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 We use this protocol and it's working

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

In 1 collection

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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.

Keywords: E. coli, T7, Protein production, Cytoplasmic expression, sHuffle strain, disulfide bond

MATERIALS

MATERIALS

⌘ SHuffle T7 Competent E.coli - 6x0.05 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 **Contributed by users**

⌘ Kanamycin **Research Products International (rpi) Catalog #K22000-25.0**

⌘ LB **Research Products International (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 INSTRUCTIONS

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 OD₆₀₀ = 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 OD₆₀₀ = 0.8 - 1.0
- 5 Induce the expression by adding IPTG to a final concentration of 1 millimolar (mM)
- 6 Grow the culture for 20:00:00 at 18 °C with 180 RPM shaking.

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