**Sporulation by Guthrie and Fink recipe**

Maitreya Dunham  
January 2005

**SPO++ media**

Small batches recommended to avoid contamination problems.

200 ml  
0.5 g  yeast extract  
3 g  potassium acetate  
water to 200 ml

Autoclave. Once cool, add:

1.25 ml  40% glucose  
20 ml  10X amino acid stock

**10X amino acid stock**

100 ml  
40 mg  adenine  
40 mg  uracil  
40 mg  tyrosine  
20 mg  histidine  
20 mg  leucine  
20 mg  lysine  
20 mg  tryptophan  
20 mg  methionine  
20 mg  arginine  
100 mg  phenylalanine  
350 mg  threonine  
water to 100 ml

Filter sterilize and store at 4C in the dark.

**Sporulation**

Streak for a fresh colony.  
Grow an overnight in YPD.  
Spin down ~250 µl cells (can vary for different strains).  
Decant supernatant and resuspend pellet in 2 ml SPO++. 
Transfer to a culture tube. Also make a no cells control to test for contamination.
Let rotate at 30C or at room temperature, depending on the strain. I use 30C for most strains.
Check the cultures under the microscope to see if they've sporulated.
SK1 strain will sporulate overnight with very high efficiency.
Y55 takes maybe 2 days, but should also be high.
S288C and CEN.PK take 3 or more days, with low efficiency.

**Dissection**

You'll need dissection plates: add 25 ml YPD with a plastic strippette to plates on a very level surface. Once solid, invert. Let dry at room temperature for ~3 days. Bag. Best after aging for a while.

Spin down 250 ul of sporulated culture.
Resuspend in 250 ul sterile water.
Mix 17 ul culture with 3 ul B-glucuronidase (can vary by strain).
Let sit 15-45 minutes (depends on strain, culture, and ambient conditions) until digested. I generally start 2 digestions and stop one after 20 minutes and the other after 30 and use which one is better. Check for digestion under the microscope.
Gently add 100 ul water. The goal is to suspend the cells without breaking up the tetrads.
Pick out your favorite dissection plate. The plate should not look wet, but when the side is deformed with your thumb, little droplets of water should squeeze out from the surface of the agar. The droplets should be immediately reabsorbed once the pressure is released.
Mark the center of the plate.
Drip 20 ul down the center.
Let absorb into the plate.
Dissect.