGCTGTCTATGTGTGACTGTTGAGCTG TAACAAGTTGTCTCAGGTGTTCAATTTCATGTTCTAGTTGCTTTGTTTTACTGGTTTCACCTGT TCTATTAGGTGTTACATGCTGTTCATCTGTTACATTGTCGATCTGTTCATGGTGAACAGCTTT AAATGCACCAAAAACTCGTAAAAGCTCTGATGTATCTATCTTTTTTACACCGTTTTCATCTGT GCATATGGACAGTTTTCCCTTTGATATCTAACGGTGAACAGTTGTTCTACTTTTGTTTGTTAG TCTTGATGCTTCACTGATAGATACAAGAGCCATAAGAACCTCAGATCCTTCCGTATTTAGCCA GTATGTTCTCTAGTGTGGTTCGTTGTTTTTGCGTGAGCCATGAGAACGAACCATTGAGATCA TGCTTACTTTGCATGTCACTCAAAAATTTTGCCTCAAAACTGGTGAGCTGAATTTTTGCAGTT AAAGCATCGTGTAGTGTTTTTCTTAGTCCGTTACGTAGGTAGGAATCTGATGTAATGGTTGTT GGTATTTTGTCACCATTCATTTTTATCTGGTTGTTCTCAAGTTCGGTTACGAGATCCATTTGT CTATCTAGTTCAACTTGGAAAATCAACGTATCAGTCGGGCGGCCTCGCTTATCAACCACCAA TTTCATATTGCTGTAAGTGTTTAAATCTTTACTTATTGGTTTCAAAACCCATTGGTTAAGCCTT TTAAACTCATGGTAGTTATTTTCAAGCATTAACATGAACTTAAATTCATCAAGGCTAATCTCTA TATTTGCCTTGTGAGTTTTCTTTTGTGTTAGTTCTTTTAATAACCACTCATAAATCCTCATAGA GTATTTGTTTTCAAAAGACTTAACATGTTCCAGATTATATTTTATGAATTTTTTTAACTGGAAAA GATAAGGCAATATCTCTTCACTAAAAACTAATTCTAATTTTTCGCTTGAGAACTTGGCATAGTT TGTCCACTGGAAAATCTCAAAGCCTTTAACCAAAGGATTCCTGATTTCCACAGTTCTCGTCAT CAGCTCTCTGGTTGCTTTAGCTAATACACCATAAGCATTTTCCCTACTGATGTTCATCATCTG AGCGTATTGGTTATAAGTGAACGATACCGTCCGTTCTTTCCTTGTAGGGTTTTCAATCGTGG GGTTGAGTAGTGCCACACAGCATAAAATTAGCTTGGTTTCATGCTCCGTTAAGTCATAGCGA CTAATCGCTAGTTCATTTGCTTTGAAAACAACTAATTCAGACATACATCTCAATTGGTCTAGG TGATTTTAATCACTATACCAATTGAGATGGGCTAGTCAATGATAATTACTAGTCCTTTTCCTTT GAGTTGTGGGTATCTGTAAATTCTGCTAGACCTTTGCTGGAAAACTTGTAAATTCTGCTAGAC CCTCTGTAAATTCCGCTAGACCTTTGTGTGTTTTTTTTGTTTATATTCAAGTGGTTATAATTTA TAGAATAAAGAAAGAATAAAAAAAGATAAAAAGAATAGATCCCAGCCCTGTGTATAACTCACT ACTTTAGTCAGTTCCGCAGTATTACAAAAGGATGTCGCAAACGCTGTTTGCTCCTCTACAAA ACAGACCTTAAAACCCTAAAGGCTTAAGTAGCACCCTCGCAAGCTCGGGCAAATCGCTGAA TATTCCTTTTGTCTCCGACCATCAGGCACCTGAGTCGCTGTCTTTTTCGTGACATTCAGTTCG CTGCGCTCACGGCTCTGGCAGTGAATGGGGGTAAATGGCACTACAGGCGCCTTTTATGGAT TCATGCAAGGAAACTACCCATAATACAAGAAAAGCCCGTCACGGGCTTCTCAGGGCGTTTTA TGGCGGGTCTGCTATGTGGTGCTATCTGACTTTTTGCTGTTCAGCAGTTCCTGCCCTCTGAT TTTCCAGTCTGACCACTTCGGATTATCCCGTGACAGGTCATTCAGACTGGCTAATGCACCCA GTAAGGCAGCGGTATCATCAACAGGCTTACCCGTCTTACTGTCCCTAGTGCTTGGATTCTCA CCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGG TCATTACTGGATCTATCAACAGGAGTCCAAGCGAGCTCTCGAACCCCAGAGTCCCGCTCAG AAGAACTCGTCAAGAAGGCGATAGAAGGCGATGCGCTGCGAATCGGGAGCGGCGATACCG TAAAGCACGAGGAAGCGGTCAGCCCATTCGCCGCCAAGCTCTTCAGCAATATCACGGGTAG CCAACGCTATGTCCTGATAGCGGTCCGCCACACCCAGCCGGCCACAGTCGATGAATCCAGA AAAGCGGCCATTTTCCACCATGATATTCGGCAAGCAGGCATCGCCATGGGTCACGACGAGA TCCTCGCCGTCGGGCATGCGCGCCTTGAGCCTGGCGAACAGTTCGGCTGGCGCGAGCCC CTGATGCTCTTCGTCCAGATCATCCTGATCGACAAGACCGGCTTCCATCCGAGTACGTGCT CGCTCGATGCGATGTTTCGCTTGGTGGTCGAATGGGCAGGTAGCCGGATCAAGCGTATGCA GCCGCCGCATTGCATCAGCCATGATGGATACTTTCTCGGCAGGAGCAAGGTGAGATGACAG

	GAGATCCTGCCCCGGCACTTCGCCCAATAGCAGCCAGTCCCTTCCCGCTTCAGTGACAACG TCGAGCACAGCTGCGCAAGGAACGCCCGTCGTGGCCAGCCACGATAGCCGCGCTGCCTCG TCCTGCAGTTCATTCAGGGCACCGGACAGGTCGGTCTTGACAAAAAGAACCGGGCGCCCCT GCGCTGACAGCCGGAACACGGCGGCATCAGAGCAGCCGATTGTCTGTTGTGCCCAGTCAT AGCCGAATAGCCTCTCCACCCAAGCGGCCGGAGAACCTGCGTGCAATCCATCTTGTTCAAT CATGCGAAACGATCCTCATCCTGTCTCTTGATCAGATCTTGATCCCCTGCGCCATCAGATCC TTGGCGGCAAGAAAGCCATCCAGTTTACTTTGCAGGGCTTCCCAACCTTACCAGAGGGCGC CCCAGCTGGCAATTCCGACGTCTGTGGCTTTTATGAAAATCACACAGTGATCACAAATTTTA AACAGAGCACAAAATGCTGCCTCGAAATGAGGGCGGGAAAATAAGGTTATCAGCCTTGTTTT CTCCCTCATTACTTGAAGGATATGAAGCTAAAACCCTTTTTTATAAAGCATTTGTCCGAATTC GGACATAATCAAAAAAGCTTAATTAAGATCAATTTGATCTACATCTCTTTAACCAACAATATGT AAGATCTCAACTATCGCATCCGTGGATTAATTCAATTATAACTTCTCTCTAACGCTGTGTATC GTAACGGTAACACTGTAGAGGGGAGCACATTGATGCGAATTCTCACACAGGAAACCGGTAC C

pZS1oxySp*-RBS30-bxbi-katGp-RBS31-PhiC-proD-oxyR	GACGTCTGTGGCTTTTATGAAAATCACACAGTGATCACAAATTTTAAACAGAGCACAAAATGC TGCCTCGAAATGAGGGCGGGAAAATAAGGTTATCAGCCTTGTTTTCTCCCTCATTACTTGAA GGATATGAAGCTAAAACCCTTTTTTATAAAGCATTTGTCCGAATTCGGACATAATCAAAAAAG CTTAATTAAGATCAATTTGATCTACATCTCTTTAACCAACAATATGTAAGATCTCAACTATCGC ATCCGTGGATTAATTCAATTATAACTTCTCTCTAACGCTGTGTATCGTAACGGTAACACTGTA GAGGGGAGCACATTGATGCGAATTCTCACACAGGAAACCGGTACCATGACACAAGGGGTTG TGACCGGGGTGGACACGTACGCGGGTGCTTACGACCGTCAGTCGCGCGAGCGCGAGAATT CGAGCGCAGCAAGCCCAGCGACACAGCGTAGCGCCAACGAAGACAAGGCGGCCGACCTT CAGCGCGAAGTCGAGCGCGACGGGGGCCGGTTCAGGTTCGTCGGGCATTTCAGCGAAGC GCCGGGCACGTCGGCGTTCGGGACGGCGGAGCGCCCGGAGTTCGAACGCATCCTGAACG AATGCCGCGCCGGGCGGCTCAACATGATCATTGTCTATGACGTGTCGCGCTTCTCGCGCCT GAAGGTCATGGACGCGATTCCGATTGTCTCGGAATTGCTCGCCCTGGGCGTGACGATTGTT TCCACTCAGGAAGGCGTCTTCCGGCAGGGAAACGTCATGGACCTGATTCACCTGATTATGC GGCTCGACGCGTCGCACAAAGAATCTTCGCTGAAGTCGGCGAAGATTCTCGACACGAAGAA CCTTCAGCGCGAATTGGGCGGGTACGTCGGCGGGAAGGCGCCTTACGGCTTCGAGCTTGT TTCGGAGACGAAGGAGATCACGCGCAACGGCCGAATGGTCAATGTCGTCATCAACAAGCTT GCGCACTCGACCACTCCCCTTACCGGACCCTTCGAGTTCGAGCCCGACGTAATCCGGTGGT GGTGGCGTGAGATCAAGACGCACAAACACCTTCCCTTCAAGCCGGGCAGTCAAGCCGCCA TTCACCCGGGCAGCATCACGGGGCTTTGTAAGCGCATGGACGCTGACGCCGTGCCGACCC GGGGCGAGACGATTGGGAAGAAGACCGCTTCAAGCGCCTGGGACCCGGCAACCGTTATGC GAATCCTTCGGGACCCGCGTATTGCGGGCTTCGCCGCTGAGGTGATCTACAAGAAGAAGC CGGACGGCACGCCGACCACGAAGATTGAGGGTTACCGCATTCAGCGCGACCCGATCACGC TCCGGCCGGTCGAGCTTGATTGCGGACCGATCATCGAGCCCGCTGAGTGGTATGAGCTTC AGGCGTGGTTGGACGGCAGGGGGCGCGGCAAGGGGCTTTCCCGGGGGCAAGCCATTCTG TCCGCCATGGACAAGCTGTACTGCGAGTGTGGCGCCGTCATGACTTCGAAGCGCGGGGAA GAATCGATCAAGGACTCTTACCGCTGCCGTCGCCGGAAGGTGGTCGACCCGTCCGCACCT GGGCAGCACGAAGGCACGTGCAACGTCAGCATGGCGGCACTCGACAAGTTCGTTGCGGAA CGCATCTTCAACAAGATCAGGCACGCCGAAGGCGACGAAGAGACGTTGGCGCTTCTGTGG GAAGCCGCCCGACGCTTCGGCAAGCTCACTGAGGCGCCTGAGAAGAGCGGCGAACGGGC GAACCTTGTTGCGGAGCGCGCCGACGCCCTGAACGCCCTTGAAGAGCTGTACGAAGACCG CGCGGCAGGCGCGTACGACGGACCCGTTGGCAGGAAGCACTTCCGGAAGCAACAGGCAG CGCTGACGCTCCGGCAGCAAGGGGCGGAAGAGCGGCTTGCCGAACTTGAAGCCGCCGAA GCCCCGAAGCTTCCCCTTGACCAATGGTTCCCCGAAGACGCCGACGCTGACCCGACCGGC CCTAAGTCGTGGTGGGGGCGCGCGTCAGTAGACGACAAGCGCGTGTTCGTCGGGCTCTTC GTAGACAAGATCGTTGTCACGAAGTCGACTACGGGCAGGGGGCAGGGAACGCCCATCGAG AAGCGCGCTTCGATCACGTGGGCGAAGCCGCCGACCGACGACGACGAAGACGACGCCCA GGACGGCACGGAAGACGTAGCGGCGAGGCCTGCAGCAAACGACGAAAACTACGCTGCAG CAGTTTAGACACATGGCATGGATGAACTATACAAATAACCCGGGGGATCCCATGGTACGCG TGCTAGAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTG TTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGCCCTAGACCTAGCACAGC TAACACCACGTCGTCCCTATCTGCTGCCCTAGGTCTATGAGTGGTTGCTGGATAACTTTACG GGCATGCATAAGGCTCGTATAATATATTCAGGGAGACCACAACGGTTTCCCTCTACAAATAA TTTTGTTTAACTTTGAATTCTTCACACAGGAAACCGGTACCATGAATATTCGTGATCTTGAGT ACCTGGTGGCATTGGCTGAACACCGCCATTTTCGGCGTGCGGCAGATTCCTGCCACGTTAG CCAGCCGACGCTTAGCGGGCAAATTCGTAAGCTGGAAGATGAGCTGGGCGTGATGTTGCT GGAGCGGACCAGCCGTAAAGTGTTGTTCACCCAGGCGGGAATGCTGCTGGTGGATCAGGC GCGTACCGTGCTGCGTGAGGTGAAAGTCCTTAAAGAGATGGCAAGCCAGCAGGGCGAGAC GATGTCCGGACCGCTGCACATTGGTTTGATTCCCACAGTTGGACCGTACCTGCTACCGCAT ATTATCCCTATGCTGCACCAGACCTTTCCAAAGCTGGAAATGTATCTGCATGAAGCACAGAC CCACCAGTTACTGGCGCAACTGGACAGCGGCAAACTCGATTGCGTGATCCTCGCGCTGGT

	GAAAGAGAGCGAAGCATTCATTGAAGTGCCGTTGTTTGATGAGCCAATGTTGCTGGCTATCT ATGAAGATCACCCGTGGGCGAACCGCGAATGCGTACCGATGGCCGATCTGGCAGGGGAAA AACTGCTGATGCTGGAAGATGGTCACTGTTTGCGCGATCAGGCAATGGGTTTCTGTTTTGAA GCCGGGGCGGATGAAGATACACACTTCCGCGCGACCAGCCTGGAAACTCTGCGCAACATG GTGGCGGCAGGTAGCGGGATCACTTTACTGCCAGCGCTGGCTGTGCCGCCGGAGCGCAAA CGCGATGGGGTTGTTTATCTGCCGTGCATTAAGCCGGAACCACGCCGCACTATTGGCCTGG TTTATCGTCCTGGCTCACCGCTGCGCAGCCGCTATGAGCAGCTGGCAGAGGCCATCCGCG CAAGAATGGATGGCCATTTCGATAAAGTTTTAAAACAGGCGGTTTAACCCGGGGGATCCCAT GGTACGCGTGCTAGAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCG TTTTATCTGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGCCCTAGACCT AGGGCCTAGGGTACGGGTTTTGCTGCCCGCAAACGGGCTGTTCTGGTGTTGCTAGTTTGTT ATCAGAATCGCAGATCCGGCTTCAGGTTTGCCGGCTGAAAGCGCTATTTCTTCCAGAATTGC CATGATTTTTTCCCCACGGGAGGCGTCACTGGCTCCCGTGTTGTCGGCAGCTTTGATTCGA TAAGCAGCATCGCCTGTTTCAGGCTGTCTATGTGTGACTGTTGAGCTGTAACAAGTTGTCTC AGGTGTTCAATTTCATGTTCTAGTTGCTTTGTTTTACTGGTTTCACCTGTTCTATTAGGTGTTA CATGCTGTTCATCTGTTACATTGTCGATCTGTTCATGGTGAACAGCTTTAAATGCACCAAAAA CTCGTAAAAGCTCTGATGTATCTATCTTTTTTACACCGTTTTCATCTGTGCATATGGACAGTTT TCCCTTTGATATCTAACGGTGAACAGTTGTTCTACTTTTGTTTGTTAGTCTTGATGCTTCACT GATAGATACAAGAGCCATAAGAACCTCAGATCCTTCCGTATTTAGCCAGTATGTTCTCTAGT GTGGTTCGTTGTTTTTGCGTGAGCCATGAGAACGAACCATTGAGATCATGCTTACTTTGCAT GTCACTCAAAAATTTTGCCTCAAAACTGGTGAGCTGAATTTTTGCAGTTAAAGCATCGTGTAG TGTTTTTCTTAGTCCGTTACGTAGGTAGGAATCTGATGTAATGGTTGTTGGTATTTTGTCACC ATTCATTTTTATCTGGTTGTTCTCAAGTTCGGTTACGAGATCCATTTGTCTATCTAGTTCAACT TGGAAAATCAACGTATCAGTCGGGCGGCCTCGCTTATCAACCACCAATTTCATATTGCTGTA AGTGTTTAAATCTTTACTTATTGGTTTCAAAACCCATTGGTTAAGCCTTTTAAACTCATGGTAG TTATTTTCAAGCATTAACATGAACTTAAATTCATCAAGGCTAATCTCTATATTTGCCTTGTGAG TTTTCTTTTGTGTTAGTTCTTTTAATAACCACTCATAAATCCTCATAGAGTATTTGTTTTCAAAA GACTTAACATGTTCCAGATTATATTTTATGAATTTTTTTAACTGGAAAAGATAAGGCAATATCT CTTCACTAAAAACTAATTCTAATTTTTCGCTTGAGAACTTGGCATAGTTTGTCCACTGGAAAA TCTCAAAGCCTTTAACCAAAGGATTCCTGATTTCCACAGTTCTCGTCATCAGCTCTCTGGTTG CTTTAGCTAATACACCATAAGCATTTTCCCTACTGATGTTCATCATCTGAGCGTATTGGTTAT AAGTGAACGATACCGTCCGTTCTTTCCTTGTAGGGTTTTCAATCGTGGGGTTGAGTAGTGCC ACACAGCATAAAATTAGCTTGGTTTCATGCTCCGTTAAGTCATAGCGACTAATCGCTAGTTCA TTTGCTTTGAAAACAACTAATTCAGACATACATCTCAATTGGTCTAGGTGATTTTAATCACTAT ACCAATTGAGATGGGCTAGTCAATGATAATTACTAGTCCTTTTCCTTTGAGTTGTGGGTATCT GTAAATTCTGCTAGACCTTTGCTGGAAAACTTGTAAATTCTGCTAGACCCTCTGTAAATTCCG CTAGACCTTTGTGTGTTTTTTTTGTTTATATTCAAGTGGTTATAATTTATAGAATAAAGAAAGA ATAAAAAAAGATAAAAAGAATAGATCCCAGCCCTGTGTATAACTCACTACTTTAGTCAGTTCC GCAGTATTACAAAAGGATGTCGCAAACGCTGTTTGCTCCTCTACAAAACAGACCTTAAAACC CTAAAGGCTTAAGTAGCACCCTCGCAAGCTCGGGCAAATCGCTGAATATTCCTTTTGTCTCC GACCATCAGGCACCTGAGTCGCTGTCTTTTTCGTGACATTCAGTTCGCTGCGCTCACGGCT CTGGCAGTGAATGGGGGTAAATGGCACTACAGGCGCCTTTTATGGATTCATGCAAGGAAAC TACCCATAATACAAGAAAAGCCCGTCACGGGCTTCTCAGGGCGTTTTATGGCGGGTCTGCT ATGTGGTGCTATCTGACTTTTTGCTGTTCAGCAGTTCCTGCCCTCTGATTTTCCAGTCTGACC ACTTCGGATTATCCCGTGACAGGTCATTCAGACTGGCTAATGCACCCAGTAAGGCAGCGGT ATCATCAACAGGCTTACCCGTCTTACTGTCCCTAGTGCTTGGATTCTCACCAATAAAAAACG CCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGATC TATCAACAGGAGTCCAAGCGAGCTCGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGT GAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGT GTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGA

	GACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAG CGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGC TAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCG TGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCG AGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTG TCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTT ACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTG AGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCG CCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTC AAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTT CAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCA AAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTAT TGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAAT AAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTTCATTATCCA TCCTCCATCGCCACGATAGTTCATGGCGATAGGTAGAATAGCAATGAACGATTATCCCTATC AAGCATTCTGACTGAGCATTGCTCACACGAATTCATTAAAGAGGAGAAAGGTACCATGAGAG CCCTGGTAGTCATCCGCCTGTCCCGCGTCACCGATGCTACGACTTCACCGGAGCGTCAGCT GGAGTCTTGCCAGCAGCTCTGCGCCCAGCGCGGCTGGGACGTCGTCGGGGTAGCGGAGG ATCTGGACGTCTCCGGGGCGGTCGATCCGTTCGACCGGAAGCGCAGACCGAACCTGGCCC GGTGGCTAGCGTTCGAGGAGCAACCGTTCGACGTGATCGTGGCGTACCGGGTAGACCGGT TGACCCGATCGATCCGGCATCTGCAGCAGCTGGTCCACTGGGCCGAGGACCACAAGAAGC TGGTCGTCTCCGCGACCGAAGCGCACTTCGATACGACGACGCCGTTTGCGGCGGTCGTCA TCGCGCTTATGGGAACGGTGGCGCAGATGGAATTAGAAGCGATCAAAGAGCGGAACCGTT CGGCTGCGCATTTCAATATCCGCGCCGGGAAATACCGAGGATCCCTGCCGCCGTGGGGAT ACCTGCCTACGCGCGTGGACGGGGAGTGGCGGCTGGTGCCGGACCCTGTGCAGCGAGAG CGCATCCTCGAGGTGTATCACCGCGTCGTCGACAACCACGAGCCGCTGCACCTGGTGGCC CACGACCTGAACCGGCGTGGTGTCCTGTCGCCGAAGGACTACTTCGCGCAGCTGCAAGGC CGCGAGCCGCAGGGCCGGGAGTGGTCGGCTACCGCGCTGAAGCGATCGATGATCTCCGA GGCGATGCTCGGGTACGCGACTCTGAACGGTAAGACCGTCCGAGACGACGACGGAGCCCC GCTGGTGCGGGCTGAGCCGATCCTGACCCGTGAGCAGCTGGAGGCGCTGCGCGCCGAGC TCGTGAAGACCTCCCGGGCGAAGCCCGCGGTGTCTACCCCGTCGCTGCTGCTGCGGGTGT TGTTCTGTGCGGTGTGCGGGGAGCCCGCGTACAAGTTCGCCGGGGGAGGACGTAAGCACC CGCGCTACCGCTGCCGCTCGATGGGGTTCCCGAAGCACTGCGGGAACGGCACGGTGGCG ATGGCCGAGTGGGACGCGTTCTGCGAGGAGCAGGTGCTGGATCTGCTCGGGGACGCGGA GCGTCTGGAGAAAGTCTGGGTAGCCGGCTCGGACTCCGCGGTCGAACTCGCGGAGGTGAA CGCGGAGCTGGTGGACCTGACGTCGCTGATCGGCTCCCCGGCCTACCGGGCCGGCTCTC CGCAGCGAGAAGCACTGGATGCCCGTATTGCGGCGCTGGCCGCGCGGCAAGAGGAGCTG GAGGGTCTAGAGGCTCGCCCGTCTGGCTGGGAGTGGCGCGAGACCGGGCAGCGGTTCGG GGACTGGTGGCGGGAGCAGGACACCGCGGCAAAGAACACCTGGCTTCGGTCGATGAACGT TCGGCTGACGTTCGACGTCCGCGGCGGGCTGACTCGCACGATCGACTTCGGGGATCTGCA GGAGTACGAGCAGCATCTCAGGCTCGGCAGCGTGGTCGAACGGCTACACACCGGGATGTC GAGGCCTGCAGCAAACGACGAAAACTACGCTGCAGCAGTTTAGACACATGGCATGGATGAA CTATACAAATAACCCGGGGGATCCCATGGTACGCGTGCTAGAGGCATCAAATAAAACGAAA GGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCCTGA GTAGGACAAATCCGCCGCCCTAGA

pZS1katGp-RBS33-TP901-proD-oxyR	ATGACTAAGAAAGTAGCAATCTATACACGAGTATCCACTACTAACCAAGCAGAGGAAGGCTT CTCAATTGATGAGCAAATTGACCGTTTAACAAAATATGCTGAAGCAATGGGGTGGCAAGTAT CTGATACTTATACTGATGCTGGTTTTTCAGGGGCCAAACTTGAACGCCCAGCAATGCAAAGA TTAATCAACGATATCGAGAATAAAGCTTTTGATACAGTTCTTGTATATAAGCTAGACCGCCTT TCACGTAGTGTAAGAGATACTCTTTATCTTGTTAAGGATGTGTTCACAAAAAATAAAATAGAC TTTATCTCGCTTAATGAAAGTATTGATACTTCTTCTGCTATGGGTAGCTTGTTTCTCACTATTC TTTCTGCAATTAATGAGTTTGAAAGAGAGAATATAAAAGAACGCATGACTATGGGTAAACTAG GGCGAGCGAAATCTGGTAAGTCTATGATGTGGACTAAGACAGCTTTTGGGTATTACCACAAC AGAAAGACAGGTATATTAGAAATTGTTCCTTTACAAGCTACAATAGTTGAACAAATATTCACT GATTATTTATCAGGAATATCACTTACAAAATTAAGAGATAAACTCAATGAATCTGGACACATC GGTAAAGATATACCGTGGTCTTATCGTACCCTAAGACAAACACTTGATAATCCAGTTTACTGT GGTTATATCAAATTTAAGGACAGCCTATTTGAAGGTATGCACAAACCAATTATCCCTTATGAG ACTTATTTAAAAGTTCAAAAAGAGCTAGAAGAAAGACAACAGCAGACTTATGAAAGAAATAAC AACCCTAGACCTTTCCAAGCTAAATATATGCTGTCAGGGATGGCAAGGTGCGGTTACTGTG GAGCACCTTTAAAAATTGTTCTTGGCCACAAAAGAAAAGATGGAAGCCGCACTATGAAATAT CACTGTGCAAATAGATTTCCTCGAAAAACAAAAGGAATTACAGTATATAATGACAATAAAAAG TGTGATTCAGGAACTTATGATTTAAGTAATTTAGAAAATACTGTTATTGACAACCTGATTGGAT TTCAAGAAAATAATGACTCCTTATTGAAAATTATCAATGGCAACAACCAACCTATTCTTGATAC TTCGTCATTTAAAAAGCAAATTTCACAGATCGATAAAAAAATACAAAAGAACTCTGATTTGTAC CTAAATGATTTTATCACTATGGATGAGTTGAAAGATCGTACTGATTCCCTTCAGGCTGAGAAA AAGCTGCTTAAAGCTAAGATTAGCGAAAATAAATTTAATGACTCTACTGATGTTTTTGAGTTA GTTAAAACTCAGTTGGGCTCAATTCCGATTAATGAACTATCATATGATAATAAAAAGAAAATC GTCAACAACCTTGTATCAAAGGTTGATGTTACTGCTGATAATGTAGATATCATATTTAAATTC CAACTCGCTAGGCCTGCAGCAAACGACGAAAACTACGCTGCAGCAGTTTAGACACATGGCA TGGATGAACTATACAAATAACCCGGGGGATCCCATGGTACGCGTGCTAGAGGCATCAAATA AAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGC TCTCCTGAGTAGGACAAATCCGCCGCCCTAGACCTAGCACAGCTAACACCACGTCGTCCCT ATCTGCTGCCCTAGGTCTATGAGTGGTTGCTGGATAACTTTACGGGCATGCATAAGGCTCGT ATAATATATTCAGGGAGACCACAACGGTTTCCCTCTACAAATAATTTTGTTTAACTTTGAATTC TTCACACAGGAAACCGGTACCATGAATATTCGTGATCTTGAGTACCTGGTGGCATTGGCTGA ACACCGCCATTTTCGGCGTGCGGCAGATTCCTGCCACGTTAGCCAGCCGACGCTTAGCGG GCAAATTCGTAAGCTGGAAGATGAGCTGGGCGTGATGTTGCTGGAGCGGACCAGCCGTAA AGTGTTGTTCACCCAGGCGGGAATGCTGCTGGTGGATCAGGCGCGTACCGTGCTGCGTGA GGTGAAAGTCCTTAAAGAGATGGCAAGCCAGCAGGGCGAGACGATGTCCGGACCGCTGCA CATTGGTTTGATTCCCACAGTTGGACCGTACCTGCTACCGCATATTATCCCTATGCTGCACC AGACCTTTCCAAAGCTGGAAATGTATCTGCATGAAGCACAGACCCACCAGTTACTGGCGCA ACTGGACAGCGGCAAACTCGATTGCGTGATCCTCGCGCTGGTGAAAGAGAGCGAAGCATTC ATTGAAGTGCCGTTGTTTGATGAGCCAATGTTGCTGGCTATCTATGAAGATCACCCGTGGGC GAACCGCGAATGCGTACCGATGGCCGATCTGGCAGGGGAAAAACTGCTGATGCTGGAAGA TGGTCACTGTTTGCGCGATCAGGCAATGGGTTTCTGTTTTGAAGCCGGGGCGGATGAAGAT ACACACTTCCGCGCGACCAGCCTGGAAACTCTGCGCAACATGGTGGCGGCAGGTAGCGGG ATCACTTTACTGCCAGCGCTGGCTGTGCCGCCGGAGCGCAAACGCGATGGGGTTGTTTATC TGCCGTGCATTAAGCCGGAACCACGCCGCACTATTGGCCTGGTTTATCGTCCTGGCTCACC GCTGCGCAGCCGCTATGAGCAGCTGGCAGAGGCCATCCGCGCAAGAATGGATGGCCATTT CGATAAAGTTTTAAAACAGGCGGTTTAACCCGGGGGATCCCATGGTACGCGTGCTAGAGGC ATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCG GTGAACGCTCTCCTGAGTAGGACAAATCCGCCGCCCTAGACCTAGGGCCTAGGGTACGGG TTTTGCTGCCCGCAAACGGGCTGTTCTGGTGTTGCTAGTTTGTTATCAGAATCGCAGATCCG GCTTCAGGTTTGCCGGCTGAAAGCGCTATTTCTTCCAGAATTGCCATGATTTTTTCCCCACG GGAGGCGTCACTGGCTCCCGTGTTGTCGGCAGCTTTGATTCGATAAGCAGCATCGCCTGTT

	TCAGGCTGTCTATGTGTGACTGTTGAGCTGTAACAAGTTGTCTCAGGTGTTCAATTTCATGTT CTAGTTGCTTTGTTTTACTGGTTTCACCTGTTCTATTAGGTGTTACATGCTGTTCATCTGTTAC ATTGTCGATCTGTTCATGGTGAACAGCTTTAAATGCACCAAAAACTCGTAAAAGCTCTGATGT ATCTATCTTTTTTACACCGTTTTCATCTGTGCATATGGACAGTTTTCCCTTTGATATCTAACGG TGAACAGTTGTTCTACTTTTGTTTGTTAGTCTTGATGCTTCACTGATAGATACAAGAGCCATA AGAACCTCAGATCCTTCCGTATTTAGCCAGTATGTTCTCTAGTGTGGTTCGTTGTTTTTGCGT GAGCCATGAGAACGAACCATTGAGATCATGCTTACTTTGCATGTCACTCAAAAATTTTGCCT CAAAACTGGTGAGCTGAATTTTTGCAGTTAAAGCATCGTGTAGTGTTTTTCTTAGTCCGTTAC GTAGGTAGGAATCTGATGTAATGGTTGTTGGTATTTTGTCACCATTCATTTTTATCTGGTTGT TCTCAAGTTCGGTTACGAGATCCATTTGTCTATCTAGTTCAACTTGGAAAATCAACGTATCAG TCGGGCGGCCTCGCTTATCAACCACCAATTTCATATTGCTGTAAGTGTTTAAATCTTTACTTA TTGGTTTCAAAACCCATTGGTTAAGCCTTTTAAACTCATGGTAGTTATTTTCAAGCATTAACAT GAACTTAAATTCATCAAGGCTAATCTCTATATTTGCCTTGTGAGTTTTCTTTTGTGTTAGTTCT TTTAATAACCACTCATAAATCCTCATAGAGTATTTGTTTTCAAAAGACTTAACATGTTCCAGAT TATATTTTATGAATTTTTTTAACTGGAAAAGATAAGGCAATATCTCTTCACTAAAAACTAATTCT AATTTTTCGCTTGAGAACTTGGCATAGTTTGTCCACTGGAAAATCTCAAAGCCTTTAACCAAA GGATTCCTGATTTCCACAGTTCTCGTCATCAGCTCTCTGGTTGCTTTAGCTAATACACCATAA GCATTTTCCCTACTGATGTTCATCATCTGAGCGTATTGGTTATAAGTGAACGATACCGTCCGT TCTTTCCTTGTAGGGTTTTCAATCGTGGGGTTGAGTAGTGCCACACAGCATAAAATTAGCTT GGTTTCATGCTCCGTTAAGTCATAGCGACTAATCGCTAGTTCATTTGCTTTGAAAACAACTAA TTCAGACATACATCTCAATTGGTCTAGGTGATTTTAATCACTATACCAATTGAGATGGGCTAG TCAATGATAATTACTAGTCCTTTTCCTTTGAGTTGTGGGTATCTGTAAATTCTGCTAGACCTTT GCTGGAAAACTTGTAAATTCTGCTAGACCCTCTGTAAATTCCGCTAGACCTTTGTGTGTTTTT TTTGTTTATATTCAAGTGGTTATAATTTATAGAATAAAGAAAGAATAAAAAAAGATAAAAAGAA TAGATCCCAGCCCTGTGTATAACTCACTACTTTAGTCAGTTCCGCAGTATTACAAAAGGATGT CGCAAACGCTGTTTGCTCCTCTACAAAACAGACCTTAAAACCCTAAAGGCTTAAGTAGCACC CTCGCAAGCTCGGGCAAATCGCTGAATATTCCTTTTGTCTCCGACCATCAGGCACCTGAGTC GCTGTCTTTTTCGTGACATTCAGTTCGCTGCGCTCACGGCTCTGGCAGTGAATGGGGGTAA ATGGCACTACAGGCGCCTTTTATGGATTCATGCAAGGAAACTACCCATAATACAAGAAAAGC CCGTCACGGGCTTCTCAGGGCGTTTTATGGCGGGTCTGCTATGTGGTGCTATCTGACTTTTT GCTGTTCAGCAGTTCCTGCCCTCTGATTTTCCAGTCTGACCACTTCGGATTATCCCGTGACA GGTCATTCAGACTGGCTAATGCACCCAGTAAGGCAGCGGTATCATCAACAGGCTTACCCGT CTTACTGTCCCTAGTGCTTGGATTCTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTT CTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGATCTATCAACAGGAGTCCAAGCGA GCTCGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATC TGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGA GGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCC AGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACT TTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGT TAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTG GTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTG TGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAG TGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGAT GCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCG AGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGT GCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGAT CCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGC GTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACAC GGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTG TCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCA

	CATTTCCCCGAAAAGTGCCACCTGACGTCTGTGGCTTTTATGAAAATCACACAGTGATCACA AATTTTAAACAGAGCACAAAATGCTGCCTCGAAATGAGGGCGGGAAAATAAGGTTATCAGCC TTGTTTTCTCCCTCATTACTTGAAGGATATGAAGCTAAAACCCTTTTTTATAAAGCATTTGTCC GAATTCGGACATAATCAAAAAAGCTTAATTAAGATCAATTTGATCTACATCTCTTTAACCAACA ATATGTAAGATCTCAACTATCGCATCCGTGGATTAATTCAATTATAACTTCTCTCTAACGCTG TGTATCGTAACGGTAACACTGTAGAGGGGAGCACATTGATGCGAATTCTCACACAGGACGG TACC

pZS1katGp-RBS31-phiC31-proD-oxyR	ATGACACAAGGGGTTGTGACCGGGGTGGACACGTACGCGGGTGCTTACGACCGTCAGTCG CGCGAGCGCGAGAATTCGAGCGCAGCAAGCCCAGCGACACAGCGTAGCGCCAACGAAGA CAAGGCGGCCGACCTTCAGCGCGAAGTCGAGCGCGACGGGGGCCGGTTCAGGTTCGTCG GGCATTTCAGCGAAGCGCCGGGCACGTCGGCGTTCGGGACGGCGGAGCGCCCGGAGTTC GAACGCATCCTGAACGAATGCCGCGCCGGGCGGCTCAACATGATCATTGTCTATGACGTGT CGCGCTTCTCGCGCCTGAAGGTCATGGACGCGATTCCGATTGTCTCGGAATTGCTCGCCCT GGGCGTGACGATTGTTTCCACTCAGGAAGGCGTCTTCCGGCAGGGAAACGTCATGGACCT GATTCACCTGATTATGCGGCTCGACGCGTCGCACAAAGAATCTTCGCTGAAGTCGGCGAAG ATTCTCGACACGAAGAACCTTCAGCGCGAATTGGGCGGGTACGTCGGCGGGAAGGCGCCT TACGGCTTCGAGCTTGTTTCGGAGACGAAGGAGATCACGCGCAACGGCCGAATGGTCAATG TCGTCATCAACAAGCTTGCGCACTCGACCACTCCCCTTACCGGACCCTTCGAGTTCGAGCC CGACGTAATCCGGTGGTGGTGGCGTGAGATCAAGACGCACAAACACCTTCCCTTCAAGCCG GGCAGTCAAGCCGCCATTCACCCGGGCAGCATCACGGGGCTTTGTAAGCGCATGGACGCT GACGCCGTGCCGACCCGGGGCGAGACGATTGGGAAGAAGACCGCTTCAAGCGCCTGGGA CCCGGCAACCGTTATGCGAATCCTTCGGGACCCGCGTATTGCGGGCTTCGCCGCTGAGGT GATCTACAAGAAGAAGCCGGACGGCACGCCGACCACGAAGATTGAGGGTTACCGCATTCA GCGCGACCCGATCACGCTCCGGCCGGTCGAGCTTGATTGCGGACCGATCATCGAGCCCGC TGAGTGGTATGAGCTTCAGGCGTGGTTGGACGGCAGGGGGCGCGGCAAGGGGCTTTCCC GGGGGCAAGCCATTCTGTCCGCCATGGACAAGCTGTACTGCGAGTGTGGCGCCGTCATGA CTTCGAAGCGCGGGGAAGAATCGATCAAGGACTCTTACCGCTGCCGTCGCCGGAAGGTGG TCGACCCGTCCGCACCTGGGCAGCACGAAGGCACGTGCAACGTCAGCATGGCGGCACTCG ACAAGTTCGTTGCGGAACGCATCTTCAACAAGATCAGGCACGCCGAAGGCGACGAAGAGAC GTTGGCGCTTCTGTGGGAAGCCGCCCGACGCTTCGGCAAGCTCACTGAGGCGCCTGAGAA GAGCGGCGAACGGGCGAACCTTGTTGCGGAGCGCGCCGACGCCCTGAACGCCCTTGAAG AGCTGTACGAAGACCGCGCGGCAGGCGCGTACGACGGACCCGTTGGCAGGAAGCACTTCC GGAAGCAACAGGCAGCGCTGACGCTCCGGCAGCAAGGGGCGGAAGAGCGGCTTGCCGAA CTTGAAGCCGCCGAAGCCCCGAAGCTTCCCCTTGACCAATGGTTCCCCGAAGACGCCGAC GCTGACCCGACCGGCCCTAAGTCGTGGTGGGGGCGCGCGTCAGTAGACGACAAGCGCGT GTTCGTCGGGCTCTTCGTAGACAAGATCGTTGTCACGAAGTCGACTACGGGCAGGGGGCA GGGAACGCCCATCGAGAAGCGCGCTTCGATCACGTGGGCGAAGCCGCCGACCGACGACG ACGAAGACGACGCCCAGGACGGCACGGAAGACGTAGCGGCGAGGCCTGCAGCAAACGAC GAAAACTACGCTGCAGCAGTTTAGACACATGGCATGGATGAACTATACAAATAACCCGGGG GATCCCATGGTACGCGTGCTAGAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGG GCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGCC CTAGACCTAGCACAGCTAACACCACGTCGTCCCTATCTGCTGCCCTAGGTCTATGAGTGGTT GCTGGATAACTTTACGGGCATGCATAAGGCTCGTATAATATATTCAGGGAGACCACAACGGT TTCCCTCTACAAATAATTTTGTTTAACTTTGAATTCTTCACACAGGAAACCGGTACCATGAATA TTCGTGATCTTGAGTACCTGGTGGCATTGGCTGAACACCGCCATTTTCGGCGTGCGGCAGA TTCCTGCCACGTTAGCCAGCCGACGCTTAGCGGGCAAATTCGTAAGCTGGAAGATGAGCTG GGCGTGATGTTGCTGGAGCGGACCAGCCGTAAAGTGTTGTTCACCCAGGCGGGAATGCTG CTGGTGGATCAGGCGCGTACCGTGCTGCGTGAGGTGAAAGTCCTTAAAGAGATGGCAAGC CAGCAGGGCGAGACGATGTCCGGACCGCTGCACATTGGTTTGATTCCCACAGTTGGACCGT ACCTGCTACCGCATATTATCCCTATGCTGCACCAGACCTTTCCAAAGCTGGAAATGTATCTG CATGAAGCACAGACCCACCAGTTACTGGCGCAACTGGACAGCGGCAAACTCGATTGCGTGA TCCTCGCGCTGGTGAAAGAGAGCGAAGCATTCATTGAAGTGCCGTTGTTTGATGAGCCAAT GTTGCTGGCTATCTATGAAGATCACCCGTGGGCGAACCGCGAATGCGTACCGATGGCCGAT CTGGCAGGGGAAAAACTGCTGATGCTGGAAGATGGTCACTGTTTGCGCGATCAGGCAATGG GTTTCTGTTTTGAAGCCGGGGCGGATGAAGATACACACTTCCGCGCGACCAGCCTGGAAAC TCTGCGCAACATGGTGGCGGCAGGTAGCGGGATCACTTTACTGCCAGCGCTGGCTGTGCC GCCGGAGCGCAAACGCGATGGGGTTGTTTATCTGCCGTGCATTAAGCCGGAACCACGCCG

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pZS1(pLtetO-TP901-aav-proA-tetR)-katGp-PhiC-proD-oxyR	ATGACACAAGGGGTTGTGACCGGGGTGGACACGTACGCGGGTGCTTACGACCGTCAGTCG CGCGAGCGCGAGAATTCGAGCGCAGCAAGCCCAGCGACACAGCGTAGCGCCAACGAAGA CAAGGCGGCCGACCTTCAGCGCGAAGTCGAGCGCGACGGGGGCCGGTTCAGGTTCGTCG GGCATTTCAGCGAAGCGCCGGGCACGTCGGCGTTCGGGACGGCGGAGCGCCCGGAGTTC GAACGCATCCTGAACGAATGCCGCGCCGGGCGGCTCAACATGATCATTGTCTATGACGTGT CGCGCTTCTCGCGCCTGAAGGTCATGGACGCGATTCCGATTGTCTCGGAATTGCTCGCCCT GGGCGTGACGATTGTTTCCACTCAGGAAGGCGTCTTCCGGCAGGGAAACGTCATGGACCT GATTCACCTGATTATGCGGCTCGACGCGTCGCACAAAGAATCTTCGCTGAAGTCGGCGAAG ATTCTCGACACGAAGAACCTTCAGCGCGAATTGGGCGGGTACGTCGGCGGGAAGGCGCCT TACGGCTTCGAGCTTGTTTCGGAGACGAAGGAGATCACGCGCAACGGCCGAATGGTCAATG TCGTCATCAACAAGCTTGCGCACTCGACCACTCCCCTTACCGGACCCTTCGAGTTCGAGCC CGACGTAATCCGGTGGTGGTGGCGTGAGATCAAGACGCACAAACACCTTCCCTTCAAGCCG GGCAGTCAAGCCGCCATTCACCCGGGCAGCATCACGGGGCTTTGTAAGCGCATGGACGCT GACGCCGTGCCGACCCGGGGCGAGACGATTGGGAAGAAGACCGCTTCAAGCGCCTGGGA CCCGGCAACCGTTATGCGAATCCTTCGGGACCCGCGTATTGCGGGCTTCGCCGCTGAGGT GATCTACAAGAAGAAGCCGGACGGCACGCCGACCACGAAGATTGAGGGTTACCGCATTCA GCGCGACCCGATCACGCTCCGGCCGGTCGAGCTTGATTGCGGACCGATCATCGAGCCCGC TGAGTGGTATGAGCTTCAGGCGTGGTTGGACGGCAGGGGGCGCGGCAAGGGGCTTTCCC GGGGGCAAGCCATTCTGTCCGCCATGGACAAGCTGTACTGCGAGTGTGGCGCCGTCATGA CTTCGAAGCGCGGGGAAGAATCGATCAAGGACTCTTACCGCTGCCGTCGCCGGAAGGTGG TCGACCCGTCCGCACCTGGGCAGCACGAAGGCACGTGCAACGTCAGCATGGCGGCACTCG ACAAGTTCGTTGCGGAACGCATCTTCAACAAGATCAGGCACGCCGAAGGCGACGAAGAGAC GTTGGCGCTTCTGTGGGAAGCCGCCCGACGCTTCGGCAAGCTCACTGAGGCGCCTGAGAA GAGCGGCGAACGGGCGAACCTTGTTGCGGAGCGCGCCGACGCCCTGAACGCCCTTGAAG AGCTGTACGAAGACCGCGCGGCAGGCGCGTACGACGGACCCGTTGGCAGGAAGCACTTCC GGAAGCAACAGGCAGCGCTGACGCTCCGGCAGCAAGGGGCGGAAGAGCGGCTTGCCGAA CTTGAAGCCGCCGAAGCCCCGAAGCTTCCCCTTGACCAATGGTTCCCCGAAGACGCCGAC GCTGACCCGACCGGCCCTAAGTCGTGGTGGGGGCGCGCGTCAGTAGACGACAAGCGCGT GTTCGTCGGGCTCTTCGTAGACAAGATCGTTGTCACGAAGTCGACTACGGGCAGGGGGCA GGGAACGCCCATCGAGAAGCGCGCTTCGATCACGTGGGCGAAGCCGCCGACCGACGACG ACGAAGACGACGCCCAGGACGGCACGGAAGACGTAGCGGCGAGGCCTGCAGCAAACGAC GAAAACTACGCTGCAGCAGTTTAGACACATGGCATGGATGAACTATACAAATAACCCGGGG GATCCCATGGTACGCGTGCTAGAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGG GCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGCC CTAGACCTAGCACAGCTAACACCACGTCGTCCCTATCTGCTGCCCTAGGTCTATGAGTGGTT GCTGGATAACTTTACGGGCATGCATAAGGCTCGTATAATATATTCAGGGAGACCACAACGGT TTCCCTCTACAAATAATTTTGTTTAACTTTGAATTCTTCACACAGGAAACCGGTACCATGAATA TTCGTGATCTTGAGTACCTGGTGGCATTGGCTGAACACCGCCATTTTCGGCGTGCGGCAGA TTCCTGCCACGTTAGCCAGCCGACGCTTAGCGGGCAAATTCGTAAGCTGGAAGATGAGCTG GGCGTGATGTTGCTGGAGCGGACCAGCCGTAAAGTGTTGTTCACCCAGGCGGGAATGCTG CTGGTGGATCAGGCGCGTACCGTGCTGCGTGAGGTGAAAGTCCTTAAAGAGATGGCAAGC CAGCAGGGCGAGACGATGTCCGGACCGCTGCACATTGGTTTGATTCCCACAGTTGGACCGT ACCTGCTACCGCATATTATCCCTATGCTGCACCAGACCTTTCCAAAGCTGGAAATGTATCTG CATGAAGCACAGACCCACCAGTTACTGGCGCAACTGGACAGCGGCAAACTCGATTGCGTGA TCCTCGCGCTGGTGAAAGAGAGCGAAGCATTCATTGAAGTGCCGTTGTTTGATGAGCCAAT GTTGCTGGCTATCTATGAAGATCACCCGTGGGCGAACCGCGAATGCGTACCGATGGCCGAT CTGGCAGGGGAAAAACTGCTGATGCTGGAAGATGGTCACTGTTTGCGCGATCAGGCAATGG GTTTCTGTTTTGAAGCCGGGGCGGATGAAGATACACACTTCCGCGCGACCAGCCTGGAAAC TCTGCGCAACATGGTGGCGGCAGGTAGCGGGATCACTTTACTGCCAGCGCTGGCTGTGCC GCCGGAGCGCAAACGCGATGGGGTTGTTTATCTGCCGTGCATTAAGCCGGAACCACGCCG

	CACTATTGGCCTGGTTTATCGTCCTGGCTCACCGCTGCGCAGCCGCTATGAGCAGCTGGCA GAGGCCATCCGCGCAAGAATGGATGGCCATTTCGATAAAGTTTTAAAACAGGCGGTTTAAC CCGGGGGATCCCATGGTACGCGTGCTAGAGGCATCAAATAAAACGAAAGGCTCAGTCGAAA GACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCC GCCGCCCTAGACCTAGGGCCTAGGGTACGGGTTTTGCTGCCCGCAAACGGGCTGTTCTGG TGTTGCTAGTTTGTTATCAGAATCGCAGATCCGGCTTCAGGTTTGCCGGCTGAAAGCGCTAT TTCTTCCAGAATTGCCATGATTTTTTCCCCACGGGAGGCGTCACTGGCTCCCGTGTTGTCGG CAGCTTTGATTCGATAAGCAGCATCGCCTGTTTCAGGCTGTCTATGTGTGACTGTTGAGCTG TAACAAGTTGTCTCAGGTGTTCAATTTCATGTTCTAGTTGCTTTGTTTTACTGGTTTCACCTGT TCTATTAGGTGTTACATGCTGTTCATCTGTTACATTGTCGATCTGTTCATGGTGAACAGCTTT AAATGCACCAAAAACTCGTAAAAGCTCTGATGTATCTATCTTTTTTACACCGTTTTCATCTGT GCATATGGACAGTTTTCCCTTTGATATCTAACGGTGAACAGTTGTTCTACTTTTGTTTGTTAG TCTTGATGCTTCACTGATAGATACAAGAGCCATAAGAACCTCAGATCCTTCCGTATTTAGCCA GTATGTTCTCTAGTGTGGTTCGTTGTTTTTGCGTGAGCCATGAGAACGAACCATTGAGATCA TGCTTACTTTGCATGTCACTCAAAAATTTTGCCTCAAAACTGGTGAGCTGAATTTTTGCAGTT AAAGCATCGTGTAGTGTTTTTCTTAGTCCGTTACGTAGGTAGGAATCTGATGTAATGGTTGTT GGTATTTTGTCACCATTCATTTTTATCTGGTTGTTCTCAAGTTCGGTTACGAGATCCATTTGT CTATCTAGTTCAACTTGGAAAATCAACGTATCAGTCGGGCGGCCTCGCTTATCAACCACCAA TTTCATATTGCTGTAAGTGTTTAAATCTTTACTTATTGGTTTCAAAACCCATTGGTTAAGCCTT TTAAACTCATGGTAGTTATTTTCAAGCATTAACATGAACTTAAATTCATCAAGGCTAATCTCTA TATTTGCCTTGTGAGTTTTCTTTTGTGTTAGTTCTTTTAATAACCACTCATAAATCCTCATAGA GTATTTGTTTTCAAAAGACTTAACATGTTCCAGATTATATTTTATGAATTTTTTTAACTGGAAAA GATAAGGCAATATCTCTTCACTAAAAACTAATTCTAATTTTTCGCTTGAGAACTTGGCATAGTT TGTCCACTGGAAAATCTCAAAGCCTTTAACCAAAGGATTCCTGATTTCCACAGTTCTCGTCAT CAGCTCTCTGGTTGCTTTAGCTAATACACCATAAGCATTTTCCCTACTGATGTTCATCATCTG AGCGTATTGGTTATAAGTGAACGATACCGTCCGTTCTTTCCTTGTAGGGTTTTCAATCGTGG GGTTGAGTAGTGCCACACAGCATAAAATTAGCTTGGTTTCATGCTCCGTTAAGTCATAGCGA CTAATCGCTAGTTCATTTGCTTTGAAAACAACTAATTCAGACATACATCTCAATTGGTCTAGG TGATTTTAATCACTATACCAATTGAGATGGGCTAGTCAATGATAATTACTAGTCCTTTTCCTTT GAGTTGTGGGTATCTGTAAATTCTGCTAGACCTTTGCTGGAAAACTTGTAAATTCTGCTAGAC CCTCTGTAAATTCCGCTAGACCTTTGTGTGTTTTTTTTGTTTATATTCAAGTGGTTATAATTTA TAGAATAAAGAAAGAATAAAAAAAGATAAAAAGAATAGATCCCAGCCCTGTGTATAACTCACT ACTTTAGTCAGTTCCGCAGTATTACAAAAGGATGTCGCAAACGCTGTTTGCTCCTCTACAAA ACAGACCTTAAAACCCTAAAGGCTTAAGTAGCACCCTCGCAAGCTCGGGCAAATCGCTGAA TATTCCTTTTGTCTCCGACCATCAGGCACCTGAGTCGCTGTCTTTTTCGTGACATTCAGTTCG CTGCGCTCACGGCTCTGGCAGTGAATGGGGGTAAATGGCACTACAGGCGCCTTTTATGGAT TCATGCAAGGAAACTACCCATAATACAAGAAAAGCCCGTCACGGGCTTCTCAGGGCGTTTTA TGGCGGGTCTGCTATGTGGTGCTATCTGACTTTTTGCTGTTCAGCAGTTCCTGCCCTCTGAT TTTCCAGTCTGACCACTTCGGATTATCCCGTGACAGGTCATTCAGACTGGCTAATGCACCCA GTAAGGCAGCGGTATCATCAACAGGCTTACCCGTCTTACTGTCCCTAGTGCTTGGATTCTCA CCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGG TCATTACTGGATCTATCAACAGGAGTCCAAGCGAGCTCGTAAACTTGGTCTGACAGTTACCA ATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTG ACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCA ATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCG GAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTG TTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTG CTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCA ACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGT CCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACT

	GCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAAC CAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGG GATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGG GCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCAC CCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGG CAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCT TTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGT ATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGT CTCTAGGGCGGCGGATTTGTCCTACTCAGGAGAGCGTTCACCGACAAACAACAGATAAAAC GAAAGGCCCAGTCTTTCGACTGAGCCTTTCGTTTTATTTGATGCCTCTAGCACGCGTACCAT GGGATCCCCCGGGTTAAGACCCACTTTCACATTTAAGTTGTTTTTCTAATCCGCATATGATCA ATTCAAGGCCGAATAAGAAGGCTGGCTCTGCACCTTGGTGATCAAATAATTCGATAGCTTGT CGTAATAATGGCGGCATACTATCAGTAGTAGGTGTTTCCCTTTCTTCTTTAGCGACTTGATGC TCTTGATCTTCCAATACGCAACCTAAAGTAAAATGCCCCACAGCGCTGAGTGCATATAATGC ATTCTCTAGTGAAAAACCTTGTTGGCATAAAAAGGCTAATTGATTTTCGAGAGTTTCATACTG TTTTTCTGTAGGCCGTGTACCTAAATGTACTTTTGCTCCATCGCGATGACTTAGTAAAGCACA TCTAAAACTTTTAGCGTTATTACGTAAAAAATCTTGCCAGCTTTCCCCTTCTAAAGGGCAAAA GTGAGTATGGTGCCTATCTAACATCTCAATGGCTAAGGCGTCGAGCAAAGCCCGCTTATTTT TTACATGCCAATACAATGTAGGCTGCTCTACACCTAGCTTCTGGGCGAGTTTACGGGTTGTT AAACCTTCGATTCCGACCTCATTAAGCAGCTCTAATGCGCTGTTAATCACTTTACTTTTATCT AATCTAGACATGGTACCGGTTTCCTGTGTGAAGAATTCAAAGTTAAACAAAATTATTTGTAGA GGGAAACCGTTGTGGTCTCCCTGAATATAGCCTACGAGCCTTATGCATGCCCGTAAAGTTAT CCAGCAACCACTCATAGACCTAGGGCAGCAGATAGGGACGACGTGGTGTTAGCTGTGGCTA GCATCTCGAGTCTAGGGCGGCGGATTTGTCCTACTCAGGAGAGCGTTCACCGACAAACAAC AGATAAAACGAAAGGCCCAGTCTTTCGACTGAGCCTTTCGTTTTATTTGATGCCTCTAGCAC GCGTACCATGGGATCCCCCGGGCTAAACTGCTGCAGCGTAGTTTTCGTCGTTTGCTGCAGG CCTAGCGAGTTGGAATTTAAATATGATATCTACATTATCAGCAGTAACATCAACCTTTGATAC AAGGTTGTTGACGATTTTCTTTTTATTATCATATGATAGTTCATTAATCGGAATTGAGCCCAAC TGAGTTTTAACTAACTCAAAAACATCAGTAGAGTCATTAAATTTATTTTCGCTAATCTTAGCTT TAAGCAGCTTTTTCTCAGCCTGAAGGGAATCAGTACGATCTTTCAACTCATCCATAGTGATAA AATCATTTAGGTACAAATCAGAGTTCTTTTGTATTTTTTTATCGATCTGTGAAATTTGCTTTTTA AATGACGAAGTATCAAGAATAGGTTGGTTGTTGCCATTGATAATTTTCAATAAGGAGTCATTA TTTTCTTGAAATCCAATCAGGTTGTCAATAACAGTATTTTCTAAATTACTTAAATCATAAGTTC CTGAATCACACTTTTTATTGTCATTATATACTGTAATTCCTTTTGTTTTTCGAGGAAATCTATTT GCACAGTGATATTTCATAGTGCGGCTTCCATCTTTTCTTTTGTGGCCAAGAACAATTTTTAAA GGTGCTCCACAGTAACCGCACCTTGCCATCCCTGACAGCATATATTTAGCTTGGAAAGGTCT AGGGTTGTTATTTCTTTCATAAGTCTGCTGTTGTCTTTCTTCTAGCTCTTTTTGAACTTTTAAA TAAGTCTCATAAGGGATAATTGGTTTGTGCATACCTTCAAATAGGCTGTCCTTAAATTTGATA TAACCACAGTAAACTGGATTATCAAGTGTTTGTCTTAGGGTACGATAAGACCACGGTATATCT TTACCGATGTGTCCAGATTCATTGAGTTTATCTCTTAATTTTGTAAGTGATATTCCTGATAAAT AATCAGTGAATATTTGTTCAACTATTGTAGCTTGTAAAGGAACAATTTCTAATATACCTGTCTT TCTGTTGTGGTAATACCCAAAAGCTGTCTTAGTCCACATCATAGACTTACCAGATTTCGCTCG CCCTAGTTTACCCATAGTCATGCGTTCTTTTATATTCTCTCTTTCAAACTCATTAATTGCAGAA AGAATAGTGAGAAACAAGCTACCCATAGCAGAAGAAGTATCAATACTTTCATTAAGCGAGAT AAAGTCTATTTTATTTTTTGTGAACACATCCTTAACAAGATAAAGAGTATCTCTTACACTACGT GAAAGGCGGTCTAGCTTATATACAAGAACTGTATCAAAAGCTTTATTCTCGATATCGTTGATT AATCTTTGCATTGCTGGGCGTTCAAGTTTGGCCCCTGAAAAACCAGCATCAGTATAAGTATC AGATACTTGCCACCCCATTGCTTCAGCATATTTTGTTAAACGGTCAATTTGCTCATCAATTGA GAAGCCTTCCTCTGCTTGGTTAGTAGTGGATACTCGTGTATAGATTGCTACTTTCTTAGTCAT GGTACCTTTCTCCTCTTTAATGAATTCGGTCAGTGCGTCCTGCTGATGTGCTCAGTATCTCTA

	TCACTGATAGGGATGTCAATCTCTATCACTGATAGGGAGACGTCTTCAGCCAAAAAACTTAA GACCGCCGGTCTTGTCCACTACCTTGCAGTAATGCGGTGGACAGGATCGGCGGTTTTCTTT TCTCTTCTCAAGACGTCTGTGGCTTTTATGAAAATCACACAGTGATCACAAATTTTAAACAGA GCACAAAATGCTGCCTCGAAATGAGGGCGGGAAAATAAGGTTATCAGCCTTGTTTTCTCCCT CATTACTTGAAGGATATGAAGCTAAAACCCTTTTTTATAAAGCATTTGTCCGAATTCGGACAT AATCAAAAAAGCTTAATTAAGATCAATTTGATCTACATCTCTTTAACCAACAATATGTAAGATC TCAACTATCGCATCCGTGGATTAATTCAATTATAACTTCTCTCTAACGCTGTGTATCGTAACG GTAACACTGTAGAGGGGAGCACATTGATGCGAATTCTCACACAGGAAACCGGTACC

TP901 GFP BAC Reporter	GGAAGCTAAAATGGAGAAAAAAATCACTGGATATACCACCGTTGATATATCCCAATGGCATC GTAAAGAACATTTTGAGGCATTTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGTTCAG CTGGATATTACGGCCTTTTTAAAGACCGTAAAGAAAAATAAGCACAAGTTTTATCCGGCCTTT ATTCACATTCTTGCCCGCCTGATGAATGCTCATCCGGAATTTCGTATGGCAATGAAAGACGG TGAGCTGGTGATATGGGATAGTGTTCACCCTTGTTACACCGTTTTCCATGAGCAAACTGAAA CGTTTTCATCGCTCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACATATATTCG CAAGATGTGGCGTGTTACGGTGAAAACCTGGCCTATTTCCCTAAAGGGTTTATTGAGAATAT GTTTTTCGTCTCAGCCAATCCCTGGGTGAGTTTCACCAGTTTTGATTTAAACGTGGCCAATAT GGACAACTTCTTCGCCCCCGTTTTCACCATGGGCAAATATTATACGCAAGGCGACAAGGTG CTGATGCCGCTGGCGATTCAGGTTCATCATGCCGTTTGTGATGGCTTCCATGTCGGCAGAA TGCTTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGGCGTAAGACGTCTAAGA AACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTTCA CCTCGAGCACAGCTAACACCACGTCGTCCCTATCTGCTGCCCTAGGTCTATGAGTGGTTGC TGGATAACTTTACGGGCATGCATAAGGCTCGTATAATATATTCAGGGAGACCACAACGGTTT CCCTCTACAAATAATTTTGTTTAACTTTTTAATTAAATGCCAACACAATTAACATCTCAATCAA GGTAAATGCTTTTTGCTTTTTTTGCATCGATTCTAGGGCGGCGGATTTGTCCTACTCAGGAG AGCGTTCACCGACAAACAACAGATAAAACGAAAGGCCCAGTCTTTCGACTGAGCCTTTCGTT TTATTTGATGCCTCTAGCACGCGTACCATGGGATCCCCCGGGCTGCAGGAATTCGATATCA AGCTTTTATTTGTAGAGATCATCCATGCCATGTGTAATCCCAGCAGCTGTTACAAACTCAAGA AGGACCATGTGGTCTCTCTTTTCGTTGGGATCTTTCGAAAGGGCAGATTGTGTGGACAGGTA ATGGTTGTCTGGTAAAAGGACAGGGCCATCGCCAATTGGAGTATTTTGTTGATAATGGTCTG CTAGTTGAACGCTTCCATCTTCAATGTTGTGTCTAATTTTGAAGTTAACTTTGATTCCATTCTT TTGTTTGTCTGCCATGATGTATACATTGTGTGAGTTATAGTTGTATTCCAATTTGTGTCCAAG AATGTTTCCATCTTCTTTAAAATCAATACCTTTTAACTCGATTCTATTAACAAGGGTATCACCT TCAAACTTGACTTCAGCACGTGTCTTGTAGTTCCCGTCATCTTTGAAAAATATAGTTCTTTCC TGTACATAACCTTCGGGCATGGCACTCTTGAAAAAGTCATGCTGTTTCATATGATCTGGGTA TCTCGCAAAGCATTGAACACCATAACCGAAAGTAGTGACAAGTGTTGGCCATGGAACAGGT AGTTTTCCAGTAGTGCAAATAAATTTAAGGGTAAGTTTTCCGTATGTTGCATCACCTTCACCC TCTCCACTGACAGAAAATTTGTGCCCATTAACATCACCATCTAATTCAACAAGAATTGGGACA ACTCCAGTGAAAAGTTCTTCTCCTTTACTCATCTTAAACCTCCTTACCTCGTAAACTATTAAAC AAAATTATTTGTAGAGGCTGTTTCGTCCTCACGGACTCATCAGACCGGAAAGCACATCCGGT GACAGCTGTGTTTAAAGGAGTTTTTTAGTTACCTTAATTGAAATAAACGAAATAAAAACTCGC CGAGCACCGTACTTGCCCTTGACAGGCATTGATGGAATCGTAGTCTCACGCTGATAGTCTG ATCGACAATACAAGTGGGACCGTGGTCCCAGACCGATAATCAGACCGACAACACGAGTGGG ATCGTGGTCCCAGACTAATAATCAGACCGACGATACGAGTGGGACCGTGGTCCCAGACTAA TAATCAGACCGACGATACGAGTGGGACCGTGGTTCCAGACTAATAATCAGACCGACGATAC GAGTGGGACCGTGGTCCCAGACTAATAATCAGACCGACGATACGAGTGGGACCATGGTCC CAGACTAATAATCAGACCGACGATACGAGTGGGACCGTGGTCCCAGTCTGATTATCAGACC GACGATACGAGTGGGACCGTGGTCCCAGACTAATAATCAGACCGACGATACGAGTGGGAC CGTGGTCCCAGACTAATAATCAGACCGACGATACGAGTGGGACCGTGGTCCCAGTCTGATT ATCAGACCGACGATACAAGTGGAACAGTGGGCCCAGAGAGAATATTCAGGCCAGTTATGCT TTCTGGCCTGTAACAAAGGACATTAAGTAAAGACAGATAAACGTAGACTAAAACGTGGTCGC ATCAGGGTGCTGGCTTTTCAAGTTCCTTAAGAATGGCCTCAATTTTCTCTATACACTCAGTTG GAACACGGGACCTGTCCAGGTTAAGCACCATTTTATCGCCCTTATACAATACTGTCGCTCCA GGAGCAAACTGATGTCGTGAGCTTAAACTAGTTCTTGATGCAGATGACGTTTTAAGCACAGA AGTTAAAAGAGTGATAACTTCTTCAGCTTCAAATATCACCCCAGCTTTTTTCTGCTCATGAAG GTTAGATGCCTGCTGCTTAAGTAATTCCTCTTTATCTGTAAAGGCTTTTTGAAGTGCATCACC TGACCGGGCAGATAGTTCACCGGGGTGAGAAAAAAGAGCAACAACTGATTTAGGCAATTTG GCGGTGTTGATACAGCGGGTAATAATCTTACGTGAAATATTTTCCGCATCAGCCAGCGCAGA AATATTTCCAGCAAATTCATTCTGCAATCGGCTTGCATAACGCTGACCACGTTCATAAGCACT

	TGTTGGGCGATAATCGTTACCCAATCTGGATAATGCAGCCATCTGCTCATCATCCAGCTCGC CAACCAGAACACGATAATCACTTTCGGTAAGTGCAGCAGCTTTACGACGGCGACTCCCATC GGCAATTTCTATGACACCAGATACTCTTCGACCGAACGCCGGTGTCTGTTGACCAGTCAGTA GAAAAGAAGGGATGAGATCATCCAGTGCGTCCTCAGTAAGCAGCTCCTGGTCACGTTCATT ACCTGACCATACCCGAGAGGTCTTCTCAACACTATCACCCCGGAGCACTTCAAGAGTAAACT TCACATCCCGACCACATACAGGCAAAGTAATGGCATTACCGCGAGCCATTACTCCTACGCG CGCAATTAACGAATCCACCATCGGGGCAGCTGGTGTCGATAACGAAGTATCTTCAACCGGT TGAGTATTGAGCGTATGTTTTGGAATAACAGGCGCACGCTTCATTATCTAATCTCCCAGCGT GGTTTAATCAGACGATCGAAAATTTCATTGCAGACAGGTTCCCAAATAGAAAGAGCATTTCT CCAGGCACCAGTTGAAGAGCGTTGATCAATGGCCTGTTCAAAAACAGTTCTCATCCGGATCT GACCTTTACCAACTTCATCCGTTTCACGTACAACATTTTTTAGAACCATGCTTCCCCAGGCAT CCCGAATTTGCTCCTCCATCCACGGGGACTGAGAGCCATTACTATTGCTGTATTTGGTAAGC AAAATACGTACATCAGGCTCGAACCCTTTAAGATCAACGTTCTTGAGCAGATCACGAAGCAT ATCGAAAAACTGCAGTGCGGAGGTGTAGTCAAACAACTCAGCAGGCGTGGGAACAATCAGC ACATCAGCAGCACATACGACATTAATCGTGCCGATACCCAGGTTAGGCGCGCTGTCAATAA CTATGACATCATAGTCATGAGCAACAGTTTCAATGGCCAGTCGGAGCATCAGGTGTGGATC GGTGGGCAGTTTACCTTCATCAAATTTGCCCATTAACTCAGTTTCAATACGGTGCAGAGCCA GACAGGAAGGAATAATGTCAAGCCCCGGCCAGCAAGTGGGCTTTATTGCATAAGTGACATC GTCCTTTTCCCCAAGATAGAAAGGCAGGAGAGTGTCTTCTGCATGAATATGAAGATCTGGTA CCCATCCGTGATACATTGAGGCTGTTCCCTGGGGGTCGTTACCTTCCACGAGCAAAACACG TAGCCCCTTCAGAGCCAGATCCTGAGCAAGATGAACAGAAACTGAGGTTTTGTAAACGCCA CCTTTATGGGCAGCAACCCCGATCACCGGTGGAAATACGTCTTCAGCACGTCGCAATCGCG TACCAAACACATCACGCATATGATTAATTTGTTCAATTGTATAACCAACACGTTGCTCAACCC GTCCTCGAATTTCCATATCCGGGTGCGGTAGTCGCCCTGCTTTCTCGGCATCTCTGATAGC CTGAGAAGAAACCCCAACTAAATCCGCTGCTTCACCTATTCTCCAGCGCCGGGTTATTTTCC TCGCTTCCGGGCTGTCATCATTAAACTGTGCAATGGCGATAGCCTTCGTCATTTCATGACCA GCGTTTATGCACTGGTTAAGTGTTTCCATGAGTTTCATTCTGAACATCCTTTAATCATTGCTTT GCGTTTTTTTATTAAATCTTGCAATTTACTGCAAAGCAACAACAAAATCGCAAAGTCATCAAA AAACCGCAAAGTTGTTTAAAATAAGAGCAACACTACAAAAGGAGATAAGAAGAGCACATACC TCAGTCACTTATTATCACTAGCGCTCGCCGCAGCCGTGTAACCGAGCATAGCGAGCGAACT GGCGAGGAAGCAAAGAAGAACTGTTCTGTCAGATAGCTCTTACGCTCAGCGCAAGAAGAAA TATCCACCGTGGGAAAAACTCCAGGTAGAGGTACACACGCGGATAGCCAATTCAGAGTAAT AAACTGTGATAATCAACCCTCATCAATGATGACGAACTAACCCCCGATATCAGGTCACATGA CGAAGGGAAAGAGAAGGAAATCAACTGTGACAAACTGCCCTCAAATTTGGCTTCCTTAAAAA TTACAGTTCAAAAAGTATGAGAAAATCCATGCAGGCTGAAGGAAACAGCAAAACTGTGACAA ATTACCCTCAGTAGGTCAGAACAAATGTGACGAACCACCCTCAAATCTGTGACAGATAACCC TCAGACTATCCTGTCGTCATGGAAGTGATATCGCGGAAGGAAAATACGATATGAGTCGTCTG GCGGCCTTTCTTTTTCTCAATGTATGAGAGGCGCATTGGAGTTCTGCTGTTGATCTCATTAA CACAGACCTGCAGGAAGCGGCGGCGGAAGTCAGGCATACGCTGGTAACTTTGAGGCAGCT GGTAACGCTCTATGATCCAGTCGATTTTCAGAGAGACGATGCCTGAGCCATCCGGCTTACG ATACTGACACAGGGATTCGTATAAACGCATGGCATACGGATTGGTGATTTCTTTTGTTTCACT AAGCCGAAACTGCGTAAACCGGTTCTGTAACCCGATAAAGAAGGGAATGAGATATGGGTTG ATATGTACACTGTAAAGCCCTCTGGATGGACTGTGCGCACGTTTGATAAACCAAGGAAAAGA TTCATAGCCTTTTTCATCGCCGGCATCCTCTTCAGGGCGATAAAAAACCACTTCCTTCCCCG CGAAACTCTTCAATGCCTGCCGTATATCCTTACTGGCTTCCGCAGAGGTCAATCCGAATATT TCAGCATATTTAGCAACATGGATCTCGCAGATACCGTCATGTTCCTGTAGGGTGCCATCAGA TTTTCTGATCTGGTCAACGAACAGATACAGCATACGTTTTTGATCCCGGGAGAGACTATATG CCGCCTCAGTGAGGTCGTTTGACTGGACGATTCGCGGGCTATTTTTACGTTTCTTGTGATTG ATAACCGCTGTTTCCGCCATGACAGATCCATGTGAAGTGTGACAAGTTTTTAGATTGTCACA CTAAATAAAAAAGAGTCAATAAGCAGGGATAACTTTGTGAAAAAACAGCTTCTTCTGAGGGC

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	AAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTG TTCCATGGCCAACACTTGTCACTACTTTCGGTTATGGTGTTCAATGCTTTGCGAGATACCCA GATCATATGAAACAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAG AACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTG ATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTG GACACAAATTGGAATACAACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGA ATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCA GACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTA CCTGTCCACACAATCTGCCCTTTCGAAAGATCCCAACGAAAAGAGAGACCACATGGTCCTTC TTGAGTTTGTAACAGCTGCTGGGATTACACATGGCATGGATGATCTCTACAAATAAAAGCTT GATATCGAATTCCTGCAGCCCGGGGGATCCCATGGTACGCGTGCTAGAGGCATCAAATAAA ACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTC TCCTGAGTAGGACAAATCCGCCGCCCTAGAATCGATGTCGGGGTTTGTACCGTACACCACT GAGACCGCGGTGGTTGACCAGACAAACCACGACACATGTCAATACTTGCCCTTGACAGGCA TTGATGGAATCGTAGTCTCACGCTGATAGTCTGATCGACAATACAAGTGGGACCGTGGTCC CAGACCGATAATCAGACCGACAACACGAGTGGGATCGTGGTCCCAGACTAATAATCAGACC GACGATACGAGTGGGACCGTGGTCCCAGACTAATAATCAGACCGACGATACGAGTGGGAC CGTGGTTCCAGACTAATAATCAGACCGACGATACGAGTGGGACCGTGGTCCCAGACTAATA ATCAGACCGACGATACGAGTGGGACCATGGTCCCAGACTAATAATCAGACCGACGATACGA GTGGGACCGTGGTCCCAGTCTGATTATCAGACCGACGATACGAGTGGGACCGTGGTCCCA GACTAATAATCAGACCGACGATACGAGTGGGACCGTGGTCCCAGACTAATAATCAGACCGA CGATACGAGTGGGACCGTGGTCCCAGTCTGATTATCAGACCGACGATACAAGTGGAACAGT GGGCCCAGAGAGAATATTCAGGCCAGTTATGCTTTCTGGCCTGTAACAAAGGACATTAAGTA AAGACAGATAAACGTAGACTAAAACGTGGTCGCATCAGGGTGCTGGCTTTTCAAGTTCCTTA AGAATGGCCTCAATTTTCTCTATACACTCAGTTGGAACACGGGACCTGTCCAGGTTAAGCAC CATTTTATCGCCCTTATACAATACTGTCGCTCCAGGAGCAAACTGATGTCGTGAGCTTAAAC TAGTTCTTGATGCAGATGACGTTTTAAGCACAGAAGTTAAAAGAGTGATAACTTCTTCAGCTT CAAATATCACCCCAGCTTTTTTCTGCTCATGAAGGTTAGATGCCTGCTGCTTAAGTAATTCCT CTTTATCTGTAAAGGCTTTTTGAAGTGCATCACCTGACCGGGCAGATAGTTCACCGGGGTGA GAAAAAAGAGCAACAACTGATTTAGGCAATTTGGCGGTGTTGATACAGCGGGTAATAATCTT ACGTGAAATATTTTCCGCATCAGCCAGCGCAGAAATATTTCCAGCAAATTCATTCTGCAATC GGCTTGCATAACGCTGACCACGTTCATAAGCACTTGTTGGGCGATAATCGTTACCCAATCTG GATAATGCAGCCATCTGCTCATCATCCAGCTCGCCAACCAGAACACGATAATCACTTTCGGT AAGTGCAGCAGCTTTACGACGGCGACTCCCATCGGCAATTTCTATGACACCAGATACTCTTC GACCGAACGCCGGTGTCTGTTGACCAGTCAGTAGAAAAGAAGGGATGAGATCATCCAGTGC GTCCTCAGTAAGCAGCTCCTGGTCACGTTCATTACCTGACCATACCCGAGAGGTCTTCTCAA CACTATCACCCCGGAGCACTTCAAGAGTAAACTTCACATCCCGACCACATACAGGCAAAGTA ATGGCATTACCGCGAGCCATTACTCCTACGCGCGCAATTAACGAATCCACCATCGGGGCAG CTGGTGTCGATAACGAAGTATCTTCAACCGGTTGAGTATTGAGCGTATGTTTTGGAATAACA GGCGCACGCTTCATTATCTAATCTCCCAGCGTGGTTTAATCAGACGATCGAAAATTTCATTG CAGACAGGTTCCCAAATAGAAAGAGCATTTCTCCAGGCACCAGTTGAAGAGCGTTGATCAAT GGCCTGTTCAAAAACAGTTCTCATCCGGATCTGACCTTTACCAACTTCATCCGTTTCACGTA CAACATTTTTTAGAACCATGCTTCCCCAGGCATCCCGAATTTGCTCCTCCATCCACGGGGAC TGAGAGCCATTACTATTGCTGTATTTGGTAAGCAAAATACGTACATCAGGCTCGAACCCTTTA AGATCAACGTTCTTGAGCAGATCACGAAGCATATCGAAAAACTGCAGTGCGGAGGTGTAGT CAAACAACTCAGCAGGCGTGGGAACAATCAGCACATCAGCAGCACATACGACATTAATCGT GCCGATACCCAGGTTAGGCGCGCTGTCAATAACTATGACATCATAGTCATGAGCAACAGTTT CAATGGCCAGTCGGAGCATCAGGTGTGGATCGGTGGGCAGTTTACCTTCATCAAATTTGCC CATTAACTCAGTTTCAATACGGTGCAGAGCCAGACAGGAAGGAATAATGTCAAGCCCCGGC CAGCAAGTGGGCTTTATTGCATAAGTGACATCGTCCTTTTCCCCAAGATAGAAAGGCAGGAG

	AGTGTCTTCTGCATGAATATGAAGATCTGGTACCCATCCGTGATACATTGAGGCTGTTCCCT GGGGGTCGTTACCTTCCACGAGCAAAACACGTAGCCCCTTCAGAGCCAGATCCTGAGCAAG ATGAACAGAAACTGAGGTTTTGTAAACGCCACCTTTATGGGCAGCAACCCCGATCACCGGT GGAAATACGTCTTCAGCACGTCGCAATCGCGTACCAAACACATCACGCATATGATTAATTTG TTCAATTGTATAACCAACACGTTGCTCAACCCGTCCTCGAATTTCCATATCCGGGTGCGGTA GTCGCCCTGCTTTCTCGGCATCTCTGATAGCCTGAGAAGAAACCCCAACTAAATCCGCTGCT TCACCTATTCTCCAGCGCCGGGTTATTTTCCTCGCTTCCGGGCTGTCATCATTAAACTGTGC AATGGCGATAGCCTTCGTCATTTCATGACCAGCGTTTATGCACTGGTTAAGTGTTTCCATGA GTTTCATTCTGAACATCCTTTAATCATTGCTTTGCGTTTTTTTATTAAATCTTGCAATTTACTGC AAAGCAACAACAAAATCGCAAAGTCATCAAAAAACCGCAAAGTTGTTTAAAATAAGAGCAAC ACTACAAAAGGAGATAAGAAGAGCACATACCTCAGTCACTTATTATCACTAGCGCTCGCCGC AGCCGTGTAACCGAGCATAGCGAGCGAACTGGCGAGGAAGCAAAGAAGAACTGTTCTGTCA GATAGCTCTTACGCTCAGCGCAAGAAGAAATATCCACCGTGGGAAAAACTCCAGGTAGAGG TACACACGCGGATAGCCAATTCAGAGTAATAAACTGTGATAATCAACCCTCATCAATGATGA CGAACTAACCCCCGATATCAGGTCACATGACGAAGGGAAAGAGAAGGAAATCAACTGTGAC AAACTGCCCTCAAATTTGGCTTCCTTAAAAATTACAGTTCAAAAAGTATGAGAAAATCCATGC AGGCTGAAGGAAACAGCAAAACTGTGACAAATTACCCTCAGTAGGTCAGAACAAATGTGAC GAACCACCCTCAAATCTGTGACAGATAACCCTCAGACTATCCTGTCGTCATGGAAGTGATAT CGCGGAAGGAAAATACGATATGAGTCGTCTGGCGGCCTTTCTTTTTCTCAATGTATGAGAGG CGCATTGGAGTTCTGCTGTTGATCTCATTAACACAGACCTGCAGGAAGCGGCGGCGGAAGT CAGGCATACGCTGGTAACTTTGAGGCAGCTGGTAACGCTCTATGATCCAGTCGATTTTCAGA GAGACGATGCCTGAGCCATCCGGCTTACGATACTGACACAGGGATTCGTATAAACGCATGG CATACGGATTGGTGATTTCTTTTGTTTCACTAAGCCGAAACTGCGTAAACCGGTTCTGTAAC CCGATAAAGAAGGGAATGAGATATGGGTTGATATGTACACTGTAAAGCCCTCTGGATGGACT GTGCGCACGTTTGATAAACCAAGGAAAAGATTCATAGCCTTTTTCATCGCCGGCATCCTCTT CAGGGCGATAAAAAACCACTTCCTTCCCCGCGAAACTCTTCAATGCCTGCCGTATATCCTTA CTGGCTTCCGCAGAGGTCAATCCGAATATTTCAGCATATTTAGCAACATGGATCTCGCAGAT ACCGTCATGTTCCTGTAGGGTGCCATCAGATTTTCTGATCTGGTCAACGAACAGATACAGCA TACGTTTTTGATCCCGGGAGAGACTATATGCCGCCTCAGTGAGGTCGTTTGACTGGACGATT CGCGGGCTATTTTTACGTTTCTTGTGATTGATAACCGCTGTTTCCGCCATGACAGATCCATG TGAAGTGTGACAAGTTTTTAGATTGTCACACTAAATAAAAAAGAGTCAATAAGCAGGGATAAC TTTGTGAAAAAACAGCTTCTTCTGAGGGCAATTTGTCACAGGGTTAAGGGCAATTTGTCACA GACAGGACTGTCATTTGAGGGTGATTTGTCACACTGAAAGGGCAATTTGTCACAACACCTTC TCTAGAACCAGCATGGATAAAGGCCTACAAGGCGCTCTAAAAAAGAAGATCTAAAAACTATA AAAAAAATAATTATAAAAATATCCCCGTGGATAAGTGGATAACCCCAAGGGAAGTTTTTTCAG GCATCGTGTGTAAGCAGAATATATAAGTGCTGTTCCCTGGTGCTTCCTCGCTCACTCGACCG GGAGGGTTCGAGAAGGGGGGGCACCCCCCTTCGGCGTGCGCGGTCACGCGCACAGGGCG CAGCCCTGGTTAAAAACAAGGTTTATAAATATTGGTTTAAAAGCAGGTTAAAAGACAGGTTAG CGGTGGCCGAAAAACGGGCGGAAACCCTTGCAAATGCTGGATTTTCTGCCTGTGGACAGCC CCTCAAATGTCAATAGGTGCGCCCCTCATCTGTCAGCACTCTGCCCCTCAAGTGTCAAGGAT CGCGCCCCTCATCTGTCAGTAGTCGCGCCCCTCAAGTGTCAATACCGCAGGGCACTTATCC CCAGGCTTGTCCACATCATCTGTGGGAAACTCGCGTAAAATCAGGCGTTTTCGCCGATTTGC GAGGCTGGCCAGCTCCACGTCGCCGGCCGAAATCGAGCCTGCCCCTCATCTGTCAACGCC GCGCCGGGTGAGTCGGCCCCTCAAGTGTCAACGTCCGCCCCTCATCTGTCAGTGAGGGCC AAGTTTTCCGCGAGGTATCCACAACGCCGGCGGCCGGCCGCGGTGTCTCGCACACGGCTT CGACGGCGTTTCTGGCGCGTTTGCAGGGCCATAGACGGCCGCCAGCCCAGCGGCGAGGG CAACCAGCCGAGGGCTTCGCCCTGTCGCTCGACTGCGGCGAGCACTACTGGCTGTAAAAG GACAGACCACATCATGGTTCTGTGTTCATTAGGTTGTTCTGTCCATTGCTGACATAATCCGC TCCACTTCAACGTAACACCGCACGAAGATTTCTATTGTTCCTGAAGGCATATTCAAATCGTTT TCGTTACCGCTTGCAGGCATCATGACAGAACACTACTTCCTATAAACGCTACACAGGCTCCT

	GAGATTAATAATGCGGATCTCTACGATAATGGGAGATTTTCCCGACTGTTTCGTTCGCTTCT CAGTGGATAACAGCCAGCTTCTCTGTTTAACAGACAAAAACAGCATATCCACTCAGTTCCAC ATTTCCATATAAAGGCCAAGGCATTTATTCTCAGGATAATTGTTTCAGCATCGCAACCGCATC AGACTCCGGCATCGCAAACTGCACCCGGTGCCGGGCAGCCACATCCAGCGCAAAAACCTT CGTGTAGACTTCCGTTGAACTGATGGACTTATGTCCCATCAGGCTTTGCAGAACTTTCAGCG GTATACCGGCATACAGCATGTGCATCGCATAGGAATGGCGGAACGTATGTGGTGTGACCGG AACAGAGAACGTCACACCGTCAGCAGCAGCGGCGGCAACCGCCTCCCCAATCCAGGTCCT GACCGTTCTGTCCGTCACTTCCCAGATCCGCGCTTTCTCTGTCCTTCCTGTGCGACGGTTAC GCCGCTCCATGAGCTTATCGCGAATAAATACCTGTGACGGAAGATCACTTCGCAGAATAAAT AAATCCTGGTGTCCCTGTTGATACCGGGAAGCCCTGGGCCAACTTTTGGCGAAAATGAGAC GTTGATCGGCACGTAAGAGGTTCCAACTTTCACCATAATGAAATAAGATCACTACCGGGCGT ATTTTTTGAGTTATCGAGATTTTCAGGAGCTAA

pZA1pLtetO-GFP	GACGTCTCCCTATCAGTGATAGAGATTGACATCCCTATCAGTGATAGAGATACTGAGCACAT CAGCAGGACGCACTGACCGAATTCATTAAAGAGGAGAAAGGTACCATGAGTAAAGGAGAAG AACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAAT TTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATT TGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTTTCGGTTATGGTGT TCAATGCTTTGCGAGATACCCAGATCATATGAAACAGCATGACTTTTTCAAGAGTGCCATGC CCGAAGGTTATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGT GCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTT AAAGAAGATGGAAACATTCTTGGACACAAATTGGAATACAACTATAACTCACACAATGTATAC ATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTGAA GATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGT CCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAAGATCCCAACGAAA AGAGAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACATGGCATGGAT GATCTCTACAAATAAAAGCTTGATATCGAATTCCTGCAGCCCGGGGGATCCCATGGTACGC GTGCTAGAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCT GTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGCCCTAGACCTAGGGGAT ATATTCCGCTTCCTCGCTCACTGACTCGCTACGCTCGGTCGTTCGACTGCGGCGAGCGGAA ATGGCTTACGAACGGGGCGGAGATTTCCTGGAAGATGCCAGGAAGATACTTAACAGGGAAG TGAGAGGGCCGCGGCAAAGCCGTTTTTCCATAGGCTCCGCCCCCCTGACAAGCATCACGA AATCTGACGCTCAAATCAGTGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTT CCCCCTGGCGGCTCCCTCGTGCGCTCTCCTGTTCCTGCCTTTCGGTTTACCGGTGTCATTC CGCTGTTATGGCCGCGTTTGTCTCATTCCACGCCTGACACTCAGTTCCGGGTAGGCAGTTC GCTCCAAGCTGGACTGTATGCACGAACCCCCCGTTCAGTCCGACCGCTGCGCCTTATCCGG TAACTATCGTCTTGAGTCCAACCCGGAAAGACATGCAAAAGCACCACTGGCAGCAGCCACT GGTAATTGATTTAGAGGAGTTAGTCTTGAAGTCATGCGCCGGTTAAGGCTAAACTGAAAGGA CAAGTTTTGGTGACTGCGCTCCTCCAAGCCAGTTACCTCGGTTCAAAGAGTTGGTAGCTCA GAGAACCTTCGAAAAACCGCCCTGCAAGGCGGTTTTTTCGTTTTCAGAGCAAGAGATTACGC GCAGACCAAAACGATCTCAAGAAGATCATCTTATTAATCAGATAAAATATTTCTAGATTTCAG TGCAATTTATCTCTTCAAATGTAGCACCTGAAGTCAGCCCCATACGATATAAGTTGTTACTAG TGCTTGGATTCTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAG ATGGAGTTCTGAGGTCATTACTGGATCTATCAACAGGAGTCCAAGCGAGCTCGTAAACTTGG TCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCA TCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTG GCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAAT AAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCAT CCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCA ACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTC AGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGG TTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATG GTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACT GGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCC CGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGA AAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTA ACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAG CAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAAT ACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGA TACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAA GTGCCACCT

PhiC GFP BAC Reporter	GGAAGCTAAAATGGAGAAAAAAATCACTGGATATACCACCGTTGATATATCCCAATGGCATC GTAAAGAACATTTTGAGGCATTTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGTTCAG CTGGATATTACGGCCTTTTTAAAGACCGTAAAGAAAAATAAGCACAAGTTTTATCCGGCCTTT ATTCACATTCTTGCCCGCCTGATGAATGCTCATCCGGAATTTCGTATGGCAATGAAAGACGG TGAGCTGGTGATATGGGATAGTGTTCACCCTTGTTACACCGTTTTCCATGAGCAAACTGAAA CGTTTTCATCGCTCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACATATATTCG CAAGATGTGGCGTGTTACGGTGAAAACCTGGCCTATTTCCCTAAAGGGTTTATTGAGAATAT GTTTTTCGTCTCAGCCAATCCCTGGGTGAGTTTCACCAGTTTTGATTTAAACGTGGCCAATAT GGACAACTTCTTCGCCCCCGTTTTCACCATGGGCAAATATTATACGCAAGGCGACAAGGTG CTGATGCCGCTGGCGATTCAGGTTCATCATGCCGTTTGTGATGGCTTCCATGTCGGCAGAA TGCTTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGGCGTAAGACGTCTAAGA AACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTTCA CCTCGAGCACAGCTAACACCACGTCGTCCCTATCTGCTGCCCTAGGTCTATGAGTGGTTGC TGGATAACTTTACGGGCATGCATAAGGCTCGTATAATATATTCAGGGAGACCACAACGGTTT CCCTCTACAAATAATTTTGTTTAACTTTTTAATTAATGCGGGTGCCAGGGCGTGCCCTTGGG CTCCCCGGGCGCGTACTCCATCGATTCTAGGGCGGCGGATTTGTCCTACTCAGGAGAGCG TTCACCGACAAACAACAGATAAAACGAAAGGCCCAGTCTTTCGACTGAGCCTTTCGTTTTAT TTGATGCCTCTAGCACGCGTACCATGGGATCCCCCGGGCTGCAGGAATTCGATATCAAGCT TTTATTTGTAGAGATCATCCATGCCATGTGTAATCCCAGCAGCTGTTACAAACTCAAGAAGG ACCATGTGGTCTCTCTTTTCGTTGGGATCTTTCGAAAGGGCAGATTGTGTGGACAGGTAATG GTTGTCTGGTAAAAGGACAGGGCCATCGCCAATTGGAGTATTTTGTTGATAATGGTCTGCTA GTTGAACGCTTCCATCTTCAATGTTGTGTCTAATTTTGAAGTTAACTTTGATTCCATTCTTTTG TTTGTCTGCCATGATGTATACATTGTGTGAGTTATAGTTGTATTCCAATTTGTGTCCAAGAAT GTTTCCATCTTCTTTAAAATCAATACCTTTTAACTCGATTCTATTAACAAGGGTATCACCTTCA AACTTGACTTCAGCACGTGTCTTGTAGTTCCCGTCATCTTTGAAAAATATAGTTCTTTCCTGT ACATAACCTTCGGGCATGGCACTCTTGAAAAAGTCATGCTGTTTCATATGATCTGGGTATCT CGCAAAGCATTGAACACCATAACCGAAAGTAGTGACAAGTGTTGGCCATGGAACAGGTAGT TTTCCAGTAGTGCAAATAAATTTAAGGGTAAGTTTTCCGTATGTTGCATCACCTTCACCCTCT CCACTGACAGAAAATTTGTGCCCATTAACATCACCATCTAATTCAACAAGAATTGGGACAACT CCAGTGAAAAGTTCTTCTCCTTTACTCATCTTAAACCTCCTTACCTCGTAAACTATTAAACAAA ATTATTTGTAGAGGCTGTTTCGTCCTCACGGACTCATCAGACCGGAAAGCACATCCGGTGAC AGCTGTGCACCCCCCAACTGAGAGAACTCAAAGGTTACCCCAGTTGGGGCACCGAGCACC GTACTTGCCCTTGACAGGCATTGATGGAATCGTAGTCTCACGCTGATAGTCTGATCGACAAT ACAAGTGGGACCGTGGTCCCAGACCGATAATCAGACCGACAACACGAGTGGGATCGTGGT CCCAGACTAATAATCAGACCGACGATACGAGTGGGACCGTGGTCCCAGACTAATAATCAGA CCGACGATACGAGTGGGACCGTGGTTCCAGACTAATAATCAGACCGACGATACGAGTGGGA CCGTGGTCCCAGACTAATAATCAGACCGACGATACGAGTGGGACCATGGTCCCAGACTAAT AATCAGACCGACGATACGAGTGGGACCGTGGTCCCAGTCTGATTATCAGACCGACGATACG AGTGGGACCGTGGTCCCAGACTAATAATCAGACCGACGATACGAGTGGGACCGTGGTCCC AGACTAATAATCAGACCGACGATACGAGTGGGACCGTGGTCCCAGTCTGATTATCAGACCG ACGATACAAGTGGAACAGTGGGCCCAGAGAGAATATTCAGGCCAGTTATGCTTTCTGGCCT GTAACAAAGGACATTAAGTAAAGACAGATAAACGTAGACTAAAACGTGGTCGCATCAGGGTG CTGGCTTTTCAAGTTCCTTAAGAATGGCCTCAATTTTCTCTATACACTCAGTTGGAACACGGG ACCTGTCCAGGTTAAGCACCATTTTATCGCCCTTATACAATACTGTCGCTCCAGGAGCAAAC TGATGTCGTGAGCTTAAACTAGTTCTTGATGCAGATGACGTTTTAAGCACAGAAGTTAAAAG AGTGATAACTTCTTCAGCTTCAAATATCACCCCAGCTTTTTTCTGCTCATGAAGGTTAGATGC CTGCTGCTTAAGTAATTCCTCTTTATCTGTAAAGGCTTTTTGAAGTGCATCACCTGACCGGG CAGATAGTTCACCGGGGTGAGAAAAAAGAGCAACAACTGATTTAGGCAATTTGGCGGTGTT GATACAGCGGGTAATAATCTTACGTGAAATATTTTCCGCATCAGCCAGCGCAGAAATATTTC CAGCAAATTCATTCTGCAATCGGCTTGCATAACGCTGACCACGTTCATAAGCACTTGTTGGG

	CGATAATCGTTACCCAATCTGGATAATGCAGCCATCTGCTCATCATCCAGCTCGCCAACCAG AACACGATAATCACTTTCGGTAAGTGCAGCAGCTTTACGACGGCGACTCCCATCGGCAATTT CTATGACACCAGATACTCTTCGACCGAACGCCGGTGTCTGTTGACCAGTCAGTAGAAAAGA AGGGATGAGATCATCCAGTGCGTCCTCAGTAAGCAGCTCCTGGTCACGTTCATTACCTGAC CATACCCGAGAGGTCTTCTCAACACTATCACCCCGGAGCACTTCAAGAGTAAACTTCACATC CCGACCACATACAGGCAAAGTAATGGCATTACCGCGAGCCATTACTCCTACGCGCGCAATT AACGAATCCACCATCGGGGCAGCTGGTGTCGATAACGAAGTATCTTCAACCGGTTGAGTAT TGAGCGTATGTTTTGGAATAACAGGCGCACGCTTCATTATCTAATCTCCCAGCGTGGTTTAA TCAGACGATCGAAAATTTCATTGCAGACAGGTTCCCAAATAGAAAGAGCATTTCTCCAGGCA CCAGTTGAAGAGCGTTGATCAATGGCCTGTTCAAAAACAGTTCTCATCCGGATCTGACCTTT ACCAACTTCATCCGTTTCACGTACAACATTTTTTAGAACCATGCTTCCCCAGGCATCCCGAAT TTGCTCCTCCATCCACGGGGACTGAGAGCCATTACTATTGCTGTATTTGGTAAGCAAAATAC GTACATCAGGCTCGAACCCTTTAAGATCAACGTTCTTGAGCAGATCACGAAGCATATCGAAA AACTGCAGTGCGGAGGTGTAGTCAAACAACTCAGCAGGCGTGGGAACAATCAGCACATCAG CAGCACATACGACATTAATCGTGCCGATACCCAGGTTAGGCGCGCTGTCAATAACTATGACA TCATAGTCATGAGCAACAGTTTCAATGGCCAGTCGGAGCATCAGGTGTGGATCGGTGGGCA GTTTACCTTCATCAAATTTGCCCATTAACTCAGTTTCAATACGGTGCAGAGCCAGACAGGAA GGAATAATGTCAAGCCCCGGCCAGCAAGTGGGCTTTATTGCATAAGTGACATCGTCCTTTTC CCCAAGATAGAAAGGCAGGAGAGTGTCTTCTGCATGAATATGAAGATCTGGTACCCATCCG TGATACATTGAGGCTGTTCCCTGGGGGTCGTTACCTTCCACGAGCAAAACACGTAGCCCCT TCAGAGCCAGATCCTGAGCAAGATGAACAGAAACTGAGGTTTTGTAAACGCCACCTTTATGG GCAGCAACCCCGATCACCGGTGGAAATACGTCTTCAGCACGTCGCAATCGCGTACCAAACA CATCACGCATATGATTAATTTGTTCAATTGTATAACCAACACGTTGCTCAACCCGTCCTCGAA TTTCCATATCCGGGTGCGGTAGTCGCCCTGCTTTCTCGGCATCTCTGATAGCCTGAGAAGA AACCCCAACTAAATCCGCTGCTTCACCTATTCTCCAGCGCCGGGTTATTTTCCTCGCTTCCG GGCTGTCATCATTAAACTGTGCAATGGCGATAGCCTTCGTCATTTCATGACCAGCGTTTATG CACTGGTTAAGTGTTTCCATGAGTTTCATTCTGAACATCCTTTAATCATTGCTTTGCGTTTTTT TATTAAATCTTGCAATTTACTGCAAAGCAACAACAAAATCGCAAAGTCATCAAAAAACCGCAA AGTTGTTTAAAATAAGAGCAACACTACAAAAGGAGATAAGAAGAGCACATACCTCAGTCACT TATTATCACTAGCGCTCGCCGCAGCCGTGTAACCGAGCATAGCGAGCGAACTGGCGAGGA AGCAAAGAAGAACTGTTCTGTCAGATAGCTCTTACGCTCAGCGCAAGAAGAAATATCCACCG TGGGAAAAACTCCAGGTAGAGGTACACACGCGGATAGCCAATTCAGAGTAATAAACTGTGA TAATCAACCCTCATCAATGATGACGAACTAACCCCCGATATCAGGTCACATGACGAAGGGAA AGAGAAGGAAATCAACTGTGACAAACTGCCCTCAAATTTGGCTTCCTTAAAAATTACAGTTCA AAAAGTATGAGAAAATCCATGCAGGCTGAAGGAAACAGCAAAACTGTGACAAATTACCCTCA GTAGGTCAGAACAAATGTGACGAACCACCCTCAAATCTGTGACAGATAACCCTCAGACTATC CTGTCGTCATGGAAGTGATATCGCGGAAGGAAAATACGATATGAGTCGTCTGGCGGCCTTT CTTTTTCTCAATGTATGAGAGGCGCATTGGAGTTCTGCTGTTGATCTCATTAACACAGACCT GCAGGAAGCGGCGGCGGAAGTCAGGCATACGCTGGTAACTTTGAGGCAGCTGGTAACGCT CTATGATCCAGTCGATTTTCAGAGAGACGATGCCTGAGCCATCCGGCTTACGATACTGACAC AGGGATTCGTATAAACGCATGGCATACGGATTGGTGATTTCTTTTGTTTCACTAAGCCGAAA CTGCGTAAACCGGTTCTGTAACCCGATAAAGAAGGGAATGAGATATGGGTTGATATGTACAC TGTAAAGCCCTCTGGATGGACTGTGCGCACGTTTGATAAACCAAGGAAAAGATTCATAGCCT TTTTCATCGCCGGCATCCTCTTCAGGGCGATAAAAAACCACTTCCTTCCCCGCGAAACTCTT CAATGCCTGCCGTATATCCTTACTGGCTTCCGCAGAGGTCAATCCGAATATTTCAGCATATT TAGCAACATGGATCTCGCAGATACCGTCATGTTCCTGTAGGGTGCCATCAGATTTTCTGATC TGGTCAACGAACAGATACAGCATACGTTTTTGATCCCGGGAGAGACTATATGCCGCCTCAGT GAGGTCGTTTGACTGGACGATTCGCGGGCTATTTTTACGTTTCTTGTGATTGATAACCGCTG TTTCCGCCATGACAGATCCATGTGAAGTGTGACAAGTTTTTAGATTGTCACACTAAATAAAAA AGAGTCAATAAGCAGGGATAACTTTGTGAAAAAACAGCTTCTTCTGAGGGCAATTTGTCACA

	GGGTTAAGGGCAATTTGTCACAGACAGGACTGTCATTTGAGGGTGATTTGTCACACTGAAAG GGCAATTTGTCACAACACCTTCTCTAGAACCAGCATGGATAAAGGCCTACAAGGCGCTCTAA AAAAGAAGATCTAAAAACTATAAAAAAAATAATTATAAAAATATCCCCGTGGATAAGTGGATA ACCCCAAGGGAAGTTTTTTCAGGCATCGTGTGTAAGCAGAATATATAAGTGCTGTTCCCTGG TGCTTCCTCGCTCACTCGACCGGGAGGGTTCGAGAAGGGGGGGCACCCCCCTTCGGCGTG CGCGGTCACGCGCACAGGGCGCAGCCCTGGTTAAAAACAAGGTTTATAAATATTGGTTTAA AAGCAGGTTAAAAGACAGGTTAGCGGTGGCCGAAAAACGGGCGGAAACCCTTGCAAATGCT GGATTTTCTGCCTGTGGACAGCCCCTCAAATGTCAATAGGTGCGCCCCTCATCTGTCAGCA CTCTGCCCCTCAAGTGTCAAGGATCGCGCCCCTCATCTGTCAGTAGTCGCGCCCCTCAAGT GTCAATACCGCAGGGCACTTATCCCCAGGCTTGTCCACATCATCTGTGGGAAACTCGCGTA AAATCAGGCGTTTTCGCCGATTTGCGAGGCTGGCCAGCTCCACGTCGCCGGCCGAAATCG AGCCTGCCCCTCATCTGTCAACGCCGCGCCGGGTGAGTCGGCCCCTCAAGTGTCAACGTC CGCCCCTCATCTGTCAGTGAGGGCCAAGTTTTCCGCGAGGTATCCACAACGCCGGCGGCC GGCCGCGGTGTCTCGCACACGGCTTCGACGGCGTTTCTGGCGCGTTTGCAGGGCCATAGA CGGCCGCCAGCCCAGCGGCGAGGGCAACCAGCCGAGGGCTTCGCCCTGTCGCTCGACTG CGGCGAGCACTACTGGCTGTAAAAGGACAGACCACATCATGGTTCTGTGTTCATTAGGTTGT TCTGTCCATTGCTGACATAATCCGCTCCACTTCAACGTAACACCGCACGAAGATTTCTATTGT TCCTGAAGGCATATTCAAATCGTTTTCGTTACCGCTTGCAGGCATCATGACAGAACACTACT TCCTATAAACGCTACACAGGCTCCTGAGATTAATAATGCGGATCTCTACGATAATGGGAGAT TTTCCCGACTGTTTCGTTCGCTTCTCAGTGGATAACAGCCAGCTTCTCTGTTTAACAGACAAA AACAGCATATCCACTCAGTTCCACATTTCCATATAAAGGCCAAGGCATTTATTCTCAGGATAA TTGTTTCAGCATCGCAACCGCATCAGACTCCGGCATCGCAAACTGCACCCGGTGCCGGGCA GCCACATCCAGCGCAAAAACCTTCGTGTAGACTTCCGTTGAACTGATGGACTTATGTCCCAT CAGGCTTTGCAGAACTTTCAGCGGTATACCGGCATACAGCATGTGCATCGCATAGGAATGG CGGAACGTATGTGGTGTGACCGGAACAGAGAACGTCACACCGTCAGCAGCAGCGGCGGCA ACCGCCTCCCCAATCCAGGTCCTGACCGTTCTGTCCGTCACTTCCCAGATCCGCGCTTTCT CTGTCCTTCCTGTGCGACGGTTACGCCGCTCCATGAGCTTATCGCGAATAAATACCTGTGAC GGAAGATCACTTCGCAGAATAAATAAATCCTGGTGTCCCTGTTGATACCGGGAAGCCCTGG GCCAACTTTTGGCGAAAATGAGACGTTGATCGGCACGTAAGAGGTTCCAACTTTCACCATAA TGAAATAAGATCACTACCGGGCGTATTTTTTGAGTTATCGAGATTTTCAGGAGCTAA

pBAC Bxbi TetR	GGAAGCTAAAATGGAGAAAAAAATCACTGGATATACCACCGTTGATATATCCCAATGGCATC GTAAAGAACATTTTGAGGCATTTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGTTCAG CTGGATATTACGGCCTTTTTAAAGACCGTAAAGAAAAATAAGCACAAGTTTTATCCGGCCTTT ATTCACATTCTTGCCCGCCTGATGAATGCTCATCCGGAATTTCGTATGGCAATGAAAGACGG TGAGCTGGTGATATGGGATAGTGTTCACCCTTGTTACACCGTTTTCCATGAGCAAACTGAAA CGTTTTCATCGCTCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACATATATTCG CAAGATGTGGCGTGTTACGGTGAAAACCTGGCCTATTTCCCTAAAGGGTTTATTGAGAATAT GTTTTTCGTCTCAGCCAATCCCTGGGTGAGTTTCACCAGTTTTGATTTAAACGTGGCCAATAT GGACAACTTCTTCGCCCCCGTTTTCACCATGGGCAAATATTATACGCAAGGCGACAAGGTG CTGATGCCGCTGGCGATTCAGGTTCATCATGCCGTTTGTGATGGCTTCCATGTCGGCAGAA TGCTTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGGCGTAAGACGTCTAAGA AACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTTCA CCTCGAGCACAGCTAACACCACGTCGTCCCTATCTGCTGCCCTAGGTCTATGAGTGGTTGC TGGATAACTTTACGGGCATGCATAAGGCTCGTATAATATATTCAGGGAGACCACAACGGTTT CCCTCTACAAATAATTTTGTTTAACTTTTTAATTAACGGCCGGCTTGTCGACGACGGCGGTCT CCGTCGTCAGGATCATCCGGGCATCGATTCTAGGGCGGCGGATTTGTCCTACTCAGGAGAG CGTTCACCGACAAACAACAGATAAAACGAAAGGCCCAGTCTTTCGACTGAGCCTTTCGTTTT ATTTGATGCCTCTAGCACGCGTACCATGGGATCCCCCGGGCTGCAGGAATTCGATATCAAG CTTTTAAGACCCACTTTCACATTTAAGTTGTTTTTCTAATCCGCATATGATCAATTCAAGGCC GAATAAGAAGGCTGGCTCTGCACCTTGGTGATCAAATAATTCGATAGCTTGTCGTAATAATG GCGGCATACTATCAGTAGTAGGTGTTTCCCTTTCTTCTTTAGCGACTTGATGCTCTTGATCTT CCAATACGCAACCTAAAGTAAAATGCCCCACAGCGCTGAGTGCATATAATGCATTCTCTAGT GAAAAACCTTGTTGGCATAAAAAGGCTAATTGATTTTCGAGAGTTTCATACTGTTTTTCTGTA GGCCGTGTACCTAAATGTACTTTTGCTCCATCGCGATGACTTAGTAAAGCACATCTAAAACTT TTAGCGTTATTACGTAAAAAATCTTGCCAGCTTTCCCCTTCTAAAGGGCAAAAGTGAGTATG GTGCCTATCTAACATCTCAATGGCTAAGGCGTCGAGCAAAGCCCGCTTATTTTTTACATGCC AATACAATGTAGGCTGCTCTACACCTAGCTTCTGGGCGAGTTTACGGGTTGTTAAACCTTCG ATTCCGACCTCATTAAGCAGCTCTAATGCGCTGTTAATCACTTTACTTTTATCTAATCTAGAC ATGTTGAACCTCCTTAATCTTATTTCCGCTAACGCTTAAACAAAATTATTTGTAGAGGCTGTTT CGTCCTCACGGACTCATCAGACCGGAAAGCACATCCGGTGACAGCTGTGCACGTCGGGGT TTGTACCGTACACCACTGAGACCGCGGTGGTTGACCAGACAAACCACGACACATGTCAATC CTTTGGTCGAAAAAAAAAGCCCGCACTGTCAGGTGCGGGCTTTTTTCTGTGTTTCCACTTGC CCTTGACAGGCATTGATGGAATCGTAGTCTCACGCTGATAGTCTGATCGACAATACAAGTGG GACCGTGGTCCCAGACCGATAATCAGACCGACAACACGAGTGGGATCGTGGTCCCAGACT AATAATCAGACCGACGATACGAGTGGGACCGTGGTCCCAGACTAATAATCAGACCGACGAT ACGAGTGGGACCGTGGTTCCAGACTAATAATCAGACCGACGATACGAGTGGGACCGTGGT CCCAGACTAATAATCAGACCGACGATACGAGTGGGACCATGGTCCCAGACTAATAATCAGA CCGACGATACGAGTGGGACCGTGGTCCCAGTCTGATTATCAGACCGACGATACGAGTGGG ACCGTGGTCCCAGACTAATAATCAGACCGACGATACGAGTGGGACCGTGGTCCCAGACTAA TAATCAGACCGACGATACGAGTGGGACCGTGGTCCCAGTCTGATTATCAGACCGACGATAC AAGTGGAACAGTGGGCCCAGAGAGAATATTCAGGCCAGTTATGCTTTCTGGCCTGTAACAA AGGACATTAAGTAAAGACAGATAAACGTAGACTAAAACGTGGTCGCATCAGGGTGCTGGCT TTTCAAGTTCCTTAAGAATGGCCTCAATTTTCTCTATACACTCAGTTGGAACACGGGACCTGT CCAGGTTAAGCACCATTTTATCGCCCTTATACAATACTGTCGCTCCAGGAGCAAACTGATGT CGTGAGCTTAAACTAGTTCTTGATGCAGATGACGTTTTAAGCACAGAAGTTAAAAGAGTGAT AACTTCTTCAGCTTCAAATATCACCCCAGCTTTTTTCTGCTCATGAAGGTTAGATGCCTGCTG CTTAAGTAATTCCTCTTTATCTGTAAAGGCTTTTTGAAGTGCATCACCTGACCGGGCAGATAG TTCACCGGGGTGAGAAAAAAGAGCAACAACTGATTTAGGCAATTTGGCGGTGTTGATACAG CGGGTAATAATCTTACGTGAAATATTTTCCGCATCAGCCAGCGCAGAAATATTTCCAGCAAA TTCATTCTGCAATCGGCTTGCATAACGCTGACCACGTTCATAAGCACTTGTTGGGCGATAAT

	CGTTACCCAATCTGGATAATGCAGCCATCTGCTCATCATCCAGCTCGCCAACCAGAACACGA TAATCACTTTCGGTAAGTGCAGCAGCTTTACGACGGCGACTCCCATCGGCAATTTCTATGAC ACCAGATACTCTTCGACCGAACGCCGGTGTCTGTTGACCAGTCAGTAGAAAAGAAGGGATG AGATCATCCAGTGCGTCCTCAGTAAGCAGCTCCTGGTCACGTTCATTACCTGACCATACCCG AGAGGTCTTCTCAACACTATCACCCCGGAGCACTTCAAGAGTAAACTTCACATCCCGACCAC ATACAGGCAAAGTAATGGCATTACCGCGAGCCATTACTCCTACGCGCGCAATTAACGAATCC ACCATCGGGGCAGCTGGTGTCGATAACGAAGTATCTTCAACCGGTTGAGTATTGAGCGTAT GTTTTGGAATAACAGGCGCACGCTTCATTATCTAATCTCCCAGCGTGGTTTAATCAGACGAT CGAAAATTTCATTGCAGACAGGTTCCCAAATAGAAAGAGCATTTCTCCAGGCACCAGTTGAA GAGCGTTGATCAATGGCCTGTTCAAAAACAGTTCTCATCCGGATCTGACCTTTACCAACTTC ATCCGTTTCACGTACAACATTTTTTAGAACCATGCTTCCCCAGGCATCCCGAATTTGCTCCTC CATCCACGGGGACTGAGAGCCATTACTATTGCTGTATTTGGTAAGCAAAATACGTACATCAG GCTCGAACCCTTTAAGATCAACGTTCTTGAGCAGATCACGAAGCATATCGAAAAACTGCAGT GCGGAGGTGTAGTCAAACAACTCAGCAGGCGTGGGAACAATCAGCACATCAGCAGCACATA CGACATTAATCGTGCCGATACCCAGGTTAGGCGCGCTGTCAATAACTATGACATCATAGTCA TGAGCAACAGTTTCAATGGCCAGTCGGAGCATCAGGTGTGGATCGGTGGGCAGTTTACCTT CATCAAATTTGCCCATTAACTCAGTTTCAATACGGTGCAGAGCCAGACAGGAAGGAATAATG TCAAGCCCCGGCCAGCAAGTGGGCTTTATTGCATAAGTGACATCGTCCTTTTCCCCAAGATA GAAAGGCAGGAGAGTGTCTTCTGCATGAATATGAAGATCTGGTACCCATCCGTGATACATTG AGGCTGTTCCCTGGGGGTCGTTACCTTCCACGAGCAAAACACGTAGCCCCTTCAGAGCCAG ATCCTGAGCAAGATGAACAGAAACTGAGGTTTTGTAAACGCCACCTTTATGGGCAGCAACCC CGATCACCGGTGGAAATACGTCTTCAGCACGTCGCAATCGCGTACCAAACACATCACGCAT ATGATTAATTTGTTCAATTGTATAACCAACACGTTGCTCAACCCGTCCTCGAATTTCCATATC CGGGTGCGGTAGTCGCCCTGCTTTCTCGGCATCTCTGATAGCCTGAGAAGAAACCCCAACT AAATCCGCTGCTTCACCTATTCTCCAGCGCCGGGTTATTTTCCTCGCTTCCGGGCTGTCATC ATTAAACTGTGCAATGGCGATAGCCTTCGTCATTTCATGACCAGCGTTTATGCACTGGTTAA GTGTTTCCATGAGTTTCATTCTGAACATCCTTTAATCATTGCTTTGCGTTTTTTTATTAAATCTT GCAATTTACTGCAAAGCAACAACAAAATCGCAAAGTCATCAAAAAACCGCAAAGTTGTTTAAA ATAAGAGCAACACTACAAAAGGAGATAAGAAGAGCACATACCTCAGTCACTTATTATCACTA GCGCTCGCCGCAGCCGTGTAACCGAGCATAGCGAGCGAACTGGCGAGGAAGCAAAGAAGA ACTGTTCTGTCAGATAGCTCTTACGCTCAGCGCAAGAAGAAATATCCACCGTGGGAAAAACT CCAGGTAGAGGTACACACGCGGATAGCCAATTCAGAGTAATAAACTGTGATAATCAACCCTC ATCAATGATGACGAACTAACCCCCGATATCAGGTCACATGACGAAGGGAAAGAGAAGGAAA TCAACTGTGACAAACTGCCCTCAAATTTGGCTTCCTTAAAAATTACAGTTCAAAAAGTATGAG AAAATCCATGCAGGCTGAAGGAAACAGCAAAACTGTGACAAATTACCCTCAGTAGGTCAGAA CAAATGTGACGAACCACCCTCAAATCTGTGACAGATAACCCTCAGACTATCCTGTCGTCATG GAAGTGATATCGCGGAAGGAAAATACGATATGAGTCGTCTGGCGGCCTTTCTTTTTCTCAAT GTATGAGAGGCGCATTGGAGTTCTGCTGTTGATCTCATTAACACAGACCTGCAGGAAGCGG CGGCGGAAGTCAGGCATACGCTGGTAACTTTGAGGCAGCTGGTAACGCTCTATGATCCAGT CGATTTTCAGAGAGACGATGCCTGAGCCATCCGGCTTACGATACTGACACAGGGATTCGTA TAAACGCATGGCATACGGATTGGTGATTTCTTTTGTTTCACTAAGCCGAAACTGCGTAAACC GGTTCTGTAACCCGATAAAGAAGGGAATGAGATATGGGTTGATATGTACACTGTAAAGCCCT CTGGATGGACTGTGCGCACGTTTGATAAACCAAGGAAAAGATTCATAGCCTTTTTCATCGCC GGCATCCTCTTCAGGGCGATAAAAAACCACTTCCTTCCCCGCGAAACTCTTCAATGCCTGCC GTATATCCTTACTGGCTTCCGCAGAGGTCAATCCGAATATTTCAGCATATTTAGCAACATGG ATCTCGCAGATACCGTCATGTTCCTGTAGGGTGCCATCAGATTTTCTGATCTGGTCAACGAA CAGATACAGCATACGTTTTTGATCCCGGGAGAGACTATATGCCGCCTCAGTGAGGTCGTTT GACTGGACGATTCGCGGGCTATTTTTACGTTTCTTGTGATTGATAACCGCTGTTTCCGCCAT GACAGATCCATGTGAAGTGTGACAAGTTTTTAGATTGTCACACTAAATAAAAAAGAGTCAATA AGCAGGGATAACTTTGTGAAAAAACAGCTTCTTCTGAGGGCAATTTGTCACAGGGTTAAGGG

	CAATTTGTCACAGACAGGACTGTCATTTGAGGGTGATTTGTCACACTGAAAGGGCAATTTGT CACAACACCTTCTCTAGAACCAGCATGGATAAAGGCCTACAAGGCGCTCTAAAAAAGAAGAT CTAAAAACTATAAAAAAAATAATTATAAAAATATCCCCGTGGATAAGTGGATAACCCCAAGGG AAGTTTTTTCAGGCATCGTGTGTAAGCAGAATATATAAGTGCTGTTCCCTGGTGCTTCCTCG CTCACTCGACCGGGAGGGTTCGAGAAGGGGGGGCACCCCCCTTCGGCGTGCGCGGTCAC GCGCACAGGGCGCAGCCCTGGTTAAAAACAAGGTTTATAAATATTGGTTTAAAAGCAGGTTA AAAGACAGGTTAGCGGTGGCCGAAAAACGGGCGGAAACCCTTGCAAATGCTGGATTTTCTG CCTGTGGACAGCCCCTCAAATGTCAATAGGTGCGCCCCTCATCTGTCAGCACTCTGCCCCT CAAGTGTCAAGGATCGCGCCCCTCATCTGTCAGTAGTCGCGCCCCTCAAGTGTCAATACCG CAGGGCACTTATCCCCAGGCTTGTCCACATCATCTGTGGGAAACTCGCGTAAAATCAGGCG TTTTCGCCGATTTGCGAGGCTGGCCAGCTCCACGTCGCCGGCCGAAATCGAGCCTGCCCC TCATCTGTCAACGCCGCGCCGGGTGAGTCGGCCCCTCAAGTGTCAACGTCCGCCCCTCAT CTGTCAGTGAGGGCCAAGTTTTCCGCGAGGTATCCACAACGCCGGCGGCCGGCCGCGGTG TCTCGCACACGGCTTCGACGGCGTTTCTGGCGCGTTTGCAGGGCCATAGACGGCCGCCAG CCCAGCGGCGAGGGCAACCAGCCGAGGGCTTCGCCCTGTCGCTCGACTGCGGCGAGCAC TACTGGCTGTAAAAGGACAGACCACATCATGGTTCTGTGTTCATTAGGTTGTTCTGTCCATT GCTGACATAATCCGCTCCACTTCAACGTAACACCGCACGAAGATTTCTATTGTTCCTGAAGG CATATTCAAATCGTTTTCGTTACCGCTTGCAGGCATCATGACAGAACACTACTTCCTATAAAC GCTACACAGGCTCCTGAGATTAATAATGCGGATCTCTACGATAATGGGAGATTTTCCCGACT GTTTCGTTCGCTTCTCAGTGGATAACAGCCAGCTTCTCTGTTTAACAGACAAAAACAGCATAT CCACTCAGTTCCACATTTCCATATAAAGGCCAAGGCATTTATTCTCAGGATAATTGTTTCAGC ATCGCAACCGCATCAGACTCCGGCATCGCAAACTGCACCCGGTGCCGGGCAGCCACATCC AGCGCAAAAACCTTCGTGTAGACTTCCGTTGAACTGATGGACTTATGTCCCATCAGGCTTTG CAGAACTTTCAGCGGTATACCGGCATACAGCATGTGCATCGCATAGGAATGGCGGAACGTA TGTGGTGTGACCGGAACAGAGAACGTCACACCGTCAGCAGCAGCGGCGGCAACCGCCTCC CCAATCCAGGTCCTGACCGTTCTGTCCGTCACTTCCCAGATCCGCGCTTTCTCTGTCCTTCC TGTGCGACGGTTACGCCGCTCCATGAGCTTATCGCGAATAAATACCTGTGACGGAAGATCA CTTCGCAGAATAAATAAATCCTGGTGTCCCTGTTGATACCGGGAAGCCCTGGGCCAACTTTT GGCGAAAATGAGACGTTGATCGGCACGTAAGAGGTTCCAACTTTCACCATAATGAAATAAGA TCACTACCGGGCGTATTTTTTGAGTTATCGAGATTTTCAGGAGCTAA

Mixed-signal integration BAC Reporter	GGAAGCTAAAATGGAGAAAAAAATCACTGGATATACCACCGTTGATATATCCCAATGGCATC GTAAAGAACATTTTGAGGCATTTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGTTCAG CTGGATATTACGGCCTTTTTAAAGACCGTAAAGAAAAATAAGCACAAGTTTTATCCGGCCTTT ATTCACATTCTTGCCCGCCTGATGAATGCTCATCCGGAATTTCGTATGGCAATGAAAGACGG TGAGCTGGTGATATGGGATAGTGTTCACCCTTGTTACACCGTTTTCCATGAGCAAACTGAAA CGTTTTCATCGCTCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACATATATTCG CAAGATGTGGCGTGTTACGGTGAAAACCTGGCCTATTTCCCTAAAGGGTTTATTGAGAATAT GTTTTTCGTCTCAGCCAATCCCTGGGTGAGTTTCACCAGTTTTGATTTAAACGTGGCCAATAT GGACAACTTCTTCGCCCCCGTTTTCACCATGGGCAAATATTATACGCAAGGCGACAAGGTG CTGATGCCGCTGGCGATTCAGGTTCATCATGCCGTTTGTGATGGCTTCCATGTCGGCAGAA TGCTTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGGCGTAAGACGTCTAAGA AACCATTAATGCCAACACAATTAACATCTCAATCAAGGTAAATGCTTTTTGCTTTTTTTGCATC GATTTGAGAAGAGAAAAGAAAACCGCCGATCCTGTCCACCGCATTACTGCAAGGTAGTGGA CAAGACCGGCGGTCTTAAGTTTTTTGGCTGAAATAGGTGAACATACAAATGCGGAAAAGTTT CTGCCCTCGCACGTAGCTGAGCAAGTCGTAGCCATGCCCCGCAAACCTGGGGTAATCACTG TATCGGGCTGTCTAAGAGCCGTTGGCTGGGCTAGTCGTCCATGGCAGTTTGTCAGTTAGGT CAAGATGCGGGTCTCTGTGTAAACCGGCCCCTGGGGTTTTGGCCCAAACTGCCTGATGCTG CATACCGAATACACGGTGGTTAATTGATGACTGCTTGTGCACTGCTGCGCCTTGCGGAGGC CGGTCCGGCGCGCGAAGCGTATAAATGAAGCTCGTCACATCCACACAGTTGCATCGTACGT CGGTGTAGGGATCGAACTTGGCCTCTAATTCCACATGCGCGCGCTGCAGGTGTAAGTGTGC CAAGTCACCTCGACCACGATGGGCTTTGCCCCAGGTATCCAAAAGGAAATCCTGATGCCGC GCTTCGATTCACCGTAGTGTGGATGTTCCCTTTGTACGGGTTTCCGACTCCTGTTGTAGCAG GCGTCTTTGTTAGCTAGCGCTATCCCCAACGTGCAACAACCCTTACCACGAAGACAGGATT GTCCGATCCTATATTACGACTTTGGCAGGGGGTTCGCAAGTCCCTGCAGGAACGATGCTGA AGGCTCAGGTTACACAGGCACAAGTACTATATATACGAGATGCATCTCTTAACCTGGATCGA ATGCAGAATCATGAATCGTACCACTGTGTTCGCCTGCAGCTCGAGCACAGCTAACACCACG TCGTCCCTATCTGCTGCCCTAGGTCTATGAGTGGTTGCTGGATAACTTTACGGGCATGCATA AGGCTCGTATAATATATTCAGGGAGACCACAACGGTTTCCCTCTACAAATAATTTTGTTTAAC TTTGCTAGCAAAGGAGTTTTTTAGTTACCTTAATTGAAATAAACGAAATAAAAACTCGCTTAG CCCAATCTTCAATACCTCGTATGCCGACAACATGGACCGGTCACCAGCGAGCCCTGTGCGG ACGGGAGGTGCGGGTGCCAGGGCGTGCCCTTGGGCTCCCCGGGCGCGTACTCCTTAATTA ACGGCCGGCTTGTCGACGACGGCGGTCTCCGTCGTCAGGATCATCCGGGCATCGATTCTA GGGCGGCGGATTTGTCCTACTCAGGAGAGCGTTCACCGACAAACAACAGATAAAACGAAAG GCCCAGTCTTTCGACTGAGCCTTTCGTTTTATTTGATGCCTCTAGCACGCGTACCATGGGAT CCCCCGGGCTGCAGGAATTCGATATCAAGCTTTTATTTGTAGAGATCATCCATGCCATGTGT AATCCCAGCAGCTGTTACAAACTCAAGAAGGACCATGTGGTCTCTCTTTTCGTTGGGATCTT TCGAAAGGGCAGATTGTGTGGACAGGTAATGGTTGTCTGGTAAAAGGACAGGGCCATCGCC AATTGGAGTATTTTGTTGATAATGGTCTGCTAGTTGAACGCTTCCATCTTCAATGTTGTGTCT AATTTTGAAGTTAACTTTGATTCCATTCTTTTGTTTGTCTGCCATGATGTATACATTGTGTGAG TTATAGTTGTATTCCAATTTGTGTCCAAGAATGTTTCCATCTTCTTTAAAATCAATACCTTTTAA CTCGATTCTATTAACAAGGGTATCACCTTCAAACTTGACTTCAGCACGTGTCTTGTAGTTCCC GTCATCTTTGAAAAATATAGTTCTTTCCTGTACATAACCTTCGGGCATGGCACTCTTGAAAAA GTCATGCTGTTTCATATGATCTGGGTATCTCGCAAAGCATTGAACACCATAACCGAAAGTAG TGACAAGTGTTGGCCATGGAACAGGTAGTTTTCCAGTAGTGCAAATAAATTTAAGGGTAAGT TTTCCGTATGTTGCATCACCTTCACCCTCTCCACTGACAGAAAATTTGTGCCCATTAACATCA CCATCTAATTCAACAAGAATTGGGACAACTCCAGTGAAAAGTTCTTCTCCTTTACTCATCTTA AACCTCCTTACCTCGTAAACTATTAAACAAAATTATTTGTAGAGGCTGTTTCGTCCTCACGGA CTCATCAGACCGGAAAGCACATCCGGTGACAGCTGTGCACGTCGGGGTTTGTACCGTACAC CACTGAGACCGCGGTGGTTGACCAGACAAACCACGACACATGTCCCCCAACTGAGAGAACT CAAAGGTTACCCCAGTTGGGGCACCGAGCACCGTACTTGCCCTTGACAGGCATTGATGGAA

	TCGTAGTCTCACGCTGATAGTCTGATCGACAATACAAGTGGGACCGTGGTCCCAGACCGAT AATCAGACCGACAACACGAGTGGGATCGTGGTCCCAGACTAATAATCAGACCGACGATACG AGTGGGACCGTGGTCCCAGACTAATAATCAGACCGACGATACGAGTGGGACCGTGGTTCCA GACTAATAATCAGACCGACGATACGAGTGGGACCGTGGTCCCAGACTAATAATCAGACCGA CGATACGAGTGGGACCATGGTCCCAGACTAATAATCAGACCGACGATACGAGTGGGACCGT GGTCCCAGTCTGATTATCAGACCGACGATACGAGTGGGACCGTGGTCCCAGACTAATAATC AGACCGACGATACGAGTGGGACCGTGGTCCCAGACTAATAATCAGACCGACGATACGAGTG GGACCGTGGTCCCAGTCTGATTATCAGACCGACGATACAAGTGGAACAGTGGGCCCAGAG AGAATATTCAGGCCAGTTATGCTTTCTGGCCTGTAACAAAGGACATTAAGTAAAGACAGATA AACGTAGACTAAAACGTGGTCGCATCAGGGTGCTGGCTTTTCAAGTTCCTTAAGAATGGCCT CAATTTTCTCTATACACTCAGTTGGAACACGGGACCTGTCCAGGTTAAGCACCATTTTATCG CCCTTATACAATACTGTCGCTCCAGGAGCAAACTGATGTCGTGAGCTTAAACTAGTTCTTGA TGCAGATGACGTTTTAAGCACAGAAGTTAAAAGAGTGATAACTTCTTCAGCTTCAAATATCAC CCCAGCTTTTTTCTGCTCATGAAGGTTAGATGCCTGCTGCTTAAGTAATTCCTCTTTATCTGT AAAGGCTTTTTGAAGTGCATCACCTGACCGGGCAGATAGTTCACCGGGGTGAGAAAAAAGA GCAACAACTGATTTAGGCAATTTGGCGGTGTTGATACAGCGGGTAATAATCTTACGTGAAAT ATTTTCCGCATCAGCCAGCGCAGAAATATTTCCAGCAAATTCATTCTGCAATCGGCTTGCAT AACGCTGACCACGTTCATAAGCACTTGTTGGGCGATAATCGTTACCCAATCTGGATAATGCA GCCATCTGCTCATCATCCAGCTCGCCAACCAGAACACGATAATCACTTTCGGTAAGTGCAGC AGCTTTACGACGGCGACTCCCATCGGCAATTTCTATGACACCAGATACTCTTCGACCGAACG CCGGTGTCTGTTGACCAGTCAGTAGAAAAGAAGGGATGAGATCATCCAGTGCGTCCTCAGT AAGCAGCTCCTGGTCACGTTCATTACCTGACCATACCCGAGAGGTCTTCTCAACACTATCAC CCCGGAGCACTTCAAGAGTAAACTTCACATCCCGACCACATACAGGCAAAGTAATGGCATTA CCGCGAGCCATTACTCCTACGCGCGCAATTAACGAATCCACCATCGGGGCAGCTGGTGTCG ATAACGAAGTATCTTCAACCGGTTGAGTATTGAGCGTATGTTTTGGAATAACAGGCGCACGC TTCATTATCTAATCTCCCAGCGTGGTTTAATCAGACGATCGAAAATTTCATTGCAGACAGGTT CCCAAATAGAAAGAGCATTTCTCCAGGCACCAGTTGAAGAGCGTTGATCAATGGCCTGTTCA AAAACAGTTCTCATCCGGATCTGACCTTTACCAACTTCATCCGTTTCACGTACAACATTTTTT AGAACCATGCTTCCCCAGGCATCCCGAATTTGCTCCTCCATCCACGGGGACTGAGAGCCAT TACTATTGCTGTATTTGGTAAGCAAAATACGTACATCAGGCTCGAACCCTTTAAGATCAACGT TCTTGAGCAGATCACGAAGCATATCGAAAAACTGCAGTGCGGAGGTGTAGTCAAACAACTC AGCAGGCGTGGGAACAATCAGCACATCAGCAGCACATACGACATTAATCGTGCCGATACCC AGGTTAGGCGCGCTGTCAATAACTATGACATCATAGTCATGAGCAACAGTTTCAATGGCCAG TCGGAGCATCAGGTGTGGATCGGTGGGCAGTTTACCTTCATCAAATTTGCCCATTAACTCAG TTTCAATACGGTGCAGAGCCAGACAGGAAGGAATAATGTCAAGCCCCGGCCAGCAAGTGG GCTTTATTGCATAAGTGACATCGTCCTTTTCCCCAAGATAGAAAGGCAGGAGAGTGTCTTCT GCATGAATATGAAGATCTGGTACCCATCCGTGATACATTGAGGCTGTTCCCTGGGGGTCGT TACCTTCCACGAGCAAAACACGTAGCCCCTTCAGAGCCAGATCCTGAGCAAGATGAACAGA AACTGAGGTTTTGTAAACGCCACCTTTATGGGCAGCAACCCCGATCACCGGTGGAAATACG TCTTCAGCACGTCGCAATCGCGTACCAAACACATCACGCATATGATTAATTTGTTCAATTGTA TAACCAACACGTTGCTCAACCCGTCCTCGAATTTCCATATCCGGGTGCGGTAGTCGCCCTG CTTTCTCGGCATCTCTGATAGCCTGAGAAGAAACCCCAACTAAATCCGCTGCTTCACCTATT CTCCAGCGCCGGGTTATTTTCCTCGCTTCCGGGCTGTCATCATTAAACTGTGCAATGGCGAT AGCCTTCGTCATTTCATGACCAGCGTTTATGCACTGGTTAAGTGTTTCCATGAGTTTCATTCT GAACATCCTTTAATCATTGCTTTGCGTTTTTTTATTAAATCTTGCAATTTACTGCAAAGCAACA ACAAAATCGCAAAGTCATCAAAAAACCGCAAAGTTGTTTAAAATAAGAGCAACACTACAAAAG GAGATAAGAAGAGCACATACCTCAGTCACTTATTATCACTAGCGCTCGCCGCAGCCGTGTAA CCGAGCATAGCGAGCGAACTGGCGAGGAAGCAAAGAAGAACTGTTCTGTCAGATAGCTCTT ACGCTCAGCGCAAGAAGAAATATCCACCGTGGGAAAAACTCCAGGTAGAGGTACACACGCG GATAGCCAATTCAGAGTAATAAACTGTGATAATCAACCCTCATCAATGATGACGAACTAACC

	CCCGATATCAGGTCACATGACGAAGGGAAAGAGAAGGAAATCAACTGTGACAAACTGCCCT CAAATTTGGCTTCCTTAAAAATTACAGTTCAAAAAGTATGAGAAAATCCATGCAGGCTGAAGG AAACAGCAAAACTGTGACAAATTACCCTCAGTAGGTCAGAACAAATGTGACGAACCACCCTC AAATCTGTGACAGATAACCCTCAGACTATCCTGTCGTCATGGAAGTGATATCGCGGAAGGAA AATACGATATGAGTCGTCTGGCGGCCTTTCTTTTTCTCAATGTATGAGAGGCGCATTGGAGT TCTGCTGTTGATCTCATTAACACAGACCTGCAGGAAGCGGCGGCGGAAGTCAGGCATACGC TGGTAACTTTGAGGCAGCTGGTAACGCTCTATGATCCAGTCGATTTTCAGAGAGACGATGCC TGAGCCATCCGGCTTACGATACTGACACAGGGATTCGTATAAACGCATGGCATACGGATTG GTGATTTCTTTTGTTTCACTAAGCCGAAACTGCGTAAACCGGTTCTGTAACCCGATAAAGAA GGGAATGAGATATGGGTTGATATGTACACTGTAAAGCCCTCTGGATGGACTGTGCGCACGT TTGATAAACCAAGGAAAAGATTCATAGCCTTTTTCATCGCCGGCATCCTCTTCAGGGCGATA AAAAACCACTTCCTTCCCCGCGAAACTCTTCAATGCCTGCCGTATATCCTTACTGGCTTCCG CAGAGGTCAATCCGAATATTTCAGCATATTTAGCAACATGGATCTCGCAGATACCGTCATGT TCCTGTAGGGTGCCATCAGATTTTCTGATCTGGTCAACGAACAGATACAGCATACGTTTTTG ATCCCGGGAGAGACTATATGCCGCCTCAGTGAGGTCGTTTGACTGGACGATTCGCGGGCTA TTTTTACGTTTCTTGTGATTGATAACCGCTGTTTCCGCCATGACAGATCCATGTGAAGTGTGA CAAGTTTTTAGATTGTCACACTAAATAAAAAAGAGTCAATAAGCAGGGATAACTTTGTGAAAA AACAGCTTCTTCTGAGGGCAATTTGTCACAGGGTTAAGGGCAATTTGTCACAGACAGGACTG TCATTTGAGGGTGATTTGTCACACTGAAAGGGCAATTTGTCACAACACCTTCTCTAGAACCA GCATGGATAAAGGCCTACAAGGCGCTCTAAAAAAGAAGATCTAAAAACTATAAAAAAAAATAAT TATAAAAATATCCCCGTGGATAAGTGGATAACCCCAAGGGAAGTTTTTTCAGGCATCGTGTG TAAGCAGAATATATAAGTGCTGTTCCCTGGTGCTTCCTCGCTCACTCGACCGGGAGGGTTC GAGAAGGGGGGGCACCCCCCTTCGGCGTGCGCGGTCACGCGCACAGGGCGCAGCCCTGG TTAAAAACAAGGTTTATAAATATTGGTTTAAAAGCAGGTTAAAAGACAGGTTAGCGGTGGCC GAAAAACGGGCGGAAACCCTTGCAAATGCTGGATTTTCTGCCTGTGGACAGCCCCTCAAAT GTCAATAGGTGCGCCCCTCATCTGTCAGCACTCTGCCCCTCAAGTGTCAAGGATCGCGCCC CTCATCTGTCAGTAGTCGCGCCCCTCAAGTGTCAATACCGCAGGGCACTTATCCCCAGGCT TGTCCACATCATCTGTGGGAAACTCGCGTAAAATCAGGCGTTTTCGCCGATTTGCGAGGCT GGCCAGCTCCACGTCGCCGGCCGAAATCGAGCCTGCCCCTCATCTGTCAACGCCGCGCCG GGTGAGTCGGCCCCTCAAGTGTCAACGTCCGCCCCTCATCTGTCAGTGAGGGCCAAGTTTT CCGCGAGGTATCCACAACGCCGGCGGCCGGCCGCGGTGTCTCGCACACGGCTTCGACGG CGTTTCTGGCGCGTTTGCAGGGCCATAGACGGCCGCCAGCCCAGCGGCGAGGGCAACCA GCCGAGGGCTTCGCCCTGTCGCTCGACTGCGGCGAGCACTACTGGCTGTAAAAGGACAGA CCACATCATGGTTCTGTGTTCATTAGGTTGTTCTGTCCATTGCTGACATAATCCGCTCCACTT CAACGTAACACCGCACGAAGATTTCTATTGTTCCTGAAGGCATATTCAAATCGTTTTCGTTAC CGCTTGCAGGCATCATGACAGAACACTACTTCCTATAAACGCTACACAGGCTCCTGAGATTA ATAATGCGGATCTCTACGATAATGGGAGATTTTCCCGACTGTTTCGTTCGCTTCTCAGTGGA TAACAGCCAGCTTCTCTGTTTAACAGACAAAAACAGCATATCCACTCAGTTCCACATTTCCAT ATAAAGGCCAAGGCATTTATTCTCAGGATAATTGTTTCAGCATCGCAACCGCATCAGACTCC GGCATCGCAAACTGCACCCGGTGCCGGGCAGCCACATCCAGCGCAAAAACCTTCGTGTAG ACTTCCGTTGAACTGATGGACTTATGTCCCATCAGGCTTTGCAGAACTTTCAGCGGTATACC GGCATACAGCATGTGCATCGCATAGGAATGGCGGAACGTATGTGGTGTGACCGGAACAGA GAACGTCACACCGTCAGCAGCAGCGGCGGCAACCGCCTCCCCAATCCAGGTCCTGACCGT TCTGTCCGTCACTTCCCAGATCCGCGCTTTCTCTGTCCTTCCTGTGCGACGGTTACGCCGCT CCATGAGCTTATCGCGAATAAATACCTGTGACGGAAGATCACTTCGCAGAATAAATAAATCC TGGTGTCCCTGTTGATACCGGGAAGCCCTGGGCCAACTTTTGGCGAAAATGAGACGTTGAT CGGCACGTAAGAGGTTCCAACTTTCACCATAATGAAATAAGATCACTACCGGGCGTATTTTT TGAGTTATCGAGATTTTCAGGAGCTAA

Bxbi GFP MCP Reporter	TTAATTAACGGCCGGCTTGTCGACGACGGCGGTCTCCGTCGTCAGGATCATCCGGGCATCG ATTCTAGGGCGGCGGATTTGTCCTACTCAGGAGAGCGTTCACCGACAAACAACAGATAAAA CGAAAGGCCCAGTCTTTCGACTGAGCCTTTCGTTTTATTTGATGCCTCTAGCACGCGTACCA TGGGATCCCCCGGGCTGCAGGAATTCGATATCAAGCTTTTATTTGTAGAGATCATCCATGCC ATGTGTAATCCCAGCAGCTGTTACAAACTCAAGAAGGACCATGTGGTCTCTCTTTTCGTTGG GATCTTTCGAAAGGGCAGATTGTGTGGACAGGTAATGGTTGTCTGGTAAAAGGACAGGGCC ATCGCCAATTGGAGTATTTTGTTGATAATGGTCTGCTAGTTGAACGCTTCCATCTTCAATGTT GTGTCTAATTTTGAAGTTAACTTTGATTCCATTCTTTTGTTTGTCTGCCATGATGTATACATTG TGTGAGTTATAGTTGTATTCCAATTTGTGTCCAAGAATGTTTCCATCTTCTTTAAAATCAATAC CTTTTAACTCGATTCTATTAACAAGGGTATCACCTTCAAACTTGACTTCAGCACGTGTCTTGT AGTTCCCGTCATCTTTGAAAAATATAGTTCTTTCCTGTACATAACCTTCGGGCATGGCACTCT TGAAAAAGTCATGCTGTTTCATATGATCTGGGTATCTCGCAAAGCATTGAACACCATAACCG AAAGTAGTGACAAGTGTTGGCCATGGAACAGGTAGTTTTCCAGTAGTGCAAATAAATTTAAG GGTAAGTTTTCCGTATGTTGCATCACCTTCACCCTCTCCACTGACAGAAAATTTGTGCCCATT AACATCACCATCTAATTCAACAAGAATTGGGACAACTCCAGTGAAAAGTTCTTCTCCTTTACT CATGGTACCTTTCTCCTCTTTAATGAATTCTTAAACAAAATTATTTGTAGAGGCTGTTTCGTCC TCACGGACTCATCAGACCGGAAAGCACATCCGGTGACAGCTGTGCACGTCGGGGTTTGTAC CGTACACCACTGAGACCGCGGTGGTTGACCAGACAAACCACGACGCTAGCCGGTCGTTCG ACTGCGGCGAGCGGAAATGGCTTACGAACGGGGCGGAGATTTCCTGGAAGATGCCAGGAA GATACTTAACAGGGAAGTGAGAGGGCCGCGGCAAAGCCGTTTTTCCATAGGCTCCGCCCC CCTGACAAGCATCACGAAATCTGACGCTCAAATCAGTGGTGGCGAAACCCGACAGGACTAT AAAGATACCAGGCGTTTCCCCCTGGCGGCTCCCTCGTGCGCTCTCCTGTTCCTGCCTTTCG GTTTACCGGTGTCATTCCGCTGTTATGGCCGCGTTTGTCTCATTCCACGCCTGACACTCAGT TCCGGGTAGGCAGTTCGCTCCAAGCTGGACTGTATGCACGAACCCCCCGTTCAGTCCGACC GCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGAAAGACATGCAAAAGCACC ACTGGCAGCAGCCACTGGTAATTGATTTAGAGGAGTTAGTCTTGAAGTCATGCGCCGGTTAA GGCTAAACTGAAAGGACAAGTTTTGGTGACTGCGCTCCTCCAAGCCAGTTACCTCGGTTCA AAGAGTTGGTAGCTCAGAGAACCTTCGAAAAACCGCCCTGCAAGGCGGTTTTTTCGTTTTCA GAGCAAGAGATTACGCGCAGACCAAAACGATCTCAAGAAGATCATCTTATTAATCAGATAAA ATATTTCTAGATTTCAGTGCAATTTATCTCTTCAAATGTAGCACCTGAAGTCAGCCCCATACG ATATAAGTTGTTACTAGTGCTTGGATTCTCACCAATAAAAAACGCCCGGCGGCAACCGAGCG TTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGATCTATCAACAGGAGTCCAAGC GAGCTCGATATCAAATTACGCCCCGCCCTGCCACTCATCGCAGTACTGTTGTAATTCATTAA GCATTCTGCCGACATGGAAGCCATCACAGACGGCATGATGAACCTGAATCGCCAGCGGCAT CAGCACCTTGTCGCCTTGCGTATAATATTTGCCCATGGTGAAAACGGGGGCGAAGAAGTTG TCCATATTGGCCACGTTTAAATCAAAACTGGTGAAACTCACCCAGGGATTGGCTGAGACGAA AAACATATTCTCAATAAACCCTTTAGGGAAATAGGCCAGGTTTTCACCGTAACACGCCACAT CTTGCGAATATATGTGTAGAAACTGCCGGAAATCGTCGTGGTATTCACTCCAGAGCGATGAA AACGTTTCAGTTTGCTCATGGAAAACGGTGTAACAAGGGTGAACACTATCCCATATCACCAG CTCACCGTCTTTCATTGCCATACGGAATTCCGGATGAGCATTCATCAGGCGGGCAAGAATGT GAATAAAGGCCGGATAAAACTTGTGCTTATTTTTCTTTACGGTCTTTAAAAAGGCCGTAATAT CCAGCTGAACGGTCTGGTTATAGGTACATTGAGCAACTGACTGAAATGCCTCAAAATGTTCT TTACGATGCCATTGGGATATATCAACGGTGGTATATCCAGTGATTTTTTTCTCCATTTTAGCT TCCTTAGCTCCTGAAAATCTCGATAACTCAAAAAATACGCCCGGTAGTGATCTTATTTCATTA TGGTGAAAGTTGGAACCTCTTACGTGCCGATCAACGTCTCATTTTCGCCAGATATCGACGTC TAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGT CTTCACCTCGAGCACAGCTAACACCACGTCGTCCCTATCTGCTGCCCTAGGTCTATGAGTG GTTGCTGGATAACTTTACGGGCATGCATAAGGCTCGTATAATATATTCAGGGAGACCACAAC GGTTTCCCTCTACAAATAATTTTGTTTAACTTT

Bxbi GFP BAC Reporter	GGAAGCTAAAATGGAGAAAAAAATCACTGGATATACCACCGTTGATATATCCCAATGGCATC GTAAAGAACATTTTGAGGCATTTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGTTCAG CTGGATATTACGGCCTTTTTAAAGACCGTAAAGAAAAATAAGCACAAGTTTTATCCGGCCTTT ATTCACATTCTTGCCCGCCTGATGAATGCTCATCCGGAATTTCGTATGGCAATGAAAGACGG TGAGCTGGTGATATGGGATAGTGTTCACCCTTGTTACACCGTTTTCCATGAGCAAACTGAAA CGTTTTCATCGCTCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACATATATTCG CAAGATGTGGCGTGTTACGGTGAAAACCTGGCCTATTTCCCTAAAGGGTTTATTGAGAATAT GTTTTTCGTCTCAGCCAATCCCTGGGTGAGTTTCACCAGTTTTGATTTAAACGTGGCCAATAT GGACAACTTCTTCGCCCCCGTTTTCACCATGGGCAAATATTATACGCAAGGCGACAAGGTG CTGATGCCGCTGGCGATTCAGGTTCATCATGCCGTTTGTGATGGCTTCCATGTCGGCAGAA TGCTTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGGCGTAAGACGTCTAAGA AACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTTCA CCTCGAGCACAGCTAACACCACGTCGTCCCTATCTGCTGCCCTAGGTCTATGAGTGGTTGC TGGATAACTTTACGGGCATGCATAAGGCTCGTATAATATATTCAGGGAGACCACAACGGTTT CCCTCTACAAATAATTTTGTTTAACTTTTTAATTAACGGCCGGCTTGTCGACGACGGCGGTCT CCGTCGTCAGGATCATCCGGGCATCGATTCTAGGGCGGCGGATTTGTCCTACTCAGGAGAG CGTTCACCGACAAACAACAGATAAAACGAAAGGCCCAGTCTTTCGACTGAGCCTTTCGTTTT ATTTGATGCCTCTAGCACGCGTACCATGGGATCCCCCGGGCTGCAGGAATTCGATATCAAG CTTTTATTTGTAGAGATCATCCATGCCATGTGTAATCCCAGCAGCTGTTACAAACTCAAGAAG GACCATGTGGTCTCTCTTTTCGTTGGGATCTTTCGAAAGGGCAGATTGTGTGGACAGGTAAT GGTTGTCTGGTAAAAGGACAGGGCCATCGCCAATTGGAGTATTTTGTTGATAATGGTCTGCT AGTTGAACGCTTCCATCTTCAATGTTGTGTCTAATTTTGAAGTTAACTTTGATTCCATTCTTTT GTTTGTCTGCCATGATGTATACATTGTGTGAGTTATAGTTGTATTCCAATTTGTGTCCAAGAA TGTTTCCATCTTCTTTAAAATCAATACCTTTTAACTCGATTCTATTAACAAGGGTATCACCTTC AAACTTGACTTCAGCACGTGTCTTGTAGTTCCCGTCATCTTTGAAAAATATAGTTCTTTCCTG TACATAACCTTCGGGCATGGCACTCTTGAAAAAGTCATGCTGTTTCATATGATCTGGGTATCT CGCAAAGCATTGAACACCATAACCGAAAGTAGTGACAAGTGTTGGCCATGGAACAGGTAGT TTTCCAGTAGTGCAAATAAATTTAAGGGTAAGTTTTCCGTATGTTGCATCACCTTCACCCTCT CCACTGACAGAAAATTTGTGCCCATTAACATCACCATCTAATTCAACAAGAATTGGGACAACT CCAGTGAAAAGTTCTTCTCCTTTACTCATCTTAAACCTCCTTACCTCGTAAACTATTAAACAAA ATTATTTGTAGAGGCTGTTTCGTCCTCACGGACTCATCAGACCGGAAAGCACATCCGGTGAC AGCTGTGCACGTCGGGGTTTGTACCGTACACCACTGAGACCGCGGTGGTTGACCAGACAAA CCACGACACATGTCAATACTTGCCCTTGACAGGCATTGATGGAATCGTAGTCTCACGCTGAT AGTCTGATCGACAATACAAGTGGGACCGTGGTCCCAGACCGATAATCAGACCGACAACACG AGTGGGATCGTGGTCCCAGACTAATAATCAGACCGACGATACGAGTGGGACCGTGGTCCCA GACTAATAATCAGACCGACGATACGAGTGGGACCGTGGTTCCAGACTAATAATCAGACCGA CGATACGAGTGGGACCGTGGTCCCAGACTAATAATCAGACCGACGATACGAGTGGGACCAT GGTCCCAGACTAATAATCAGACCGACGATACGAGTGGGACCGTGGTCCCAGTCTGATTATC AGACCGACGATACGAGTGGGACCGTGGTCCCAGACTAATAATCAGACCGACGATACGAGTG GGACCGTGGTCCCAGACTAATAATCAGACCGACGATACGAGTGGGACCGTGGTCCCAGTCT GATTATCAGACCGACGATACAAGTGGAACAGTGGGCCCAGAGAGAATATTCAGGCCAGTTA TGCTTTCTGGCCTGTAACAAAGGACATTAAGTAAAGACAGATAAACGTAGACTAAAACGTGG TCGCATCAGGGTGCTGGCTTTTCAAGTTCCTTAAGAATGGCCTCAATTTTCTCTATACACTCA GTTGGAACACGGGACCTGTCCAGGTTAAGCACCATTTTATCGCCCTTATACAATACTGTCGC TCCAGGAGCAAACTGATGTCGTGAGCTTAAACTAGTTCTTGATGCAGATGACGTTTTAAGCA CAGAAGTTAAAAGAGTGATAACTTCTTCAGCTTCAAATATCACCCCAGCTTTTTTCTGCTCAT GAAGGTTAGATGCCTGCTGCTTAAGTAATTCCTCTTTATCTGTAAAGGCTTTTTGAAGTGCAT CACCTGACCGGGCAGATAGTTCACCGGGGTGAGAAAAAAGAGCAACAACTGATTTAGGCAA TTTGGCGGTGTTGATACAGCGGGTAATAATCTTACGTGAAATATTTTCCGCATCAGCCAGCG CAGAAATATTTCCAGCAAATTCATTCTGCAATCGGCTTGCATAACGCTGACCACGTTCATAA

	GCACTTGTTGGGCGATAATCGTTACCCAATCTGGATAATGCAGCCATCTGCTCATCATCCAG CTCGCCAACCAGAACACGATAATCACTTTCGGTAAGTGCAGCAGCTTTACGACGGCGACTC CCATCGGCAATTTCTATGACACCAGATACTCTTCGACCGAACGCCGGTGTCTGTTGACCAGT CAGTAGAAAAGAAGGGATGAGATCATCCAGTGCGTCCTCAGTAAGCAGCTCCTGGTCACGT TCATTACCTGACCATACCCGAGAGGTCTTCTCAACACTATCACCCCGGAGCACTTCAAGAGT AAACTTCACATCCCGACCACATACAGGCAAAGTAATGGCATTACCGCGAGCCATTACTCCTA CGCGCGCAATTAACGAATCCACCATCGGGGCAGCTGGTGTCGATAACGAAGTATCTTCAAC CGGTTGAGTATTGAGCGTATGTTTTGGAATAACAGGCGCACGCTTCATTATCTAATCTCCCA GCGTGGTTTAATCAGACGATCGAAAATTTCATTGCAGACAGGTTCCCAAATAGAAAGAGCAT TTCTCCAGGCACCAGTTGAAGAGCGTTGATCAATGGCCTGTTCAAAAACAGTTCTCATCCGG ATCTGACCTTTACCAACTTCATCCGTTTCACGTACAACATTTTTTAGAACCATGCTTCCCCAG GCATCCCGAATTTGCTCCTCCATCCACGGGGACTGAGAGCCATTACTATTGCTGTATTTGGT AAGCAAAATACGTACATCAGGCTCGAACCCTTTAAGATCAACGTTCTTGAGCAGATCACGAA GCATATCGAAAAACTGCAGTGCGGAGGTGTAGTCAAACAACTCAGCAGGCGTGGGAACAAT CAGCACATCAGCAGCACATACGACATTAATCGTGCCGATACCCAGGTTAGGCGCGCTGTCA ATAACTATGACATCATAGTCATGAGCAACAGTTTCAATGGCCAGTCGGAGCATCAGGTGTGG ATCGGTGGGCAGTTTACCTTCATCAAATTTGCCCATTAACTCAGTTTCAATACGGTGCAGAG CCAGACAGGAAGGAATAATGTCAAGCCCCGGCCAGCAAGTGGGCTTTATTGCATAAGTGAC ATCGTCCTTTTCCCCAAGATAGAAAGGCAGGAGAGTGTCTTCTGCATGAATATGAAGATCTG GTACCCATCCGTGATACATTGAGGCTGTTCCCTGGGGGTCGTTACCTTCCACGAGCAAAAC ACGTAGCCCCTTCAGAGCCAGATCCTGAGCAAGATGAACAGAAACTGAGGTTTTGTAAACG CCACCTTTATGGGCAGCAACCCCGATCACCGGTGGAAATACGTCTTCAGCACGTCGCAATC GCGTACCAAACACATCACGCATATGATTAATTTGTTCAATTGTATAACCAACACGTTGCTCAA CCCGTCCTCGAATTTCCATATCCGGGTGCGGTAGTCGCCCTGCTTTCTCGGCATCTCTGATA GCCTGAGAAGAAACCCCAACTAAATCCGCTGCTTCACCTATTCTCCAGCGCCGGGTTATTTT CCTCGCTTCCGGGCTGTCATCATTAAACTGTGCAATGGCGATAGCCTTCGTCATTTCATGAC CAGCGTTTATGCACTGGTTAAGTGTTTCCATGAGTTTCATTCTGAACATCCTTTAATCATTGC TTTGCGTTTTTTTATTAAATCTTGCAATTTACTGCAAAGCAACAACAAAATCGCAAAGTCATCA AAAAACCGCAAAGTTGTTTAAAATAAGAGCAACACTACAAAAGGAGATAAGAAGAGCACATA CCTCAGTCACTTATTATCACTAGCGCTCGCCGCAGCCGTGTAACCGAGCATAGCGAGCGAA CTGGCGAGGAAGCAAAGAAGAACTGTTCTGTCAGATAGCTCTTACGCTCAGCGCAAGAAGA AATATCCACCGTGGGAAAAACTCCAGGTAGAGGTACACACGCGGATAGCCAATTCAGAGTA ATAAACTGTGATAATCAACCCTCATCAATGATGACGAACTAACCCCCGATATCAGGTCACAT GACGAAGGGAAAGAGAAGGAAATCAACTGTGACAAACTGCCCTCAAATTTGGCTTCCTTAAA AATTACAGTTCAAAAAGTATGAGAAAATCCATGCAGGCTGAAGGAAACAGCAAAACTGTGAC AAATTACCCTCAGTAGGTCAGAACAAATGTGACGAACCACCCTCAAATCTGTGACAGATAAC CCTCAGACTATCCTGTCGTCATGGAAGTGATATCGCGGAAGGAAAATACGATATGAGTCGTC TGGCGGCCTTTCTTTTTCTCAATGTATGAGAGGCGCATTGGAGTTCTGCTGTTGATCTCATT AACACAGACCTGCAGGAAGCGGCGGCGGAAGTCAGGCATACGCTGGTAACTTTGAGGCAG CTGGTAACGCTCTATGATCCAGTCGATTTTCAGAGAGACGATGCCTGAGCCATCCGGCTTA CGATACTGACACAGGGATTCGTATAAACGCATGGCATACGGATTGGTGATTTCTTTTGTTTC ACTAAGCCGAAACTGCGTAAACCGGTTCTGTAACCCGATAAAGAAGGGAATGAGATATGGG TTGATATGTACACTGTAAAGCCCTCTGGATGGACTGTGCGCACGTTTGATAAACCAAGGAAA AGATTCATAGCCTTTTTCATCGCCGGCATCCTCTTCAGGGCGATAAAAAACCACTTCCTTCC CCGCGAAACTCTTCAATGCCTGCCGTATATCCTTACTGGCTTCCGCAGAGGTCAATCCGAAT ATTTCAGCATATTTAGCAACATGGATCTCGCAGATACCGTCATGTTCCTGTAGGGTGCCATC AGATTTTCTGATCTGGTCAACGAACAGATACAGCATACGTTTTTGATCCCGGGAGAGACTAT ATGCCGCCTCAGTGAGGTCGTTTGACTGGACGATTCGCGGGCTATTTTTACGTTTCTTGTGA TTGATAACCGCTGTTTCCGCCATGACAGATCCATGTGAAGTGTGACAAGTTTTTAGATTGTC ACACTAAATAAAAAAGAGTCAATAAGCAGGGATAACTTTGTGAAAAAACAGCTTCTTCTGAG

	GGCAATTTGTCACAGGGTTAAGGGCAATTTGTCACAGACAGGACTGTCATTTGAGGGTGATT TGTCACACTGAAAGGGCAATTTGTCACAACACCTTCTCTAGAACCAGCATGGATAAAGGCCT ACAAGGCGCTCTAAAAAAGAAGATCTAAAAACTATAAAAAAAATAATTATAAAAATATCCCCG TGGATAAGTGGATAACCCCAAGGGAAGTTTTTTCAGGCATCGTGTGTAAGCAGAATATATAA GTGCTGTTCCCTGGTGCTTCCTCGCTCACTCGACCGGGAGGGTTCGAGAAGGGGGGGCAC CCCCCTTCGGCGTGCGCGGTCACGCGCACAGGGCGCAGCCCTGGTTAAAAACAAGGTTTA TAAATATTGGTTTAAAAGCAGGTTAAAAGACAGGTTAGCGGTGGCCGAAAAACGGGCGGAA ACCCTTGCAAATGCTGGATTTTCTGCCTGTGGACAGCCCCTCAAATGTCAATAGGTGCGCC CCTCATCTGTCAGCACTCTGCCCCTCAAGTGTCAAGGATCGCGCCCCTCATCTGTCAGTAG TCGCGCCCCTCAAGTGTCAATACCGCAGGGCACTTATCCCCAGGCTTGTCCACATCATCTG TGGGAAACTCGCGTAAAATCAGGCGTTTTCGCCGATTTGCGAGGCTGGCCAGCTCCACGTC GCCGGCCGAAATCGAGCCTGCCCCTCATCTGTCAACGCCGCGCCGGGTGAGTCGGCCCCT CAAGTGTCAACGTCCGCCCCTCATCTGTCAGTGAGGGCCAAGTTTTCCGCGAGGTATCCAC AACGCCGGCGGCCGGCCGCGGTGTCTCGCACACGGCTTCGACGGCGTTTCTGGCGCGTTT GCAGGGCCATAGACGGCCGCCAGCCCAGCGGCGAGGGCAACCAGCCGAGGGCTTCGCCC TGTCGCTCGACTGCGGCGAGCACTACTGGCTGTAAAAGGACAGACCACATCATGGTTCTGT GTTCATTAGGTTGTTCTGTCCATTGCTGACATAATCCGCTCCACTTCAACGTAACACCGCAC GAAGATTTCTATTGTTCCTGAAGGCATATTCAAATCGTTTTCGTTACCGCTTGCAGGCATCAT GACAGAACACTACTTCCTATAAACGCTACACAGGCTCCTGAGATTAATAATGCGGATCTCTA CGATAATGGGAGATTTTCCCGACTGTTTCGTTCGCTTCTCAGTGGATAACAGCCAGCTTCTC TGTTTAACAGACAAAAACAGCATATCCACTCAGTTCCACATTTCCATATAAAGGCCAAGGCAT TTATTCTCAGGATAATTGTTTCAGCATCGCAACCGCATCAGACTCCGGCATCGCAAACTGCA CCCGGTGCCGGGCAGCCACATCCAGCGCAAAAACCTTCGTGTAGACTTCCGTTGAACTGAT GGACTTATGTCCCATCAGGCTTTGCAGAACTTTCAGCGGTATACCGGCATACAGCATGTGCA TCGCATAGGAATGGCGGAACGTATGTGGTGTGACCGGAACAGAGAACGTCACACCGTCAG CAGCAGCGGCGGCAACCGCCTCCCCAATCCAGGTCCTGACCGTTCTGTCCGTCACTTCCCA GATCCGCGCTTTCTCTGTCCTTCCTGTGCGACGGTTACGCCGCTCCATGAGCTTATCGCGA ATAAATACCTGTGACGGAAGATCACTTCGCAGAATAAATAAATCCTGGTGTCCCTGTTGATA CCGGGAAGCCCTGGGCCAACTTTTGGCGAAAATGAGACGTTGATCGGCACGTAAGAGGTTC CAACTTTCACCATAATGAAATAAGATCACTACCGGGCGTATTTTTTGAGTTATCGAGATTTTC AGGAGCTAA

Bxbi + TP901 GFP bandpass BAC Reporter	GGAAGCTAAAATGGAGAAAAAAATCACTGGATATACCACCGTTGATATATCCCAATGGCATC GTAAAGAACATTTTGAGGCATTTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGTTCAG CTGGATATTACGGCCTTTTTAAAGACCGTAAAGAAAAATAAGCACAAGTTTTATCCGGCCTTT ATTCACATTCTTGCCCGCCTGATGAATGCTCATCCGGAATTTCGTATGGCAATGAAAGACGG TGAGCTGGTGATATGGGATAGTGTTCACCCTTGTTACACCGTTTTCCATGAGCAAACTGAAA CGTTTTCATCGCTCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACATATATTCG CAAGATGTGGCGTGTTACGGTGAAAACCTGGCCTATTTCCCTAAAGGGTTTATTGAGAATAT GTTTTTCGTCTCAGCCAATCCCTGGGTGAGTTTCACCAGTTTTGATTTAAACGTGGCCAATAT GGACAACTTCTTCGCCCCCGTTTTCACCATGGGCAAATATTATACGCAAGGCGACAAGGTG CTGATGCCGCTGGCGATTCAGGTTCATCATGCCGTTTGTGATGGCTTCCATGTCGGCAGAA TGCTTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGGCGTAAGACGTCTAAGA AACCATTAATGCCAACACAATTAACATCTCAATCAAGGTAAATGCTTTTTGCTTTTTTTGCATC GATTTGAGAAGAGAAAAGAAAACCGCCGATCCTGTCCACCGCATTACTGCAAGGTAGTGGA CAAGACCGGCGGTCTTAAGTTTTTTGGCTGAAATAGGTGAACATACAAATGCGGAAAAGTTT CTGCCCTCGCACGTAGCTGAGCAAGTCGTAGCCATGCCCCGCAAACCTGGGGTAATCACTG TATCGGGCTGTCTAAGAGCCGTTGGCTGGGCTAGTCGTCCATGGCAGTTTGTCAGTTAGGT CAAGATGCGGGTCTCTGTGTAAACCGGCCCCTGGGGTTTTGGCCCAAACTGCCTGATGCTG CATACCGAATACACGGTGGTTAATTGATGACTGCTTGTGCACTGCTGCGCCTTGCGGAGGC CGGTCCGGCGCGCGAAGCGTATAAATGAAGCTCGTCACATCCACACAGTTGCATCGTACGT CGGTGTAGGGATCGAACTTGGCCTCTAATTCCACATGCGCGCGCTGCAGGTGTAAGTGTGC CAAGTCACCTCGACCACGATGGGCTTTGCCCCAGGTATCCAAAAGGAAATCCTGATGCCGC GCTTCGATTCACCGTAGTGTGGATGTTCCCTTTGTACGGGTTTCCGACTCCTGTTGTAGCAG GCGTCTTTGTTAGCTAGCGCTATCCCCAACGTGCAACAACCCTTACCACGAAGACAGGATT GTCCGATCCTATATTACGACTTTGGCAGGGGGTTCGCAAGTCCCTGCAGGAACGATGCTGA AGGCTCAGGTTACACAGGCACAAGTACTATATATACGAGATGCATCTCTTAACCTGGATCGA ATGCAGAATCATGAATCGTACCACTGTGTTCGCCTGCAGCTCGAGCACAGCTAACACCACG TCGTCCCTATCTGCTGCCCTAGGTCTATGAGTGGTTGCTGGATAACTTTACGGGCATGCATA AGGCTCGTATAATATATTCAGGGAGACCACAACGGTTTCCCTCTACAAATAATTTTGTTTAAC TTTGCTAGCAAAGGAGTTTTTTAGTTACCTTAATTGAAATAAACGAAATAAAAACTCGCTTAG CCCAATCTTCAATACCTCGTATGCCGACAACATGGACCGGTCACCAGCGAGCCCTGTGCGG ACGGGAGGTTAATTAACGGCCGGCTTGTCGACGACGGCGGTCTCCGTCGTCAGGATCATC CGGGCATCGATTCTAGGGCGGCGGATTTGTCCTACTCAGGAGAGCGTTCACCGACAAACAA CAGATAAAACGAAAGGCCCAGTCTTTCGACTGAGCCTTTCGTTTTATTTGATGCCTCTAGCA CGCGTACCATGGGATCCCCCGGGCTGCAGGAATTCGATATCAAGCTTTTATTTGTAGAGATC ATCCATGCCATGTGTAATCCCAGCAGCTGTTACAAACTCAAGAAGGACCATGTGGTCTCTCT TTTCGTTGGGATCTTTCGAAAGGGCAGATTGTGTGGACAGGTAATGGTTGTCTGGTAAAAGG ACAGGGCCATCGCCAATTGGAGTATTTTGTTGATAATGGTCTGCTAGTTGAACGCTTCCATC TTCAATGTTGTGTCTAATTTTGAAGTTAACTTTGATTCCATTCTTTTGTTTGTCTGCCATGATG TATACATTGTGTGAGTTATAGTTGTATTCCAATTTGTGTCCAAGAATGTTTCCATCTTCTTTAA AATCAATACCTTTTAACTCGATTCTATTAACAAGGGTATCACCTTCAAACTTGACTTCAGCAC GTGTCTTGTAGTTCCCGTCATCTTTGAAAAATATAGTTCTTTCCTGTACATAACCTTCGGGCA TGGCACTCTTGAAAAAGTCATGCTGTTTCATATGATCTGGGTATCTCGCAAAGCATTGAACA CCATAACCGAAAGTAGTGACAAGTGTTGGCCATGGAACAGGTAGTTTTCCAGTAGTGCAAAT AAATTTAAGGGTAAGTTTTCCGTATGTTGCATCACCTTCACCCTCTCCACTGACAGAAAATTT GTGCCCATTAACATCACCATCTAATTCAACAAGAATTGGGACAACTCCAGTGAAAAGTTCTTC TCCTTTACTCATCTTAAACCTCCTTACCTCGTAAACTATTAAACAAAATTATTTGTAGAGGCTG TTTCGTCCTCACGGACTCATCAGACCGGAAAGCACATCCGGTGACAGCTGTGCACGTCGGG GTTTGTACCGTACACCACTGAGACCGCGGTGGTTGACCAGACAAACCACGACACATGTCAA TACTTGCCCTTGACAGGCATTGATGGAATCGTAGTCTCACGCTGATAGTCTGATCGACAATA CAAGTGGGACCGTGGTCCCAGACCGATAATCAGACCGACAACACGAGTGGGATCGTGGTC

	CCAGACTAATAATCAGACCGACGATACGAGTGGGACCGTGGTCCCAGACTAATAATCAGAC CGACGATACGAGTGGGACCGTGGTTCCAGACTAATAATCAGACCGACGATACGAGTGGGAC CGTGGTCCCAGACTAATAATCAGACCGACGATACGAGTGGGACCATGGTCCCAGACTAATA ATCAGACCGACGATACGAGTGGGACCGTGGTCCCAGTCTGATTATCAGACCGACGATACGA GTGGGACCGTGGTCCCAGACTAATAATCAGACCGACGATACGAGTGGGACCGTGGTCCCA GACTAATAATCAGACCGACGATACGAGTGGGACCGTGGTCCCAGTCTGATTATCAGACCGA CGATACAAGTGGAACAGTGGGCCCAGAGAGAATATTCAGGCCAGTTATGCTTTCTGGCCTG TAACAAAGGACATTAAGTAAAGACAGATAAACGTAGACTAAAACGTGGTCGCATCAGGGTGC TGGCTTTTCAAGTTCCTTAAGAATGGCCTCAATTTTCTCTATACACTCAGTTGGAACACGGGA CCTGTCCAGGTTAAGCACCATTTTATCGCCCTTATACAATACTGTCGCTCCAGGAGCAAACT GATGTCGTGAGCTTAAACTAGTTCTTGATGCAGATGACGTTTTAAGCACAGAAGTTAAAAGA GTGATAACTTCTTCAGCTTCAAATATCACCCCAGCTTTTTTCTGCTCATGAAGGTTAGATGCC TGCTGCTTAAGTAATTCCTCTTTATCTGTAAAGGCTTTTTGAAGTGCATCACCTGACCGGGCA GATAGTTCACCGGGGTGAGAAAAAAGAGCAACAACTGATTTAGGCAATTTGGCGGTGTTGA TACAGCGGGTAATAATCTTACGTGAAATATTTTCCGCATCAGCCAGCGCAGAAATATTTCCA GCAAATTCATTCTGCAATCGGCTTGCATAACGCTGACCACGTTCATAAGCACTTGTTGGGCG ATAATCGTTACCCAATCTGGATAATGCAGCCATCTGCTCATCATCCAGCTCGCCAACCAGAA CACGATAATCACTTTCGGTAAGTGCAGCAGCTTTACGACGGCGACTCCCATCGGCAATTTCT ATGACACCAGATACTCTTCGACCGAACGCCGGTGTCTGTTGACCAGTCAGTAGAAAAGAAG GGATGAGATCATCCAGTGCGTCCTCAGTAAGCAGCTCCTGGTCACGTTCATTACCTGACCAT ACCCGAGAGGTCTTCTCAACACTATCACCCCGGAGCACTTCAAGAGTAAACTTCACATCCCG ACCACATACAGGCAAAGTAATGGCATTACCGCGAGCCATTACTCCTACGCGCGCAATTAAC GAATCCACCATCGGGGCAGCTGGTGTCGATAACGAAGTATCTTCAACCGGTTGAGTATTGA GCGTATGTTTTGGAATAACAGGCGCACGCTTCATTATCTAATCTCCCAGCGTGGTTTAATCA GACGATCGAAAATTTCATTGCAGACAGGTTCCCAAATAGAAAGAGCATTTCTCCAGGCACCA GTTGAAGAGCGTTGATCAATGGCCTGTTCAAAAACAGTTCTCATCCGGATCTGACCTTTACC AACTTCATCCGTTTCACGTACAACATTTTTTAGAACCATGCTTCCCCAGGCATCCCGAATTTG CTCCTCCATCCACGGGGACTGAGAGCCATTACTATTGCTGTATTTGGTAAGCAAAATACGTA CATCAGGCTCGAACCCTTTAAGATCAACGTTCTTGAGCAGATCACGAAGCATATCGAAAAAC TGCAGTGCGGAGGTGTAGTCAAACAACTCAGCAGGCGTGGGAACAATCAGCACATCAGCA GCACATACGACATTAATCGTGCCGATACCCAGGTTAGGCGCGCTGTCAATAACTATGACATC ATAGTCATGAGCAACAGTTTCAATGGCCAGTCGGAGCATCAGGTGTGGATCGGTGGGCAGT TTACCTTCATCAAATTTGCCCATTAACTCAGTTTCAATACGGTGCAGAGCCAGACAGGAAGG AATAATGTCAAGCCCCGGCCAGCAAGTGGGCTTTATTGCATAAGTGACATCGTCCTTTTCCC CAAGATAGAAAGGCAGGAGAGTGTCTTCTGCATGAATATGAAGATCTGGTACCCATCCGTG ATACATTGAGGCTGTTCCCTGGGGGTCGTTACCTTCCACGAGCAAAACACGTAGCCCCTTC AGAGCCAGATCCTGAGCAAGATGAACAGAAACTGAGGTTTTGTAAACGCCACCTTTATGGG CAGCAACCCCGATCACCGGTGGAAATACGTCTTCAGCACGTCGCAATCGCGTACCAAACAC ATCACGCATATGATTAATTTGTTCAATTGTATAACCAACACGTTGCTCAACCCGTCCTCGAAT TTCCATATCCGGGTGCGGTAGTCGCCCTGCTTTCTCGGCATCTCTGATAGCCTGAGAAGAA ACCCCAACTAAATCCGCTGCTTCACCTATTCTCCAGCGCCGGGTTATTTTCCTCGCTTCCGG GCTGTCATCATTAAACTGTGCAATGGCGATAGCCTTCGTCATTTCATGACCAGCGTTTATGC ACTGGTTAAGTGTTTCCATGAGTTTCATTCTGAACATCCTTTAATCATTGCTTTGCGTTTTTTT ATTAAATCTTGCAATTTACTGCAAAGCAACAACAAAATCGCAAAGTCATCAAAAAACCGCAAA GTTGTTTAAAATAAGAGCAACACTACAAAAGGAGATAAGAAGAGCACATACCTCAGTCACTT ATTATCACTAGCGCTCGCCGCAGCCGTGTAACCGAGCATAGCGAGCGAACTGGCGAGGAA GCAAAGAAGAACTGTTCTGTCAGATAGCTCTTACGCTCAGCGCAAGAAGAAATATCCACCGT GGGAAAAACTCCAGGTAGAGGTACACACGCGGATAGCCAATTCAGAGTAATAAACTGTGAT AATCAACCCTCATCAATGATGACGAACTAACCCCCGATATCAGGTCACATGACGAAGGGAAA GAGAAGGAAATCAACTGTGACAAACTGCCCTCAAATTTGGCTTCCTTAAAAATTACAGTTCAA

	AAAGTATGAGAAAATCCATGCAGGCTGAAGGAAACAGCAAAACTGTGACAAATTACCCTCAG TAGGTCAGAACAAATGTGACGAACCACCCTCAAATCTGTGACAGATAACCCTCAGACTATCC TGTCGTCATGGAAGTGATATCGCGGAAGGAAAATACGATATGAGTCGTCTGGCGGCCTTTC TTTTTCTCAATGTATGAGAGGCGCATTGGAGTTCTGCTGTTGATCTCATTAACACAGACCTG CAGGAAGCGGCGGCGGAAGTCAGGCATACGCTGGTAACTTTGAGGCAGCTGGTAACGCTC TATGATCCAGTCGATTTTCAGAGAGACGATGCCTGAGCCATCCGGCTTACGATACTGACACA GGGATTCGTATAAACGCATGGCATACGGATTGGTGATTTCTTTTGTTTCACTAAGCCGAAAC TGCGTAAACCGGTTCTGTAACCCGATAAAGAAGGGAATGAGATATGGGTTGATATGTACACT GTAAAGCCCTCTGGATGGACTGTGCGCACGTTTGATAAACCAAGGAAAAGATTCATAGCCTT TTTCATCGCCGGCATCCTCTTCAGGGCGATAAAAAACCACTTCCTTCCCCGCGAAACTCTTC AATGCCTGCCGTATATCCTTACTGGCTTCCGCAGAGGTCAATCCGAATATTTCAGCATATTTA GCAACATGGATCTCGCAGATACCGTCATGTTCCTGTAGGGTGCCATCAGATTTTCTGATCTG GTCAACGAACAGATACAGCATACGTTTTTGATCCCGGGAGAGACTATATGCCGCCTCAGTG AGGTCGTTTGACTGGACGATTCGCGGGCTATTTTTACGTTTCTTGTGATTGATAACCGCTGT TTCCGCCATGACAGATCCATGTGAAGTGTGACAAGTTTTTAGATTGTCACACTAAATAAAAAA GAGTCAATAAGCAGGGATAACTTTGTGAAAAAACAGCTTCTTCTGAGGGCAATTTGTCACAG GGTTAAGGGCAATTTGTCACAGACAGGACTGTCATTTGAGGGTGATTTGTCACACTGAAAGG GCAATTTGTCACAACACCTTCTCTAGAACCAGCATGGATAAAGGCCTACAAGGCGCTCTAAA AAAGAAGATCTAAAAACTATAAAAAAAATAATTATAAAAATATCCCCGTGGATAAGTGGATAA CCCCAAGGGAAGTTTTTTCAGGCATCGTGTGTAAGCAGAATATATAAGTGCTGTTCCCTGGT GCTTCCTCGCTCACTCGACCGGGAGGGTTCGAGAAGGGGGGGCACCCCCCTTCGGCGTG CGCGGTCACGCGCACAGGGCGCAGCCCTGGTTAAAAACAAGGTTTATAAATATTGGTTTAA AAGCAGGTTAAAAGACAGGTTAGCGGTGGCCGAAAAACGGGCGGAAACCCTTGCAAATGCT GGATTTTCTGCCTGTGGACAGCCCCTCAAATGTCAATAGGTGCGCCCCTCATCTGTCAGCA CTCTGCCCCTCAAGTGTCAAGGATCGCGCCCCTCATCTGTCAGTAGTCGCGCCCCTCAAGT GTCAATACCGCAGGGCACTTATCCCCAGGCTTGTCCACATCATCTGTGGGAAACTCGCGTA AAATCAGGCGTTTTCGCCGATTTGCGAGGCTGGCCAGCTCCACGTCGCCGGCCGAAATCG AGCCTGCCCCTCATCTGTCAACGCCGCGCCGGGTGAGTCGGCCCCTCAAGTGTCAACGTC CGCCCCTCATCTGTCAGTGAGGGCCAAGTTTTCCGCGAGGTATCCACAACGCCGGCGGCC GGCCGCGGTGTCTCGCACACGGCTTCGACGGCGTTTCTGGCGCGTTTGCAGGGCCATAGA CGGCCGCCAGCCCAGCGGCGAGGGCAACCAGCCGAGGGCTTCGCCCTGTCGCTCGACTG CGGCGAGCACTACTGGCTGTAAAAGGACAGACCACATCATGGTTCTGTGTTCATTAGGTTGT TCTGTCCATTGCTGACATAATCCGCTCCACTTCAACGTAACACCGCACGAAGATTTCTATTGT TCCTGAAGGCATATTCAAATCGTTTTCGTTACCGCTTGCAGGCATCATGACAGAACACTACT TCCTATAAACGCTACACAGGCTCCTGAGATTAATAATGCGGATCTCTACGATAATGGGAGAT TTTCCCGACTGTTTCGTTCGCTTCTCAGTGGATAACAGCCAGCTTCTCTGTTTAACAGACAAA AACAGCATATCCACTCAGTTCCACATTTCCATATAAAGGCCAAGGCATTTATTCTCAGGATAA TTGTTTCAGCATCGCAACCGCATCAGACTCCGGCATCGCAAACTGCACCCGGTGCCGGGCA GCCACATCCAGCGCAAAAACCTTCGTGTAGACTTCCGTTGAACTGATGGACTTATGTCCCAT CAGGCTTTGCAGAACTTTCAGCGGTATACCGGCATACAGCATGTGCATCGCATAGGAATGG CGGAACGTATGTGGTGTGACCGGAACAGAGAACGTCACACCGTCAGCAGCAGCGGCGGCA ACCGCCTCCCCAATCCAGGTCCTGACCGTTCTGTCCGTCACTTCCCAGATCCGCGCTTTCT CTGTCCTTCCTGTGCGACGGTTACGCCGCTCCATGAGCTTATCGCGAATAAATACCTGTGAC GGAAGATCACTTCGCAGAATAAATAAATCCTGGTGTCCCTGTTGATACCGGGAAGCCCTGG GCCAACTTTTGGCGAAAATGAGACGTTGATCGGCACGTAAGAGGTTCCAACTTTCACCATAA TGAAATAAGATCACTACCGGGCGTATTTTTTGAGTTATCGAGATTTTCAGGAGCTAA

Bxbi + PhiC31 GFP bandpass BAC Reporter	GGAAGCTAAAATGGAGAAAAAAATCACTGGATATACCACCGTTGATATATCCCAATGGCATC GTAAAGAACATTTTGAGGCATTTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGTTCAG CTGGATATTACGGCCTTTTTAAAGACCGTAAAGAAAAATAAGCACAAGTTTTATCCGGCCTTT ATTCACATTCTTGCCCGCCTGATGAATGCTCATCCGGAATTTCGTATGGCAATGAAAGACGG TGAGCTGGTGATATGGGATAGTGTTCACCCTTGTTACACCGTTTTCCATGAGCAAACTGAAA CGTTTTCATCGCTCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACATATATTCG CAAGATGTGGCGTGTTACGGTGAAAACCTGGCCTATTTCCCTAAAGGGTTTATTGAGAATAT GTTTTTCGTCTCAGCCAATCCCTGGGTGAGTTTCACCAGTTTTGATTTAAACGTGGCCAATAT GGACAACTTCTTCGCCCCCGTTTTCACCATGGGCAAATATTATACGCAAGGCGACAAGGTG CTGATGCCGCTGGCGATTCAGGTTCATCATGCCGTTTGTGATGGCTTCCATGTCGGCAGAA TGCTTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGGCGTAAGACGTCTAAGA AACCATTATGCGGGTGCCAGGGCGTGCCCTTGGGCTCCCCGGGCGCGTACTCCATCGATT TGAGAAGAGAAAAGAAAACCGCCGATCCTGTCCACCGCATTACTGCAAGGTAGTGGACAAG ACCGGCGGTCTTAAGTTTTTTGGCTGAAATAGGTGAACATACAAATGCGGAAAAGTTTCTGC CCTCGCACGTAGCTGAGCAAGTCGTAGCCATGCCCCGCAAACCTGGGGTAATCACTGTATC GGGCTGTCTAAGAGCCGTTGGCTGGGCTAGTCGTCCATGGCAGTTTGTCAGTTAGGTCAAG ATGCGGGTCTCTGTGTAAACCGGCCCCTGGGGTTTTGGCCCAAACTGCCTGATGCTGCATA CCGAATACACGGTGGTTAATTGATGACTGCTTGTGCACTGCTGCGCCTTGCGGAGGCCGGT CCGGCGCGCGAAGCGTATAAATGAAGCTCGTCACATCCACACAGTTGCATCGTACGTCGGT GTAGGGATCGAACTTGGCCTCTAATTCCACATGCGCGCGCTGCAGGTGTAAGTGTGCCAAG TCACCTCGACCACGATGGGCTTTGCCCCAGGTATCCAAAAGGAAATCCTGATGCCGCGCTT CGATTCACCGTAGTGTGGATGTTCCCTTTGTACGGGTTTCCGACTCCTGTTGTAGCAGGCGT CTTTGTTAGCTAGCGCTATCCCCAACGTGCAACAACCCTTACCACGAAGACAGGATTGTCCG ATCCTATATTACGACTTTGGCAGGGGGTTCGCAAGTCCCTGCAGGAACGATGCTGAAGGCT CAGGTTACACAGGCACAAGTACTATATATACGAGATGCATCTCTTAACCTGGATCGAATGCA GAATCATGAATCGTACCACTGTGTTCGCCTGCAGCTCGAGCACAGCTAACACCACGTCGTC CCTATCTGCTGCCCTAGGTCTATGAGTGGTTGCTGGATAACTTTACGGGCATGCATAAGGCT CGTATAATATATTCAGGGAGACCACAACGGTTTCCCTCTACAAATAATTTTGTTTAACTTTGC TAGCCCCCCAACTGAGAGAACTCAAAGGTTACCCCAGTTGGGGCACTTAGCCCAATCTTCA ATACCTCGTATGCCGACAACATGGACCGGTCACCAGCGAGCCCTGTGCGGACGGGAGGTT AATTAACGGCCGGCTTGTCGACGACGGCGGTCTCCGTCGTCAGGATCATCCGGGCATCGA TTCTAGGGCGGCGGATTTGTCCTACTCAGGAGAGCGTTCACCGACAAACAACAGATAAAAC GAAAGGCCCAGTCTTTCGACTGAGCCTTTCGTTTTATTTGATGCCTCTAGCACGCGTACCAT GGGATCCCCCGGGCTGCAGGAATTCGATATCAAGCTTTTATTTGTAGAGATCATCCATGCCA TGTGTAATCCCAGCAGCTGTTACAAACTCAAGAAGGACCATGTGGTCTCTCTTTTCGTTGGG ATCTTTCGAAAGGGCAGATTGTGTGGACAGGTAATGGTTGTCTGGTAAAAGGACAGGGCCA TCGCCAATTGGAGTATTTTGTTGATAATGGTCTGCTAGTTGAACGCTTCCATCTTCAATGTTG TGTCTAATTTTGAAGTTAACTTTGATTCCATTCTTTTGTTTGTCTGCCATGATGTATACATTGT GTGAGTTATAGTTGTATTCCAATTTGTGTCCAAGAATGTTTCCATCTTCTTTAAAATCAATACC TTTTAACTCGATTCTATTAACAAGGGTATCACCTTCAAACTTGACTTCAGCACGTGTCTTGTA GTTCCCGTCATCTTTGAAAAATATAGTTCTTTCCTGTACATAACCTTCGGGCATGGCACTCTT GAAAAAGTCATGCTGTTTCATATGATCTGGGTATCTCGCAAAGCATTGAACACCATAACCGA AAGTAGTGACAAGTGTTGGCCATGGAACAGGTAGTTTTCCAGTAGTGCAAATAAATTTAAGG GTAAGTTTTCCGTATGTTGCATCACCTTCACCCTCTCCACTGACAGAAAATTTGTGCCCATTA ACATCACCATCTAATTCAACAAGAATTGGGACAACTCCAGTGAAAAGTTCTTCTCCTTTACTC ATCTTAAACCTCCTTACCTCGTAAACTATTAAACAAAATTATTTGTAGAGGCTGTTTCGTCCTC ACGGACTCATCAGACCGGAAAGCACATCCGGTGACAGCTGTGCACGTCGGGGTTTGTACC GTACACCACTGAGACCGCGGTGGTTGACCAGACAAACCACGACACATGTCAATACTTGCCC TTGACAGGCATTGATGGAATCGTAGTCTCACGCTGATAGTCTGATCGACAATACAAGTGGGA CCGTGGTCCCAGACCGATAATCAGACCGACAACACGAGTGGGATCGTGGTCCCAGACTAAT

	AATCAGACCGACGATACGAGTGGGACCGTGGTCCCAGACTAATAATCAGACCGACGATACG AGTGGGACCGTGGTTCCAGACTAATAATCAGACCGACGATACGAGTGGGACCGTGGTCCCA GACTAATAATCAGACCGACGATACGAGTGGGACCATGGTCCCAGACTAATAATCAGACCGA CGATACGAGTGGGACCGTGGTCCCAGTCTGATTATCAGACCGACGATACGAGTGGGACCG TGGTCCCAGACTAATAATCAGACCGACGATACGAGTGGGACCGTGGTCCCAGACTAATAAT CAGACCGACGATACGAGTGGGACCGTGGTCCCAGTCTGATTATCAGACCGACGATACAAGT GGAACAGTGGGCCCAGAGAGAATATTCAGGCCAGTTATGCTTTCTGGCCTGTAACAAAGGA CATTAAGTAAAGACAGATAAACGTAGACTAAAACGTGGTCGCATCAGGGTGCTGGCTTTTCA AGTTCCTTAAGAATGGCCTCAATTTTCTCTATACACTCAGTTGGAACACGGGACCTGTCCAG GTTAAGCACCATTTTATCGCCCTTATACAATACTGTCGCTCCAGGAGCAAACTGATGTCGTG AGCTTAAACTAGTTCTTGATGCAGATGACGTTTTAAGCACAGAAGTTAAAAGAGTGATAACTT CTTCAGCTTCAAATATCACCCCAGCTTTTTTCTGCTCATGAAGGTTAGATGCCTGCTGCTTAA GTAATTCCTCTTTATCTGTAAAGGCTTTTTGAAGTGCATCACCTGACCGGGCAGATAGTTCA CCGGGGTGAGAAAAAAGAGCAACAACTGATTTAGGCAATTTGGCGGTGTTGATACAGCGGG TAATAATCTTACGTGAAATATTTTCCGCATCAGCCAGCGCAGAAATATTTCCAGCAAATTCAT TCTGCAATCGGCTTGCATAACGCTGACCACGTTCATAAGCACTTGTTGGGCGATAATCGTTA CCCAATCTGGATAATGCAGCCATCTGCTCATCATCCAGCTCGCCAACCAGAACACGATAATC ACTTTCGGTAAGTGCAGCAGCTTTACGACGGCGACTCCCATCGGCAATTTCTATGACACCA GATACTCTTCGACCGAACGCCGGTGTCTGTTGACCAGTCAGTAGAAAAGAAGGGATGAGAT CATCCAGTGCGTCCTCAGTAAGCAGCTCCTGGTCACGTTCATTACCTGACCATACCCGAGA GGTCTTCTCAACACTATCACCCCGGAGCACTTCAAGAGTAAACTTCACATCCCGACCACATA CAGGCAAAGTAATGGCATTACCGCGAGCCATTACTCCTACGCGCGCAATTAACGAATCCAC CATCGGGGCAGCTGGTGTCGATAACGAAGTATCTTCAACCGGTTGAGTATTGAGCGTATGT TTTGGAATAACAGGCGCACGCTTCATTATCTAATCTCCCAGCGTGGTTTAATCAGACGATCG AAAATTTCATTGCAGACAGGTTCCCAAATAGAAAGAGCATTTCTCCAGGCACCAGTTGAAGA GCGTTGATCAATGGCCTGTTCAAAAACAGTTCTCATCCGGATCTGACCTTTACCAACTTCAT CCGTTTCACGTACAACATTTTTTAGAACCATGCTTCCCCAGGCATCCCGAATTTGCTCCTCC ATCCACGGGGACTGAGAGCCATTACTATTGCTGTATTTGGTAAGCAAAATACGTACATCAGG CTCGAACCCTTTAAGATCAACGTTCTTGAGCAGATCACGAAGCATATCGAAAAACTGCAGTG CGGAGGTGTAGTCAAACAACTCAGCAGGCGTGGGAACAATCAGCACATCAGCAGCACATAC GACATTAATCGTGCCGATACCCAGGTTAGGCGCGCTGTCAATAACTATGACATCATAGTCAT GAGCAACAGTTTCAATGGCCAGTCGGAGCATCAGGTGTGGATCGGTGGGCAGTTTACCTTC ATCAAATTTGCCCATTAACTCAGTTTCAATACGGTGCAGAGCCAGACAGGAAGGAATAATGT CAAGCCCCGGCCAGCAAGTGGGCTTTATTGCATAAGTGACATCGTCCTTTTCCCCAAGATA GAAAGGCAGGAGAGTGTCTTCTGCATGAATATGAAGATCTGGTACCCATCCGTGATACATTG AGGCTGTTCCCTGGGGGTCGTTACCTTCCACGAGCAAAACACGTAGCCCCTTCAGAGCCAG ATCCTGAGCAAGATGAACAGAAACTGAGGTTTTGTAAACGCCACCTTTATGGGCAGCAACCC CGATCACCGGTGGAAATACGTCTTCAGCACGTCGCAATCGCGTACCAAACACATCACGCAT ATGATTAATTTGTTCAATTGTATAACCAACACGTTGCTCAACCCGTCCTCGAATTTCCATATC CGGGTGCGGTAGTCGCCCTGCTTTCTCGGCATCTCTGATAGCCTGAGAAGAAACCCCAACT AAATCCGCTGCTTCACCTATTCTCCAGCGCCGGGTTATTTTCCTCGCTTCCGGGCTGTCATC ATTAAACTGTGCAATGGCGATAGCCTTCGTCATTTCATGACCAGCGTTTATGCACTGGTTAA GTGTTTCCATGAGTTTCATTCTGAACATCCTTTAATCATTGCTTTGCGTTTTTTTATTAAATCTT GCAATTTACTGCAAAGCAACAACAAAATCGCAAAGTCATCAAAAAACCGCAAAGTTGTTTAAA ATAAGAGCAACACTACAAAAGGAGATAAGAAGAGCACATACCTCAGTCACTTATTATCACTA GCGCTCGCCGCAGCCGTGTAACCGAGCATAGCGAGCGAACTGGCGAGGAAGCAAAGAAGA ACTGTTCTGTCAGATAGCTCTTACGCTCAGCGCAAGAAGAAATATCCACCGTGGGAAAAACT CCAGGTAGAGGTACACACGCGGATAGCCAATTCAGAGTAATAAACTGTGATAATCAACCCTC ATCAATGATGACGAACTAACCCCCGATATCAGGTCACATGACGAAGGGAAAGAGAAGGAAA TCAACTGTGACAAACTGCCCTCAAATTTGGCTTCCTTAAAAATTACAGTTCAAAAAGTATGAG

	AAAATCCATGCAGGCTGAAGGAAACAGCAAAACTGTGACAAATTACCCTCAGTAGGTCAGAA CAAATGTGACGAACCACCCTCAAATCTGTGACAGATAACCCTCAGACTATCCTGTCGTCATG GAAGTGATATCGCGGAAGGAAAATACGATATGAGTCGTCTGGCGGCCTTTCTTTTTCTCAAT GTATGAGAGGCGCATTGGAGTTCTGCTGTTGATCTCATTAACACAGACCTGCAGGAAGCGG CGGCGGAAGTCAGGCATACGCTGGTAACTTTGAGGCAGCTGGTAACGCTCTATGATCCAGT CGATTTTCAGAGAGACGATGCCTGAGCCATCCGGCTTACGATACTGACACAGGGATTCGTA TAAACGCATGGCATACGGATTGGTGATTTCTTTTGTTTCACTAAGCCGAAACTGCGTAAACC GGTTCTGTAACCCGATAAAGAAGGGAATGAGATATGGGTTGATATGTACACTGTAAAGCCCT CTGGATGGACTGTGCGCACGTTTGATAAACCAAGGAAAAGATTCATAGCCTTTTTCATCGCC GGCATCCTCTTCAGGGCGATAAAAAACCACTTCCTTCCCCGCGAAACTCTTCAATGCCTGCC GTATATCCTTACTGGCTTCCGCAGAGGTCAATCCGAATATTTCAGCATATTTAGCAACATGG ATCTCGCAGATACCGTCATGTTCCTGTAGGGTGCCATCAGATTTTCTGATCTGGTCAACGAA CAGATACAGCATACGTTTTTGATCCCGGGAGAGACTATATGCCGCCTCAGTGAGGTCGTTT GACTGGACGATTCGCGGGCTATTTTTACGTTTCTTGTGATTGATAACCGCTGTTTCCGCCAT GACAGATCCATGTGAAGTGTGACAAGTTTTTAGATTGTCACACTAAATAAAAAAGAGTCAATA AGCAGGGATAACTTTGTGAAAAAACAGCTTCTTCTGAGGGCAATTTGTCACAGGGTTAAGGG CAATTTGTCACAGACAGGACTGTCATTTGAGGGTGATTTGTCACACTGAAAGGGCAATTTGT CACAACACCTTCTCTAGAACCAGCATGGATAAAGGCCTACAAGGCGCTCTAAAAAAGAAGAT CTAAAAACTATAAAAAAAATAATTATAAAAATATCCCCGTGGATAAGTGGATAACCCCAAGGG AAGTTTTTTCAGGCATCGTGTGTAAGCAGAATATATAAGTGCTGTTCCCTGGTGCTTCCTCG CTCACTCGACCGGGAGGGTTCGAGAAGGGGGGGCACCCCCCTTCGGCGTGCGCGGTCAC GCGCACAGGGCGCAGCCCTGGTTAAAAACAAGGTTTATAAATATTGGTTTAAAAGCAGGTTA AAAGACAGGTTAGCGGTGGCCGAAAAACGGGCGGAAACCCTTGCAAATGCTGGATTTTCTG CCTGTGGACAGCCCCTCAAATGTCAATAGGTGCGCCCCTCATCTGTCAGCACTCTGCCCCT CAAGTGTCAAGGATCGCGCCCCTCATCTGTCAGTAGTCGCGCCCCTCAAGTGTCAATACCG CAGGGCACTTATCCCCAGGCTTGTCCACATCATCTGTGGGAAACTCGCGTAAAATCAGGCG TTTTCGCCGATTTGCGAGGCTGGCCAGCTCCACGTCGCCGGCCGAAATCGAGCCTGCCCC TCATCTGTCAACGCCGCGCCGGGTGAGTCGGCCCCTCAAGTGTCAACGTCCGCCCCTCAT CTGTCAGTGAGGGCCAAGTTTTCCGCGAGGTATCCACAACGCCGGCGGCCGGCCGCGGTG TCTCGCACACGGCTTCGACGGCGTTTCTGGCGCGTTTGCAGGGCCATAGACGGCCGCCAG CCCAGCGGCGAGGGCAACCAGCCGAGGGCTTCGCCCTGTCGCTCGACTGCGGCGAGCAC TACTGGCTGTAAAAGGACAGACCACATCATGGTTCTGTGTTCATTAGGTTGTTCTGTCCATT GCTGACATAATCCGCTCCACTTCAACGTAACACCGCACGAAGATTTCTATTGTTCCTGAAGG CATATTCAAATCGTTTTCGTTACCGCTTGCAGGCATCATGACAGAACACTACTTCCTATAAAC GCTACACAGGCTCCTGAGATTAATAATGCGGATCTCTACGATAATGGGAGATTTTCCCGACT GTTTCGTTCGCTTCTCAGTGGATAACAGCCAGCTTCTCTGTTTAACAGACAAAAACAGCATAT CCACTCAGTTCCACATTTCCATATAAAGGCCAAGGCATTTATTCTCAGGATAATTGTTTCAGC ATCGCAACCGCATCAGACTCCGGCATCGCAAACTGCACCCGGTGCCGGGCAGCCACATCC AGCGCAAAAACCTTCGTGTAGACTTCCGTTGAACTGATGGACTTATGTCCCATCAGGCTTTG CAGAACTTTCAGCGGTATACCGGCATACAGCATGTGCATCGCATAGGAATGGCGGAACGTA TGTGGTGTGACCGGAACAGAGAACGTCACACCGTCAGCAGCAGCGGCGGCAACCGCCTCC CCAATCCAGGTCCTGACCGTTCTGTCCGTCACTTCCCAGATCCGCGCTTTCTCTGTCCTTCC TGTGCGACGGTTACGCCGCTCCATGAGCTTATCGCGAATAAATACCTGTGACGGAAGATCA CTTCGCAGAATAAATAAATCCTGGTGTCCCTGTTGATACCGGGAAGCCCTGGGCCAACTTTT GGCGAAAATGAGACGTTGATCGGCACGTAAGAGGTTCCAACTTTCACCATAATGAAATAAGA TCACTACCGGGCGTATTTTTTGAGTTATCGAGATTTTCAGGAGCTAA

ADC BAC Reporter	TCTTAAGTTTTTTGGCTGAACTCGAGCACAGCTAACACCACGTCGTCCCTATCTGCTGCCCT AGGTCTATGAGTGGTTGCTGGATAACTTTACGGGCATGCATAAGGCTCGTATAATATATTCA GGGAGACCACAACGGTTTCCCTCTACAAATAATTTTGTTTAACTTTTTAATTAACGGCCGGCT TGTCGACGACGGCGGTCTCCGTCGTCAGGATCATCCGGGCATCGATTCTAGGGCGGCGGA TTTGTCCTACTCAGGAGAGCGTTCACCGACAAACAACAGATAAAACGAAAGGCCCAGTCTTT CGACTGAGCCTTTCGTTTTATTTGATGCCTCTAGCACGCGTACCATGGGATCCCCCGGGCT GCAGGAATTCGATATCAAGCTTTTATTTGTAGAGATCATCCATGCCATGTGTAATCCCAGCA GCTGTTACAAACTCAAGAAGGACCATGTGGTCTCTCTTTTCGTTGGGATCTTTCGAAAGGGC AGATTGTGTGGACAGGTAATGGTTGTCTGGTAAAAGGACAGGGCCATCGCCAATTGGAGTA TTTTGTTGATAATGGTCTGCTAGTTGAACGCTTCCATCTTCAATGTTGTGTCTAATTTTGAAGT TAACTTTGATTCCATTCTTTTGTTTGTCTGCCATGATGTATACATTGTGTGAGTTATAGTTGTA TTCCAATTTGTGTCCAAGAATGTTTCCATCTTCTTTAAAATCAATACCTTTTAACTCGATTCTA TTAACAAGGGTATCACCTTCAAACTTGACTTCAGCACGTGTCTTGTAGTTCCCGTCATCTTTG AAAAATATAGTTCTTTCCTGTACATAACCTTCGGGCATGGCACTCTTGAAAAAGTCATGCTGT TTCATATGATCTGGGTATCTCGCAAAGCATTGAACACCATAACCGAAAGTAGTGACAAGTGT TGGCCATGGAACAGGTAGTTTTCCAGTAGTGCAAATAAATTTAAGGGTAAGTTTTCCGTATG TTGCATCACCTTCACCCTCTCCACTGACAGAAAATTTGTGCCCATTAACATCACCATCTAATT CAACAAGAATTGGGACAACTCCAGTGAAAAGTTCTTCTCCTTTACTCATCTTAAACCTCCTTA CCTCGTAAACTATTAAACAAAATTATTTGTAGAGGCTGTTTCGTCCTCACGGACTCATCAGAC CGGAAAGCACATCCGGTGACAGCTGTGCACGTCGGGGTTTGTACCGTACACCACTGAGAC CGCGGTGGTTGACCAGACAAACCACGACACATGTCAATACTTGCCCTTGACAGGCATTGAT GGAATCGTAGTCTCACGCTGATAGTCTGATCGACAATACAAGTGGGACCGTGGTCCCAGAC CGATAATCAGACCGACAACACGAGTGGGATCGTGGTCCCAGACTAATAATCAGACCGACGA TACGAGTGGGACCGTGGTCCCAGACTAATAATCAGACCGACGATACGAGTGGGACCGTGG TTCCAGACTAATAATCAGACCGACGATACGAGTGGGACCGTGGTCCCAGACTAATAATCAG ACCGACGATACGAGTGGGACCATGGTCCCAGACTAATAATCAGACCGACGATACGAGTGGG ACCGTGGTCCCAGTCTGATTATCAGACCGACGATACGAGTGGGACCGTGGTCCCAGACTAA TAATCAGACCGACGATACGAGTGGGACCGTGGTCCCAGACTAATAATCAGACCGACGATAC GAGTGGGACCGTGGTCCCAGTCTGATTATCAGACCGACGATACAAGTGGAACAGTGGGCC CAGAGAGAATATTCAGGCCAGTTATGCTTTCTGGCCTGTAACAAAGGACATTAAGTAAAGAC AGATAAACGTAGACTAAAACGTGGTCGCATCAGGGTGCTGGCTTTTCAAGTTCCTTAAGAAT GGCCTCAATTTTCTCTATACACTCAGTTGGAACACGGGACCTGTCCAGGTTAAGCACCATTT TATCGCCCTTATACAATACTGTCGCTCCAGGAGCAAACTGATGTCGTGAGCTTAAACTAGTT CTTGATGCAGATGACGTTTTAAGCACAGAAGTTAAAAGAGTGATAACTTCTTCAGCTTCAAAT ATCACCCCAGCTTTTTTCTGCTCATGAAGGTTAGATGCCTGCTGCTTAAGTAATTCCTCTTTA TCTGTAAAGGCTTTTTGAAGTGCATCACCTGACCGGGCAGATAGTTCACCGGGGTGAGAAA AAAGAGCAACAACTGATTTAGGCAATTTGGCGGTGTTGATACAGCGGGTAATAATCTTACGT GAAATATTTTCCGCATCAGCCAGCGCAGAAATATTTCCAGCAAATTCATTCTGCAATCGGCTT GCATAACGCTGACCACGTTCATAAGCACTTGTTGGGCGATAATCGTTACCCAATCTGGATAA TGCAGCCATCTGCTCATCATCCAGCTCGCCAACCAGAACACGATAATCACTTTCGGTAAGTG CAGCAGCTTTACGACGGCGACTCCCATCGGCAATTTCTATGACACCAGATACTCTTCGACC GAACGCCGGTGTCTGTTGACCAGTCAGTAGAAAAGAAGGGATGAGATCATCCAGTGCGTCC TCAGTAAGCAGCTCCTGGTCACGTTCATTACCTGACCATACCCGAGAGGTCTTCTCAACACT ATCACCCCGGAGCACTTCAAGAGTAAACTTCACATCCCGACCACATACAGGCAAAGTAATG GCATTACCGCGAGCCATTACTCCTACGCGCGCAATTAACGAATCCACCATCGGGGCAGCTG GTGTCGATAACGAAGTATCTTCAACCGGTTGAGTATTGAGCGTATGTTTTGGAATAACAGGC GCACGCTTCATTATCTAATCTCCCAGCGTGGTTTAATCAGACGATCGAAAATTTCATTGCAGA CAGGTTCCCAAATAGAAAGAGCATTTCTCCAGGCACCAGTTGAAGAGCGTTGATCAATGGC CTGTTCAAAAACAGTTCTCATCCGGATCTGACCTTTACCAACTTCATCCGTTTCACGTACAAC ATTTTTTAGAACCATGCTTCCCCAGGCATCCCGAATTTGCTCCTCCATCCACGGGGACTGAG

	AGCCATTACTATTGCTGTATTTGGTAAGCAAAATACGTACATCAGGCTCGAACCCTTTAAGAT CAACGTTCTTGAGCAGATCACGAAGCATATCGAAAAACTGCAGTGCGGAGGTGTAGTCAAA CAACTCAGCAGGCGTGGGAACAATCAGCACATCAGCAGCACATACGACATTAATCGTGCCG ATACCCAGGTTAGGCGCGCTGTCAATAACTATGACATCATAGTCATGAGCAACAGTTTCAAT GGCCAGTCGGAGCATCAGGTGTGGATCGGTGGGCAGTTTACCTTCATCAAATTTGCCCATT AACTCAGTTTCAATACGGTGCAGAGCCAGACAGGAAGGAATAATGTCAAGCCCCGGCCAGC AAGTGGGCTTTATTGCATAAGTGACATCGTCCTTTTCCCCAAGATAGAAAGGCAGGAGAGTG TCTTCTGCATGAATATGAAGATCTGGTACCCATCCGTGATACATTGAGGCTGTTCCCTGGGG GTCGTTACCTTCCACGAGCAAAACACGTAGCCCCTTCAGAGCCAGATCCTGAGCAAGATGA ACAGAAACTGAGGTTTTGTAAACGCCACCTTTATGGGCAGCAACCCCGATCACCGGTGGAA ATACGTCTTCAGCACGTCGCAATCGCGTACCAAACACATCACGCATATGATTAATTTGTTCAA TTGTATAACCAACACGTTGCTCAACCCGTCCTCGAATTTCCATATCCGGGTGCGGTAGTCGC CCTGCTTTCTCGGCATCTCTGATAGCCTGAGAAGAAACCCCAACTAAATCCGCTGCTTCACC TATTCTCCAGCGCCGGGTTATTTTCCTCGCTTCCGGGCTGTCATCATTAAACTGTGCAATGG CGATAGCCTTCGTCATTTCATGACCAGCGTTTATGCACTGGTTAAGTGTTTCCATGAGTTTCA TTCTGAACATCCTTTAATCATTGCTTTGCGTTTTTTTATTAAATCTTGCAATTTACTGCAAAGC AACAACAAAATCGCAAAGTCATCAAAAAACCGCAAAGTTGTTTAAAATAAGAGCAACACTACA AAAGGAGATAAGAAGAGCACATACCTCAGTCACTTATTATCACTAGCGCTCGCCGCAGCCG TGTAACCGAGCATAGCGAGCGAACTGGCGAGGAAGCAAAGAAGAACTGTTCTGTCAGATAG CTCTTACGCTCAGCGCAAGAAGAAATATCCACCGTGGGAAAAACTCCAGGTAGAGGTACAC ACGCGGATAGCCAATTCAGAGTAATAAACTGTGATAATCAACCCTCATCAATGATGACGAAC TAACCCCCGATATCAGGTCACATGACGAAGGGAAAGAGAAGGAAATCAACTGTGACAAACT GCCCTCAAATTTGGCTTCCTTAAAAATTACAGTTCAAAAAGTATGAGAAAATCCATGCAGGCT GAAGGAAACAGCAAAACTGTGACAAATTACCCTCAGTAGGTCAGAACAAATGTGACGAACCA CCCTCAAATCTGTGACAGATAACCCTCAGACTATCCTGTCGTCATGGAAGTGATATCGCGGA AGGAAAATACGATATGAGTCGTCTGGCGGCCTTTCTTTTTCTCAATGTATGAGAGGCGCATT GGAGTTCTGCTGTTGATCTCATTAACACAGACCTGCAGGAAGCGGCGGCGGAAGTCAGGCA TACGCTGGTAACTTTGAGGCAGCTGGTAACGCTCTATGATCCAGTCGATTTTCAGAGAGACG ATGCCTGAGCCATCCGGCTTACGATACTGACACAGGGATTCGTATAAACGCATGGCATACG GATTGGTGATTTCTTTTGTTTCACTAAGCCGAAACTGCGTAAACCGGTTCTGTAACCCGATAA AGAAGGGAATGAGATATGGGTTGATATGTACACTGTAAAGCCCTCTGGATGGACTGTGCGC ACGTTTGATAAACCAAGGAAAAGATTCATAGCCTTTTTCATCGCCGGCATCCTCTTCAGGGC GATAAAAAACCACTTCCTTCCCCGCGAAACTCTTCAATGCCTGCCGTATATCCTTACTGGCT TCCGCAGAGGTCAATCCGAATATTTCAGCATATTTAGCAACATGGATCTCGCAGATACCGTC ATGTTCCTGTAGGGTGCCATCAGATTTTCTGATCTGGTCAACGAACAGATACAGCATACGTT TTTGATCCCGGGAGAGACTATATGCCGCCTCAGTGAGGTCGTTTGACTGGACGATTCGCGG GCTATTTTTACGTTTCTTGTGATTGATAACCGCTGTTTCCGCCATGACAGATCCATGTGAAGT GTGACAAGTTTTTAGATTGTCACACTAAATAAAAAAGAGTCAATAAGCAGGGATAACTTTGTG AAAAAACAGCTTCTTCTGAGGGCAATTTGTCACAGGGTTAAGGGCAATTTGTCACAGACAGG ACTGTCATTTGAGGGTGATTTGTCACACTGAAAGGGCAATTTGTCACAACACCTTCTCTAGA ACCAGCATGGATAAAGGCCTACAAGGCGCTCTAAAAAAGAAGATCTAAAAACTATAAAAAAA ATAATTATAAAAATATCCCCGTGGATAAGTGGATAACCCCAAGGGAAGTTTTTTCAGGCATC GTGTGTAAGCAGAATATATAAGTGCTGTTCCCTGGTGCTTCCTCGCTCACTCGACCGGGAG GGTTCGAGAAGGGGGGGCACCCCCCTTCGGCGTGCGCGGTCACGCGCACAGGGCGCAGC CCTGGTTAAAAACAAGGTTTATAAATATTGGTTTAAAAGCAGGTTAAAAGACAGGTTAGCGGT GGCCGAAAAACGGGCGGAAACCCTTGCAAATGCTGGATTTTCTGCCTGTGGACAGCCCCTC AAATGTCAATAGGTGCGCCCCTCATCTGTCAGCACTCTGCCCCTCAAGTGTCAAGGATCGC GCCCCTCATCTGTCAGTAGTCGCGCCCCTCAAGTGTCAATACCGCAGGGCACTTATCCCCA GGCTTGTCCACATCATCTGTGGGAAACTCGCGTAAAATCAGGCGTTTTCGCCGATTTGCGA GGCTGGCCAGCTCCACGTCGCCGGCCGAAATCGAGCCTGCCCCTCATCTGTCAACGCCGC

	GCCGGGTGAGTCGGCCCCTCAAGTGTCAACGTCCGCCCCTCATCTGTCAGTGAGGGCCAA GTTTTCCGCGAGGTATCCACAACGCCGGCGGCCGGCCGCGGTGTCTCGCACACGGCTTCG ACGGCGTTTCTGGCGCGTTTGCAGGGCCATAGACGGCCGCCAGCCCAGCGGCGAGGGCA ACCAGCCGAGGGCTTCGCCCTGTCGCTCGACTGCGGCGAGCACTACTGGCTGTAAAAGGA CAGACCACATCATGGTTCTGTGTTCATTAGGTTGTTCTGTCCATTGCTGACATAATCCGCTC CACTTCAACGTAACACCGCACGAAGATTTCTATTGTTCCTGAAGGCATATTCAAATCGTTTTC GTTACCGCTTGCAGGCATCATGACAGAACACTACTTCCTATAAACGCTACACAGGCTCCTGA GATTAATAATGCGGATCTCTACGATAATGGGAGATTTTCCCGACTGTTTCGTTCGCTTCTCA GTGGATAACAGCCAGCTTCTCTGTTTAACAGACAAAAACAGCATATCCACTCAGTTCCACAT TTCCATATAAAGGCCAAGGCATTTATTCTCAGGATAATTGTTTCAGCATCGCAACCGCATCA GACTCCGGCATCGCAAACTGCACCCGGTGCCGGGCAGCCACATCCAGCGCAAAAACCTTC GTGTAGACTTCCGTTGAACTGATGGACTTATGTCCCATCAGGCTTTGCAGAACTTTCAGCGG TATACCGGCATACAGCATGTGCATCGCATAGGAATGGCGGAACGTATGTGGTGTGACCGGA ACAGAGAACGTCACACCGTCAGCAGCAGCGGCGGCAACCGCCTCCCCAATCCAGGTCCTG ACCGTTCTGTCCGTCACTTCCCAGATCCGCGCTTTCTCTGTCCTTCCTGTGCGACGGTTACG CCGCTCCATGAGCTTATCGCGAATAAATACCTGTGACGGAAGATCACTTCGCAGAATAAATA AATCCTGGTGTCCCTGTTGATACCGGGAAGCCCTGGGCCAACTTTTGGCGAAAATGAGACG TTGATCGGCACGTAAGAGGTTCCAACTTTCACCATAATGAAATAAGATCACTACCGGGCGTA TTTTTTGAGTTATCGAGATTTTCAGGAGCTAAGGAAGCTAAAATGGAGAAAAAAATCACTGGA TATACCACCGTTGATATATCCCAATGGCATCGTAAAGAACATTTTGAGGCATTTCAGTCAGTT GCTCAATGTACCTATAACCAGACCGTTCAGCTGGATATTACGGCCTTTTTAAAGACCGTAAA GAAAAATAAGCACAAGTTTTATCCGGCCTTTATTCACATTCTTGCCCGCCTGATGAATGCTCA TCCGGAATTTCGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTCACCCTT GTTACACCGTTTTCCATGAGCAAACTGAAACGTTTTCATCGCTCTGGAGTGAATACCACGAC GATTTCCGGCAGTTTCTACACATATATTCGCAAGATGTGGCGTGTTACGGTGAAAACCTGGC CTATTTCCCTAAAGGGTTTATTGAGAATATGTTTTTCGTCTCAGCCAATCCCTGGGTGAGTTT CACCAGTTTTGATTTAAACGTGGCCAATATGGACAACTTCTTCGCCCCCGTTTTCACCATGG GCAAATATTATACGCAAGGCGACAAGGTGCTGATGCCGCTGGCGATTCAGGTTCATCATGC CGTTTGTGATGGCTTCCATGTCGGCAGAATGCTTAATGAATTACAACAGTACTGCGATGAGT GGCAGGGCGGGGCGTAAGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAAT AGGCGTATCACGAGGCCCTTTCGTCTTCACCTCGAGCACAGCTAACACCACGTCGTCCCTA TCTGCTGCCCTAGGTCTATGAGTGGTTGCTGGATAACTTTACGGGCATGCATAAGGCTCGTA TAATATATTCAGGGAGACCACAACGGTTTCCCTCTACAAATAATTTTGTTTAACTTTTTAATTA AATGCCAACACAATTAACATCTCAATCAAGGTAAATGCTTTTTGCTTTTTTTGCATCGATTCTA GGGCGGCGGATTTGTCCTACTCAGGAGAGCGTTCACCGACAAACAACAGATAAAACGAAAG GCCCAGTCTTTCGACTGAGCCTTTCGTTTTATTTGATGCCACGCGTACCATGGGATCCCCCG GGTTATTTGTACAATTCATCCATACCATGGGTAATACCAGCAGCAGTCCTAAATTCTAACAGG ACCATGTGGTCTCTCTTTTCGTTTGGATCTTTGGATAAGGCTGATTGGGTGGATAAGTAATG GTTGTCTGGTAACAAGACTGGACCATCACCAATTGGAGTATTTTGTTGATAATGGTCAGCTA ATTGAACAGAACCATCTTCAATGTTGTGTCTAATTTTGAAGTTCACTTTGATACCATTCTTTTG TTTGTCAGCCATGATGTATATATTGTGAGAGTTGAAGTTGTATTCCAATTTGTGACCTAAAAT GTTACCATCTTCTTTAAAATCAATACCTTTTAATTCGATTCTATTAACTAAGGTATCACCTTCA AACTTGACTTCAGCTCTGGTCTTGTAGTTACCGTCATCTTTGAAAAAAATAGTTCTTTCTTGA ACATAACCTTCTGGCATGGCAGACTTGAAAAAGTCATGTTGTTTCATATGATCTGGGTATCTA GAAAAACATTGAACACCATGGCTCAAAGTAGTTACTAAGGTTGGCCATGGAACTGGCAATTT ACCAGTAGTACAAATAAATTTTAAGGTCAATTTACCGTACGTAGCATCACCTTCACCTTCACC GGAGACAGAAAATTTGTGACCATTAACATCACCATCTAATTCAACCAAAATTGGGACAACAC CAGTGAATAATTCTTCACCTTTAGACATTTTTAACCTCCTCCCTACGTACTCATTAAACAAAAT TATTTGTAGAGGCTGTTTCGTCCTCACGGACTCATCAGACCGGAAAGCACATCCGGTGACA GCTGTGTTTAAAGGAGTTTTTTAGTTACCTTAATTGAAATAAACGAAATAAAAACTCGCCGAG

	CACCGCCTTTGGTCGAAAAAAAAAAGCCCGCACTGTCAGGTGCGGGCTTTTTTCTGTGTTTCC CTCGAGCACAGCTAACACCACGTCGTCCCTATCTGCTGCCCTAGGTCTATGAGTGGTTGCT GGATAACTTTACGGGCATGCATAAGGCTCGTATAATATATTCAGGGAGACCACAACGGTTTC CCTCTACAAATAATTTTGTTTAACTTTTTAATTAATGCGGGTGCCAGGGCGTGCCCTTGGGCT CCCCGGGCGCGTACTCCATCGATTCTAGGGCGGCGGATTTGTCCTACTCAGGAGAGCGTT CACCGACAAACAACAGATAAAACGAAAGGCCCAGTCTTTCGACTGAGCCTTTCGTTTTATTT GATGCCTCTAGCACGCGTACCATGGGATCCCCCGGGTTAGTTCAGTTTGTGGCCCAGTTTG GAAGGCAGATCGCAATAGCGTGCCACTGCCACTTCATGCTGTTCGACATAGGTTTCTTTGTC CGCTTCTTTGATACGTTCCAGACGGCGGTCGACATAATATACGCCCGGCATTTTCAGGTTTT TAGCCGGTTTTTTGCTGCGGTAAGTAGTTTTCAGGTTACAGATCAGGTGGCCGCCACCAAC CAGTTTCAGCGCCATATCAGAACGGCCTTCCAGGCCGCCGTCAGCCGGGTACAGCATTTCG GTGGATGCTTCCCAGCCCAGCGTTTTTTTCTGCATAACCGGGCCGTTGCTCGGGAAATTAA CACCACGAATTTTAACGTTATAGATCAGACAGCCATCCTGCAGAGACGTGTCCTGCGTAGC GGTCAGGACGCCACCATCTTCATACGTCGTAACGCGTTCCCAGGTAAAGCCCTCCGGGAAG CTCTGTTTGAAAAAATCCGGGATACCTTGAGTGTGGTTGATGAAGGTTTTGCTGCCGTACAT GAAGCTGGTCGCCAGGATGTCGAATGCAAACGGCAGCGGACCGCCTTCCACAACTTTGATA CGCATGGTTTGGGTGCCCTCATACGGTTTGCCTTCGCCTTCGCTGGTACATTTGAAGTGGT GGTTGTTTACAGTACCTTCCATGTACAGCTTCATGTGCATGTTTTCTTTAATCAGTTCTTCAC CTTTGGAAACCATTTTTACCTCCTTATTAATTTTATGTCGTTTAAACAAAATTATTTGTAGAGG CTGTTTCGTCCTCACGGACTCATCAGACCGGAAAGCACATCCGGTGACAGCTGTGCACCCC CCAACTGAGAGAACTCAAAGGTTACCCCAGTTGGGGCACCGAGCATTGAGAAGAGAAAAGA AAACCGCCGATCCTGTCCACCGCATTACTGCAAGGTAGTGGACAAGACCGGCGG

pZS2oxySp-GFP-proD-oxyR	ACACCGCCATTTTCGGCGTGCGGCAGATTCCTGCCACGTTAGCCAGCCGACGCTTAGCGG GCAAATTCGTAAGCTGGAAGATGAGCTGGGCGTGATGTTGCTGGAGCGGACCAGCCGTAA AGTGTTGTTCACCCAGGCGGGAATGCTGCTGGTGGATCAGGCGCGTACCGTGCTGCGTGA GGTGAAAGTCCTTAAAGAGATGGCAAGCCAGCAGGGCGAGACGATGTCCGGACCGCTGCA CATTGGTTTGATTCCCACAGTTGGACCGTACCTGCTACCGCATATTATCCCTATGCTGCACC AGACCTTTCCAAAGCTGGAAATGTATCTGCATGAAGCACAGACCCACCAGTTACTGGCGCA ACTGGACAGCGGCAAACTCGATTGCGTGATCCTCGCGCTGGTGAAAGAGAGCGAAGCATTC ATTGAAGTGCCGTTGTTTGATGAGCCAATGTTGCTGGCTATCTATGAAGATCACCCGTGGGC GAACCGCGAATGCGTACCGATGGCCGATCTGGCAGGGGAAAAACTGCTGATGCTGGAAGA TGGTCACTGTTTGCGCGATCAGGCAATGGGTTTCTGTTTTGAAGCCGGGGCGGATGAAGAT ACACACTTCCGCGCGACCAGCCTGGAAACTCTGCGCAACATGGTGGCGGCAGGTAGCGGG ATCACTTTACTGCCAGCGCTGGCTGTGCCGCCGGAGCGCAAACGCGATGGGGTTGTTTATC TGCCGTGCATTAAGCCGGAACCACGCCGCACTATTGGCCTGGTTTATCGTCCTGGCTCACC GCTGCGCAGCCGCTATGAGCAGCTGGCAGAGGCCATCCGCGCAAGAATGGATGGCCATTT CGATAAAGTTTTAAAACAGGCGGTTTAACCCGGGGGATCCCATGGTACGCGTGCTAGAGGC ATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCG GTGAACGCTCTCCTGAGTAGGACAAATCCGCCGCCCTAGACCTAGGGCCTAGGGTACGGG TTTTGCTGCCCGCAAACGGGCTGTTCTGGTGTTGCTAGTTTGTTATCAGAATCGCAGATCCG GCTTCAGGTTTGCCGGCTGAAAGCGCTATTTCTTCCAGAATTGCCATGATTTTTTCCCCACG GGAGGCGTCACTGGCTCCCGTGTTGTCGGCAGCTTTGATTCGATAAGCAGCATCGCCTGTT TCAGGCTGTCTATGTGTGACTGTTGAGCTGTAACAAGTTGTCTCAGGTGTTCAATTTCATGTT CTAGTTGCTTTGTTTTACTGGTTTCACCTGTTCTATTAGGTGTTACATGCTGTTCATCTGTTAC ATTGTCGATCTGTTCATGGTGAACAGCTTTAAATGCACCAAAAACTCGTAAAAGCTCTGATGT ATCTATCTTTTTTACACCGTTTTCATCTGTGCATATGGACAGTTTTCCCTTTGATATCTAACGG TGAACAGTTGTTCTACTTTTGTTTGTTAGTCTTGATGCTTCACTGATAGATACAAGAGCCATA AGAACCTCAGATCCTTCCGTATTTAGCCAGTATGTTCTCTAGTGTGGTTCGTTGTTTTTGCGT GAGCCATGAGAACGAACCATTGAGATCATGCTTACTTTGCATGTCACTCAAAAATTTTGCCT CAAAACTGGTGAGCTGAATTTTTGCAGTTAAAGCATCGTGTAGTGTTTTTCTTAGTCCGTTAC GTAGGTAGGAATCTGATGTAATGGTTGTTGGTATTTTGTCACCATTCATTTTTATCTGGTTGT TCTCAAGTTCGGTTACGAGATCCATTTGTCTATCTAGTTCAACTTGGAAAATCAACGTATCAG TCGGGCGGCCTCGCTTATCAACCACCAATTTCATATTGCTGTAAGTGTTTAAATCTTTACTTA TTGGTTTCAAAACCCATTGGTTAAGCCTTTTAAACTCATGGTAGTTATTTTCAAGCATTAACAT GAACTTAAATTCATCAAGGCTAATCTCTATATTTGCCTTGTGAGTTTTCTTTTGTGTTAGTTCT TTTAATAACCACTCATAAATCCTCATAGAGTATTTGTTTTCAAAAGACTTAACATGTTCCAGAT TATATTTTATGAATTTTTTTAACTGGAAAAGATAAGGCAATATCTCTTCACTAAAAACTAATTCT AATTTTTCGCTTGAGAACTTGGCATAGTTTGTCCACTGGAAAATCTCAAAGCCTTTAACCAAA GGATTCCTGATTTCCACAGTTCTCGTCATCAGCTCTCTGGTTGCTTTAGCTAATACACCATAA GCATTTTCCCTACTGATGTTCATCATCTGAGCGTATTGGTTATAAGTGAACGATACCGTCCGT TCTTTCCTTGTAGGGTTTTCAATCGTGGGGTTGAGTAGTGCCACACAGCATAAAATTAGCTT GGTTTCATGCTCCGTTAAGTCATAGCGACTAATCGCTAGTTCATTTGCTTTGAAAACAACTAA TTCAGACATACATCTCAATTGGTCTAGGTGATTTTAATCACTATACCAATTGAGATGGGCTAG TCAATGATAATTACTAGTCCTTTTCCTTTGAGTTGTGGGTATCTGTAAATTCTGCTAGACCTTT GCTGGAAAACTTGTAAATTCTGCTAGACCCTCTGTAAATTCCGCTAGACCTTTGTGTGTTTTT TTTGTTTATATTCAAGTGGTTATAATTTATAGAATAAAGAAAGAATAAAAAAAGATAAAAAGAA TAGATCCCAGCCCTGTGTATAACTCACTACTTTAGTCAGTTCCGCAGTATTACAAAAGGATGT CGCAAACGCTGTTTGCTCCTCTACAAAACAGACCTTAAAACCCTAAAGGCTTAAGTAGCACC CTCGCAAGCTCGGGCAAATCGCTGAATATTCCTTTTGTCTCCGACCATCAGGCACCTGAGTC GCTGTCTTTTTCGTGACATTCAGTTCGCTGCGCTCACGGCTCTGGCAGTGAATGGGGGTAA ATGGCACTACAGGCGCCTTTTATGGATTCATGCAAGGAAACTACCCATAATACAAGAAAAGC CCGTCACGGGCTTCTCAGGGCGTTTTATGGCGGGTCTGCTATGTGGTGCTATCTGACTTTTT

	GCTGTTCAGCAGTTCCTGCCCTCTGATTTTCCAGTCTGACCACTTCGGATTATCCCGTGACA GGTCATTCAGACTGGCTAATGCACCCAGTAAGGCAGCGGTATCATCAACAGGCTTACCCGT CTTACTGTCCCTAGTGCTTGGATTCTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTT CTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGATCTATCAACAGGAGTCCAAGCGA GCTCTCGAACCCCAGAGTCCCGCTCAGAAGAACTCGTCAAGAAGGCGATAGAAGGCGATG CGCTGCGAATCGGGAGCGGCGATACCGTAAAGCACGAGGAAGCGGTCAGCCCATTCGCCG CCAAGCTCTTCAGCAATATCACGGGTAGCCAACGCTATGTCCTGATAGCGGTCCGCCACAC CCAGCCGGCCACAGTCGATGAATCCAGAAAAGCGGCCATTTTCCACCATGATATTCGGCAA GCAGGCATCGCCATGGGTCACGACGAGATCCTCGCCGTCGGGCATGCGCGCCTTGAGCCT GGCGAACAGTTCGGCTGGCGCGAGCCCCTGATGCTCTTCGTCCAGATCATCCTGATCGACA AGACCGGCTTCCATCCGAGTACGTGCTCGCTCGATGCGATGTTTCGCTTGGTGGTCGAATG GGCAGGTAGCCGGATCAAGCGTATGCAGCCGCCGCATTGCATCAGCCATGATGGATACTTT CTCGGCAGGAGCAAGGTGAGATGACAGGAGATCCTGCCCCGGCACTTCGCCCAATAGCAG CCAGTCCCTTCCCGCTTCAGTGACAACGTCGAGCACAGCTGCGCAAGGAACGCCCGTCGT GGCCAGCCACGATAGCCGCGCTGCCTCGTCCTGCAGTTCATTCAGGGCACCGGACAGGTC GGTCTTGACAAAAAGAACCGGGCGCCCCTGCGCTGACAGCCGGAACACGGCGGCATCAGA GCAGCCGATTGTCTGTTGTGCCCAGTCATAGCCGAATAGCCTCTCCACCCAAGCGGCCGGA GAACCTGCGTGCAATCCATCTTGTTCAATCATGCGAAACGATCCTCATCCTGTCTCTTGATC AGATCTTGATCCCCTGCGCCATCAGATCCTTGGCGGCAAGAAAGCCATCCAGTTTACTTTGC AGGGCTTCCCAACCTTACCAGAGGGCGCCCCAGCTGGCAATTCCGACGTCTTCATTATCCA TCCTCCATCGCCACGATAGTTCATGGCGATAGGTAGAATAGCAATGAACGATTATCCCTATC AAGCATTCTGACTGATAATTGCTCACACGAATTCATTAAAGAGGAGAAAGGTACCATGAGTA AAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATG GGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTT AAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTTTCGGT TATGGTGTTCAATGCTTTGCGAGATACCCAGATCATATGAAACAGCATGACTTTTTCAAGAGT GCCATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAA GACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTA TTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAATTGGAATACAACTATAACTCACACA ATGTATACATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTTCAAAATTAGACACA ACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGAT GGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAAGATCC CAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACATG GCATGGATGATCTCTACAAATAACCCGGGGGATCCCATGGTACGCGTGCTAGAGGCATCAA ATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAA CGCTCTCCTGAGTAGGACAAATCCGCCGCCCTAGACCTAGCACAGCTAACACCACGTCGTC CCTATCTGCTGCCCTAGGTCTATGAGTGGTTGCTGGATAACTTTACGGGCATGCATAAGGCT CGTATAATATATTCAGGGAGACCACAACGGTTTCCCTCTACAAATAATTTTGTTTAACTTTGA ATTCTTCACACAGGAAACCGGTACCATGAATATTCGTGATCTTGAGTACCTGGTGGCATTGG CTGA

Table B.3| List of synthetic parts

Part Name	**Description and Source**
oxySp	Promoter for *E. coli oxySp* RNA[287]
katGp	Promoter for *E. coli katG*[287]
ahpCp	Promoter for *E. coli ahpC*[287]

proD	Strong constitutive promoter[288]
proA	Weak constitutive promoter[288]
pLtetO	tetR-regulated lambda phage promoter[222]
RBS30	Ribosome binding site. BBa_B0030[289]
RBS29	Ribosome binding site. BBa_B0029[289]
RBS33	Ribosome binding site. BBa_B0033[289]
RBS31	Ribosome binding site. BBa_B0031[289]
"RBS" with no number	RBS with maximized strength using computational method[225]
RiboJ	Ribozyme-insulator[224]
oxyR	*oxyR* protein-coding sequence[287]
mCherry	mCherry fluorescent protein coding sequence. BBa_J06504[289]
mKate	mKate fluorescent protein coding sequence[290]
azurite	Azurite fluorescent protein coding sequence[291]
gfp	Gfpmut3 fluorescent protein coding sequence. BBa_K863120[289]
Bxb1	Bxb1 serine integrase protein coding sequence[219]
phiC31	PhiC31 serine integrase protein coding sequence[219]
tp901	TP901 serine integrase protein coding sequence[220]
Bxb1B/P	Bxb1 AttB and Bxbi AttP DNA recombination sites[219]
PhiCB/P	PhiC31 AttB and Bxbi AttP DNA recombination sites[219]
TP901B/P	TP901 AttB and Bxbi AttP DNA recombination sites[220]
ECK120029600	Synthetic transcriptional terminator[292]
ECK120033737	Synthetic transcriptional terminator[292]
AAV	AAV degradation tag[289]
TermT1	Transcriptional Terminator T1[222]
TermT0	Transcriptional Terminator T0[222]
p15A	Medium-copy number plasmid origin of replication[222]

pSC101	Low-copy number plasmid origin of replication[222]
ampR	Ampicillin-resistance cassette[222]
kanR	Kanamycin-resistance cassette[222]
cmR	Spectinomycin-resistance cassette[222]
oriV	Trfa-activated plasmid origin of replication[223]
BAC/F/RepE *incW* *parA/B/C*	Bacterial artificial chromosome replication factors and origin[223]

Data Processing and Calculations

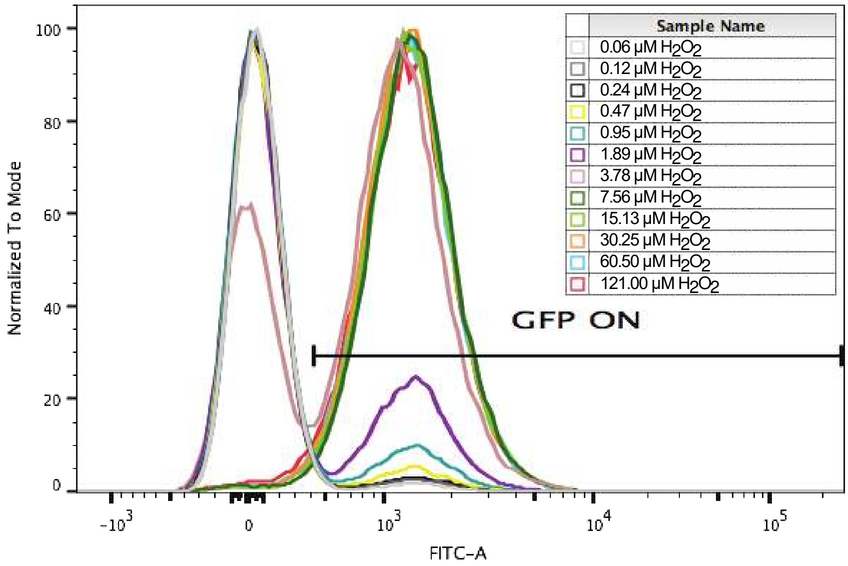
Table B.4| Fitting parameters used in this study

Data	ON_{Max}	*n*	K_{on}	ON_{Min}
Figure 3.2-b, red	93.90	2.603	2.650	4.587
Figure 3.2-d, red	90.22	4.245	11.73	3.208
Figure 3.2-f, red	92.83	3.138	30.61	0.7300
Figure 3.3-b, highpass parameters	94.29	2.623	5.328	1.173
Figure 3.3-b, lowpass parameters	94.88	4.550	19.01	0.3330
Figure 3.3-d, highpass parameters	91.49	2.519	4.519	1.457
Figure 3.3-d, lowpass parameters	94.01	2.434	38.14	0.01933
Figure 3.4-b, green	91.62	-2.512	3.782	0.00889
Figure 3.4-b, red	94.89	3.144	11.04	1.330

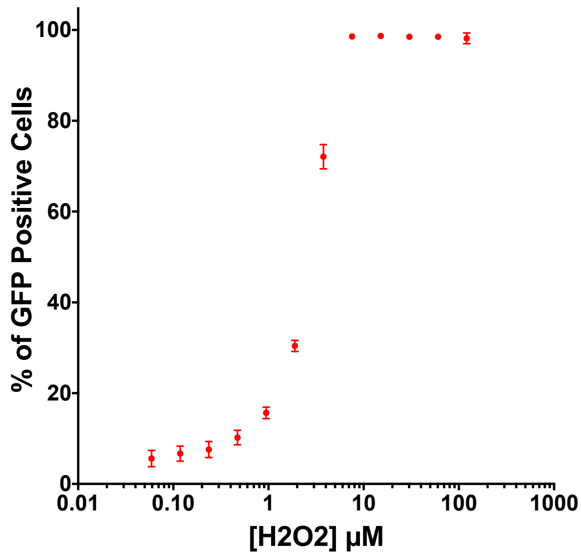
Figure 3.4-d, green	90.89	2.684	5.545	4.780
Figure 3.4-d, red	94.24	3.098	15.29	1.720
Figure 3.4-d, blue	91.88	2.900	41.65	2.727
Figure 3.5-b	Same as Figure 3.3-b	Same as Figure 3.3-b	Same as Figure 3.3-b	Same as Figure 3.3-b
Figure B.1-b	28628	1.515	163.2	13.50
Figure B.2-b, black	82.31	2.155	1.719	18.07
Figure B.2-b, red	93.99	2.669	2.676	5.613
Figure B.3b	92.96	-3.008	2.861	0.2667
Figure B.14, green	90.94	2.461	2.051	2.287
Figure B.14, red	94.75	2.085	4.983	-0.3013
Figure B.14, blue	86.67	2.468	18.80	2.814

Calculating the sigmoidal fit, input threshold, and relative input range

The data from the BAC circuit in Figure B.2 is shown as an example.



Calculate % of cells at each concentration of H_2O_2 that fall within the "GFP ON" gate. The "GFP ON" gate is drawn for each experiment at the FITC fluorescence level where the fluorescence distribution of uninduced cells intersects with the fluorescence distribution of induced cells, or in between the uninduced and induced distributions when the fluorescence distributions are well-resolved and do not overlap. Take the average %ON of biological replicates to calculate the

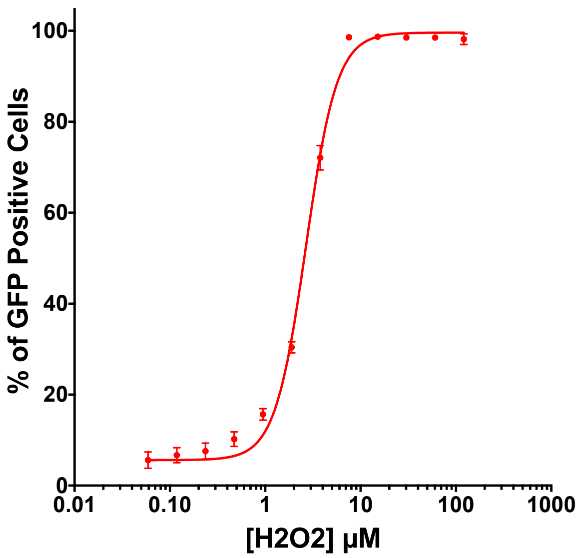


mean and standard deviation (plotted).

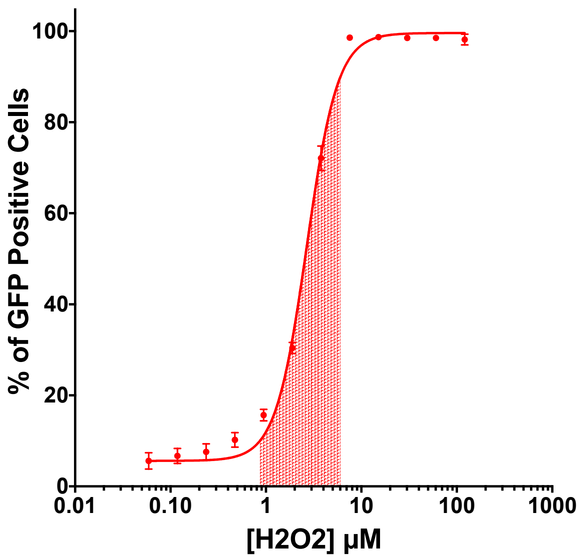
To derive the transfer function, fit the mean %ON vs. H_2O_2 concentration data to a Hill-like sigmoidal function (solid line below):

$$\%ON = ON_{Max}\frac{[H_2O_2]^n}{[H_2O_2]^n + (K_{on})^n} + ON_{Min}$$

Where $[H_2O_2]$ is the independent variable, ON_{Min} is the empirically observed minimum percent ON, and ON_{Max}, K_{on}, and *n* are fit to the data.



The input dynamic range (transition band, shaded red below) is defined as the input H_2O_2 concentration span that yields 10% ON to 90% ON, as interpolated from the transfer function:



Calculate the relative input range from the 10% ON and 90% ON input values:

$$Relative\ Input\ Range\ (RIR) = \frac{[H_2O_2]_{90\%}}{[H_2O_2]_{10\%}}$$

Calculating the fit to a bandpass filter circuit

The fit to a bandpass filter circuit (black line in Figure 3.3-b,d and Figure 3.5-b) was derived by subtracting the transfer function of the low-pass comparator (Figure B.6-f or Figure B.7-f) from the transfer function of the high-pass comparator (Figure B.6-c or Figure B.7-c):

$$\%ON = ON_{Max,hp} \cdot \frac{H_2O_2^{n,hp}}{H_2O_2^{n,hp} + (K_{on,hp})^{n,hp}} + ON_{Min,hp} - ON_{Max,lp} \cdot \frac{H_2O_2^{n,lp}}{H_2O_2^{n,lp} + (K_{on,hp})^{n,lp}} - ON_{Min,lp}$$

Where *hp* subscript denotes a variable from the “high pass” circuit and *lp* subscript denotes a variable from the “low pass” circuit.

Calculating the relative resolution of a genetic analog-to-digital converter circuit

We defined the relative resolution (*RQ*) as:

$$RQ = \frac{ADC\ RIR}{2^{bits} - 2}$$

Where the *ADC RIR* is:

$$\frac{[H_2O_2]_{50\%,high}}{[H_2O_2]_{50\%,low}}$$

$[H_2O_2]_{50\%,high}$ is the concentration of H_2O_2 necessary for 50% of cells to turn ON for the highest threshold comparator in the ADC, and $[H_2O_2]_{50\%,low}$ is the concentration of H_2O_2 necessary for 50% of cells to turn ON for the lowest threshold comparator in the ADC.

The number of bits is the total number of bits encoded by the ADC (in the case of Figure 3.4-d,f it is 2 bits). We subtract 2 in the denominator because 2 of the states are encoded outside of the ADC RIR (i.e., below the $[H_2O_2]_{50\%,low}$concentration and above the $[H_2O_2]_{50\%,high}$ concentration, states 000 and 111).

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