OptimumGene™ Codon Optimization

Most Cited Genetic Codon Designer for Protein Expression

GenScript
Make Research Easy

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GenScript
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Still struggling with your protein expression?

My protein does not express in heterologous systems

My protein has a low expression level

My protein cannot fold properly

My protein loses it proper function

...

Now,

GenScript proprietary OptimumGene™ technology can conquer all of these obstacles.

GenScript
Make Research Easy

GenScript USA Inc. is a global integrated biology service company providing custom services, custom reagents, catalog reagents, and bulk quantity biological components. Headquartered in Piscataway, New Jersey, US, the company has sold her service and products to more than 30,000 customers from top 20 global pharmaceutical giants, research scientists in academic institutes and biotech companies in over 70 countries.

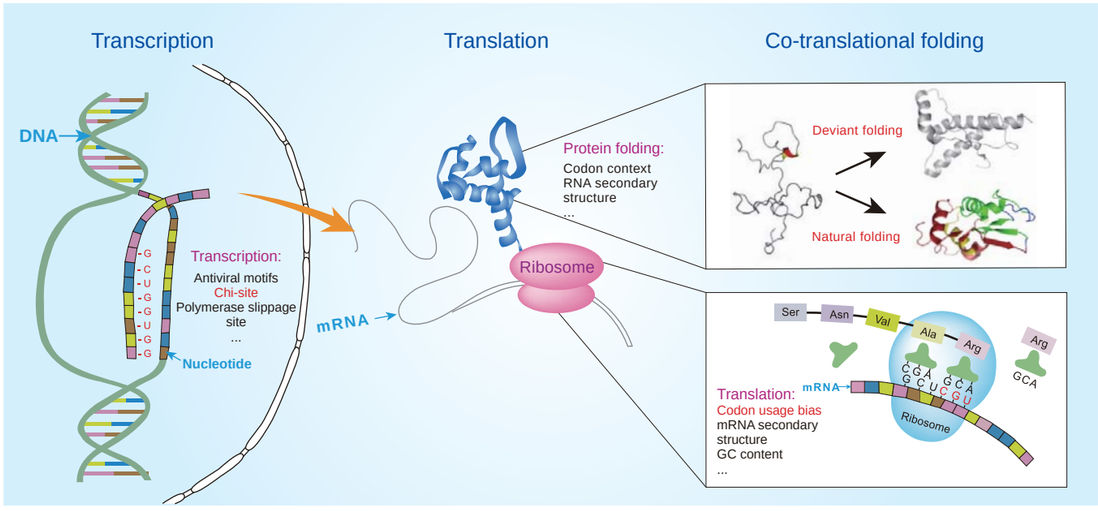
GenScript OptimumGene™ Codon Optimization

—Most Cited Genetic Codon Designer in the Industry

- Most cited gene optimization algorithm – OptimumGene™
- Increase protein express level up to 100-fold
- Comprehensive usage tables for optimization in any host species
- Professional service from PhD level customer service representatives

GenScript's proprietary OptimumGene™ codon optimization is the most cited technology for gene design. The technology takes the entire mRNA structure, various cis-elements and codon adaptability into consideration, as opposed to traditional optimization methodologies which only partially consider the codon usage frequency and the structure of mRNA.

OptimumGene™ codon optimization typically increases protein expression level in *E. coli* up to 100-fold. Using proteins with the highest levels of expression, you can quickly obtain meaningful results while saving time and money.



Track Record in Each Expression System

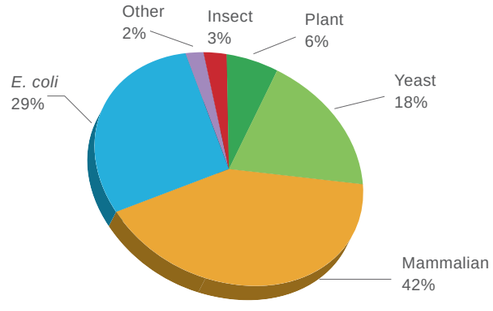
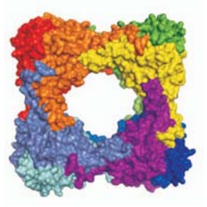


Fig. 1. Based on our records, the OptimumGene™ codon optimization has successfully optimized over **60,000** sequences in almost all major expression systems.

Papers Published in Nature Cited OptimumGene™ Codon Optimization

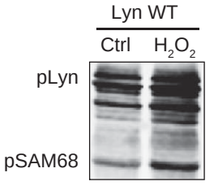
- Story 1. An enzyme allowing an acid-loving bacterium feeding CS_2



Many bacteria (e.g. *Acidianus*) living in volcanic hot wells obtain energy from CS_2 instead of CO_2. Scientists from Radboud University Nijmegen (Netherlands) found the unique structure of an enzyme that allows the microbe to efficiently utilize CS_2 as the main energy source. GenScript used OptimumGene™ technology to maximize the expression level of the gene in *E. coli*, making it possible to study the structure biology of this key enzyme.

(**Reference:** M. J. Smeulders, *et al*. Evolution of a new enzyme for carbon disulphide conversion by an acidothermophilic archaeon. **Nature. 2011. 478:** 412-416)

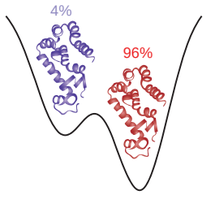
- Story 2. Lyn protein mediates leukocyte wound attraction



Little is known how immunity cells were activated by wounding signals, such as H_2O_2. Scientists from UW-Madison (USA) found that a neutrophil protein, Lyn, serves as a sensor in this process. GenScript used OptimumGene™ technology to optimize the expression of zebrafish *Lyn* gene in HEK293 cells, making it possible to have the protein available in bulk for *in vitro* assays.

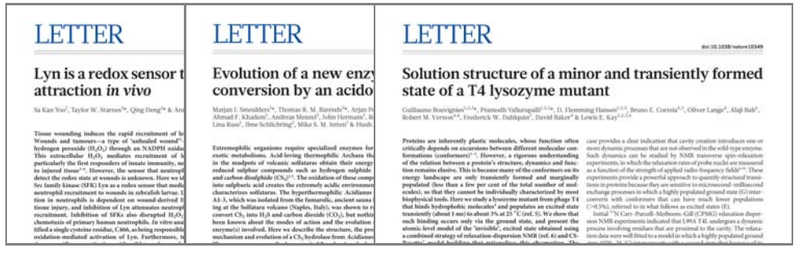
(**Reference:** S. K. Yoo, *et al*. Lyn is a redox sensor that mediates leukocyte wound attraction *in vivo*. **Nature. 2011. 480:** 109-114)

- Story 3. Structures of T4 lysozyme in solution



Proteins undergo structural conformations transitions in solution. Some proteins' structural conformations exist only for short periods of time, making them extremely difficult to study. Scientists from University of Toronto (Canada) established a robust method to model the structures of T4 lysozyme in solution. GenScript used OptimumGene™ technology to optimize the expression of the T4 lysozyme gene in *E. coli*, thereby maximizing the protein production of T4 lysozyme for structural biology study.

(**Reference:** G. Bouvignies, *et al*. Solution structure of a minor and transiently formed state of a T4 lysozyme mutant. **Nature. 2011. 477:** 111-117)


LETTER

Lyn is a redox sensor t
attraction *in vivo*

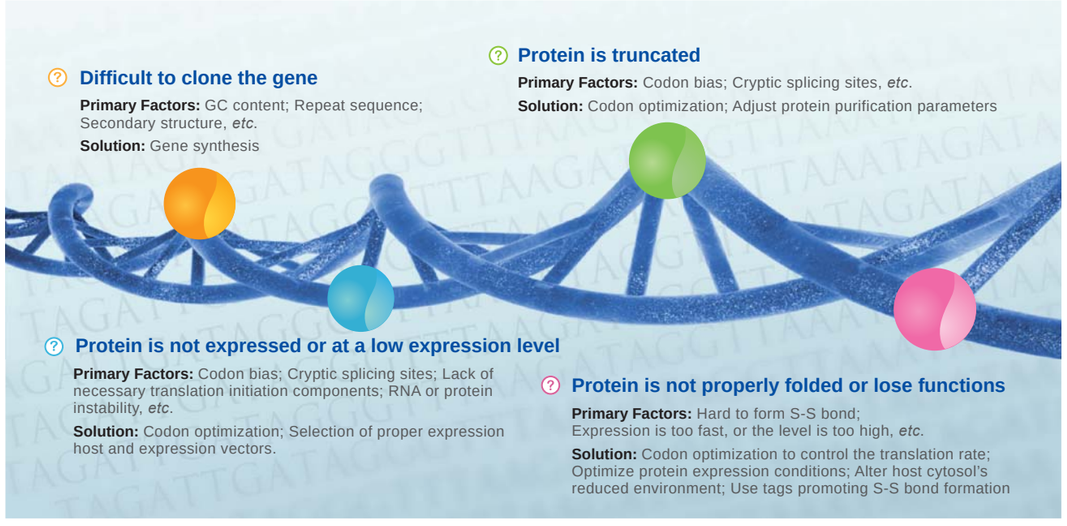
LETTER

Evolution of a new enzy
conversion by an acido

LETTER

Solution structure of a minor and transiently formed state of a T4 lysozyme mutant

Gateway to Smooth Molecular Biology and Protein Research!



What Our Customers Say…

"I think GenScript provides absolutely excellent service in a timely fashion. I have primarily used GenScript to order codon optimized artificial genes. Ordering artificial genes on their website or via Email makes this especially facile because I can simply inquire about a gene, get a quote and then get a PO number. I also think their technical support are wonderful people, they have been most helpful in facilitating the process. I am so impressed with them that I have recommended them to several of my colleagues."

— Dr. Maria Schumacher, The University of Texas MD Anderson Cancer Center, USA

"GenScript is the company that I have entrusted my project with. The delivery of your gene synthesis is on time and the OptimumGene™ technology on your Gene-on-Demand™ gene synthesis platform has increased my gene expression dramatically. I am very happy with my results and GenScript is always my first choice for gene services."

— Dr. Bin He, Bristol-Myers Squibb, USA

"GenScript provides fast, professional protein synthesis services at very reasonable prices. By making it cost-effective to outsource protein production, GenScript has made it possible for my lab to focus on our own area of expertise and get more research done. The detailed planning, updates, and reports that GenScript provides all of the quality control that one could ask for. I strongly recommend GenScript's protein production service."

— Dr. Barry Bradford, Kansas State University Department of Animal Sciences & Industry

Proven Increase in Protein Expression & Protein Solubility

Gene Name	Native			Synthetic			Expression	Solubility
	1	2	3	1	2	3	Syn vs Nat	Syn vs Nat
CBR1	■	■	■	■	■	■	▲	▲
CBR3	■	■	■	■	■	■	▲	▲
GMDS					■	■	▲	▲
HADH2		■	■		■	■	▲	▲
HSD17B2	■	■		■	■		▲	
HSD17B4		■	■	■	■	■	▲	▲
MGC4172	■	■	■	■	■	■		
PECR				■	■	■	▲	▲
RETSDR2	■	■	■	■	■	■	▲	
SPR	■	■	■	■	■	■		

■ Expressed
■ Expressed, Soluble and Purified
□ Not Expressed

▲ Targets shown improvement of expression and/or solubility with synthetic gene after codon optimization

1. Total Cellular Protein
2. Soluble Fraction
3. Eluted Fraction

Fig. 2. GenScript OptimumGene™ codon optimization genes increased the yield of expression (8 out of 10 genes) and the degree of solubility in some cases (6 out of 10 genes) compared to the native genes.

(**Reference:** N. A. Burgess-Brown, *et al*. Codon optimization can improve expression of human genes in *Escherichia coli*: A multi-gene study. **Protein Expression and Purification.** 2008. 59: 94-102)

Codon Optimized Synthetic Gene Enable Functional Folding of Recombinant Enzymes

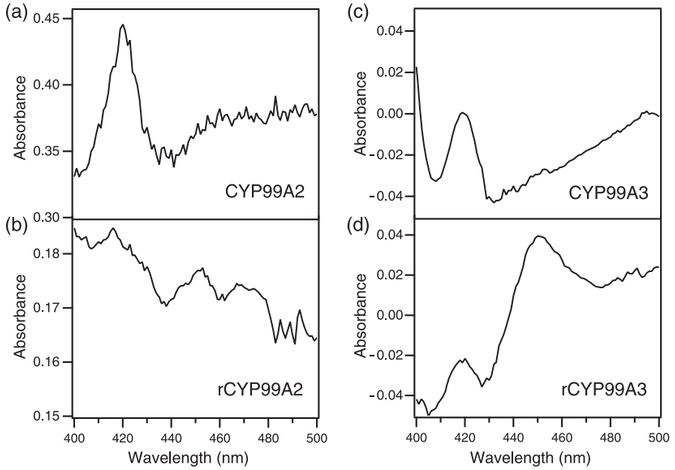
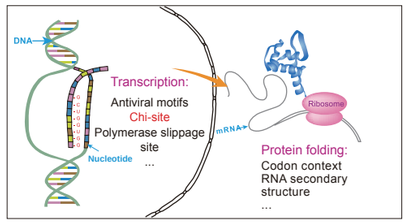


Fig. 3. The recombinant enzymes (CYP99A2 & CYP99A3) expressed from native gene sequences are mis-refolded and lose their functions (a) &(c); while those expressed from codon optimized synthetic genes are properly folded and have proper functions (b) & (d).

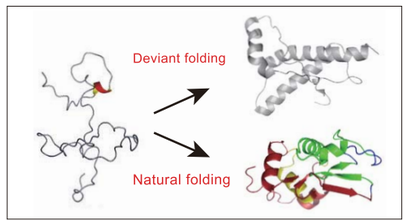
(**Reference:** Q. Wang, *et al*. CYP99A3: functional identification of a diterpene oxidase from the momilactone biosynthetic gene cluster in rice. **Plant Journal.** 2011. 65: 87-95)

Factors Considered for Codon Optimization



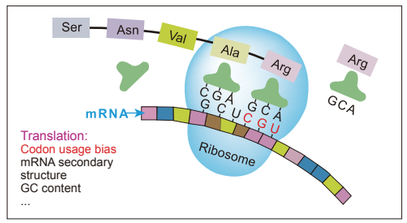
Transcription:

GC content, CpG dinucleotides content, Cryptic splicing sites, Negative CpG islands, SD/kozak sequence, TATA box, Terminal signal



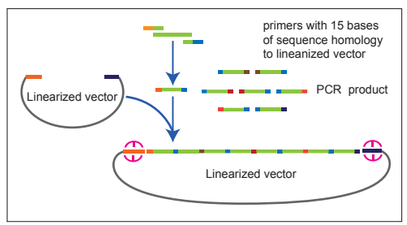
Protein Refolding:

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



Translation:

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

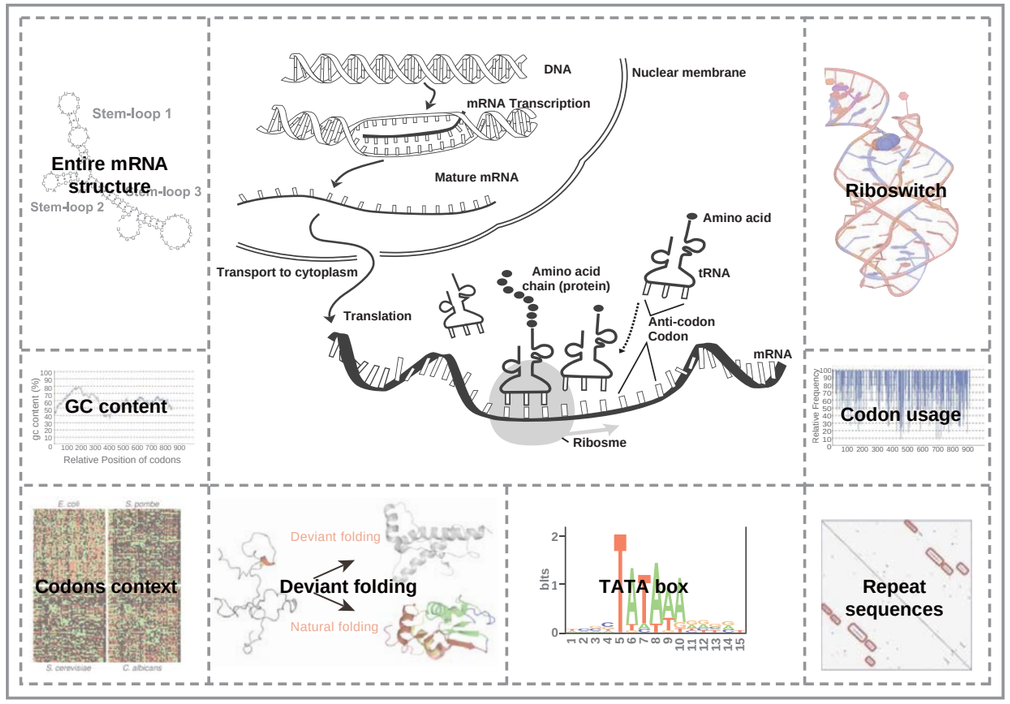


Gene Synthesis:

GC content, Repeat sequence, Secondary structures

Codon Optimization Algorithm

Multi-parameter Optimization



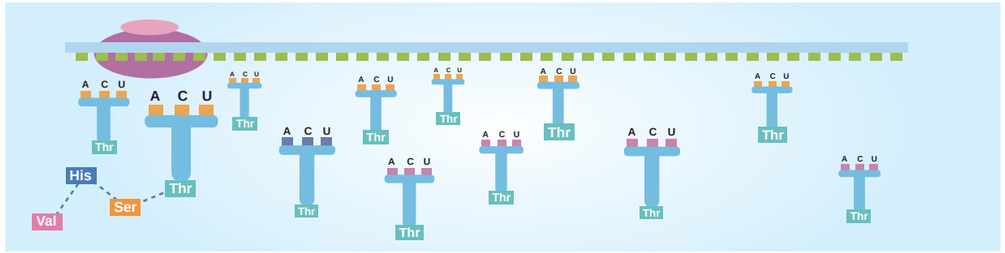
- Premature PolyA sites
- Negative CpG islands
- Internal chi sites and ribosomal binding sites
- Interaction of codon and anti-codon
- Terminal signal

And more…

1. tRNA Abundance:

Translational efficiency depends on availability of tRNAs. tRNA content is the major determinant of the bias between groups of codons recognized by different synonymous tRNAs. Codons recognized by abundant tRNAs are used more often than those recognised by rare tRNAs, particularly in highly expressed genes, probably owing to selection at the level of translation against codons recognized by rare tRNAs.

(**Reference:** M. Bulmer. Coevolution of codon usage and transfer RNA abundance. **Nature.** 1987. 325:728-730)



2. Codon Bias

The use of synonymous codons is strongly biased in the bacterium *Escherichia coli*, yeast and some higher organisms, which directly affect the translation efficiency. In table 1, the codon usage frequencies of four organisms are partially listed, which sufficiently illustrates how important it is to consider codon bias when expressing genes in a heterologous system.

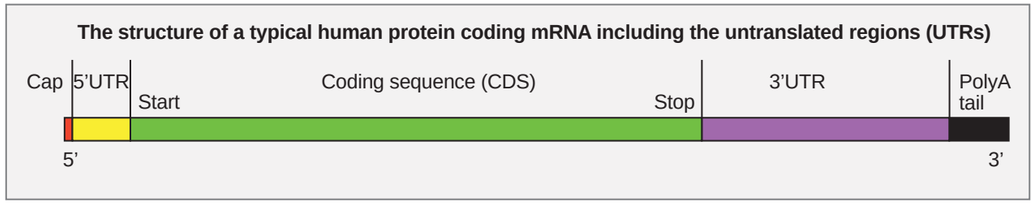
Table 1. Part of the codon usage table for insect, yeast, *E. coli*, and human.




		Sf9	*Pichia pastoris*	*E.coli*	*Homo.sapiens*
Gly	GGT	0.34	0.44	0.35	0.16
	GGC	0.31	0.13	0.37	0.34
	GGA	0.28	0.33	0.13	0.25
	GGG	0.07	0.10	0.15	0.25
Leu	TTA	0.09	0.16	0.02	0.07
	TTG	0.19	0.32	0.03	0.13
	CTT	0.12	0.17	0.15	0.13
	CTC	0.21	0.08	0.13	0.20
	CTA	0.09	0.11	0.05	0.07
	CTG	0.30	0.16	0.62	0.41
Arg	CGT	0.24	0.16	0.36	0.07
	CGC	0.24	0.05	0.36	0.19
	CGA	0.08	0.10	0.07	0.11
	CGG	0.06	0.05	0.11	0.21
	AGA	0.19	0.48	0.06	0.20
	AGG	0.19	0.16	0.04	0.20
Phe	UUU	0.27	0.54	0.57	0.46
	UUC	0.73	0.46	0.43	0.54
Ile	AUU	0.30	0.50	0.58	0.36
	AUC	0.54	0.32	0.35	0.47
	AUA	0.16	0.18	0.07	0.17
Val	GUU	0.20	0.42	0.25	0.18
	GUC	0.29	0.23	0.18	0.24
	GUA	0.17	0.15	0.17	0.12
	GUG	0.34	0.20	0.40	0.46
Ser	UCU	0.17	0.29	0.11	0.19
	UCC	0.21	0.20	0.11	0.22
	UCA	0.17	0.18	0.15	0.15
	UCG	0.13	0.09	0.16	0.05
	AGU	0.14	0.15	0.14	0.15
	AGC	0.18	0.09	0.33	0.24

3. Entire mRNA Structure

Besides 5' UTR and 3' UTR, the entire mRNA structure has an influence on the protein translation process. Recently, in a systematic investigation of determinants of protein expression, the predominant role of mRNA folding, especially the mRNA structure around RBS has been highlighted. There are plenty of reports on significantly increased protein expression by reducing the secondary structure around the start codon.



(**Reference:** G. Kudl, *et al*. Coding-sequence determinants of gene expression in *Escherichia coli*. **Science.** 2009. 324:255-258)

4. Gene Design Indexes

Codon adaptation index (CAI)

The parameter CAI (codon adaptation index) describes how well codons match the codon usage preference of the target organism. The range of CAI is 0-1.0. A CAI of 1.0 would be perfect and a CAI of > 0.8 is considered as good.

(**Reference:** G. A. Gutman and G. Wesley Hatfield. Nonrandom utilization of codon pairs in *Escherichia coli*. **Proceeding of the National Academy of Sciences USA. 1989. 86:** 3699–3703)

Codons context index (CCI)

Many investigations have shown that the expression of a heterologous gene is influenced dramatically by the genetic context of a specific host. Codon context such as codon usage, dicodons usage, translational kinetics and GC content, characterizes an integrative evolutional environment of codon selection of a species.

(**Reference:** S. Boycheva, *et al*. Codon pairs in the genome of *Escherichia coli*. **Bioinformatics. 2003. 19:** 987–998)

OptimumGene™ Codon Optimization is designed for proteins:

- Which do not express in heterologous systems
- Which have low expression levels
- Which cannot fold properly
- Which lose their proper functions

OptimumGene™ Codon Optimization Analysis Report

- The one cited by thousands of research publications

Start your project with the best gene sequence optimized for protein expression

FREE genetic codon optimization service is available from GenScript PhD level customer service representatives. Call 1-877-436-7274 (Toll-Free), or send your sequence and your requirements to gene@genscript.com for professional assistance.

Technical Support

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