TSS Transformation (after Chung et al., 1989, PNAS 86:2172-2175)

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Abstract

Transformation & Storage Solution (2X TSS) enables researchers to take advantage of the simple system described by Chung et al. [1] for the preparation, long-term storage and transformation of competent E. coli. Early log-phase cells are suspended in 1X TSS: a solution containing polyethylene glycol, dimethyl sulfoxide, and divalent cations in a bacterial growth medium.

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Protocol

Preparing TSS

Step 1.

2 x TSS (Transformation and Storage Solution):

- 20 g PEG (3350 or 8000) 20% (w/v)
- 10 mL DMSO (after autoclave) 10% (v/v)
- 2,03 g MgCl2 * 6H2O 100 mM
- ad 100mL LB medium (→ pH 6.5)

Transformation

Step 2.

• Inoculate starter culture of 1.5 mL LB medium

• After 1.5 – 2 hours culture should become turbid (→ exponential growth)

Transformation

Step 3.

• Add 200 µL of culture to 1.5 mL tube and mix with 200 µL 2xTSS (work on ice)

• Add 0.5 – 1.0 µL plasmid (high copy: rather 0.3 µL)

Incubation

Step 4.

• Incubate on ice for 20-30 min

• Incubate 45-60 min at 37°C on shaking device (> 200 rpm) – addition of LB is not requested

• Plate on LB-Agar (incl. appropriate antibiotic)