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**

<table>
<thead>
<tr>
<th>Component</th>
<th>For 100ml</th>
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<tbody>
<tr>
<td>0.1 M NaPO4, pH 6.5</td>
<td>10 ml 1M NaPO4, pH 6.5</td>
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<tr>
<td>1.2 M Sorbitol</td>
<td>21.9g Sorbital</td>
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<tr>
<td>40% Glycerol</td>
<td>80 ml 50% Glycerol</td>
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<tr>
<td>DW</td>
<td>Up to 100ml</td>
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Filter Steralize. (Allow time because thick)
Dissolve 5mg Zymolyase 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.