Nov 23, 2020

CK111 comp cells electroporation

Elizabeth Fozo[1]

[1]In-house protocol

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

Eadewunm

PROTOCOL CITATION

Elizabeth Fozo 2020. CK111 comp cells electroporation. **protocols.io** https://protocols.io/view/ck111-comp-cells-electroporation-bpz2mp8e

CREATED

Nov 23, 2020

LAST MODIFIED

Nov 23, 2020

PROTOCOL INTEGER ID

44826

GUIDELINES

Lysozyme solution:

- 100µL 1M Tris-HCl
- 2.5 g sucrose
- 200 µL 0.5M EDTA
- 100 µL 1M Tris-HCl
- 8.6 mL of water
- Autoclave solution. Add lysozyme just before use

Electroporation buffer:

- 8.56 g sucrose
- 5 mL 10% glycerol
- Water to 50 mL
- Autoclave solution.

DISCLAIMER:

DISCLAIMER: THIS IS A WORK IN PROGRESS. IT IS FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer-reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

How to generate competent CK111/pCF10-101 cells for transformation

1 In 10 mL BHI with 100 µg/mL erythromycin, start an overnight culture of EC1000 containing your plasmid of interest.

2 In 10 mL BHI with 1 mg/mL spectinomycin, start an overnight culture of CK111/pCF10-101.

3 Next morning, dilute the overnight culture of CK111 10-fold after determining the amount of culture that will be required (25 mL for 2 transformations).

Rachel suggests that the pCF10-101 plasmid is stable; adding spectinomycin is unnecessary.

4 Grow cells at 37°C until OD600 is between 0.5 and 1.0. Suggest 0.75. This takes ~2 hours.

5 Isolate plasmid of interest from the overnight culture of EC1000 containing your plasmid of interest.

Rachel suggests that the plasmid isolation does not need to be done the same day; can be done at an earlier time.

6 Once CK111 has reached the proper OD600, aliquot your cultures into 25 mL conicals which have been chilled on ice.

7 Chill cells on ice for 15-20 minutes.

8 Pellet cells at 3500 RPM at 4°C for 10 minutes.

9 Pour off supernatant and wash with cold water. Spin again.

10 Resuspend each pellet in 500µL lysozyme solution containing 30µg/mL lysozyme. Add lysozyme to the solution fresh just before use. Leave cells in the same conical.

11 Incubate cells for 20 min at 37°C.

12 Flood cells with cold water. Wash two times.

Resuspend pellet in 800µL of electroporation solution, and transfer to a chilled Eppendorf tube. Spin cells at 13K RPM

13 for 3 minutes at 4°C.

14 Resuspend the pellet in 85µL electroporation solution.

15 Transfer 50µL of these competent cells to a new Eppendorf tube.

16 To your competent cells, add 50 ng, 100 ng, or 150 ng DNA (this is variable; attempt different concentrations).

17 Add cells to the prechilled electrocurvette and use the EC2 settings on the electroporator.

18 After electroporation, rescue cells in 500µL plain BHI for 2 hours using a snap cap tube. Incubate statically or shaking at 100 RPM at 37°C.

19 Transfer 100-125µL cells to a pre-warmed BHI plate containing 10µg/mL ERM and 250µg/mL X-gal.

> Rachel suggests plate 100µLof cells on 5 plates; do NOT spin cells down to concentrate. It is not many plates and eliminates the difficulty of resuspending the cells (it is very difficult after lysozyme treatment and electroporation).

20 Incubate plates for 24-48 hours and look for blue colonies.

21 Restreak blue colonies on the same selective media. Freeze good clones.

22 Optional: isolate plasmid from CK111 and do a PCR to ensure the correct plasmid is in the cells.