Yeast Sporulation (adapted from Guthrie and Fink recipe)

**SPO++ Media**

Add .5g of yeast extract

Add 3g of potassium acetate

Add sterile water to 200mL

Autoclave and cool

Add 1.25mL of 40% glucose (autoclaved)

Add 20mL of 10X amino acid stock (filter sterilized)

Note: make small batches (50mL) of SPO++ media to avoid contamination problems.

**10X Amino Acid Stock**

40mg of adenine

40mg of uracil

40mg of tyrosine

20mg of histidine

20mg of leucine

20mg of lysine

20mg of tryptophan

20mg of methionine

20mg of arginine

100mg of phenylalanine

350mg of threonine

Add water to 100mL

Filter sterile and store at 4°C in the dark
Dissection Plates

Add 25mL of YPD agar with plastic strippette to plates on level surface

Invert plates once agar solidifies

Let plates dry at room temperature for ~3 days before use

Sporulation Protocol

1. Grow overnight liquid YPD culture from single colonies.
2. Spin down 250µL of cells.
3. Remove supernatant, resuspend pellet in 2-3mL of SPO++, and transfer to culture tubes.
4. Make a no cells control to test for contamination in SPO++ media.
5. Place tubes at 30°C on roller drum for 2-5 days.
6. Check sporulation efficiency under microscope.

Dissection Protocol

1. Spin down 250µL of sporulated culture
2. Resuspend in 250µL of sterile water
3. Mix 17µL of culture with 3µL of β-glucuronidase
4. Let sit for 15-45 minutes, or until digested. (check culture for digestion at 20min, 30, and 40min by microscopy)
5. Add 100µL of sterile water
6. Mark and drip 20µL of digested culture down the center of the aged agar plate
7. Let absorb into plate and dissect