Optimizing soluble protein expression: codon optimization, RBS design, and expression vector

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Molecular Biology Specialist

GenScript
Make Research Easy

3 Easy Ways to Optimize Soluble Protein Expression

1. Codon Optimization – *2015 Updates!*
2. Ribosomal Binding Site Design
3. Expression Vector Optimization
4. Resources

Protein Expression Workflow

Select/Design the end product (amino acid sequence)

↓

Choose expression system

↓

Design expression clone (DNA construct)

↓

Express the protein

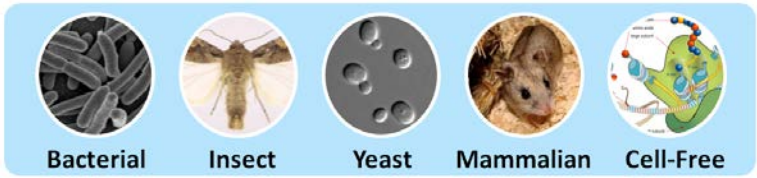
↓

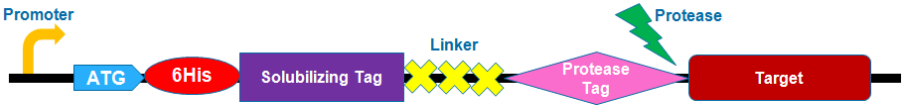
Purify the protein

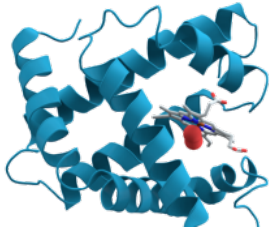
↓

Characterize the protein

MGVHECPAWLWLLLSLLSLPLGLPVLGAPPRLIC…







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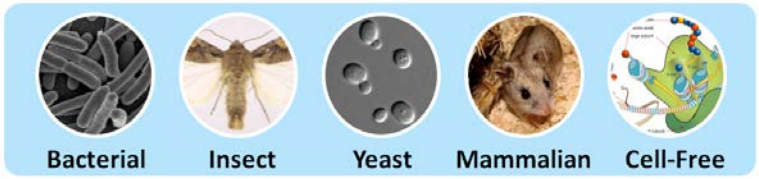
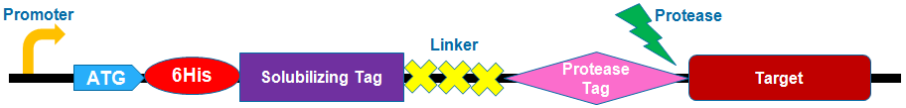
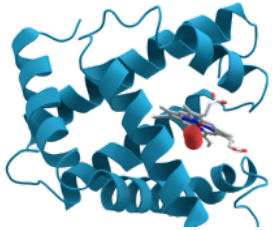
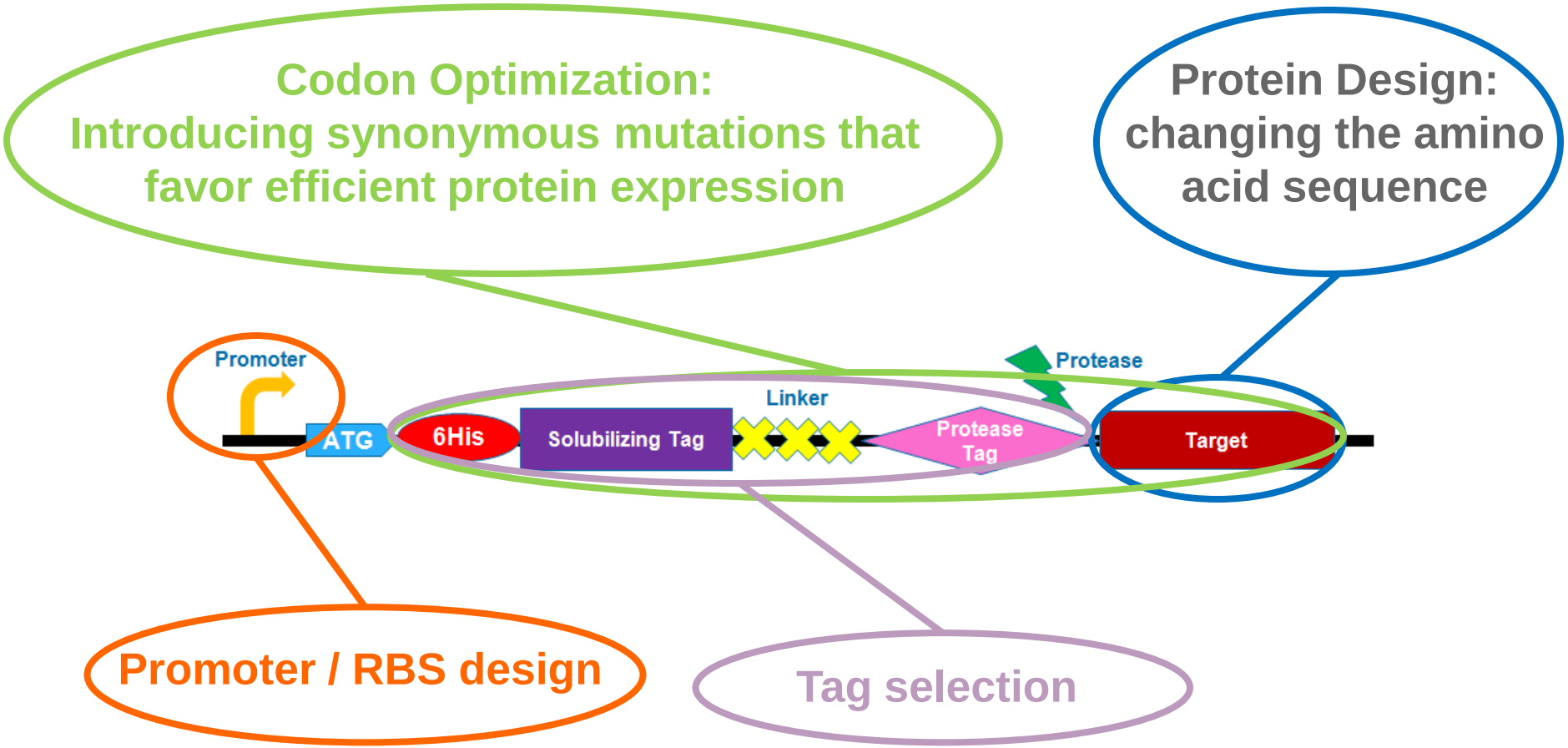






What is Codon Optimization?



Why do Codons Matter? The Facts

- Redundancy in the genetic code
- Synonymous mutations affect protein expression rates up to 1000-fold.
- Synonymous mutations can also alter protein conformation, PTM, stability, and function.

1st letter	Second Letter: U	Second Letter: C	Second Letter: A	Second Letter: G	3rd letter
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

Codon Optimization:

Introducing synonymous mutations that favor efficient soluble protein expression

```
Optimized AGTTTTCCAGGTTGAGGTCCGCCCGTT
                || ||  ||  ||  ||||| ||  ||  |||
Original  AGCTTCCCGGGATGAGGGCCCCCGGTT
```

Evidence-Based Codon Optimization

Transcriptional Efficacy:

- GC content
- CpG dinucleotides content
- Cryptic splicing sites
- Negative CpG islands
- SD sequence
- TATA boxes
- Terminal signal

Translation Efficiency:

- Codon usage bias
- GC content
- mRNA secondary structure
- Premature PolyA sites
- RNA instability motif (ARE)
- Stable free energy of mRNA
- Internal chi sites and ribosomal binding sites

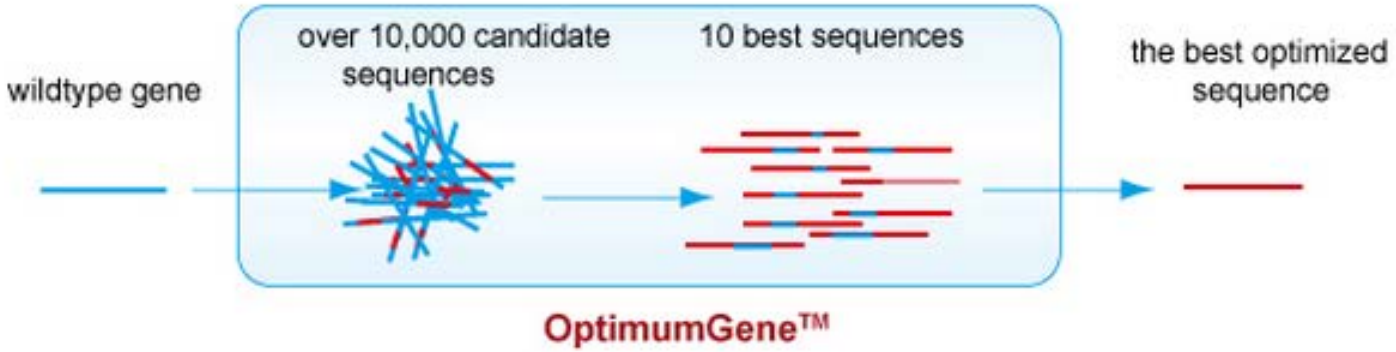
Protein Refolding:

- Codon usage bias
- Interaction of codon and anti-codon
- Codon-context
- RNA secondary structures

Flexibility to adjust the weight of different factors or add customized constraints:

- Filter out restriction sites
- Reduce similarity between library members
- Alternative codon tables / condition-specific codon preferences

Patented PSO Bioinformatic Algorithm powers OptimumGene



(12) **United States Patent**
Liu et al.

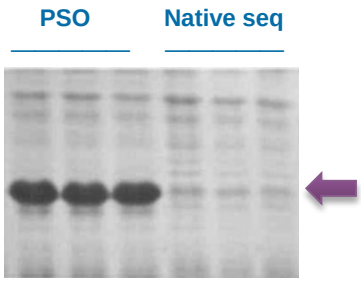
(10) **Patent No.: US 8,326,547 B2**
(45) **Date of Patent: Dec. 4, 2012**

(54) **METHOD OF SEQUENCE OPTIMIZATION FOR IMPROVED RECOMBINANT PROTEIN EXPRESSION USING A PARTICLE SWARM OPTIMIZATION ALGORITHM**

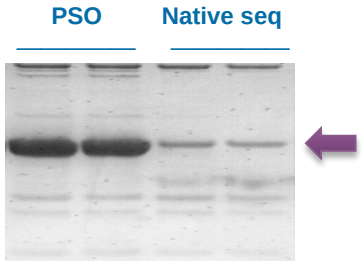
Khalid et al. (Prosiding Simposium Kebangsaan Sains Matematik ke-16 (2008) Jun. 205; pp. 1-11).*
Shen et al.(Computational Biology and Chemistry (2008) vol. 32; pp. 53-60).*
Xiao et al. (Concurrency and Computation: Practice and Experience

Codon Optimization improves expression in all host systems

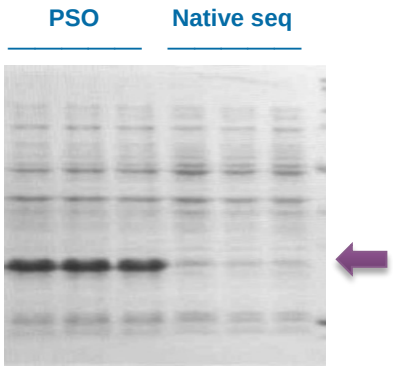
- *Production of proteins with single domain or subunit*



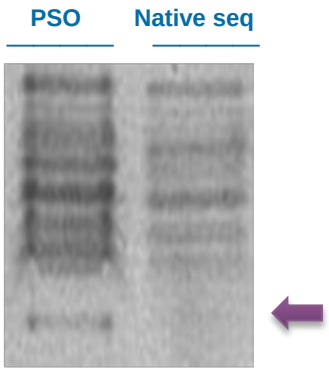
Expressed in ***E. coli***



Expressed in ***P. pastoris***

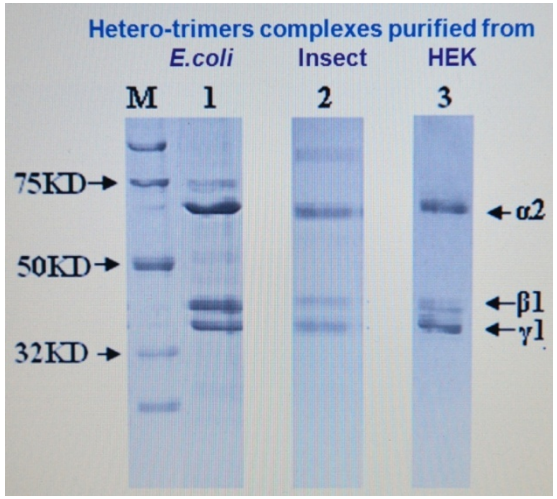


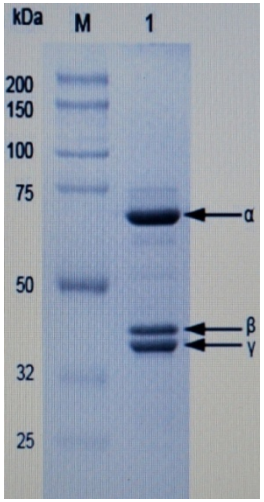
Expressed in ***insect cells***



Expressed in ***mammalian cells***

Codon Optimization aids production of protein complexes





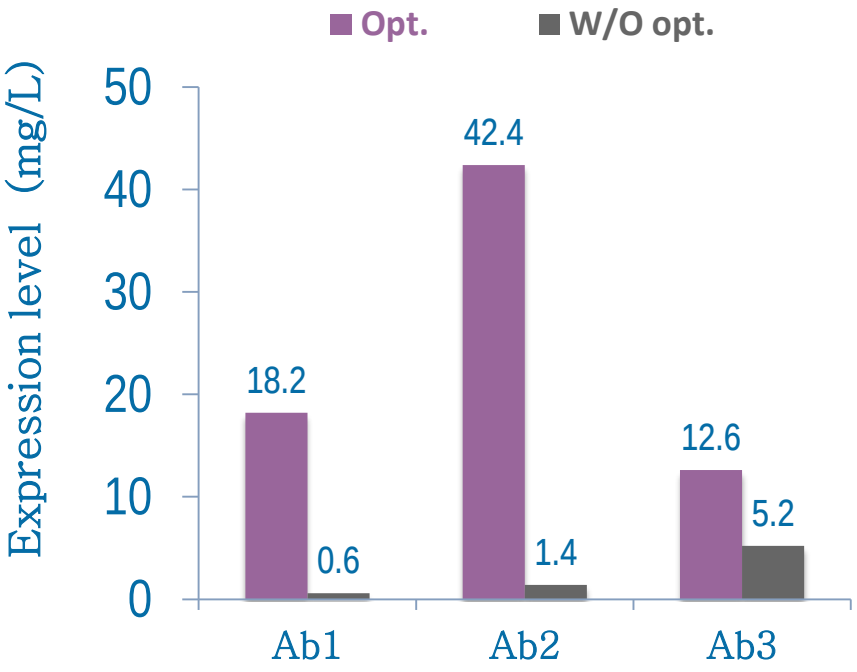
ONE transcript for THREE subunits of a protein produced from HEK293,
gene optimization was the key.

Production of LBD of a nuclear receptor associated with its ligand in correct conformation from *E. coli*,
PSO contributed to success.

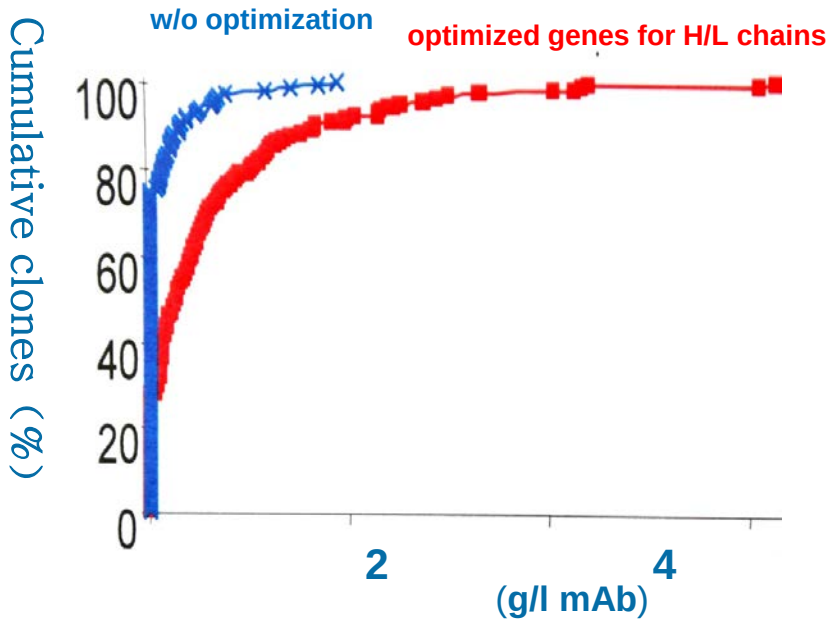
Codon Optimization improves mAb production yield

- ***Production of monoclonal antibodies***

Transient expression

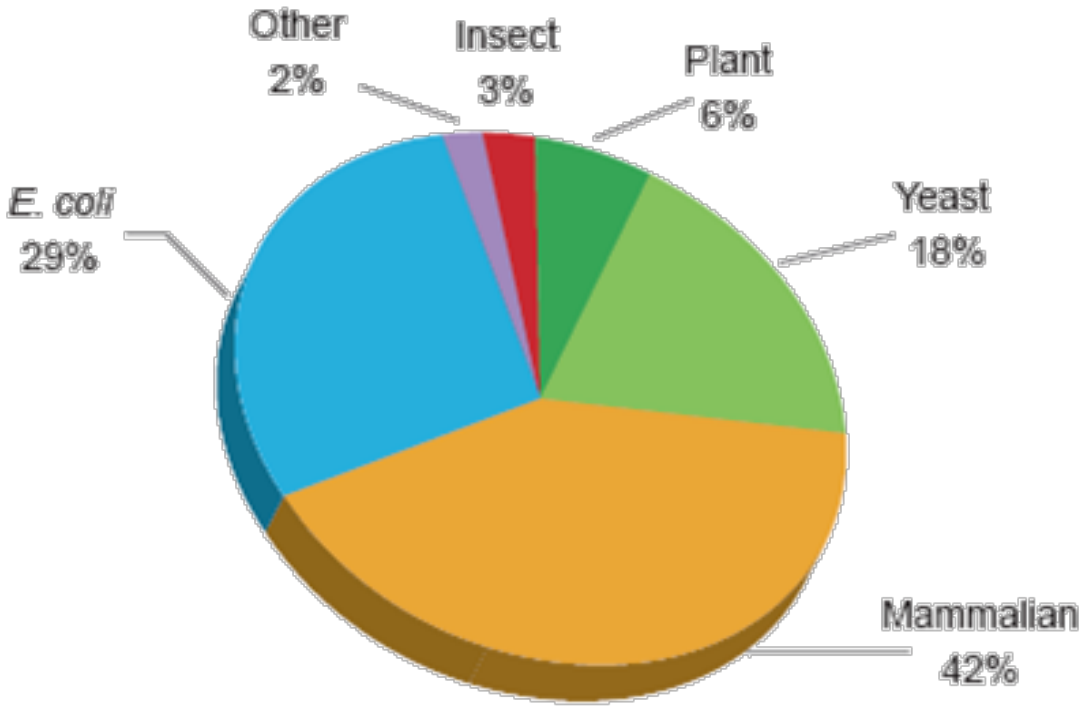


Stable expression

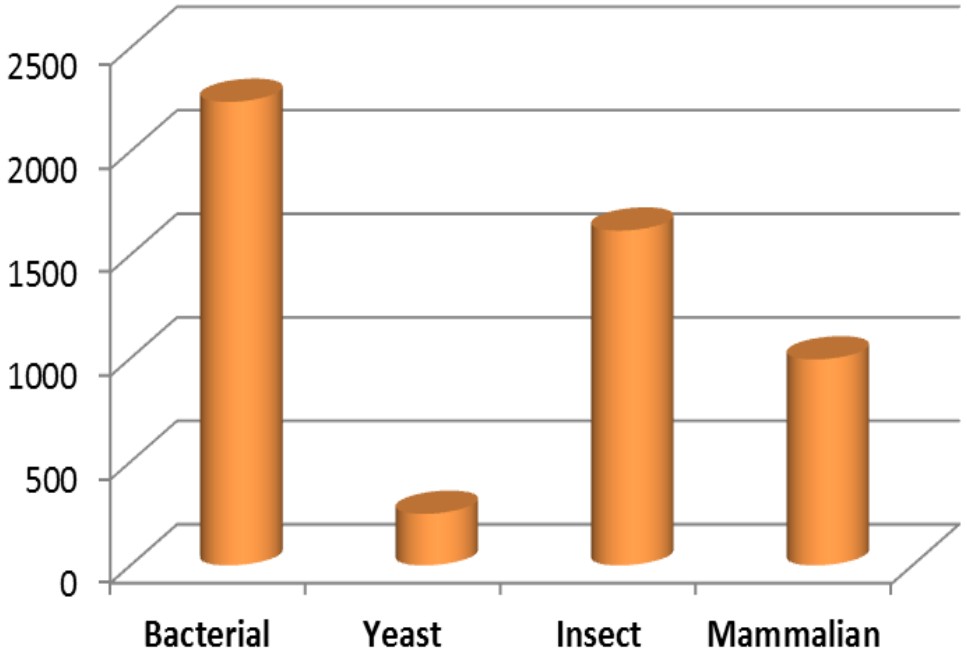


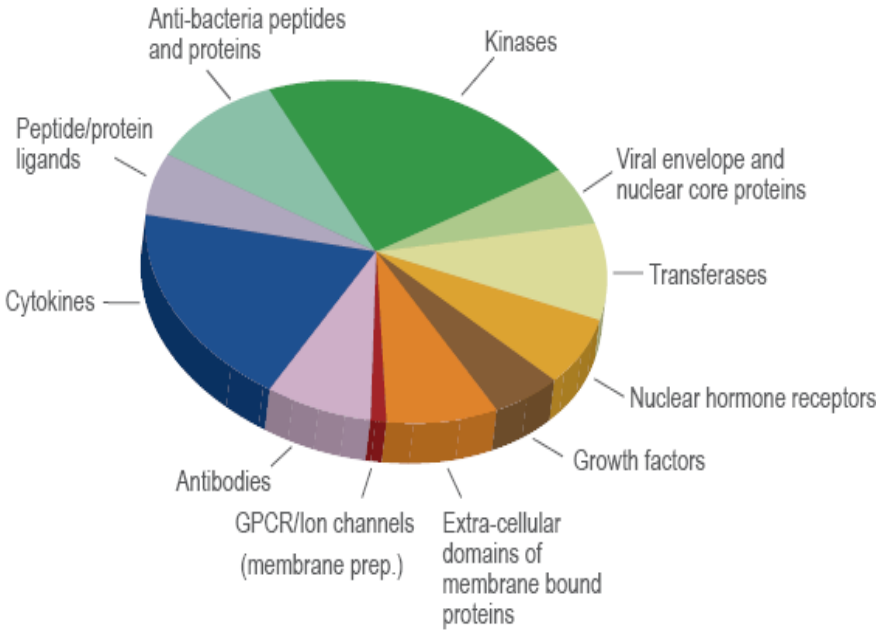
Performance of OptimumGene™

- **Over 500,000 sequences have been optimized by GenScript in all major expression systems.**



Performance of OptimumGene™





GenScript has delivered over **5,000** proteins in four expression systems. Statistics showed **95%** success rate for all protein projects.

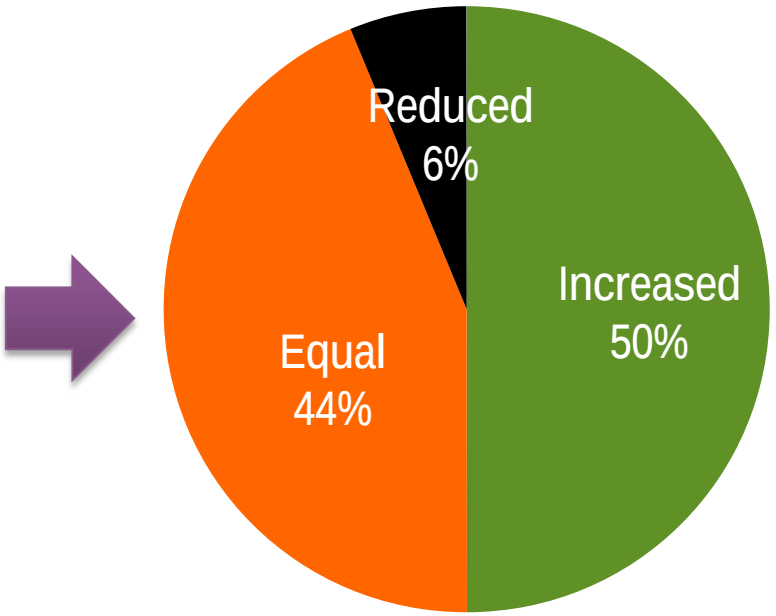
OptimumGene™ - Apricot

- 2015 Update for *E. coli expression system*

Item	*Opti-10*	*2015*
1	Genome codon table	High-productivity codon table
2	Global codon optimization	Global codon optimization & N-terminal codon bias

OptimumGene™ 2015 performance

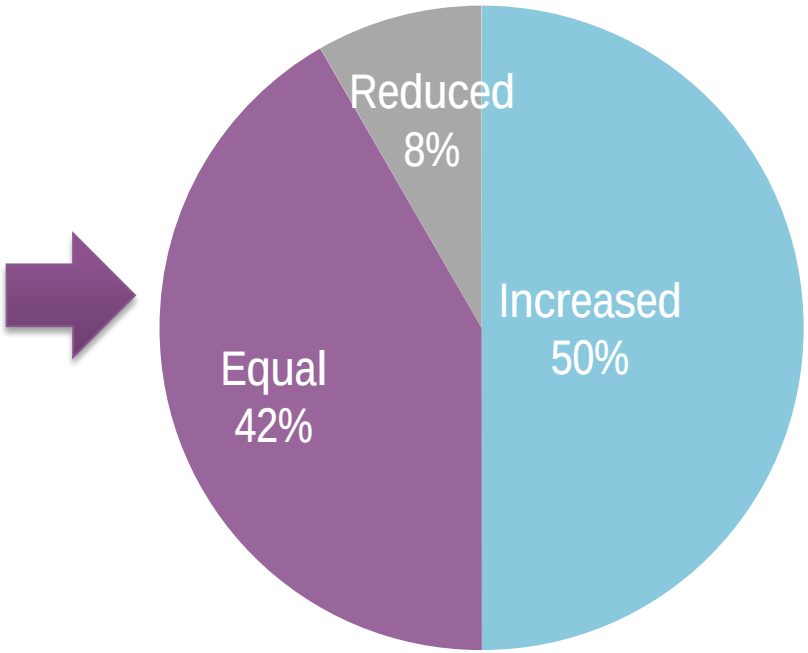
#	Protein	Opti-10	2015	Note
1	PD_Capsid	2.3	3	+
2	ALAD-L-Apo	17.7	23.2	+
3	Ng22	0.9	1.2	+
4	α-Gliadin	6.32	9.3	+
5	Human IGF-1	1.8	5.6	+
6	Foxn1	1.05	7.25	+
7	OSEIL2	0	2	+
8	Mu1c	0	13.9	+
9	ApxII	20.4	18.4	=
10	OspA	9.7	10	=
11	Human cMycΔC	27.7	29.5	=
12	BRPF1BD	17.5	18.9	=
13	FGF19-Apo	4	4.1	=
14	PcrV_144-257	5.9	6.1	=
15	PBANKA_061260	0	0	=
16	Mouse FGF 6	3.5	2.1	-



GenScript vs. Other Algorithms

Expression level (mg/L)

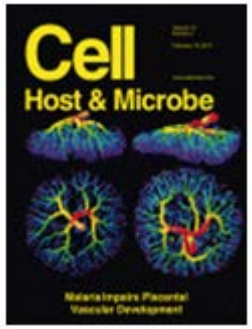
#	Gene	GenScript	Competitor	Note
1	IGF-1	5.6	5.8	=
2	ALAD	23.2	0	+
3	BRPF1BD	18.9	18.8	=
4	cMyc	29.5	30.9	=
5	Ng22	1.2	1.2	=
6	Mu1c	13.9	6.8	+
7	PD_Capsid	3	0.9	+
8	Foxn1	7.25	4.04	+
9	ApxII	18.4	9.65	+
10	α-Gliadin	9.3	4.91	+
11	OspA	10	10	=
12	FGF 6	2.1	7	-



Hundreds of papers cite GenScript for codon-optimized gene synthesis

"Humanization and optimization of codon usage was performed (GenScript) owing to poor expression of the original zebrafish lyn in HEK293 cells."

...The following genes were codon optimized and synthesized (Genscript):



"were generated in eukaryotic mycoplasma-free HEK-293 cells, **using cDNAs optimized to fit eukaryotic codon usage (Genscript…**

"…Zebrafish codon optimized cerulean (**GenScript**, Piscataway, NJ, USA)…"

"…IFP1.4 gene was de novo synthesized by **GenScript** Company, based on the available protein sequence. The DNA sequence was **optimized with proprietary OptimumGene algorithm (GenScript**)..."

...The mangosteen stearoyl-ACP thioesterase (GenBank accession AAB51523.1) was codon-optimized (**GenScript**, Piscataway, NJ)...

3 Easy Ways to Optimize Soluble Protein Expression

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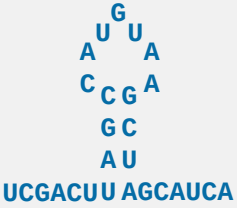
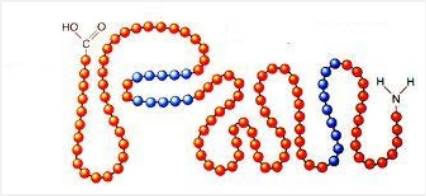
Protein Expression in a Nutshell

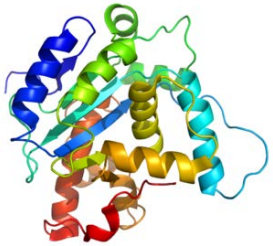
RATE OF Protein Production

Transcription → Translation →

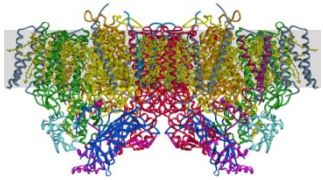




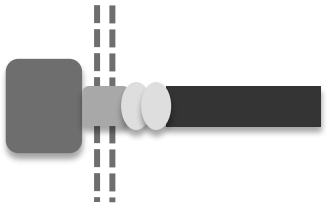
RATE OF
Protein Folding



RATE OF
Membrane Insertion

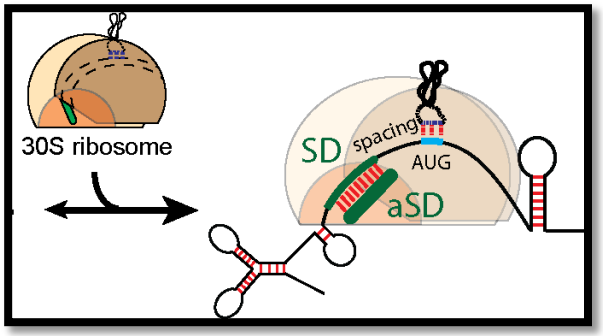


RATE OF
Secretion

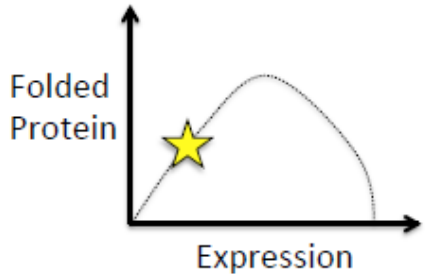
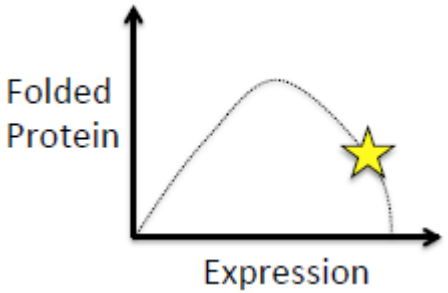
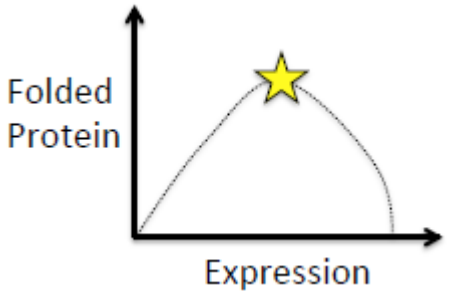
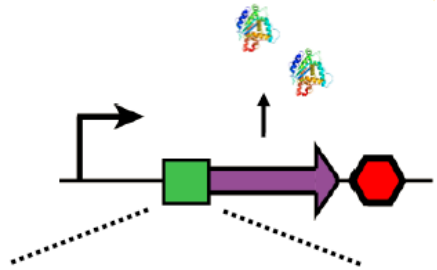
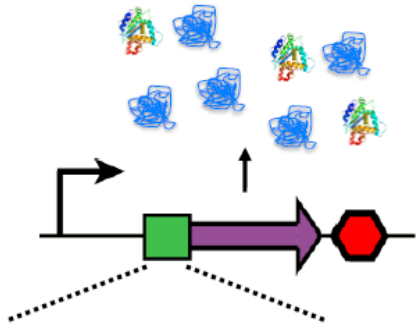
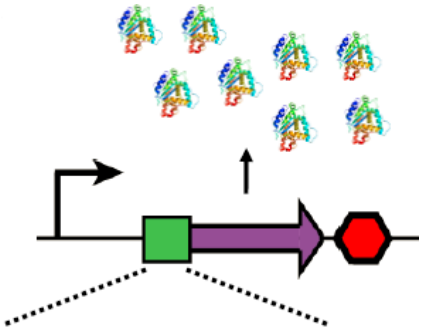


Rate-Limiting Step of Protein Production:

Translation Initiation



Maximizing Protein Titer: a balancing act

Translation Initiation Rate / Protein produced		
Too low rate: Low Protein Titers	High rate: mis-folded Protein	Just right rate: active and folded Protein
		
 ribosome binding site sequence	 ribosome binding site sequence	 ribosome binding site sequence

➢**RBS design can *slow down* translation for better solubility of *E. coli*-expressed proteins**

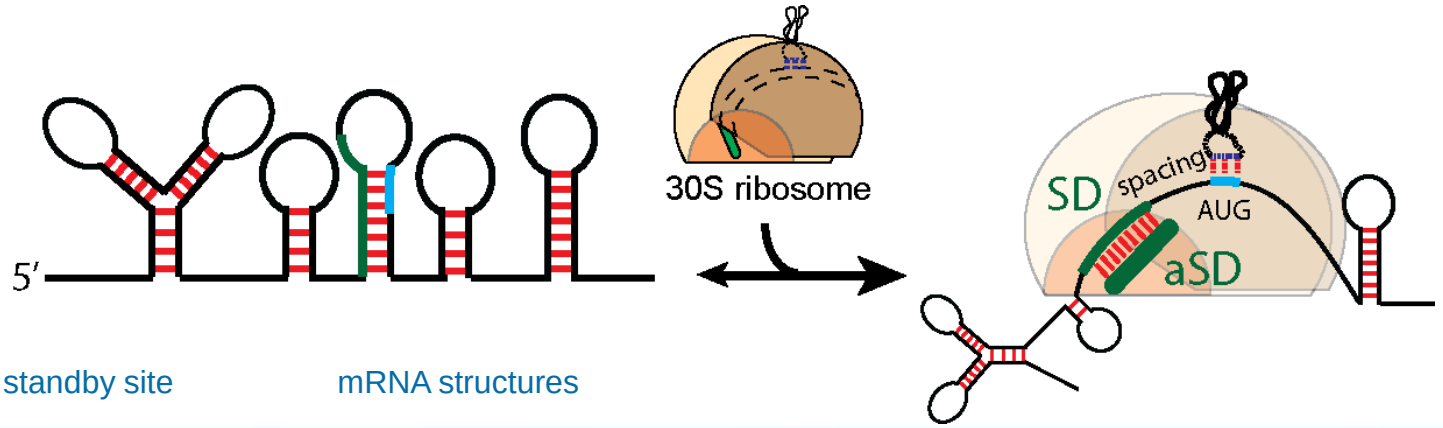
Factors that Affect Translation Initiation

RBS sequence CDS sequence

AUCCGAUAACUAGUCACACAGUAAAAAUUUAGUUAUGACCACCUUUCACAAAGCAAGCGGAGUGCUUC
UUGUGCCGACUUACGAGCAUCUAGCGAGCAUCUAGCGACUACUGAC . . . **UAA**

Molecular interactions controlling translation initiation

1. Hybridization between the mRNA and 3' end of the 16S rRNA @ the "Shine-Dalgarno"
2. The unfolding of mRNA structures that overlap with the ribosome's footprint
3. Hybridization between the start codon and tRNAfMet
4. Ribosome stretching or compression, due to long or short spacer regions
5. mRNA structures in standby sites that block ribosome binding
6. The time-scale of RNA folding kinetics vs. ribosome assembly kinetics



blocked standby site mRNA structures

Factors that Affect Translation Initiation

RBS sequence **CDS sequence**

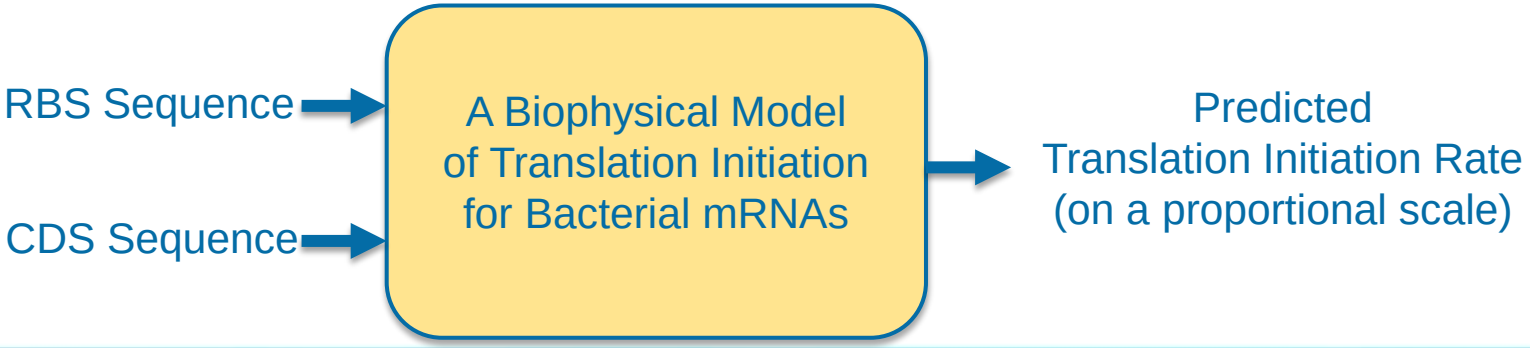
AUCCGAUAACUAGUCACACAGUAAAAAUUUAGUUAUGACCACCUUUCACAAAGCAAGCGGAGUGCUUC
UUGUGCCGACUUACGAGCAUCUAGCGAGCAUCUAGCGACUACUGAC . . . **UAA**

Let's insert a consensus Shine-Dalgarno sequence for maximum mRNA-rRNA hybridization
Uh Oh! We created a new mRNA structure that will inhibit translation rate.

AUCCGAUAACUAGUCACACAUAAGGAGGUAAGUUAUGACCACCUUCACAAAGCAAGCGGAGUGCUUCU
UGUGCCGACUUACGAGCAUCUAGCGAGCAUCUAGCGACUACUGAC . . . **UAA**

Many overlapping causes = difficult to design RBS sequences "by eye"

Need to use a Quantitative Model that Calculates Causes & Predicts their Effect



Automated Design to Optimize Expression

Objective: design an RBS sequence
to decrease translation initiation of a specific protein *by 20-fold*

Your initial sequence

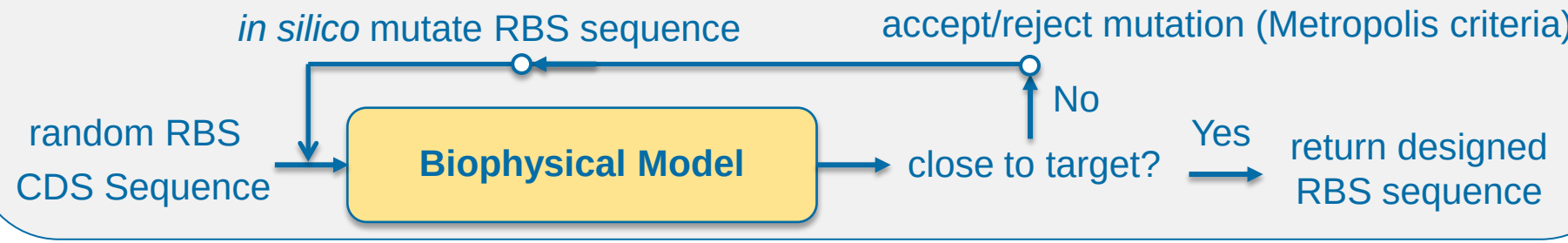
RBS sequence **CDS sequence**

AUCCGAUAACUAGUCACACAGUAAAAAUUUAGUUAUGACCACCUUUCACAAAGCAAGCGGAGUGCUUC
UUGUGCCGACUUACGAGCAUCUAGCGAGCAUCUAGCGACUACUGAC . . . **UAA**

How many choices? 35 nt 5' UTR … $4^{35} = 1.1 \times 10^{21}$

Computational Optimization

a target Protein production rate → ***a target*** translation initiation rate → ***a designed*** RBS Sequence



Automated Design to Optimize Expression

Objective: design an RBS sequence

to decrease translation initiation of a specific protein *by 20-fold*

RBS sequence **CDS sequence**

AUCCGAUAACUAGUCACACAGUAAAAAUUUAGUUAUGACCACCUUUCACAAAGCAAGCGGAGUGCUUC
UUGUGCCGACUUACGAGCAUCUAGCGAGCAUCUAGCGACUACUGAC . . . **UAA**

RBS Calculator

predict translation init. rate

400,000 au

400,000 au / 20 = 20 000 au

design a new RBS

CGAACCGCUAUUCUAGAUAGUUCAAAAACAGAAC	100 au
UAUGCCUUCACAUUCACCAUUCAGAGACCGGUCG	300 au
AUCCGAUAACUAGUCUUUAAGUAAAAAUUUAGUU	3 000 au
UACCACUAAAACUAACCUAACGAGUAGGUUAUAA	10 000 au
AAAAUUUUCAUAAACAAGGUCGGGGGAUAUCAAG	**20 000 au**
UAUCAUUAUAUUCAUCGAAUAAGGGGAAUCUACU	100 000 au

The Ribosome Binding Site Calculator

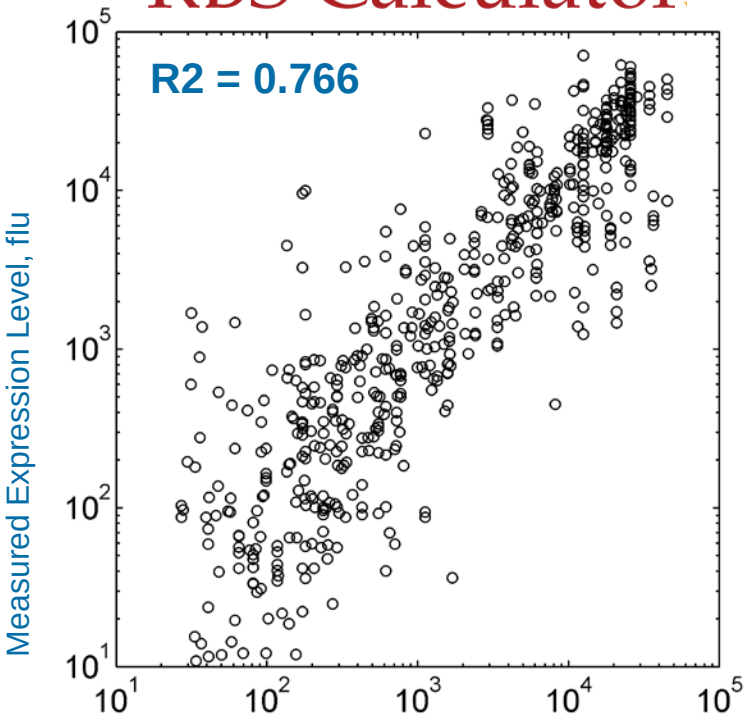
RBS Sequence

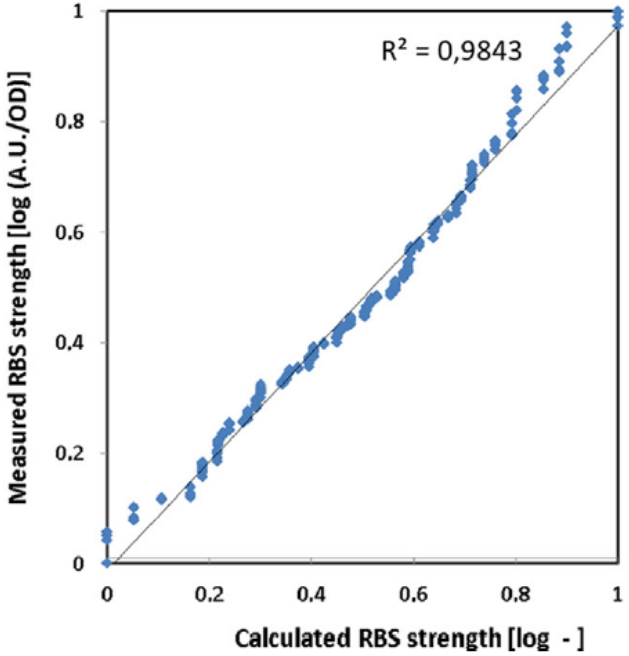
CDS Sequence

A Biophysical Model of Translation Initiation for Bacterial mRNAs

Predicted Translation Initiation Rate (on a proportional scale)

RBS Calculator



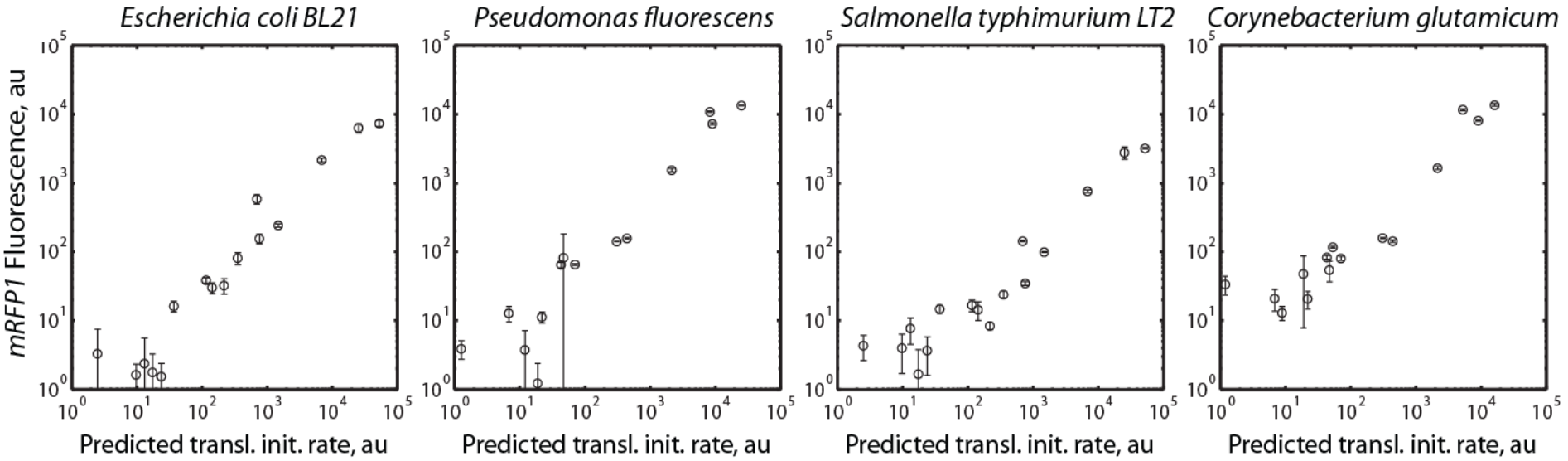


Coussement et. al,*Metabolic Engineering*, 2014
Salis et. al., *Nature Biotechnology*, 2009
Farasat et. al., Molecular Systems Biology, 2014

The RBS Calculator in Different Hosts

Different organisms, same RNA biophysics

Slightly different ribosomes



Farasat et. al., *Molecular Systems Biology*, 2014

How does the RBS Calculator Work?

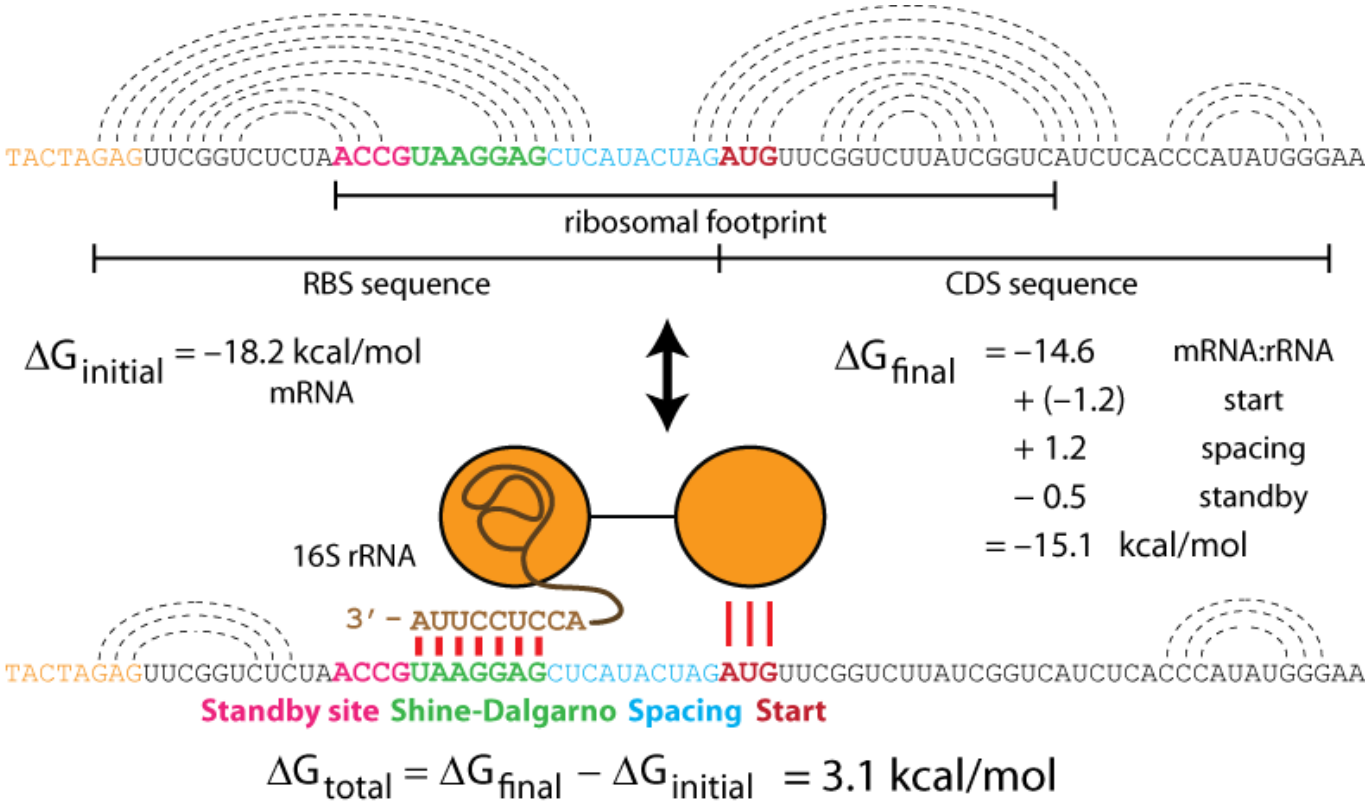
mRNA sequence

Thermodynamic Model

ΔGtotal = 3.1 kcal/mol

r = 620 au
(proportional scale)

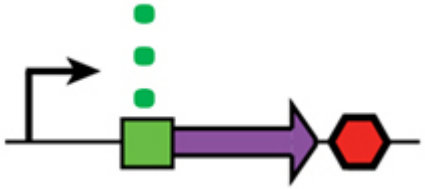
dotted lines = nucleotide base pairing

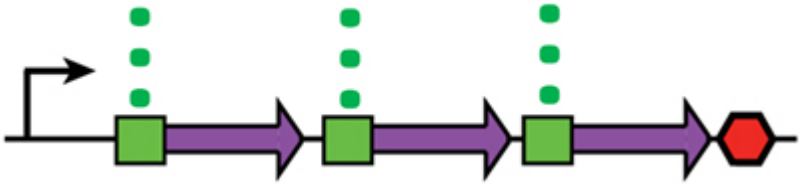


$$\Delta G_{total} = \Delta G_{final} - \Delta G_{initial} = 3.1 \text{ kcal/mol}$$

Ribosome Binding Site Design

- Sequence-dependent; design RBS *after* codon optimization
- Useful for single proteins or multi-enzyme pathways





Extremely Low ~ Low	Medium	High	Extremely High
1~500 au	1,000~ 10,000 au	10,000~100,000 au	100,000~200,000 au
yield low protein titers	Suitable for trans-membrane protein or protein with enzymatic activity that inhibits cellular metabolism.	For non-cytotoxic protein, 100,000 au TIR will produce a large amount of soluble protein. Rich media is required	For inert and fast folding protein, extremely high TIRs will express a huge amount of protein. Rich media is required

Optimizing soluble protein expression

1. Codon Optimization – 2015 Updates!
2. Ribosomal Binding Site Design
3. Expression Vector Optimization
4. Resources

Expression Vector Components

Goal	Component
1. Insert cargo into the plasmid and verify the insert sequence accuracy	•**MCS** – restriction sites OR recombination regions **5' and 3' Primer sites** for sequence verification
2. Insert plasmid into cells, enable the plasmid to replicate inside the host, & select for cells carrying the plasmid	•Backbone compatible with **cloning method** • **Origin of replication** • **Selection marker and/or screening marker**
3. Transcribe mRNA from the plasmid	•**Promoter** (constitutive or inducible) operator, terminator
4. Translate mRNA into protein	•**Ribosome Binding Site,** start codon, stop codon
5. Promote proper folding of nascent protein	•**co-expression of chaperones** •**Solubilization tags** •**custom-designed synthetic RBS** •**Codon-optimized ORF**
6. Detect or Purify target protein	•**Epitope tags (His)** •**reporters (GFP)**

Transcriptional Promoters

Host	Commonly Used Promoters
Bacteria	Lac, T7, araBAD
Yeast	GAL4, PGK, ADH1, ADE2, TRP1
Mammalian	Constitutive: CMV, SV40, EF1a, CAG Inducible: Tet *Tissue-specific for in vivo work: varied*

Epitope Tags / Fusion Proteins

Goal	Commonly Use Tag
Detect target protein (common Ab epitope tags)	FLAG (DYKDDDK) HA (YPYDVPDYA)
Purify target protein (affinity tags)	His6 glutathione-S-transferase (GST)
Improve solubility of target protein	maltose-binding protein (MBP) glutathione-S-transferase (GST)

Tags can be removed at cleavage sites place between target ORF and tag:

Enzyme	Cleavage site
Thrombin	Leu-Val-Pro-Arg-Gly-Ser
Enterokinase	Asp-Asp-Asp-Asp-Lys
Factor Xa	Ile-Glu/Asp-Gly-Arg
TEV	Glu-Asn-Leu-Tyr-Phe-Gln-Gly

Learn More about Expression Vectors

http://www.genscript.com/webinars.html

Expression vectors: how to choose, or customize, vectors for gene & protein expression

On Demand

View Now

Download PDF

Do you make new DNA constructs only using the old expression vectors you're most familiar with? This webinar will help you make your experimental design more efficient and powerful by learning how to select or design an expression vector that is optimized for your experiments. We will walk through how to read a plasmid map and what key features to look for in an expression vector depending on your research goal. Using case studies from published literature, we'll discuss why and how you might want to make custom changes to elements already included in commercial available vectors (e.g. RBS or tags). Along with handy reference guides for popular vectors used in different eukaryotic and prokaryotic species, this webinar will introduce you to GenScript's gene synthesis and cloning services that can help you get expression-ready clones most efficiently to accelerate your research.

How to optimize protein expression

Remember: optimize ≠ maximize; more is not always better!

- Optimize the Expression Vector
  - Choose/Reorder ORI, promoter, solubilization tags
  - RBS must be customized for each cargo
- Optimize the Cargo (insert DNA)
  - codon optimization
- Optimize the Host
  - Platform (species) and strain
- Optimize your Methods
  - transformation, selection, growth, induction, purification

Optimizing soluble protein expression

1. Codon Optimization – 2015 Updates!
2. Ribosomal Binding Site Design
3. Expression Vector Optimization
4. Resources

Related GenScript Webinars

Clone less, know more: efficient expression optimization of proteins and pathways using the RBS calculator

Presented by: Prof. Howard Salis, Penn State University

On Demand

View now

Codon optimization: Why & how to design DNA sequences for optimal soluble protein expression

Presented by: Rachel Speer, Ph.D.
Originally aired October 29, 2014

On Demand

View now

Optimizing conditions for recombinant soluble protein production in *E. coli*

Presented by: Keshav Vasanthavada
Originally aired May 8th and June 24th, 2014

On Demand

View now

Recombinant protein expression & purification: challenges and solutions

Presented by: Liyan Pang, Ph.D.
Originally aired June 11th and June 12th, 2014

On Demand

View now

Strategies to Promote Proper Folding

- Folding may be improved by chaperone co-expression

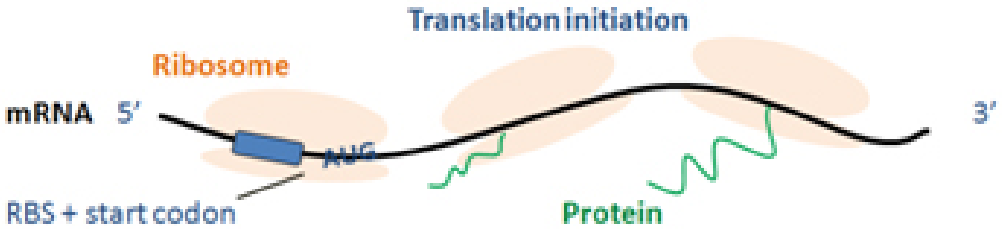
Chaperone co-expression strategies for recombinant soluble protein production in *E. coli*

Presented by: Bo Wu, Ph.D, Senior Scientist, GenScript

On Demand

View now

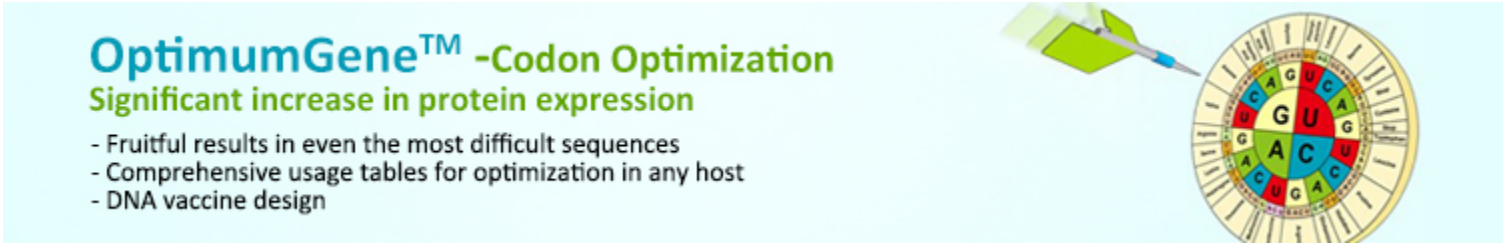
- Folding is coupled with translation initiation and elongation.



  - Initiation: RBS Calculator
  - Elongation: codon choice (“optimization” or “de-optimization”)

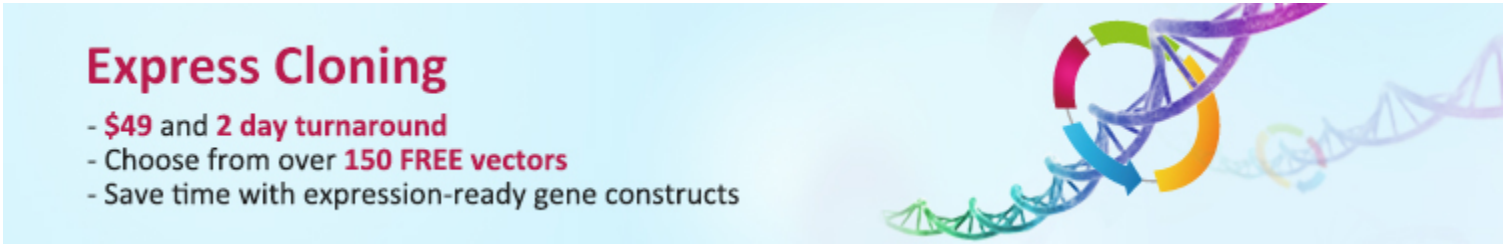
GenScript Toolkit to Optimize Expression

- **Codon optimization:** boost transcription & translation in any host



- **RBS design:** tune translation in prokaryotes

- **Expression Vector Selection**



Gene Synthesis to create any custom insert

Recommended Services for your needs:	Low Price	Fast Turnaround	High-Volume	Long Genes
Custom Gene Synthesis Cat No. SC1010	✓ $0.35/bp	✓ 8+ business days	No min / max	≤8 kb
Rush Gene Synthesis Cat No. SC1575	Request a quote	✓ 4+ business days	No min / max	≤2 kb
GenPlus™ High-Throughput Gene Synthesis Cat No. SC 1645	✓ $0.23/bp	10+ business days	✓ ≥25 genes	✓ ≤10 kb
GenPlus™ Economy Gene Synthesis Cat No. SC1681	✓ $0.23/bp	25+ business days	No min / max	✓ ≤10 kb
GenBrick™ Synthesis Cat No. SC1584	$0.45/bp	23 business days	No min / max	✓ 8 - 15kb or more

Express Cloning – free vectors!
$49, 2-day cloning

Mammalian
pcDNA3.1(+)
pcDNA3.1(-)
pcDNA3.1(+)_myc-His A
pcDNA3.1(+)_myc-His B
pcDNA3.1(+)_myc-His C
pcDNA3.1(-)_myc-His A
pcDNA3.1(-)_myc-His B
pcDNA3.1(-)_myc-His C
pCI-Neo
pcDNA3.1+C-DYK
pcDNA3.1+C-HA
pcDNA3.1+C-6His
pcDNA3.1+C-Myc
pcDNA3.1+N-DYK
pcDNA3.1+N-HA
pcDNA3.1+N-6His
pcDNA3.1+N-Myc
pcDNA3.1+N-GST(Thrombin)
pcDNA3.1+N-GST(TEV)
pcDNA3.1/Hygro(+)
pcDNA3.1/Hygro(-)
pcDNA3.1/Zeo (+)
pcDNA3.1/Zeo (-)
pCMV-3Tag-1a
pCMV-3Tag-2a
pCMV-3Tag-3a
pCMV-3Tag-4a
pcDNA3.1-C-eGFP
pcDNA3.1-N-eGFP
pcDNA3.1-P2A-eGFP
pcDNA3.1-P2A
pcDNA3.1+N-DYK-P2A
pcDNA3.1+C-DYK-P2A
pCMV-3Tag-1a-P2A
pCMV-3Tag-3a-P2A

Yeast
pAO815
pPIC 3.5k
pPIC9
pPIC9K
pPICZA
pPICZB
pPICZC
pPICZalphaA
pPICZalphaB
pPICZalphaC
pESC-TRP
pESC-URA
pESC-HIS
pESC-LEU

Baculovirus/Insect
pBacPAK8
pBacPAK9
pAcG2T
pAcHLT A
pAcHLT B
pAcHLT C
pAcGHLT A
pAcGHLT B
pAcGHLT C
pAcSG2
pBAC-1
pFastBac1
pFastBacHT-A
pFastBacHT-B
pFastBacHT-C
pFastBac-Dual

Bacterial
pBluescript II KS(-)
pBluescript II KS(+)
pBluescript II SK(-)
pBluescript II SK(+)
pET-3a
pET-3b
pET-3c
pET-3d
pET-9a
pET-11a
pET-11b
pET-11c
pET-11d
pET-14b
pET-15b
pET-16b
pET-17b
pET-19b
pET-20b(+)
pET-21a(+)
pET-21b(+)
pET-21d(+)
pET-22b(+)
pET-23a(+)
pET-24a(+)
pET-24b(+)
pET-24c(+)
pET-24d(+)
pET-25b(+)
pET-26b(+)
pET-27b(+)
pET-28a(+)
pET-28b(+)
pET-28c(+)
pET-29a(+)
pET-29b(+)
pET-29c(+)
PET-30a(+)
PET-30b(+)
PET-30c(+)
PET-31b(+)
pET-32a(+)
pET-32b(+)
pET-41a(+)
pET-41b(+)
pET-41c(+)
pET-42a(+)
pET-42b(+)
pET-42c(+)
pET-43.1a(+)
pET-43.1b(+)
pET-45b(+)
pET-50b(+)
pET-51b(+)
pET-52b(+)
pGEX-2TK
pGEX-4T-1
pGEX-4T-2
pGEX-4T-3
pGEX-5X-1
pGEX-5X-2
pGEX-5X-3
pGEX-6P-1
pGEX-6P-2
pGEX-6P-3
pMAL-c4x
pMAL-c5E
pMAL-c5x
pMAL-p5E
pMAl-p5g
pMAl-p5x
pQE-1
pQE-60
pGS-21a
pETDuet-1
pCDFDuet-1
pRSFDuet-1
pCOLADuet-1
pGEX-4T-1-H(RBS)
pGEX-4T-1-M(RBS)
pGEX-5X-1-H(RBS)
pGEX-5X-1-M(RBS)
pGEX-6P-1-H(RBS)
pGEX-6P-1-M(RBS)
pMAL-c4x-1-H(RBS)
pMAL-c4x-1-M(RBS)

Cloning & Mutagenesis Services

Express Cloning

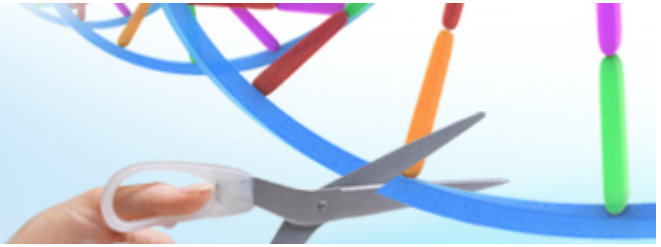
- starting from **$49** and **2 day turnaround**
- save time with expression-ready gene constructs

VectorArk

- Secure, free storage of vector plasmids and data
- **$49**, 5-day cloning for your archived vectors

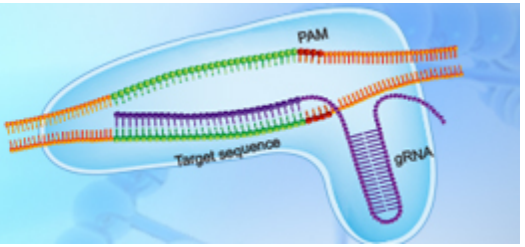
Express Mutagenesis

- starts at **$99**/mutation
- **5** business days
- **100%** sequence accuracy guaranteed



GenCRISPR™ gRNA constructs

- gRNA constructs designed by Zhang lab at Broad
- all-in-one vectors for KO, KI, or transcriptional activation (SAM)



GenScript Toolkit For Optimizing Protein Expression

Design & manufacture expression clone (plasmid DNA construct)

↓

Choose expression system (host organism)

↓

Express the protein

↓

Purify the protein

↓

Characterize the protein

Codon Optimization RBS Design	Gene Synthesis Mutagenesis Cloning
**CRISPR gRNA & Cas9 expression constructs*	
PROTential™ protein expression evaluation service	
BacPower™ YeastHIGH™ MamPower™ InsectPower™	FragPower™ Recombinant Antibody
FoldArt™ Refolding ToxinEraser™ Endotoxin Removal	
Protein Characterization Services	

GenScript – The most cited biology CRO

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- RBS design, codon optimization or quotes: gene@genscript.com

References

Chu D *et al.* **Translation elongation can control translation initiation on eukaryotic mRNAs**. *EMBO J.* 2014 Jan 7;33(1):21-34.

Farasat *et. al.* (2014) **Efficient search, mapping, and optimization of multi-protein genetic systems in diverse bacteria**,*Molecular Systems Biology*, v10(6).

Kramer G, Boehringer D, Ban N, Bukau B. **The ribosome as a platform for co-translational processing, folding and targeting of newly synthesized proteins.** *Nat Struct Mol Biol.* 2009;16:589–597.

Kudla G, Murray AW, Tollervey D, Plotkin JB. **Coding-sequence determinants of gene expression in Escherichia coli.** *Science.* 2009 Apr 10;324(5924):255-8. Free Full Text

Li GW, Oh E, Weissman JS. **The anti-Shine-Dalgarno sequence drives translational pausing and codon choice in bacteria.** *Nature.* 2012 Mar 28;484(7395):538-41. Free Full Text

Plotkin JB, Kudla G. **Synonymous but not the same: the causes and consequences of codon bias.** *Nat Rev Genet.* 2011;12:32–42. Free Full Text

Rosano GL, Ceccarelli EA. **Recombinant protein expression in Escherichia coli: advances and challenges.** *Front Microbiol.* 2014 Apr 17;5:172. Free Full Text

Salis, H.M., Mirsky, E.A. & Voigt, C.A. (2009) **Automated Design of Synthetic Ribosome Binding Sites to Control Protein Expression**, *Nature Biotechnology*, v27 (10).

Shine J, Dalgarno L. **The 3′-terminal sequence of Escherichia coli 16S ribosomal RNA: complementarity to nonsense triplets and ribosome binding sites.** *Proc. Natl Acad. Sci. USA* 1974;71:1342-1346. Free Full Text

Studier FW **Use of bacteriophage T7 lysozyme to improve an inducible T7 expression system.** *J Mol Biol.* 1991 May 5;219(1):37-44.

Ueki T, Nevin KP, Woodard TL, Lovley DR. **Converting Carbon Dioxide to Butyrate with an Engineered Strain of Clostridium ljungdahlii** *MBio.* 2014 Oct 21;5(5):e01636-14. Free Full Text

Welch M et al. **Design parameters to control synthetic gene expression in Escherichia coli**. *PLoS One*. 2009 Sep 14;4(9):e7002. doi: 10.1371/journal.pone.0007002.