Protocol

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IPTG Induction and Extraction of Proteins

Modified from IPTG Induction and Extraction of Proteins from Bacteria by Arur and Nayak, Schedl Lab Washington University, St. Louis

Introduction

Generation of recombinant proteins through the lac operon system in *E. coli* has become a widely used and necessary method for the large-scale production of proteins for experiments and downstream analysis in biological research. In the lac operon system, the absence of an inducer or lactose results in a lac repressor (encoded by *lacI*) binding the lac operon and repressing gene transcription. However, in the presence of a chemical inducer such as β-D-1-thiogalactopyranoside (IPTG), which allosterically binds the lac repressor, the repressor disassociates from the lac operon (lacO), leading to transcription of genes under lac operon control (Figure 1). The resulting recombinant proteins can then be purified for downstream analysis. Induction in bacteria can be performed using one of two basic methods: slow or fast induction. Fast induction will not work for all proteins and may give suboptimal yields, whereas using slow induction may enhance the solubility of some proteins. The method used will depend on your particular protein and its eventual application. For optimal solubility, test both methods before scaling up. Here, we describe a general protocol that can be optimized for the specific bacterial strain, recombinant protein and parent plasmid.

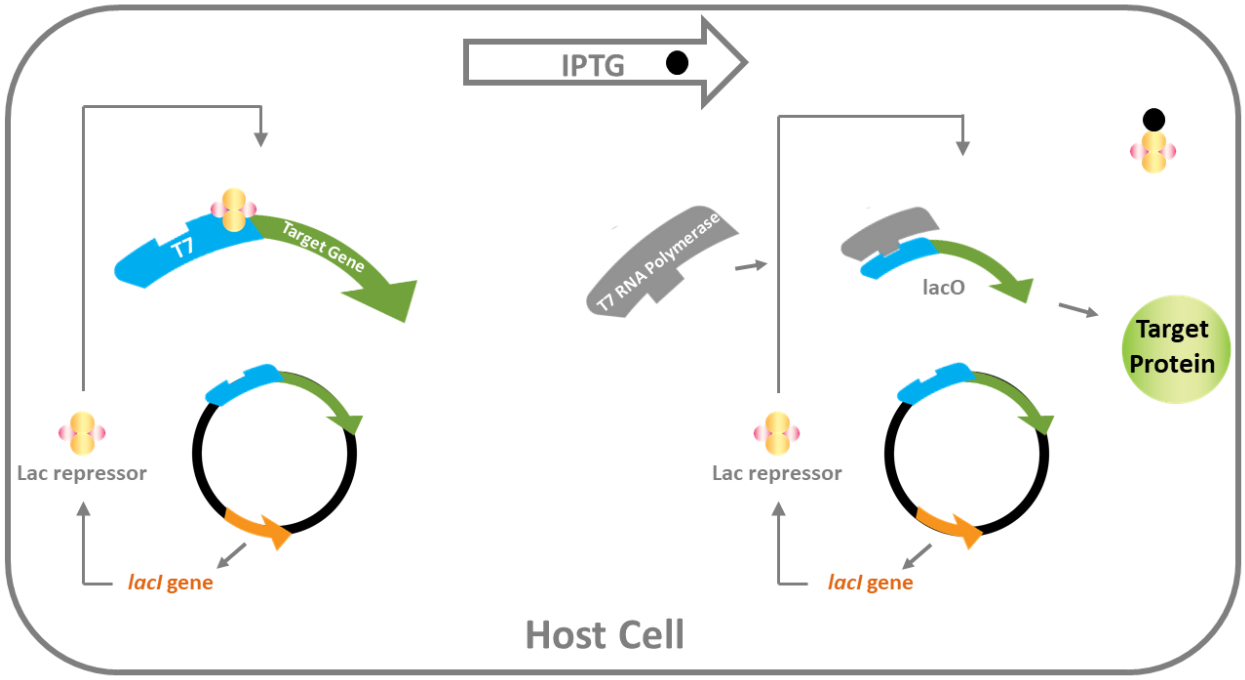

Figure 1. IPTG induction of protein expression.

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Materials

- LB Antibiotic (Ampicillin, Kanamycin, Carbenicillin, etc.)
- IPTG (GoldBio Catalog # I2481)
- Lysozyme (GoldBio Catalog # L-040)
- Ice
- DTT (GoldBio Catalog # DTT)
- Protease Inhibitors
- N-laurylsarcosine (Sarkosyl)
- Triton X-100
- Nickle Agarose Beads (GoldBio Catalog # H-320)
- Ice-cold PBS (GoldBio Catalog # P-271)
- Imidazole (GoldBio Catalog # I-902)

For Loading Buffer:

- Tris-HCl (GoldBio Catalog # T-095)
- SDS
- Glycerol
- EDTA Disodium (GoldBio Catalog # E-210)
- Bromophenol Blue (GoldBio Catalog # B-092)
- dH_2O
- β-mercaptoethanol

Preparation of Loading Buffer:
Loading Buffer – 4X Stock (to make total volume 40 ml)

1. 2.0 ml 1M Tris-HCl, pH 6.8 (final concentration 50mM Tris-HCl, pH 6.8)
2. 0.8 g SDS (final concentration 2% SDS)
3. 4.0 ml 100% Glycerol (final concentration 10% Glycerol)
4. 1.0 ml 0.5M EDTA (final concentration 12.5mM EDTA)
5. 8 mg Bromophenol Blue (final concentration 0.02% Bromophenol Blue)
6. Fill to volume with dH_2O

Note: Add fresh β-mercaptoethanol (BME) to 1% before use.

Method

Fast Induction

1. From a relatively fresh plate, pick a colony and place in a 15 ml tube containing 1-2 ml Luria Broth (LB) + Antibiotic (e.g. Ampicillin, Kanamycin, Carbenicillin, etc.).

2. Grow overnight at 30°C (or 37°C) on a rotator or shaker.

3. Dilute to 1:50 (1:100 if grown at 37°C overnight) in 2 ml LB + Antibiotic in a 15 ml tube and grow for 3-4 hours at 37°C in a rotator.

Note: After 3-4 hours of growth, a 1 ml aliquot of this bacterial culture will be used for the uninduced control (in step 5) and the rest will be used for induction (in step 6).

4. Prepare 1 ml LB + Antibiotic + 1mM IPTG in a 15 ml conical and prewarm to 37°C about 10 minutes before use.

   Note: IPTG concentration can vary from 0.1 to 1M.

5. After growing for 3-4 hours, remove 1 ml from tubes prepared in step 3 and place in labeled 1.5 ml tubes. Centrifuge at maximum speed for 30 seconds at room temperature and remove supernatant. Freeze pellet at -20°C until needed. **THIS IS THE UNINDUCED CONTROL.**

   Note: For slow induction, skip to the slow induction section.

6. Add 1 ml prewarmed (37°C) LB + Antibiotic + 1mM IPTG prepared in step 4 to 15 ml tubes prepared in step 3, and return to 37°C for 3-4 hours.

   Note: This will get the final volume back to 2 ml and the final concentration of IPTG to 0.5mM.

   Note: Fast induction times may vary from 2 to 5 hours.

7. After 3-4 hours, transfer 1 ml from the induced sample to labeled 1.5 ml tubes and centrifuge at maximum speed for 30 seconds at room temperature and remove the supernatant. Freeze pellet at -20°C until needed. **THIS IS THE INDUCED SAMPLE.**

8. Sample preparation for SDS-PAGE.
   a. Add 100 µl of 1x Loading Buffer with 1% BME to uninduced and induced samples.
   b. Vortex the samples for 10 seconds to 1 minute or until there are no clumps of bacteria.
   c. Boil the samples for 3 to 5 minutes.
   d. Centrifuge at maximum speed for 30 seconds at room temperature and load 5-25 µL (usually 10 µl), depending on the gel, amount of protein, size of pellet and Western, etc.

   Note: If you boil your sample too long, it will become viscous from the total release of cellular DNA. You can still use them if you can find an area of low viscosity. However, it is usually better to repeat the experiment.

Slow Induction

For slow induction of protein, follow the fast induction protocol with the following changes.

1. In step 6 of the fast induction protocol, add 1 ml LB + Antibiotic + 1mM IPTG (prewarmed to 20°C) to 15 ml tube and incubate rotating or shaking at **20°C for 12 to 16 hours.**

Note: This will get the final volume back to 2 ml and the final concentration of IPTG to 0.5mM.

2. After 12-16 hours, transfer 1 ml from induced sample to labeled 1.5 ml tubes and centrifuge at maximum speed for 30 seconds at room temperature and remove the supernatant. Freeze pellet at -20°C until needed. **THIS IS THE INDUCED SAMPLE.**

Extraction of Soluble Proteins

This basic protocol will work for FLAG, GST and His-tags. It has not been tested for MBP, which does not respond well to detergents.

1. Wash the bacterial pellet with 2 ml of ice cold Tris-EDTA (STE) Buffer (10mM Tris at pH 8.0, 150mM NaCl and 1mM EDTA) once.

2. Resuspend the bacterial pellet (from a 10 ml induced culture) in 800 µl of STE buffer containing 100 µg/ml of Lysozyme (added immediately prior to resuspension).

3. Incubate on ice for 15 minutes.

4. Add DTT (see 1M DTT Stock Solution Protocol) to a final concentration of 5mM.

5. Add protease inhibitors (e.g. EDTA or PMSF).

Note: Suggested concentration of PMSF is 100mM (see 1M PMSF Stock Solution Protocol).

6. Bacteria are then lysed by the addition of N-laurylsarcosine (Sarkosyl) from a 10% (w/v) stock in STE buffer. The final concentration of N-laurylsarcosine should be 1.5%.

7. Sonicate the cells for 2 cycles (6 minutes each).

8. Centrifuge the lysate for 5 minutes at ~100 g, 4°C.

9. Transfer the supernatant to a new 1.5 ml tube and add Triton X-100 (from a 10% stock made in STE buffer) to a final concentration of 2%.

10. Take 100 µl of Nickel Agarose Beads and wash twice by centrifugation with ice-cold PBS at ~100 g for 1 minute each.

    Note: The inclusion of Nickel Agarose Beads and Imidazole is specific to His-Tags

11. Add the beads to the 1.5 ml tube containing the lysate and the Triton X-100 tube and incubate on a rotator or rocker at room temperature for 30-60 minutes.

12. Wash the beads 4X with 1 ml ice-cold PBS containing 20mM imidazole at ~100 g for 1 minute.

    Note: TWEEN 20 at 0.1-1% can also be incorporated into the wash buffer to reduce background if required.

13. Add 1X Loading Buffer with 1% BME, boil for 3 minutes and analyze on SDS-PAGE.

Tips

- Ensure that the appropriate bacterial strain for the appropriate level of protein production is chosen. Specific strains are more appropriate for large scale recombinant protein production.
- The use of tagged proteins is recommended because it facilitates the purification process.
- One recommended tag is the N-terminal polyhistidine tag because it allows for easy protein purification and does not interfere with protein folding or function. Thus, it does not have to be cleaved before use of the protein in downstream applications.
- When attempting to produce eukaryotic proteins, ensure that the bacterial strain used contains additional tRNAs.
- Carbenicillin is more stable than penicillin. Thus, it might be advantageous to use in protein production.
- Lower induction temperatures may be advantageous since they result in slower rate of protein production and allows the proteins to fold properly.

Associated Products

- LB Antibiotic (Ampicillin, Kanamycin, Carbenicillin, etc.)

- IPTG (GoldBio Catalog # I2481)
- DTT (GoldBio Catalog # DTT)
- Protease Inhibitors
- Nickel Agarose Beads (GoldBio Catalog # H-320)
- PBS (GoldBio Catalog # P-271)
- Imidazole (GoldBio Catalog # I-902)
- Tris-HCl (GoldBio Catalog # T-095)
- EDTA Disodium (GoldBio Catalog # E-210)
- Bromophenol Blue (GoldBio Catalog # B-092)
- Lysozyme (GoldBio Catalog # L-040)

References

Arur, S. and Nayak, S. (n.d.). *IPTG Induction*. Retrieved June 8, 2018 from http://genetics.wustl.edu/tslab/protocol/protein-stuff/iptg-induction/

Graslund, S., Nordlund, P., Weigelt, J., Hallberg, M., Bray, J., Gileadi, O., . . . Gunsalus, K. (2008). Protein production and purification. *Nature Methods, 5*(4), 369-369. Doi:10.1038/nmeth0408-369.

Rabinovsky, E. D., Browder, D. P., & Mcmanaman, J. L. (1994). Preparation and affinity purification of a novel, biologically active, CNTF fusion protein. *Journal of Neuroscience Research, 38*(2), 127-133. Doi:10.1002/jnr.490380202.