Mimic pathogen

Aim of the experiment

The purpose of this experiment is to express viral nucleic acid in Escherichia coli to simulate the state that viral nucleic acid is wrapped in a protein shell under real conditions. We constructed a plasmid with a virus sequence (about 100bp). It was introduced into E. coli and induced with IPTG to produce target RNA for subsequent experiments.
(Note: The virus plasmid only contains a small segment of the virus sequence and is not pathogenic. It is safe and legal.)

Materials

- *E. coli* BL21 competent cells
- LB medium
- 500ml flasks
- Kanachloramphenicol（Km，100ug/ml）：Dissolve 0.1g of Km 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.
- Shaker

Procedure

1. Thaw one vial of *E. coli* BL21 competent cells on ice for 30 min, and then add 1 μL of 50 ng/μL of H1N1 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 Kanachloramphenicol. 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 overnight at 300 r.p.m. in apre-chilled 28°C biological shaker to make E. coli transcribe RNA.