



## TITLE: PREPARATION OF COMPETENT BACTERIA

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1. Strick competent bacteria on LB agar plate with the appropriate antibiotics [e.g. DH5 $\alpha$  - NA<sup>R</sup>; TOP10 – Strep<sup>R</sup>; XL10Gold – Cm<sup>R</sup> & Tet<sup>R</sup>]; BL21 – has no antibiotic resistance, hence strick on a plain LB agar plate.
2. Pick a single colony and grow overnight starter (in 2-3 ml LB). If the bacteria strain has antibiotic resistance, grow in the appropriate antibiotics.
3. Put into 100ml LB (dilution 1:100), and save 1ml clean LB for Blank. BL21-SI requires LB without NaCl.
4. Grow (shaker – 250 rpm @37°C) until OD600=0.3-0.4. If OD600 is Over 0.45 dilute the bacterial suspension using LB to OD600 0.1 and grow again to 0.3-0.4. [Strain DH5 $\alpha$  took 2.5h-3h to reach OD600=0.3].
5. Put the 100 ml bacteria in appropriate tubes (good option: 4 tubes of 50ml (25ml in each), spin 1000xg for 10 min (4°C).
6. Prepare ~100 very cold (on ice) Eppendorf tubes in advance.
7. Remove the sup and resuspend in 1/10 of the original volume (If made 100ml use 10ml TSS) of very cold TSS buffer freshly made. (All four tubes of 25 ml bacterial LB resuspend with the same 10 ml TSS).
8. Aliquot into Eppendorf tubes at least 110 $\mu$ l in each. Freeze immediately on dry ice, or directly into -80 (less recommended). It is good enough to work quickly on the ice.
9. Store at -70°C. Cells are best for use in 4-6 months.

### TSS Buffer preparing:

- LB
- 10% w/v PEG (size 3350 or 8000) \*Pay attention the powder is dry, PEG is very hygroscopic, and using an old powder can reduce the efficiency of competent cells.  
**Different sizes of PEGs are not suitable for this protocol!**
- 5% v/v DMSO
- 50 mM MgCl<sub>2</sub> (or MgSO<sub>4</sub>)
- pH should be below 7 (6.5-6.7). Usually, there is no need to titrate, leave it with pH 6.7.
- Filter sterilize (0.2  $\mu$ M filter)
  
- \*For the culture of 100ml LB with competent bacteria recommended to prepare at least 11 ml TSS buffer.
- \*Use TSS buffer for 1-2 days, keep it at 4°C, do not freeze.