Protein Expression of Cas 13a Lwa,Lba and Cas 13b Psm,Cca

Aim of the experiment

The experiment is aimed to express the Cas13a Lwa、Lba and the Cas13b Psm、Cca. *E. coli* strains suitable for protein expression after transformed with the respective plasmids. For the usage of this protocol, the expression plasmids were all under the control of an IPTG-inducible. So, we can use IPTG to induce protein expression at low temperature.

Materials

- *E. coli* Rosetta 2（DE3）pLysS、*E. coli* BL21
- 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.
- Isopropyl-β-D-1-thiogalactopyranoside （IPTG）：

  Dissolve 1.19 g of IPTG 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.
- 37℃ constant temperature incubator
- Biological shaker

- Centrifuge
- Clean bench

Procedure

1. Thaw one vial of Rosetta 2(DE3)pLysS competent cells on ice for 30 min, and then add 1 μL of 50 ng/μL of LwaCas13a expression 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 45 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.

4. The next day, inoculate 5 mL of LB medium containing two antibiotics with a single colony and incubate the culture overnight at 37 °C in a biological shaker at 300 r.p.m.

5. Inoculate 500 mL of LB medium, containing two antibiotics and determine the optical density (OD, 600 nm). Shake cultures at 37 °C, 300 r.p.m.

6. Monitor the OD every hour until the cells reach an OD of 0.4–0.6, and then induce expression by adding 1 mL/L 0.5 M IPTG and shake the cultures for 16 h at 300 r.p.m. in apre-chilled 15°C biological shaker.

7. Harvest the cells by spinning the culture down at (5,200g) for 15 min at 4 °C.The collected bacterial pellets are directly sonicated for purification or stored at -20℃.