One Shot® TOP10 Chemically Competent E. coli Transformation

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Abstract

This procedure allows you to transform DNA into chemically competetent *E. coli*.

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Guidelines

Be extremely gentle when working with competent cells. Competent cells are highly sensitive to changes in temperature or mechanical lysis caused by pipetting. Transformation should be started immediately following the thawing of the cells on ice. Mix by swirling or tapping the tube gently, not by pipetting.

Before start

• Equilibrate a water bath to 42°C.
• Warm the vial of S.O.C medium to room temperature.
• Spread X-Gal onto LB agar plates with antibiotic, if desired for blue/white selection.
• Warm the selective plates in a 37°C incubator for 30 minutes (use one plate for each transformation).
IMPORTANT! It is essential that LB plates containing 100 µg/mL ampicillin are pre-warmed if you are performing the rapid chemical transformation procedure

Protocol

Step 1.
Centrifuge the vial(s) containing the ligation reaction(s) briefly and place on ice.

Step 2.
Thaw, on ice, one 50 µL vial of One Shot® cells for each ligation/transformation.

DURATION
00:05:00

Step 3.
Pipet 1–5 µL of each ligation reaction directly into the vial of competent cells and mix by tapping gently. Do not mix by pipetting up and down. The remaining ligation mixture(s) can be stored at −20°C.

Step 4.

Incubate the vial(s) on ice for 30 minutes.

DURATION
00:30:00

Step 5.
Incubate for exactly 30 seconds in the 42°C water bath. Do not mix or shake.

DURATION
00:00:30

Step 6.
Remove vial(s) from the 42°C bath and place them on ice.

Step 7.
Add 250 µL of pre-warmed S.O.C medium to each vial. S.O.C is a rich medium; sterile technique must be practiced to avoid contamination.

AMOUNT
250 µl Additional info:

PROTOCOL

. SOC Media

CONTACT: New England Biolabs

NOTES
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LB can also be used in place of S.O.C.
Step 7.1.
SOB Media

PROTOCOL

. SOB Media

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Step 1.1.
2% tryptone

Step 1.2.
0.5% yeast extract

Step 1.3.
10 mM NaCl

Step 1.4.
2.5 mM KCl

Step 1.5.
10 mM MgCl2

Step 1.6.
10 mM MgSO4

Step 7.2.
20 mM glucose

Step 8.
Place the vial(s) in a microcentrifuge rack on its side and secure with tape to avoid loss of the vial(s).

Shake the vial(s) at 37°C for exactly 1 hour at 225 rpm in a shaking incubator.

DURATION

01:00:00

Step 9.

Spread 20–200 µL from each transformation vial on separate, labeled LB agar plates. The remaining transformation mix may be stored at 4°C and plated out the next day, if desired.

Step 10.

Invert the plate(s) and incubate at 37°C overnight.

Step 11.

Select colonies and analyze by plasmid isolation, PCR, or sequencing.