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Electroporation Transformation Protocol in S. Cerevisiae

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ABSTRACT

Protocol adapted by Stephanie Hood, 09/2022, from:

An improved yeast transformation method for the generation of very large human antibody libraries

Overview:

This protocol calls for ~800-900 ul of cell pellet and is optimized for **1 ug** of plasmid DNA in the **lab strain** (BY4742).

PROTOCOL CITATION

Stephanie Hood 2022. Electroporation Transformation Protocol in S. Cerevisiae. **protocols.io** https://protocols.io/view/electroporation-transformation-protocol-in-s-cerev-cizquf5w

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MATERIALS TEXT

Reagent List:

- 1X YPD
- 2X YPD
- Sterile Water
- 2M LiOAC
- 1 mM DTT
- 1M Sorbitol
- 2M Sorbitol
- 100 mM CaCl2
- SD Ura- liquid
- SD Ura plates

2-3 Days before starting experiment

2d

2d

1

- Streak out yeast strains for electroporation protocol.
- Depending on how many cultures you are setting up you may need to streak more than 1 plate to get enough isolated colonies. Grow at 30°C for 2 days.

Day before experiment

16h

2

- Grow 5 ml culture(s) of *S. cerevisiae* cells overnight to stationary phase (OD600 to or about 3) in 1XYPD media on a platform shaker at 270 rpm and 30°C.
- Need enough cells to set up 100 ml culture(s).

Day of experiment: Preparation of electrocompetent cells

4h 15m

15m

3

- Turn on spectrophotometer (cuvette)

4
- If you have multiple tubes from the same strain, pool the 5 mL O/N cultures into a sterile 50 mL conical tube
- Vortex to mix

5
- Spec the O/N cells
- 1:50 dilution (20 ul cells: 980 ul 1X YPD)

6
- Set up the culture(s) in 500 mL flasks.

Example:
100 ml 2X YPD culture — starting OD600 of 2.0

4h

7
- Use C1V1=C2V2 to calculate the amount of O/N cells you need to add to 100 ml for a starting OD600 of _____.
- Grow the inoculated cultures on a platform shaker at 30°C and 270 rpm for 4 hours.

While cultures are incubating: prepare media

8
- Put **sterile water** on ice and place at 4°C.
- ~100 mL of sterile water needed per reaction.

9
- Prepare **electroporation buffe**r and put in ice at 4°C.

500 mL Electroporation buffer: ~100 mL needed per reaction

- Add 250 mL 2M Sorbitol to a sterile 500 mL bottle
- Add 5 mL 100 mM CaCl2
- Bring volume to 500 mL with sterile water.

39m

After 4 hour incubation

10
- Collect yeast cells by centrifugation at **1500 g for 3 minutes** and remove the media.
- 2-50 ml conical tubes - 50 ml each

11 ▪ Prepare **conditioning buffer** during spins- **0.1 M LiAc/10 mM DTT**.

Conditioning Buffer: 20 mL needed per reaction

- Add 50 mL sterile water to a sterile 250 mL sterile bottle
- Add 5 mL 2M Lithium Acetate
- Add 1000 ul 1M DTT
- Bring volume to 100 mL (44 mL sterile water added with a serological pipette)

Put any remaining conditioning buffer in a well labeled 50 mL conical tube for disposal with EHS.

3m

12 ▪ Wash the cell pellets with 25 mL ice-cold water.
▪ 50 ml conical tubes - 25 ml each
▪ Spin at **1500 g for 3 minutes** and remove the media.

3m

13 ▪ Wash the cell pellets again with 25 mL ice-cold water.
▪ 50 ml conical tubes - 25 ml each
▪ Spin at **1500 g for 3 minutes** and remove the media.

3m

14 ▪ Wash the cell pellet with 25 mL of ice-cold electroporation buffer (**1M Sorbitol / 1 mM CaCl2**)
▪ 50 ml conical tubes - 25 ml each
▪ Spin at **1500 g for 3 minutes** and remove the media.

30m

15 ▪ Condition the yeast cells by re-suspending the cell pellet in **10 mL 0.1 M LiAc/10 mM DTT.**
▪ **50 ml** conical tubes - **10 ml each**
▪ Pool the tubes culture into one flask for the outgrowth.
▪ 10 ml of conditioning buffer for each tube, then pool in a 125 mL flask.
▪ **Shake at 270 rpm in a culture flask for 30 minutes at 30 °C**.

3m

16 ▪ Pour cells into a 50 mL conical tube.
▪ Collect the conditioned cells by centrifugation (**1500g for 3 minutes**).

3m

17 ▪ Wash the cells once with 50 mL ice-cold electroporation buffer.
▪ Spin at **1500 g for 3 minutes** and remove the media.

18 ▪ Re-suspended the cell pellet in **100 to 200 µL** electroporation buffer to reach a final volume of 1 mL.
▪ Record the volume needed to get to 1 mL

Example: 700 ul cell pellet + 300 ul electroporation buffer = 1 mL
2 electroporation reactions of 400 µl each.

You may need to add more electroporation buffer to reach 1 mL cell mixture.
If only doing 1 transformation, less volume can be added as only 400 ul is needed per transformation.

19 **The cells are kept on ice until electroporation.**

DNA preparation and electroporation and plating

20
- Pre-chill BioRad GenePulser cuvette(s) (0.2 cm electrode gap).
- 1 cuvette per transformation

21
- Prepare enough outgrowth media for all your samples: **1:1 1M sorbitol: 2X YPD**

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

22
- Use **1 ug** of plasmid DNA per 400 ul of cells.
- Add ____ uL of plasmid DNA to the 1 mL of cells = 1 ug per 400 uL.
- 2.5 ug of plasmid DNA in 1 mL of cells.

5m

23
- Gently mix **400 µl** electrocompetent cells and DNA and transfer to a pre-chilled BioRad GenePulser cuvette (0.2 cm electrode gap).
- Kept on ice for 5 minutes until electroporation.

24
- **Electroporate the cells** at 2.5 kV and 25 µF.
- Typical time constant ranges from 3.0 to 4.5 milliseconds.
- 200 Ohms

25
- Using a sterile pasteur pipette, transfer electroporated cells from each cuvette into **8 mL of 1:1 mix of 1 M sorbitol : 2X YPD media**.

1h

26
- Incubate the 50 mL conical tube containing the 8 mL of recovery media in the 30°C standing incubator for 1 hour.
- Mix by gentle inversion (~4x) every 15 mins.
- Use a rack to store 50 mL conical tubes in the incubator. Do not store conical tubes upside down as they tend to leak.

3m

27
- Collect cells by centrifugation (**1500g for 3 minutes**) and resuspend in SD URA- + 2% glucose.

- Use 1 mL SD URA- + 2% glucose media for every 400 µl electroporated cells.

28

- Prepare 10-fold serial dilutions from the cell suspension and plate 100 µl of 10-2, 10-3, and 10-4 dilutions onto selection plates and incubate at 30 °C incubator for 2 days.
- If using an antibiotic marker for selection, let the cells grow on just YPD for 1 days and then replica print onto YPD + antibiotic the following day.

2d

2-3 days after experiment

29

- Count the number of colonies on each plate to determine the CFUs.