Sporulation and Tetrad Dissection
From S. Silverman and M. Hickman

SPORULATION
Mark Hickman uses this protocol and regularly has sporulation efficiencies of >50%.

1. Grow a 5 mL overnight culture; dilute 1:50 in the morning and grow for 4 hours at 30°C (to log phase).

2. Pellet cells, wash in 1 mL of 1% potassium acetate, and resuspend in 3-4 mL of 1% potassium acetate.

3. Incubate at room temperature on a roller wheel for 3 day (can incubate longer, if desired).

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

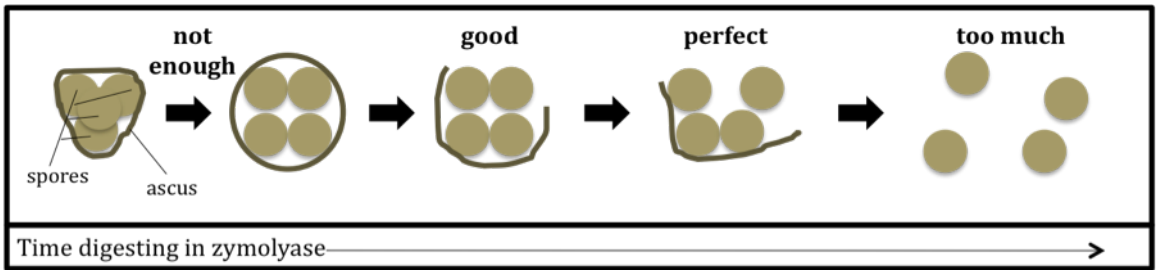
1. Make a solution of zymolyase (0.25-2.5 mg/mL) in 1M sorbitol (can leave at 4°C for at least a month). Lower concentration is slower but may help to avoid overdigestion, especially with very freshly sporulated cultures.

2. Examine culture cell density and remove 200 - 400 μL of sporulated cells (OD_{600} = 2-4).

3. Spin down cells, resuspend in 200 - 400 μL of zymolyase solution.

4. Incubate at room temperature for 5 minutes. Check cells under microscope to determine zymolyase efficiency (see below).

5. Repeat #4 until asci are digested to the correct level (with fresh zymolyase, 5-10 minutes is usually good).



6. After tetrads are appropriately digested, GENTLY add and GENTLY (by inversion) mix 600 μL – 1000 μL of ddH_20 (stops zymolyase by dilution).

7. Spread cells on a plate in a line across the center of the plate (either with a loop, or by adding 50 μL and tilting the plate to form a line of cells).

8. 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).

9. Dissect approximately 20 tetrads per plate (10 on each side of the line)(see below).

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

11. 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.