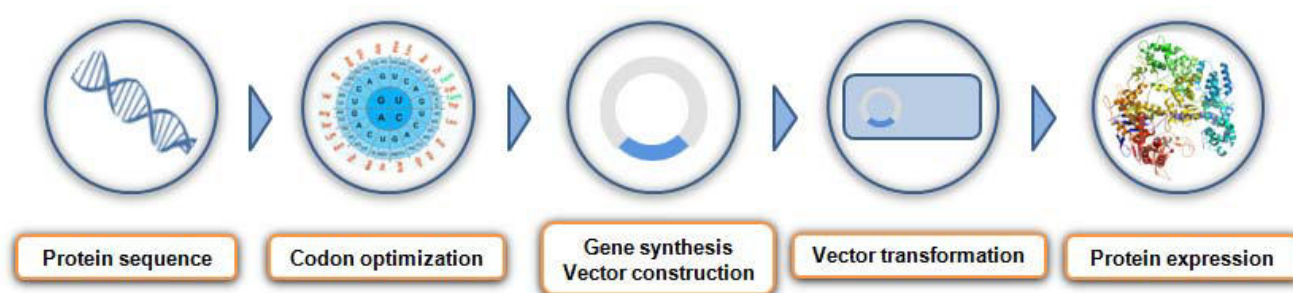


Protein Expression Protocol & Troubleshooting in *E. coli*

In terms of recombinant expression, *E. coli* has always been the preferred microbial cell factory as it has multiple, significant benefits over other expression systems including cost, ease-of-use, and scale. Here, we present a general protocol of protein expression as well as a list of possible solutions when facing the challenge of expressing a new protein in *E. coli*.

I. Protein Expression Protocol in *E. coli*

General protocol of protein expression process from gene to protein is given below.



Phase 1: Codon Optimized Gene Synthesis and Vector Construction

- Codon and mRNA structure optimization
- Fuse gene to a protein tag and insert them into an expression vector
- Verify the correctness of construction by sequencing

Phase 2: Transform Expression Vector into *E. coli* Competent Cells

- Add expression vector to thawed competent cells
- Add heat-shocked cells to LB broth and shake
- Plate cell culture onto LB agar plates with appropriate antibiotic

Phase 3: Starter Culture

- Pick single colony of expression strain into 5 ml of LB with appropriate antibiotic
- Shake at 37°C for 3 to 5 hours

Phase 4: Expansion of Starter Culture

- Expand the culture by adding the starter culture to larger volume LB with antibiotic (room temp)
- Incubate for 1-4 hours until culture density of OD₆₀₀ reaches 0.5-0.6

Note: Top the flask with cotton or a culture flask cap to allow culture to oxygenate without getting environmental contamination.

Phase 5: Protein Expression Induction

Option 1: 37°C Induction

- Induce expression by adding IPTG to a final concentration of 0.5 mM after culture has reached OD₆₀₀ 0.5-0.6
- Induce for 3-4 hours at 37°C with shaking

Note: IPTG is a frozen solution in the -20 °C freezer.

Option 2: Room Temp (20°C) Induction

- Cool down the culture to room temperature by placing in fridge or iced water bath after it has reached OD₆₀₀ 0.5-0.6
- Induce expression by adding IPTG to a final concentration of 0.1 to 1.0 mM
- Induce overnight (12-18 hours) at room (20°C) temp with shaking

Phase 6: Cells Collection and Lysis

- Centrifuge the cells at 3,500 x g for 20 min
- Resuspend cells in ice cold PBS and re-centrifuge in an appropriate sized tube
- Remove the supernatant and freeze pellet for later processing
- Lyse cells using appropriate protocol

II. Protein Expression Troubleshooting in *E. coli*

In this section, we present different strategies for optimizing recombinant protein production in *E. coli* when encountering expression obstacles. Possible reasons and solutions in each case are discussed in the following tables.

1) No / Low protein expression

When the protein of interest cannot be detected through a sensitive technique (e.g., Westernblot) or it is detected but at very low levels (less than micrograms per liter of culture), the problem often lies in a harmful effect that the heterologous protein exerts on the cell.

Reasons	Solutions		
	Vector	Host strain	Growth conditions
Incorrect vector construction	Confirm vector by sequencing		

Rare codons	Codon optimization	Use strains supplementing rare codons (Rosetta, Codon Plus)	• Lower induction temperature • Grow in poor media
Protein toxicity	• Use promoters with tighter regulation • Lower plasmid copy number	• Use pLysS/pLysE bearing strains in T7-based systems • Use strains that are better for the expression of toxic proteins (C41 or C43)	• Start induction at high OD • Shorten induction time • Add glucose when using expression vectors containing lac-based promoters • Use defined media with glucose as source of carbon

2) Protein aggregation

The buildups of protein aggregates are known as inclusion bodies (IBs). IB formation results from an unbalanced equilibrium between protein aggregation and solubilization. So, it is possible to obtain a soluble recombinant protein by strategies that ameliorate the factors leading to IB formation.

Reasons	Solution		
	Vector	Host strain	Growth conditions
Incorrect disulfide bond formation	• Add fusion partners, including thioredoxin, DsbA, DsbC • Clone in a vector containing secretion signal to cell periplasm	Use <i>E. coli</i> strains with oxidative cytoplasmic environment	• Lower inducer concentration • Lower induction temperature
Incorrect folding	• Use a solubilizing partner • Co-express with molecular chaperones	Use strains with cold-adapted chaperones	• Supplement media with chemical chaperones and cofactors • Remove inducer and add fresh media • Lower inducer concentration • Lower temperature

Proteins with high hydrophobicity or transmembrane domains	• Add fusion tags, including GST, MBP, SUMO, etc. • Generate truncated forms of protein	Use membrane rich strains (C41/C43)	• Lower induction temperature • Shorten induction time • Grow in poor medium • Add heat shock chaperones
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3) Truncated protein

Sometimes a truncated form of protein is expressed rather than a complete wild protein. Reasons of the phenomenon and possible solutions are given below.

Reasons	Solution		
	Vector	Host strain	Growth conditions
Rare codon	Codon optimization	Use strains supplementing rare codons (Rosetta, Codon Plus)	• Lower induction temperature • Grow in poor media
Protein degradation	Replace specific protease sites	Use low protease strains	• Induce at high OD • Induce at low temperature • Shorten induction time • Use protease inhibitors when breaking cells
Imbalanced translation process of fusion protein	• Change another fusion protein • Move fusion protein to C-terminal		• Induce at low temperature • Shorten induction time • Change to poor media

4) Protein inactivity

Obtaining a nice amount of soluble protein is not the end of the road. The protein may still be of bad quality; i.e., it does not have the activity it should.

Reasons	Solutions		
	Vector	Host strain	Growth conditions

Low solubility of the protein	Fuse desired protein to a solubility enhancer (fusion partners)		Lower temperature
Lack of essential post translational modification	Change expression system		
Incomplete folding	• Use a solubilizing partner • Co-express with molecular chaperones	Use strains with cold-adapted chaperones	• Monitor disulfide bond formation and allow further folding in vitro • Lower temperature
Mutations in cDNA	Sequence plasmid before and after induction	Use a <i>recA</i> ⁻ strain to ensure plasmid stability	Transform <i>E. coli</i> before each expression round

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Francis DM, Page R (2010). Strategies to optimize protein expression in *E. coli*. *Current Protocols in Protein Science*, Chapter 5:Unit 5.24.1-29.



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