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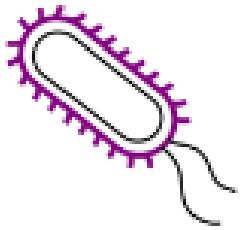
Protein expression on the surface of Escherichia coli



In 1 collection

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

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Abstract

Display of proteins on the bacterial cell surface has always been an attractive technique for the production of functional cell-anchored proteins, thereby reducing the cost, time and effort related to enzyme purification. Thus, surface display can enable fast and easy screening of protein libraries, e.g. activity variants, or screening of different enzyme substrates, while maintaining a connection between the phenotype and the genotype. Additionally, cells displaying functional enzymes can potentially be used as whole-cell catalysts.

This protocol describes how to express proteins on the surface of *E. coli* BL21 (DE3) using a L-rhamnose inducible expression system.

Guidelines

Display of proteins on the bacterial cell surface is very attractive for fast and easy screening of protein variants, because it maintains a connection between the phenotype and the genotype. For some enzymes to be active, it is crucial whether they are cell-anchored via the C- or N-terminus. The choice of display construct is therefore of great importance. In our lab, we use two different constructs:
pBAD42-Lpp^{SP}-OmpA-TEV-*ccdB* and pBAD42-*ccdB*-TEV-C-IgAP

The constructs are adapted from Wendel et al. (2016) A nanobody:GFP bacterial platform that enables functional enzyme display and easy quantification of display capacity. Microb Cell Fact 15:71, DOI 10.1186/s12934-016-0474-y

Materials

MATERIALS

- BL21(DE3) Competent E.coli - 6×0.2 ml **New England Biolabs** **Catalog #**C2527I
- Trizma® base **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**93350
- Kanamycin **Research Products International Corp (RPI)** **Catalog #**K22000-25.0
- LB **Research Products International Corp (RPI)** **Catalog #**L24400-2000.0
- L-rhamnose **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**W373011-100G-K

Safety warnings

This protocol describes the handling 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 Induce expression by adding L-rhamnose to a final concentration of [M] 5 millimolar (mM)

5 Let the culture grow at 30 °C with 250 RPM shaking for up to 20:00:00

Assaying surface displayed proteins - Day 3

6 Spin the culture down at 4000 x g, 4°C, 00:05:00 and discard the supernatant

7 Resuspend pellet in the desired assay buffer (e.g. [M] 10 millimolar (mM) - [M] 50 millimolar (mM) Tris-HCl)

8 Run activity assay in liquid or on agar plates.

Alternatively: Shaving-off of surface displayed proteins - Day 3

9 Spin the culture down at 4000 x g, 4°C, 00:05:00 and discard the supernatant

10 Resuspend pellet in [M] 10 millimolar (mM) Tris-HCl or 100 µL /ODU TEV cleavage buffer ([M] 10 millimolar (mM) Tris-HCl pH 7.5 , [M] 150 millimolar (mM) $NaCl_2$, [M] 0.5 millimolar (mM) EDTA)

11 Add purified and activated TEV protease

12 Incubate at Room temperature Overnight (~ 16:00:00)

> Note
>
> It is also possible to shorten the incubation time, so that the protein harvest can still be performed on the same day.

Protein harvest - Day 4

13 Spin the cells at 5000 x g, 4°C, 00:10:00 and collect the supernatant

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