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Duplication and transformation of the *Schizosaccharomyces pombe* collection of deletion strains

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Abstract

We present an efficient and organized method of lithium acetate and polyethylene glycol-based transformation of plasmid DNA into the commercially available collection of *Schizosaccharomyces pombe* with single gene deletions. We also describe how to prepare a duplicate collection of the deletion strains in order to preserve the longevity of the master set. These protocols are adapted to the 96-well format of the 3,004 strains of the Version 2.0 Bioneer set but can also be used for later releases of the collection. This transformation method typically yields efficiencies in the range between 1.0×10^3 and 1.0×10^4 transformants per microgram of plasmid DNA. However some deletion strains transformed with significantly lower efficiencies. We provide a list of these difficult to transform strains. Applications for this methodology include the transformation of the deletion set with plasmids necessary for genetic screens.

Keywords

Tf1; *Schizosaccharomyces pombe*; fission yeast; lithium acetate; polyethylene glycol; transformation; commercial deletion library

2. Introduction

Unicellular organisms such as the budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe* have become important genetic models for molecular biology research, and as such have gained enormous popularity as tools for genomic and proteomic research. The whole genome sequence and the genome database have greatly accelerated genetic studies of *S. pombe* [1, 2]. The availability of the genome sequence provided the information necessary to generate a collection of deletion strains in which each gene was replaced with *kanMX4* [3]. A particularly valuable resource that resulted from the deletion project was a collection of haploid strains each lacking a single non-essential gene. The haploid collection of deletion strains is commercially available (Bioneer Corporation) and regularly used to evaluate the function of specific genes.

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The collection of haploid deletions is a valuable tool for genetic screens designed to identify lists of genes that contribute to specific processes. Often these screens require reporter or expression plasmids be introduced into each strain. We recently completed a screen of the Version 2.0 haploid set of deletions to identify genes that contribute to retrotransposition of Tfl1 [4]. For this screen we introduced a Tfl1 expression plasmid (Fig. 1) into each strain using a transformation protocol adapted to the 96 well format used for the distribution of the deletion strains. Our protocol affords sterile, efficient, and simple transformation of 96 strains at once. We also describe below methods for producing duplicate copies of the deletion collection. We recommend that labs copy the deletion collection to avoid the reduced viability that results from repeated freeze-thaw cycles of the original master stocks.

3. Materials

Elements of these methods related to the transformation of individual strains were also reported in an accompanying manuscript that describes single strain transformation {Rai, In Press, Ref to be included when available}.

3.1 Instruments

Centrifuges for cultures (5 to 250 ml) and microfuge tubes (0.5 to 2.0 ml); a centrifuge to spin deep-well 96-well plates; culture tube roller; spectrophotometer; microtube incubator; a programmable water bath or heat block; sterile glass beads (3 mm, Sigma cat# Z143928-1EA) for spreading cells. Library replication tools from V&P Scientific including: 96-pin long-pin multi-blot replicator, spring-loaded for frozen specimens (cat #VP 408FS2AS-1); library copier guide pin plates for shallow (cat # VP 381A) and deep-well (cat # VP 381B) microtiter dishes; pin cleaning brush (cat# VP 425); pin cleaning solution (cat# VP 110).

3.2 Fission Yeast Media

1. YES (yeast extract plus supplements) (per liter): 5 g yeast extract (Difco), 30 g glucose and 2 g complete dropout amino acid mix (see 3.3.1 below, and Note 1).
2. Rich media containing Geneticin (YES+G418): To YES medium, add 500 ug/mL G418 (#11811-031, Life Technologies, Rockville, MD). Most commercially available Geneticin (G418) has batch details from the manufacturer that indicate purity level, usually between 60 and 90%. Be sure to correct for purity when weighing for stock solutions, in order to produce accurate final concentrations.
3. PMG (pombe glutamate medium) (per liter): 3 g potassium hydrogen phthalate [14.7 mM]; 2.2 g Na₂PO₄ [15.5 mM]; 3.75 g L-glutamic acid; monosodium salt (#G-5889, Sigma-Aldrich); 20 g glucose (2% w/v); 20 ml of 50x salt stock (see 3.3.2 below); 1 ml of 1000x vitamin stock (see 3.3.3 below); 0.1 ml of 10,000X mineral stock (see 3.3.4 below); 2 g of amino acid dropout mix lacking uracil and leucine (see Note 1).
4. PMG supplemented with leucine and vitamin B1 (PMG-U+L+B1): To PMG medium add (to a final concentration) 250 µg/ml L-Leucine, and 10 µM vitamin B1 (see Note 2).

3.3 Stock Solutions (Table 1)

1. Complete amino acid dropout powder: 5 g adenine sulfate and 2 g each of the following amino acids: alanine, arginine HCl, aspartic acid, asparagine H₂O, cysteine HCl.H₂O, glutamic acid, glutamine, glycine, histidine HCl.H₂O, isoleucine, leucine, lysine HCl, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, uracil, and valine. Mix thoroughly (*see Note 3*). When preparing amino acid drop out mix to be added to PMG media, exclude whatever amino acid, adenine, or uracil your experimental design dictates as these will be produced by selectable markers on the plasmid (*see Note 1*).
2. 50x salt stock (per liter): 2 g Na₂SO₄ [14.1 mM], 50 g KCl [0.67 M], 0.735 g CaCl₂.2H₂O [4.99 mM], and 52.5 g MgCl₂.6H₂O [0.26 M]. Dissolve in deionized water and autoclave. May be stored at room temperature.
3. 1000x vitamin stock (per liter): 1 g pantothenic acid [4.20 mM], 10 g nicotinic acid [81.2 mM], 10 g inositol [55.5 mM], and 10 mg biotin [40.8 μM]. Dissolve in deionized water and filter sterilize.
4. 10,000x mineral stock (per liter): 5 g boric acid [80.9 mM], 4 g MnSO₄ [23.7 mM], 4 g ZnSO₄.7H₂O [13.9 mM], 2 g FeCl₂.6H₂O [7.40 mM], 0.4 g molybdic acid [2.47 mM], 1 g KI [6.02 mM], 0.4 g CuSO₄.5H₂O [1.60 mM], and 10 g citric acid [47.6 mM]. Dissolve in deionized water and filter sterilize. May be aliquoted into 50 mL portions. Wrap tubes in foil to prevent damage from light.

3.4 Preparation of equilibrated Phenol:Chloroform:Isoamylalcohol solution

UltraPure™ phenol:chloroform:isoamyl alcohol (25:24:1, v/v) is commercially available (ThermoFisher #15593049). If you make your own phenol:chloroform:isoamyl solution equilibrate it by adding an equal volume of 0.1 M Tris-HCl (pH 8.0); stir the mixture on a magnetic stirrer for 15 minutes, and then let sit to allow layers to separate. May be stored in a dark bottle for several months at 4°C. The phenolic layer is on the bottom. (See Note 4).

3.5 Preparation of Carrier DNA

1. Large quantities of DNA are available from various sources; we used Sigma Aldrich (D6898) herring sperm DNA. Dissolve 1 g DNA in 100 mL of 1x TE buffer overnight at 4 °C with slow constant stirring. (See Note 5).
2. Treat with Proteinase K (New England Biolabs #P8107S) [final concentration of 10 ug/mL] for 2 hours at 37 °C.
3. Precipitate DNA by adding 0.6 volumes isopropanol at room temperature and mix well.
4. Resuspend DNA in 50 mL TE buffer and extract with the 50 mL phenol:chloroform:isoamyl solution. Precipitate aqueous phase with 0.6 volumes isopropanol.

5. Wash pellet with 25 mL 70% cold ethanol to remove traces of phenol and isopropanol. Remove excess ethanol and let dry for a few hours. Resuspend DNA in 50 mL TE buffer, determine concentration (1.0 OD at 260 nm is 50 mg/mL), and adjust to 10 mg/mL.
6. Place tube containing herring sperm DNA in beaker with ice and shear by sonication using a high power setting and cycles of 30 seconds on and 30 seconds off to prevent DNA from getting hot. After 6 minutes of sonication time determine the size of fragments using a 0.7% TBE agarose gel. The desired size range is approximately 2.0 kb to 6.0 kb.
7. When the desired size range is achieved aliquot DNA into 1 mL eppendorf tubes and store in freezer. Before using for transformation heat tube at 95°C for 5 minutes. Once denatured there is no need to re-boil for subsequent use.

3.6 Preparation of 100x Tris (per liter)

Dissolve 121.1g Tris base and 37.2 g Na₂ EDTA (Ethylenediamine tetra acetic acid disodium salt dihydrate) in 800ml distilled water; adjust pH to 7.6 with 2N Hydrochloric acid before bringing volume to a final 1 liter. Aliquot into 100 mL bottles, and autoclave to sterilize. (See **Note 6**).

3.7 Preparation of Lithium Acetate (per liter)

Dissolve 102 g of LiOAc (dihydrate, Sigma L-6883) in deionized water and autoclave sterilize. (See **Note 6**).

3.8 Preparation of 44 % Polyethylene Glycol-3350

Dissolve 440 g PEG 3350 (polyethylene glycol, Sigma P-4338) in deionized water and bring volume up to 1 liter. Filter sterilize and store in 50 ml tubes. (See **Notes 6 and 7**).

3.9 Aliquot preparation of experimental transformation plasmid with carrier DNA

Because we have adapted this transformation protocol for a 96-well format, we pre-mix standardized quantities of the experimental plasmid and carrier (herring sperm) DNA. We combine 500 uL of the plasmid (1 ug/uL) with 500 uL of herring sperm carrier DNA (preparation described above) in sterile microfuge tubes for storage at -20°C until use in transformation. Each of the 96 strains per plate will receive 10 uL of this mixture.

3.10 Plasmids and strains

1. This method was developed for high-throughput transformation of a single plasmid into the collection of deletion strains. We transformed a wild-type version of a Tfl expression plasmid pHL2282 (Fig. 1) into each deletion strain of the nonessential, haploid deletion library Version 2.0 (Bioneer, M2030H). The construction of pHL2282 has been described previously [4].

4. Methods

Part A: Replication and amplification of the commercial deletion library

1. Since the Bioneer deletion library is commercially distributed in a low-volume, shallow-well, 96-well format, we found it important, in order to protect both the integrity and the viability of the 3000+ library strains, to make duplicate copies of the library. This allowed us to increase the volume of the frozen glycerol stock for each strain. We strongly recommend storing frozen glycerol stock libraries in deep-well (2 mL) 96-well format microtiter plates.

2. To replicate and amplify the Bioneer deletion set (provided in 32 standard-depth, 96-well microtiter plates), we began with a light-spin (2200 x g; 2 minutes) using the microtiter plate adaptors for the JS 5.3 rotor in a Beckman J26-SI centrifuge. This pulls condensation droplets from the film-lid of each plate, and thereby avoids cross-contamination from well-to-well when the lids are removed (Note - Because of the low volume (50-100 uL) of strains, no initial “thaw” time is required. However, to maintain the viability of strains, take caution to keep plates on ice, and use pre-cooled centrifuges.

3. Following this short, low speed centrifugation, the sealing lid (either plastic or foil) should be removed most carefully. This is best done by using one hand to very slowly peel back the seal-lid from whichever corner seems most accessible; the other hand should be used to apply firm pressure onto the plate, to hold it securely on the (level) bench surface, so that the plate does not slip, or flip, and the small volume of each strain is not unintentionally aspirated causing cross-contamination of other wells in the plate. (See **Note 8**). Upon opening, the user will note that each plate in the deletion set has a characteristic pattern of empty wells that serve to distinguish each plate from every other, as well as to orient the plate, identifying top, bottom, right and left sides. (See **Note 9**).

4. The lightly spun and sterilely opened plate is now ready for “pinning” material from the master plate to non-selective ‘revival’ plates for amplification and replication. This is best accomplished using the spring-loaded 96-pin long-pin, multi-blot replicator, specially designed for transfer of frozen specimens (VP 408FS2AS-1) along with the library copier “guide pin plate” (also from V&P; cat #381A), designed for shallow-well microtiter dishes. Prior to use from the manufacturer, and between uses, the 96-pin replicator should be carefully cleaned and sterilized. (See **Note 10**). The frozen specimen pin tool has “guide pins” that align with specific holes in the guide pin plate, which helps to direct and align each of the 96-pins directly into each of the corresponding 96-wells of the library plate, so a guide pin plate should be used to stabilize both the 96-well master plate and the single-well revival plate. The freshly sterilized pin tool is directed by the guide plate into the 96-wells of the master plate. The 96 pins are uniformly and lightly depressed into all of the frozen strains across the plate, and then the pin-tool, now carrying a small amount of cell mass on each pin from each well, is lightly stamped onto a single-well rectangular plate (Thermo Scientific, cat# 267060) containing solid, non-selective (YES) media. These single-well plates should also be stably positioned within a duplicate 381-A guide pin plate, so that the spatial position and orientation of the 96 pinned strains is maintained from its original 96-well plate format onto the single-well plate format. Only a very light “touch” of the pins to the plate should be used, in order to transfer sufficient cellular material from the pins to the

plate. The pins should not break the agar surface. The pin tool should be very carefully re-cleaned before use with the next plate, (see **Note 10**).

5. Once cells have been “pinned” (or transferred) from master plate to revival plate, the original master plate should be carefully re-covered with a fresh aluminum sealing-film lid (E&K Scientific, cat. #T596600), by using a gloved finger to apply firm pressure across the complete surface of the lid. (See **Notes 11 and 12**). The rectangular, single-well revival plates (recommended above) come sterilely wrapped, with their own lids, which should be used to maintain sterility during the revival incubation.

6. “Pinned” single-well YES “revival” plates should be grown 3–5 days at 32°C, and strains failing to revive should be recorded. Across the library, we experienced that 10–15% of the strains failed to revive at first attempt. (See **Note 13**). When strains did not revive in the 96-pin format, we went back to revive them “manually” using pipet tips or sterile toothpicks to transfer larger amounts of cellular material from the bottom of the wells, directly streaking them onto YES plates.

7. Once revived on the single well rectangular plate, strains are then “re-pinned” from the non-selective revival plate, (again using the spring-loaded, long-pin “pinner” [VP 408FS2AS-1]) into sterile, “deep-well” 96-well plates [E&K Scientific, 2.2 mL, cat # EK-2440-S; See **Note 14**], in which each well contains 700 uL of YES G418 media. (See **Note 15**). These plates should also be covered during this grow-out phase, and we found that Nunc lids (cat. #264623) possess several features (condensation rings over each well; an evaporation barrier around the perimeter of the plate; long-edge corner cut outs that fit lids snugly over the recommended E&K deep-well plates) which make them favorable for this application. Revived and pinned cells are then grown with lids, without shaking, at 32°C for another 3–5 days, and as before, strains failing to grow are carefully recorded, and once noted, can be “back-filled” at a later time. (See **Note 16**).

8. Following the grow-out phase on selective media (YES G418), we used a multi-channel pipettor (Eppendorf Research Pro 1200, 8-channel) to sterilely add an equal volume (700 uL) of 50% glycerol to each well, pipetting up and down several times for adequate mixing of the cells and glycerol before covering with a sterile foil film-lid (E&K Scientific, cat# T596600), and carefully labeling each deep-well plate. A quick freeze on a bed of dry-ice is recommended before long-term storage at –80°C.

Part B: Preparation of competent *S. pombe* Strains from Amplified Library Plates

Figure 2 provides a checklist of steps in the following transformation protocol.

1. To revive strains from the amplified, deep-well library dishes, these plates are first thawed 25 minutes on a bed of dry ice and spun lightly.
2. A sterile (see **Note 10**) 96-pin long-pin (spring-loaded) multi-blot replicator (V&P Scientific; cat #VP 408FS2AS) is depressed directly into all 96 strains with a single action. Strains are then pinned onto sterile YES agar in shallow, single-well plates and grown three days at 32°C. (See **Note 17**). As discussed in the library replication section, the accessory

guide pin plates should be used (#VP381B for the deep-well replicated library master plate, and #VP 381A for the shallow, single-well solid YES plate) to stabilize the plates, and maintain the spatial alignment of the 96-pins during the pinning process. Figure 3 is an example of revived strains that also illustrates the system of empty wells that identifies the plate number, orientation, and version of the library. Strains with integrated selectable drug markers revive with much higher efficiency if first grown on non-selective, rich solid media (such as YES); they can later be replica printed onto selective media to confirm their phenotypic markers.

3. Following “pinned” revival of 96 strains onto a single-well YES plate, use a toothpick to collect as much of each “pinned” cell patch as possible, and resuspend each into 1 mL of YES media in a microfuge tube. Measure and record the OD (600nm) of each 1 mL resuspension. Calculate the volume of each resuspension needed to make a new 5 mL (YES) culture at a starting OD of 0.01. We use a 14-mL culture tube and prefer Falcon “snap cap” tubes, ref 3522059).

4. Grow inoculates in shaking incubator at 32°C, 180 rpm until they reach OD 0.3 –0.5 at 600 nm. (See **Note 18**).

5. Harvest cultures with a 10-minute centrifugation of ~1700 x g (3000 rpm Beckman, Model J6-MI, JS 4.2 swinging bucket rotor).

6. Discard supernatant, and resuspend cell pellets in half the original culture volume using 1x TE with 0.1 M LiOAc. Spin at 1700 x g, for five minutes. To remove all traces of growth media this step is repeated two times more.

7. Following the third (final) wash and centrifugation, discard the supernatant and resuspend each cell pellet in 0.2 ml of 1x TE 0.1M LiOAc.

8. Transfer each sample to a sterile 1.5 mL microfuge tube and incubate all 96 tubes on a roller drum for 60-minutes at 32°C.

Part C: Transformation of competent *S. pombe* Cells*

* Once the strains have been made competent, and transferred from the 96-well plates to 1.5 mL microfuge tubes, the transformation method is very similar to the protocol outlined in the accompanying manuscript which describes single strain Lithium Acetate transformation {Rai, add ref once details are available}.

8. Divide each 0.2 mL sample evenly between 2 sterile 1.5 mL microfuge tubes. This provides duplicate 0.1 mL samples for each strain to be transformed; one for experimental transformation reaction and the other for a parallel “No DNA” negative control. For each strain add 10 uL of the plasmid carrier DNA mixture to the tube to be transformed and 5 uL of carrier DNA to the no DNA tube. (See **Note 19**).

9. On a roller drum at 32°C, incubate all 192 “transformation” reactions (both experimental and negative control reactions for each strain) for 30 minutes.

10. Following incubation, 0.7 ml of PEG solution (40% PEG 3,350 in 1x TE with 0.1M LiOAc) should be added to all reactions (experimental as well as parallel negative controls). Follow with thorough mixing (either by end-over-end inversion or gentle vortexing).

11. Careful mixing of samples should be followed by another 32°C incubation, this time for 60 minutes and agitation on the roller drum is no longer necessary at this step.

12. All samples should next be subjected to a 5-minute heat shock (42°C). (See **Note 20**).

13. Cells should then be pelleted with a 2-minute centrifugation at 1,000 x g (3,000 rpm, Model Eppendorf 5430, Rotor: FA-45–30-11). The final cell pellet is then resuspended with vigorous hand-pipetting or vortexing in 200µl of YES media.

14. Once resuspended, cells are spread on selective medium. (See **Note 21**). If transformed cells are auxotrophic and the appropriate selectable gene marker is present in the transformed DNA, the growth medium can lack the appropriate constituent. If an antibiotic resistance marker was engineered on the transformation plasmid, growth media supplemented with that antibiotic can be used. (See **Note 22**). Instead of sterile, disposable spreaders we recommend using 3mm glass beads for cell spreading (See **Note 23**). We add ~20 sterile glass beads to each plate, replace the lid, and shake the plate about 30 seconds from side-to-side by hand, to disburse the cell suspensions evenly across the surface area of the plate.

15. Well-spread transformation plates should be wrapped with parafilm, and incubated agar side up, at 32 °C for up to 5 days. Transformation frequency is calculated by counting colony numbers on the selective media relative to the microgram amount of input DNA. To assess contamination examine all negative control plates (no DNA) for colonies. (See **Note 24**, and Fig. 4).

16. Although most strains produced substantial number of transformed colonies, the efficiencies of transformation did vary (Fig. 4). In addition, some strains had very poor transformation efficiencies (Table 2).

17. A trouble shooting guide that addresses poor transformation efficiencies is provided in our accompanying chapter {Rai, add ref once details are available}.

6. Notes

1. Dropout mixture contains equal weights of all amino acids and uracil, while adenine is present in 2.5 fold excess relative to the amount of the other components [5]. For YES medium, we use the complete drop out mixture. To select for cells that take up the transforming DNA, exclude from the drop out mixture the component corresponding to the marker gene and auxotroph that is used to make the PMG (or EMM) media.

2. To select for cells that have taken in the transformed expression plasmid marked with the URA3 gene marker, Uracil must be excluded from the selection media.

3. Mixing of all components is best accomplished with a milling jar and either steel balls or ceramic pellets.
4. When handling phenol, always employ protective measures such as lab coat, gloves and eye protection.
5. To ensure that DNA has been adequately resuspended after overnight stirring, use careful visual inspection. A viscous suspension, lacking fibers visible to the naked eye indicates adequate resuspension.
6. Since accidental contamination of large-volume solutions is a common problem with transformations, we have found that it works best to aliquot such stocks (100 mL bottles for 100X TE and 50 mL screw cap disposable tubes for the PEG solution) to protect sterility.
7. By storing 45 mL aliquots in 50 mL disposable tubes the PEG transformation solution can easily be made by the addition of 5 mL 1M LiOAc and 0.5 mL 100 X TE.
8. This should be done sterilely near a flame to avoid environmental contamination.
9. For instance, well A1 is empty in plate 1; A12 is empty in plate 12; and B1 is empty in plate 13; etc. Wells H2, H3 and H12 are empty in all but the last plate of the library, to indicate that the plates are from Version 2.0, as well as to orient the directionality of the plate. Newer versions of the library are labeled with writing on the side of the plates. This is also explained in the legend for Fig. 3.
10. To clean, the “frozen specimen pin tool” should first be immersed (make 3–5 repeated “dips” to the top of the pin but not up to the top plate) into a 10% bleaching solution, followed by an immersion wash in 100% ethyl alcohol, followed by a final immersion in 70% ethyl alcohol. Each successive immersion should consist of 3–5 repeated, 1-second “dips” nearly to the tops of the pins but not as high as the top plate. Each solution used in the cleaning progression should be deeper (higher volume level) than the preceding solution. Following the final immersion, the excess ethanol can be “burned off” by holding over the flame from a Bunsen burner for a brief fraction of a second; too much burning can eventually corrode the steel pins. Allow the pins to cool down to room temperature (only another several seconds) before use with cells.
11. These foil “lids” have a sticky backing that must be removed, and the edges of the unbacked foil lids are very sharp, so caution should be used to avoid cutting one’s fingers.
12. The E&K Foil lids are recommended because they maintain adherence to the master plate even at -80°C , and they remain pliable enough at -80°C to unseal while plates are still frozen.)
13. An initial failure to revive on non-selective, rich YES media required secondary, tertiary, and sometimes quaternary rounds of attempts for some “straggling” strains. Careful record keeping is critical at all stages of the project. The commercial Bioneer libraries are formatted with unique patterns of empty wells to facilitate identification of specific plates of the library (See Note 9). To maintain the integrity of the library, it is essential that, for example, the

strain in plate1, position B4 is replicated into the same position (plate 1, position B4) in the replicated library plates.

14. A taller, “footed” guide pin plate, VP 381A, is also available from V&P, and should be used to facilitate the “guidance” of the long pins into the desired and corresponding 2.2 mL wells.

15. In our case, since the Bioneer library was made by substituting each non-essential gene with a Kan cassette, we used YES G418 for selection.

16. Depending on efficiency rates of revival growth on any one plate (and personal organizational preferences) back-filling of non-revived strains can be accomplished a couple of ways. To maintain positional integrity (for instance, the strain in plate 1, position A5, is correctly replicated into position A5 in the deep-well replicated plate 1), we found it simplest to always use the same alpha-numeric position for each replicated strain. As long as accurate records are maintained, strains from Plate 1, position A5; Plate 2, position E4; and Plate 3, position G9 could all be revived in the same, single deep-well dish. Alternatively, a file like an excel table could be used to create a new plate map for secondary, tertiary, quaternary revival rounds (eg. the strain originally housed in plate1, position A5 could be “picked” into position A1 on the secondary revival dish, as long as it gets pipetted back into position A5 of replica plate 1 following the final grow-out and mixing with glycerol. Once a strain has been back-filled into an already frozen plate, the plate should also receive a second “quick freeze” on a bed of dry ice.

17. As with the library replication process, some strains fail to revive from the initial “pin” onto solid YES. Care must be taken at this experimental step to record which strains are slow to revive, or do not grow at all from the initial revival step, before proceeding steps are taken to make them competent and assess their transformation efficiency.

18. Over-night inoculants for making library strains competent to transform should not grow to an OD600 higher than 0.5. For *S. pombe*, with a doubling time of roughly 3 hours, a culture of starting OD 0.01 usually reaches OD 0.5 after an overnight growth at 32°C, or 12–16 hours of growth.)

19. To test and validate the sterility of technique, always manipulate the “NO DNA” negative control reaction tube after the experimental (plus DNA) reaction tube, for each pair of reactions.

20. We suggest use of a heat-block for this step because it results in less contamination than a water bath. Particularly when manipulating a high number of tubes for 96-well format transformation, sterility is of critical importance.

21. Since the calculation of transformation efficiency requires good plating efficiency and the presence of well-spaced single colonies, we spread two plates for each transformation reaction: 180 microliters onto one plate and the remaining 20 microliters onto a second plate.

22. It has been our experience that for these applications PMG media is superior, but the more commonly used *S. pombe* EMM media can also be used if preferred.

23. Glass beads (3 mm, Sigma #Z143928–1EA) can be washed and re-used, so after use they should be stored in 70% ethanol until they can be cleaned. They should be acid washed before re-use, as well as before use from the manufacturer. We wash beads in a glass beaker by covering with sufficient volume of concentrated sulfuric or nitric acid, and soak 30 minutes to overnight. (Remember to wear gloves, lab coat, goggles and face-mask when decanting acid.) Following soaking, carefully decant the acid into an appropriate waste container; we do this by pouring the beads into a large Buchner funnel on a 3-legged stand, over the waste bin. Rinse the beads with purified water, periodically measuring the pH of the run-off, until the run-off pH is close to that of water. Drain beads and autoclave portions in foil-covered 100 mL glass bottles. Keeping the plastic lids separate (as they will melt), heat the bottles in a hot drying oven for one or two days. Beads must be completely dry, but cooled to room temperature before use.

24. If low numbers of transformants are observed for any single strain, the transforming DNA should be assessed for concentration, purity and genetic selection. The negative (no DNA) control is critical to test for contamination at many steps, including in the transformation solutions, the carrier DNA, the cultures, as well as to test generally for good sterile handling technique. This is especially important when transforming large libraries such as a deletion set with manual manipulation of as many as 192 tubes.

8. References

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Figure 1. pHL2882, the high copy expression plasmid of Tf1-*NatAI* that we transformed into the deletion set. Tf1, whose expression is under control of the inducible *nmt1* promoter, is expressed from a multi-copy plasmid that is also marked with the selectable marker *URA3*. Some of the single cutting restriction sites are shown.

Figure 2.

Graphic guide for the transformation procedure. This is a tool that can be copied and used for each transformation to track your progress in the protocol.

Figure 3.

Example (Plate 11, Bioneer V2.0) of 96 pinned and revived deletion strains on rich agar media. Frozen cells were transferred with the sterile long-pin multi-blot replicator onto a single-well YES solid media plate, and grown 4 days at 32°C. Four positions from the master plate (in this instance, Plate 11) are left empty. The yellow circle highlights position A11, indicating that this is Plate 11 (of 32). Two orange circles highlight positions H2 and H3, identifying this as a plate from Version 2.0 of the Bioneer deletion set. The green circle in position H12 is left empty in plates 1-31 of the Version 2.0 deletion set to establish directionality (top/bottom/left/right) of each plate. Note also the comparatively low growth rates of some deletion strains such as those in positions C3 and D10.

Figure 4.

Relative transformation efficiencies of pHL2882 in four deletion strains from the Bioneer Version 2.0 set using the 96-well format, lithium acetate transformation protocol. Four representative deletion strains (*Rec25*, *Trm5* , *Vp1302* and *Mus7*) are shown following transformation with a 5 day, 32°C grow-out on PMG-ura+leu+B1. Both the experimental plate (transformed with plasmid pHL2882) and the matched negative control (no DNA) are shown in parallel. Strains with relatively high (*Rec25*) and low (*Mus7*) transformation efficiency are shown to illustrate the range present in the deletion collection.

Table1:

Transformation Solutions

Solution	Stock Concentration	Working concentration
(A). Tris-EDTA with Lithium Acetate		
1. Tris-base ethylenediamine tetra acetic acid (dissodium salt dihydrate)	100X (1M Tris-HCl, pH 8.0) (100 mM EDTA)	1X (10 mM Tris-HCl, pH 8.0) (1 mM EDTA)
2. Lithium Acetate	1M	100 mM
(B). Polyethylene Glycol with Lithium Acetate and Tris-EDTA		
1. Poly-ethylene Glycol (PEG) 3,350	44%	40%
2. Lithium Acetate	1M	100 mM
3. Tris-EDTA	100X	1X

Table 2:

Strains of the Deletion library Version 2.0 that transformed with very low efficiency {Rai, add ref once details are available}.

Bioneer M-2030H Position	Gene ID	Gene name	Gene description
V2-01-A08	SPAC13G7.11		mitochondrial inner membrane protein
V2-03-D02	SPCC1682.01	qcr9	ubiquinol-cytochrome-c reductase complex subunit 9 (predicted)
V2-03-G05	SPAC22G7.06c	ura1	carbamoyl-phosphate synthase (glutamine hydrolyzing), aspartate carbamoyltransferase Ura1 (This was due to the –ura selection)
V2-04-B02	SPBP4H10.03	oxa102	mitochondrial inner membrane translocase Oxa102
V2-04-E05	SPCC965.10		transcription factor (predicted)
V2-05-G12	SPAC27D7.14c	tpr1	RNA polymerase II associated Paf1 complex subunit Tpr1
V2-07-A08	SPBC19G7.04		HMG box protein
V2-09-D05	SPCC970.10c	brl2	ubiquitin-protein ligase E3 Brl2
V2-09-D10	SPAC23C4.06c		methytransferase, human Fam119 ortholog (predicted)
V2-10-A05	SPAC56F8.04c	ppt1	para-hydroxybenzoate--polyprenyltransferase Ppt1
V2-10-A08	SPAC3A11.10c		dipeptidyl peptidase (predicted)
V2-10-A09	SPAC1805.01c	ppk6	serine/threonine protein kinase Ppk6
V2-10-B08	SPAC9.07c		GTPase Rbg1
V2-10-D08	SPAC31A2.16	gef2	RhoGEF Gef2
V2-10-D11	SPBPJ4664.06	gpt1	UDP-glucose-glycoprotein glucosyltransferase Gpt1
V2-10-H08	SPCPIE11.02	ppk38	Ark1/Prk1 family protein kinase Ppk38
V2-11-F03	SPBC18H10.06c	swd2	Set1C complex subunit Swd2.1
V2-12-G02	SPAC24H6.03	cul3	cullin 3
V2-13-A10	SPAC57A10.12c	ura3	dihydroorotate dehydrogenase Ura3 (This was due to the –ura selection)
V2-15-G03	SPBC2G2.01c	liz1	pantothenate transporter Liz1
V2-15-G11	SPCC553.01c	dbl2	meiotic chromosome segregation protein Dbl2
V2-16-F06	SPAC25H1.05	meu29	calcium transport regulatory factor (predicted)
V2-17-A11	SPCC31H12.05c	sds21	serine/threonine protein phosphatase PP1 subfamily, Sds21
V2-17-E12	SPACUNK4.12c	iph1	insulinase pombe homologue 1
V2-17-H04	SPAC688.11	end4	Huntingtin-interacting protein homolog
V2-18-B11	SPCC162.11c		uridine kinase/uracil phosphoribosyltransferase (predicted)
V2-18-F12	SPAC1687.12c	coq4	ubiquinone biosynthesis protein Coq4
V2-20-D06	SPBC16D10.07c	sir2	Sir2 family histone deacetylase Sir2
V2-22-A04	SPBPB10D8.07c		membrane transporter (predicted)
V2-24-B08	SPAC10F6.12c	mam4	protein-S isoprenylcysteine O-methyltransferase Mam4
V2-24-E04	SPBC725.15	ura5	orotate phosphoribosyltransferase Ura5 (This was due to the –ura selection)
V2-25-C06	SPBP35G2.07	ilv1	acetolactate synthase catalytic subunit
V2-25-F08	SPBC3H7.14	mug176	BRCT domain protein
V2-26-A10	SPAC3F10.04	gsa1	glutathione synthetase large subunit Gsa1
V2-26-D12	SPAC17A5.14	exo2	exonuclease II Exo2

Bioneer M-2030H Position	Gene ID	Gene name	Gene description
V2-27-A03	SPBC31F10.15c	atp15	F0-ATPase epsilon subunit (predicted)
V2-27-A12	SPAC23A1.16c	rtr1	RNA polymerase II CTD phosphatase Rtr1 (predicted)
V2-27-H08	SPBC409.20c	psh3	ER chaperone SHR3 homologue Psh3
V2-28-C07	SPAC1952.17c		GTPase activating protein (predicted)
V2-28-E06	SPBC25H2.11c	spt7	SAGA complex bromodomain subunit Spt7
V2-28-G10	SPCC1450.12		PXA domain protein
V2-28-H04	SPAC17G8.13c	mst2	histone acetyltransferase Mst2
V2-29-C01	SPAC8F11.02c	dph3	diphthamide biosynthesis protein Dph3 (predicted)
V2-29-E06	SPCC1672.04c		mitochondrial copper chaperone (predicted)
V2-30-D08	SPCC1235.09	hif2	Set3 complex subunit Hif2
V2-30-E02	SPBC725.09c	hob3	BAR adaptor protein Hob3
V2-30-G08	SPBC1703.12	ubp9	ubiquitin C-terminal hydrolase Ubp9
V2-32-A08	SPCC794.02	wtf5	wtf element Wtf5
V2-32-D07	SPAC26F1.12c	hgh1	conserved eukaryotic protein Hgh1
V2-32-G10	SPCC4G3.04c	coq5	C-methyltransferase (predicted)