TSS TRANSFORMATION

Transformation and storage solution (TSS)

- LB containing 10 % PEG8000 (w/v), 5 % DMSO (v/v), and 30 mM MgSO4.
- Adjust the pH to 6.5, and sterilize by filtration through a 0.22 µM filter.
    - TSS solution can also be sterilized by autoclaving.
- Store in aliquots at −20 °C.

Preparation of TSS competent cells

- Start a fresh **overnight** culture (2 mL in LB) of the target strain
- Next day, dilute 1:100 into 50 mL LB media in a 500 mL flask.
- Incubate at 37 °C with shaking (at approximately 200 rpm). When the diluted culture has grown to an OD600 between 0.4 and 0.6 (**~ 2-3h**), place the culture on ice for **10 min**.
- Centrifuge the cells at 4000 × g for **10 min** at 4 °C to pellet. Discard the supernatant, and gently resuspend the cells in 1/10 volume of ice-cold TSS buffer (5 mL). It is crucial to keep the cells cold at all times.
- Aliquot the cells (50 µL) into precooled Eppendorf tubes and use on the same day or freeze at −80 °C for later use.
- Aliquote 100 µL of TSS suspended cells to prechilled sterile 2 mL tubes (mae 10 - 20 aliquots, freeze the rest in one stock tube). Use on the same day or freeze at −80 °C for later use.

TSS Transformation

- Gently mix a 3 µL aliquot of the plasmid with a 100 µL aliquot of TSS chemically competent cells in a precooled 1.5 mL Eppendorf tube on ice.
- Incubate on ice for **20–30 min**.
- 1 mL of LB media (or SOC is better) is added immediately and the cells incubated at 37 °C for **1 h** with shaking (150 rpm).
- Spread 200 µL of the transformation mix on an LB-agar plus kanamycin (20 µg/mL) plate.
    - For larger plasmid sizes where integration efficiency may be lower, the whole transformation mix can be plated out on a side and streaked.
- Incubate the plate at 37 °C **overnight**.

Tip: Protocol adapted for double plasmid TSS transformation
DAY1: attempt transforming 2 plasmids, and transform one plasmid only in parallel
DAY2: If double plasmid transfo worked, all good. If only single plasmid transfo worked, use the colony to follow protocol below (preparation of TSS competent cell + Transformation of the second plasmid on the same day)

- From a colony on plate, inoculate 500 µL of LB + Cm
- After ~**4h**, dilute 1:50 into 10 mL LB + Cm in a 100 mL flask.
- Incubate at 37 °C with shaking (at approximately 200 rpm). When the diluted culture has grown to an OD600 between 0.4 and 0.6 (~ **2h**), place the culture on ice for 5 min.
- Centrifuge the cells at 4000 × g for 10 min at 4 °C to pellet. Discard the supernatant, and gently resuspend the cells in 1/10 volume of ice-cold TSS buffer (1 mL). It is crucial to keep the cells cold at all times.
- Aliquot 100 µL of TSS suspended cells to prechilled sterile 2 mL tubes. Use aliquots to transform plasmid #2 right away, and freeze the rest at −80 °C for later use.