Sporulation Protocol

Sporulation

1. Grow up a small (2mL) overnight culture in YEPD (rich glucose media), 30°C.
2. Back dilute cells 1 to 50 into 2 mL YPD.
3. Let the new culture grow at 30 deg C for around 6 hours (about two to three doublings in cell number somewhere between 2-8e7 cells/mL).
4. Spin down 1.5 mLs of the cells and wash them once with sterile water.
5. Resuspend the cells in sporulation media (2% potassium acetate, pH 7.0) supplemented with necessary nutrients. Set up this culture so that the cells are at a concentration of 1-5E7 cells/mL, and a total volume of 2.5 mL.
6. Let the cultures sporulate 5-7 days at 23 deg C.

Lazy Sporulation

1. Inoculate diploid into super-SPO media.
2. Let the cultures sporulate 5-7 days at 23C

<table>
<thead>
<tr>
<th>1L Super SPO Media</th>
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<tbody>
<tr>
<td>15g Potassium Acetate</td>
</tr>
<tr>
<td>2.5g Yeast Extract</td>
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<tr>
<td>0.5g Glucose</td>
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<tr>
<td>0.7g SC Amino Acid Mix</td>
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Yeast Vivisection

1. Spin down 100uL of sporulation product @ 6,000g for 1 minute
2. Remove supernatant and resuspend in 100uL Zymolase solution
3. Incubate @37°C for 12 minutes
4. Gently add 200uL cold sterile water put onto ice
5. Drip 20uL of cells down the center of a plate
6. Dissect tetrads
7. Record Results on Dissection Sheet