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TSS competent cells and transformation

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ABSTRACT

This protocol allows the efficient transformation of plasmid into E. coli and related strains

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MANUSCRIPT CITATION:

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Protocol status: In development
We are still developing and optimizing this protocol

Created: Apr 11, 2023

Last Modified: Jun 19, 2023

PROTOCOL integer ID:

80304

Transformation Storage Solution (TSS) buffer

- PEG3350 10%. Sigma. Ref: 202444
- DMSO 5%.
 - DMSO (dimethyl sulfoxide) Merck MilliporeSigma (Sigma-Aldrich) Catalog #D8418
- Glycerol 10%
 - Glycerol for molecular biology, ≥99% Merck MilliporeSigma (Sigma-Aldrich) Catalog #G5516
- MgCl2 20 mM.
 - 1 M Magnesium Chloride (MgCl2) Merck MilliporeSigma (Sigma-Aldrich) Catalog #M8266
- LB medium 2x.

Adjust to pH 6.1 with 6 M HCl.

Autoclave.

Add previously sterilized MnCl2 4 M to a final concentration of 140 mM.

Add water to adjust the modified LB medium to 1x.

Prepare aliquots of 1 ml and store at -20°C.

5x KCM solution

- KCI 0.5 M.
 - Potassium Chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog**#P9541
- Cacl2 150 mM.
 - Calcium chloride dihydrate Merck MilliporeSigma (Sigma-Aldrich) Catalog
 #C7902
- MgCl2 250 mM.
 - Magnesium chloride Merck MilliporeSigma (Sigma-Aldrich) Catalog #M8266

Sterilize at 121°C for 20 min. Store at 4°C.

Prepare solution TSS and KCM.

When prepared, TSS has a cloudy appearance that disappears once autoclaved. Also, over time, TSS can seem to be contaminated without being so, since the polyethylene glycol tends to precipitate.

Preparing competent cells

2d

1 Grow o/n a 5 mL culture of the strain to be transformed.







2 Dilute 1:100 of the preinoculum in 50 mL prewarmed LB ($OD_{600} = 0.05$).

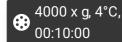


3 Grow at 37°C with agitation (170 rpm) until it reaches $OD_{600} = 0.5$. 1h 30m

(5) 170 rpm, 37°C

4 Centrifuge the culture at 4000 g and 4°C for 10 min.





5 Discard supernatant and wash with 1 mL of chilled TSS.





6 Centrifuge at 4000 g and 4°C for 3 min. 3m

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4000 x g, 4°C,
00:03:00
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7 Discard supernatant and resuspend in 1 mL of TSS.



8 Incubate 10 min on ice.

10m



9 Store 30 μ L aliquots at -80°C.



Transformation protocol

1h 40m

Mix 5 μ L of 5x KCM solution with DNA (around 1-5 μ L) and H₂O to a total of a 25 μ L mixture.

Δ 25 μL

11 Mix the mixture with 25 μ L of competent cells.

12 Incubate 30 min on ice.

30m

13 Thermal shock: incubate at 42°C for 90 s.

\$ 42 °C **♦** 00:01:30

1m 30s

14 Incubate 2 min on ice.

2m

15 Recovery: add 250 μl of LB and incubate at 37°C for 1 h.

1h



16 Plate 100 μ l of the solution on LB agar plates supplemented with the indicated antibiotic and incubate o/n at 37°C.

12h

\$ 37 °C Overnight