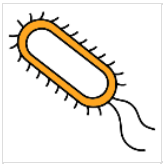

Oct 27, 2020

Protein expression and extraction of hard-to-produce proteins in the periplasmic space of *Escherichia coli*

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1 *Works for me* dx.doi.org/10.17504/protocols.io.bdr2i58e

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ABSTRACT

E. coli is a gram-negative bacteria used mainly in academia and in some industrial scenarios, as a protein production workhorse. This is due to its ease of manipulation and the range of genetic tools available.

This protocol describes how to express proteins in the periplasm *E. coli* with the strain BL21 (DE3) using a T7 expression system. Specifically, it describes a series of steps and tips to express "hard-to-express" proteins in *E. coli*, as for instance, LPMOs.

The protocol is adapted from 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.
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KEYWORDS

LyGo, LPMO, Periplasmic expression, E. coli, protein expression, Periplasmic extraction

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GUIDELINES

The periplasm of *E. coli* is often the preferred strategy to produce heterologous proteins in this bacterium as it provides the means for disulfide bond formation.

The choice of the signal peptide is of great importance to ensure correct and efficient translocation to the periplasm. In our lab, we routinely screen five different signal peptides: MalE^{SP}, OmpA^{SP}, PhoA^{SP}, DsBA^{SP}, and PelB^{SP}.

MATERIALS TEXT

MATERIALS

BL21(DE3) Competent E.coli - 6x0.2 ml **New England Biolabs Catalog #C2527I**

IPTG **Bio Basic Inc. Catalog #IB0168.SIZE.100g**

EDTA **Fisher Scientific Catalog #16 004Y**

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

LB **Research Products International (rpi) Catalog #L24400-2000.0**

Sucrose **Fisher Scientific Catalog #S25590B**

Trizma® base **Merck Millipore Sigma Catalog #93362**

SAFETY WARNINGS

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

ABSTRACT

E. coli is a gram-negative bacteria used mainly in academia and in some industrial scenarios, as a protein production workhorse. This is due to its ease of manipulation and the range of genetic tools available.

This protocol describes how to express proteins in the periplasm *E. coli* with the strain BL21 (DE3) using a T7 expression system. Specifically, it describes a series of steps and tips to express "hard-to-express" proteins in *E. coli*, as for instance, LPMOs.

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BEFORE STARTING

Prepare a fresh transformation of your expression vector in *E. coli* BL21 DE3 cells.

Pre culture - Day 1

1 Pick a fresh colony of your BL21 (DE3) strain with your expression vector, and inoculate it in LB 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

Inoculation, Induction and expression - Day 2

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

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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 grow the culture to OD_{600} = 0.8 - 1.0

5 Induce the expression by adding IPTG to a final concentration of **1 Milimolar (mM)**

6 Let the culture grow at **18 °C** with 180 RPM shaking for **20:00:00**

Expression at low temperatures is recommended to enhance the solubility of some proteins.

Harvesting and periplasmic extraction - Day 3 10m

7 Spin the culture down at **8000 x g, 4°C, 00:20:00** and discard the supernatant

Remove as much of the remaining liquid as possible from the centrifuge tube. This helps greatly in periplasmic extraction.

8 Resuspend the pellet in **3 mL** of buffer TSE buffer (200 mM Tris-HCl pH 8, 500mM sucrose, 1mM EDTA) per gram of cells (this normalization can also be based on OD_{600} where **12 µl** TSE per OD Unit is added)

Carefully resuspend the cells in the TSE buffer to avoid breaking the cells. A good tip is to use a sterile inoculation plastic loop to resuspend the pellet in the buffer before using a pipette tip.

9 Incubate the suspension at **Room temperature** for **00:10:00** 10m

10 Cold-shock the cells by adding **3 mL** of ice-cold sterile MQ water per gram of cells (or alternatively, **12 µl** of ice-cold water per every OD_{600} Unit can be added).

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11 Incubate the suspension **On ice** for **00:10:00**

12 Spin down the cells at **8000 x g, 4°C, 00:20:00** and collect the supernatant

The supernatant contains the periplasmic extraction

13 Keep the extraction **On ice** when working with it and at **4 °C** for storage

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