Protocol

Setting up *Schizosaccharomyces pombe* Crosses/Matings

Karl Ekwall^{1,3} and Geneviève Thon²

¹Department of Biosciences and Nutrition, Karolinska Institute, Stockholm SE-141 83, Sweden; ²Department of Biology, University of Copenhagen, Copenhagen DK-2200, Denmark

Here we provide methods for setting up standard crosses with *Schizosaccharomyces pombe* strains. All strain genotypes and pedigrees should be recorded in a laboratory strain book. Matings between two haploid strains of interest are induced on solid medium poor in nitrogen. Usually, sporulation agar (SPA) plates are preferred, but for difficult matings it is advisable to try several mating media in parallel because one medium might allow for more efficient mating. Protoplast fusion can be used to produce zygotes from sterile mutants that fail to mate.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at <http://cshprotocols.cshlp.org/site/recipes>.

Reagents

Agar

CaCl₂ (0.1 M)

Glucanex (β-glucanase; Novozymes)

Prepare a fresh 3 mg/mL solution in 0.65 M KCl for each application.

KCl (0.65 M)

Media

Selection medium containing 1.2 M sorbitol appropriate for experimental strains

Sporulation agar (SPA) medium <R>

Supplements may be needed for auxotrophic strains (see Step 2).

Yeast extract with supplements (YES) <R>

Omit lysine from YES if growing arginine auxotrophs.

Polyethylene glycol (PEG 6000; 30%)

S. pombe strains appropriate for experimental goals

Genetic manipulations require a precise knowledge of the genotypes of parental and progeny strains. It is essential to keep track of these by giving each strain a name (usually a number in a strain collection) and by writing down its complete genotype, stock date, PubMed references for parental strains, investigators name, and other notes of interest, such as temperature sensitivity, etc. This information should be kept in a strain book (and/or electronically). This greatly simplifies the communication of strain information and of handling strain requests after the publication of new strains.

³Correspondence: karl.ekwall@ki.se

From the Fission Yeast collection, edited by Iain M. Hagan, Antony M. Carr, Agnes Grallert, and Paul Nurse.

© 2017 Cold Spring Harbor Laboratory Press

Cite this protocol as *Cold Spring Harb Protoc*; doi:10.1101/pdb.prot091694

K. Ekwall and G. Thon

Sorbitol (1.2 M)
Water (distilled, sterile)

Equipment

Applicator sticks (long, wooden, autoclaved; or disposable plastic inoculation loops, sterile)
Centrifuge to hold swing out rotor for 50-mL screw-cap tubes
Flasks (250 mL)
Hemocytometer
Phase-contrast light microscope equipped with 40× objective and 10× eyepiece
Plate incubator
Shaking incubator
Toothpicks (flat and sharp, autoclaved)
Tubes (50 mL, screw-cap, sterile)

METHOD

Standard Crosses

1. Grow strains of opposite mating type that are to be crossed.

*Strains should be freshly grown overnight, either in 5 mL of YES liquid cultures or as a patch on a YES plate. Usually h^+ and h^- mating-type strains are used. For details on culturing *S. pombe*, see Introduction: **Growth and the Environment of Schizosaccharomyces pombe** (Petersen and Russell 2016).*

2. Mix equal amounts of cells of the two strains in a drop of sterile water on an SPA plate using an inoculating loop or a flat toothpick.

Cells should be taken directly from the YES plate or, for liquid cultures, after harvesting by centrifugation. Several crosses can be set up on the same 9-cm diameter plate.

When crossing homothallic h^{90} strains with h^- or h^+ strains, use ratios of 1:3 for h^{90} to h^+ or h^- cells to minimize self-mating.

If strains are auxotrophic, add the necessary supplements to the sporulation plate to a final concentration of 40 µg/mL. Do not exceed this level or mate on plates containing all amino acids because the nitrogen from the amino acids will be catabolized, which reduces the efficiency of G_1 arrest and, therefore, mating efficiency.

3. Allow the cell suspensions to dry completely. Invert the plate and incubate for 2–3 d below 30°C until asci have formed.
4. Monitor the formation of asci by removing some cells and examining them under a light microscope.

See Troubleshooting.

Crossing by Protoplast Fusion

Some mutants are completely sterile and cannot be studied by routine crosses. However, it is possible to force such crosses by digesting the cell wall to form protoplasts that can then fuse to form a zygote (Nadin-Davis and Nasim 1990).

5. Grow each of the two strains in 50 mL of YES medium in 250-mL flasks with shaking at 30°C to logarithmic phase ($2\text{--}3 \times 10^6$ cells/mL).

*For details on culturing *S. pombe*, see Introduction: **Growth and the Environment of Schizosaccharomyces pombe** (Petersen and Russell 2016).*

6. Transfer cells to 50-mL screw-cap tubes and harvest cells by centrifugation for 3 min at 3200g.
7. Wash cells by resuspension in 10 mL of 0.65 M KCl followed by centrifugation for 3 min at 3200g.

8. Discard supernatant and resuspend cells in 5 mL of 0.65 M KCl containing 3 mg/mL Glucanex.
Do not vortex the cells/protoplasts.
9. Incubate cells for 20–30 min at 30°C until protoplasts form. Check for protoplast formation by observing a 5-μL aliquot on a hemocytometer under a phase-contrast microscope.
Protoplasts appear rounded.
10. When >90% of cells have formed protoplasts, add 10 mL of 1.2 M sorbitol and then harvest by centrifugation at 1250g for 3 min.
11. Discard the supernatant, and resuspend protoplasts in 2 mL of 1.2 M sorbitol.
If the two strains are ready at slightly different times, the first strain can be left in sorbitol at room temperature.
12. Combine the two protoplast samples and mix by gentle pipetting up and down.
Cut the end of a 1-mL pipette tip to reduce sheer forces during mixing
13. Harvest the protoplasts by centrifugation for 3 min at 1250g.
14. Mix nine parts 30% PEG 6000 with one part 0.1 M CaCl₂. Resuspend the protoplasts in 1 mL of the PEG–CaCl₂ mixture.
15. Incubate for 30 min at room temperature.
16. Melt 1% agar in 10 mL of an appropriate selective growth medium made with 1.2 M sorbitol. Cool to <45°C. Add to the protoplast mixture.
17. Pour the 1% agar growth medium incorporating the protoplasts onto an appropriate selection plate containing 1.2 M sorbitol.
Do not use any physical means of spreading (beads or glass rod) as this will burst the protoplasts.
18. Incubate the plate for 2–3 d below 30°C until asci have formed.

TROUBLESHOOTING

Problem: (Step 4): Some mutants mate inefficiently using the standard protocol.

Solution: Mix equal numbers of cells of each strain from mid-log phase cultures grown in minimal medium or MSL medium ($2\text{--}5 \times 10^6$ cells/mL). Place droplets of the cell mixture onto different types of mating medium, that is, ME, MSA, SPA, or EMM-N plates (see Introduction: **Growth and the Environment of *Schizosaccharomyces pombe*** [Petersen and Russell 2016]) and incubate overnight. Efficiency of mating is often improved on one or more of these media. It may also be helpful to use a higher ratio of mutant to wild-type cells in the cross (e.g., a 3:1 ratio). A further way to increase efficiency can be to reduce the number of auxotrophic markers in the strains.

RECIPES

Sporulation Agar (SPA) Medium

Reagent	Quantity (for 1 L)	Final concentration
Glucose	10 g	55.5 mM
KH ₂ PO ₄	1 g	7.3 mM
Vitamins (1000×) <R>	1 mL	1×
Agar	20 g	20 g/L
H ₂ O	to 1 L	

To prepare SPA medium (Munz and Leupold 1970), combine the ingredients listed above. Sterilize by autoclaving at 10 psi for 15 min. Store at 4°C.

K. Ekwall and G. Thon

Vitamins (1000×)

Reagent	Quantity (for 1 L)	Final concentration
Pantothenic acid	1 g	4.20 mM
Nicotinic acid	10 g	81.2 mM
Inositol	10 g	55.5 mM
Biotin	10 mg	40.8 μM

Store at 4°C.

Yeast Extract with Supplements (YES)

Reagent	Amount to add (1 L)
Glucose	30 g
Yeast extract	5 g
Adenine	0.2 g
Uracil	0.2 g
Histidine	0.2 g
Leucine	0.2 g
Lysine	0.2 g
Agar (for solid medium only)	20 g
H ₂ O	to 1 L

Prepare 1 L of YES by combining the reagents listed above. Sterilize by autoclaving at 10 psi for 15 min. Store at 4°C. (Note that the yeast extract contains sufficient thiamine to repress *nmt1* promoters.)

ACKNOWLEDGMENTS

We acknowledge Agnes Grallert for providing the protoplast fusion protocol. K.E. was supported by the Swedish Cancer Society and the Swedish Research Council. G.T. was supported by the Novo Nordisk Foundation and the University of Copenhagen Center of Excellence MolPhysX.

REFERENCES

- Munz P, Leupold U. 1970. Characterization of ICR-170-induced mutations in *Schizosaccharomyces pombe*. *Mutat Res* 9: 199–212.
- Nadin-Davis SA, Nasim A. 1990. *Schizosaccharomyces pombe ras1* and *byr1* are functionally related genes of the *ste* family that affect starvation-induced transcription of mating-type genes. *Mol Cell Biol* 10: 549–560.
- Petersen J, Russell P. 2016. Growth and the environment of *Schizosaccharomyces pombe*. *Cold Spring Harb Protoc* doi: 10.1101/pdb.top079764.

Cold Spring Harbor Protocols

Setting up *Schizosaccharomyces pombe* Crosses/Matings

Karl Ekwall and Geneviève Thon

Cold Spring Harb Protoc; doi: 10.1101/pdb.prot091694

Email Alerting Service

Receive free email alerts when new articles cite this article - [click here](#).

Subject Categories

Browse articles on similar topics from *Cold Spring Harbor Protocols*.

[Yeast](#) (288 articles)

[Yeast Genetics](#) (135 articles)

To subscribe to *Cold Spring Harbor Protocols* go to:
<http://cshprotocols.cshlp.org/subscriptions>