Transformation electroporation

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[1]In-house protocol

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1 *Works for me* This protocol is published without a DOI.

Eadewunm

ABSTRACT

Preparation of component cells and their transformation by electroporation

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- Preparation of component cells
- Transformation by electroporation

DISCLAIMER:

ABSTRACT

Preparation of component cells and their transformation by electroporation

BEFORE STARTING

Once cells are grown up, keep on ice!

Preparation of component cells

1 Inoculate a single colony into appropriate media with antibiotics as needed. Grow overnight at the appropriate temperature and shaking conditions.

2 Dilute ON culture back to an optical density of 0.01 in the morning in 25 mL of cells for every two transformations (4 transformations would be 50 mL).

3 row at appropriate conditions until OD_{600} is ~0.4 to 0.6. Do not grow past this point.

4 Pour cells into 50 mL conicals. Spin down cells at 3500 RPM at 4°C for 10 minutes (program 1 in swinging bucket centrifuge)

5 Resuspend pellet in an equal volume of ice-cold water as the amount of culture placed in the conical. Spin cells down again.

6 For every 25 mL of culture, resuspend pellet in 800 µL of ice-cold water, place into Eppendorf tube that has been chilled on ice.

7 Spin down cells at 13,000 RPM at 4°C for 3 minutes.

8 Aspirate supernatant.

9 Resuspend pellet in 100 µL ice-cold water per 25 mL of culture.

Transformation by electroporation

10 Place the appropriate number of gene pulse cuvettes and Eppendorf tubes on ice in advance.

11 To the chilled Eppendorf tube, mix 40-50 µL of competent cells, and the appropriate amount of ligation or plasmid DNA.

12 Transfer to a chilled cuvette; make sure you tap so there are no air bubbles!

13 Wipe off any water/ice from the cuvette.

14 Electroporate using EC 2 on electroporator.

15 Immediately place back on ice; read the time constant and note this in your notebook.

16 Once complete, add 0.5 mL SOC media to rescue cells.

17 Transfer cell mixture to a 14 mL snap-cap tube.

18 Recover at appropriate growth conditions for 1 hour.

19 For ligations, transfer recovery to an Eppendorf tube and spin down at 13000 RPM for 5 minutes. Resuspend pellets in 100 µL and plate on the appropriate plate with appropriate antibiotics.

20 For whole plasmids, plate a dilution series. 10^{-3} to 10^{-6} suggested.

21 Incubate overnight at the appropriate temperature.