Transformation of competent E.coli cells with plasmid DNA

Nat Brown

Abstract

Transformation of heat-shock competent E. coli cells

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Before start

For incubation on ice, make sure the tubes are standing in an ice-water mix, because without water, the cooling effect of ice is not reproducible due to the air between the ice fragments, especially if you have to incubate for a certain period of time.

Protocol

Step 1.
Thaw the appropriate amount of competent cells on ice.

NOTES
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The appropriate amount refers to the amount you will need to all transformations you need to do. If you need to do one, thaw 100 µl (or the next biggest aliquot size). If you need to do six, thaw 600 µl (minimum, or the closest volume you can from the size of the aliquots you have).

Step 2.
Pre-chill the required number of empty 1.5 ml microcentrifuge tubes.

Step 3.
Pipet 100 µl aliquots of cells into the pre-chilled tubes.

Step 4.
Add 5-10 µl of a ligation reaction mix or 5 ng of pure plasmid DNA to each tube. Mix gently!

Step 5.
Incubate the tubes of ice for 30 min

DURATION
00:30:00

Step 6.
Heat shock the cells for 45 sec at 42°C

DURATION
00:00:45

Step 7.

Place the tubes immediately on ice for at least 2 min

DURATION
00:02:00

Step 8.
Add 1000 µl of SOC medium to each tube and incubate for 1 hour at 37°C with shaking.

DURATION
01:00:00

NOTES
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LB medium can be used instead on SOC with a small loss in efficiency.

Step 9.
Transfer the cultures to 1.5 ml microcentrifuge tubes and spin for 1 min at 10000 x g.

DURATION
00:01:00

NOTES
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Before proceeding with this step, consider how many transformants you expect. Unless you expect a low yield (<200) plate 100 µl (or less) before concentrating the bacteria. By plating two or more amounts of bacteria you increase your chances of having well isolated colonies on your plates the next day.

Step 10.
Remove 800 µl of the supernatant and resuspend the pellet.

NOTES
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This can be achieved, with just a little practice, by gently decanting the supernatant without trying to fully remove all the liquid. With a little experience, decanting and leaving 100 - 200 µl remaining in the tube will be routinely achieved.

Step 11.
Plate out the suspension on a LB agar plate containing the appropriate antibiotic.

Step 12.
Incubate the plates overnight at 37°C.

Warnings

If you notice a significant drop in colony numbers after several transformations with plasmid DNA it's time to prepare fresh competent cells.