Protocol 1

Mating, Spore Dissection, and Selection of Diploid Cells in *Schizosaccharomyces japonicus*

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Haploid yeast cells mate to form heterozygotes and subsequently undergo meiosis to form spores. This process can be used to produce gene combinations and variants that are useful for genetic analysis. For example, these spores can be used to generate double mutants or to measure genetic distances in a mutational analysis. Here, we describe mating and spore dissection procedures for *Schizosaccharomyces japonicus* cells. Although the overall procedures resemble those used in *Schizosaccharomyces pombe*, some differences exist, including the use of EMM2 medium without nitrogen (EMM-N) for mating and the shorter incubation time of 16–20 h for *S. japonicus* cells. Furthermore, the *S. japonicus* zygotes produce eight spores and thus require an "octad" analysis.

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

EMM-N <R>

Prepare EMM-N liquid medium. (EMM-N is EMM2 medium without nitrogen.)

EMM2 <R>

Prepare EMM2 agar plates without adenine.

Schizosaccharomyces japonicus strains

Strains are available from the web home of the S. japonicas *bioresource center, JapoNet (http://night.nig.ac.jp/labs/MicroGen/japonet/).*

ade6-domK and *ade6-domE* mutant strains (for selection of diploid cells; see Steps 9–12)
Heterothallic cells of opposite mating types (for induction of meiosis; see Steps 1–8)
YE medium <R>

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Prepare liquid medium and 2% agar plates. If necessary, the YE medium can be supplemented with 0.1 mg/mL adenine and 0.1 mg/mL uracil.

Zymolyase 100 T (1 mg/mL)

Equipment

Heat block at 37°C
Micromanipulator
Sonicator (optional; see Step 11)
Tabletop centrifuge
Test tubes (18-mm)
Vortex
Water bath incubator with shaker at 30°C

METHOD

Induction of Meiosis by Nitrogen Starvation

Nitrogen deficiency is effective for inducing sporulation. The best way to sporulate is by using liquid medium. Portions of this procedure were previously described in Furuya and Niki (2009).

1. Inoculate each mating type of heterothallic cells in 3 mL of YE liquid medium in 18-mm test tubes, and shake overnight at 30°C.

   The cells must be saturated the next morning.

2. Centrifuge the cells to harvest (1100*g* for 1 min in a tabletop centrifuge at room temperature). Remove the supernatant.
3. Resuspend cells in 0.5 mL of EMM-N liquid medium. Vortex well.
4. Prepare a new test tube (18-mm diameter) with 0.5 mL of EMM-N liquid medium, and add 0.05 mL of both mating types of heterothallic cells.
5. Incubate the mixture of heterothallic cells for 16–20 h at 30°C.

   It is not necessary to shake the mixture in the test tube; the tube can simply be left standing for incubation. Overnight incubation in EMM-N liquid medium at 30°C provides a sufficient number of spores to work on.

6. Check for sporulation by microscopy.

   A dumbbell-shaped ascus includes eight spores.

   See Troubleshooting.

7. Harvest and centrifuge the cells in a tabletop centrifuge (1100*g* for 1 min at room temperature). Add 0.1 mL of 1 mg/mL Zymolyase 100 T to the cell pellet. Place in a heat block for 5 min at 37°C.

   Fresh asci are hard to dissect. Digesting the ascus wall with Zymolyase 100 T helps to subsequently release the eight spores for dissection using the micromanipulator needle. If the mixture is standing at room temperature for an extended period of time, the asci are autonomously lysed so that the spores are released. Spore viability is maintained for 1 wk at 4°C.

8. Spread the ascospores onto YE 2% agar plates for dissection. Perform spore dissection using a micromanipulator.

Selection of Diploid Cells

The ade6-domK *and* ade6-domE *alleles show interallelic complementation when they are simultaneously expressed in the same cell. As with* S. pombe ade6-M210 *and* ade6-M216*, this facilitates the construction of diploid cells (Furuya and Niki 2011).*

9. Mix the two *ade6* mutants, one of each mating type, following Steps 1–4.

10. Incubate the mixture for 5 h at 30°C.

    Because heterozygotes are formed in 4–5 h, cell aggregation, namely sexual flocculation, can be seen in the tube.

11. Spread cells in the mixture onto EMM2 agar plates lacking adenine.

    If necessary, aggregated cells can be gently sonicated.

12. Check for diploid cells under a microscope.

    The diploid cells are twice as large as the haploid cells.

TROUBLESHOOTING

Problem (Step 6): Some of the selected clones of diploid cells lose their ability to sporulate.
Solution: Multiple clones of diploid cells should be isolated and tested.

RECIPES

EMM2

Reagent	Quantity (for 1 L)	Final concentration
Ammonium chloride	5 g	93.5 mM
Potassium hydrogen phthalate	3 g	14.7 mM
Na_2HPO_4	2.2 g	15.5 mM
Glucose	20 g	111 mM
Salt stock (50×)	20 mL	1×
Vitamins (1000×)	1 mL	1×
Minerals (10,000×)	0.1 mL	1×
H_2O	to 1 L	

Prepare 1 L of EMM2 by combining the reagents above. To prepare solid medium, include agar (20 g/L). Sterilize by autoclaving at 10 psi for 10 min (for liquid medium) or 15 min (for solid medium). (For liquid medium, low pressure and a short cycle is essential to avoid caramelization of glucose and breakdown of vitamins and minerals. For live cell imaging, filter-sterilized medium has lower background fluorescence than autoclaved medium. Use a 0.22-μm pore size for filtration.) Store at 4°C. If desired, add 12 μM thiamine (364 μL of a filter-sterilized stock solution of 10 mg/mL on H_2O) after autoclaving to fully repress expression from *nmt1*-derived promoters (Maundrell 1990).

EMM−N

Reagent	Quantity (for 1 L)	Final concentration
Potassium hydrogen phthalate	3 g	14.7 mM
Na_2HPO_4	2.2 g	15.5 mM
Glucose	20 g	111 mM
Salt stock (50×) <R>	20 mL	1×
Vitamins (1000×) <R>	1 mL	1×
Minerals (10,000×) <R>	0.1 mL	1×
H_2O	to 1 L	

Prepare 1 L of EMM−N (EMM2 without nitrogen) (Nurse 1975) by combining the reagents above. To prepare solid medium, include agar (20 g/L). Sterilize by autoclaving at 10 psi for 10 min (for liquid medium) or 15 min (for solid medium). Store at 4°C. If desired, add 12 μM thiamine (364 μL of a filter-sterilized stock solution of 10 mg/mL on H_2O) after autoclaving to fully repress expression from *nmt1*-derived promoters (Maundrell 1990).

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Minerals (10,000×)

Reagent	Quantity (for 1 L)	Final concentration
Boric acid	5 g	80.9 mM
$MnSO_4$	4 g	23.7 mM
$ZnSO_4 \cdot 7H_2O$	4 g	13.9 mM
$FeCl_2 \cdot 6H_2O$	2 g	7.40 mM
Molybdic acid	0.4 g	2.47 mM
KI	1 g	6.02 mM
$CuSO_4 \cdot 5H_2O$	0.4 g	1.60 mM
Citric acid	10 g	47.6 mM

Store at 4°C.

Salt Stock (50×)

Reagent	Quantity (for 1 L)	Final concentration
$MgCl_2 \cdot 6H_2O$	52.5 g	0.26 M
$CaCl_2 \cdot 2H_2O$	0.735 g	4.99 mM
KCl	50 g	0.67 M
Na_2SO_4	2 g	14.1 mM

Store at 4°C.

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.

YE Medium

Reagent	Quantity (for 1 L)	Final concentration (w/v)
Yeast extract	5 g	0.5%
Glucose	30 g	3%
Agar (for plates only)	20 g	2%

Store for up to 3 mo at room temperature, or for up to 6 mo at 4°C.

REFERENCES

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