Screening for Optimal Membrane Protein Expression Conditions using E. coli Expression Systems

Overview

The yield of membrane proteins expressed in heterologous expression systems is limited by the capability of the producing organism to import additional protein or replace its own proteins within the cell membrane or subcellular compartments. On the one hand, overwhelming the capacity of transport systems and limiting processing steps (e.g., rate of synthesis, rate of folding) leads to the formation of inclusion bodies. On the other hand, different cell types have advantages and disadvantages—prokaryotic cells have a quick doubling time but lack post-translational modification systems; yeast performs post-translational modification but has a strong cell wall that must be disrupted to obtain the target membrane protein. Therefore, developing a robust and optimal research protocol for a given membrane protein requires a systematic screening of expression systems and conditions.

This protocol describes the screening of 3 *E. coli* expression systems—*E. coli* BL21(DE3), C43(DE3), and C41(DE3)—to optimize conditions for the expression of a membrane protein. Similar screenings can be set up for alternative heterologous expression systems. Expression scale is kept low for this screen and results are analyzed by Western blot, using a detection antibody specific to the target protein or the fused tag (e.g., His, GST, Strep®-tag, rho-1D4). The clone and expression conditions that yield the highest expression level, as determined with this screen, are then used for a scaled up expression round to screen for the best solubilzation detergent (see Protocol "Screening Detergents for Optimal Solubilization and Purification of Membrane Proteins").

Equipment

- UV/VIS Spectrophotometer
- SDS-PAGE and Western blotting equipment
- pH-meter
- Incubator shaker
- Waterbath
- 100 mL culture flasks
- 2mL microcentrifuge tubes
- 0.45 µm filters

Materials

- Tryptone
- Yeast extract
- Sodium chloride (NaCl)
- Glycerol
- Potassium phosphate monobasic (KH_2PO_4)
- Potassium phosphate dibasic (K_2HPO_4)
- Isopropyl-β-1-thiogalactopyranoside (IPTG)
- Tris base
- Hydrochloric acid (HCl)
- Sodium dodecyl sulfate (SDS)
- Bromophenol blue
- Dithiothreitol (DTT)
- Competent cells transformed with the tested expression constructs (e.g., OverExpress™ C41(DE3) and C43(DE3) competent cells from Lucigen®, BL21(DE3) competent cells from Novagen®) .
- A detection antibody that binds to the target protein or protein tag (for Western blot analysis).

Solutions and buffers

2X YT Media, 1000 mL

Component	Amount needed for stock	Instructions
Tryptone	16 g	Dissolve in 900 mL deionized water. Adjust pH to 7.0 using NaOH, then add deionized water to a total of 1000 mL. Sterilize by autoclaving and allow to cool.
Yeast extract	10 g	
NaCl	5 g	

Terrific Broth (TB) Media, 1000 mL

Component	Amount needed for stock	Instructions
Tryptone	12 g	Dissolve in 800 mL deionized water, then adjust the final volume to 900 mL. Sterilize by autoclaving and allow to cool. Add 100 mL 10X TB Salts (see next recipe).
Yeast extract	24 g	
Glycerol	4 mL	

10X TB Salts, 1000 mL

Component	Amount needed for stock	Instructions
KH_2PO_4	23.12 g	Dissolve in 1000 mL deionized water. Sterilize by autoclaving and allow to cool.
K_2HPO_4	125.41 g	

IPTG, 1 mL

Component	Stock concentration	Amount needed for stock	Instructions
IPTG	1 M	238 mg	Dissolve in 1 mL deionized water. Pass through a 0.45 µm filter. Store in aliquots at –20°C.

50% Glycerol, 10 mL

Component	Stock concentration	Amount needed for stock	Instructions
Glycerol	50% (v/v)	5 mL	Dissolve in 5 mL deionized water. Pass through a 0.45 µm filter.

5X SDS-PAGE Buffer, 10 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for final buffer
Tris-HCl, pH 6.8–7.0	300 mM	121.14	1 M	121.14 g/1 L	3 mL
Glycerol	50% (v/v)	—	100%	—	5 mL
SDS	5 % (w/v)	—	—	—	0.5 g
Bromophenol blue	0.05% (v/v)	—	4%	—	125 µL
DTT	250 mM	154.25	1 M	1.54 g/10 mL	125 µL/aliquot

Instructions: Make sure to prepare a 1M Tris-HCl stock by dissolving Tris base in 500 mL deionized water, adding HCl to a pH of 6.8–7.0, and adding water to a final volume of 1 L. For the SDS-PAGE Buffer, mix all components listed **except DDT** and add water to a total of 10 mL. Freeze 20 aliquots (0.5 mL each) at –20°C. Before use, add DTT to the needed single aliquots.

Procedure

1. For each combination of expression construct and bacterial strain (e.g., *E. coli* BL21 (DE3), C43 (DE3), and C41 (DE3)) pick 3 fresh bacterial colonies and inoculate 3–5 mL cultures of 2X YT containing the appropriate antibiotic. Grow at 37°C overnight, shaking at 150 rpm.

These are the precultures.
Note: Because individual colonies can differ in expression level, picking 3 colonies from each transformation increases the likelihood of obtaining a clone with high expression level. This increases the number of screening cultures threefold (see Fig. 1).

2. Prepare a glycerol stock of each preculture by mixing 100 µL cell culture with 100 µL 50% glycerol. Snap-freeze the glycerol stocks in liquid nitrogen and store at –80°C.

Note: The glycerol stocks will be used to inoculate further expression cultures.

3. Determine optical density of the precultures by spectrophotometry (600 nm).

Measure OD_{600} within linear range. If the reading exceeds 0.5, dilute the sample.

4. Prepare 9 mL of media in 100 mL flasks for each screening culture (Fig. 1). Add the appropriate antibiotic(s) and warm to room temperature (15–25°C).

These are the screening cultures.
Note: This culture volume and flask size maximize protein expression.

5. Calculate the volume of each preculture needed to inoculate the corresponding 9 mL screening cultures at a starting OD_{600} of 0.1. Inoculate the screening cultures.

6. Grow the screening cultures at 37°C, 150 rpm, until they reach an OD_{600} of 0.4.

7. Remove a 20 µL aliquot from each screening culture immediately before induction. Store each aliquot in 5 µL 5X SDS-PAGE Buffer at –20°C.

This is the uninduced control.

9. Add 1.8 µL IPTG (200 µM final concentration) to induce protein expression. Return each flask to a shaking incubator at the corresponding temperature (37°C or 16°C; see Fig. 1).

10. Induce expression of the protein for the indicated lenght of time (4 or 10 h).

11. Remove 20 µL aliquots from the induced screening cultures at suitable time points (e.g., every 2 h). Mix each aliquot with 5 µL 5X SDS-PAGE Buffer and store at –20°C.

The aliquots serve as a time course of protein expression induction.

12. Thaw all aliquots taken from uninduced and induced screening cultures and heat for 30 min at 46°C.

Note: To avoid membrane protein aggregation, do not boil the samples.

13. Analyze the samples by SDS-PAGE and Western blot.

The strongest signal on the blot indicates the best expression conditions.

14. Using the clone and expression conditions that yielded the highest expression levels, scale up the expression of the target protein for the detergent screen (e.g., 150 mL in a 2 L culture flask; see Protocol "Screening Detergents for Optimal Solubilization and Purification of Membrane Proteins").

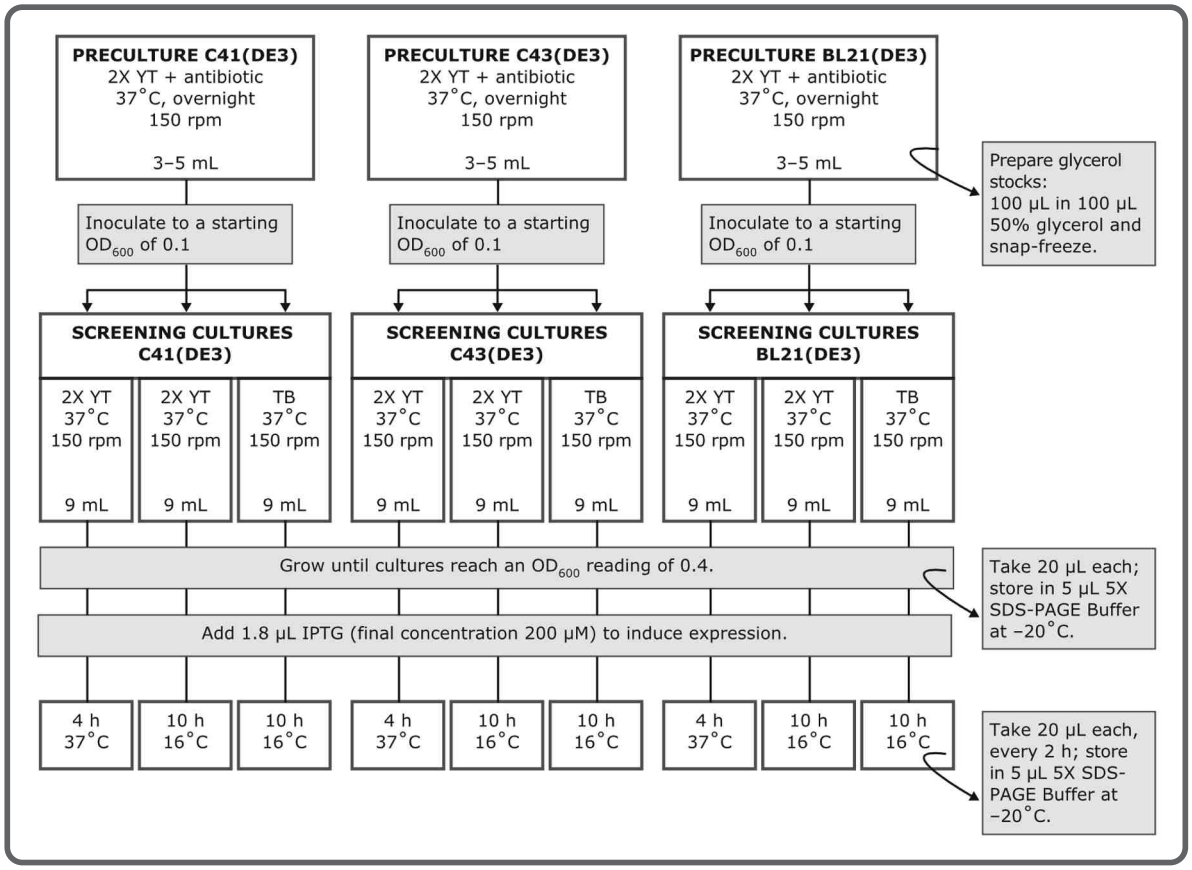

Fig.1: Overview of screening protocol. Precultures are initiated from transformed clones and used to inoculate the screening cultures needed to test all combinations of expression conditions (expression host, media, temperature, and length of induction). It is recommended to sample at least 3 colonies of each transformation, which would increase the number of screening cultures depicted threefold.

References:

Spriestersbach, A., Kubicek, J., Schaefer, F., Block, H., and Maertens, B. 2011. Purification of His-tagged proteins. Methods Navigator.