Jul 10, 2019

08 Exploration of expression condition

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1 Works for me dx.doi.org/10.17504/protocols.io.5cfg2tn

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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 monoclone 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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