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We are still developing and optimizing this protocol

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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.

MATERIALS

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 -20ºC.

5x KCM solution

- KCl 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 121ºC for 20 min. Store at 4ºC.

BEFORE START INSTRUCTIONS

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. 12h

5 mL | 37 °C | Overnight

2 Dilute 1:100 of the preinoculum in 50 mL prewarmed LB (OD_{600} = 0,05).

50 mL | 37 °C

3 Grow at 37ºC with agitation (170 rpm) until it reaches OD_{600} = 0,5. 1h 30m

170 rpm, 37°C

4 Centrifuge the culture at 4000 g and 4ºC for 10 min. 10m

4000 x g, 4°C, 00:10:00

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

1 mL | On ice

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

4000 x g, 4°C, 00:03:00

7 Discard supernatant and resuspend in 1 mL of TSS.

1 mL On ice

8 Incubate 10 min on ice. 10m
On ice 00:10:00

9 Store 30 µL aliquots at -80ºC.
30 µL -80 °C

Transformation protocol 1h 40m

10 Mix 5 µL of 5x KCM solution with DNA (around 1-5 µL) and H_2O to a total of a 25 µL mixture.
25 µL

11 Mix the mixture with 25 µL of competent cells.
25 µL

12 Incubate 30 min on ice. 30m
On ice 00:30:00

13 Thermal shock: incubate at 42ºC for 90 s. 1m 30s
42 °C 00:01:30

14 Incubate 2 min on ice. 2m
On ice 00:02:00

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

37 °C 01:00:00

16 Plate 100 µl of the solution on LB agar plates supplemented with the indicated antibiotic and incubate o/n at 37ºC. 12h

37 °C Overnight