Yeast Spore Enrichment for Random Spore Analysis

1. Start a 5ml culture of cells in Sporulation Media. Leave at RT in shaker for 1 week.

2. Prepare a suspension from ~ 1X10^8 cells and asci of sporulated culture in a polypropylene 1.5ml μfuge tube containing 180μl sterile DW. (1 ODU=3 X 10^7). Wash cells and asci 2X in 1ml sterile DW before suspending in 180μl DW.

3. Add 20μl of 5mg/ml solution of Zymolyase 20T in ZL buffer to the suspension and mix. Incubate at 30°C for 1 hour

ZL Buffer	For 100ml
0.1 M NaPO4, pH 6.5	10 ml 1M NaPO4, pH 6.5
1.2 M Sorbital	21.9g Sorbital
40% Glycerol	80 ml 50% Glycerol
DW	Up to 100ml

   Filter Steralize. (Allow time because thick)
   Dissolve 5mg Zymolyse 20T in 1 ml ZL buffer
   Store solution at -20°C

4. Spin 30 seconds FS, discard supernatant.

5. Suspend pellet in 1 ml DW and respin 30 seconds FS, discard supernatant.

6. Suspend pellet in 100μl DW and vortex on high speed for 2 minutes.

7. Pour the liquid out of the μfuge tube and rinse the tube 2X with 1ml DW. Pour liquid out of tube each time. (Note: The hydrophobic spores will stick to the μfuge tube wall while the cells and debris will remain mostly in suspension.)

8. Add 1.0ml sterile 0.01% NP-40 to the tube and sonicate for 30 seconds-1 minute.

9. Prepare 4X 1:10 serial dilutions of the sonicated suspension (50μl into 450μl 0.01% NP-40).

10. Spread 200μl of each dilution onto YPD plate or selective plate.