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TSS transformation of non-competent *E. coli* cells

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1 Works for me dx.doi.org/10.17504/protocols.io.gtabwie



ABSTRACT

Transformation & Storage Solution (2X TSS) enables researchers to take advantage of the simple system described by Chung et al., 1989 (DOI:[10.1073/pnas.86.7.2172](https://doi.org/10.1073/pnas.86.7.2172)) for the transformation of *E. coli* cells. Early log-phase cells are suspended in 1X TSS, a solution containing polyethylene glycol, dimethyl sulfoxide, and divalent cations in a bacterial growth medium. This protocol is an alternative to the transformation method with chemically CaCl_2 -competent cells.

Advantage: You can use any fresh *E. coli* cells

Disadvantage: Low efficiency. Use only for transformation of plasmids, not for ligation mixtures in molecular cloning. It is possible to transform at least two plasmids at the same time.

CaCl_2 -Transformation does not work for *E. coli* strains W3110 and W3110 Z1, thus TSS transformation is a method of choice.

MATERIALS TEXT

2 x TSS (Transformation and Storage Solution):

- 20 g PEG (3350 or 8000) 20% (w/v)
 - 10 mL DMSO, 10% (v/v)
 - 2,03 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 100 mM
 - ad 100mL LB medium (\rightarrow pH 6.5)
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- prepare solution without DMSO and autoclave
 - add DMSO
 - make 10 ml aliquots and store at 4 °C

Transformation

- 1 Inoculate 3 ml LB-medium with colonies from a fresh agar plate.
- 2 Incubate at 37 °C and 230 rpm for 1.5 to 2.0 h (exponential growth). Culture should become turbid.
🕒 02:00:00
- 3 Meanwhile prepare 1.5 ml tubes with 200 μl of 2x TSS-buffer and keep on ice.
Add 200 μl cells and 0.5 - 1.0 μl Plasmid. Vortex and incubate on ice for 20 - 30 min. Longer incubation time will reduce transformation efficiency!
🕒 00:20:00

- 4 Incubate 45-60 min at 37 °C on the Thermomixer with shaking (900 rpm). Addition of LB is not required.

🕒 01:00:00

- 5 Plate on agar plates containing the appropriate antibiotic



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