Jun 19, 2023

TSS competent cells and transformation

DOI
dx.doi.org/10.17504/protocols.io.5jyl8jzbrg2w/v1

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Protocol Citation: David Ruano Gallego 2023. TSS competent cells and transformation . **protocols.io** **https://dx.doi.org/10.17504/protocols.io.5jyl8jzbrg2w/v1**

Manuscript citation:

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

Created: April 11, 2023

Last Modified: June 19, 2023

Protocol Integer ID: 80304

Keywords: efficient transformation of plasmid, tss competent cell, plasmid, competent cell, efficient transformation

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
- CacI2 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.

Troubleshooting

Before start

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

Protocol references

https://pubmed.ncbi.nlm.nih.gov/2648393/

https://pubmed.ncbi.nlm.nih.gov/36317996/