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

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We use this protocol and it's working

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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

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)
- 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