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Electroporation & Electrocompetent Cell Preparation Protocol for DAP auxotroph E.coli WM3064

Forked from Electroporation Protocol

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We regularly use this protocol.

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Abstract

This is a protocol for making electrocompetent E.coli WM3064 cells and transforming them with DNA through electroporation. This protocol produces 30 µL aliquots of electrocompetent cells, each enough for one prep of electroporation.

Guidelines

Electroporation Protocol
The electroporation protocol will vary depending on the strain so this protocol may need to be optimized.

Transformation efficiency calculation
If the culture was diluted 1000-fold when plated, the total cfu per ml is 1000 times the number of colonies counted. The cfu is divided by the amount of plasmid (e.g. 10 pg per ml)

Transformation efficiency = cfu/ µg = (colonies counted*1000) / (0.00001 µg plasmid)

Materials

MATERIALS

- Magnesium sulfate heptahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M2773
- NaCl **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**53014
- Tryptone **Fisher Scientific Catalog #**BP1421-500
- Glucose **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**G8270
- Magnesium chloride hexahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M2670
- Potassium chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P9333
- Glycerol **Thermo Fisher Scientific Catalog #**17904
- Yeast Extract **Thermo Fisher Catalog #**211930
- 2,6-Diaminopimelic acid **Thermo Scientific**

Media

SOB + DAP:
2% tryptone
0.5% yeast extract
10 mM NaCl
2.5 mM KCl
10 mM MgCl2
10 mM MgSO4
0.3 mM DAP

SOC + DAP :
SOB + 20 mM glucose + 0.3 mM DAP

LB agar + DAP + antibiotic
LB agar + 0.3 mM DAP + appropriate antibiotic

Appropriate Antibiotics for Your Application
Antibiotics for Plasmid selection

	A	B
	Antibiotic	**Working Concentration**

	A	B
	Ampicillin	100 µg/ml
	Carbenicillin	100 µg/ml
	Chloramphenicol	33 µg/ml
	Kanamycin	50 µg/ml
	Streptomycin	25 µg/ml
	Tetracycline	15 µg/ml

Protocol materials

- 2,6-Diaminopimelic acid **Thermo Scientific**
- Glycerol **Thermo Fisher Scientific** **Catalog #**17904
- Potassium chloride **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**P9333
- Yeast Extract **Thermo Fisher** **Catalog #**211930
- Magnesium sulfate heptahydrate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**M2773
- Tryptone **Fisher Scientific** **Catalog #**BP1421-500
- Glucose **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**G8270
- Magnesium chloride hexahydrate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**M2670
- NaCl **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**53014

Safety warnings

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

Before start

Quite a bit of glycerol gets left behind in the tube during the washing steps while discarding the supernatant. Note that this dilutes the cells a bit. Prepare aliquots based on the cell quantity required specifically for your use.

Dilute the plasmid to have 1 - 3 µL of DNA (<100 ng) per electroporation prep. Calculate the amount of DNA used per prep to determine the transformation efficiency later.

The electroporation mixture ratio will vary depending on the strain and DNA size so this protocol may need to be optimized.

Make sure to include a negative control which also serves as a "survival control". This mixture should contain only electrocompetent cells and no plasmid. Plate this mixture on LB+DAP agar to assess whether the cells survive the electroporation pulse.

This protocol was developed as part of a master's thesis affiliated with the Microalgae & Microbiomes Research Group at NFH, UiT, Norway.
M2RG Website : https://uit.no/research/micro

Growing overnight culture

1 Add a loopful of bacterial culture to 5 mL LB broth.

2 Incubate Overnight at 37 °C with shaking.

Making electrocompetent cells

3 Add 10 mL SOB medium containing [M] 0.3 millimolar (mM) 2,6-Diaminopimelic acid **Thermo Scientific** in a 50 mL conical flask.

4 Inoculate each flask with overnight culture to OD600 = 0.05

5 Grow cells until mid-exponential phase (OD600 = 0.6) or 02:00:00

6 Transfer the cells to a 15 mL Falcon tube

7 Chill the culture On ice for 00:10:00 .

8 Centrifuge the cells at 6000 rpm, 4°C, 00:05:00 .

9 Discard the supernatant.

10 Wash the pellet by adding 10 mL chilled [M] 10 % volume Glycerol **Thermo Fisher Scientific Catalog #**17904 and vortex vigourously.

11 Centrifuge 6000 rpm, 4°C, 00:03:30 and discard the supernatant.

12 go to step #10 Repeat for a total of 4 wash cycles .

13 Resuspend the washed pellet in 100 µL [M] 10 % volume Glycerol **Thermo Fisher Scientific Catalog #**17904

14 Make 30 µL aliquots in sterile microcentrifuge tubes. Proceed to electroporation or store at -80 °C . One tube allows for one prep of electroporation.

Thawing and preparation

15 Turn on electroporator and set to **1.5 kv**, **200 ohms** and **25 µF**.

16 Pre-warm recovery SOC+DAP medium and LB-antibiotic plates at 37 °C .

17 Thaw microcentrifuge tubes with electrocompetent cells On ice or use freshly made cells.

18 Place appropriate number of 1 mm-electroporation cuvettes On ice .

Electroporation and recovery

19 Add 1 µL DNA solution (<100 ng) to 30 µL electrocompetent cells in microcentrifuge tubes.

Note

This mixture can be optimized for your strain and plasmid. Trying a few combination ratios is recommended in order to determine which one yields the highest transformation efficiency. A lower amount of DNA usually yields higher efficiency.

20 Transfer the DNA-cell mixture to the cold cuvette, tap on countertop 2X, wipe water from exterior of cuvette and place in the electroporation module and press pulse (**don't hold**

the button down).

21 Immediately add 970 µL 37°C SOC + DAP , mix by pipetting up and down once and transfer to a microcentrifuge tube.

Note

Try to add the recovery medium as soon as possible. Transformation efficiency sharply declines with increase in time between pulse and addition of recovery medium.

22 Incubate at 37 °C for 01:00:00 .

Dilution plating and efficiency calculation

23 Make appropriate dilutions and plate 100 µL on LB agar + DAP + antibiotic plates.

24 Incubate Overnight at 37 °C .

Note

Transformation efficiency calculation:

If the culture was diluted 1000-fold when plated, the total cfu per ml is 1000 times the number of colonies counted. The cfu is divided by the amount of plasmid (e.g. 10 pg per ml)

Transformation efficiency = cfu/ µg = (colonies counted*1000) / (0.00001 µg plasmid)

Protocol references

1. Short Protocols in Molecular Biology, Chapter 1.
2. Datsenko, K.A., and Wanner, B.L. 2000. One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. Proc. Natl. Acad. Sci. U. S. A. 97: 6640-6645.
3. https://barricklab.org/twiki/bin/view/Lab/ProtocolsElectrocompetentCells

Acknowledgements

This protocol was developed as part of a master's thesis affiliated with the Microalgae & Microbiomes Research Group at NFH, UiT, Norway.
M2RG Website : https://uit.no/research/micro

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