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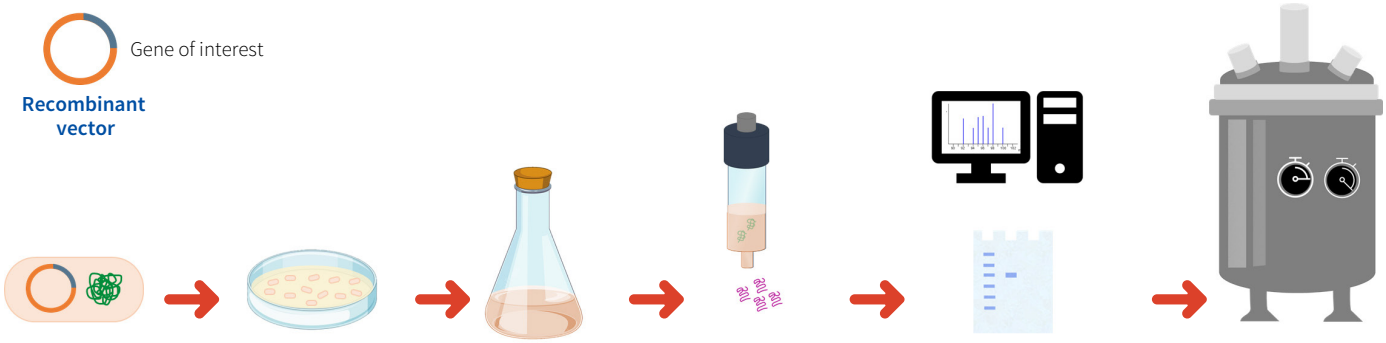
Aragen's Solutions for Mitigating Challenges in Recombinant Protein Production

Recombinant proteins (RP), produced through genetic engineering, include a diverse range of proteins including antibodies, enzymes, and vaccines, widely used in the biopharmaceutical industry. These proteins are successfully produced with *E. coli*-based expression systems that offer rapid, scalable, and cost-effective synthesis of RPs. However, inherent challenges can impede efficient protein production, leading to low yields.

At Aragen, our experienced scientists offer a streamlined protein expression and purification process (Figure 1) that helps in overcoming the challenges encountered with *E. coli* protein expression systems through effective mitigation strategies to optimize yield, quality, and functionality.

Process Development



Transformation	Expression	Culture	Purification	Characterization & QC	Large-scale production
	• Optimal temperature • Appropriate inducer concentration • Suitable growth media		• Optimization of chromatography parameters optimization-column, elution conditions at 3 levels.	• Identity confirmation by mass spectrometry, peptide mass fingerprinting and Western blot analysis. • Purity analysis by SDS-PAGE, analytical chromatography (size-exclusion, reverse phase).	• Enhanced scalability up to >10L. • QC analysis for product qualification. • Reproducible batch–to–batch yield and quality recombinant protein production phase).

1. Improper protein folding and solubility: Recombinant protein expression in *E. coli* often encounters hurdles primarily related to protein folding, which directly impacts protein solubility and functionality.

Mitigation strategies:

- **Use of fusion tags** like maltose-binding protein (MBP), glutathione S-transferase (GST) or small ubiquitin-like modifier (SUMO) facilitates proper folding and improves solubility of target proteins.
- **Optimize growth conditions** like temperature, inducer concentration, or culture medium composition, promoting proper protein folding and solubility, thereby improving the yield of functional, soluble protein.
- **Co-expression with molecular chaperones** (eg., GroEL/GroES or DnaK/DnaJ/GrpE) facilitates proper folding, prevents misfolding, and minimizes aggregation.
- **Protein expression in the periplasmic space** using the pelB leader sequence provides an oxidizing environment optimal for disulfide bonds formation and reduces the risk of proteolytic degradation, allowing for a higher proportion of properly folded and soluble protein production.

2. Lack of specific protein folding conditions: Some proteins require post-translational modifications or specific folding conditions that are absent in the *E. coli* cytoplasm.

Mitigation strategies:

- **Use of specialized strains** like the SHuffle strain, promotes disulfide bond formation via constitutive expression of the disulfide bond isomerase (DsbC), or strains with engineered chaperone expression.
- **Co-expression with folding factors or enzymes** involved in post-translational modifications.

3. Codon Bias: Preferential usage of the target gene's synonymous codons causes inefficient expression in *E. coli* expression systems.

Mitigation strategies:

- **Codon design optimization** typically by synonymous codon substitution.
- **Use of rare codon-supplemented strains, like Rosetta strains** facilitate enhanced protein expression.

4. Protein Degradation: *E. coli* contains proteases that might degrade recombinant proteins, reducing overall yield and purity.

Mitigation strategies:

- **Use of protease-deficient strains** [e.g., BL21 (DE3), BL21 Star (DE3) or Rosetta (DE3) strains].
- **Protease inhibitors or expressing proteins** at lower temperatures reduce protein degradation.
- **Implementing rapid purification protocols** minimizes exposure to proteases.

5. Toxicity: Some recombinant proteins may be toxic to *E. coli* cells, leading to poor growth or low protein expression levels.

Mitigation strategies:

- **Using tightly regulated promoters** to control expression levels and minimize toxicity during growth. For example, BL21(DE3) pLysS strain expresses T7 phage lysozyme that inhibits T7 RNA polymerase activity and hence, reduces target protein's basal expression.
- **Lower expression temperature** can slow down protein synthesis, reducing toxicity.
- **Tuning expression levels by** adjusting inducer concentration or promoter strength can optimize expression levels without causing toxicity.

Outcome: Maximized soluble protein expression in *E. coli* system, reduced production costs by 20-30% (reduction in both FTE hours and material cost) with a substantial drop in turn-around time by 20%.

Aragen's Leadership in Protein Expression

At Aragen, we have over 10 years extensive experience in the field of recombinant protein production. Our clientele comprises biopharma and biotechnology companies focused on protein engineering and targeting to create multifunctional, precision therapies for various indications such as cancer, hematology, immunology, and rare diseases. We also extend our services to animal health and agroscience companies.

- Track record of producing ≥ 1000 functionally active recombinant proteins, including monoclonal antibodies and complex fusion proteins.
- World class facilities for recombinant protein generation in microbial, insect, and mammalian expression systems.
- Experienced team of protein biotechnologists with expertise in delivering proteins with desired activity, purity, and other analytical characteristics.
- Industry-leading developability assessment capability to identify and mitigate liabilities associated with protein candidates.

Ready to elevate your game in the recombinant protein industry? Write to us at info@aragen.com to speak to our expert.