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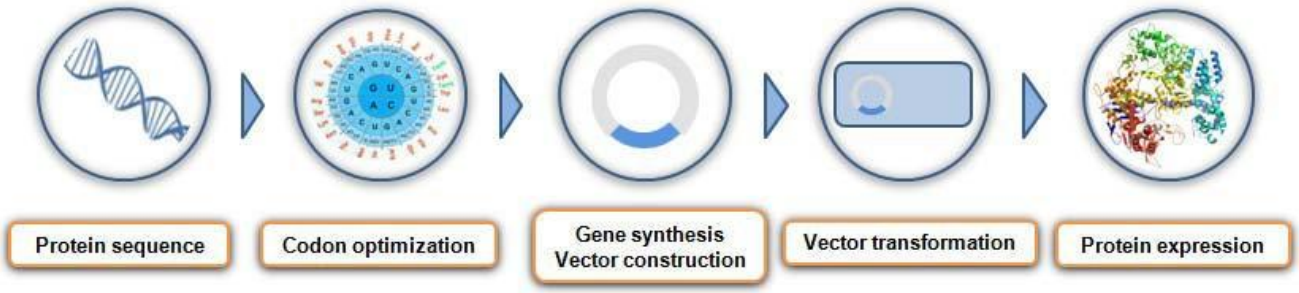
# Protein Expression Protocol & Troubleshooting in *E. coli*

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.




**Step 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

**Step 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

**Step 3: Starter Culture**

- Pick single colony of expression strain into 5-10 ml of LB with appropriate antibiotic
- Shake at 37℃ for 3 to 5 hours

**Step 4: Expansion of Starter Culture**

- Expand the culture by adding the starter culture to larger volume LB with antibiotic
- Incubate for 4-6 hours until culture density of $OD_{600}$ reaches 0.6-0.8

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**Step 5: Protein Expression Induction**

Option 1: 37℃ Induction

- Induce expression by adding IPTG to a final concentration of 0.5 mM-1mM after culture has reached $OD_{600}$ 0.6-0.8
- Induce for 4 hours at 37℃ with shaking

Option 2: 16℃Induction

- Cool down the culture to 16℃ by placing in fridge or iced water bath after it has reached $OD_{600}$ 0.6-0.8
- Induce expression by adding IPTG to a final concentration of 0.1 to 1.0 mM
- Induce overnight (12-18 hours) at 16℃with shaking

**Step 6: Cells Collection and Lysis**

- Centrifuge the cells at 5,000 x g for 10 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 can't 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 | | |

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| | | | |
|---|---|---|---|
| **Rare codons** | Codon optimization | Use strains Supplementing rare codons (Rosetta, Codon Plus) | • Lower induction temperature<br>• Grow in poor media |
| **Protein toxicity** | • Use promoters with tighter regulation<br>• Lower plasmid copy number | • Use pLysS/pLysE bearing strains in T7-based systems<br>• Use strains that are better for the expression of toxic proteins (C41 or C43) | • Start induction at high OD<br>• Shorten induction time<br>• Add glucose when using expression vectors containing lac-based promoters<br>• 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<br>• Clone in a vector containing secretion signal to cell periplasm | Use *E. coli* strains with oxidative cytoplasmic environment | • Lower inducer concentration<br>• Lower induction temperature |
| **Incorrect folding** | • Use a solubilizing partner<br>• Co-express with molecular chaperones | Use strains with cold-adapted chaperones | • Supplement media with chemical chaperones and cofactors<br>• Remove inducer and add fresh media<br>• Lower inducer concentration<br>• Lower temperature |

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

### 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 supplement in rare codons (Rosetta, Codon Plus) | • Lower induction temperature<br>• Grow in poor media |
| **Protein degradation** | Replace specific protease sites | Use low protease strains | • Induce at high OD<br>• Induce at low temperature<br>• Shorten induction time<br>• Use protease inhibitors when breaking cells |
| **Imbalanced translation process of fusion protein** | • Change another fusion protein<br>• Move fusion protein to C-terminal | | • Induce at low temperature<br>• Shorten induction time<br>• 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** |

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| | | | |
|---|---|---|---|
| **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<br>• Co-express with molecular chaperones | Use strains with cold-adapted chaperones | • Monitor disulfide bond formation and allow further folding in vitro<br>• Lower temperature |
| **Mutations in cDNA** | Sequence plasmid before and after induction | Use a *recA*$^-$ strain to ensure plasmid stability | Transform *E. coli* before each expression round |