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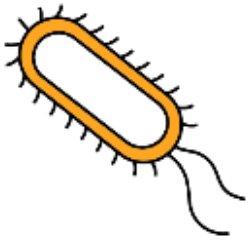
Protein expression and extraction of hard-to-produce proteins in the periplasmic space of *Escherichia coli*



In 1 collection

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

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Abstract

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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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

MATERIALS

- BL21(DE3) Competent E.coli - 6×0.2 ml **New England Biolabs** **Catalog #**C2527I
- IPTG **Bio Basic Inc.** **Catalog #**IB0168.SIZE.100g
- EDTA **Fisher Scientific** **Catalog #**16 004Y
- Kanamycin **Research Products International Corp (RPI)** **Catalog #**K22000-25.0
- LB **Research Products International Corp (RPI)** **Catalog #**L24400-2000.0
- Sucrose **Fisher Scientific** **Catalog #**S25590B
- Trizma® base **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**93362

Troubleshooting

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.

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

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 [M] 1 millimolar (mM)

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

Note

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

Note

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)

Note

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).

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

Note

The supernatant contains the periplasmic extraction

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