Transformation of Chemical Competent Cells

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Version: 1.0 - Print Version (.PDF)

The efficiency depends on how much time you have. The better the results should be the longer it takes.

Required:

- Plasmid DNA (0,1 ng/µl, pBlue or similar plasmid of 3 kb size with ampicilin resistance)
- LB medium agar plate with ampicilin
- LB medium, no antibiotics

Protocol A

1. Defreeze the competent cells in the hand and leave them on ice
2. Put 0.25 ng (2.5 µl) plasmid in eppendorf tube and store on ice
3. Add 50 µl competent cell solution to the tube with the DNA
4. Incubate 30 min on ice
5. Heatshock for 90 sec at 42 °C in water bath
6. Leave for 2 min on ice
7. Add 1 ml LB without antibiotics
8. Incubate for 45 min at 37 °C
9. Plate 100 µl

I got ca. 1354 colonies on per plate: 5.4 x 10^7 Col/µg DNA

Protocol B:

1. Defreeze the competent cells in the hand and leave on ice
2. Put 0.25 ng (2.5 µl) plasmid in eppendorf tube and store on ice
3. Add 50 µl competent cell solution to the tube with the DNA
4. Incubate 5 min on ice
5. Heatshock for 90 sec at 42 °C in water bath
6. Leave for 2 min on ice
7. Add 1 ml off LB without antibiotics
8. Incubate for 30 min at 37 °C
9. Plate 100 µl

I got ca. 672 colonies per plate: 2.7 x 10^7 Col/µg DNA

Protocol C:

1. Defreeze the competent cells in the hand and store on ice
2. Put 0.25 ng (2.5 µl) plasmid in eppendorf tube and leave on ice
3. Add 50 µl competent cell solution to the tube with the DNA
4. Incubate 5 min on ice
5. Heatshock for 90 sec at 42 °C in water bath
6. Plate 5 µl

I got ca. 278 colonies per plate: 1.1 x 10^7 Col/µg DNA

Protocol D

1. Defreeze the competent cells in the hand and leave on ice
2. Put 0.25 ng (2.5 µl) plasmid in eppendorf tube and store on ice
3. Add 50 µl competent cell solution to the tube with the DNA
4. Incubate 5 min on ice
5. Plate 50 µl

I got ca. 133 colonies per plate: 0.5 x 10^6 Col/µg DNA

References and Comments:

This is the common protocol heat shock. I rewrote the protocol to stress critical steps and give some ideas about efficiency. I transformed my competent cells with this protocol for over 3 years with constant good results.

How to cite this page in publications:

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<http://www.untergasser.de/lab/protocols/competent_cells_chemical_trafo_v1_0.htm>.

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