Transformation of competent E.coli cells with plasmid DNA

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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.

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

Step 3.
Pipet 50 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 800 µl of SOC medium to each tube and incubate for 1 hour at 37°C

DURATION
01:00:00

Step 9.
Transfer the cultures to 1.5 ml microcentrifuge tubes and spin for 1 min at 6000 rpm.

DURATION
00:01:00

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

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.