08 Exploration of expression condition

Tjusls China

1Tianjin University

MATERIALS

- IPTG Bio Basic Inc. Catalog #IB0168.SI.ZE.100g
- SDS-PAGE Tricine Loading Buffer Boster Bio Catalog #AR1143
- LB medium Contributed by users Catalog #/

BEFORE START INSTRUCTIONS

Set the gradient of condition to explore how to express it best. For example, we often use 0.5mM IPTG, 16°C/0.5mM IPTG, 37°C/1mM IPTG, 16°C/1mM IPTG, 37°C as different conditions.
1. Transform the plasmid into bacteria used to express target protein (e.g. E.coli BL21(DE3)).

2. Take monoclonal in the culture plate into LB tube and cultivate in shaking incubator overnight (10-12h) to activate bacteria.

3. Test the OD600 number of bacteria, then pipe 5-10μl into each new 5 mL LB tube. Don't forget to add antibiotic into tubes and mark them.

4. Cultivate in shaking incubator for 3-4 hours until the OD600 of bacteria range from 0.6 to 0.8.

5. Pipet 200μl bacterial liquid as uninduced sample, and take another 600μl to mix with 400μl 50%glycerol to store. Then add inducer IPTG into each tube in different concentration, and incubate at 16°C for 16 hours or at 37°C for 4 hours shaking at 200-300rpm.

6. After cultivating, pipet 200μl for each as induced sample. (The method to make samples
   1) Centrifuge the taken bacterial liquid at 12,000rpm for 3 minutes
   2) Drop the supernatant and resuspend the precipitate using 100μl ddw.
   3) Pipet 50μl resuspending liquid to mix with 10μl 6XSDS Loading buffer
   4) Boil it in dry bath at 100°C for 10 minutes)

Use SDS-PAGE to check whether the target protein express or not and what the most suitable condition for its expression is.