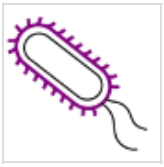

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Protein expression on the surface of Escherichia coli

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1 Works for me dx.doi.org/10.17504/protocols.io.bfh4jj8w

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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.

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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 TEXT

MATERIALS

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

Trizma® base **Sigma Aldrich Catalog #93350**

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

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

L-rhamnose **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.

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.

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

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 **5 Milimolar (mM)**

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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. **10 Milimolar (mM)** - **50 Milimolar (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 **10 Milimolar (mM)** Tris-HCl or **100 µl** /ODU TEV cleavage buffer (**10 Milimolar (mM)** Tris-HCl **pH 7.5**, **150 Milimolar (mM)** $NaCl_2$, **0.5 Milimolar (mM)** EDTA)

11 Add purified and activated TEV protease

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

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

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