Recipe: Preparation of high performance chemical competent Cell

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1 Introduction

2 Protocol

- Streak glycerol stocks in plate with appropriate antibiotics;
- Pick one to three colonies into LB medium with appropriate antibiotics;
- Overnight culture dilute 100 fold in 50 mL SOB medium with appropriate antibiotics;
- Vigorously shaking before OD_{600} reach to about 0.6 (It is recommended having pre-culture in SOB medium for OD_{600} reach to 0.3.);
- Transform 50 mL medium into 50 mL centrifuge tube and stall it in ice for 10 min;
- 4 °C, 2500 ×g centrifuge 10 min;
- Discard supernatant, re-suspend pellet by 15 mL TB buffer (Table **??**.)and stall it in ice for 10 min;
- 4 °C, 2500 ×g centrifuge 10 min;
- Discard supernatant and use 4 mL TB buffer re-suspend pellet, add 300 μL DMSO finally(final concentration: 7% (v/v)).
- Add 5 μL plasmid solution into 100 μL fresh chemical competent cell.
- Stall on ice for 30 min.
- 42 °C heat-shocked for 45s and chilled on ice for 2 min.
- Add 900 μL SOC medium and shake the culture vigorously.
- Coat 100 μL medium on plate with appropriate antibiotics.

Component	Volume
ddH_2O	12.5 mL
1 M KCl	4 mL
0.45M $MnCl_2$	2.4 mL
0.5 M $CaCl_2$	0.6 mL
0.5 M K-MES(1)	0.5 mL

Table 1: 20 mL K-MES Buffer Recipe

Note:
(1) Use KOH adjusting the K-MES solution to pH 6.3, store at −20°C for long term storage and split into aliquots avoiding repeated freezing and thawing.
(2) For a 5 mL system. It can be performed *via* reducing volume of each reagent proportionally.