Factors in soluble protein expression optimization

- **Codon optimization**
- Strains
- Vectors
- Protein synthesis rate
- Growth medium
- Fusion tags
- Co-expression proteins
CODON OPTIMIZATION

- Adjust codon usage frequency and enhance mRNA stability
- State-of-art algorithms
- Pre-computed software from a predicted group of highly expressed genes

**Parameters**

- Codon usage bias
- GC content
- mRNA secondary structure
- Repeat sequence

- Avoided restriction enzymes
- Non-coding regions
- Motifs
**STRAINS**

### BL21 (DE3)
- Routine protein expression
- Deficient in proteases Lon and OmpT, which increase protein stability

### BL21 Star (DE3)
- Promotes high mRNA stability and protein yield
- T7 promoter-based expression systems

### Rosetta (DE3)
- Contain rare codons (AUA, AGG, AGA, CU A, CC, GGA) in E. coli
- Adjust codon usage bias and enhance protein expression

### BL21 (DE3) pLysS
- Suppress basal expression
- Ideal for toxic protein expression

### BL21-CodonPlus (DE3)
- Contain extra copies of tRNA genes
- Improve expression of heterologous proteins from organisms without AT- or GC-rich genomes

### OrigamiB (DE3)
- Combine desirable characteristics of BL21, Tuner™, and Origami strains in one strain
- Ideal for expressing protein with disulfide bonds
VECTORS

**pET vector**
- Powerful: high expression levels, tight control over basal expression
- Versatile: choices of tags and multiple cloning sites
- Rapid: convenient restriction sites for sub cloning, one-step purification

**pCold vector**
- Efficient: improved protein expression at low temperatures

**In-house fusion vector**
- Multiple vectors available to improve protein expression level
Multiple strategies slows down translation process for optimized protein solubility.

- Use a weak promoter
- Use a low copy number plasmid
- Lower growth temperature
- Lower inducer concentration
Special chemicals in media serve important roles in protein proper folding and protein stability.

- **Add buffer**: Control pH fluctuation in the growth medium.
- **Add Glucose**: Repress induction of the lac promoter by lactose.
- **Add polyols**: Work as osmo-prectants and stabilize protein structure.
- **Add prosthetic groups or cofactors**: Trace metals, minerals, and vitamins may contribute to protein proper folding and stability.
A large variety of protein tags are available for improving protein stability and facilitating soluble protein expression.

<table>
<thead>
<tr>
<th>Tag</th>
<th>Size</th>
<th>Protein</th>
<th>Key features</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST</td>
<td>211 aa</td>
<td>Glutathione-S-transferase</td>
<td>Easy to manipulate</td>
</tr>
<tr>
<td>MBP</td>
<td>396 aa</td>
<td>Maltose-binding protein</td>
<td>Available for purification</td>
</tr>
<tr>
<td>NusA</td>
<td>495 aa</td>
<td>N-utilization substance</td>
<td>Increase membrane protein solubility</td>
</tr>
<tr>
<td>Trx</td>
<td>109 aa</td>
<td>Thioredoxin</td>
<td>Simple initial purification step</td>
</tr>
<tr>
<td>SUMO</td>
<td>~100 aa</td>
<td>Small ubiquitin modifier</td>
<td>High specific cleavage sites</td>
</tr>
</tbody>
</table>
**Chaperones:** transiently interact with protein folding intermediates and contribute to protein expression
- GroES-GroEL
- DnaK-DnaJ-GrpE
- ClpB
- Trigger factor
- Heat shock proteins

**Foldases:** accelerate rate-limiting steps in the folding process
- Peptidylprolylcis/trans isomerases (PPI's)
- Disulfide oxidoreductase (DsbA) and disulfide isomerase (DsbC)
- Protein disulfide isomerase (PDI)
Soluble protein expression optimization process

- Codon optimized gene synthesis
- Expression condition optimization
- Scale-up production
- Purification and delivery
BiologicsCorp (BIC) owns a self contained and complete optimization platform for supernatant protein expression. We also offer tag-free protein production, endotoxin removal, and high-throughput protein production services. Please feel free to contact us for more information about the services.

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REFERENCES