Protocol : Generating Competent Cell

Before Starting
Pour minimal media plates. 5x M9 salts etc overleaf. Prepare 100ml LB per strain. Prepare 50ml ice cold 0.1M $CaCl_2$ / 15% glycerol per strain Pre-chill eppendorf tubes

Day 1

1	Streak cells on minimal agar plate. Incubate 37°C overnight.

Day 2

2	Pick a colony into 5 ml LB + 100µl 1M $MgSO_4$ Incubate 37°C in shaker overnight.

Day 3

3	Inoculate 100ml LB in pre-warmed conical with 1 ml of the 5 ml O/N culture from Day 2.
4	Incubate 2hrs in 37°C shaker until the cells at early log phase of growth curve (A_{600} ~ 0.3)
5	Transfer to chilled, sterile 50 ml Falcon tube and incubate on ice 10 min
6	Centrifuge at 3300g for 5 min at room temperature.
7	Resuspend in 10ml ice cold 0.1M $CaCl_2$ / 15% glycerol and incubate on ice 30 min
8	Centrifuge at 3300g for 5 min at room temperature.
9	Resuspend in 1ml ice cold 0.1M $CaCl_2$ / 15% glycerol. Transfer 100µl aliquots to pre-chilled, pre-labelled eppendorf tubes. Store at -70°C.

Solution Recipe

- 5x M9 salts in 500 ml dH_20.

Na_2HPO_4	32g
KH_2PO_4	7.5g
NaCl	1.25g
NH_4Cl	2.5g

- Minimal media plates for competence

In 50 ml Falcon;

39 ml	melted Bacteriological Agar solution (≤50□C)
10 ml	5x M9 salt solution
1 ml	20% (w/v) D-glucose
50 µl	2mg ml^{-1} thiamine
5 µl	1M $CaCl_2$
100 µl	1M $MgSO_4$

- 0.1M $CaCl_2$ / 15% glycerol

In 50 ml Falcon;

5 ml	1M $CaCl_2$
7.5 ml	100% glycerol