Title: Methods for studying the zebrafish brain: past, present and future

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Abstract
The zebrafish (*Danio rerio*) is one of the most promising new model organisms. The increasing popularity of this amazing small vertebrate is evident **from** the exponentially growing numbers of research articles, funded projects and new discoveries associated with the use of zebrafish for studying development, brain function, human diseases and screening for new drugs. Thanks to the development of novel technologies, the range of zebrafish research is constantly expanding with new tools synergistically enhancing traditional techniques. In this review we will highlight the past and present techniques which have made, and continue to make, zebrafish an attractive model organism for various fields of biology, with a specific focus on neuroscience.

1. History

Use of the zebrafish as a model organism was pioneered in the 1960s by George Streisinger, one of the founders of modern genetics who sought a genetically tractable vertebrate model organism. To understand the genetics of a vertebrate was a remarkably ambitious goal for a time when the study of the molecular genetics of invertebrates was in its early stages. While *Drosophila* was becoming the mainstream organism for genetics and others were establishing *Caenorhabditis elegans*, Streisinger was experimenting with a variety of tropical fishes, ultimately selecting zebrafish as the most advantageous. Streisinger's specific interest was in uncovering the genetics of neurodevelopment, which continues to be one of the main areas of zebrafish research today. However zebrafish research is not limited to genetics, as from the beginning other advantageous properties were recognised, such as the large and easily identifiable Mauthner cells, responsible for the stereotyped escape response, which were perfect for eletrophysiological study(Eaton & Farley, 1975). Zebrafish are ideally suited to developmental biology due to a range of properties. The rapid, external development of the transparent egg and embryo allow embryogenesis to be studied longitudinally in the living embryo. This is further enhanced by the compact size of the embryo and the brain combined with its self-sustaining nature, as it subsists on an internal yolk for the first week of life, by which time the organs are formed, the nervous system is active and the animal is capable of a range of behaviours. Conveniently, this entire process can take place in only a few hundred microlitres of water. These properties were exploited by pioneering researchers in the 1980s to reveal the development and patterning of the nervous system in a vertebrate, particularly in the work on motoneurons of the developing spinal cord by Westerfield and Eisen, (Myers *et al.*, 1986; Liu & Westerfield, 1988; Westerfield & Eisen, 1988), which proved foundational for many later studies(Saint-Amant & Drapeau, 1998).

Zebrafish occupy a midpoint niche in the roster of model organisms, somewhere between the invertebrates and mammals. They belong to the infraclass teleosti which account for almost half of all extant vertebrate species, therefore zebrafish could be described as the 'average' vertebrate. Using zebrafish has the advantages of working with a vertebrate model with the addition of high-throughput approaches that are more economic and rapid, especially when designing new experiments. In the modern scientific and social climate, zebrafish, as a ‘lower vertebrate’, contributes to the 3Rs (replacement, refinement, reduction) by replacing certain mammalian experiments and reducing the numbers of mammals required. Initially there was doubt as to the relevance of zebrafish to translational research as the level of genetic conservation between species was unclear. This was assuaged by cross species similarities in mutants such as the zebrafish mutant, no tail, which shares both phenotype and affected gene with the well known mouse mutant, Brachyury (Schulte-Merker *et al.*, 1994). Advances in genetics, particularly genome mapping, and molecular biology, have continued to highlight the similarities between the zebrafish and mammalian genomes and proteomes(Howe *et al.*, 2013). An early area of zebrafish research was creating homozygous diploid lines and haploid embryos which, given the methods available at the time, would greatly improve the process of screening

for mutants by preventing masking by wild type alleles. This task may have been more difficult than anticipated as we now know that zebrafish do not tolerate inbreeding, which has resulted in a lack of well characterised inbred strains. Nonetheless, in 1981, after many years, the work to establish germline mutations in lethal mutation free clonal lines in a vertebrate was published as the cover article of Nature(Streisinger *et al.*, 1981). Zebrafish had arrived as the new genetic model.

Today a vast catalogue of mutant and transgenic zebrafish lines exist, with a bias towards mutations that affect development. This bias arises due to the ease with which developmental mutants can be identified and the not insignificant proportion of mutations which are post embryonic lethal, often due to a failure of swim bladder inflation around 5 days post fertilisation. Early forward genetic approaches were continued by large scale N-ethyl-N-nitrosourea (ENU) chemical mutagenesis screens, the most well-known being the Tubingen and Boston screens which have created thousands of developmental and behavioural mutants by random chemical mutagenesis and earned a dedicated issue of Development(Development, 1996; Nusslein-Volhard, 2012). In more recent years, the Zebrafish Mutation Project has generated mutations for 12,000 genes, which is 45% of the final target to generate knockouts for every protein coding gene in the zebrafish genome(Miller *et al.*, 2011; Hwang *et al.*, 2013b; Kettleborough *et al.*, 2013). **However,** validating and thoroughly cataloguing all of these potential mutants is arguably a more daunting task than generating them. This project is in addition to the thousands of mutants which already exist and the 27,000 transgenics currently listed on the zebrafish database site, ZFIN (zfin.org). It is clear that the zebrafish has always been a powerful organism for forward genetics and now also reverse genetics with the advent of genome editing tools which allow targeted mutation in a relatively easy and rapid manner(Gonzalez *et al.*, 2010). Such approaches are made possible by the sequencing and annotation of the zebrafish genome which was completed by the Sanger Institute in 2010, with ongoing improvements and variations being added continuously(Howe *et al.*, 2013). These genome editing techniques will be outlined below, along with a wide range of other techniques which are used successfully in zebrafish.

2. Mutagenesis

Although radiation has been used in the past to create random germline mutations(Walker & Streisinger, 1983), ENU mutagenesis has dominated zebrafish mutagenesis until recently. This was mainly due to the ease of using chemical mutagenesis and its compatibility with high throughput generation and screening methodologies(Kalueff *et al.*, 2014). The large clutch size (up to 200 eggs per female), frequency of mating (a mutagenised male can be mated daily), rapid early development, small size, density at which they can be raised and transparency all contribute to an efficient, rapid and cheap method to screen large numbers of potential founders for any developmental phenotype in any of the observable organs or behavioural phenotypes(Mullins *et al.*, 1994; Driever *et al.*, 1996; Haffter *et al.*, 1996). This has led to the zebrafish catalogue of mutants being particularly enriched for mutations which are more readily identified, such as those of the motor and visual systems. By establishing methods to analyse behavioural consequences in

these mutants, such as optomotor responses, to identify key genes, important steps were made towards genetically dissecting vertebrate vision(Neuhauss *et al.*, 1999). Many mutants exist which have been used as disease models, including for disorders of the CNS (**Table 1**). These range from high profile disorders such as Alzheimer's disease(Paquet *et al.*, 2009) to less known disorders such as Menkes disease(Madsen & Gitlin, 2008) to complex psychiatric disorders such as schizophrenia(Morris, 2009) and addiction(Darland & Dowling, 2001).

A drawback of ENU techniques was their reliance on linkage analysis based positional cloning to locate the site of mutagenesis and affected gene. As the point mutations generated by ENU are random, it can be difficult to locate the mutated gene. Due to this, some mutants have yet to be cloned, including those with phenotypes which imply the biological significance of the gene. This remains true, despite the sequencing of the genome and with the availability of TILLING(Moens *et al.*, 2008) and, later, whole genome sequencing techniques(Leshchiner *et al.*, 2012), as some mutations known since the early 1990s are listed on ZFIN as location "ambiguous"(Stainier *et al.*, 1995). This is further complicated by the fact that a zebrafish ancestor species underwent genome duplication 200 million years ago resulting in zebrafish having 2 paralogues for many genes found in mammals as a single orthologue (Santini *et al.*, 2009). This can present problems, as to achieve comparable knockout to a mammalian mutant, 2 zebrafish genes may have to be knocked out, which was relatively difficult when mutants were being randomly generated. However it can provide opportunities as the original gene function may be split between paralogues, which have diverged during evolution, allowing elements of gene function to be dissected by manipulating each paralogue separately. While the forms of mutagenesis outlined so far produce permanent, and potentially heritable, genetic alterations, they are untargeted. Other techniques were developed to allow for the targeting of specific genetic sequences, at the cost of these effects being transient and therefore not heritable.

3. Transient alteration of gene expression

3a. Morpholinos

Morpholino oligonucleotides are a synthetic form of DNA in which the deoxyribose ring has been replaced with a morpholine ring(Summerton & Weller, 1997). Antisense morpholinos are injected into the fertilised egg where they decrease the expression of the endogenous target gene (**Fig.1A**) (Nasevicius & Ekker, 2000; Bill *et al.*, 2009). Injection of morpholinos into a zebrafish zygote is an easy technique as zebrafish eggs are robust, large (1mm diameter), transparent and can be collected daily in large numbers at the single cell stage. Morpholinos work most efficiently during the first 2 days of development, but effective duration is dependent on protein turnover rate(Kimmel *et al.*, 2003; Bill *et al.*, 2008). The effect of morpholinos is known as knockdown because it is a transitory and usually incomplete form of knockout. The 2 forms of morpholino are start codon directed morpholinos which prevent translation leading to a reduction in protein expression (**Fig.2B**)(Summerton & Weller, 1997; Nasevicius & Ekker, 2000; Eisen & Smith, 2008; Bill *et al.*, 2009), and splice site directed morpholinos which sterically block target pre-

mRNA to prevent normal processing by spliceosomes, resulting in alternatively spliced mRNA (**Fig.2C**). The efficacy of splice site morpholinos can be determined by the ratio of normally spliced mRNA to alternatively spliced mRNA(Morcos, 2007; Wyatt *et al.*, 2010). Morpholinos continue to evolve with new forms such as Vivo-Morpholinos which are readily taken into cells by endocytosis and protected from enzymatic degradation so increasing their activity, period of usefulness and ease with which they can be introduced into mature cells(Morcos *et al.*, 2008). Photo-uncaging of morpholinos offers strict spatial and temporal control over morpholino activation(Deiters *et al.*, 2010). The original advantage of morpholinos, generating knockdown phenotypes in wildtype embryos, has largely been superceded by cutting edge targeted genome editing techniques which can make stable, heritable changes to the genome (described in section 4). However morpholinos remain useful for rapid knockdown of a chosen gene, which can inform choices before the investment of time is made to create a mutant line. This does not mean that morphant phenotypes will exactly match that of mutants, as recent work suggests that a majority of morpholino phenotypes differ in some way from the corresponding mutant phenotype (Kok *et al.*, 2014). These discrepancies may result from the differing levels of knockdown/knockout, off-target-like effects in either the morphants or mutants and the existence of hypomorphic and gain-of-function mutations. Morpholinos are not solely useful for studying development as the ability to transiently knockdown a gene during development can allow the study of animals which underwent abnormal development but currently have normal gene expression due to having no permanent mutations(Wyatt *et al.*, 2010). Additionally, the incomplete knockdown achieved with morpholinos can be useful when a full knockout mutation proves to be lethal. The challenge of introducing morpholinos into adult tissues has been addressed in a variety of manners, using Vivo-Morpholinos, electroporation(Thummel *et al.*, 2011) and surgical implantation of morpholino beads(Becker *et al.*, 2004). A major caveat when using morpholinos is the need for vigilance for off target and toxic effects, which often manifest as developmental retardation and deformity, particularly of the eyes and body length(Bedell *et al.*, 2011). This can be controlled by titration of morpholino concentration or using a different morpholino sequence which targets the same gene. The most convincing proof of the specificity of a morpholino is the rescue of the phenotype by co-injection of mRNA for the target gene, provided the mRNA does not contain the morpholino target sequence(Bill *et al.*, 2009). This can help to test cross species differences in protein function by attempting to rescue a zebrafish morphant with mRNA derived from another genome. Such experiments have shown that a zebrafish morphant for the paralogue of an Alzheimer's disease linked gene, APPb, can be rescued by mRNA coding for the human protein, APP(Song & Pimplikar, 2012).

3b. mRNA and DNA

mRNA injection is a standard technique in zebrafish developmental studies. mRNA is first synthesised from cDNAs which code for proteins of interest. When injected into the single cell zygote, mRNA sequences are distributed to all cells subsequently formed. The endogenous translation machinery expresses the proteins coded for by the mRNA as early as the 256 cell stage,

around 3 hours post fertilisation (hpf)(Kane & Kimmel, 1993). Thus an advantage of mRNA injection is very rapid expression of the transgene, whose expression levels can be adjusted by controlling the concentration of mRNA injected (**Fig.2D**). A downside is that mRNA activity is correspondingly brief because of degradation by normal cellular mechanisms or dilution due to the ever increasing number of cells. Generally mRNA is most effective during the first day of development(Erter *et al.*, 1998; Gritsman *et al.*, 1999). Thus mRNA is a tool used to study early development through ubiquitous misexpression of transgenes.

A related technique involves the injection of DNA constructs. Unlike morpholinos and mRNA which will pass into the cell following injection into the large embryonic yolk, DNA constructs must be directly injected into the cell, preferably at the single cell stage. Expression of the transgene will not occur before the shield stage (6hpf), after the maternal-zygotic transition, but may remain for weeks. Generally, translation occurs using the construct as a template, without permanent genome integration. Expression tends to be intense but highly mosaic. Such highly mosaic expression can be an advantage when studying the anatomy or activity of individual cells, such as following the projections of individual neurons. An advantage of DNA injection is that tissue specific promoters can be incorporated into the construct to give spatial control of expression(Westerfield, 2000).

An adaption of the transient expression techniques introduced above is electroporation which uses an electrical charge to drive charged molecules into cells(Haas *et al.*, 2001). This includes DNA, RNA, morpholinos and synthetic dyes such as calcium indicators. Electroporation can be carried out later in development, to target cells belonging to specific tissues, and can be as precise as electroporating a single cell (from 12hpf to larval stages)(Bianco *et al.*, 2008; Tawk *et al.*, 2009). It can also be applied in the adult to move extracellular morpholino into cells of choice which are already part of a fully developed organ such as the zebrafish eye(Thummel *et al.*, 2011). Even when used in the adult, these techniques have limited effective durations. To achieve longer lasting transgenic effects, heritable transgenesis must occur.

4. Transgenesis

Now that the zebrafish genome has been sequenced(Howe *et al.*, 2013), insertional mutagenesis, through either transposon or viral based methods, offers a more attractive method to mutate random genes than ENU mutagenesis. With insertional mutagenesis, as the sequence of the insert is known, the location of the insert can be readily identified through polymerase chain reaction (PCR) and sequencing. Inspired by work in *Drosophila*(Bellen, 1999), transposon mutagenesis systems were successfully adapted to zebrafish. Transposons are sequences of DNA that can jump into or out of the genome, catalysed by transposase enzymes. The properties of transposons can be co-opted for transgenesis by flanking the sequence to be integrated with transposon sites and then co-injecting the DNA construct and transposase mRNA into a single cell embryo (**Fig.1B**). Arguably the most widely used transposon system in zebrafish is the Tol2 system(Kwan *et al.*, 2007), but other forms have been used such as Sleeping Beauty(Davidson *et al.*, 2003). By preceding an inserted transgene with a constitutive promoter, such as a modified heatshock

promoter (hsp), when the insert is in proximity to an endogenous enhancer, the transgene will be expressed in cells in which the enhancer is active. This allows identification of the group of cells which express this enhancer and, generally, the endogenous gene which would share that expression pattern. By incorporating transgenic fluorescent reporters into the inserts these methods further capitalise on the transparency of zebrafish to identify the cells which express the mutated gene, even when no gross phenotype is observable. One of the first, and still most widely used transgenically expressed fluorophores, green fluorescent protein (GFP), was expressed in *C.elegans*(Chalfie *et al.*, 1994) and not long after in zebrafish(Higashijima *et al.*, 1997). As a proof of concept, GFP in zebrafish was first expressed under the actin promoter, resulting in widespread expression. Later studies built on this by using a specific promoter of choice in place of the actin promoter, to achieve spatial and temporal expression patterns of GFP in specific cells or tissues where the promoter is expressed. The transparent nature of the young zebrafish allowed these patterns to be studied longitudinally throughout development making visualising patterns of gene expression easier than ever before. The CNS was no exception, with transgenic reporters soon created which labelled neurons, starting with the well known islet1:GFP line(Higashijima *et al.*, 2000). A drawback of the enhancer trap system is that expression patterns do not necessarily correspond to an endogenous gene. As the native promoter is not used there are often multiple inserts per line and the use of constitutive promoters can cause expression in non-specific tissue. Due to the random placement of their insertion, enhancer trap lines may succumb to silencing over time, most probably due to chromatin conformations and methylation of DNA, making the line unstable in the long term(Balciuniene *et al.*, 2013). For these reasons, gene traps are becoming more favoured over enhancer traps. The key difference with gene trapping is that the insert makes use of both the endogenous enhancer and promoter so must be inserted into the endogenous gene(Trinh le & Fraser, 2013). The transposon system in zebrafish continues to evolve new methods such as Gene Breaking Transposons which have been developed to improve levels of fidelity and mutagenicity while being expressed only when disrupting an endogenous gene(Balciuniene *et al.*, 2013). The relative ease with which transgenesis can be achieved in zebrafish and the utility of expressing fluorescent transgenes in a transparent animal accounts for the tens of thousands of transgenic zebrafish lines which currently exist (zfin.org). Transgenes are not limited to marking cells, but can also be used to manipulate, reveal the activity or kill cells. These novel tools continue to transform zebrafish research and will be discussed in more detail in the later sections.

4a. Targeted transgenesis

Random insertion is suitable for forward genetic screens as it makes it possible to knock out and hijack the promoter of any gene, which can then be detected and identified through screening and sequencing. However, for reverse genetics approaches targeted genome editing is required as mutating a specific gene by random mutagenesis is insurmountably time consuming and labour intensive. Over the past several years this shortcoming has been addressed through the development of chimeric nucleases which consist of a non-specific DNA cleavage domain and

sequence specific DNA binding domains(Urnov *et al.*, 2010; Carroll, 2011; Tesson *et al.*, 2011). It has long been known that inducing double strand breaks in DNA leads to error-prone repair mechanisms, such as non-homologous end joining, which can introduce small insertions or deletions leading to frameshift mutations(Santiago *et al.*, 2008). By controlling the locations of these double strand breaks, specific genes can be knocked out. To generate founders all that is required is the injection of the mRNA transcript of the nuclease into the cell of a fertilised egg, which can be performed readily and on a large scale in zebrafish due to the ease with which large clutches of single cell embryos can be obtained(Doyon *et al.*, 2008; Meng *et al.*, 2008).

4a. i, Zinc finger nucleases (ZFNs)

The original tools for genome editing were ZFNs, which are fusions of specific zinc finger DNA binding domains and the nonspecific DNA cleavage domain from the FokI restriction endonuclease (**Fig.3A**). Each zinc finger binding domain binds a specific triplet of DNA base pairs. When 2 ZFNs bind to DNA in close proximity, the Fok1 nuclease activity leads to a double stranded break in the DNA, followed by DNA damage response activity. Zinc finger domains are some of the most commonly encoded protein domains in eukaryotes which are highly conserved(Beerli & Barbas, 2002), allowing the adaption to other model organisms, including zebrafish where they have been used to great effect(Doyon *et al.*, 2008; Meng *et al.*, 2008). Following the construction of synthetic ZFNs pairs that are specific for binding an 18bp (base pair) sequence rather than the endogenous ZFNs which have a 3bp binding region(Liu *et al.*, 1997), it was possible to construct libraries of ZFNs which bind to almost all possible 18bp sequences (Beerli & Barbas, 2002; Gonzalez *et al.*, 2010; Kim *et al.*, 2011; Bhakta *et al.*, 2013). Thus allowing the targeting of any known sequence in the genome.

4a. ii, Transcription activator-like effector nucleases (TALENs)

ZFNs dominance in genome editing was later challenged by TALENs(Boch *et al.*, 2009; Moscou & Bogdanove, 2009). TALENs operate by similar principles but are inherently easier to design for specific DNA targets. TALENs contain 15-19 repeated domains that each bind a specific single base pair, unlike the triplet base pair specificity of zinc finger domains (**Fig.3B**). This allows for sufficient target length to target almost any sequence of choice(Deng *et al.*, 2012), with reduced off target effects(Ding *et al.*, 2013; Kim *et al.*, 2013). TALENs have benefited from their compatibility with many of the effector domains developed for use with ZFNs, such as a variety of nucleases(Miller *et al.*, 2011), which has accelerated the adoption of TALENs. This has been aided by the development of a variety of methods to generate custom TALENs, further accelerating the generation of TALEN resources(Cermak *et al.*, 2011; Miller *et al.*, 2011; Yang *et al.*, 2013). Due to these properties, TALENs have been widely used in zebrafish, despite only being introduced to zebrafish as recently as 2011(Huang *et al.*, 2011; Sander *et al.*, 2011). However, TALENs do not entirely eclipse ZFNs as their binding sites must start with a thymine nucleotide(Boch & Bonas, 2010) and the presence of identical repeat sequences in the DNA binding domains can make cloning more difficult. Both systems continue to be improved for a

variety of properties, such as reduced toxic effects and increased cleavage specificity(Szczepek *et al.*, 2007; Guo *et al.*, 2010).

4a. iii, Clustered regularly interspaced short palindromic repeats (CRISPRs)

Most recently, the CRISPR system has provided yet further options for fast, efficient genome editing. The CRISPR system is modified from a defence mechanism in bacteria which uses RNA strands to guide CRISPR-associated 9 (Cas9) nuclease to cleave foreign DNA(Barrangou *et al.*, 2007; Sashital *et al.*, 2012; Wiedenheft *et al.*, 2012). The CRISPR system achieves similar results to ZFNs and TALENs by a different method. CRISPR specificity is defined by small RNAs, rather than binding domains, which are more amenable to customisation to target any genome sequence of choice. This system has been rapidly adapted for use in eukaryotic models through the addition of nuclear localization signals for the expression of Cas9 and simplification of the guide RNA system by fusing the multiple forms of RNA required by the natural system into a single strand dubbed single guide RNA (sgRNA) (**Fig.3C**) (Jinek *et al.*, 2012). In this way only a 20bp sequence of RNA needs to be designed, based on simple Watson-Crick pairing rules, to target cleavage to a sequence of choice. Thus, this is the easiest gene editing system to customise. A caveat is the requirement for a specific 3 nucleotide sequence, commonly NGG, in close proximity to the target region, known as a protospacer adjacent motif (PAM). As the DNA binding specificity is governed by only 20bps, there have been concerns in the scientific community regarding potential off target effects of CRISPRs. The lessons learned from previous technologies have therefore been rapidly incorporated into the CRISPR toolkit. This includes converting Cas9 nuclease into a nickase to improve the specificity of cleaving events(Cong *et al.*, 2013; Mali *et al.*, 2013; Kim *et al.*, 2014), at the cost of requiring 2 sgRNAs to be used simultaneously for neighbouring target sites. While still a relatively new system, CRISPR has already been used successfully in zebrafish *in vivo*(Hruscha *et al.*, 2013; Hwang *et al.*, 2013a; Hwang *et al.*, 2013b; Jao *et al.*, 2013), in addition to a variety of eukaryotes, including human cells(Cong *et al.*, 2013; Li *et al.*, 2013a; Li *et al.*, 2013b; Li *et al.*, 2013c; Wang *et al.*, 2013), demonstrating the cross-species compatibility of this system. In zebrafish, CRISPRs(Hruscha *et al.*, 2013; Hwang *et al.*, 2013a; Hwang *et al.*, 2013b; Jao *et al.*, 2013) have shown increased effectiveness and germline transmission stability over TALEN mediated genome changes(Huang *et al.*, 2011; Sander *et al.*, 2011; Cade *et al.*, 2012; Dahlem *et al.*, 2012). A further advantage of the CRISPR system, which has been employed in zebrafish, is the ease of editing multiple target loci simultaneously by co-injecting multiple sgRNAs(Jao *et al.*, 2013). Despite only being in use in zebrafish for 2 years, the CRISPR system has already undergone substantial optimisation, including enhancement of Cas9 production in the target cell(Jao *et al.*, 2013) and methods to co-inject the Cas9 enzyme to bypass the need for transcription in the target cell(Sung *et al.*, 2014). One of the most recent CRISPR advances, achieved in zebrafish, combines the Tol2 system with the CRISPR system in a single DNA construct(Ablain *et al.*, 2015). By customising the promoters for the CRISPR system in the construct it can be injected into zebrafish eggs to generate heritable, tissue specific, targeted knockouts, thus allowing the study of mutant tissues or cell lineages of interest while maintaining unaltered expression of the target gene in all other cells.

4b. Insertional genome editing

The genome editing tools outlined so far have mirrored traditional knockout mutagenesis, with the added benefit of precise, selective targeting. However, the systems discussed above can also be employed to insert transgenic sequences into the site of directed cleavage. Co-injection of a DNA template, which has homology arms to the target site, along with the nuclease system of choice can lead to integration of the template sequence due to inherent homology directed repair mechanisms which repair the genome double strand break based on the template sequence(Moehle *et al.*, 2007). Depending on the template, resulting inserts can be only a few bases, e.g. to introduce standard nuclease cutting sites such as EcoRV(Bedell *et al.*, 2012; Chang *et al.*, 2013; Hruscha *et al.*, 2013; Hwang *et al.*, 2013a) or entire open reading frames for transgene insertion(Zu *et al.*, 2013). This template can be single stranded DNA oligonucleotides(Chen *et al.*, 2011; Bedell *et al.*, 2012; Chang *et al.*, 2013; Hwang *et al.*, 2013a) or double stranded plasmid DNA(Zu *et al.*, 2013). An alternative method being pioneered in zebrafish makes use of the non-homologous end joining pathway, wherein the insert and the genomic target are cleaved and then directly integrated into the genome at the cleavage site(Auer *et al.*, 2014). Using this method whole plasmid vectors have been inserted into the genome containing large transgenes exceeding 10kb in length(Cristea *et al.*, 2013; Maresca *et al.*, 2013; Auer *et al.*, 2014). This method is ideal for zebrafish as it is already known that the non-homologous end joining pathway is the more active of the 2 DNA repair pathways in the developing zebrafish(Hagmann *et al.*, 1998; Dai *et al.*, 2010). A prime advantage of this system is the obviation of the need for homology arms in the template, instead requiring only the incorporation of nuclease cutting sites, which allow for a more modular system which requires less customisation. A further optional refinement of this system, which has been shown in zebrafish, is to match the nuclease cutting sites in the template to those of the genomic target site. Thus co-injection of additional nuclease enzymes or nuclease templates is unnecessary as the linearisation of the template is carried out by the same enzyme (TALEN or Cas) that cleaves the genomic target (**Fig.1B**)(Auer *et al.*, 2014).

5. Binary expression systems

5a. Gal4/UAS

Transgenesis typically involves inserting a DNA cassette containing an activator and responder into the genome. This means the chosen responder (e.g. GFP) will be expressed in cells where the promoter (e.g. HuC) is acted upon by an endogenous enhancer. This means the activator governs the temporal and spatial selectivity and the responder governs the protein produced. To change either component requires making a new transgenic, which can be labour intensive. The Gal4/UAS binary expression system offers a rapid way to mix and match existing transgenes in a modular fashion. The Gal4, yeast transcription activator protein, when expressed in a cell drives expression of genes with the Upstream Activating Sequence (UAS) in their promoter region(Ma & Ptashne, 1987; Traven *et al.*, 2006) with no requirement for proximity of the inserts in the genome. Once a

stable Gal4 line has been established, it can be combined with any UAS responder line through breeding alone, without the need for further transgenesis. Offspring of such a cross must inherit both the activator and responder to lead to expression of the responder. Assuming heterozygous parents, this means a theoretical maximum of 25% of offspring will be positive for both transgenes. Therefore, this system synergises with the properties of large clutches of embryonic zebrafish as their transparent nature and external development allow rapid, reliable, efficient sorting of large numbers of transgene expressing embryos by fluorescence microscope observation. In mammalian models this would typically require postnatal genotyping with a 25% success rate being less favourable given small litter sizes. There are already 600 Gal4 promoter lines and 250 UAS responder lines listed on the zebrafish database site, ZFIN (zfin.org). These responder lines are not limited to simple markers (GFP, YFP, RFP), but also express photoconvertible proteins (dendra(Lombardo *et al.*, 2012)), systems to selectively kill cells (KillerRed(Teh *et al.*, 2010), nitroreductase(White & Mumm, 2013)), monitor cell activity (GCaMPs(Muto & Kawakami, 2011)), alter cell electrical activity (ChR, NpHR(Arrenberg *et al.*, 2010)) and many more applications. While the ease of mixing activator and responder lines is the key advantage of the Gal4 system it offers other advantages. The binary system introduces an amplification step. Instead of directly expressing 1 (e.g.) GFP molecule there will be 1 Gal4 molecule expressed, which will activate UAS which will lead to the expression of multiple GFP molecules. The most commonly used Gal4 systems incorporate the VP16 activating region(Sadowski *et al.*, 1988) and a multiple repeat version of UAS to enhance amplification(Koster & Fraser, 2001) which leads to a 100-fold increase in activity(Sadowski *et al.*, 1988). To give finer control over Gal4 expression patterns, Gal80 can be expressed under a different promoter to suppress Gal4 in cells where both promoters are active(Faucherre & Lopez-Schier, 2011; Fujimoto *et al.*, 2011), which allows the labelling of a smaller subset of cells. Chemically inducible Gal4 systems grant temporal control of Gal4 expression through the fusion of Gal4 with the ecdysone receptor (EcR)(Esengil *et al.*, 2007) or, recently, the Gal4-ER fusion(Akerberg *et al.*, 2014), operating on a similar principle to the well used CreER system (see section 5c).

5b. Tetracycline-controlled transcriptional activation (Tet)

Due to limitations of the Gal4 system, such as vulnerability to silencing by methylation and potential toxic side effects (Scott *et al.*, 2007; Distel *et al.*, 2009), other binary amplification systems have been developed. These include the Tet system(Gossen & Bujard, 1992; Huang *et al.*, 2005), the LexPr system(Emelyanov & Parinov, 2008) and the QUAS system(Subedi *et al.*, 2014). The Tet system, derived from *Escherichia coli*, consists of a transactivator tTa and the Ptet tTa-responder element, fulfilling similar roles to Gal4 and UAS, respectively(Gossen & Bujard, 1992; Luo *et al.*, 2008). The most common configuration is the Tet-Off system in which tTA binds to Ptet and initiates transcription of the transgene regulated by Ptet. Addition of the Tetracycline derivative Doxycycline, leads to Doxycycline occupying the binding site of tTA and prevents it from binding Ptet, thus eliminating transgene expression(Gossen & Bujard, 1992; Luo *et al.*, 2008). Doxycycline can be used in zebrafish for at least 1 month without resulting toxicity(Zhu *et*

al., 2009). The advantage of this system over the Gal/UAS system is that not only is spatial control achieved by the activator element as with Gal4, but temporal control is also possible through the addition of Doxycycline. In the case of zebrafish, this can be added to their aquatic environment at a controlled concentration and can be removed at any time. The Tet-On system works in a similar but opposite manner. Expression is activated only in the presence of Doxycycline. As has been established in the mouse and now also in zebrafish, the Tet system tends to express only in subsets of the cells in which the promoter component is active. While this subset is reproducible and well defined within a line, it varies between different lines with different inserts of the same promoter(Mayford *et al.*, 1996; Zhu *et al.*, 2009), offering the opportunity to study subsets of a gene expression pattern. Caged Doxycylcine can be used to provide further spatial and temporal control using optical techniques to release Doxycylcine in a highly controlled manner(Cambridge *et al.*, 2009). Enhanced versions of tTA, improved tTA (itTA), are available in zebrafish which give enhanced levels of expression with minimal toxicity(Zhu *et al.*, 2009).

5c. Cre/Lox recombination

While the Gal4/UAS system is the most common binary expression system in zebrafish, another form of binary expression system, Cre/Lox, offers additional properties. Cre/Lox is more suitable for experiments requiring temporal control and permanent de/activation of a transgene, such as lineage tracing. Cyclic recombinase (Cre), discovered as a naturally occurring component of a bacteriophage life cycle, catalyses site specific recombination at the location of Lox sites (34bp sequences)(Sauer, 1987; Sauer & Henderson, 1988). Any sequence flanked by Lox sites, orientated in the same direction, will be excised from the genome in the presence of Cre. This can be used to stop expression of a gene by excising it, to express a gene by excising a stop site which precedes the gene (known as the cargo gene), or both simultaneously so switching off one gene and on another(Sauer, 1998; Bailey *et al.*, 2009). This excision is permanent and heritable by any daughter cells, thus making this a perfect tool for lineage tracing. A key difference with the Gal4/UAS system is that Cre does not drive the responder transgene, but rather unmasks it. Therefore the responder will have a promoter which is independent of subsequent Cre activity. Originally in zebrafish, Cre was expressed under a constitutive promoter such as hsp or ubiquitin so the spatial specificity was governed by the promoter of the Lox flanked gene(Thummel *et al.*, 2005; Le *et al.*, 2007; Mosimann *et al.*, 2011), although some studies also made use of a constitutive promoter for the Lox cassette(Langenau *et al.*, 2005). A key advantage of having a restricted Cre promoter and a constitutive Lox flanked promoter, is that the restricted promoter defines the cell population and the constitutive promoter promotes long term, stable expression of the transgene, meaning the cells will continue to express even when the restricted Cre promoter is no longer active. However, it is increasingly common to use specific promoters with a more defined expression. The Cre/Lox system can be combined with Gal4/UAS to give permanent activation or inactivation of the transgene(Dong & Stuart, 2004; Le *et al.*, 2007) such as by heatshock driven Cre recombinase expression which will allow Gal4 to be permanently inactivated or activated(Collins *et al.*, 2010). It can also be used to enhance precision by further restricting

Gal4 expression patterns to subsets of cells(Sato *et al.*, 2007; Okamoto *et al.*, 2008). The chemically inducible CreER system has been successfully employed in zebrafish, taking advantage of the ease with which the activation drugs can be added to the living medium(Jungke *et al.*, 2013). Due to the small size and permeability of zebrafish embryos, Cre can be reliably and rapidly temporally controlled through drugs introduced to the aquatic environment(Hans *et al.*, 2011; Mosimann *et al.*, 2011). In addition to the commonly used LoxP, there are other forms of Lox such as LoxN and Lox2272, which allows for the simultaneous use of multiple different recombination patterns within the same line(Livet *et al.*, 2007). The Zebrabow/Brainbow system employs this to achieve stochastic multicolour labelling which allows the simultaneous tracing of multiple lineages within a single transgenic animal(Pan *et al.*, 2013b). While Cre/Lox is used commonly in vertebrate models such as the mouse, it has been less common in zebrafish, largely due to historical reasons which have been overcome by the current availability of genome editing tools, starting with the Tol2 transposon system which led to a rapid increase in Cre/Lox lines(Jungke *et al.*, 2013). As with other binary systems, the more activators and responders that are widely available, the greater the synergy and the more useful the system becomes. On a practical note, the ease with which zebrafish eggs can be shipped between laboratories and the general willingness of the zebrafish community to share new lines, enhances this availability and accelerates the breeding of new binary combinations.

6. Cell transplantation

While genetic techniques are a key feature of zebrafish research, they are not the only techniques which benefit from the properties of zebrafish. Cell transplantation, a traditional developmental technique which predates the genetic age, was used in pioneering work to establish fate maps of cell lineages in the zebrafish nervous system(Eisen, 1991; Eisen & Pike, 1991; Westerfield, 2000). Historically, cell transplantation has been an important technique in Xenopus larvae and chick embryos, but it is optimal in the transparent, accessible zebrafish embryo. While the technique requires practice and finesse, it is methodologically simple. A single cell, few cells or many cells can be aspirated from an embryo using a microcapillary pipette, and then injected into a recipient embryo. The developing chimera can then be studied as a standard embryo. The location of the cells and timing of transplantation reveal the state of commitment of cells. Additionally, transplanting cells from an embryo of one genotype into another of a different genotype can distinguish cell autonomous from non-autonomous properties of genes(Ho & Kane, 1990; Ho & Kimmel, 1993). This traditional technique continues to be enhanced by modern genetic techniques. Following transplanted cells *in vivo* is straightforward in the transparent zebrafish benefitting from the wide range of transgenic markers currently available, allowing for longitudinal study and further manipulation of the transplanted cells.

7. Regeneration

A property of zebrafish which can be viewed as an inherent tool for scientists is that of extensive regeneration. Just as development demonstrates how to build a brain, regeneration in an inherently

regenerative organism reveals how to rebuild a brain. In contrast to adult mammals, the zebrafish is one of the most regenerative of all vertebrates, showing extensive regeneration in the majority of tissues and organs, including the CNS, heart, liver, retina, barbels, fins and more (Otteson & Hitchcock, 2003; Raya *et al.*, 2004; Schweitzer *et al.*, 2007; Curado & Stainier, 2010; LeClair & Topczewski, 2010; Becker & Becker, 2014). The tissue in which zebrafish regenerative abilities are most remarkable, in comparison to humans, is the CNS. While paralysis following human spinal injury is permanent, zebrafish show complete functional recovery mere weeks after spinal cord transection(Becker *et al.*, 1997; van Raamsdonk *et al.*, 1998; Becker *et al.*, 2004). This restoration of function has also been shown in other CNS areas such as the optic system(Zou *et al.*, 2013; Sherpa *et al.*, 2014). To understand zebrafish regeneration, and ultimately apply it to mammals, the main approaches have been genetic and cellular. Many screens have been carried out to identify genes that play essential roles in regeneration and development(Poss *et al.*, 2002; Nechiporuk *et al.*, 2003; Whitehead *et al.*, 2005), both those that support or inhibit regenerative processes, such as Fgf(Ganz *et al.*, 2010),(Lepilina *et al.*, 2006), Notch(Chapouton *et al.*, 2010), Wnt(Grandel *et al.*, 2006) and others(Ma *et al.*, 2012; Pan *et al.*, 2013a; Yu & Schachner, 2013). Through manipulation of local compounds, such as by adding cyclic AMP to lesioned neurons, non-regenerating classes of zebrafish neurons have been enticed to begin regenerating(Bhatt *et al.*, 2004).

Approaches focussing on the activity of cells, especially progenitor cells, are also a rich area of study. The zebrafish CNS continues to grow throughout life with widespread neurogenesis and the constant extension of new axons to their targets in other CNS areas. Whereas in mammals these progenitor zones are limited to the subventricular zone and dentate gyrus (Ming & Song, 2011), in zebrafish there are 16 known progenitor zones which are active throughout life and contribute to ongoing growth of the brain (**Fig.4**) (Adolf *et al.*, 2006; Grandel *et al.*, 2006; Zupanc & Zupanc, 2006; Kaslin *et al.*, 2009; Marz *et al.*, 2010). It is possible that the high regenerative capacity of the zebrafish may be a byproduct of their continuous growth, but this relationship remains to be explored. However, it is known that these neurogenic processes can be influenced by neural activity(Lindsey *et al.*, 2014) and injury states. Following CNS injury in zebrafish, the progenitor cells that reside in progenitor zones increase their rate of proliferation and differentiate into multiple cell types required to restore the tissue(Adolf *et al.*, 2006; Grandel *et al.*, 2006). As with mammals, a zebrafish CNS lesion is marked by apoptosis, inflammation and proliferation of glial cells(Fitch & Silver, 2008). However, a key difference is that mammalian lesion sites form permanent scars, called the glial scar. This scar is composed of non-neurogenic, reactive astrocytes which block axonal growth and prevent neuronal cell infiltration(Sofroniew, 2009; Lang *et al.*, 2014). Whereas zebrafish show limited scarring, with axons able to more freely penetrate and exit the lesion site to ultimately restore lost connections(Becker & Becker, 2014). Not only is the glial scar a physical barrier to regeneration, but also a molecular one. The lesion site in the mammalian CNS is inhibitory to axon outgrowth, while the lesion site in zebrafish is permissive to axon growth. Identifying the molecular cues which contribute to this property is an important step

towards revealing why regeneration succeeds in zebrafish and if these cues can enhance mammalian regeneration.

While adult regeneration is limited to specific organisms, all species must undergo a process which is superficially similar: development. Therefore one view of regeneration is that it is a recapitulation of development and that dormant developmental programs could be reactivated to initiate regeneration in adult tissues. Thus zebrafish is an ideal model to answer this as it lends itself to both developmental and regeneration based studies. There is a growing wealth of evidence that while development and regeneration in zebrafish share various properties, with regulation of overlapping families of genes, the precise expression patterns and specific gene family members involved vary greatly(Kizil *et al.*, 2009; Stewart *et al.*, 2009; Millimaki *et al.*, 2010). Only a proportion of regeneration associated genes have been shown to play a role in development(Raya *et al.*, 2003; Lepilina *et al.*, 2006; Lien *et al.*, 2006; Reimer *et al.*, 2009). In fin regeneration it has been shown that fgf20a, an fgf ligand, is essential for regeneration(Whitehead *et al.*, 2005) but plays only a minor role in embryogenesis(Gonzalez-Quevedo *et al.*, 2010). In the CNS, it has been shown that robo2 plays an important role in developmental axon guidance, whereas adult CNS regeneration occurs with only minor disruption in the absence of robo2(Wyatt *et al.*, 2010). These findings challenge the idea that regeneration can be invoked by duplicating development and reveals the situation to be more complex than a direct recapitulation of development. Insights such as these, which may one day guide mammalian regeneration, can only be gained from a regenerative vertebrate model such as the zebrafish.

8. Chemical screens

In the simplest terms, a chemical screen involves exposing cells or organisms to a bank of small molecules and screening for possible effects in various properties ranging from gene expression to development to behaviour(Tamplin *et al.*, 2012; Rennekamp & Peterson, 2014). This technique is most widely used for drug discovery when screening the effects of chemical libraries consisting of known or unknown compounds, with many libraries commercially available(Mathias *et al.*, 2012). These screens can be carried out using *in vitro* or *in vivo* methods. *In vitro* screening is relatively cheap, simple and compatible with high throughput methods. However, cultured cells do not share the same physiological processes as a living organism, so effects seen in cells may not apply *in vivo*. The zebrafish embryo shares many of the advantages of both *in vitro* and *in vivo* methods. Rapid, external development allows all stages of development to be studied, which is extremely challenging to perform *in utero* in mammals. Large clutches of eggs with synchronised development allow different molecules to be screened side by side, to give the most comparable results. The aquatic nature of zebrafish allows drugs to be administered in defined concentrations in their living medium, providing strict control over dose and timing. The corollary being that non-water soluble drugs can be more difficult to deliver, requiring dissolution in a suitable vehicle. The rate of development, size and permeability of zebrafish embryos means that only small amounts of the compound are required and repeated doses may not be necessary. Due to their small size, immunohistochemistry and *in situ* hybridisation can be performed on whole intact embryos as an

end point to a screen to reveal protein or mRNA patterns, respectively. Due to their transparent nature and the availability of many transgenic lines, changes in gene expression can be readout directly through simple fluorescent observation at multiple time points, rather than a single endpoint(Reimer *et al.*, 2013), including physiological observation such as heart rate(Burns *et al.*, 2005). Since the first zebrafish molecular screens over a decade ago, they have been used to study many conditions relevant to human health, including cardiovascular development, regeneration, cancer, kidney disease and muscular dystrophy(Kalev-Zylinska *et al.*, 2002; Peterson *et al.*, 2004; Oppedal & Goldsmith, 2010; Kawahara *et al.*, 2011; Poureetezadi & Wingert, 2013). Screens which use behavioural readouts are becoming increasingly popular in the search for neuroactive drugs for human neurological disorders due to the zebrafish's repertoire of stereotyped behaviours which can be readily elicited and are amenable to automated observation (Rihel *et al.*, 2010; Kokel & Peterson, 2011; Wolman *et al.*, 2011; Rihel & Schier, 2012; Kermen *et al.*, 2013; Bruni *et al.*, 2014). Behaviours such as swimming, escape response and spontaneous motor output begin as early as 19hpf, providing insight into chemical effects from, relatively, early development onwards. With recent advances in whole brain functional imaging, with individual neuron resolution, it can be expected that a growing target of future screens will be functional brain activity, visualised through the use of calcium indicators (see section 9). Despite the genetic, anatomical and physiological similarities between zebrafish and mammals, it remains to be seen what percentage of drugs that are effective in zebrafish can be applied successfully to humans. Some studies have found up to half of compounds active in zebrafish have comparable effects on human cells(Li *et al.*, 2014). It should be noted that only 10% of drugs which pass full *in vivo* mammalian screens are effective in humans so such loss of hits can be expected at each stage of drug discovery(Hay *et al.*, 2014). A further consideration is that, while such screens can be carried out on adult zebrafish(Stewart *et al.*, 2015), the key advantages of zebrafish based chemical screens derive from developmental studies, whereas the majority of commercially targeted disorders affect adult humans. Due to these factors, zebrafish chemical screens should not be seen as a replacement for other forms of drug screening but are an ideal complement to mammalian screens. High throughput zebrafish screens can identify potential candidates from vast libraries to be subsequently applied in mammalian screens, greatly accelerating the drug discovery process. This method could prove vital as currently drug discovery rates are declining, while the cost of developing new drugs increases(Hughes *et al.*, 2011; Hay *et al.*, 2014).

9. Monitoring activity of neural circuits in the zebrafish brain

The computations performed by neuronal circuits cannot be derived from the functions of the individual elements (neurons and synapses). The analysis of circuit function is therefore one of the central questions in neurobiology. This requires simultaneous recordings from many neurons, which has been technically difficult. Multi-electrode arrays have been successfully used to monitor the activity of multiple neurons in the mammalian brain, simultaneously(Nicolelis *et al.*, 1993; Wilson & McNaughton, 1993; Nicolelis *et al.*, 1995; Welsh *et al.*, 1995). However, the need for invasive surgery, the low spatial resolution, and the requirement for advanced technical skills are

still important challenges. Alternatively, the activity of neuronal populations can be measured by imaging methods. Methods such as functional magnetic resonance imaging or optical imaging of intrinsic signals have low spatial and temporal resolution and, thus, are of limited use for the study of neuronal circuit function. Voltage sensitive dyes are fast indicators of membrane potential, can easily be loaded into neurons, and have been successfully used to monitor the activity of neuronal populations(Cinelli *et al.*, 1995; Shoham *et al.*, 1999; Zochowski *et al.*, 2000; Spors & Grinvald, 2002). However, the low signal to noise ratio and the lack of cellular resolution in intact tissue limits the use of voltage-sensitive dyes for circuit analysis. Calcium sensitive dye imaging has also been widely used by many neurophysiologists(Friedrich & Korsching, 1997; Yuste & Majewska, 2001; Euler *et al.*, 2002; Froemke *et al.*, 2002; Wachowiak *et al.*, 2002). Due to their high signal to noise ratios, they are, in principle, convenient to monitor neuronal activity. In combination with two-photon microscopy, single neuron resolution can be achieved in the intact brain(Helmchen *et al.*, 1999; Delaney *et al.*, 2001).

With its transparency and small brain, the zebrafish is a perfect model animal for monitoring brain activity with calcium imaging. The first calcium imaging studies in zebrafish were performed using synthetic calcium sensing fluorescent dyes that require creative ways to load into the tissues of interest(Fetcho & O'Malley, 1995; O'Malley *et al.*, 1996; Friedrich & Korsching, 1997; Li *et al.*, 2005; Yaksi, 2006; Mack-Bucher *et al.*, 2007). The first generation transgenic calcium indicators (such as inverse pericam(Higashijima *et al.*, 2003; Li *et al.*, 2005)) were not very effective mainly due to their low sensitivity and low signal to noise ratios. However thanks to the development of next generation transgenic calcium indicators such as GCaMPs(Nakai *et al.*, 2001; Tallini *et al.*, 2006; Tian *et al.*, 2009; Ohkura *et al.*, 2012; Muto *et al.*, 2013), we have entered a new era of zebrafish experiments that can be performed non-invasively with relatively little technical training or surgical skills.

In a small, transparent animal such as the zebrafish, optical imaging was a transformative step. While zebrafish larvae are known to be optically transparent, they still have pigments which can absorb light, leading to photo damage and reduced image quality. Early studies in zebrafish larvae dealt with this pigmentation problem using chemical agents such as Phenylthiourea (PTU) which is commonly used for inhibiting melanisation(Li *et al.*, 2005). However PTU also interferes with several developmental processes and therefore can affect the experimental results. It is now more common to use the reduced pigmentation mutant zebrafish lines, nacre(Lister *et al.*, 1999) or casper(White *et al.*, 2008), which allow imaging of zebrafish at different developmental stages from a few days old larvae(Ahrens *et al.*, 2012; Ahrens *et al.*, 2013b) up to 3-4 weeks old juveniles(Jetti *et al.*, 2014) without the requirement of surgery.

As it was for the entire neuroscience community, the introduction of two-photon microscopy to calcium imaging in zebrafish(Yaksi & Friedrich, 2006; Yaksi *et al.*, 2007) was a revolution, which allowed optical sectioning of brain tissue non-invasively(Mack-Bucher *et al.*, 2007; Orger, 2008; Ahrens *et al.*, 2012; Ahrens *et al.*, 2013b). This approach was subsequently combined with automated detection of neurons(Ohki *et al.*, 2005; Jetti *et al.*, 2014) and high throughput two-photon microscopy recordings(Orger *et al.*, 2008; Grama & Engert, 2012), which

lead to exhaustive data sets of brain activity. Most of these early experiments were performed in restrained or paralyzed embryos to measure sensory responses in the brain, which was one of the major questions that was under investigation. Fictive swimming behaviour, which is essentially measuring motor neuron activity in paralyzed animals under a microscope, was the first solution for imaging zebrafish brain activity while animals perform fictive motor tasks(Masino & Fetcho, 2005), controlling locomotion gain(Ahrens *et al.*, 2012) and virtual navigation(Ahrens *et al.*, 2013a). However fictive swimming does not provide animals with the full proprioceptive feedback of motion and it is likely that the perception of locomotion or navigation is limited. Currently several groups are working on optimizing preparations where the zebrafish larvae are not paralyzed but they are semi-restrained. These preparations involve embedding the embryo's head in low melting agarose gels, while freeing the tail or the eyes for motor activity. Coupled with closed loop virtual reality, such preparations can now be used for studying naturalistic behaviours such as the visio-motor reflex(Kubo *et al.*, 2014; Portugues *et al.*, 2014) and prey-capture(Bianco, 2011; Preuss *et al.*, 2014).

The introduction of light sheet microscopy was another transformative event for many different fields of life sciences that use transparent samples(Huisken *et al.*, 2004; Truong *et al.*, 2011; Schmid *et al.*, 2013). The speed of image acquisition using this approach is currently difficult to match. Hence, not surprisingly, light sheet microscopy combined with calcium imaging provided us the most exhaustive data set of a vertebrate brain, by imaging the entire volume of the brain of the larval zebrafish (~80,000 neurons) *in vivo* at 0.8 Hz, capturing more than 80% of all neurons at single cell resolution(Ahrens *et al.*, 2013b). This was the first time the activity of an entire vertebrate brain was measured with such spatial and temporal resolution, which is very important for the understanding of how brain circuits function as a whole rather than isolated brain regions. Currently many zebrafish laboratories around the world are using volumetric imaging approaches such as light sheet microscopy(Vladimirov *et al.*, 2014) and patterned illumination(Prevedel, 2014), which are generating neural data sets that are bigger than have ever been handled before. These great achievements, however, present a great challenge in handling the immense size of generated data, while lacking a framework for analysing such data sets. One way to deal with such large data sets is to combine the multi-disciplinary expertise from different fields, especially of data management and applied mathematics(Freeman *et al.*, 2014). As these advanced imaging methods will become more widely available to a broad community, we will all be using similar frameworks to deal with the large and complex data sets of brain activity, collected in different animal models including zebrafish.

10. Perturbing neural circuit activity in the zebrafish brain

While the optical monitoring of brain activity was transforming neurophysiology, another revolution in neuroscience was taking place: optogenetics, a novel technique that uses light to activate or silence genetically altered neurons. Soon after the discovery and the first use of the genetically expressed blue light sensitive cation channel *channelrhodopsin*(Nagel *et al.*, 2002; Boyden *et al.*, 2005) and the yellow light sensitive chloride pump *halorhodopsin*(Han & Boyden,

2007; Zhang *et al.*, 2007; Zhao *et al.*, 2008) in other model systems, optogenetic perturbations became standard methods in the zebrafish toolbox for manipulating neural activity *in vivo*. The first successful demonstration for the use of enhanced *channelrhodopsin2* (Chr2) in the zebrafish brain was for activating neurons that mediate escape response(Douglass *et al.*, 2008). Later it was possible to use the Gal4/UAS binary expression system, in order to achieve high levels of cell specific expression in different brain regions(Scott *et al.*, 2007; Asakawa & Kawakami, 2008; Asakawa *et al.*, 2008). Recently it was shown that very high levels of Chr2 expression can be achieved by using viral gene delivery and the iTet-Off system, which allowed two-photon optogenetic stimulation in the zebrafish brain(Zhu *et al.*, 2009). Combining Gal4/UAS with iTet-Off system in the future would allow manipulation of separate genetically defined neuronal populations simultaneously.

The optogenetic silencer protein *halorhodopsin* (NpHR) was demonstrated to be able to silence neural activity(Hegemann *et al.*, 1985; Oesterhelt *et al.*, 1985). This tool was later improved for a more effective version, eNPhR, by trafficking to the cell membrane(Zhao *et al.*, 2008). eNpHR was first used in zebrafish to silence caudal hindbrain neurons, which are shown to be sufficient to initiate locomotion(Arrenberg, 2009). The same study also showed that both Chr2 and eNpHR can be used in combination in the same animal by carefully selecting the wavelengths of light that control Chr2 and eNpHR separately.

While in practice the spatial and temporal patterns of light are relatively straightforward to generate (using televisions or image projectors), most of the early optogenetic perturbations in zebrafish, had little or no spatial control of light due to using a narrow optical fibre(Arrenberg *et al.*, 2009; Arrenberg *et al.*, 2010). The introduction of digital micro-mirror devices for patterning the optogenetic light stimulation was therefore an important step in achieving both spatial and temporal control for optogenetic perturbations in the zebrafish brain(Zhu, 2012). While patterned illumination using visible light can be used to successfully generate two-dimensional patterns of light, this method lacks axial (z) resolution. It is however now possible to use light sculpting methods to control ultrafast infra-red lasers to generate three-dimensional patterns of light(Andrasfalvy *et al.*, 2010; Vaziri & Emiliani, 2012), which can be used for multi-photon excitation of optogenetic proteins. The optogenetic toolkit of zebrafish continues to grow everyday with the development of new light sensitive actuators of neural activity, more effective and cell type specific expression of optogenetic proteins and more precise spatial and temporal control of light stimulation. All these methods combined in transparent zebrafish larvae or juveniles provide researchers with tools that were unimaginable only a few years ago in order to perturb neural activity and test the effects of these perturbations on neural activity and animal behaviour.

11. Monitoring and quantifying zebrafish behaviour in adults and larvae

Originally evolved to live in the shallow waters of South Asia, zebrafish exhibit a wide variety(Spence *et al.*, 2008; Kalueff, 2013) of social behaviours at the adult stage. Zebrafish are shoaling fish (Miller & Gerlai, 2007), preferring each other's company(Al-Imari & Gerlai, 2008), but can also display aggression towards each other(Oliveira *et al.*, 2011). They are capable of

forming complex hierarchical structures(Wright *et al.*, 2003). They exhibit robust and stereotyped behaviours such as odour induced alarm response (Magurran *et al.*, 1996; Jesuthasan & Mathuru, 2008; Mathuru *et al.*, 2012) as well as reproductive(Darrow & Harris, 2004) and appetitive(Valentincic *et al.*, 2005) behaviours. Moreover, zebrafish have been shown to display cognitive behaviours such as associative learning(Colwill *et al.*, 2005; Braubach *et al.*, 2009; Sison & Gerlai, 2010) and avoidance learning(Blank *et al.*, 2009; Agetsuma *et al.*, 2010).

Manual quantification of adult zebrafish behaviour was the most common method until only a few years ago. Thanks to the efforts of many individual groups, custom written video tracking and behavioural classification software(Gomez-Marin *et al.*, 2012; Maaswinkel *et al.*, 2013; Pittman & Ichikawa, 2013) are now available for a wide range of researchers with different levels of computer skills. A recent methods article(Perez-Escudero *et al.*, 2014) now provides the possibility to track the identity of individual adult zebrafish within a group, which can be used to study social interactions among several fish.

While most behavioural assays were originally designed to be used in adult zebrafish, zebrafish larvae are now attracting a lot more attention for behavioural assays, thanks to the popularity of optogenetic tools as well as the potential for high-throughput genetic and chemical screens. Combined with genetic and optogenetic tools, zebrafish larvae can also be used effectively for studying the neural basis for complex behaviours such as sleep(Rihel *et al.*, 2010), feeding(Clift *et al.*, 2014), prey capture(Gahtan *et al.*, 2005; Bianco *et al.*, 2011; Preuss *et al.*, 2014), locomotion(Saint-Amant & Drapeau, 1998; Budick & O'Malley, 2000; Kimura *et al.*, 2013; Mirat *et al.*, 2013), startle response(Eaton & Farley, 1975; Bhatt *et al.*, 2004; Burgess & Granato, 2007; Kohashi *et al.*, 2012) and optokinetic response(Rinner *et al.*, 2005; Brockerhoff, 2006) which are simple to elicit in the laboratory. The behaviour of larval zebrafish in all these assays can be captured with high speed video monitoring in both freely behaving zebrafish(Bianco, 2011; Mirat *et al.*, 2013) with complete behavioural repertoires or semi-restrained animals(Arrenberg *et al.*, 2009; Goncalves *et al.*, 2014; Preuss *et al.*, 2014) that have less behavioural freedom but allow simultaneous imaging of brain activity as well as optogenetic manipulations in order to perturb neural activity during behaviour(Kimura *et al.*, 2013). Fictive swimming, which involves recording the motor neuron activity in paralyzed animals, was also successfully used for studying naturalistic behaviours such as navigation(Ahrens *et al.*, 2013a), while simultaneously imaging brain activity with optical methods.

It is certain that both larval and adult zebrafish exhibit a rich behavioural repertoire which can be digitized and quantified using inventive approaches and elegantly designed experiments. Automated quantification tools for more precise experiments are now becoming widely available for a broader community through open-source hardware and software implementations as well as private companies which are selling off-the-shelf systems. This recent boom in behavioural studies of zebrafish adults and larvae will most certainly result in crucial advances in our understanding of how brain circuits control or regulate animal behaviour. The next frontier in such behavioural assays will be to monitor or perturb brain activity in freely behaving animals with sufficient spatial and temporal resolution in order to functionally dissect these miniature vertebrate brains.

12. Conclusion

Streisinger's untimely death in 1984 threatened to derail the rise of a promising new model vertebrate. Fortunately the collaborative nature of the fledgling zebrafish community helped it to survive and grow, from rocky beginnings to an assured future. The zebrafish field continues to grow from strength to strength, with rate of publication growing faster than any other model and an ever expanding resource base particularly rich in mutant and transgenic lines. With the timely convergence of innate properties and the emerging novel technologies and approaches outlined here, this trend seems certain to continue.

References

Ablain, J., Durand, E.M., Yang, S., Zhou, Y. & Zon, L.I. (2015) A CRISPR/Cas9 Vector System for Tissue-Specific Gene Disruption in Zebrafish. *Developmental cell*.

Adolf, B., Chapouton, P., Lam, C.S., Topp, S., Tannhauser, B., Strahle, U., Gotz, M. & Bally-Cuif, L. (2006) Conserved and acquired features of adult neurogenesis in the zebrafish telencephalon. *Developmental biology*, **295**, 278-293.

Agetsuma, M., Aizawa, H., Aoki, T., Nakayama, R., Takahoko, M., Goto, M., Sassa, T., Amo, R., Shiraki, T., Kawakami, K., Hosoya, T., Higashijima, S. & Okamoto, H. (2010) The habenula is crucial for experience-dependent modification of fear responses in zebrafish. *Nat Neurosci*, **13**, 1354-1356.

Ahrens, M.B., Huang, K.H., Narayan, S., Mensh, B.D. & Engert, F. (2013a) Two-photon calcium imaging during fictive navigation in virtual environments. *Front Neural Circuits*, **7**, 104.

Ahrens, M.B., Li, J.M., Orger, M.B., Robson, D.N., Schier, A.F., Engert, F. & Portugues, R. (2012) Brain-wide neuronal dynamics during motor adaptation in zebrafish. *Nature*, **485**, 471-477.

Ahrens, M.B., Orger, M.B., Robson, D.N., Li, J.M. & Keller, P.J. (2013b) Whole-brain functional imaging at cellular resolution using light-sheet microscopy. *Nat Methods*, **10**, 413-420.

Akerberg, A.A., Stewart, S. & Stankunas, K. (2014) Spatial and temporal control of transgene expression in zebrafish. *PloS one*, **9**, e92217.

Al-Imari, L. & Gerlai, R. (2008) Sight of conspecifics as reward in associative learning in zebrafish (Danio rerio). *Behav Brain Res*, **189**, 216-219.

Andrasfalvy, B.K., Zemelman, B.V., Tang, J. & Vaziri, A. (2010) Two-photon single-cell optogenetic control of neuronal activity by sculpted light. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 11981-11986.

Arrenberg, A.B., Del Bene, F. & Baier, H. (2009) Optical control of zebrafish behavior with halorhodopsin. *Proc Natl Acad Sci U S A*, **106**, 17968-17973.

Arrenberg, A.B., et al (2009) Optical control of zebrafish behavior with halorhodopsin. *PNAS*, **106**, 17968 –17973

Arrenberg, A.B., Stainier, D.Y., Baier, H. & Huisken, J. (2010) Optogenetic control of cardiac function. *Science*, **330**, 971-974.

Asakawa, K. & Kawakami, K. (2008) Targeted gene expression by the Gal4-UAS system in zebrafish. *Development, growth & differentiation*, **50**, 391-399.

Asakawa, K., Suster, M.L., Mizusawa, K., Nagayoshi, S., Kotani, T., Urasaki, A., Kishimoto, Y., Hibi, M. & Kawakami, K. (2008) Genetic dissection of neural circuits by Tol2 transposon-mediated Gal4 gene and enhancer trapping in zebrafish. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 1255-1260.

Auer, T.O., Duroure, K., De Cian, A., Concordet, J.P. & Del Bene, F. (2014) Highly efficient CRISPR/Cas9-mediated knock-in in zebrafish by homology-independent DNA repair. *Genome research*, **24**, 142-153.

Bailey, J.M., Creamer, B.A. & Hollingsworth, M.A. (2009) What a fish can learn from a mouse: principles and strategies for modeling human cancer in mice. *Zebrafish*, **6**, 329-337.

Balciuniene, J., Nagelberg, D., Walsh, K.T., Camerota, D., Georlette, D., Biemar, F., Bellipanni, G. & Balciunas, D. (2013) Efficient disruption of Zebrafish genes using a Gal4-containing gene trap. *BMC genomics*, **14**, 619.

Baraban, S.C., Dinday, M.T., Castro, P.A., Chege, S., Guyenet, S. & Taylor, M.R. (2007) A large-scale mutagenesis screen to identify seizure-resistant zebrafish. *Epilepsia*, **48**, 1151-1157.

Baraban, S.C., Dinday, M.T. & Hortopan, G.A. (2013) Drug screening in Scn1a zebrafish mutant identifies clemizole as a potential Dravet syndrome treatment. *Nat Commun*, **4**, 2410.

Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., Romero, D.A. & Horvath, P. (2007) CRISPR provides acquired resistance against viruses in prokaryotes. *Science*, **315**, 1709-1712.

Becker, C.G., Lieberoth, B.C., Morellini, F., Feldner, J., Becker, T. & Schachner, M. (2004) L1.1 is involved in spinal cord regeneration in adult zebrafish. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **24**, 7837-7842.

Becker, T. & Becker, C.G. (2014) Axonal regeneration in zebrafish. *Current opinion in neurobiology*, **27**, 186-191.

Becker, T., Wullimann, M.F., Becker, C.G., Bernhardt, R.R. & Schachner, M. (1997) Axonal regrowth after spinal cord transection in adult zebrafish. *The Journal of comparative neurology*, **377**, 577-595.

Bedell, V.M., Wang, Y., Campbell, J.M., Poshusta, T.L., Starker, C.G., Krug, R.G., 2nd, Tan, W., Penheiter, S.G., Ma, A.C., Leung, A.Y., Fahrenkrug, S.C., Carlson, D.F., Voytas, D.F., Clark, K.J., Essner, J.J. & Ekker, S.C. (2012) In vivo genome editing using a high-efficiency TALEN system. *Nature*, **491**, 114-118.

Bedell, V.M., Westcot, S.E. & Ekker, S.C. (2011) Lessons from morpholino-based screening in zebrafish. *Briefings in functional genomics*, **10**, 181-188.

Beerli, R.R. & Barbas, C.F., 3rd (2002) Engineering polydactyl zinc-finger transcription factors. *Nature biotechnology*, **20**, 135-141.

Bellen, H.J. (1999) Ten years of enhancer detection: lessons from the fly. *The Plant cell*, **11**, 2271-2281.

Bhakta, M.S., Henry, I.M., Ousterout, D.G., Das, K.T., Lockwood, S.H., Meckler, J.F., Wallen, M.C., Zykovich, A., Yu, Y., Leo, H., Xu, L., Gersbach, C.A. & Segal, D.J. (2013) Highly active zinc-finger nucleases by extended modular assembly. *Genome research*, **23**, 530-538.

Bhatt, D.H., Otto, S.J., Depoister, B. & Fetcho, J.R. (2004) Cyclic AMP-induced repair of zebrafish spinal circuits. *Science*, **305**, 254-258.

Bianco, I.H., Carl, M., Russell, C., Clarke, J.D. & Wilson, S.W. (2008) Brain asymmetry is encoded at the level of axon terminal morphology. *Neural development*, **3**, 9.

Bianco, I.H., et al, (2011) Prey capture behavior evoked by simple visual stimuli in larval zebrafish. *Front. Syst. Neurosci.*, **5**.

Bianco, I.H., Kampff, A.R. & Engert, F. (2011) Prey capture behavior evoked by simple visual stimuli in larval zebrafish. *Front Syst Neurosci*, **5**, 101.

Bill, B.R., Balciunas, D., McCarra, J.A., Young, E.D., Xiong, T., Spahn, A.M., Garcia-Lecea, M., Korzh, V., Ekker, S.C. & Schimmenti, L.A. (2008) Development and Notch signaling requirements of the zebrafish choroid plexus. *PloS one*, **3**, e3114.

Bill, B.R., Petzold, A.M., Clark, K.J., Schimmenti, L.A. & Ekker, S.C. (2009) A primer for morpholino use in zebrafish. *Zebrafish*, **6**, 69-77.

Blank, M., Guerim, L.D., Cordeiro, R.F. & Vianna, M.R. (2009) A one-trial inhibitory avoidance task to zebrafish: rapid acquisition of an NMDA-dependent long-term memory. *Neurobiol Learn Mem*, **92**, 529-534.

Boch, J. & Bonas, U. (2010) Xanthomonas AvrBs3 family-type III effectors: discovery and function. *Annual review of phytopathology*, **48**, 419-436.

Boch, J., Scholze, H., Schornack, S., Landgraf, A., Hahn, S., Kay, S., Lahaye, T., Nickstadt, A. & Bonas, U. (2009) Breaking the code of DNA binding specificity of TAL-type III effectors. *Science*, **326**, 1509-1512.

Boyden, E.S., Zhang, F., Bamberg, E., Nagel, G. & Deisseroth, K. (2005) Millisecond-timescale, genetically targeted optical control of neural activity. *Nat Neurosci*, **8**, 1263-1268.

Braubach, O.R., Wood, H.D., Gadbois, S., Fine, A. & Croll, R.P. (2009) Olfactory conditioning in the zebrafish (Danio rerio). *Behav Brain Res*, **198**, 190-198.

Brockerhoff, S.E. (2006) Measuring the optokinetic response of zebrafish larvae. *Nature protocols*, **1**, 2448-2451.

Bruni, G., Lakhani, P. & Kokel, D. (2014) Discovering novel neuroactive drugs through high-throughput behavior-based chemical screening in the zebrafish. *Frontiers in pharmacology*, **5**, 153.

Budick, S.A. & O'Malley, D.M. (2000) Locomotor repertoire of the larval zebrafish: swimming, turning and prey capture. *J Exp Biol*, **203**, 2565-2579.

Burgess, H.A. & Granato, M. (2007) Sensorimotor gating in larval zebrafish. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **27**, 4984-4994.

Burns, C.G., Milan, D.J., Grande, E.J., Rottbauer, W., MacRae, C.A. & Fishman, M.C. (2005) High-throughput assay for small molecules that modulate zebrafish embryonic heart rate. *Nature chemical biology*, **1**, 263-264.

Cade, L., Reyon, D., Hwang, W.Y., Tsai, S.Q., Patel, S., Khayter, C., Joung, J.K., Sander, J.D., Peterson, R.T. & Yeh, J.R. (2012) Highly efficient generation of heritable zebrafish gene mutations using homo- and heterodimeric TALENs. *Nucleic acids research*, **40**, 8001-8010.

Cambridge, S.B., Geissler, D., Calegari, F., Anastassiadis, K., Hasan, M.T., Stewart, A.F., Huttner, W.B., Hagen, V. & Bonhoeffer, T. (2009) Doxycycline-dependent photoactivated gene expression in eukaryotic systems. *Nature methods*, **6**, 527-531.

Carroll, D. (2011) Genome engineering with zinc-finger nucleases. *Genetics*, **188**, 773-782.

Cermak, T., Doyle, E.L., Christian, M., Wang, L., Zhang, Y., Schmidt, C., Baller, J.A., Somia, N.V., Bogdanove, A.J. & Voytas, D.F. (2011) Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. *Nucleic acids research*, **39**, e82.

Chalfie, M., Tu, Y., Euskirchen, G., Ward, W.W. & Prasher, D.C. (1994) Green fluorescent protein as a marker for gene expression. *Science*, **263**, 802-805.

Chang, N., Sun, C., Gao, L., Zhu, D., Xu, X., Zhu, X., Xiong, J.W. & Xi, J.J. (2013) Genome editing with RNA-guided Cas9 nuclease in zebrafish embryos. *Cell research*, **23**, 465-472.

Chapouton, P., Skupien, P., Hesl, B., Coolen, M., Moore, J.C., Madelaine, R., Kremmer, E., Faus-Kessler, T., Blader, P., Lawson, N.D. & Bally-Cuif, L. (2010) Notch activity levels control the balance between quiescence and recruitment of adult neural stem cells. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **30**, 7961-7974.

Chen, F., Pruett-Miller, S.M., Huang, Y., Gjoka, M., Duda, K., Taunton, J., Collingwood, T.N., Frodin, M. & Davis, G.D. (2011) High-frequency genome editing using ssDNA oligonucleotides with zinc-finger nucleases. *Nature methods*, **8**, 753-755.

Cinelli, A.R., Hamilton, K.A. & Kauer, J.S. (1995) Salamander olfactory bulb neuronal activity observed by video rate, voltage-sensitive dye imaging. III. Spatial and temporal properties of responses evoked by odorant stimulation. *J. Neurophysiol.*, **73**, 2053-2071.

Clift, D., Richendrfer, H., Thorn, R.J., Colwill, R.M. & Creton, R. (2014) High-throughput analysis of behavior in zebrafish larvae: effects of feeding. *Zebrafish*, **11**, 455-461.

Collins, R.T., Linker, C. & Lewis, J. (2010) MAZe: a tool for mosaic analysis of gene function in zebrafish. *Nature methods*, **7**, 219-223.

Colwill, R.M., Raymond, M.P., Ferreira, L. & Escudero, H. (2005) Visual discrimination learning in zebrafish (Danio rerio). *Behav Processes*, **70**, 19-31.

Cong, L., Ran, F.A., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, P.D., Wu, X., Jiang, W., Marraffini, L.A. & Zhang, F. (2013) Multiplex genome engineering using CRISPR/Cas systems. *Science*, **339**, 819-823.

Cristea, S., Freyvert, Y., Santiago, Y., Holmes, M.C., Urnov, F.D., Gregory, P.D. & Cost, G.J. (2013) In vivo cleavage of transgene donors promotes nuclease-mediated targeted integration. *Biotechnology and bioengineering*, **110**, 871-880.

Curado, S. & Stainier, D.Y. (2010) deLiver'in regeneration: injury response and development. *Seminars in liver disease*, **30**, 288-295.

Da Costa, M.M., Allen, C.E., Higginbottom, A., Ramesh, T., Shaw, P.J. & McDermott, C.J. (2014) A new zebrafish model produced by TILLING of SOD1-related amyotrophic lateral sclerosis replicates key features of the disease and represents a tool for in vivo therapeutic screening. *Disease models & mechanisms*, **7**, 73-81.

Dahlem, T.J., Hoshijima, K., Jurynec, M.J., Gunther, D., Starker, C.G., Locke, A.S., Weis, A.M., Voytas, D.F. & Grunwald, D.J. (2012) Simple methods for generating and detecting locus-specific mutations induced with TALENs in the zebrafish genome. *PLoS genetics*, **8**, e1002861.

Dai, J., Cui, X., Zhu, Z. & Hu, W. (2010) Non-homologous end joining plays a key role in transgene concatemer formation in transgenic zebrafish embryos. *International journal of biological sciences*, **6**, 756-768.

Darland, T. & Dowling, J.E. (2001) Behavioral screening for cocaine sensitivity in mutagenized zebrafish. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 11691-11696.

Darrow, K.O. & Harris, W.A. (2004) Characterization and development of courtship in zebrafish, Danio rerio. *Zebrafish*, **1**, 40-45.

Davidson, A.E., Balciunas, D., Mohn, D., Shaffer, J., Hermanson, S., Sivasubbu, S., Cliff, M.P., Hackett, P.B. & Ekker, S.C. (2003) Efficient gene delivery and gene expression in zebrafish using the Sleeping Beauty transposon. *Developmental biology*, **263**, 191-202.

Deiters, A., Garner, R.A., Lusic, H., Govan, J.M., Dush, M., Nascone-Yoder, N.M. & Yoder, J.A. (2010) Photocaged morpholino oligomers for the light-regulation of gene function in zebrafish and Xenopus embryos. *Journal of the American Chemical Society*, **132**, 15644-15650.

Delaney, K., Davison, I. & Denk, W. (2001) Odour-evoked [Ca2+] transients in mitral cell dendrites of frog olfactory glomeruli. *Eur J Neurosci*, **13**, 1658-1672.

Deng, D., Yan, C., Pan, X., Mahfouz, M., Wang, J., Zhu, J.K., Shi, Y. & Yan, N. (2012) Structural basis for sequence-specific recognition of DNA by TAL effectors. *Science*, **335**, 720-723.

Development (1996) Development Volume 123. *Development*, **123**, 153-203.

Ding, Q., Lee, Y.K., Schaefer, E.A., Peters, D.T., Veres, A., Kim, K., Kuperwasser, N., Motola, D.L., Meissner, T.B., Hendriks, W.T., Trevisan, M., Gupta, R.M., Moisan, A., Banks, E., Friesen, M., Schinzel, R.T., Xia, F., Tang, A., Xia, Y., Figueroa, E., Wann, A., Ahfeldt, T., Daheron, L., Zhang, F., Rubin, L.L., Peng, L.F., Chung, R.T., Musunuru, K. & Cowan, C.A. (2013) A TALEN genome-editing system for generating human stem cell-based disease models. *Cell stem cell*, **12**, 238-251.

Distel, M., Wullimann, M.F. & Koster, R.W. (2009) Optimized Gal4 genetics for permanent gene expression mapping in zebrafish. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 13365-13370.

Dong, J. & Stuart, G.W. (2004) Transgene manipulation in zebrafish by using recombinases. *Methods in cell biology*, **77**, 363-379.

Douglass, A.D., Kraves, S., Deisseroth, K., Schier, A.F. & Engert, F. (2008) Escape behavior elicited by single, channelrhodopsin-2-evoked spikes in zebrafish somatosensory neurons. *Curr Biol*, **18**, 1133-1137.

Doyon, Y., McCammon, J.M., Miller, J.C., Faraji, F., Ngo, C., Katibah, G.E., Amora, R., Hocking, T.D., Zhang, L., Rebar, E.J., Gregory, P.D., Urnov, F.D. & Amacher, S.L. (2008) Heritable targeted gene disruption in zebrafish using designed zinc-finger nucleases. *Nature biotechnology*, **26**, 702-708.

Driever, W., Solnica-Krezel, L., Schier, A.F., Neuhauss, S.C., Malicki, J., Stemple, D.L., Stainier, D.Y., Zwartkruis, F., Abdelilah, S., Rangini, Z., Belak, J. & Boggs, C. (1996) A genetic screen for mutations affecting embryogenesis in zebrafish. *Development*, **123**, 37-46.

Eaton, R.C. & Farley, R.D. (1975) Mauthner neuron field potential in newly hatched larvae of the zebra fish. *J Neurophysiol*, **38**, 502-512.

Eisen, J.S. (1991) Determination of primary motoneuron identity in developing zebrafish embryos. *Science*, **252**, 569-572.

Eisen, J.S. & Pike, S.H. (1991) The spt-1 mutation alters segmental arrangement and axonal development of identified neurons in the spinal cord of the embryonic zebrafish. *Neuron*, **6**, 767-776.

Eisen, J.S. & Smith, J.C. (2008) Controlling morpholino experiments: don't stop making antisense. *Development*, **135**, 1735-1743.

Emelyanov, A. & Parinov, S. (2008) Mifepristone-inducible LexPR system to drive and control gene expression in transgenic zebrafish. *Developmental biology*, **320**, 113-121.

Erter, C.E., Solnica-Krezel, L. & Wright, C.V. (1998) Zebrafish nodal-related 2 encodes an early mesendodermal inducer signaling from the extraembryonic yolk syncytial layer. *Developmental biology*, **204**, 361-372.

Esengil, H., Chang, V., Mich, J.K. & Chen, J.K. (2007) Small-molecule regulation of zebrafish gene expression. *Nature chemical biology*, **3**, 154-155.

Euler, T., Detwiler, P.B. & Denk, W. (2002) Directionally selective calcium signals in dendrites of starburst amacrine cells. *Nature*, **418**, 845-852.

Faucherre, A. & Lopez-Schier, H. (2011) Delaying Gal4-driven gene expression in the zebrafish with morpholinos and Gal80. *PloS one*, **6**, e16587.

Fetcho, J.R. & O'Malley, D.M. (1995) Visualization of active neural circuitry in the spinal cord of intact zebrafish. *J Neurophysiol*, **73**, 399-406.

Fitch, M.T. & Silver, J. (2008) CNS injury, glial scars, and inflammation: Inhibitory extracellular matrices and regeneration failure. *Exp Neurol*, **209**, 294-301.

Freeman, J., Vladimirov, N., Kawashima, T., Mu, Y., Sofroniew, N.J., Bennett, D.V., Rosen, J., Yang, C.T., Looger, L.L. & Ahrens, M.B. (2014) Mapping brain activity at scale with cluster computing. *Nature methods*, **11**, 941-950.

Friedrich, R.W. & Korsching, S.I. (1997) Combinatorial and chemotopic odorant coding in the zebrafish olfactory bulb visualized by optical imaging. *Neuron*, **18**, 737-752.

Froemke, R.C., Kumar, V.S., Czkwianianc, P. & Yuste, R. (2002) Analysis of multineuronal activation patterns from calcium-imaging experiments in brain slices. *Trends Cardiovasc Med*, **12**, 247-252.

Fujimoto, E., Gaynes, B., Brimley, C.J., Chien, C.B. & Bonkowsky, J.L. (2011) Gal80 intersectional regulation of cell-type specific expression in vertebrates. *Developmental dynamics : an official publication of the American Association of Anatomists*, **240**, 2324-2334.

Gahtan, E., Tanger, P. & Baier, H. (2005) Visual prey capture in larval zebrafish is controlled by identified reticulospinal neurons downstream of the tectum. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **25**, 9294-9303.

Ganz, J., Kaslin, J., Hochmann, S., Freudenreich, D. & Brand, M. (2010) Heterogeneity and Fgf dependence of adult neural progenitors in the zebrafish telencephalon. *Glia*, **58**, 1345-1363.

Gomez-Marin, A., Partoune, N., Stephens, G.J., Louis, M. & Brembs, B. (2012) Automated tracking of animal posture and movement during exploration and sensory orientation behaviors. *PLoS One*, **7**, e41642.

Goncalves, P.J., Arrenberg, A.B., Hablitzel, B., Baier, H. & Machens, C.K. (2014) Optogenetic perturbations reveal the dynamics of an oculomotor integrator. *Front Neural Circuits*, **8**, 10.

Gonzalez-Quevedo, R., Lee, Y., Poss, K.D. & Wilkinson, D.G. (2010) Neuronal regulation of the spatial patterning of neurogenesis. *Developmental cell*, **18**, 136-147.

Gonzalez, B., Schwimmer, L.J., Fuller, R.P., Ye, Y., Asawapornmongkol, L. & Barbas, C.F., 3rd (2010) Modular system for the construction of zinc-finger libraries and proteins. *Nature protocols*, **5**, 791-810.

Gossen, M. & Bujard, H. (1992) Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proceedings of the National Academy of Sciences of the United States of America*, **89**, 5547-5551.

Grama, A. & Engert, F. (2012) Direction selectivity in the larval zebrafish tectum is mediated by asymmetric inhibition. *Frontiers in neural circuits*, **6**, 59.

Grandel, H., Kaslin, J., Ganz, J., Wenzel, I. & Brand, M. (2006) Neural stem cells and neurogenesis in the adult zebrafish brain: origin, proliferation dynamics, migration and cell fate. *Developmental biology*, **295**, 263-277.

Gritsman, K., Zhang, J., Cheng, S., Heckscher, E., Talbot, W.S. & Schier, A.F. (1999) The EGF-CFC protein one-eyed pinhead is essential for nodal signaling. *Cell*, **97**, 121-132.

Guo, J., Gaj, T. & Barbas, C.F., 3rd (2010) Directed evolution of an enhanced and highly efficient FokI cleavage domain for zinc finger nucleases. *Journal of molecular biology*, **400**, 96-107.

Haas, K., Sin, W.C., Javaherian, A., Li, Z. & Cline, H.T. (2001) Single-cell electroporation for gene transfer in vivo. *Neuron*, **29**, 583-591.

Haffter, P., Granato, M., Brand, M., Mullins, M.C., Hammerschmidt, M., Kane, D.A., Odenthal, J., van Eeden, F.J., Jiang, Y.J., Heisenberg, C.P., Kelsh, R.N., Furutani-Seiki, M., Vogelsang, E., Beuchle, D., Schach, U., Fabian, C. & Nusslein-Volhard, C. (1996) The identification of genes with unique and essential functions in the development of the zebrafish, Danio rerio. *Development*, **123**, 1-36.

Hagmann, M., Bruggmann, R., Xue, L., Georgiev, O., Schaffner, W., Rungger, D., Spaniol, P. & Gerster, T. (1998) Homologous recombination and DNA-end joining reactions in zygotes and early embryos of zebrafish (Danio rerio) and Drosophila melanogaster. *Biological chemistry*, **379**, 673-681.

Han, X. & Boyden, E.S. (2007) Multiple-color optical activation, silencing, and desynchronization of neural activity, with single-spike temporal resolution. *PloS one*, **2**, e299.

Hans, S., Freudenreich, D., Geffarth, M., Kaslin, J., Machate, A. & Brand, M. (2011) Generation of a non-leaky heat shock-inducible Cre line for conditional Cre/lox strategies in zebrafish. *Developmental dynamics : an official publication of the American Association of Anatomists*, **240**, 108-115.

Hao le, T., Burghes, A.H. & Beattie, C.E. (2011) Generation and Characterization of a genetic zebrafish model of SMA carrying the human SMN2 gene. *Molecular neurodegeneration*, **6**, 24.

Hay, M., Thomas, D.W., Craighead, J.L., Economides, C. & Rosenthal, J. (2014) Clinical development success rates for investigational drugs. *Nature biotechnology*, **32**, 40-51.

Hegemann, P., Oesterbelt, D. & Steiner, M. (1985) The photocycle of the chloride pump halorhodopsin. I: Azide-catalyzed deprotonation of the chromophore is a side reaction of photocycle intermediates inactivating the pump. *EMBO J*, **4**, 2347-2350.

Helmchen, F., Svoboda, K., Denk, W. & Tank, D.W. (1999) In vivo dendritic calcium dynamics in deep-layer cortical pyramidal neurons. *Nat Neurosci*, **2**, 989-996.

Higashijima, S., Hotta, Y. & Okamoto, H. (2000) Visualization of cranial motor neurons in live transgenic zebrafish expressing green fluorescent protein under the control of the islet-1 promoter/enhancer. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **20**, 206-218.

Higashijima, S., Masino, M.A., Mandel, G. & Fetcho, J.R. (2003) Imaging neuronal activity during zebrafish behavior with a genetically encoded calcium indicator. *J Neurophysiol*, **90**, 3986-3997.

Higashijima, S., Okamoto, H., Ueno, N., Hotta, Y. & Eguchi, G. (1997) High-frequency generation of transgenic zebrafish which reliably express GFP in whole muscles or the whole body by using promoters of zebrafish origin. *Developmental biology*, **192**, 289-299.

Ho, R.K. & Kane, D.A. (1990) Cell-autonomous action of zebrafish spt-1 mutation in specific mesodermal precursors. *Nature*, **348**, 728-730.

Ho, R.K. & Kimmel, C.B. (1993) Commitment of cell fate in the early zebrafish embryo. *Science*, **261**, 109-111.

Howe, K. & Clark, M.D. & Torroja, C.F. & Torrance, J. & Berthelot, C. & Muffato, M. & Collins, J.E. & Humphray, S. & McLaren, K. & Matthews, L. & McLaren, S. & Sealy, I. & Caccamo, M. & Churcher, C. & Scott, C. & Barrett, J.C. & Koch, R. & Rauch, G.J. & White, S. & Chow, W. & Kilian, B. & Quintais, L.T. & Guerra-Assuncao, J.A. & Zhou, Y. & Gu, Y. & Yen, J. & Vogel, J.H. & Eyre, T. & Redmond, S. & Banerjee, R. & Chi, J. & Fu, B. & Langley, E. & Maguire, S.F. & Laird, G.K. & Lloyd, D. & Kenyon, E. & Donaldson, S. & Sehra, H. & Almeida-King, J. & Loveland, J. & Trevanion, S. & Jones, M. & Quail, M. & Willey, D. & Hunt, A. & Burton, J. & Sims, S. & McLay, K. & Plumb, B. & Davis, J. & Clee, C. & Oliver, K. & Clark, R. & Riddle, C. & Elliot, D. & Threadgold, G. & Harden, G. & Ware, D. & Begum, S. & Mortimore, B. & Kerry, G. & Heath, P. & Phillimore, B. & Tracey, A. & Corby, N. & Dunn, M. & Johnson, C. & Wood, J. & Clark, S. & Pelan, S. & Griffiths, G. & Smith, M. & Glithero, R. & Howden, P. & Barker, N. & Lloyd, C. & Stevens, C. & Harley, J. & Holt, K. & Panagiotidis, G. & Lovell, J. & Beasley, H. & Henderson, C. & Gordon, D. & Auger, K. & Wright, D. & Collins, J. & Raisen, C. & Dyer, L. & Leung, K. & Robertson, L. & Ambridge, K. & Leongamornlert, D. & McGuire, S. & Gilderthorp, R. & Griffiths, C. & Manthravadi, D. & Nichol, S. & Barker, G. & Whitehead, S. & Kay, M. & Brown, J. & Murnane, C. & Gray, E. & Humphries, M. & Sycamore, N. & Barker, D. & Saunders, D. & Wallis, J. & Babbage, A. & Hammond, S. & Mashreghi-Mohammadi, M. & Barr, L. & Martin, S. & Wray, P. & Ellington, A. & Matthews, N. & Ellwood, M. & Woodmansey, R. & Clark, G. & Cooper, J. & Tromans, A. & Grafham, D. & Skuce, C. & Pandian, R. & Andrews, R. & Harrison, E. & Kimberley, A. & Garnett, J. & Fosker, N. & Hall, R. & Garner, P. & Kelly, D. & Bird, C. & Palmer, S. & Gehring, I. & Berger, A. & Dooley, C.M. & Ersan-Urun, Z. & Eser, C. & Geiger, H. & Geisler, M. & Karotki, L. & Kirn, A. & Konantz, J. & Konantz, M. & Oberlander, M. & Rudolph-Geiger, S. & Teucke, M. & Lanz, C. & Raddatz, G. & Osoegawa, K. & Zhu, B. & Rapp, A. & Widaa, S. & Langford, C. & Yang, F. & Schuster, S.C. & Carter, N.P. & Harrow, J. & Ning, Z. & Herrero, J. & Searle, S.M. & Enright, A. & Geisler, R. & Plasterk, R.H. & Lee, C. & Westerfield, M. & de Jong, P.J. & Zon, L.I. & Postlethwait, J.H. & Nusslein-Volhard, C. & Hubbard, T.J. & Roest Crollius, H. & Rogers, J. & Stemple, D.L. (2013) The zebrafish reference genome sequence and its relationship to the human genome. *Nature*, **496**, 498-503.

Hruscha, A., Krawitz, P., Rechenberg, A., Heinrich, V., Hecht, J., Haass, C. & Schmid, B. (2013) Efficient CRISPR/Cas9 genome editing with low off-target effects in zebrafish. *Development*, **140**, 4982-4987.

Huang, C.J., Jou, T.S., Ho, Y.L., Lee, W.H., Jeng, Y.T., Hsieh, F.J. & Tsai, H.J. (2005) Conditional expression of a myocardium-specific transgene in zebrafish transgenic lines. *Developmental dynamics : an official publication of the American Association of Anatomists*, **233**, 1294-1303.

Huang, P., Xiao, A., Zhou, M., Zhu, Z., Lin, S. & Zhang, B. (2011) Heritable gene targeting in zebrafish using customized TALENs. *Nature biotechnology*, **29**, 699-700.

Hughes, J.P., Rees, S., Kalindjian, S.B. & Philpott, K.L. (2011) Principles of early drug discovery. *British journal of pharmacology*, **162**, 1239-1249.

Huisken, J., Swoger, J., Del Bene, F., Wittbrodt, J. & Stelzer, E.H. (2004) Optical sectioning deep inside live embryos by selective plane illumination microscopy. *Science*, **305**, 1007-1009.

Hwang, W.Y., Fu, Y., Reyon, D., Maeder, M.L., Kaini, P., Sander, J.D., Joung, J.K., Peterson, R.T. & Yeh, J.R. (2013a) Heritable and precise zebrafish genome editing using a CRISPR-Cas system. *PloS one*, **8**, e68708.

Hwang, W.Y., Fu, Y., Reyon, D., Maeder, M.L., Tsai, S.Q., Sander, J.D., Peterson, R.T., Yeh, J.R. & Joung, J.K. (2013b) Efficient genome editing in zebrafish using a CRISPR-Cas system. *Nature biotechnology*, **31**, 227-229.

Jao, L.E., Wente, S.R. & Chen, W. (2013) Efficient multiplex biallelic zebrafish genome editing using a CRISPR nuclease system. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 13904-13909.

Jesuthasan, S.J. & Mathuru, A.S. (2008) The alarm response in zebrafish: innate fear in a vertebrate genetic model. *Journal of neurogenetics*, **22**, 211-228.

Jetti, S.K., Vendrell-Llopis, N. & Yaksi, E. (2014) Spontaneous activity governs olfactory representations in spatially organized habenular microcircuits. *Curr Biol*, **24**, 434-439.

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A. & Charpentier, E. (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*, **337**, 816-821.

Jungke, P., Hans, S. & Brand, M. (2013) The zebrafish CreZoo: an easy-to-handle database for novel CreER(T2)-driver lines. *Zebrafish*, **10**, 259-263.

Kalev-Zylinska, M.L., Horsfield, J.A., Flores, M.V., Postlethwait, J.H., Vitas, M.R., Baas, A.M., Crosier, P.S. & Crosier, K.E. (2002) Runx1 is required for zebrafish blood and vessel development and expression of a human RUNX1-CBF2T1 transgene advances a model for studies of leukemogenesis. *Development*, **129**, 2015-2030.

Kalueff, A.V., et al (2013) Towards a Comprehensive Catalog of Zebrafish Behavior 1.0 and Beyond. *Zebrafish*, **10**.

Kalueff, A.V., Stewart, A.M. & Gerlai, R. (2014) Zebrafish as an emerging model for studying complex brain disorders. *Trends in pharmacological sciences*, **35**, 63-75.

Kane, D.A. & Kimmel, C.B. (1993) The zebrafish midblastula transition. *Development*, **119**, 447-456.

Kaslin, J., Ganz, J., Geffarth, M., Grandel, H., Hans, S. & Brand, M. (2009) Stem cells in the adult zebrafish cerebellum: initiation and maintenance of a novel stem cell niche. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **29**, 6142-6153.

Kawahara, G., Karpf, J.A., Myers, J.A., Alexander, M.S., Guyon, J.R. & Kunkel, L.M. (2011) Drug screening in a zebrafish model of Duchenne muscular dystrophy. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 5331-5336.

Kermen, F., Franco, L.M., Wyatt, C. & Yaksi, E. (2013) Neural circuits mediating olfactory-driven behavior in fish. *Frontiers in neural circuits*, **7**, 62.

Kettleborough, R.N., Busch-Nentwich, E.M., Harvey, S.A., Dooley, C.M., de Bruijn, E., van Eeden, F., Sealy, I., White, R.J., Herd, C., Nijman, I.J., Fenyes, F., Mehroke, S., Scahill, C., Gibbons, R., Wali, N., Carruthers, S., Hall, A., Yen, J., Cuppen, E. & Stemple, D.L. (2013) A systematic genome-wide analysis of zebrafish protein-coding gene function. *Nature*, **496**, 494-497.

Kim, S., Kim, D., Cho, S.W., Kim, J. & Kim, J.S. (2014) Highly efficient RNA-guided genome editing in human cells via delivery of purified Cas9 ribonucleoproteins. *Genome research*, **24**, 1012-1019.

Kim, S., Lee, M.J., Kim, H., Kang, M. & Kim, J.S. (2011) Preassembled zinc-finger arrays for rapid construction of ZFNs. *Nature methods*, **8**, 7.

Kim, Y., Kweon, J., Kim, A., Chon, J.K., Yoo, J.Y., Kim, H.J., Kim, S., Lee, C., Jeong, E., Chung, E., Kim, D., Lee, M.S., Go, E.M., Song, H.J., Kim, H., Cho, N., Bang, D., Kim, S. & Kim, J.S. (2013) A library of TAL effector nucleases spanning the human genome. *Nature biotechnology*, **31**, 251-258.

Kimmel, C.B., Ullmann, B., Walker, M., Miller, C.T. & Crump, J.G. (2003) Endothelin 1-mediated regulation of pharyngeal bone development in zebrafish. *Development*, **130**, 1339-1351.

Kimura, Y., Satou, C., Fujioka, S., Shoji, W., Umeda, K., Ishizuka, T., Yawo, H. & Higashijima, S. (2013) Hindbrain V2a neurons in the excitation of spinal locomotor circuits during zebrafish swimming. *Current biology : CB*, **23**, 843-849.

Kizil, C., Otto, G.W., Geisler, R., Nusslein-Volhard, C. & Antos, C.L. (2009) Simplet controls cell proliferation and gene transcription during zebrafish caudal fin regeneration. *Developmental biology*, **325**, 329-340.

Kohashi, T., Nakata, N. & Oda, Y. (2012) Effective sensory modality activating an escape triggering neuron switches during early development in zebrafish. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **32**, 5810-5820.

Kok, F.O., Shin, M., Ni, C., Gupta, A., Grosse, A.S., van Impel, A., Kirchmaier, B.C., Peterson-Maduro, J., Kourkoulis, G., Male, I., DeSantis, D.F., Sheppard-Tindell, S., Ebarasi, L., Betsholtz, C., Schulte-Merker, S., Wolfe, S.A. & Lawson, N.D. (2014) Reverse Genetic Screening Reveals Poor Correlation between Morpholino-Induced and Mutant Phenotypes in Zebrafish. *Developmental cell*.

Kokel, D. & Peterson, R.T. (2011) Using the zebrafish photomotor response for psychotropic drug screening. *Methods in cell biology*, **105**, 517-524.

Koster, R.W. & Fraser, S.E. (2001) Tracing transgene expression in living zebrafish embryos. *Developmental biology*, **233**, 329-346.

Kubo, F., Hablitzel, B., Dal Maschio, M., Driever, W., Baier, H. & Arrenberg, A.B. (2014) Functional architecture of an optic flow-responsive area that drives horizontal eye movements in zebrafish. *Neuron*, **81**, 1344-1359.

Kwan, K.M., Fujimoto, E., Grabher, C., Mangum, B.D., Hardy, M.E., Campbell, D.S., Parant, J.M., Yost, H.J., Kanki, J.P. & Chien, C.B. (2007) The Tol2kit: a multisite gateway-based construction kit for Tol2 transposon transgenesis constructs. *Developmental dynamics : an official publication of the American Association of Anatomists*, **236**, 3088-3099.

Lang, B.T., Cregg, J.M., DePaul, M.A., Tran, A.P., Xu, K., Dyck, S.M., Madalena, K.M., Brown, B.P., Weng, Y., Li, S., Karimi-Abdolrezaee, S., Busch, S.A., Shen, Y. & Silver, J. (2014) Modulation of the proteoglycan receptor PTPsigma promotes recovery after spinal cord injury. *Nature*.

Langenau, D.M., Feng, H., Berghmans, S., Kanki, J.P., Kutok, J.L. & Look, A.T. (2005) Cre/lox-regulated transgenic zebrafish model with conditional myc-induced T cell acute lymphoblastic leukemia. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 6068-6073.

Le, X., Langenau, D.M., Keefe, M.D., Kutok, J.L., Neuberg, D.S. & Zon, L.I. (2007) Heat shock-inducible Cre/Lox approaches to induce diverse types of tumors and hyperplasia in transgenic zebrafish. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 9410-9415.

LeClair, E.E. & Topczewski, J. (2010) Development and regeneration of the zebrafish maxillary barbel: a novel study system for vertebrate tissue growth and repair. *PloS one*, **5**, e8737.

Lepilina, A., Coon, A.N., Kikuchi, K., Holdway, J.E., Roberts, R.W., Burns, C.G. & Poss, K.D. (2006) A dynamic epicardial injury response supports progenitor cell activity during zebrafish heart regeneration. *Cell*, **127**, 607-619.

Leshchiner, I., Alexa, K., Kelsey, P., Adzhubei, I., Austin-Tse, C.A., Cooney, J.D., Anderson, H., King, M.J., Stottmann, R.W., Garnaas, M.K., Ha, S., Drummond, I.A., Paw, B.H., North, T.E., Beier, D.R., Goessling, W. & Sunyaev, S.R. (2012) Mutation mapping and identification by whole-genome sequencing. *Genome research*, **22**, 1541-1548.

Li, D., Qiu, Z., Shao, Y., Chen, Y., Guan, Y., Liu, M., Li, Y., Gao, N., Wang, L., Lu, X., Zhao, Y. & Liu, M. (2013a) Heritable gene targeting in the mouse and rat using a CRISPR-Cas system. *Nature biotechnology*, **31**, 681-683.

Li, J., Mack, J.A., Souren, M., Yaksi, E., Higashijima, S., Mione, M., Fetcho, J.R. & Friedrich, R.W. (2005) Early development of functional spatial maps in the zebrafish olfactory bulb. *J Neurosci*, **25**, 5784-5795.

Li, J.F., Norville, J.E., Aach, J., McCormack, M., Zhang, D., Bush, J., Church, G.M. & Sheen, J. (2013b) Multiplex and homologous recombination-mediated genome editing in Arabidopsis and Nicotiana benthamiana using guide RNA and Cas9. *Nature biotechnology*, **31**, 688-691.

Li, W., Teng, F., Li, T. & Zhou, Q. (2013c) Simultaneous generation and germline transmission of multiple gene mutations in rat using CRISPR-Cas systems. *Nature biotechnology*, **31**, 684-686.

Li, Y., Cheng, C.N., Verdun, V.A. & Wingert, R.A. (2014) Zebrafish nephrogenesis is regulated by interactions between retinoic acid, mecom, and Notch signaling. *Developmental biology*, **386**, 111-122.

Lien, C.L., Schebesta, M., Makino, S., Weber, G.J. & Keating, M.T. (2006) Gene expression analysis of zebrafish heart regeneration. *PLoS biology*, **4**, e260.

Lindsey, B.W., Di Donato, S., Kaslin, J. & Tropepe, V. (2014) Sensory-specific modulation of adult neurogenesis in sensory structures is associated with the type of stem cell present in the neurogenic niche of the zebrafish brain. *The European journal of neuroscience*, **40**, 3591-3607.

Lister, J.A., Robertson, C.P., Lepage, T., Johnson, S.L. & Raible, D.W. (1999) nacre encodes a zebrafish microphthalmia-related protein that regulates neural-crest-derived pigment cell fate. *Development*, **126**, 3757-3767.

Liu, D.W. & Westerfield, M. (1988) Function of identified motoneurones and co-ordination of primary and secondary motor systems during zebra fish swimming. *J Physiol*, **403**, 73-89.

Liu, Q., Segal, D.J., Ghiara, J.B. & Barbas, C.F., 3rd (1997) Design of polydactyl zinc-finger proteins for unique addressing within complex genomes. *Proceedings of the National Academy of Sciences of the United States of America*, **94**, 5525-5530.

Livet, J., Weissman, T.A., Kang, H., Draft, R.W., Lu, J., Bennis, R.A., Sanes, J.R. & Lichtman, J.W. (2007) Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system. *Nature*, **450**, 56-62.

Lombardo, V.A., Sporbert, A. & Abdelilah-Seyfried, S. (2012) Cell tracking using photoconvertible proteins during zebrafish development. *Journal of visualized experiments : JoVE*.

Luo, L., Callaway, E.M. & Svoboda, K. (2008) Genetic dissection of neural circuits. *Neuron*, **57**, 634-660.

Ma, J. & Ptashne, M. (1987) The carboxy-terminal 30 amino acids of GAL4 are recognized by GAL80. *Cell*, **50**, 137-142.

Ma, L., Yu, Y.M., Guo, Y., Hart, R.P. & Schachner, M. (2012) Cysteine- and glycine-rich protein 1a is involved in spinal cord regeneration in adult zebrafish. *The European journal of neuroscience*, **35**, 353-365.

Maaswinkel, H., Zhu, L. & Weng, W. (2013) Using an automated 3D-tracking system to record individual and shoals of adult zebrafish. *Journal of visualized experiments : JoVE*, 50681.

Mack-Bucher, J.A., Li, J. & Friedrich, R.W. (2007) Early functional development of interneurons in the zebrafish olfactory bulb. *Eur J Neurosci*, **25**, 460-470.

Madsen, E.C. & Gitlin, J.D. (2008) Zebrafish mutants calamity and catastrophe define critical pathways of gene-nutrient interactions in developmental copper metabolism. *PLoS genetics*, **4**, e1000261.

Magurran, A.E., Irving, P.W. & Henderson, P.A. (1996) Is there a fish alarm pheromone? A wild study and critique. *Proc R Soc Lond B*, **263**, 1551-1556.

Mali, P., Aach, J., Stranges, P.B., Esvelt, K.M., Moosburner, M., Kosuri, S., Yang, L. & Church, G.M. (2013) CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering. *Nature biotechnology*, **31**, 833-838.

Maresca, M., Lin, V.G., Guo, N. & Yang, Y. (2013) Obligate ligation-gated recombination (ObLiGaRe): custom-designed nuclease-mediated targeted integration through nonhomologous end joining. *Genome research*, **23**, 539-546.

Marz, M., Chapouton, P., Diotel, N., Vaillant, C., Hesl, B., Takamiya, M., Lam, C.S., Kah, O., Bally-Cuif, L. & Strahle, U. (2010) Heterogeneity in progenitor cell subtypes in the ventricular zone of the zebrafish adult telencephalon. *Glia*, **58**, 870-888.

Masino, M.A. & Fetcho, J.R. (2005) Fictive swimming motor patterns in wild type and mutant larval zebrafish. *J Neurophysiol*, **93**, 3177-3188.

Mathias, J.R., Saxena, M.T. & Mumm, J.S. (2012) Advances in zebrafish chemical screening technologies. *Future medicinal chemistry*, **4**, 1811-1822.

Mathuru, A.S., Kibat, C., Cheong, W.F., Shui, G., Wenk, M.R., Friedrich, R.W. & Jesuthasan, S. (2012) Chondroitin fragments are odorants that trigger fear behavior in fish. *Curr Biol*, **22**, 538-544.

Mayford, M., Bach, M.E., Huang, Y.Y., Wang, L., Hawkins, R.D. & Kandel, E.R. (1996) Control of memory formation through regulated expression of a CaMKII transgene. *Science*, **274**, 1678-1683.

Meng, X., Noyes, M.B., Zhu, L.J., Lawson, N.D. & Wolfe, S.A. (2008) Targeted gene inactivation in zebrafish using engineered zinc-finger nucleases. *Nature biotechnology*, **26**, 695-701.

Miller, J.C., Tan, S., Qiao, G., Barlow, K.A., Wang, J., Xia, D.F., Meng, X., Paschon, D.E., Leung, E., Hinkley, S.J., Dulay, G.P., Hua, K.L., Ankoudinova, I., Cost, G.J., Urnov, F.D., Zhang, H.S., Holmes, M.C., Zhang, L., Gregory, P.D. & Rebar, E.J. (2011) A TALE nuclease architecture for efficient genome editing. *Nature biotechnology*, **29**, 143-148.

Miller, N. & Gerlai, R. (2007) Quantification of shoaling behaviour in zebrafish (Danio rerio). *Behav Brain Res*, **184**, 157-166.

Millimaki, B.B., Sweet, E.M. & Riley, B.B. (2010) Sox2 is required for maintenance and regeneration, but not initial development, of hair cells in the zebrafish inner ear. *Developmental biology*, **338**, 262-269.

Ming, G.L. & Song, H. (2011) Adult neurogenesis in the mammalian brain: significant answers and significant questions. *Neuron*, **70**, 687-702.

Mirat, O., Sternberg, J.R., Severi, K.E. & Wyart, C. (2013) ZebraZoom: an automated program for high-throughput behavioral analysis and categorization. *Front Neural Circuits*, **7**, 107.

Moehle, E.A., Rock, J.M., Lee, Y.L., Jouvenot, Y., DeKelver, R.C., Gregory, P.D., Urnov, F.D. & Holmes, M.C. (2007) Targeted gene addition into a specified location in the human genome using designed zinc finger nucleases. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 3055-3060.

Moens, C.B., Donn, T.M., Wolf-Saxon, E.R. & Ma, T.P. (2008) Reverse genetics in zebrafish by TILLING. *Brief Funct Genomic Proteomic*, **7**, 454-459.

Morcos, P.A. (2007) Achieving targeted and quantifiable alteration of mRNA splicing with Morpholino oligos. *Biochemical and biophysical research communications*, **358**, 521-527.

Morcos, P.A., Li, Y. & Jiang, S. (2008) Vivo-Morpholinos: a non-peptide transporter delivers Morpholinos into a wide array of mouse tissues. *BioTechniques*, **45**, 613-614, 616, 618 passim.

Morris, J.A. (2009) Zebrafish: a model system to examine the neurodevelopmental basis of schizophrenia. *Progress in brain research*, **179**, 97-106.

Moscou, M.J. & Bogdanove, A.J. (2009) A simple cipher governs DNA recognition by TAL effectors. *Science*, **326**, 1501.

Mosimann, C., Kaufman, C.K., Li, P., Pugach, E.K., Tamplin, O.J. & Zon, L.I. (2011) Ubiquitous transgene expression and Cre-based recombination driven by the ubiquitin promoter in zebrafish. *Development*, **138**, 169-177.

Mullins, M.C., Hammerschmidt, M., Haffter, P. & Nusslein-Volhard, C. (1994) Large-scale mutagenesis in the zebrafish: in search of genes controlling development in a vertebrate. *Current biology : CB*, **4**, 189-202.

Muto, A. & Kawakami, K. (2011) Imaging functional neural circuits in zebrafish with a new GCaMP and the Gal4FF-UAS system. *Commun Integr Biol*, **4**, 566-568.

Muto, A., Ohkura, M., Abe, G., Nakai, J. & Kawakami, K. (2013) Real-time visualization of neuronal activity during perception. *Current biology : CB*, **23**, 307-311.

Myers, P.Z., Eisen, J.S. & Westerfield, M. (1986) Development and axonal outgrowth of identified motoneurons in the zebrafish. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **6**, 2278-2289.

Nagel, G., Ollig, D., Fuhrmann, M., Kateriya, S., Musti, A.M., Bamberg, E. & Hegemann, P. (2002) Channelrhodopsin-1: a light-gated proton channel in green algae. *Science*, **296**, 2395-2398.

Nakai, J., Ohkura, M. & Imoto, K. (2001) A high signal-to-noise Ca(2+) probe composed of a single green fluorescent protein. *Nat Biotechnol*, **19**, 137-141.

Nasevicius, A. & Ekker, S.C. (2000) Effective targeted gene 'knockdown' in zebrafish. *Nature genetics*, **26**, 216-220.

Nechiporuk, A., Poss, K.D., Johnson, S.L. & Keating, M.T. (2003) Positional cloning of a temperature-sensitive mutant emmental reveals a role for sly1 during cell proliferation in zebrafish fin regeneration. *Developmental biology*, **258**, 291-306.

Neuhauss, S.C., Biehlmaier, O., Seeliger, M.W., Das, T., Kohler, K., Harris, W.A. & Baier, H. (1999) Genetic disorders of vision revealed by a behavioral screen of 400 essential loci in zebrafish. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **19**, 8603-8615.

Nicolelis, M.A., Baccala, L.A., Lin, R.C. & Chapin, J.K. (1995) Sensorimotor encoding by synchronous neural ensemble activity at multiple levels of the somatosensory system. *Science*, **268**, 1353-1358.

Nicolelis, M.A., Lin, R.C., Woodward, D.J. & Chapin, J.K. (1993) Induction of immediate spatiotemporal changes in thalamic networks by peripheral block of ascending cutaneous information. *Nature*, **361**, 533-536.

Nusslein-Volhard, C. (2012) The zebrafish issue of Development. *Development*, **139**, 4099-4103.

O'Malley, D.M., Kao, Y.H. & Fetcho, J.R. (1996) Imaging the functional organization of zebrafish hindbrain segments during escape behaviors. *Neuron*, **17**, 1145-1155.

Oesterhelt, D., Hegemann, P. & Tittor, J. (1985) The photocycle of the chloride pump halorhodopsin. II: Quantum yields and a kinetic model. *EMBO J*, **4**, 2351-2356.

Ohki, K., Chung, S., Ch'ng, Y.H., Kara, P. & Reid, R.C. (2005) Functional imaging with cellular resolution reveals precise micro-architecture in visual cortex. *Nature*, **433**, 597-603.

Ohkura, M., Sasaki, T., Sadakari, J., Gengyo-Ando, K., Kagawa-Nagamura, Y., Kobayashi, C., Ikegaya, Y. & Nakai, J. (2012) Genetically encoded green fluorescent Ca2+ indicators with improved detectability for neuronal Ca2+ signals. *PloS one*, **7**, e51286.

Okamoto, H., Sato, T. & Aizawa, H. (2008) Transgenic technology for visualization and manipulation of the neural circuits controlling behavior in zebrafish. *Development, growth & differentiation*, **50 Suppl 1**, S167-175.

Oliveira, R.F., Silva, J.F. & Simoes, J.M. (2011) Fighting zebrafish: characterization of aggressive behavior and winner-loser effects. *Zebrafish*, **8**, 73-81.

Oppedal, D. & Goldsmith, M.I. (2010) A chemical screen to identify novel inhibitors of fin regeneration in zebrafish. *Zebrafish*, **7**, 53-60.

Orger, M.B., et al (2008) Control of visually guided behavior by distinct populations of spinal projection neurons. *Nat. Neurosci.*, **11**, 327-333.

Orger, M.B., Kampff, A.R., Severi, K.E., Bollmann, J.H. & Engert, F. (2008) Control of visually guided behavior by distinct populations of spinal projection neurons. *Nat Neurosci*, **11**, 327-333.

Otteson, D.C. & Hitchcock, P.F. (2003) Stem cells in the teleost retina: persistent neurogenesis and injury-induced regeneration. *Vision research*, **43**, 927-936.

Pan, H.C., Lin, J.F., Ma, L.P., Shen, Y.Q. & Schachner, M. (2013a) Major vault protein promotes locomotor recovery and regeneration after spinal cord injury in adult zebrafish. *The European journal of neuroscience*, **37**, 203-211.

Pan, Y.A., Freundlich, T., Weissman, T.A., Schoppik, D., Wang, X.C., Zimmerman, S., Ciruna, B., Sanes, J.R., Lichtman, J.W. & Schier, A.F. (2013b) Zebrabow: multispectral cell labeling for cell tracing and lineage analysis in zebrafish. *Development*, **140**, 2835-2846.

Paquet, D., Bhat, R., Sydow, A., Mandelkow, E.M., Berg, S., Hellberg, S., Falting, J., Distel, M., Koster, R.W., Schmid, B. & Haass, C. (2009) A zebrafish model of tauopathy allows in vivo imaging of neuronal cell death and drug evaluation. *J Clin Invest*, **119**, 1382-1395.

Perez-Escudero, A., Vicente-Page, J., Hinz, R.C., Arganda, S. & de Polavieja, G.G. (2014) idTracker: tracking individuals in a group by automatic identification of unmarked animals. *Nature methods*, **11**, 743-748.

Peterson, R.T., Shaw, S.Y., Peterson, T.A., Milan, D.J., Zhong, T.P., Schreiber, S.L., MacRae, C.A. & Fishman, M.C. (2004) Chemical suppression of a genetic mutation in a zebrafish model of aortic coarctation. *Nature biotechnology*, **22**, 595-599.

Pietri, T., Roman, A.C., Guyon, N., Romano, S.A., Washbourne, P., Moens, C.B., de Polavieja, G.G. & Sumbre, G. (2013) The first mecp2-null zebrafish model shows altered motor behaviors. *Frontiers in neural circuits*, **7**, 118.

Pittman, J.T. & Ichikawa, K.M. (2013) iPhone(R) applications as versatile video tracking tools to analyze behavior in zebrafish (Danio rerio). *Pharmacol Biochem Behav*, **106**, 137-142.

Portugues, R., Feierstein, C.E., Engert, F. & Orger, M.B. (2014) Whole-brain activity maps reveal stereotyped, distributed networks for visuomotor behavior. *Neuron*, **81**, 1328-1343.

Poss, K.D., Nechiporuk, A., Hillam, A.M., Johnson, S.L. & Keating, M.T. (2002) Mps1 defines a proximal blastemal proliferative compartment essential for zebrafish fin regeneration. *Development*, **129**, 5141-5149.

Poureetezadi, S.J. & Wingert, R.A. (2013) Congenital and Acute Kidney Disease: Translational Research Insights from Zebrafish Chemical Genetics. *General medicine*, **1**, 112.

Preuss, S.J., Trivedi, C.A., Vom Berg-Maurer, C.M., Ryu, S. & Bollmann, J.H. (2014) Classification of object size in retinotectal microcircuits. *Current biology : CB*, **24**, 2376-2385.

Prevedel, R., et al (2014) Simultaneous whole-animal 3D imaging of neuronal activity using light-field microscopy. *Nature methods*, **11**, 727-730.

Raya, A., Consiglio, A., Kawakami, Y., Rodriguez-Esteban, C. & Izpisua-Belmonte, J.C. (2004) The zebrafish as a model of heart regeneration. *Cloning and stem cells*, **6**, 345-351.

Raya, A., Koth, C.M., Buscher, D., Kawakami, Y., Itoh, T., Raya, R.M., Sternik, G., Tsai, H.J., Rodriguez-Esteban, C. & Izpisua-Belmonte, J.C. (2003) Activation of Notch signaling pathway precedes heart regeneration in zebrafish. *Proceedings of the National Academy of Sciences of the United States of America*, **100 Suppl 1**, 11889-11895.

Reimer, M.M., Kuscha, V., Wyatt, C., Sorensen, I., Frank, R.E., Knuwer, M., Becker, T. & Becker, C.G. (2009) Sonic hedgehog is a polarized signal for motor neuron regeneration in adult zebrafish. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **29**, 15073-15082.

Reimer, M.M., Norris, A., Ohnmacht, J., Patani, R., Zhong, Z., Dias, T.B., Kuscha, V., Scott, A.L., Chen, Y.C., Rozov, S., Frazer, S.L., Wyatt, C., Higashijima, S., Patton, E.E., Panula, P., Chandran, S., Becker, T. & Becker, C.G. (2013) Dopamine from the brain promotes spinal motor neuron generation during development and adult regeneration. *Developmental cell*, **25**, 478-491.

Rennekamp, A.J. & Peterson, R.T. (2014) 15 years of zebrafish chemical screening. *Current opinion in chemical biology*, **24C**, 58-70.

Rihel, J., Prober, D.A., Arvanites, A., Lam, K., Zimmerman, S., Jang, S., Haggarty, S.J., Kokel, D., Rubin, L.L., Peterson, R.T. & Schier, A.F. (2010) Zebrafish behavioral profiling links drugs to biological targets and rest/wake regulation. *Science*, **327**, 348-351.

Rihel, J. & Schier, A.F. (2012) Behavioral screening for neuroactive drugs in zebrafish. *Developmental neurobiology*, **72**, 373-385.

Rinner, O., Rick, J.M. & Neuhauss, S.C. (2005) Contrast sensitivity, spatial and temporal tuning of the larval zebrafish optokinetic response. *Invest Ophthalmol Vis Sci*, **46**, 137-142.

Sadowski, I., Ma, J., Triezenberg, S. & Ptashne, M. (1988) GAL4-VP16 is an unusually potent transcriptional activator. *Nature*, **335**, 563-564.

Saint-Amant, L. & Drapeau, P. (1998) Time course of the development of motor behaviors in the zebrafish embryo. *J Neurobiol*, **37**, 622-632.

Sander, J.D., Cade, L., Khayter, C., Reyon, D., Peterson, R.T., Joung, J.K. & Yeh, J.R. (2011) Targeted gene disruption in somatic zebrafish cells using engineered TALENs. *Nature biotechnology*, **29**, 697-698.

Santiago, Y., Chan, E., Liu, P.Q., Orlando, S., Zhang, L., Urnov, F.D., Holmes, M.C., Guschin, D., Waite, A., Miller, J.C., Rebar, E.J., Gregory, P.D., Klug, A. & Collingwood, T.N. (2008) Targeted gene knockout in mammalian cells by using engineered zinc-finger nucleases. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 5809-5814.

Santini, F., Harmon, L.J., Carnevale, G. & Alfaro, M.E. (2009) Did genome duplication drive the origin of teleosts? A comparative study of diversification in ray-finned fishes. *BMC evolutionary biology*, **9**, 194.

Sashital, D.G., Wiedenheft, B. & Doudna, J.A. (2012) Mechanism of foreign DNA selection in a bacterial adaptive immune system. *Molecular cell*, **46**, 606-615.

Sato, T., Hamaoka, T., Aizawa, H., Hosoya, T. & Okamoto, H. (2007) Genetic single-cell mosaic analysis implicates ephrinB2 reverse signaling in projections from the posterior tectum to the hindbrain in zebrafish. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **27**, 5271-5279.

Sauer, B. (1987) Functional expression of the cre-lox site-specific recombination system in the yeast Saccharomyces cerevisiae. *Molecular and cellular biology*, **7**, 2087-2096.

Sauer, B. (1998) Inducible gene targeting in mice using the Cre/lox system. *Methods*, **14**, 381-392.

Sauer, B. & Henderson, N. (1988) Site-specific DNA recombination in mammalian cells by the Cre recombinase of bacteriophage P1. *Proceedings of the National Academy of Sciences of the United States of America*, **85**, 5166-5170.

Schmid, B., Shah, G., Scherf, N., Weber, M., Thierbach, K., Campos, C.P., Roeder, I., Aanstad, P. & Huisken, J. (2013) High-speed panoramic light-sheet microscopy reveals global endodermal cell dynamics. *Nat Commun*, **4**, 2207.

Schulte-Merker, S., van Eeden, F.J., Halpern, M.E., Kimmel, C.B. & Nusslein-Volhard, C. (1994) no tail (ntl) is the zebrafish homologue of the mouse T (Brachyury) gene. *Development*, **120**, 1009-1015.

Schweitzer, J., Gimnopoulos, D., Lieberoth, B.C., Pogoda, H.M., Feldner, J., Ebert, A., Schachner, M., Becker, T. & Becker, C.G. (2007) Contactin1a expression is associated with oligodendrocyte differentiation and axonal regeneration in the central nervous system of zebrafish. *Molecular and cellular neurosciences*, **35**, 194-207.

Scott, E.K., Mason, L., Arrenberg, A.B., Ziv, L., Gosse, N.J., Xiao, T., Chi, N.C., Asakawa, K., Kawakami, K. & Baier, H. (2007) Targeting neural circuitry in zebrafish using GAL4 enhancer trapping. *Nature methods*, **4**, 323-326.

Sherpa, T., Lankford, T., McGinn, T.E., Hunter, S.S., Frey, R.A., Sun, C., Ryan, M., Robison, B.D. & Stenkamp, D.L. (2014) Retinal regeneration is facilitated by the presence of surviving neurons. *Developmental neurobiology*, **74**, 851-876.

Shoham, D., Glaser, D.E., Arieli, A., Kenet, T., Wijnbergen, C., Toledo, Y., Hildesheim, R. & Grinvald, A. (1999) Imaging cortical dynamics at high spatial and temporal resolution with novel blue voltage-sensitive dyes. *Neuron*, **24**, 791-802.

Sison, M. & Gerlai, R. (2010) Associative learning in zebrafish (Danio rerio) in the plus maze. *Behav Brain Res*, **207**, 99-104.

Sofroniew, M.V. (2009) Molecular dissection of reactive astrogliosis and glial scar formation. *Trends in neurosciences*, **32**, 638-647.

Song, P. & Pimplikar, S.W. (2012) Knockdown of amyloid precursor protein in zebrafish causes defects in motor axon outgrowth. *PloS one*, **7**, e34209.

Spence, R., Gerlach, G., Lawrence, C. & Smith, C. (2008) The behaviour and ecology of the zebrafish, Danio rerio. *Biol Rev Camb Philos Soc*, **83**, 13-34.

Spors, H. & Grinvald, A. (2002) Spatio-temporal dynamics of odor representations in the mammalian olfactory bulb. *Neuron*, **34**, 301-315.

Stainier, D.Y., Weinstein, B.M., Detrich, H.W., 3rd, Zon, L.I. & Fishman, M.C. (1995) Cloche, an early acting zebrafish gene, is required by both the endothelial and hematopoietic lineages. *Development*, **121**, 3141-3150.

Stewart, A.M., Gerlai, R. & Kalueff, A.V. (2015) Developing highER-throughput zebrafish screens for in-vivo CNS drug discovery. *Front Behav Neurosci*, **9**, 14.

Stewart, A.M., Nguyen, M., Wong, K., Poudel, M.K. & Kalueff, A.V. (2014) Developing zebrafish models of autism spectrum disorder (ASD). *Prog Neuropsychopharmacol Biol Psychiatry*, **50**, 27-36.

Stewart, S., Tsun, Z.Y. & Izpisua Belmonte, J.C. (2009) A histone demethylase is necessary for regeneration in zebrafish. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 19889-19894.

Streisinger, G., Walker, C., Dower, N., Knauber, D. & Singer, F. (1981) Production of clones of homozygous diploid zebra fish (Brachydanio rerio). *Nature*, **291**, 293-296.

Subedi, A., Macurak, M., Gee, S.T., Monge, E., Goll, M.G., Potter, C.J., Parsons, M.J. & Halpern, M.E. (2014) Adoption of the Q transcriptional regulatory system for zebrafish transgenesis. *Methods*, **66**, 433-440.

Summerton, J. & Weller, D. (1997) Morpholino antisense oligomers: design, preparation, and properties. *Antisense & nucleic acid drug development*, **7**, 187-195.

Sundvik, M., Chen, Y.C. & Panula, P. (2013) Presenilin1 regulates histamine neuron development and behavior in zebrafish, danio rerio. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **33**, 1589-1597.

Sung, Y.H., Kim, J.M., Kim, H.T., Lee, J., Jeon, J., Jin, Y., Choi, J.H., Ban, Y.H., Ha, S.J., Kim, C.H., Lee, H.W. & Kim, J.S. (2014) Highly efficient gene knockout in mice and zebrafish with RNA-guided endonucleases. *Genome research*, **24**, 125-131.

Szczepek, M., Brondani, V., Buchel, J., Serrano, L., Segal, D.J. & Cathomen, T. (2007) Structure-based redesign of the dimerization interface reduces the toxicity of zinc-finger nucleases. *Nature biotechnology*, **25**, 786-793.

Tallini, Y.N., Ohkura, M., Choi, B.R., Ji, G., Imoto, K., Doran, R., Lee, J., Plan, P., Wilson, J., Xin, H.B., Sanbe, A., Gulick, J., Mathai, J., Robbins, J., Salama, G., Nakai, J. & Kotlikoff, M.I. (2006) Imaging cellular signals in the heart in vivo: Cardiac expression of the high-signal Ca2+ indicator GCaMP2. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 4753-4758.

Tamplin, O.J., White, R.M., Jing, L., Kaufman, C.K., Lacadie, S.A., Li, P., Taylor, A.M. & Zon, L.I. (2012) Small molecule screening in zebrafish: swimming in potential drug therapies. *Wiley interdisciplinary reviews. Developmental biology*, **1**, 459-468.

Tawk, M., Bianco, I.H. & Clarke, J.D. (2009) Focal electroporation in zebrafish embryos and larvae. *Methods in molecular biology*, **546**, 145-151.

Teh, C., Chudakov, D.M., Poon, K.L., Mamedov, I.Z., Sek, J.Y., Shidlovsky, K., Lukyanov, S. & Korzh, V. (2010) Optogenetic in vivo cell manipulation in KillerRed-expressing zebrafish transgenics. *BMC developmental biology*, **10**, 110.

Tesson, L., Usal, C., Menoret, S., Leung, E., Niles, B.J., Remy, S., Santiago, Y., Vincent, A.I., Meng, X., Zhang, L., Gregory, P.D., Anegon, I. & Cost, G.J. (2011) Knockout rats generated by embryo microinjection of TALENs. *Nature biotechnology*, **29**, 695-696.

Thummel, R., Bailey, T.J. & Hyde, D.R. (2011) In vivo electroporation of morpholinos into the adult zebrafish retina. *Journal of visualized experiments : JoVE*, e3603.

Thummel, R., Burket, C.T., Brewer, J.L., Sarras, M.P., Jr., Li, L., Perry, M., McDermott, J.P., Sauer, B., Hyde, D.R. & Godwin, A.R. (2005) Cre-mediated site-specific recombination in zebrafish embryos. *Developmental dynamics : an official publication of the American Association of Anatomists*, **233**, 1366-1377.

Tian, L., Hires, S.A., Mao, T., Huber, D., Chiappe, M.E., Chalasani, S.H., Petreanu, L., Akerboom, J., McKinney, S.A., Schreiter, E.R., Bargmann, C.I., Jayaraman, V., Svoboda, K. & Looger, L.L. (2009) Imaging neural activity in worms, flies and mice with improved GCaMP calcium indicators. *Nat Methods*, **6**, 875-881.

Traven, A., Jelicic, B. & Sopta, M. (2006) Yeast Gal4: a transcriptional paradigm revisited. *EMBO reports*, **7**, 496-499.

Trinh le, A. & Fraser, S.E. (2013) Enhancer and gene traps for molecular imaging and genetic analysis in zebrafish. *Development, growth & differentiation*, **55**, 434-445.

Truong, T.V., Supatto, W., Koos, D.S., Choi, J.M. & Fraser, S.E. (2011) Deep and fast live imaging with two-photon scanned light-sheet microscopy. *Nature methods*, **8**, 757-760.

Urnov, F.D., Rebar, E.J., Holmes, M.C., Zhang, H.S. & Gregory, P.D. (2010) Genome editing with engineered zinc finger nucleases. *Nature reviews. Genetics*, **11**, 636-646.

Valentincic, T., Miklavc, P., Dolenek, J. & Pliberek, K. (2005) Correlations between olfactory discrimination, olfactory receptor neuron responses and chemotopy of amino acids in fishes. *Chem Senses*, **30 Suppl 1**, i312-314.

van Raamsdonk, W., Maslam, S., de Jong, D.H., Smit-Onel, M.J. & Velzing, E. (1998) Long term effects of spinal cord transection in zebrafish: swimming performances, and metabolic properties of the neuromuscular system. *Acta histochemica*, **100**, 117-131.

Vaziri, A. & Emiliani, V. (2012) Reshaping the optical dimension in optogenetics. *Current opinion in neurobiology*, **22**, 128-137.

Vladimirov, N., Mu, Y., Kawashima, T., Bennett, D.V., Yang, C.T., Looger, L.L., Keller, P.J., Freeman, J. & Ahrens, M.B. (2014) Light-sheet functional imaging in fictively behaving zebrafish. *Nat Methods*, **11**, 883-884.

Wachowiak, M., Cohen, L.B. & Zochowski, M.R. (2002) Distributed and concentration-invariant spatial representations of odorants by receptor neuron input to the turtle olfactory bulb. *J Neurophysiol*, **87**, 1035-1045.

Walker, C. & Streisinger, G. (1983) Induction of Mutations by gamma-Rays in Pregonial Germ Cells of Zebrafish Embryos. *Genetics*, **103**, 125-136.

Wang, H., Yang, H., Shivalila, C.S., Dawlaty, M.M., Cheng, A.W., Zhang, F. & Jaenisch, R. (2013) One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. *Cell*, **153**, 910-918.

Welsh, J.P., Lang, E.J., Suglhara, I. & Llinas, R. (1995) Dynamic organization of motor control within the olivocerebellar system. *Nature*, **374**, 453-457.

Westerfield, M. (2000) The zebrafish book. A guide for the laboratory use of zebrafish (Danio rerio). 4th ed. *Univ. of Oregon Press, Eugene.*

Westerfield, M. & Eisen, J.S. (1988) Neuromuscular specificity: pathfinding by identified motor growth cones in a vertebrate embryo. *Trends in neurosciences*, **11**, 18-22.

White, D.T. & Mumm, J.S. (2013) The nitroreductase system of inducible targeted ablation facilitates cell-specific regenerative studies in zebrafish. *Methods*, **62**, 232-240.

White, R.M., Sessa, A., Burke, C., Bowman, T., LeBlanc, J., Ceol, C., Bourque, C., Dovey, M., Goessling, W., Burns, C.E. & Zon, L.I. (2008) Transparent adult zebrafish as a tool for in vivo transplantation analysis. *Cell stem cell*, **2**, 183-189.

Whitehead, G.G., Makino, S., Lien, C.L. & Keating, M.T. (2005) fgf20 is essential for initiating zebrafish fin regeneration. *Science*, **310**, 1957-1960.

Wiedenheft, B., Sternberg, S.H. & Doudna, J.A. (2012) RNA-guided genetic silencing systems in bacteria and archaea. *Nature*, **482**, 331-338.

Wilson, M.A. & McNaughton, B.L. (1993) Dynamics of the hippocampal ensemble code for space. *Science*, **261**, 1055-1058.

Wolman, M.A., Jain, R.A., Liss, L. & Granato, M. (2011) Chemical modulation of memory formation in larval zebrafish. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 15468-15473.

Wright, D., Rimmer, L.B., Pritchard, V.L., Krause, J. & Butlin, R.K. (2003) Inter and intra-population variation in shoaling and boldness in the zebrafish (Danio rerio). *Naturwissenschaften*, **90**, 374-377.

Wyatt, C., Ebert, A., Reimer, M.M., Rasband, K., Hardy, M., Chien, C.B., Becker, T. & Becker, C.G. (2010) Analysis of the astray/robo2 zebrafish mutant reveals that degenerating tracts do not provide strong guidance cues for regenerating optic axons. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **30**, 13838-13849.

Xi, Y., Ryan, J., Noble, S., Yu, M., Yilbas, A.E. & Ekker, M. (2010) Impaired dopaminergic neuron development and locomotor function in zebrafish with loss of pink1 function. *The European journal of neuroscience*, **31**, 623-633.

Yaksi, E. & Friedrich, R.W. (2006) Reconstruction of firing rate changes across neuronal populations by temporally deconvolved Ca2+ imaging. *Nat Methods*, **3**, 377-383.

Yaksi, E., Friedrich, R. (2006) Reconstruction of firing rate changes across neuronal populations by temporally deconvolved Ca2+ imaging. *Nature methods*, **3**, 377-383.

Yaksi, E., Judkewitz, B. & Friedrich, R.W. (2007) Topological reorganization of odor representations in the olfactory bulb. *PLoS Biol*, **5**, e178.

Yang, J., Yuan, P., Wen, D., Sheng, Y., Zhu, S., Yu, Y., Gao, X. & Wei, W. (2013) ULtiMATE system for rapid assembly of customized TAL effectors. *PloS one*, **8**, e75649.

Yu, Y. & Schachner, M. (2013) Syntenin-a promotes spinal cord regeneration following injury in adult zebrafish. *The European journal of neuroscience*, **38**, 2280-2289.

Yuste, R. & Majewska, A. (2001) On the function of dendritic spines. *Neuroscientist*, **7**, 387-395.

Zhang, F., Wang, L.P., Brauner, M., Liewald, J.F., Kay, K., Watzke, N., Wood, P.G., Bamberg, E., Nagel, G., Gottschalk, A. & Deisseroth, K. (2007) Multimodal fast optical interrogation of neural circuitry. *Nature*, **446**, 633-639.

Zhao, S., Cunha, C., Zhang, F., Liu, Q., Gloss, B., Deisseroth, K., Augustine, G.J. & Feng, G. (2008) Improved expression of halorhodopsin for light-induced silencing of neuronal activity. *Brain Cell Biol*, **36**, 141-154.

Zhu, P., Friedrich, R.W., et al (2012) High-resolution optical control of spatiotemporal neuronal activity patterns in zebrafish using a digital micromirror device. *Nature protocols*, **7**, 1410-1425.

Zhu, P., Narita, Y., Bundschuh, S.T., Fajardo, O., Scharer, Y.P., Chattopadhyaya, B., Bouldoires, E.A., Stepien, A.E., Deisseroth, K., Arber, S., Sprengel, R., Rijli, F.M. & Friedrich, R.W. (2009) Optogenetic Dissection of Neuronal Circuits in Zebrafish using Viral Gene Transfer and the Tet System. *Frontiers in neural circuits*, **3**, 21.

Zochowski, M., Wachowiak, M., Falk, C.X., Cohen, L.B., Lam, Y.W., Antic, S. & Zecevic, D. (2000) Imaging membrane potential with voltage-sensitive dyes. *Biol Bull*, **198**, 1-21.

Zou, S., Tian, C., Ge, S. & Hu, B. (2013) Neurogenesis of retinal ganglion cells is not essential to visual functional recovery after optic nerve injury in adult zebrafish. *PloS one*, **8**, e57280.

Zu, Y., Tong, X., Wang, Z., Liu, D., Pan, R., Li, Z., Hu, Y., Luo, Z., Huang, P., Wu, Q., Zhu, Z., Zhang, B. & Lin, S. (2013) TALEN-mediated precise genome modification by homologous recombination in zebrafish. *Nature methods*, **10**, 329-331.

Zupanc, G.K. & Zupanc, M.M. (2006) New neurons for the injured brain: mechanisms of neuronal regeneration in adult teleost fish. *Regenerative medicine*, **1**, 207-216.

Figure Legends

Figure 1. Methods of transient alteration of expression and permanent transgenesis.
A. Diagram of pressure injection of oligonucleotides into a single cell stage zebrafish embryo (zygote) via glass microcapillary needle.
B. Transient misexpression can be achieved by injection of mRNA (widespread misexpression in the majority of cells), DNA (stochastic misexpression in few cells) or morpholino (reduction in production of a specific wild type protein).
Randomly integrated permanent transgenesis can be achieved by injection of a DNA template (construct in a plasmid vector flanked by Tol sites) along with a transposase enzyme, leading to random integration of the construct into the genome.
Targeted permanent transgenesis can be achieved by injection of a DNA template (construct in a plasmid vector flanked by nuclease cutting sites) along with nucleases to cleave the construct and a target sequence of the genome, leading to targeted integration of the construct. If this occurs in the germline, the transgenic modification will be heritable.

Scale bar in A is 250μm.

Figure 2. Transient manipulation of the expression pathway from genome to protein.
A. The control column shows a standard expression pathway from genomic DNA transcribed to pre-mRNA, spliced to mRNA and then translated to a protein.
B. Start codon directed morpholinos bind to mRNA at the 5'-untranslated region, near the region of the start codon, interfering with the progression of the ribosomal initiation complex. This prevents translation, leading to a reduction in production of wild type protein.
C. Splice site directed morpholinos bind to pre-mRNA at intron/exon boundaries thus interfering with the progression of the spliceosome along the pre-mRNA. This results in aberrantly spliced mRNA and subsequently aberrant proteins can be formed, while reducing the production of wild type protein.
D. Injection of synthesised mRNA transcripts provides additional targets for translation so increasing the production of the protein of interest.

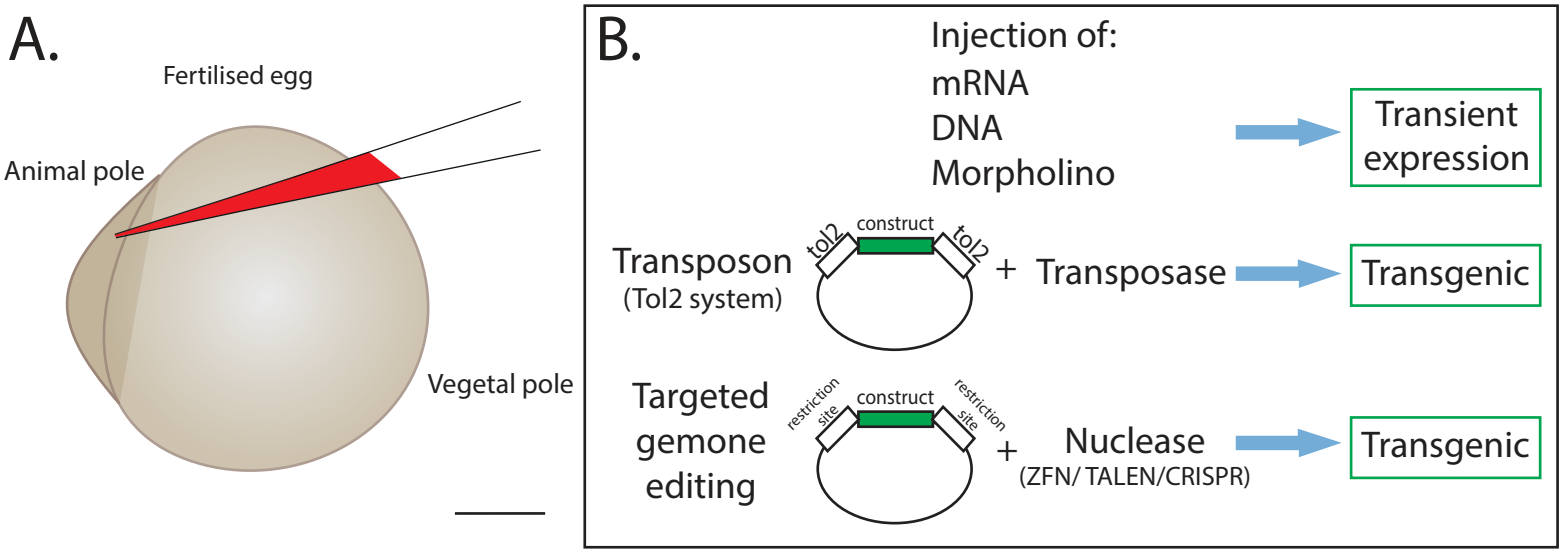
Ex (Exon), I (Intron).

Figure 3. Targeted, permanent transgenesis using chimeric nucleases, ZFNs and TALENs, or the CRISPR/Cas9 system.
A. ZFNs' nuclease activity is targeted by zinc finger DNA binding domains that bind to specific base pair triplets in genomic DNA. When 2 ZFNs bind to DNA in close proximity, Fok1 nuclease activity is induced leading to a double stranded break in the DNA, followed by DNA damage response activity. This activity, such as non-homologous end joining, can introduce small insertions or deletions leading to frameshift mutations or the incorporation of DNA constructs co-injected with the nuclease.
B. TALENs operate in a similar manner to ZFNs, with the additional benefit that each binding domain is specific to a single base pair, making it easier to design TALENs against specific genome sequences.
C. The CRISPR/Cas9 binding specificity is governed not by protein to DNA binding, but by Watson-Crick pairing rules as the single guide RNA (sgRNA) binds to the target genomic DNA. The bound sgRNA guides the Cas9 nuclease to the desired target resulting in cleaving of the DNA.

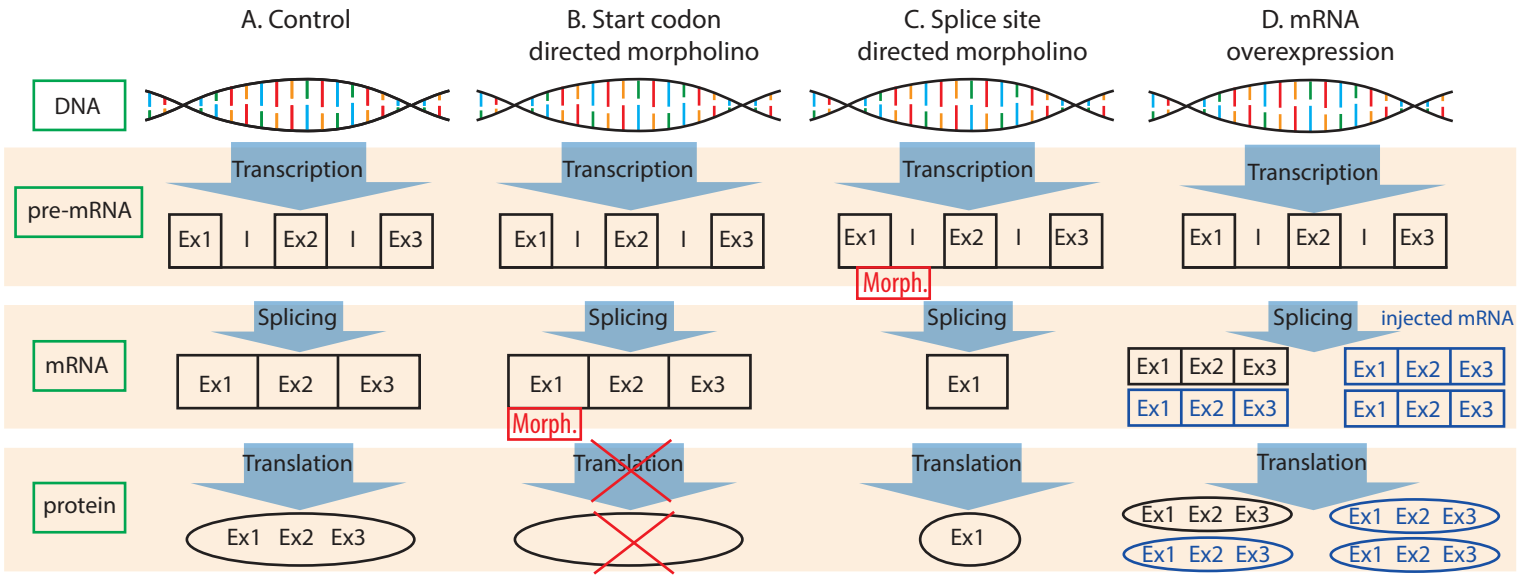
Figure 4. Adult zebrafish brain, with key brain regions and neurogenic zones labelled.
The adult zebrafish brain shows widespread neurogenesis in multiple regions (blue) with numerous adult stems cell niches (red).
Parasagittal view, rostral is left. Typical adult brain length (left to right) is 4mm.

E (Epiphysis), Hb (Habenula), HT (Hypothalamus), IPN (Interpeduncular nucleus), OB (Olfactory bulb), OT (Optic tectum), R (Raphe).

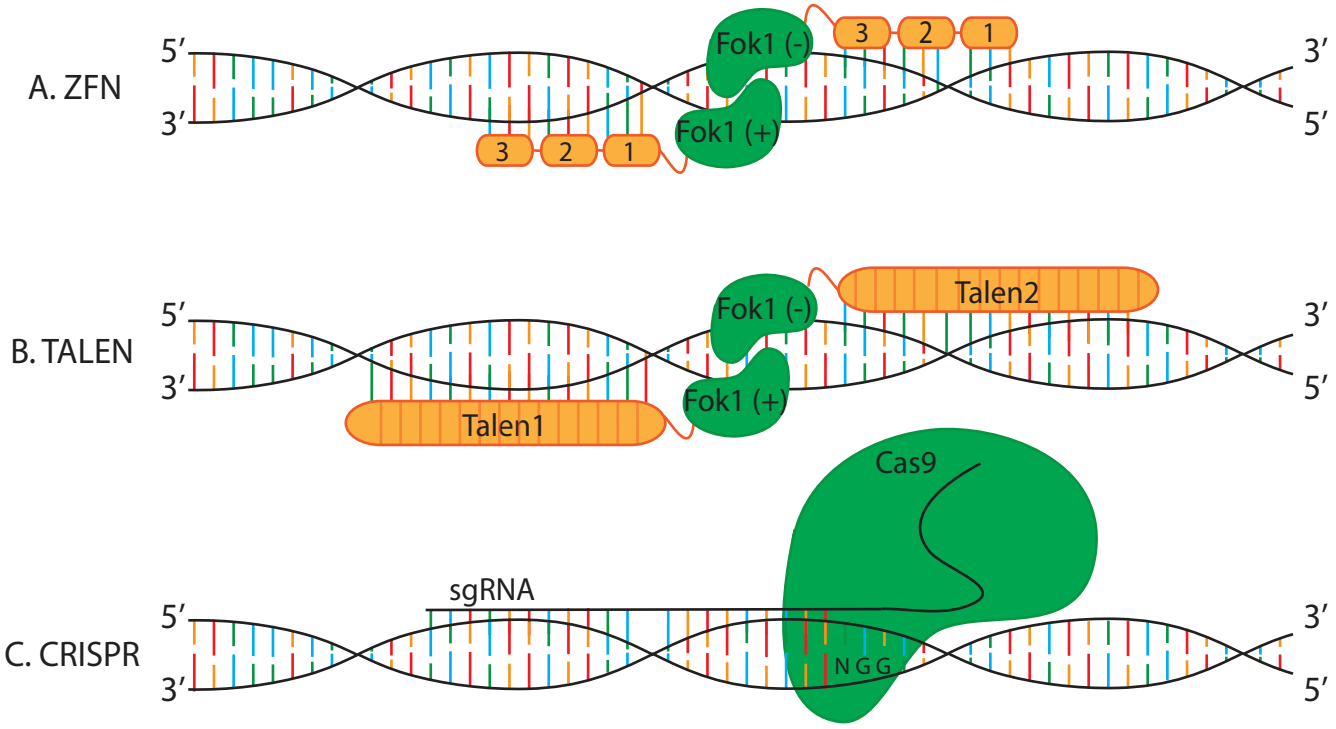
Table 1. A selection of CNS disorders studied in the zebrafish using mutant lines.

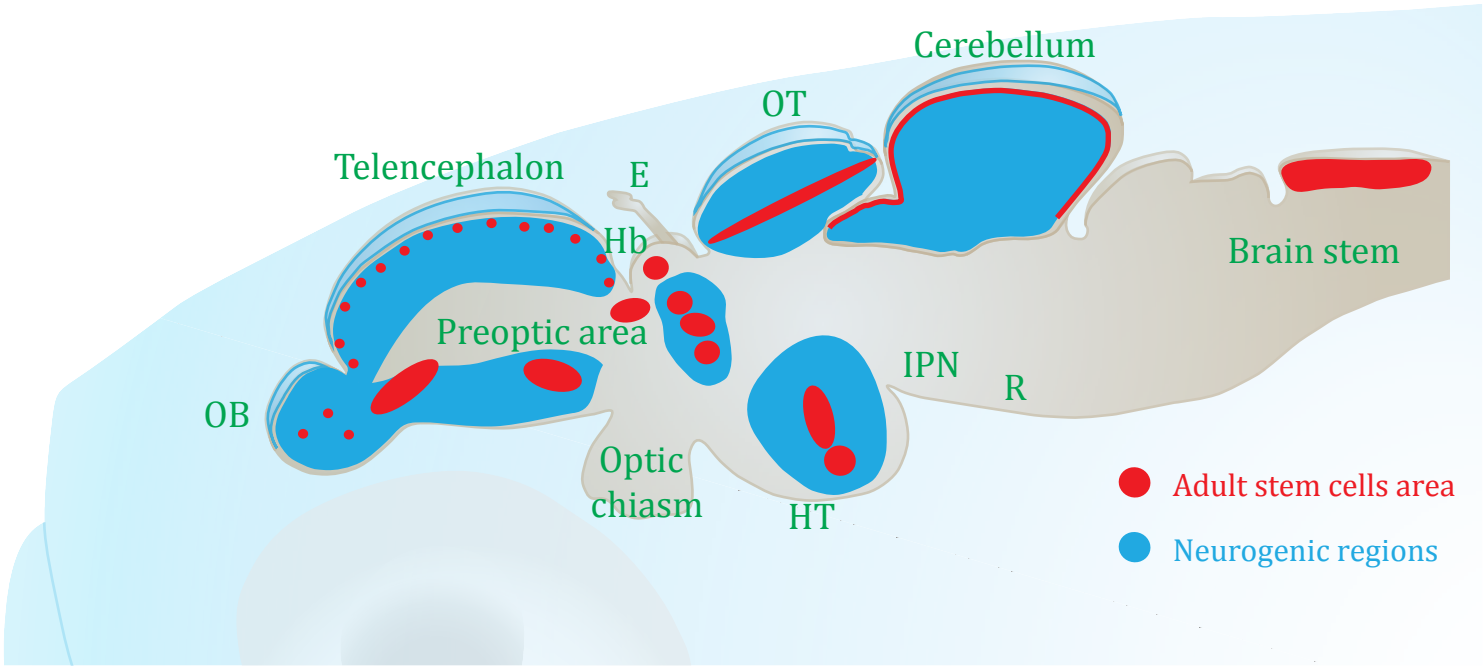

A.
Fertilised egg
Animal pole
Vegetal pole
B.
Injection of:
mRNA
DNA
Morpholino
Transient expression
Transposon
(Tol2 system)
tol2
construct
tol2
+
Transposase
Transgenic
Targeted gemone editing
restriction site
construct
restriction site
+
Nuclease
(ZFN/ TALEN/CRISPR)
Transgenic

Transient misexpression



Transgenics methods




Cerebellum
OT
Telencephalon
E
Hb
Brain stem
Preoptic area
IPN
R
OB
Optic
chiasm
HT
Adult stem cells area
Neurogenic regions