**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 H2O (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 H2O (~4-4.5mL H2O 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