BISHOP LAB
LIQUID SPORULATION OF YEAST

Day 1

1. Streak diploid fresh from freezer stock
2. If making diploids, mate fresh haploids and pull zygotes

Day 3

1. Select single colonies or zygotes (at least 2 per strain)
2. Patch 1/3 of colony onto YPG plate, 1/3 colony onto SPM plate
3. Inoculate 5mL YPDA with remainder of colony
4. Incubate plates at 30ºC and rotate culture overnight at 30ºC

Day 4

1. Check for growth on YPG plates and make sure diploids sporulated on SPM plates using a light or phase contrast microscope (if using diploids that do not sporulate, check for diploids by growing pattern or other method)
   Note: Do not use a colony for sporulation that either does not grow on YPG (indicating a petite) or did not sporulate on SPM plates!
2. Inoculate 25mL SPS with 1mL of overnight culture
3. Shake at 30ºC (250-300rpm) for 5-6 hours
4. Check OD_{600} of cultures (to increase efficiency of growth, aim for OD_{600} to be 0.5-1.0)
5. Calculate I (a number that corresponds to the amount of overday culture that needs to be inoculated into overnight culture for a final cell density of 0.7 at OD_{600})
   Equation: $I = (\text{Final Volume})/OD_{600}) \times (0.7/2^n)$
   n = (Growing Hours x 60)/142; 142 = doubling time
6. Inoculate overnight culture (SPS) with amount calculated by I
   Note: Overnight volume of SPS is equal to final volume of sporulation
7. To increase chances of getting a culture at the right density, set up two additional overnight cultures by inoculating 1/4 I, and 1/2 I
8. Shake overnight at 30ºC at 250-300 rpm

Day 5

1. In the morning, near the set time, check density of cultures at OD_{600}
2. Ideally, cultures should be at density between 0.7-1.0
   Note: Cultures at density above 1.4 are too overgrown; cultures at density of 0.5 or lower are not grown enough. If necessary, grow cultures longer to harvest at optimal density.
3. Pour cells into centrifuge bottles and spin for 3 minutes at 5000 rpm at room temperature
4. Resuspend cell pellet in 25-50mL sterile dH_2O
5. Spin as above
6. Resuspend cell pellet in 10mL SPM+1/5 COM, then add to the remainder of SPM +1/5 COM already aliquoted into flask
7. Shake at 30ºC (250-300rpm)
   General notes: To ensure the most efficient, synchronous sporulation, work quickly after harvesting the overnight SPS cultures and use reagents that are pre-heated to the correct temperature and equipment that is at room temperature. Also, set up sporulations in flasks that are 5-10 times the volume of the sporulating culture.

YPDA
10g Yeast extract
20g Bacto peptone
10mL 0.1% Adenine solution

Add dH_20 to 1L
Adjust pH to 7.0 with NaOH
Autoclave
Add 50mL of sterile 40% glucose per liter after autoclaving

SPS
5g Ammonium sulfate
1.7g Yeast nitrogen base
10g Bacto peptone
10g Potassium acetate
5g Yeast extract
10.2g Potassium bipthalate (same thing as Potassium hydrogen pthalate)

Add dH_20 to 1L
Adjust pH to 5.5 with KOH
Autoclave

SPM +1/5 COM
3g Potassium acetate
0.12g COM powder

Dissolve ingredients in 1L dH_20
Adjust pH to 7.0 HCl or KOH
Autoclave
Add 2mL sterile10% raffinose per liter after autoclaving