BULK SEGREGANT PROTOCOL

Grow 10mL YPD overnight

Spin down and resuspend in 20mL SPO++ until ~75% sporulation efficiency is reached

Spin down and resuspend in 200λ H_2O (Final volume ~500-750λ)

Add 5λ zymolyase (1,000x, 150 mg/mL)

** Note tetrad prevalence after each of the following steps**

Incubate at 30°C for 1hr

Add 50λ glass beads and 50λ 10% Triton

Vortex for 2mins

Incubate at 30°C for 40mins

Vortex for 2mins

Bring up to 5mL with H_2O (~4-4.5mL H_2O needed)

Sonicate at power 4 for 4secs

Start two 5mL YPD holds (500λ sample into 5mL YPD)

After 24hrs plate 4 BSM plates: two 2mL (BSP's) and two 1mL (BSI's)

Grow for ~3 days and pick two 96well plates of singles from the 1mL plates and pool the 2mL plates:

Spread 5mL YPD (collected volume ~2-3mL)
Spread 2.5mL YPD (additional collected volume ~1-2mL)
End volume ~4mL
Add 1mL 75% Glycerol
Freeze down in single row of 96well BSP plate and 1mL in cryo tube

BULK SEGREGANT MEDIA (BSM)

500mL SD agar
Add 0.4g CSM –arg and 0.5g 5FOA before autoclaving
Add 500λ ClonNat (100mg/mL) and 500λ CAN (60mg/mL) to melted agar

YIELD: ~7-8 Big plates