

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

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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 - procaryotic 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(DE), and C41(DE3) - to optimize conditions for the expression of a membrane protein. Similar screening 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 solubilization 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
- 100mL culture flasks
- 2mL microcentrifuge tubes
- 0.45µm filters

Materials

- Tryptone
- Yeast extract
- Sodium chloride (NaCl)
- Glycerol
- Potassium phosphate monobasic (KH₂PO₄)
- Potassium phosphate dibasic (K₂HPO₄)
- 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 construct (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, 1000mL

| Component | Amount needed for stock | Instructions |
|---------------|-------------------------|---|
| Tryptone | 16g | Dissolve in 900mL deionized water. Adjust to pH7.0 using NaOH, then add deionized water to a total of 1000mL. Sterilize by autoclaving and allow to cool. |
| Yeast extract | 10g | |
| NaCl | 5g | |


Terrific Broth (TB) Media, 1000mL

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

10X TB salts, 1000mL

| Component | Amount needed for stock | Instructions |
|---------------------------------|-------------------------|---|
| KH ₂ PO ₄ | 23.12g | Dissolve in 1000mL deionized water. Sterilize by autoclaving and allow to cool. |
| K ₂ HPO ₄ | 125.41g | |

IPTG, 1mL

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

50% Glycerol, 10mL

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

5X SDS-PAGE buffer, 10mL

| Component | Final concentration | Molecular weight (g/mol) | Stock concentration | Amount needed for stock | Stock needed for final buffer |
|---------------------|---------------------|--------------------------|---------------------|-------------------------|-------------------------------|
| Tris/HCl, pH6.8-7.0 | 300mM | 121.14 (Tris base) | 1M | 121.14g/L | 3mL |
| Glycerol | 50% (v/v) | - | 100% | - | 5mL |
| SDS | 5% (w/v) | - | - | - | 0.5g |
| Bromophenol blue | 0.05% (w/v) | - | 4% | - | 125µL |
| DTT | 250mM | 154,25 | 1M | 1.54g/10mL | 125µL/aliquot |

Instructions: Make sure to prepare a 1M Tris/HCl stock by dissolving Tris base in 500mL deionized water, adding HCl to a pH of 6.8-7.0, and adding water to a final volume of 1L. For the SDS-PAGE buffer, mix all components listed **except DDT** and add water to a total of 10mL. Freeze 20 aliquots (0.5mL 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 3-5mL cultures of 2X YT containing the appropriate antibiotic. Grow at 37°C overnight, shaking at 150 rpm.
2. Prepare a glycerol stock of each pre-culture by mixing 100µL cell culture with 100µL 50% glycerol. Snap-freeze the glycerol stocks in liquid nitrogen and store at -80°C.
3. Determine optical density of the pre-cultures by spectrophotometry (600nm).
4. Prepare 9mL of media in 100mL flasks for each screening culture (Fig. 1). Add the appropriate antibiotic(s) and warm to room temperature (15°C - 25°C)
5. Calculate the volume of each pre-culture needed to inoculate the corresponding 9mL screening cultures at a starting OD₆₀₀ of 0.1. Inoculate the screening cultures.
6. Grow the screening cultures at 37°C, shake at 150 rpm, until they reach an OD₆₀₀ of 0.4.
7. Remove a 20µL aliquot from each screening culture immediately before induction. Store each aliquot in 5µL 5X SDS-PAGE buffer at -20°C.
8. Add 1.8µL IPTG (200µM 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).
9. Induce expression of the protein for the indicated length of time (4h to 10h).
10. Remove 20µL aliquots from the induced screening cultures at suitable time points (e.g., every 2h). Mix each aliquot with 5µL 5X SDS-PAGE buffer and store at -20°C.
11. Thaw all aliquots taken from uninduced and induced screening cultures and heat for 30min. at 46°C.
12. Analyze the samples by SDS-PAGE and Western blot.
13. 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., 150mL in a 2L culture flask; see Protocol "Screening Detergents for Optimal Solubilization and Purification of Membrane Proteins.

These are the pre-cultures.

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 (Fig.1).

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

Measure OD₆₀₀ within linear range. If the reading exceeds 0.5, dilute the sample.

These are the screening cultures.

Note: This culture volume and flask size maximize protein expression.

This is the uninduced control.

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

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

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

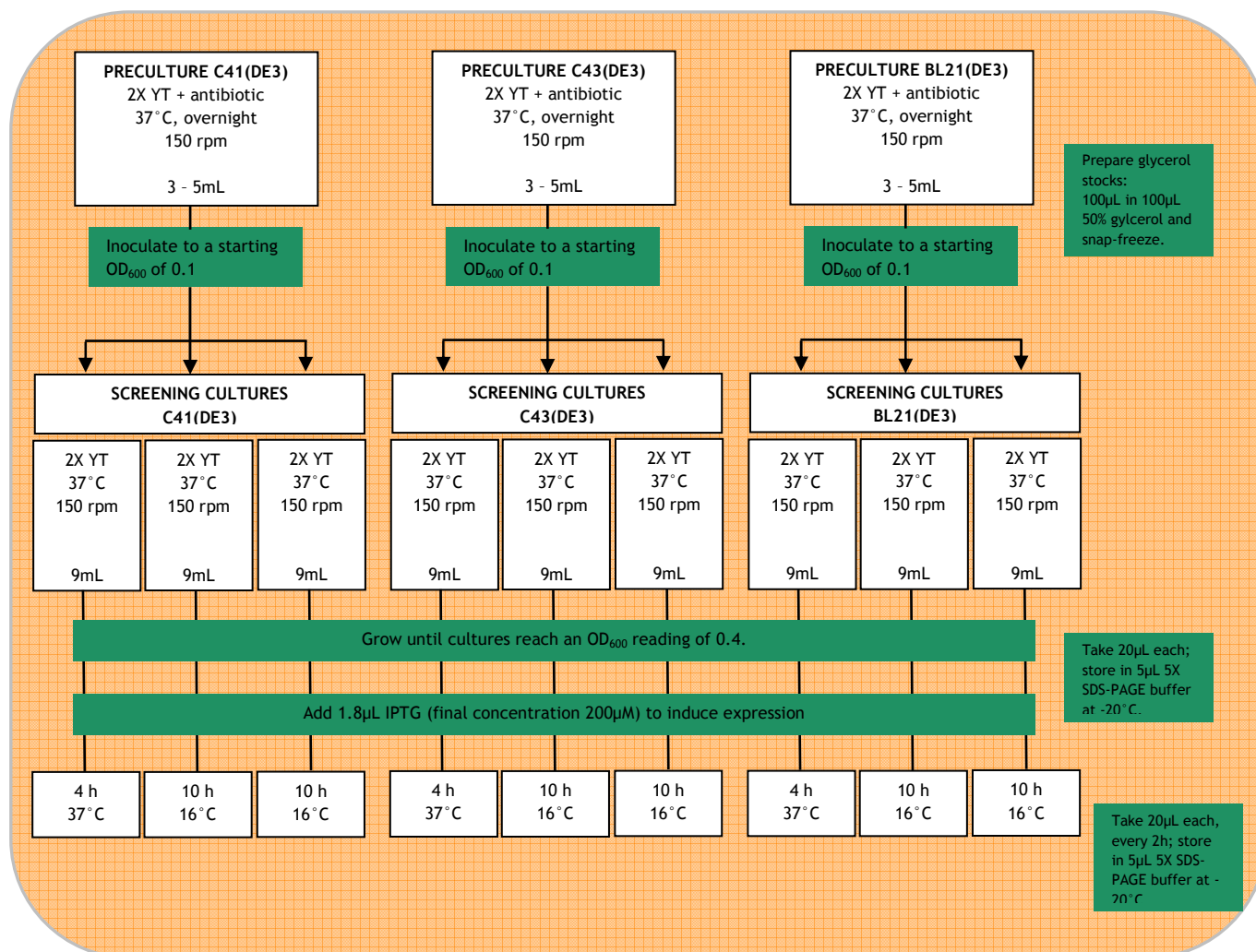


Fig. 1: Overview of screening protocol. Pre-cultures are initiated from transformed clones and use to inoculate the screening cultures needed to test all combinations of expression conditions (expression host, media, temperature, and length of induction). It is recommended to sample at least 3 colonies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

References:

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

9. Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online as pdf-file or on request (info@genaxxon.com).