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E. coli protein expression and purification V.1

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Protocol status: In development
We are still developing and optimizing this protocol

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Abstract

Protocol for recombinant protein expression in E. coli for enzyme assays, protein crystallography etc.

Guidelines

This protocol will take a few days so be sure to have all buffers, cell strains, and plasmids on hand. It can be tricky to organise, practice and familiarity are the best tools. Different sections do not need to be performed immediately after each other - there are various safe stopping steps where cells can be stored at -20/-80 ºC until you are ready to continue with the protocol. However, for convenience, the entire protocol is described here.

Adjust volumes, taking care to ensure appropriate vessels are used to allow proper aeration (e.g. Grow 800mL culture in 2L flasks, 1.3L culture in 3L flasks and 2L culture in 5L flasks), depending on the desired downstream application and expected protein yield (e.g. trialling expression vs bulk production for crystallography). We commonly use BL21 (DE3) strains for T7 expression (i.e. IPTG induction).

Materials

MATERIALS

- Potassium chloride **P212121**
- Petri Dish **P212121 Catalog #**LI-PD01100
- Luria-Bertani (LB) broth, makes 1L **Amresco Catalog #**K488
- EDTA
- 1.5 mL Eppendorf tubes
- Electroporation System Gene Pulser XCell **Bio-Rad Laboratories**
- 37˚C Incubator
- DTT **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D0632
- 2.5ml SDS-PAGE Sample Loading Buffer [2X] **G-Biosciences Catalog #**786-025
- 14ml Polystyrene Cell Culture Tubes **Alkali Scientific Catalog #**CT5250
- 4X Bolt LDS Sample Buffer **Invitrogen - Thermo Fisher Catalog #**B0007
- NaCl **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**53014
- Heat transfer block **Promega Catalog #**Z3271
- IPTG **Bio Basic Inc. Catalog #**IB0168.SIZE.100g
- Coomassie Blue Staining & Destaining Solution **Boster Bio Catalog #**AR0140
- BL21(DE3) or BL21-Star(DE3) or Rosetta2(DE3) or etc for protein purification
- Magnesium chloride hexahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M2670
- Electroporation Cuvette 1mm **Bio-Rad Laboratories Catalog #**1652089
- Falcon® Conical Tubes, 50 mL 500 Tubes **STEMCELL Technologies Inc. Catalog #**38010
- Tris-HCl **Life Technologies Catalog #**AM9855
- 28°C incubator without CO2 **Thermo Fisher Scientific**
- Disodium phosphate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S7907
- Monopotassium phosphate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P9791
- 42°C water bath
- Imidazole **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**I5513
- UV/Vis spectrophotometer
- GelCode™ Blue Stain Reagent **Thermo Fisher Scientific Catalog #**24590

Safety warnings

Ensure use of appropriate asceptic technique. Use caution if using a bunsen burner and ethanol.

Before start

Make sure you have your verified plasmid transformed into your desired E. coli strain for protein expression e.g. BL21 Star (DE3). These should be plated on selective LB media to produce positive colonies for starter cultures. Prepare all the buffers described in Step 1, except make fresh IPTG stocks (ideally).

Prepare buffers

1 **10X PBS:**

- Dissolve the following in 800ml distilled H_2O.
  - 80 g of NaCl
  - 2.0 g of KCl
  - 14.4 g of Na_2HPO_4
  - 2.4 g of KH_2PO_4

- Adjust pH to 7.4.
- Add H_2O to 1L.
- Autoclave

Store 10X stock at 4 °C from which you can dilute 1:10 to make 1X working stock to keep at room temp.

Resuspension buffer:
50 mM Tris-HCl pH 8
2 mM EDTA

1X Binding Buffer:
50 mM NaH_2P0_4, pH 8
300 mM NaCl
10 mM Imidazole

1X Wash Buffer:
50 mM NaH_2P0_4, pH 8
300 mM NaCl
20 mM Imidazole

1X Elute Buffer:
50 mM NaH_2P0_4, pH 8
300 mM NaCl
250 mM Imidazole

Digestion / Storage buffer:
50 mM Tris-HCl pH 8
150 mM NaCl
20 mM KCl

Transformation

2 Transform desired E. coli cell strain with plasmid to be expressed using desired method (e.g. heat shock or electroporation depending on type of competency). 14h

3 For electrocompetent cells:
- Add 0.5 - 1 µL purified plasmid to 50 µL cells (thawing on ice, 15 minutes)
- Gently flick with finger to mix
- Transfer mixer to chilled electroporation cuvette ensuring there are no bubbles. Keep on ice until ready to electroporate
- Set machine to 1.8 kV, 25 µF, 200-400 Ω
- Dry the outside of the cuvette and place into electroporation chamber.
- Prepare p1000 and p200 pipettes to be ready
- Close chamber and electroporate
- Immediately remove cuvette and add 1 mL LB. Transfer contents to microfuge tube using both p1000 and p200.
- Let cells recover at 37 ºC with ~200 rpm shaking for > 1 hour.

4 For chemically competent cells:
- Add 0.5 - 1 µL purified plasmid to 50 µL cells (thawing on ice, 15 minutes)
- Gently flick with finger to mix
- Sit on ice for 30 minutes, set water bath to heat to 42 ºC
- Depending on cells, incubate in water bath for 30 - 90 seconds.
- Return to ice for 5 minutes
- Add 1 mL LB and let cells recover at 37 ºC with ~200 rpm shaking for > 1 hour.

5 Plate recovered transformed cells (~100 µL of transformed cells) onto selective LB media and grow O/N @ 37 ºC. Adjust volume as needed in order to obtain single colonies that can be picked for subsequent innoculation. 1h

Protein expression

6 Innoculate bacterial colony from selective media into LB + antibiotic of choice. Use p100-200 pipette tip to scrape colony and drop into 3-5 mL broth in 10 mL culture tube. Grow O/N @ 37 ºC with ~200 rpm shaking. 1h

7 Innoculate larger culture using the starter culture generated from Step 3 at 1:100 dilution (e.g. 0.5 mL in 50 mL LB + antibiotic). Larger culture volume will depend on what you plan to do with the protein. For example, for large-scale protein production you may use 1 - 2 L cultures - ensure you use an appropriate vessel to allow appropriate aeration (see suggestions in guidelines). For smaller scale tests for protein induction and solubility, you may prefer 10 - 15 mL cultures in 50 mL falcon tubes. 1m

8 Grow larger culture at 37 ºC and check OD_{600} after 2.5 - 3 hours (times may vary depending on total culture volume and quality of cells). 3h

9 When OD_{600} is between 0.6 - 0.8, take an aliquot of culture (up to 15 mL) as non-induced control. To the remaining culture, add induction media. We typically use BL21 (DE3) derived strains and, thus, add 100 mM IPTG to achieve $[IPTG]_{final}$ = 1 mM (i.e. 1:100 dilution, 100 µL IPTG to 10 mL culture). 1m

10 Grow cultures for approx. 5 hours, then check OD for difference between induced vs non-induced. Non-induced should be higher by at least 0.1.

QC protein induction

11 If you are running small scale test induction (e.g. testing constructs, induction, protein solubility), we can run SDS-page gels on the crude lysate (~1-2 mL culture) before trying larger cultures. With larger cultures, you may want to take an aliquot of the culture to check induction before proceeding with purification. If you are confident, proceed to step 21 for washing and storage. 2m

12 Spin down culture (~1 - 3 mL) at max speed for 3 minutes and remove supernatant. This pellet can be stored O/N @ -20 ºC until you are ready to proceed with checking induction of your expressed recombinant protein.

13 Resuspend cells in 100 µL 1X PBS (per 1 mL culture). 1m

14 Calculate how much crude lysate to load onto gel based on OD (µL = 180/OD). 1m

15 To these volumes, add the appropriate amount of 4X LDS (or required SDS-PAGE sample buffer), DTT (final concentration = 50 mM) and $MgCl_2$ (final concentration = 100 mM). These should be calculated first, and a mastermix of LDS, DTT and $MgCl_2$ can be prepared and added to samples accordingly.

16 Heat sample @ 72 ºC for 10 minutes on heat transfer block. 1m

17 Place samples on ice for 5 minutes, then spin for 15 minutes max speed. 2m

18 Transfer supernatant to new tubes, taking care to avoid any "sticky" DNA coating the tube.

19 Run supernatant on SDS-PAGE gel (160V, 40-45 minutes).

20 Stain gel with Coomasie or Gel Code Blue (or other stain of choice). Alternatively, perform Western blot if antibodies are available or recombinant proteins are epitope tagged (e.g. MYC).

Store cells for purification

21 If you are confident with your protein induction (see QC) and want to preserve the cell culture for protein purification, spin down cells at 4 ºC (~7000 rcf for 5 minutes with gentle/no brake). Remove supernatant. 1m

22 Wash cells in resuspension buffer, spin down and remove supernatant.

23 Snap-freeze pellet in LN_2 and store at -80 ºC.

Likely, this will be done pre-emptively until results from the QC are obtained, then cells can be discarded or retained accordingly.

This is a safe stopping point where you can continue with protein purification as needed.

Protein purification

24 Take stored cells out of -80 ºC and let thaw on ice. 3m