High-Efficiency Yeast Electroporation V.2

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WORKS FOR ME 1

ABSTRACT

For when extremely high transformation efficiencies are needed (e.g., for transforming plasmid DNA libraries). Adapted from An improved yeast transformation method for the generation of very large human antibody libraries.

Overview:
Using strain BY4742, transformation efficiencies were optimal using 400 µl of concentrated cells and 1 µg of plasmid DNA, yielding ~3.5 million transformants per electroporation. It is possible that other strains may have different optima. The protocol is designed to allow for 2 electroporations per culture (yields ~1ml of concentrated cells) and can be scaled up for producing libraries.

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GUIDELINES

The OD_{600} values are based on those obtained from a Unico 1100RS Spectrophotometer.

MATERIALS TEXT

Growth Media:
YPD (1% yeast extract, 2% peptone, 2% dextrose)
2x YPD (2% yeast extract, 4% peptone, 4% dextrose)

Reagents:

- 2M Lithium Acetate (LiOAc)
- 1 mM Dithiothreitol (DTT)
- 1M Sorbitol
- 2M Sorbitol
- 100 mM $CaCl_2$
- Sterile ddH_2O

Electroporation Materials:
2 mm gap electroporation cuvettes (VWR Cat# 89047-208)
Bio-Rad Gene Pulser Electroporation System

Recipes:

Electroporation Buffer (500 ml):

- 250 ml 2M Sorbitol
- 5 ml 100 mM $CaCl_2$
- 150 ml ddH_2O

Conditioning Buffer (100 ml) :

- 50 ml sterile ddH_2O
- 5 ml 2M LiOAc
- 1 ml 1M DTT
- 44 mL sterile ddH_2O

Note: Add in order, as precipitation will occur if undiluted LiOAc and DTT are added together

Outgrowth Media (100 ml):

- 50 ml 2x YPD
- 50 ml 1M Sorbitol

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SAFETY WARNINGS

Lithium acetate is harmful if ingested and irritating to the eyes and skin. Wear appropriate personal protective equipment, including eye protection, lab coat, long pants, closed toed shoes, and nitrile gloves. Be sure to follow your Institution's guidelines for proper disposal.

2 days before starting experiment

2d

2d

1 Streak out yeast strains to be transformed onto YPD agar and grow at 30°C for 2 days.

Day before experiment

16h

16h

2 For each transformation, inoculate cells into 4 separate tubes containing 5 ml of YPD, and grow to saturation overnight at 30°C with 270 rpm orbital shaking.

Day of experiment: preparation of electrocompetent cells

4h 15m

5m

3 Pool the individual 5-ml overnight cultures into a sterile 50-ml conical tube and vortex to mix.

5m

4 Measure the OD_{600} of the saturated cells.

- Generally need to make a 1:20 or 1:50 dilution to be within the linear range of the spectrophotometer.

5m

5 In a 500-ml flask, dilute cultures to be transformed to a starting OD_{600} of 2 in 100 ml of 2x YPD.

Example: If the overnight culture has an OD_{600} of 10, use the solution dilution equation ($C_1V_1 = C_2V_2$) to solve for the amount of cells to add:

- C_1 = 2 OD_{600} units (target OD_{600}), V_1 = 100 ml (target volume), C_2 = 10 OD_{600} units (saturated OD_{600}), V_2 = ? (what we are solving for)
- Rearrange the equation: $V_2 = C_1V_1 / C_2$
- V2 = (2 OD_{600} units x 100 ml) / 10 OD_{600} units = **20-ml saturated cells**
- For 100 ml total, add 20-ml saturated cells to 80-ml 2x YPD.

4h

6 Grow the 100-ml inoculated cultures at 30°C with 270 rpm orbital shaking for 4 hours.

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15m

While cultures are incubating: prepare media

5m

7 Place sterile water on ice and store at **4°C until ready to use.**

- 100 ml of sterile water per transformation

10m

8 Prepare electroporation buffer, place on ice, and store at **4°C until ready to use.**

- 100 ml of electroporation buffer per transformation.

Electroporation Buffer (500 ml):

- 250 ml 2M Sorbitol
- 5 ml 100 mM $CaCl_2$
- 150 ml ddH_2O

9 Prepare conditioning buffer, and store **at room temperature until ready to use.**

- 20 ml of conditioning buffer per transformation.

Conditioning Buffer (100 ml) :

- 50 ml sterile ddH_2O
- 5 ml 2M LiOAc
- 1 ml 1M DTT
- 44 mL sterile ddH_2O

Note: Add in order, as precipitation will occur if undiluted LiOAc and DTT are added together.

51m

After 4 hour incubation

3m

10 Split yeast culture into two 50-ml conical tubes, collect cells by gentle centrifugation at 1500 rcf for 3 minutes, and decant the media.

3m

11 Resuspend (wash) each cell pellet by gently pipetting with 25-ml ice-cold **sterile water**, centrifuge at 1500 rcf for 3 minutes, and decant the media.

3m

12 Wash the cell pellets again with 25-ml ice-cold **sterile water**, centrifuge at 1500 rcf for 3 minutes, and decant

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the media.

13 Wash the cell pellets with 25-ml of ice-cold **electroporation buffer**, centrifuge at 1500 rcf for 3 minutes, and decant the media. 3m

14 Resuspend the cell pellets in 10-ml **conditioning buffer** and pool into a single 125-ml flask. Incubate at 30°C with 270 rpm orbital shaking for 30 minutes. 30m

15 Pour cells into a 50-ml conical tube, centrifuge at 1500 rcf for 3 minutes, and decant the media. 3m

Note: follow institutional waste disposal procedures for unused or 'spent' conditioning buffer.

16 Wash the cells with 50-ml ice-cold **electroporation buffer**, centrifuge at 1500 rcf for 3 minutes, and decant the media. 3m

17 Resuspend the cell pellet to a final volume of 1 ml by adding 100-200 µl electroporation buffer. 3m

- If you need to add more or less electroporation buffer than this, you likely need to optimize your initial inoculation OD_{600}.

18 Keep cells on ice until electroporation.

Electroporation, outgrowth, and plating

1h 8m

19 Pre-chill electroporation cuvette(s) on ice.

- 1 cuvette per transformation.

20 Prepare Outgrowth Media.

- 8 ml per sample, aliquoted into 50-ml conical tubes.

Outgrowth Media:

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- 50 ml 2x YPD
- 50 ml 1M Sorbitol

21 For each electroporation, transfer 1 µg of DNA and 400 µl of concentrated cells to a microcentrifuge tube and gently mix by pipetting.

5m

22 Pipet concentrated cells plus DNA into a pre-chilled Gently electroporation cuvette and keep on ice for 5 minutes.

23 Electroporate the cells with the following instrument settings:

- Voltage: 2.5 kV
- Capacitance: 25 µF
- Resistance: 200 Ohms

- Typical time constant ranges from 3.0 to 4.5 milliseconds.

24 Using a sterile Pasteur pipette, transfer electroporated cells into the 50-ml conical tube containing 8 ml of **outgrowth media.**

- To reduce the amount of cells left in cuvette, ~500 µl of the outgrowth media can be pipetted into the cuvette and then transferred back into the 50-ml conical with the rest of the outgrowth media.

1h

25 Incubate the 50-ml conical tube containing the cells plus 8-ml conditioning media at 30°C **without shaking** for 1 hour, mixing every 15 minutes by gentle inversion.

- Orbital shaking reduces transformation efficiency, possibly due to increased fragility of electroporated cells.

3m

26 Following outgrowth, collect cells by gentle centrifugation at 1500 rcf for 3 minutes, and decant the media.

27 For auxotrophic marker selection, resuspend cells in the appropriate yeast dropout media (e.g., for uracil selection, resuspend cells in SC -Ura). For drug marker selection, resuspend cells in YPD.

28 For auxotrophic marker selection, plate directly on selective media. For drug marker selection, can either plate directly to selective media, or can plate to YPD and replica print to selective media the next day.

- Replica printing often increases the number of transformants recovered when using drug markers.
- Alternatively, can perform selection in liquid or semi-solid (low-percentage agarose) media and then pellet the transformants.
- To determine the total number of transformants (e.g., if plating to confluency or performing a liquid or

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semi-solid selection), plate 100 µl of 10-fold serial dilutions down to a 10^{-5} dilution.

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