Transformation of Saccharomyces cerevisiae by the lithium acetate/single-stranded carrier DNA/polyethylene glycol protocol

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Introduction

Transformation of bacteria was first suggested by Griffiths in 1928. It was not until fifty years later that a system was reported by Hinnen et al. (Ref. 1) and Beggs (Ref. 2) for the induction of transformation in Saccharomyces cerevisiae. Further development by Ito et al. (Ref. 3) allowed transformation of intact yeast cells following exposure to alkali cations. This procedure was less complicated, but it yielded only 400 transformants/µg of plasmid DNA. Schiestl and Gietz (Ref. 4) increased the efficiency of the alkali cation protocol to 100 000 transformants/µg plasmid DNA by using single-stranded carrier DNA in the transformation mixture. Since this time, we have streamlined and optimized this protocol to give yields as high as $2.2 \times 10^7$ transformants/µg DNA (Ref. 5, 6, 7, 8). High efficiency is essential for transformation of cDNA expression libraries for the two-hybrid system (Ref. 9, 10) as well as other similar systems (Ref. 11, 12, 13).

Three transformation protocols are listed here. The ‘standard high-efficiency’ version of the lithium acetate (LiAc)/single-stranded DNA (ss-DNA)/polyethylene glycol (PEG) protocol is used when a large number of transformants are required. The ‘large-scale high efficiency’ version is used to obtain the millions of transformants needed to screen complex cDNA libraries. The ‘quick and easy’ version can be used when large numbers of transformants are not required.

Solutions

Lithium acetate (1.0 M)
Dissolve 5.1 g of lithium acetate dihydrate (Sigma Chemical Co. L-6883) in 50 ml sterile distilled deionized water (ddH2O). Autoclave the solution and store it at room temperature. To make 100 mM stock LiAc, dilute 1 ml of 1.0 M into 9 mls of sterile ddH2O.

Polyethylene glycol (PEG) MW 3350 (50% w/v)
Dissolve 50 g of PEG MW 3350 (Sigma Chemical Co. P-3640) in 30 ml sterile ddH2O on a stirring plate. Adjust the volume to 100 ml and autoclave. Store it in a securely capped bottle at room temperature. Evaporation from poorly sealed containers will increase the concentration of PEG and dramatically reduce transformation efficiencies.

Single-stranded carrier DNA (2 mg/ml)
Dissolve 200 mg salmon sperm DNA (Sigma catalogue number L-1626) in 100 ml sterile TE buffer (10 mM Tris-HCl, 1 mM Na2 EDTA, pH 8.0) by stirring overnight at 4°C. Dispense 0.5 ml samples of the solution into 1.5 ml microfuge tubes. Incubate the tubes in a boiling water bath for 5 min and then chill in ice water. Store the samples at −20°C. Alternatively, the double-stranded carrier DNA can be stored at −20°C and when needed, boiled for 5 min and chilled in ice water before use. Carrier DNA can be boiled, frozen and thawed three or four times with negligible effect on transformation efficiency, after which it should be discarded.

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Media
To condition the cells for transformation the yeast cells are grown in YPD + adenine (YPAD) medium. Transformed yeast cells are plated onto synthetic complete (SC) dropout media to select the transformed plasmid. Recipes for both are described in Gietz et al. (Ref. 10).

Standard high-efficiency transformation protocol (1 × scale)
The following procedure yields standard efficiencies of greater than 1 × 10^6 transformants/µg plasmid DNA when 10^8 cells are transformed with 100 ng plasmid DNA. Increasing the amount of plasmid DNA reduces the transformation efficiency but increases the transformation yield or total number of transformants produced. Plasmid DNA concentrations of up to 10 µg per transformation reaction can be used, but it is usually more productive to ‘scale up’ the transformation reactions for these quantities of DNA (Ref. 10).

Protocol 1: standard high-efficiency transformation (1 × scale)
Day 1
1. Inoculate yeast strain into 5 ml of YPAD or 10 ml of the appropriate SC-dropout medium and incubate overnight at 30°C on a shaker at 200 rpm.

Day 2
2. Determine the titre of the overnight culture. This can be done by measuring the OD_{600} of a 1 in 10 dilution into water. For most strains an OD_{600} of 0.1 corresponds to approximately 1 × 10^8 cells/ml. Alternatively, the titre can be determined using a haemocytometer.
3. Calculate the volume of overnight culture that contains 2.5 × 10^8 cells and add to prewarmed (30°C) YPAD medium, to give a total volume of 50 ml in a 250 ml flask. This will give a starting cell titre of about 5 × 10^6 cells / ml.
4. Incubate the diluted culture at 30°C on a shaker at 200 rpm until the cells have gone through two divisions (2 × 10^2 cells/ml, which is sufficient for 10 transformations). This may take 3–4 h, depending on the yeast strain.
5. Harvest the cells in a sterile 50 ml disposable centrifuge tube by centrifugation at 3000 × g for 5 min and remove the supernatant.
6. Resuspend the cells in 25 ml of sterile ddH2O to wash the cells, pellet again by centrifugation, and remove the supernatant.
7. Resuspend the cell pellet in a approximately 900 µl of sterile ddH2O and transfer to a 1.5 ml microcentrifuge tube.
8. Pellet the cells by centrifugation at 13 000 rpm for 1 min and resuspend the pellet in 100 mM LiAc to a final volume of 1 ml. (Add approximately 700 µl 100 mM LiAc.) Incubate at 30°C for 10 min.
9. For each 1 × transformation reaction aliquot 100 µl of the LiAc cell suspension into a new 1.5 ml microcentrifuge tube. Pellet the cells at top speed in a microcentrifuge for 1 min. Remove the supernatant.
10. Add the following reagents in the order listed to make the transformation mix for each transformation planned:

   50% PEG, 240 µl
   1.0 M LiAc, 36 µl
   ss-DNA (2 mg/ml), 50 µl
   plasmid DNA, X µl
   sterile ddH2O, 34 µl – X µl
   total volume, 360 µl

   *Note: the total volume of the 4th and 5th components is 34 µl.

   Vortex to mix and add the transformation mix to the cell pellet and vortex vigorously until the pellet is fully resuspended.
11. Incubate the transformation mixture at 30°C for 30 minutes.
12. Heat shock the cells in a 42°C water bath for 30 minutes.
13. Pellet the cells at top speed in a microcentrifuge for 1 min and remove the transformation mixture.
14. Add 1.0 ml of sterile ddH2O and resuspend by stirring the cell pellet with micropipette tip or gently pipetting the water up and down. Vortex briefly to ensure even resuspension and plate each sample onto the appropriate SC-dropout medium.
15. Incubate the plates at 30°C for 2–4 days until colonies appear.

Large-scale, high-efficiency transformation protocol
One and two-hybrid screens require a high yield of transformants for good coverage of complex cDNA libraries. For screens we use either a 30 × or a 60 × scale-up to obtain an adequate number of transformants. Before a large-scale transformation it is necessary to perform an experiment at the 1 × scale with increasing amounts of library DNA to determine the amount of plasmid DNA and the specific scale of transformation needed for good library coverage. Table 1 shows that it is more effective to do a 10 × scaled up with 10 × 1 µg of plasmid DNA than a 1 × transformation with 10 µg of plasmid DNA. In addition, this data shows that a 30 × scale transformation with 30 µg of plasmid DNA will generate approximately 3.0 × 10^7 transformants, which is good coverage for a library with a complexity of 1–2 × 10^6 independent clones.
In most one- and two-hybrid screens two different plasmids, such as the GAL4 binding (GAL4BD) and GAL4 activating domain (GAL4AD) fusion plasmids, are ultimately transformed into your yeast strain. The best strategy to optimize transformation yield is to initially transform the yeast strain with the bait or target plasmid. This innoculum is then transformed into the yeast strain containing the bait or target plasmid. This is done by growing an inoculum of the plasmid-containing yeast selecting for maintenance of the bait or target plasmid. This innoculum is then used to produce yeast cells that are competent for transformation by growth for at least two generations in a rich non-selective medium such as YPAD. This short growth in non-selective medium does not usually result in significant plasmid loss. These cells are then transformed with library plasmid DNA (up to 200 µg) to produce enough transformants for most screens. The following protocol is for a 30 °C scale-up, however a 120 °C scale-up, use a 100 mM LiAc and remove the supernatant.

Protocol 2: one or two-hybrid screening

Day 1
1. Inoculate the yeast strain containing the first plasmid into the appropriate SC-drop-out medium (usually SC-Trp) in an appropriate sized flask and incubate at 30 °C overnight (16–24 h) on a shaker at 200 rpm. For a 30 × scale-up, use 50 ml medium in a 500 ml flask; for a 60 × scale-up, use 100 ml medium in a 1 l flask.

Day 2
2. Determine the titre of the overnight culture. This can be done by measuring the OD_{600} of a 1 in 10 dilution. For most strains an OD_{600} of 0.1 corresponds to approximately 1 × 10^6 cells/ml. Alternatively, the titre can be determined using a haemocytometer.
3. Calculate the volume of overnight culture that contains the number of cells needed (Table 2). Harvest that volume of SC-dropout culture by centrifugation and resuspend the cells in the appropriate volume of YPAD medium which has been pre-warmed to 30 °C. This will give a starting cell titre of 5 × 10^6 cells/ml. In most cases the SC-Trp culture will give a cell titre of approximately 2 × 10^7 cells/ml which corresponds to an OD_{600} of approximately 2.0.
4. Incubate the YPAD culture at 30 °C and 200 rpm until the cells have gone through 2 divisions, which may take 3–4 h, depending on the strain. The cell titre should be at least 2 × 10^7 cells/ml which corresponds to an OD_{600} of approximately 2.0.
5. Harvest the cells in 3 or 6 sterile 50 ml disposable centrifuge tubes by centrifugation at 3000 × g for 5 min and discard the supernatant.
6. Resuspend the cells from each 50 ml centrifuge tube with 25 ml of sterile ddH_2O and pellet again by centrifugation and remove the supernatant.
7. Resuspend and pool the washed cells in the appropriate volume of 100 mM LiAc (Table 3) and incubate in a water bath at 30 °C for 15 min.
8. Transfer the cells in a 50 ml disposable centrifuge tube, harvest by centrifugation at 3000 × g for 5 min, and discard the supernatant.
9. Add, in the order indicated, the transformation mix components listed in (Table 4) to a separate tube and mix thoroughly by vortexing. Add the mixed transformation mix to the cell pellet and vortex vigorously to resuspend the cell pellet in the transformation mix.
10. Incubate in a water bath at 30 °C for 30 min. Mix the tube gently several times after 10 and 20 min to mix the contents thoroughly.

Table 1. Transformation efficiency vs transformation yield

<table>
<thead>
<tr>
<th>Plasmid DNA</th>
<th>TRAFO efficiency</th>
<th>TRAFO yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 µg</td>
<td>9.5 × 10^2</td>
<td>9.5 × 10^4</td>
</tr>
<tr>
<td>1.0 µg</td>
<td>1.1 × 10^6</td>
<td>1.1 × 10^6</td>
</tr>
<tr>
<td>10 µg</td>
<td>2.7 × 10^8</td>
<td>2.7 × 10^8</td>
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</tbody>
</table>

Yeast strain PJ69-4a was transformed with increasing amounts of YEplac181 plasmid DNA (Ref. 15).

<table>
<thead>
<tr>
<th>Number of cells needed</th>
<th>30 × scale</th>
<th>60 × scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of SC-Trp culture</td>
<td>37.5 ml</td>
<td>75 ml</td>
</tr>
<tr>
<td>Volume of YPAD</td>
<td>150 ml</td>
<td>300 ml</td>
</tr>
<tr>
<td>Volume of flask</td>
<td>1 l</td>
<td>2 l</td>
</tr>
</tbody>
</table>

Volume of YPAD = number cells needed / cell titer (cells/ml) of SC-Trp culture.

<table>
<thead>
<tr>
<th>Lithium acetate volumes</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mM LiAc</td>
</tr>
<tr>
<td>Centrifuge tube</td>
</tr>
</tbody>
</table>

Note: always measure the OD_{600} of this culture after a 1/10 dilution.
the cells in a total volume of either 20 or 40 mls and plate of selective medium. For two hybrid screens we resuspend listed in (Table 5) and plate 400 transformation mix.

11. Heat shock the cells in a 42°C water bath for 45 min. Invert the tube gently several times at 5 min intervals to mix the contents and aid in temperature equilibration.

12. Centrifuge at 3000 × g for 5 minutes and remove the transformation mix.

13. Resuspend the cells in the volume of sterile water listed in (Table 5) and plate 400 µl samples onto each plate of selective medium. For two hybrid screens we resuspend the cells in a total volume of either 20 or 40 mls and plate 400µl onto each of either 50 (30 ×) or 100 (60 ×) 150 mm plates of SC-Trp, Leu, His (containing 3AT) medium, respectively.

14. Incubate at 30°C for 4–7 days at 30°C.

We have successfully scaled up this protocol to 120 × scale. With the large volumes of cells and reagents it is important to mix the tubes during all incubations at 30°C and 42°C to ensure good temperature transfer.

Table 4. Transformation mix components

<table>
<thead>
<tr>
<th>Order</th>
<th>30 × scale</th>
<th>60 × scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st * 50% PEG</td>
<td>7.20 ml</td>
<td>14.40 ml</td>
</tr>
<tr>
<td>2nd* 1.0 M LiAc</td>
<td>1.08 ml</td>
<td>2.16 ml</td>
</tr>
<tr>
<td>3rd* ss-DNA (2 mg/ml)</td>
<td>1.50 ml</td>
<td>3.00 ml</td>
</tr>
<tr>
<td>4th* Plasmid DNA</td>
<td>A ml</td>
<td>8 ml</td>
</tr>
<tr>
<td>5th* sterile ddH2O</td>
<td>1.02 – A ml*</td>
<td>2.04 – B ml*</td>
</tr>
</tbody>
</table>

Note: the amount of sterile ddH2O, plasmid DNA can each vary but the total amount must not exceed the volume listed in the 5th row.

Table 5. Volume of sterile ddH2O for resuspension and plating

<table>
<thead>
<tr>
<th>Volume of ddH2O</th>
<th>30 × scale</th>
<th>60 × scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2O</td>
<td>20 ml</td>
<td>40 ml</td>
</tr>
<tr>
<td>Plating volume</td>
<td>400 µl</td>
<td>400 µl</td>
</tr>
<tr>
<td>Number of 150 mm plates</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

11. Heat shock the cells in a 42°C water bath for 45 min. Invert the tube gently several times at 5 min intervals to mix the contents and aid in temperature equilibration.

12. Centrifuge at 3000 × g for 5 minutes and remove the transformation mix.

13. Resuspend the cells in the volume of sterile water listed in (Table 5) and plate 400 µl samples onto each plate of selective medium. For two hybrid screens we resuspend the cells in a total volume of either 20 or 40 mls and plate 400µl onto each of either 50 (30 ×) or 100 (60 ×) 150 mm plates of SC-Trp, Leu, His (containing 3AT) medium, respectively.

14. Incubate at 30°C for 4–7 days at 30°C.

We have successfully scaled up this protocol to 120 × scale. With the large volumes of cells and reagents it is important to mix the tubes during all incubations at 30°C and 42°C to ensure good temperature transfer.

Quick and easy transformation

This protocol is used when only a few transformants for a specific plasmid are desired, such as for a GAL4 binding domain plasmid or a one-hybrid target plasmid. Yeast grown overnight on agar medium can be transformed up to an efficiency of 1 × 10^4 transformants/µg plasmid DNA. This is usually more than adequate for the isolation of a transformant for subsequent work.

1. Grow the culture to be transformed overnight on YPAD, YPD or appropriate SC-minus medium.

2. Scrape approximately a 25 µl volume of cells from the plate with a sterile toothpick or inoculating loop and resuspend in 1.0 ml of sterile water in a 1.5 ml microfuge tube.

Note: To determine the size of a 25 µl volume of yeast, scrape a blob of yeast using a sterile toothpick or loop into 100 µl of sterile water and resuspend by vortexing. Measure the resulting volume with a micropipette to determine the volume contributed by the yeast cells.

3. Vortex vigorously to wash the cells.

4. Pellet the cells at top speed in a microcentrifuge for 30 sec.

5. Add to the cell pellet in the following order:

- 240 µl PEG
- 36 µl 1.0 M LiAc
- 50 µl ss-DNA
- 34 µl containing plasmid DNA (0.1–5.0 µg) and sterile ddH2O, giving a final volume of 360 µl.

Vortex vigorously until resuspended.

6. Heat shock the cells in a 42°C water bath for 30 min.

7. Pellet the cells at top speed in a microcentrifuge for 1 min and remove the transformation mixture.

8. Add 400 µl of sterile ddH2O to the pellet and incubate at room temperature for 5 min. Resuspend by vortexing or gently pipetting the fluid up and down with a micropipette. Plate 200 µl samples onto 2 plates of the appropriate SC-dropout medium.

9. Incubate at 30°C for 2–4 days.

This protocol can be applied to cultures which have been stored for several days or weeks. However, the yield of transformants is best with fresh cultures.

Common problems and their solutions

Transformation efficiency and yield can be affected by a number of factors. Perhaps the most important is the yeast strain. Some strains transform well while others transform with a much reduced efficiency (Ref. 6). Efficiency can be improved by optimizing the amount of plasmid and ss-carrier DNA and by increasing or decreasing the duration of the heat shock at 42°C (Ref. 7). If these fail to produce an increase in transformation efficiency or yield, it is best to consider using another yeast strain.

The growth of a culture to logarithmic phase is also important. Two cell divisions appear to be required for optimal transformation competence in yeast, however efficiency remains high for an additional two divisions. Yeast cells should be grown with vigorous aeration. Baffle flasks (Bellco™) produce better aeration than standard conical flasks but are not essential.

The transformation efficiency and yield can also be affected by the preparation and storage of the SC-drop-out
medium. The pH of the medium should be adjusted to 5.6 before the addition of the agar and it should be autoclaved for no more than 20 min. Some components of SC medium are light sensitive and liquid and plates should be stored in the dark at all times.

Plasmid DNA can be purified by numerous methods without affecting its transformation characteristics. The same amount of plasmid DNA purified by the miniprep method transforms just as effectively as that purified by the CsCl density gradient method. The size of the plasmid also affects transformation, smaller plasmids are more effective than large ones. We have not tested our protocols for YAC transformation.

References

Products Used
- lithium acetate dihydrate: lithium acetate dihydrate from Sigma
- PEG MW 3350: PEG MW 3350 from Sigma
- sperm DNA: sperm DNA from Sigma
- Baffle flasks: Baffle flasks from Bellco Glass Inc