High Efficiency Transformation Protocol

The day before, start at least a 2ml overnight culture from a single colony.

(**If your transformation efficiency is low or non-existent, make fresh 1M LiAc)

1. Dilute 1 mL of cultured cells into 50 mL of YPD in baffled 250 mL culture flask.
2. Incubate the flask on a rotary shaker at 30°C and 200 rpm. Cells should complete at least 2 divisions (about 4 hours).
3. When the cell titer is at least 2 x 10^7 cells/mL, harvest by centrifugation at 5000 RPM for 5 min.
4. Wash the cells with 25 mL of sterile water and spin down again.
   (Boil a 1.0 mL sample of carrier DNA for 5 min. and put on ice.)
5. Resuspend in 1 mL of sterile water, and transfer to an eppendorf tube. Centrifuge for 30 sec. at 6000 RPM, discard supernatent.
6. Add water to a final volume of 1.0 mL and vortex vigorously to resuspend (maintain at 2x 10^9 cells/mL).
7. Pipette 100 µL samples into 1.5 mL microfuge tubes, one for each transformation including the negative controls.
8. Centrifuge at top speed (15000 RPM) for 30 seconds and remove supernatent.
9. Make Transformation Mix and keep on ice:
   a. 240 µL PEG 3500 50% w/v
   b. 36 µL LiAc 1.0 M
   c. 50 µL Boiled SS-carrier DNA (2mg/mL)
   d. 34 µL Plasmid DNA plus water, or just water for negative control
   360 µL TOTAL
10. Add 360 µL of Mix to each transformation (100 µL of cells) and resuspend cells by vortexing vigorously.
11. Incubate the tubes in a 42°C water bath for 40 min.
12. Microcentrifuge at top speed for 30 sec. and remove supernatent w/ micropipette.
13. Pipette 1 mL of media into each tube, stir the pellet with a micropipette tip and vortex (gently).
14. For transformations using a nutritional marker proceed to step 15. For transformation using an antibiotic marker let incubate for 4 hours in rotary drum, spin down and discard supernatent and resuspend in 1 ml sterile water.
15. Plate 50 µL of each transformation on a selective plate.
16. Spin down the remaining 950 µL of each transformation, resuspend in 200 ul of sterile water and plate on to another selective plate.
17. Incubate the plates for 2-3 days at 30°C.

Frozen Competent Yeast Cells

<table>
<thead>
<tr>
<th>FCC Solution (50 mL)</th>
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<tbody>
<tr>
<td>10% DMSO</td>
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<tr>
<td>5% Glycerol</td>
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<tr>
<td>H2O</td>
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</tbody>
</table>

Preparing Competent Cells

1. Inoculate 5 mL YPD and grow overnight at 30C
2. Subculture 50 OD600 units (10^9 cells; Usually about 2.5mL of overnight culture) into 500 mL of pre-warmed YPD in a 1-2L flask
3. Incubate on a rotary shaker at 30C until titer is 2 x 10^7 cells/mL (~4-4.5 hours)
4. Harvest by centrifugation
5. Wash cells twice with 50+ mL of sterile water
6. Wash cells once with 5 mL of FCC solution
7. Resuspend cells in 2 mL FCC solution
8. Aliquot 50 uL of cell suspension into sterile microfuge tubes
Slowly cool tubes to -80°C
  a. Use a Mr. Frosty OR
  b. Put tubes into a styrofoam box and cover with room-temperature ice packs (do not put a lid on the box)
10. Transfer the tubes to a cardboard freezer box after they are completely frozen (~3 hours to overnight)

Transforming Competent Cells

1. Prepare transformation mix:

<table>
<thead>
<tr>
<th>Transformation Mix (1x)</th>
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<tbody>
<tr>
<td>100 mM LiOAc</td>
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<tr>
<td>10 µL 1M LiOAc</td>
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<tr>
<td>1 mg/mL ssDNA</td>
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<tr>
<td>10 µL 10 mg/mL ssDNA</td>
</tr>
<tr>
<td>DNA</td>
</tr>
<tr>
<td>1-5 µL DNA (PCR product or plasmid)</td>
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<tr>
<td>H2O</td>
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<tr>
<td>Volume to 50 µL with sterile water</td>
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2. Add 50 µL of transformation mix to 50 µL of competent cell suspension and mix thoroughly
3. Incubate at 30°C for 30 minutes
4. Add 300 µL of 40% PEG:

<table>
<thead>
<tr>
<th>40% PEG</th>
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<tbody>
<tr>
<td>40% PEG</td>
</tr>
<tr>
<td>240 µL 50% PEG 3350</td>
</tr>
<tr>
<td>100 mM LiOAc</td>
</tr>
<tr>
<td>30 µL 1M LiOAc</td>
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<tr>
<td>H2O</td>
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<tr>
<td>30 µL Sterile water</td>
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</tbody>
</table>

5. Incubate at 30°C for 30 minutes
6. Add 35 µL 100% DMSO
7. Incubate at 42°C for 15 minutes
8. Microcentrifuge at top speed for 30 sec. and remove supernatant w/ micropipette.
9. Pipette 1 mL of media into each tube, stir the pellet with a micropipette tip and vortex (gently).
10. For transformations using a nutritional marker proceed to step 11. For transformations using an antibiotic marker let incubate for 4 hours in rotary drum, spin down and discard supernatant and resuspend in 1 ml sterile water.
11. Plate 50 µL of each transformation on a selective plate.
12. Spin down the remaining 950 µL of each transformation, resuspend in 200 µL of sterile water and plate on to another selective plate.
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