Heat shock competent cells

Aim of the experiment

The purpose of this experiment is to transfer the plasmid into E.coli competent cells by heat shock.

Materials

- E.coli Rosetta 2（DE3）pLysS、E.coli BL21、E.coli DH5alpha Competent bacteria；
- pC013-Twinstrep-SUMO-huLwCas13a、
  pC0061 PsmCas13（B05）His6-TwinStrep-SUMO-Bsal、
  p2CT-His-MBP-Lba_Cas13a_WT；
- LB medium；
- Chloramphenicol（Cm, 35ug/ml）：
  Dissolve 0.35g of Amp by adding it to 8 mL of absolute ethanol and then vortexing. Add absolute ethanol to bring the volume to 10 mL. Store at−20 °C；
- Ampicillin（Amp,100ug/ml）：
  Dissolve 0.1g of Amp by adding it to 8 mL of deionized water and then vortexing. Add deionizedwater to bring the volume to 10 mL andfilter-sterilize with a 0.22-μm syringefilter. Store at -20 °C.

Procedure

1. Thaw one vial of E.coli competent cells on ice for 30 min, and then add 2 μL of plasmid. Incubate on ice for 5 min.

2. Heat-shock the cells by placing the vial into a 42 °C pre-heated water bath for 90 s, and then cold-shock the cells on ice for 2 min.

3. Add 200 μL of LB medium to the cells and plate 100 μL of cell suspension on a pre-warmed LB plate containing 100 μg/mL ampicillin and 35ug/ml Chloramphenicol. Incubate the plate overnight in a 37 °C incubator.