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08 Exploration of expression condition

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Works for me

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MATERIALS

NAME ▾	CATALOG # ▾	VENDOR ▾
IPTG	IB0168.SIZE.100g	Bio Basic Inc.
SDS-PAGE Tricine Loading Buffer	AR1143	Boster Bio
LB medium	/	

BEFORE STARTING

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 5-10ul 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 200ul bacterial liquid as uninduced sample, and take another 600ul to mix with 400ul 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.
 **600 µl**
- 6 After cultivating, pipet 200ul 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 100ul ddw.
 - 3) Pipet 50ul resuspending liquid to mix with 10ul 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.

 **200 µl**


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