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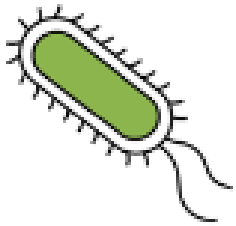
Protein expression of hard-to-produce proteins in the cytoplasm of *Escherichia coli*



In 1 collection

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol describes the expression and extraction of heterologously cytoplasmically expressed proteins in the bacterium *E.coli*, for T7 based expression systems.

Specifically, it describes how to produce "hard-to-produce", as for example, LPMOs.

It is based on a modified version of:
"IPTG Induction and Extraction of Proteins from Bacteria" by Swathi Arur and Sudhir Nayak, Schedl Lab. Washington University Genetics, St. Louis.
Hemsworth, G. R., Henrissat, B., Davies, G. J., and Walton, P. H. (2014) Discovery and characterization of a new family of lytic polysaccharide monooxygenases. Nat. Chem. Biol.10, 122–126.

The extraction of cytoplasmatic proteins is followed by a chemical lysis protocol, alternatively, sonication can also be employed.

Guidelines

Make sure to clone your favorite gene for expression in the appropriate expression vector.

Materials

MATERIALS

- SHuffle T7 Competent E.coli - 6×0.05 ml **New England Biolabs Catalog #**C3026H
- IPTG **Bio Basic Inc. Catalog #**IB0168.SIZE.100g
- BL21(DE3) or BL21-Star(DE3) or Rosetta2(DE3) or etc for protein purification
- Kanamycin **Research Products International Corp (RPI) Catalog #**K22000-25.0
- LB **Research Products International Corp (RPI) Catalog #**L24400-2000.0

Safety warnings

This protocol describes the construction of GMO classified organisms. Make sure that the local GMO and safety legislations are respected.

Before start

Prepare a fresh transformation of your expression vector in *E. coli* BL21 (DE3) cells or other appropriate strain (e.g. sHuffle strain, Rosetta, etc.)

Day 1 - Culture inoculation

1 Pick a fresh colony of your BL21 (DE3) strain previously transformed with your expression vector, and inoculate it in LB medium supplemented with relevant antibiotics. Grow the culture at 37 °C at 250 RPM shaking Overnight. The volume of the overnight culture depends on the volume of the expression culture and should be at least 1/100 of the expression culture

Day 2 - Induction

2 Dilute the overnight culture 1:100 in fresh LB supplemented with relevant antibiotics

3 Grow the culture at 37 °C with 250 RPM shaking until an OD_{600} = 0.5 - 0.6

4 Move the culture into an incubator set to 18 °C with 180 RPM of shaking and allow the culture to reach OD_{600} = 0.8 - 1.0

5 Induce the expression by adding IPTG to a final concentration of [M] 1 millimolar (mM)

6 Grow the culture for 20:00:00 at 18 °C with 180 RPM shacking.

> Note
>
> The temperature can be lowered further allowing for a slower induction in order to increase the production of soluble product.

Day 3 - Collection and lysis

7 Collect induced samples by centrifugation at 8000 x g, 4°C, 00:20:00 and carefully discard the supernatant

Note

Pellets can be frozen here at -80 °C or -20 °C until protein extraction

8 Lyse the cells using a lysis protocol or a commercially available kit

9 Keep the extractions On ice when working with it and at 4 °C for storage