Soft Agarose

Soft Agarose Protocol

Nathan Brandt, 9/01/2017

Adapted from Panasyuk, Nemazanyy, et. al. Biotechniques 2004

Soft Agar is used to easily grow up and collect a large-scale yeast transformation with a library. It is currently recommended to use 1 L of Soft Agar media per 2-8e6 transformed colonies you hope to obtain. This does mean you should do a small-scale transformation of your library to estimate your transformation efficiency.

1) Perform the transformation of your yeast cells. Remember a negative.
2) Prepare your soft agarose media, it should be cooled to room temperature by the time you are ready to plate your cells.
3) Prepare an ice water bath in the 4C room. You need to be able to completely submerge your media bottle into the ice water bath.
4) Plate 1e1, 1e2, 1e3, and 1e4 estimated transformants of your positive transformation, and all of your negative on a regular selective plate. Place plates in 30C incubator.
5) Add the remaining positive transformation to the soft agarose selective media. Stir with magnetic stir bar very gently.
6) Seal media bottle with lid and then wrap with parafilm.
7) Submerge into ice-water bath at 4C. Let sit for a full hour. Transfer gently to 30C.
8) After 2-3 days transformants should form. Calculate number of transformants from plates.
9) Check soft agarose container to make sure colonies look uniform throughout. An uneven distribution means it was either not stirred properly or not chilled properly. 10) Mix soft agarose and decant steriley into centrifluge tubes. Spin Down at 5000 rpm for 5 minutes. Remove Supernatant.
11) Resuspend cells in a minimal amount of the selective media. Add glycerol to bring it to 15%.
12) Aliquot Samples and store in the -80 C

Soft Agarose Media

    Use Media Bottle
    Make Media as normal.
    Add 3 g/L SeaPrep ultralow gelling soft agarose or equivalent
    Add a magnetic stir bar.
Autoclave.

Add any post-autoclave reagents. Mix without causing foaming.

Bring to Room Temp.

Ex. SC-URA Soft Agarose Media – 1 L

In 1 L media bottle

1.7 g Yeast Nitrogen Base without Amino Acids and without Ammonium Sulfate
5 g Ammonium Sulfate
2 g SC-URA Dropout Mix
Bring Volume to 950 mL with Milli-Q H2O
3 g SeaPrep ultralow gelling soft agarose

Add magnetic stir bar.
Mix Gently
Autoclave

50 mL 40% Glucose
Mix without causing foaming.

Bring to Room Temp

Example Run.
Transformed 1.51e9 cells with 9ug of MoBy-ORF plasmid library.

Calculating Transformation Efficiency

= # of Colonies on Plate

* Plating Factor (Proportion of resuspended cells plated)
* Plasmid/PCR Factor (Proportion of Plasmid/PCR out 1 ug transformed)
* # cells transformed/10^8

On the Plates

<table>
<thead>
<tr>
<th>Estimated Transformation Range</th>
<th>Number of Colonies</th>
<th>Plating Factor</th>
<th>Plasmid Factor</th>
<th># Cells per Transformation * 10^8</th>
<th>Transformation Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1e1</td>
<td>89</td>
<td>9000 (9 ml / 1 uL)</td>
<td>0.111 (1 ug / 9 ug)</td>
<td>15.1 (1.51e9/1e8)</td>
<td>1.34e6</td>
</tr>
<tr>
<td>1e2</td>
<td>478</td>
<td>900 (9 ml / 10 uL)</td>
<td>0.111 (1 ug / 9 ug)</td>
<td>15.1 (1.51e9/1e8)</td>
<td>7.22e5</td>
</tr>
<tr>
<td>1e3</td>
<td>3657</td>
<td>90 (9 ml / 100 uL)</td>
<td>0.111 (1 ug / 9 ug)</td>
<td>15.1 (1.51e9/1e8)</td>
<td>5.52e5</td>
</tr>
<tr>
<td>1e4</td>
<td>Unreadable</td>
<td>9 (9 ml / 1 mL)</td>
<td>0.111 (1 ug / 9 ug)</td>
<td>15.1 (1.51e9/1e8)</td>
<td>NA</td>
</tr>
<tr>
<td>Neg</td>
<td>0</td>
<td>1 (200 uL / 200 uL)</td>
<td>0</td>
<td>1 (1e8/1e8)</td>
<td>0</td>
</tr>
</tbody>
</table>

Average of Readable Plates = Transformation efficiency was 8.73e5 transformants/ug plasmid/1e8 cells.

Calculated transformants recovered

= Plate Count

* Dilution Factor

Average of (Plate Count * Dilution Factor) = 5.2e5 colonies formed * Volume of Transformation Added to Media (7889 ul) / Total Volume of Transformation (9000 uL) = 4.56e5 colonies