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Basic Methods for Fission Yeast

Susan L Forsburg and

Program in Molecular & Computational Biology, University of Southern California, 1050 Child's Way MCB201, Los Angeles CA 90089-2910 tel. 213-740-7342 fax. 213-740-8631

Nicholas Rhind

Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, 364 Plantation Street LRB904, Worcester MA 01605 USA (508) 856-8316, fax: 1 (508) 856-8358

Susan L Forsburg: forsburg@usc.edu; Nicholas Rhind: nick.rhind@umassmed.edu

Abstract

The fission yeast *S. pombe* is a popular model system, and has been particularly influential in studies of the cell cycle and chromosome dynamics. Despite its differences from *Sacch. cerevisiae*, the tools and methods for fission yeast are conceptually similar to those used in budding yeast. Here, we present basic methods sufficient for a beginner in this system to carry out most required manipulations for genetic analysis or molecular biology.

I. Introduction

The fission yeast *S. pombe* is a popular model eukaryote that has become particularly useful in studies of cell cycle and chromosome dynamics. *S. pombe* and *Sacch. cerevisiae* are separated by an estimated 1000 million years of evolution (11). The genomes share no obvious synteny, and *S. pombe* does not show evidence for genome duplications as does *Sacch. cerevisiae* (23, 24). Increasingly, investigators from mammalian cells are using *S. pombe* because their gene of interest may be present in only one copy. Its rising popularity in recent years, especially in labs studying mammalian cell biology, has led to the nickname "micro-mammal". With a haploid genome size of just 13.8 Mb, distributed amongst 4,824 ORFs (compared to over 5500 for *Sacch. cerevisiae*), *S. pombe* has the smallest sequenced eukaryotic genome (24). There are approximately 145 genes in *S. pombe* that have metazoan homologues but are not found in *Sacch. cerevisiae*, and a similar number *vice versa* (24).

Like budding yeast, fission yeast is genetically tractable, and lends itself to easy molecular manipulation. Most tools available in *Sacch. cerevisiae* are available in *S. pombe* versions that accommodate the distinct biology of the fission yeast, and similar genetic strategies are available for both systems (e.g. (8)). Plasmids containing *S. pombe*-specific replication origins and markers are strongly recommended and a large variety now exist (20). Genomics resources are still being developed, since its genome sequence was only completed in 2002. Commercial microarrays or oligonucleotide libraries are not widely available, although opportunities for academic collaborations exist (14, 18). A complete collection of deletion alleles is being constructed by a Korean consortium, and individual heterozygous deletion strains can be purchased (eng.bioneer.com/Spombe.html). Other community-wide resources have been developed, including libraries of temperatures sensitive mutants and insertion

mutants at FYSSION at the University of Sussex, UK (pombe.biols.susx.ac.uk), which are freely available for screening. A large expression project is underway at the Sanger Centre and the data are fully searchable (www.sanger.ac.uk/PostGenomics/S_pombe). There is also a community web site at the University of Southern California that provides protocols, lists of meetings, investigators, and plasmids, and other information (www.Pombe.net).

Approximately 43% of genes have at least one intron, and multiple introns are common. However, the introns are usually short, and most are less than 100 bp in length (24). They do not have the rigid splicing consensus observed in *Sacch. cerevisiae*. TATA boxes and other consensus sequences for transcription are more proximal to the ATG than observed in budding yeast (19).

Most protocols for *Sacch. cerevisiae* are broadly applicable to fission yeast with only minor changes. Recent collections of methods for *S. pombe* are recommended (1, 9, 10, 17). General handling of the organism, microbial culture, and overall purification of nucleic acids and proteins are similar to budding yeast. Generally, an investigator who has handled *E. coli* in the course of performing standard molecular biology manipulations will have no trouble working with yeast of either species.

Unless otherwise stated, all cultures are grown at 32°C and other steps are performed at room temperature.

II. GROWTH AND MEDIA

S. pombe cells are rod-shaped and grow by increasing length while maintaining a constant diameter. The length of a cell is a sensitive indicator of its position within the cell cycle. They divide by medial fission, producing two essentially identical daughter cells. The nuclear cell cycle is divided into distinct G1 (10%), S (10%), G2 (70%) and M (10%) phases. Following mitosis, the newly replicated nuclei enter the next cell cycle and undergo G1 and S phase prior to completion of the previous cycle's cytokinesis. This quirk means that a single cell almost always has a 2C DNA content: either as a G2 cell, or as a binucleate G1 or S phase cell. Fission yeast is generally grown in either YES, a rich yeast extract based medium, or EMM2 (often simply called EMM) a defined medium. It grows about 50% slower in EMM2 – a wild type strain doubles in about 2 hours in YES and 3 hours in EMM2. EMM2 is used mainly for selecting auxotrophic markers and for *nmt1* promoter expression (see below). The optimal media differ from those used for budding yeast, although *S. pombe* will grow on budding yeast media if necessary. Commercially available media mixes are also available from a number of companies including Qbiogene, Inc. (www.qbiogene.com) and United States Biological (www.usbio.net).

The *nmt1* promoter, which is the most commonly used promoter for heterologous gene expression (15, 16), is repressed by the addition of 15 μM thiamine (15μM or 5μg/ml; from filter sterilized stock at 10mg/ml) immediately prior to use. The levels of expression can also be titrated using intermediate amounts of thiamine (0.05μM or 0.016μg/ml; (12)). SD medium, used for budding yeast, contains thiamine and therefore it cannot be used for fission yeast when *nmt1* expression is desired. YE or YES also contain thiamine.

The concentration of yeast cells in a culture is often measured in OD units. One OD is the amount of cells required to give 1 ml of culture an optical density of 1 at 600 nm. Thus, a 5 ml culture at OD_{600}=2.0, a 10 ml culture at OD_{600}=1.0 and a 20 ml culture at OD_{600}=0.5 all contain 10 ODs of cells.

Media recipes are in Table 1 and stock solutions in Table 2. Mating media are described in Table 3.

Phloxin B

Phloxin B (Magdala Red; Sigma No. P 4030) is added to a final concentration of 5 mg/l from a stock of 10 g/l made up in water, filter sterilized, and kept covered at room temperature. If the plates start to dull or look brownish, they are past their usefulness.

This vital stain is commonly used to determine cell ploidy and temperature sensitivity of *S. pombe*. It colors solid media vivid pink and the colonies will range in color according to the health of the cells. However, because it is mildly toxic, it should only be used for testing and not for long-term storage. Cells on phloxin B media die rapidly if the plates are refrigerated. Wild type haploids form pale pink colonies on phloxin B. Diploid colonies are slightly darker. Dying cells form darker colonies or patches, and lethal mutants are often dark red. Phloxin B is also very useful for conditional or synthetic lethal screens that involve replica plating, because it makes the dead replica colonies readily apparent, greatly facilitating the screening. The colony color is not apparent at the single-cell level (any dead cells will stain vivid pink), but relies on macroscopic examination of colony color.

III. SYNCHRONY

Fission yeast can be cell-cycle synchronized by selection or arrest. Selection synchrony involves selecting a synchronous subpopulation of the smallest cells from an asynchronous culture. The preferred method is centrifugal elutriation, but it requires a specialized centrifuge. An alternative method selects the smallest cells from a lactose gradient. Both techniques benefit from the fact that the cells suffer relatively little physiological disruption. Arrest synchronization involve blocking cells at various points in the cell cycle using temperature sensitive cell cycle mutations or drugs. The standard blocks are: *cdc10* in G1, hydroxyurea in S, *cdc25* in G2 and *nuc2* in metaphase. For the *ts* mutations, cells are generally held at 35°C for four hours to allow all cells to reach the block. Since pombe is quite sick at 37°C and dead at 38°C, it is best not to use temperatures above 35°C if not absolutely necessary. The use of microtubule poisons is complicated in pombe by the fact that at least some of them seem to block cells in G2 (2). Pheromone synchronization of pombe has been reported, but requires a specific genetic background and simultaneous nitrogen starvation (21). *S. pombe* can also be synchronized in G1 by nitrogen starvation, though the release is not as synchronous as other methods. Progress through a synchronization experiment can be monitored in real time by assessing the septation index of the culture, counting the fraction of cells with a complete septum but no invagination. The maximum percentage of septated cells in the population determines the degree of synchronization.

Cell cycle block and release

The key to temperature arrest is rapid increase and decrease of temperature. All water baths should be pre-warmed to the correct temperature. The volume of culture should be low for the size of the flask, to ensure the most efficient heat exchange.

1. Grow cells to early/mid log phase (OD_{600} 0.2–1.0) at permissive temperature. Sample if necessary for an exponentially growing cell timepoint.
2. Shift to 35°C. Incubate 4 to 6 hours.
3. Sample for a t=0 timepoint. Release cells to 25°C by rapidly swirling the flask in ice water. Monitor with a thermometer that has been cleaned with ethanol. Place in 25°C water bath.
4. To follow synchrony: sample timepoints every 20 minutes for septation index (correlates with S phase). This should reach 60 to 80% for an effective temperature-driven block and release. Samples can be fixed for simple microscopy, or pellets can be harvested and frozen for RNA or protein analysis.

Selection synchronization

Because fission yeast cells are very regular in size, size selection to isolate newborn G2 cells is an effective means to synchronize a population. Centrifugal elutriation is a frequent method in fission yeast research, but requires an expensive and highly specialized centrifuge rotor, and working with more than one strain at a time is difficult. Lactose gradient centrifugation (e.g., 7) offers a convenient alternative that can be performed in most laboratories on multiple strains. Both these methods rely on separating cells based on cell size, so the strains must not show elongation or variation in cell morphology. Mutants that have heterogeneous phenotypes or cause elongation are much more difficult to synchronize using size selection methods.

Lactose gradient synchronization

1. Prepare a 45 ml 10%–40% lactose gradient using a commercially available gradient maker or by freezing 45 ml of 20% lactose in growth media for at least 4 hours at −80°C and thawing for 3 hours at 30°C. The thawing spontaneously creates an approximately 10% – 30% gradient.
2. Grow cell to mid-log phase, harvest 100 ODs, resuspend in 3 ml medium.
3. Layered onto gradient.
4. Spin at 500 × g for 8 min in a swinging bucket rotor.
5. Remove 2 ml fractions from the top of the gradient.
6. Pool those containing uniformly small cells, usually in the first five fractions.
7. Wash 1× in fresh medium to remove the lactose.

8. 5–10% of the starting cells should be recovered, and fewer than 1% of those should be septated. A second gradient (10mls) can be used for additional synchronization.

Flow Cytometry on Isolated Nuclei

Flow cytometry is commonly used to determine the cell cycle state of fission yeast and the synchrony of cultures. The common method fixes cells in 70% v/v ethanol, treats with RNase, and stains for analysis. Two major problems are often encountered. First, as cells arrest and elongate, they accumulate cytoplasmic background staining, which causes increased background signal. Second, due to the very short G1 in rapidly growing pombe, replication and cytokinesis are coincident. Therefore, 1C cells are not seen, complicating the explanation of cell cycle experiments. The protocol below, adapted from (5), solves both problems by using isolated nuclei, thus removing cytoplasmic background and releasing 1C nuclei from binucleate cells.

1. Pellet 1.0 OD cells, resuspend cells in 70% v/v ethanol to fix.
2. Wash cells in 1ml 0.6M KCl.
3. Resuspend cells in 1 ml 0.6M KCl, 1 mg/ml Novozym 234 (Sigma L1412), 0.3 mg/ml Zymolyase 20T, incubate cells 30 min at 37°C.
4. Pellet cells, resuspend in 1ml 0.1 M KCl 0.1% triton-X100, incubate 3min at room temperature.
5. Wash cells 1× with, and resuspend in 1 ml PBS (or other FACS sheath buffer).
6. Add 10 µl 20 mg/ml RNase A, incubate cells 2 hours at 37°C.
7. Sonicate with microtip at maximum power for 5 seconds. Sonication parameters will vary for different sonicators. Adjust power and time to disrupt cells and release nuclei without fragmenting nuclei.
8. Add 300 ml of disrupted cells to 300 ml of 2 µm Sytox Green in FACS sheath buffer.
9. Analyze by flow cytometry

IV. GENETICS

Fission yeast has two mating types: h^+ and h^-, which are alleles of the *mat1* locus (*mat1-M* for *h-minus*, *mat1-P* for *h-plus*). Fully wild type cells switch between h^+ and h^- mating types; these are called h^{90} (because 90% of cells can typically switch). In a culture of h^{90} cells, approximately half of the cells will be functionally h^+, and half functionally h^- because of switching. Laboratory investigators typically use strains with rearrangements of the silent mating loci to provide a stable mating type and prevent switching. These *heterothallic* strains will only mate if a partner of the opposite mating type is provided to them. Since the silent loci are closely linked to the expressed *mat1* locus, it is convenient (but not quite accurate) to think of mating type in *S. pombe* as having three alleles: h^{90}, h^+,

and h^-. The common h^- is stable, but most laboratory h^+ strains revert to h^{90} at a low frequency ($\leq 10^{-3}$/generation). For review, see (13).

Fission yeast is generally haploid even in the wild. Following conjugation, newly formed zygotes immediately enter meiosis and sporulate to produce four spores in a linear tetrad ascus. Fission yeast haploids must be starved for nitrogen in order to undergo mating. Once they mate, they normally proceed immediately through sporulation, generating a curved, zygotic ascus. Diploids can be recovered in the laboratory by interrupting this process, and can be maintained vegetatively. However, they are prone to sporulate and when they do, they form a linear, azygotic ascus that is easily distinguished from the zygotic ascus.

Crossing strains

Construction of new strains, or analysis of meiotic products requires that the parent strains cross efficiently, under conditions where the spores are viable. Successful mating can be determined microscopically by observing the formation of zygotes and asci.

1. Take a toothpick full of cells from Strain 1 and make a small patch on a mating plate (ca. 5 mm in diameter). Take an equivalent amount of Strain 2 and add to the previous patch, mix well.
2. Incubate at 25–29°C for 2–4 days. Monitor microscopically for formation of asci. Mating (and sporulation – are you sure?) are inherently temperature sensitive and should be carried out at 25°C (maximum of 29°C).

Assaying mating by iodine staining

As a quick way to determine if two strains mated, the mating patch can be stained with iodine vapors. The starch in the spore wall stains darkly with iodine, so a successful mating patch will stain dark purple; an unsuccessful mating patch will stain light yellow. To stain a plate, turn it upside down over a dish with a few crystals of iodine in a fume hood; the iodine vapors will stain the cells in about 10 minutes at room temperature. The process can be accelerated by warming the iodine on a hot plate in a fume hood, but one must be very careful not to boil off all of the iodine in a thick toxic purple cloud. This technique is usually used when testing a large number of strains for mating type. However, the iodine kills the cells, so it should not be used to assay successful matings for strain constructions.

Isolation of diploids

Although fission yeast diploids are unstable, they can be recovered by interrupting the mating process. The diploid strains must be selected by complementation of nutritional markers. However, after they are identified, they should be maintained on YES to suppress sporulation. Diploids are important for many assays and take only a little advance planning to be useful in the laboratory.

A particularly convenient pair of markers for diploid construction is provided by two complementing *ade6* alleles, *ade6-M210* and *ade6-M216*. When present in the same cell, these alleles complement intragenically, leading to an Ade^+ phenotype. This also provides a

color screen: the *ade6*$^{+}$ gene is the ortholog of budding yeast *ADE2*, and mutants cause the accumulation of a pink or red color in the cell in the presence of low adenine (see media). Ade^{+} strains are white. The two *ade6* alleles can be distinguished by their degree of color: *ade6-M210* is usually darker pink than *ade6-M216*. Neither is as dark as a null allele. However, their color can be affected by the strain background, so the marker is frequently mis-assigned. Assignment can be verified by attempting to make a diploid against a strain with a known *ade6* allele.

If the appropriate *ade6* markers are unavailable, a diploid can be constructed by complementation of any two auxotrophic markers. In this case, the investigator must use care to verify that the strain recovered is diploid, and not a recombinant haploid spore.

1. Follow the steps for mating using strains with complementing mating types and *ade6* markers. At 6 hours after mating, and again at 12 hours, pick a swatch of cells and streak to EMM medium lacking adenine (but containing any other required supplements). Incubate at 32°C (25°C for temperature sensitive diploids).
2. Once white colonies are visible, pick and streak several white colonies to YES + phloxin B. Incubate for 2–4 days at 32°C (25°C for temperature sensitive mutants). The cells in the middle of a colony on EMM may have sporulated; these will form pale pink haploid colonies on YES + phloxin B. The darker pink colonies are still diploids. It is useful to streak a known haploid on the YES + phloxin B plates to verify its color. In addition, diploids are longer and wider than haploids when viewed under the microscope.
3. Pick several independent colonies and streak to YES plates. Patch the same colony in a spot of about 5–10mm on mating plates (ME or SPAS) and incubate at 25°C for 1–2 days. Expose the mating plates to iodine vapor as described in mating type testing to verify clones are sporulation competent. Diploids are stable for 1–2 weeks at 4°C if maintained on YES. Prolonged maintenance selects for sporulation deficient mutants; therefore, diploids should be constructed freshly whenever possible.

Tetrad dissection vs Random Spore Analysis (RSA)

Both tetrad dissection and random spore analysis are used to construct new strains and for analysis of meiotic products. Because mating and meiosis are linked in *S. pombe*, it is generally not necessary to isolate a diploid prior to characterizing offspring. Instead, the haploid parents are crossed on mating plates and allowed to proceed all the way through sporulation. The only exception is generally when very efficient sporulation is required, or when one parent is homothallic (h^{90}), and would otherwise cross with itself.

The logic of tetrad dissection is exactly the same for *S. pombe* as for *Sacch. cerevisiae*, and the manipulations are similar. Random spore analysis (RSA) in fission yeast is much simpler than for budding yeast because vegetative cells are easily killed by the enzyme glusulase (snail gut enzyme), and the spores do not stick to one another, so they are readily dispersed.

RSA provides certain advantages because it allows the analysis of a large population of offspring with relatively little labor, and is very commonly used. On the other hand, tetrad dissection uniquely allows the analysis of the products of an individual meiosis, and the unambiguous identification of double mutants. *S. pombe* tetrads are generally somewhat linear in shape, and therefore ordered, but it is seldom possible to dissect the spores in such as way as to maintain the order.

For general strain construction, in which the desired double mutant can be unambiguously identified, RSA is the method of choice. It is particularly useful if the two mutations are linked, e.g., *leu1* and *his7*, because a large number of offspring can be analyzed to identify a rare event. RSA can be used for linkage analysis in genetic screens, since large numbers of crosses can be processed easily, and it is commonly used to determine recombination frequency in large mapping studies. The only caveat is that all the genotypes must be equally viable, and the desired recombinant must be unambiguously identified. In cases where there is some doubt about the genotype of a putative double mutant, the candidates can be backcrossed to each parent to verify linkage. Most common strain construction requirements are satisfied by RSA. When used in bulk liquid culture, RSA allows spore germination experiments to analyze large populations of marked, lethal alleles.

Tetrad analysis provides an enormous amount of information, even in ten tetrads (6). Linkage trends are usually visible in just a few tetrads (but meaningful numbers for weak linkage may take many more). Most importantly, tetrad analysis allows the unambiguous identification of double mutants and is essential if the phenotype of a double mutant is unknown or potentially lethal. The method is similar to that used for *Sacch. cerevisiae*, but easier, as the asci break down by themselves and digestion with glusulase is not required.

Wild-type fission yeast are efficient at germinating on YES plates. Germination is modestly reduced on EMM plates, so unless selection for specific markers is required, YES plates are preferred both for RSA and tetrad dissection.

Random Spore Analysis

1. Pick a toothpick full of cells from the mating/sporulation plate into 100 µl of sterile water. Add 10µl of 5% glusulase (PerkinElmer) and incubate overnight at room temperature.
2. Using a hemacytometer, count the number of spores in 10 µl of a 10:1 dilution in sterile water. Verify that no complete asci remain.
3. Plate 500 spores per plate, assuming all spores are viable. Because plasmids are quite unstable during meiosis, when selecting directly for spores containing a plasmid, plate 1000 to 10,000 spores per plate. The dilutions can be stored at 4°C for several days. Alternatively, spores in the glusulase suspension can be washed free of the enzyme and stored at 4°C in water for weeks in case further plating is required.
4. Colonies will appear in 2–5 days, depending on the temperature, and plates can be replica plated to assign markers.

V. MICROSCOPY

Fission yeast has proven a tractable model for cell biology, with its regular cell size and small chromosome complement. As with *Sacch. cerevisiae*, the limiting factor is ensuring access across the cell wall. Because the structure of the walls differ somewhat between the two species, somewhat different digestion methods are required for fission yeast, but apart from that similar methods can be employed. (3)

Ethanol fixation

Samples fixed in 70% v/v ethanol may be stored indefinitely at 4°C. This is particularly useful if FACS analysis or DAPI staining will also be performed as the fixation is identical. Ethanol fixation is particularly good for maintaining nuclear structure. Samples can be stained with DAPI to visualize the nucleus, with Calcofluor to visualize the septa, or processed for FACS analysis.

1. Harvest 1 OD unit of cells from an exponentially growing culture (see GROWTH AND MEDIA, section II.)
2. Resuspend pellet by vortexing tube while adding 1.0 ml cold 70% EtOH. Store at 4 °C.
3. Rehydrate 100 µl cells by adding 1 ml water. Pellet and resuspend the cells in 0.1 ml water.
4. Pipette 5 µl of cell suspension onto a cleaned microscope slide. Allow to air dry, or place on heating plate at low temperature until liquid is just evaporated. To ensure a monolayer of cells, pre-treat the slides with poly-L-lysine (Sigma P8920) or use a positively charged slide.
5. Add 5 µl of DAPI + Mount (with or without Calcofluor) and immediately cover with cover slip.
6. Examine by epifluorescence microscopy.

Mounting medium:

50 mg n-propyl gallate (Sigma P02370)

50 mg p-phenylenediamine (PPD; Sigma P1519)

Dissolve in 5mls PBS. Add glycerol to 50mls.

Aliquot and store in freezer in the dark.

Mount + DAPI

Dilute DAPI to 0.66 µg/ml in mounting medium

Store frozen in the dark. Discard if it turns brown.

DAPI stock: 1 mg/ml in DMSO, stored in small aliquots at −20°C

Mount + Calcofluor (with or without DAPI)

Dilute Calcofluor to 50 μg/ml in mounting medium. Adjust concentration to balance with DAPI signal. Use fresh.

Calcofluor (Sigma F-3397) stock: 1 mg/ml stock in 50 mM sodium citrate, 100mM sodium phosphate pH 6.0, stored in the dark at 4°C

Whole Cell Immunofluorescence

1. Grow cells 10 ml cells to mid-log phase.
2. Wash with PBS, resuspend in 1ml of PBS 0.2 mg/ml Zymolyase 20T.
3. Incubate until spheroplasts form, about10 minutes. Spheroplasts lose their refractivity and become dark under phase microscopy when treated with 0.1% SDS.
4. Wash cells 3 times in PBS.
5. Fix the cells in any of the following fixation solutions:
   a. 0.1 M potassium phosphate, pH 6.5, 10 % v/v methanol, 3.7 % w/v formaldehyde for 20 minutes,
   b. 70% v/v ethanol for 15 minutes at 4°C or
   c. ice cold methanol for 8 minutes in the freezer. Different antibodies and antigens respond differently to the types of fixative, so you may have to determine empirically.
6. Wash cells 3 times in PBS.
7. Resuspend cells in 200 μl of PBS and place onto a poly-lysine treated slide. Let sit for 30–60 minutes, then tip the slide to drain the excess.
8. Heat fix the slide (low heat on a heat block) for 2 minutes.
9. Place the slide in a humid chamber.
10. Block the slides with PBS 3% BSA by putting 100 microliters of the solution on the slide, cover with a cover slip for 60 minutes.
11. Tip the slide and let the cover slip and the excess solution run off.
12. Place 100 microliters of primary antibody diluted in PBS 3% BSA on the slide, cover with a cover slip. Antibody dilution and time of staining (usually 1 hour to overnight) will vary.
13. Wash the slides in PBS. Either pipet the solution onto the slide and drain off, or wash in a Coplin jar.
14. Incubate slides with secondary antibody dilution.
15. Wash slides as in step 11.
16. Let the slides air dry. Then add mounting medium containing DAPI.

17. Observe by epifluorescence microscopy.

VI. DNA TRANSFORMATION

Transformation of fission yeast is straight forward. We offer two chemical transformation protocols. The short protocol is fine for plasmid transformation; it yields around 10^3 colonies/μg DNA. The long protocol is used for transforming PCR products for homologous recombination and for transforming libraries, and can yield 10^4 colonies/μg. The most common problem for PCR-based transformation seems to be insufficient PCR product. Using at least 10 μg greatly improves yields. Higher transformation efficiencies can be obtained by electroporation. Efficiencies of between 10^5 to 10^6 have been reported, but more commonly we observe around 10^3 to 10^4 per microgram of DNA. Nonsense suppressor strains have been suggested to be osmotically unstable, possibly explaining the anecdotal evidence that *sup3-5* strains do not always electroporate as efficiently. However, there is usually not a problem in getting sufficient colonies if you are simply putting a plasmid into a strain. Whatever the method of transformation, for selection with kanamycin and other cytotoxic drugs, plate on nonselective plates for 18 hours to allow for resistance to develop, and then print onto selective plates.

Short protocol for fission yeast transformation

1. Resuspend a toothpick full of a fresh (less than a week old) colony in 150 μl 0.1M lithium acetate (LiOAc) pH 4.9.
2. Incubate at room temperature for 60 min.
3. Add ~1 μg DNA and mix.
4. Add 350 μl 50% PEG8000 and mix.
5. Incubate at room temperature for 60 min.
6. Wash 1× with water and plate on appropriate media.

Long protocol for fission yeast transformation (4)

1. Grow yeast in liquid culture to mid-exponential phase.
2. Pellet 20 ODs, resuspend yeast in 1ml water and transfer to 1.5 ml tube.
3. Wash in 1 ml LiOAc/TE (0.1M LiOAc in 10 mM Tris-Cl 1 mM EDTA pH 7.5)
4. Resuspend in 100 μl LiOAc/TE.
5. Add 10 μl DNA and 2 μl 10 mg/ml sonicated salmon sperm DNA as carrier.
6. Incubate 10 min.
7. Add 260 μl 40% polyethylene glycol (PEG) 8000 in LiOAc/TE, mix well.
8. Incubate 30 min.

9. Add 43 µl dimethylsulfoxide (DMSO), mix well.
10. Incubate 5 min at 42°C.
11. Wash 1× in water, plate on appropriate media.

Electroporation

1. Grow cells in liquid culture to mid exponential phase (OD_{600} 0.5–1.0) in minimal medium.
2. Harvest cells. Wash once in ice-cold water, once in ice-cold 1M sorbitol. It has been reported that 15 minute incubation of these cells in the presence of 25 mM dithiothreitol (DTT) increases electrocompetence (22).
3. Resuspend in ice-cold 1M sorbitol at a density of $1 – 5 \times 10^9$/ml.
4. Add 40 µl of cell suspension to chilled 1.5 ml tubes containing 100 ng DNA, incubate on ice for 5 minutes.
5. The electroporator is set as follows:

   1.5 kV, 200 ohms, 25µF (Biorad)

   1.5 kV, 132 ohms, 40 µF (Jensen/Flowgen).

   Use your manufacturer's suggested settings if in doubt.
6. Transfer cells and DNA to a pre-chilled cuvette and pulse.
7. Immediately add 0.9 ml of ice-cold 1M sorbitol and return the cell suspension to the 1.5 ml tube on ice while other electroporations are carried out.
8. Plate cell as soon as possible onto minimal selective medium. Do not to put sorbitol into the agar; it will retard growth. Transformants appear in 4 – 6 days at 32°C

VII. BIOCHEMISTRY AND MOLECULAR BIOLOGY

For the most part, any purification protocol can be adapted to fission yeast. In general, the only *S. pombe* specific part of these protocols is the lysis step. *S. pombe* can be lysed mechanically or enzymatically. For protein purification, mechanical lysing is usually prefer because lysing enzymes can be contaminated with proteases. We have included small-scale preps of protein, DNA and RNA that lyse the cells by vortexing with glass beads. These protocols can be scaled up using a bead-beater (BioSpec Products, www.biospec.com). For large scale lysis, mechanical grinding in liquid nitrogen (eg, with a Retsch RM 100 Mortar Grinder; http://www.retsch.com/) or micro-fluidizing (eg, with a Microfluidics M110-S; http://www.microfluidicscorp.com) are more efficient, but require expensive hardware. In addition several companies make kits for pombe protein and nucleic acid purification.

Small scale protein prep

1. Grow yeast in liquid culture to desired density; pellet 5–20 ODs. All subsequent steps should be carried out with cold solutions on ice.
2. Wash once in water or lysis buffer; resuspend pellet in 200 µl protein lysis buffer (or other appropriate buffer) in a 1.5 ml screwcap tube.
3. Add 0.5 mm glass beads (BioSpec Products, www.biospec.com) to lysis buffer meniscus.
4. Vortex aggressively at 4°C for 5 min using a multi-tube holder. The buffer should be yellow and most yeast appear broken by microscopy.
5. Puncture screwcap tube bottom with needle. The needle can by heated with a flame to facilitate puncturing.
6. Place the punctured tube in a fresh 1.5 ml tube.
7. Spin 10 seconds at maximum speed in a microcentrofuge to drain lysate into the fresh tube.
8. Spin 5 min at maximum speed in a microcentrofuge to pellet insoluble material.
9. Supernatant can be used as a clear cell lysate for subsequent biochemistry.

Protein Lysis Buffer

50 mM Tris pH 7.5

150 mM NaCl

5 mM EDTA

10% Glycerol

1 mM phenylmethylsulfonylfluoride (PMSF), added fresh

Small scale DNA prep

1. Grow a 5 ml culture to saturation.
2. Harvest cells and resuspend in 250 µl of DNA lysis buffer.
3. Transfer to 1.5 ml screw cap tube, add 250 µl of phenol/chloroform and 250 µl 0.5 mm glass beads, vortex for 1 minute.
4. Spin 5 min., move aqueous phase to new tube, add 250 µl TE.
5. Repeat phenol/chloroform extraction until interface is fairly clear.
6. Add 1 ml ethanol, spin for 5 minutes to pellet.
7. Wash pellet with 70% v/v ethanol.
8. Resuspend in 100 µl of 20 mg/ml RNase A in TE.

9. Expect yields of about 1 µg/OD.

Plasmids can by recovered from this prep by transforming the DNA into *E. coli*. Efficiency may be increased by using electroporation for bacterial transformation.

DNA Lysis Buffer

100 mM Tris pH 8.0

100 mM NaCl

1 mM EDTA pH 8.0

1% SDS

Small scale RNA prep

1. Grow yeast to desired density; pellet 20 ODs, resuspend in 250 µl of RNA lysis buffer.
2. Transfer to 1.5 ml screw cap tube, add 250 µl of phenol pH4.7 and 250 µl 0.5 mm glass beads, vortex for 1 minute.
3. Incubate 10 min at 65°C, spin for 5 min., transfer aqueous phase to new tube, add 250 µl TE.
4. Repeat acidic phenol extraction.
5. Extract with phenol/chloroform, pH 6.7.
6. Add 1 ml EtOH, spin for 5 minutes to pellet.
7. Wash pellet with 70% v/v EtOH.
8. Resuspend in appropriate buffer for subsequent use.
9. Expect total RNA yields of about 20 µg/OD.

RNA Lysis Buffer

50 mM NaOAc pH 4.2

10 mM EDTA

1% SDS

Colony PCR: Zymolyase Method

1. Touch a yeast colony with a plastic pipette tip. Wooden toothpicks should be avoided because they may interfere either release of DNA from yeast cells or PCR reaction itself.
2. Rinse the tip in 10 µl Zymolyase solution
3. Incubate for 10 min at 37°C.
4. Use 2 µl spheroplasted yeast cells for 50 µl PCR reaction.

Zymolyase Solution

2.5 mg/ml Zymolyase (20T)

1.2 M sorbitol

0.1 M Na phosphate, pH 7.4

Aliquots of Zymolyase Solution can be stored at −20°C for at least 6 months.

VII. SUMMARY

Fission yeast has proven to be an important and increasingly popular model for cell biology. Its relative ease of manipulation makes it an attractive system for anyone with basic molecular biology skills, and it can be a useful adjunct to labs working in tissue culture or other genetically less tractable species. With these basic protocols, any lab can begin to work with this versatile organism and join the thriving *S. pombe* community.

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Table 1

growth media for fission yeast

Rich medium – YES (YE + Supplements)

Per liter:	**Final concentration**:
5 g yeast extract	0.5% yeast extract
30 g glucose	3% glucose
225 mg Adenine	
225 mg L-histidine	
225 mg L-leucine	
225 mg Uracil	
225 mg L-lysine	

Solid medium is made by adding 2% (w/v) Difco Bacto Agar prior to autoclaving.

Minimal media EMM2 (Edinburgh Minimal Medium)

Per liter	**Final concentration**
3 g potassium hydrogen phthallate	14.7mM
2.2 g Na_2HPO_4	15.5 mM
5 g NH_4Cl	93.5 mM
20 g glucose	2% w/v
20 ml salt stock (see Table 2)	
1 ml vitamin stock (see Table 2)	
0.1 ml mineral stock (see Table 2)	

Autoclave. Solid medium is made by adding 2% w/v Difco Bacto Agar. Required supplements for auxotrophies (e.g., adenine, uracil) are added to a final concentration of 225 mg/l as required. These can be maintained as sterile stock solutions at 7.5 mg/ml in water (3.75 mg/ml for uracil). Low adenine media, which allows the development of a red color in Ade- strains, reduces the amount of adenine to 7.5 mg/l.

Table 2

Stock solutions made in water, filter sterilized, and stored at 4°C

50× salt stock (per liter)	
Per liter	**Final concentration**
52.5 g $MgCl_2$•$6H_2O$	0.26 M
0.735 g $CaCl_2$•$2H_2O$	4.99 mM
50 g KCl	0.67 M
2 g Na_2SO_4	14.l mM
1000× Vitamin stock (per liter)	
Per liter	**Final concentration**
1 g pantothenic acid	4.20 mM
10 g nicotinic acid	8l.2 mM
10 g inositol	55.5 mM
10 mg biotin	40.8 uM
10,000× Mineral stock (per liter)	
Per liter	**Final concentration**
5 g boric acid	80.9 mM
4 g $MnSO_4$	23.7 mM
4 g $ZnSO_4$•$7H_2O$	13.9 mM
2 g $FeCl_2$•$6H_2O$	7.40 mM
0.4 g molybdic acid	2.47 mM
1 g KI	6.02 mM
0.4 g $CuSO_4$•$5H_2O$	1.60 mM
10 g citric acid	47.6 mM

Table 3

Mating media

ME: Malt extract (per liter)

Per liter	Final concentration
30 g Bacto-malt extract	3% (w/v)

Adjust to pH 5.5 with NaOH. Supplements added as for YES (except for lysine). Solid media is made by adding 2% w/v Difco Bacto Agar. ME is the most common mating media, but may show batch-to-batch variation. More defined media can be used instead, such as SPAS, EMM-N, or EMM with 1 g/l L-glutamate instead of NH_4Cl.

SPAS (per liter)

Per liter	Final concentration
10 g glucose	1% (w/v)
1 g KH_2PO_4	7.3 mM
1 ml 1000× vitamin stock	

supplements: 45 mg/l adenine, histidine, leucine, uracil and lysine hydrochloride (1/5 normal). Solid media is made by adding 3% (w/v) Difco Bacto Agar