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.