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Transformation electroporation

Elizabeth Fozo¹¹In-house protocol

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Works for me

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ABSTRACT

Preparation of component cells and their transformation by electroporation

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GUIDELINES

- Preparation of component cells
- Transformation by electroporation

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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 Grow at appropriate conditions until OD₆₀₀ 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 μ L 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.