SOLUBLE PROTEIN EXPRESSION OPTIMIZATION

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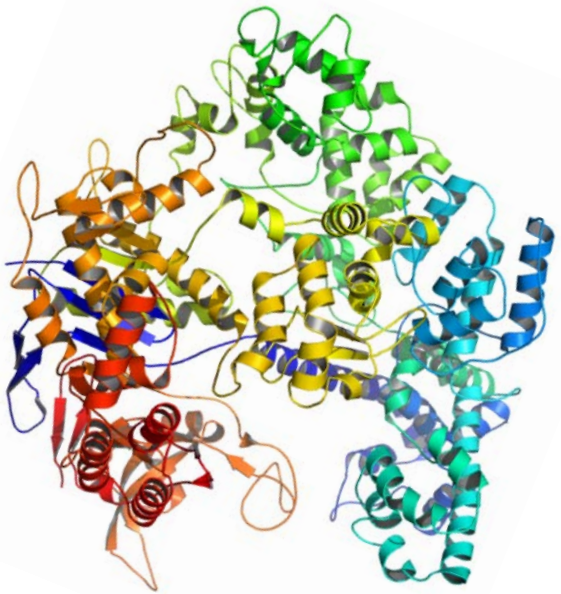
OUTLINE

- Factors in soluble protein expression optimization
  - Codon optimization
  - Strains
  - Vectors
  - Protein synthesis rate
  - Growth medium
  - Fusion tags
  - Co-expression proteins



CODON OPTIMIZATION

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- 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

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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,CUA,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

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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

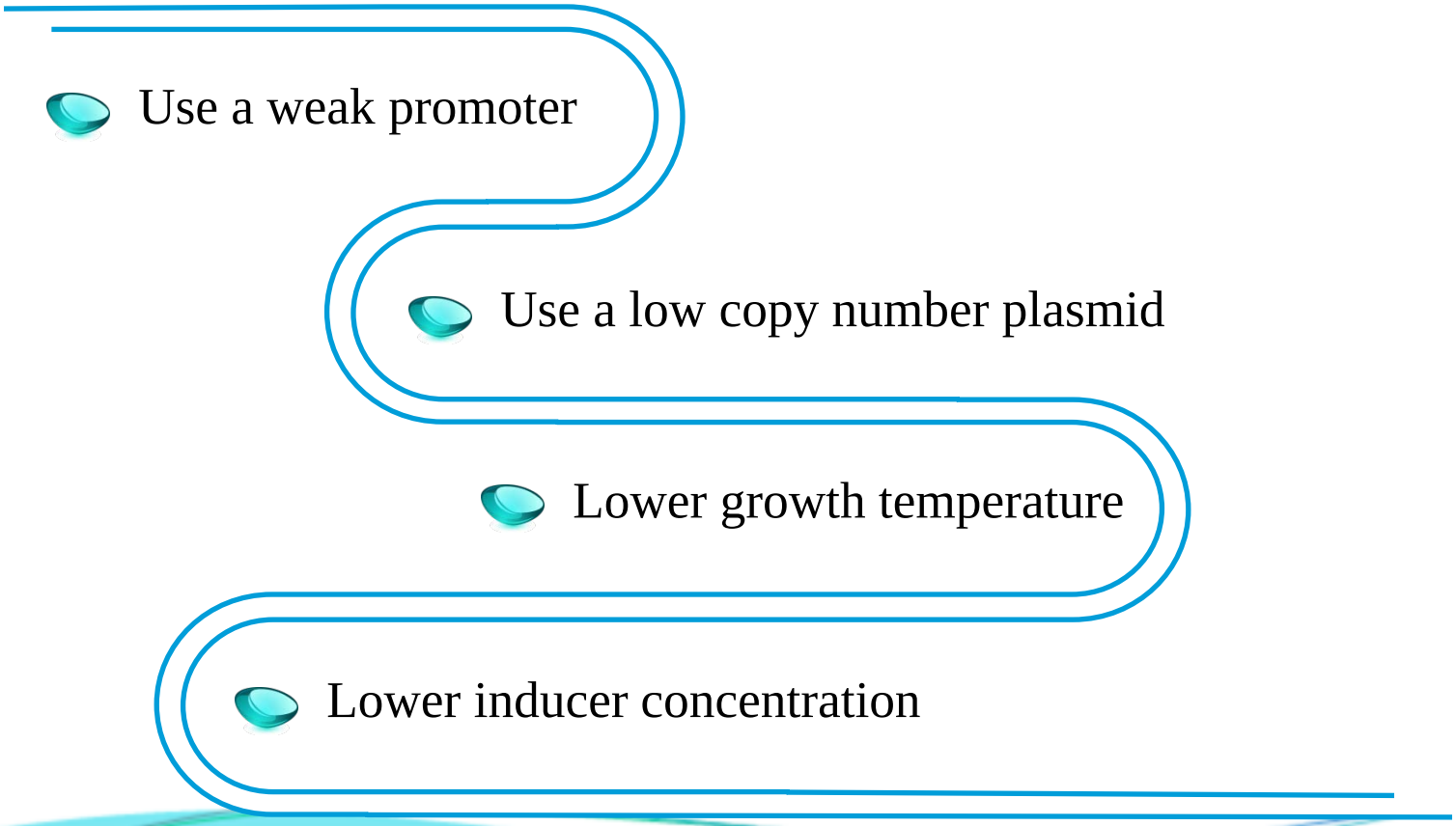
- Efficient: improved protein expression at low temperatures

In-house fusion vector

- Multiple vectors available to improve protein expression level

PROTEIN SYNTHESIS RATE

- Multiple strategies slows down translation process for optimized protein solubility.



GROWTH MEDIUM

- Special chemicals in media serve play 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 co-factors	Trace metals, minerals, and vitamins may contribute to protein proper folding and stability.

FUSION TAGS

- A large variety of protein tags are available for improving protein stability and facilitating soluble protein expression.

Tag	Size	Protein	Key features
GST	211 aa	Glutathione-S-transferase	Easy to manipulate
MBP	396 aa	Maltose-binding protein	Available for purification
NusA	495 aa	N-utilization substance	Increase membrane protein solubility
Trx	109 aa	Thioredoxin	Simple initial purification step
SUMO	~100 aa	Small ubiquitin modifier	High specific cleavage sites

CO-EXPRESSION PROTEINS

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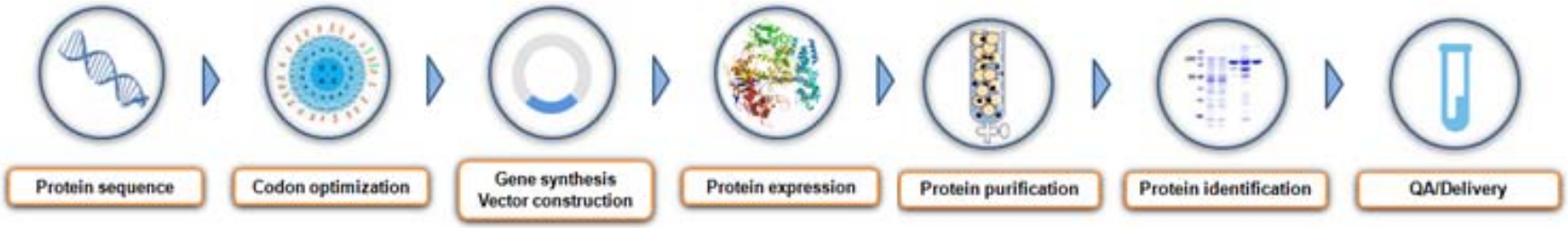
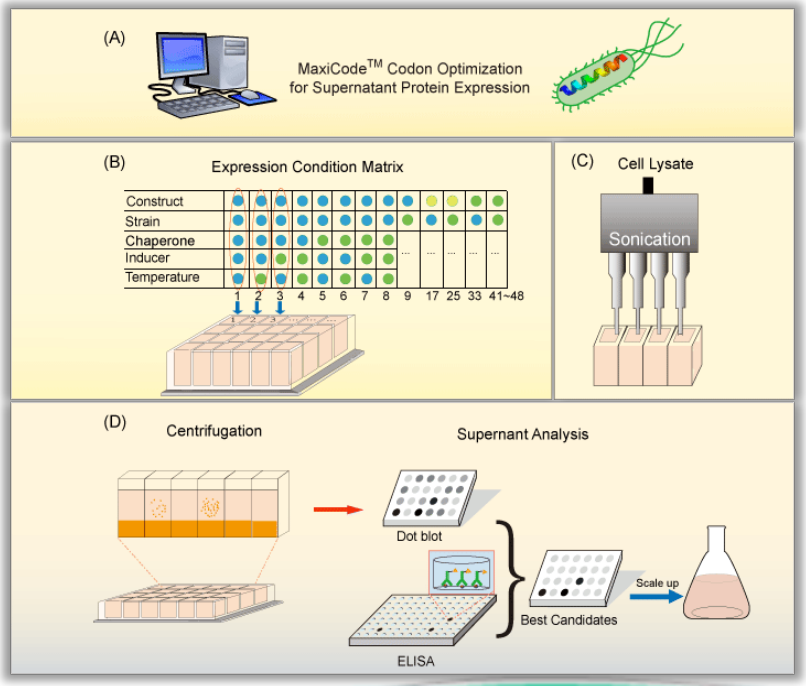
- **Chaperones:** transiently interact with protein folding intermediates and contribute to protein expression
  - GroES-GroEL
  - DnaK-DnaJ-GrpE
  - ClpB
  - Trigger factor
  - Heat shock proteins
- **Foldases:** accelerate rate-limiting steps in the folding process
  - Peptidylprolylcis/trans isomerases (PPI's)
  - Disulfide oxidoreductase (DsbA) and disulfide isomerase (DsbC)
  - Protein disulfide isomerase (PDI)

PROJECT PROCESS





- **Soluble protein expression optimization process**
  - **Codon optimized gene synthesis**
  - **Expression condition optimization**
  - **Scale-up production**
  - **Purification and delivery**

CONTACT US

- 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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