Sporulation and Tetrads Dissection
From S. Silverman and P. Gibney

SPORULATION

1. Resuspend one large colony (from YPD) in 2 mL of 1% potassium acetate.
2. Incubate at room temperature on a roller wheel for 1 day.
3. Incubate at 30°C for 2 more days (can also leave at room temperature for a few days, if desired) on a roller wheel.

NOTE: When sporulating, if diploid is homozygous for an auxotrophic mutation, add that nutrient to the potassium acetate (about ¼ the amount listed for addition to SD media… any more and it could be used as a nitrogen source).

NOTE: To sporulate yeast cells need to be starved for nitrogen in the presence of a non-fermentable carbon source.

TETRAD DISSECTION

1. Make a solution of zymolyase (final concentration = 0.25 mg/mL) in Qiagen Y1 buffer (1M sorbitol, 100 mM EDTA, 14 mM β-mercaptoethanol).
2. Examine culture cell density and remove 200 µL of dense, sporulated cells (OD₆₀₀ = 2-4).
3. Spin down cells, resuspend in 200 µL of zymolyase solution.
4. Incubate at room temperature for 5 minutes. Check cells under microscope to determine zymolyase efficiency (see below).
5. Incubate at 37°C for 5 minutes. Check cells under microscope to determine zymolyase efficiency (see below).
6. Repeat until asci are digested to desired level.

7. After tetrads are appropriately digested, GENTLY add and GENTLY mix 600 µL – 1000 µL of ddH₂O (stops zymolyase by dilution).
8. Spread cells on a plate in a line across the center of the plate.
9. Remove the four spores of a tetrad away from the line, and drop one at a time using the micromanipulator guides (preset clicks will separate spores by 5 mm).
10. Dissect approximately 20 tetrads per plate (10 on each side of the line)(see below).

11. Incubate at 30°C for 2-3 days until spore-derived colonies are about medium size.

12. Replica plate sporulation plate to desired selection media and/or mating type tester plates.

NOTE: Ascus digestion is much faster with fresh spores than with older spores.

NOTE: For tetrad dissection success, you need: dry, level plates, good ascus digestion, and good sporulation efficiency.