



OptimumGene™密码子优化技术

——为蛋白表达而设计

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仍在为您的蛋白表达而烦恼吗？

- 蛋白不能在异源系统中表达？
- 蛋白表达水平很低？
- 蛋白不能正确折叠？
- 蛋白失去功能活性？
-

现在，
金斯瑞专利的OptimumGene™密码子优化技术帮您攻克蛋白表达的各种难题！



金斯瑞是一家具有全球经营规模和国际领先地位的综合性生物服务公司，主要提供科研定制服务，生物研究试剂，目录产品及批量生物试剂。公司总部位于美国新泽西州的Piscataway，已为30,000多名来源于世界级的大规模制药公司、生物技术公司以及全球70多个国家的著名科研院校的客户 提供多项服务及产品。

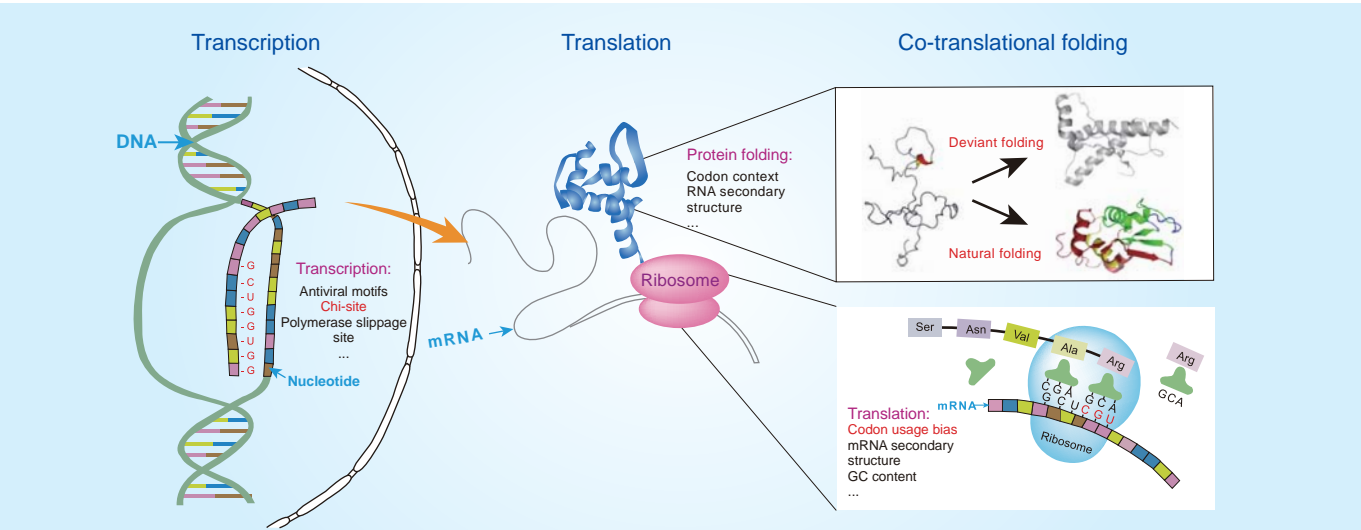
金斯瑞OptimumGene™密码子优化
——行业内引用最多的密码子优化技术

作为全球最大的基因合成供应商，金斯瑞自主研发了OptimumGene™密码子优化技术，并已成功运用到数万条基因的表达中：

- 被引用最多的基因优化算法
- 显著提高蛋白表达水平，最大可高达100倍
- 全面的密码子使用频率表适用于任何寄主
- 有效优化复杂序列

金斯瑞专利的OptimumGene™密码子优化技术可以优化任何自然或者重组的基因序列，在任何给定的表达系统中达到最高表达水平。相比传统的优化技术仅考虑密码子使用频率及mRNA结构，OptimumGene™基因优化算法充分考虑到蛋白表达不同阶段可能遇到的多种复杂因素，如：密码子偏爱性、mRNA结构以及转录和翻译过程中涉及的各种顺式元件。

运用OptimumGene™密码子优化技术，可使*E.coli*表达系统的蛋白表达量提高达100倍。拥有高表达量的蛋白，您可快速获得有意义的实验结果，并可节省实验时间和经费。



OptimumGene™密码子优化的成功记录

金斯瑞拥有多年的密码子优化和基因合成经验，密码子优化技术在所有表达系统中优化了超过60,000条序列。

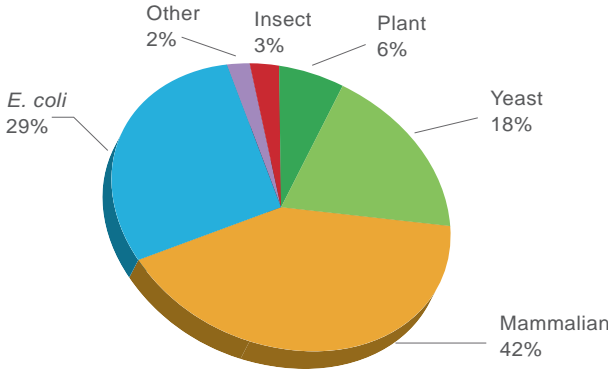


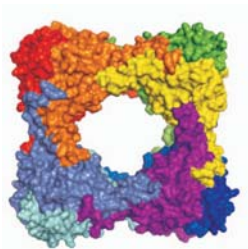
图1. 据统计，OptimumGene™密码子优化技术几乎在所有表达系统中实现超过60,000条序列的优化。

OptimumGene™密码子优化客户应用实证

2011年，金斯瑞已在150多种期刊中被引用2300多次，成为同行杂志期刊提及最多的生物研究合作机构。专利的OptimumGene™密码子优化技术已被大量实验证明可最大化提高*E. coli*等的基因表达水平，使得一些蛋白或关键酶生物学结构研究成为可能。

2011年发表在《Nature》上应用金斯瑞OptimumGene™密码子优化技术的部分文章

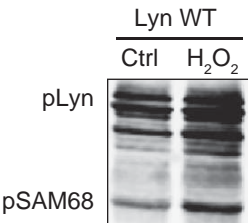
- Story 1. An enzyme allowing an acid-loving bacterium feeding CS₂



Many bacteria (e.g. *Acidithiobacillus*) living in volcanic hot wells obtain energy from CS₂ instead of CO₂. Scientists from Radboud University Nijmegen (Netherlands) found the unique structure of an enzyme that allows the microbe to efficiently utilize CS₂ 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)

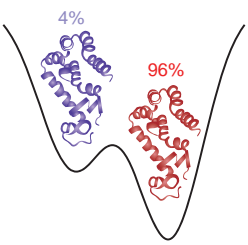
- Story 2. Lyn protein mediates leukocyte wound attraction



Little is known how immunity cells were activated by wounding signals, such as H₂O₂. 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

Evolution of a new enzyme for carbon disulphide conversion by an acidothermophilic archaeon

Marjan J. Smeulders^{1,2}, Thomas R. M. Barends^{1,2}, Arjan Prof. Anna Scherer², Marcel H. Zandvoort¹, Antko Alhouti¹, Khalid¹, Anissa Mouton¹, John Hermann¹, Robert L. Shewmaker¹, Hans J. C. T. Wessels¹, Ludo Lina Ruse¹, Hans Schilling¹, Mike S. M. Smit¹ & Huihui J. M. Opden Camp¹

Extremophilic organisms require specialized enzymes for their exotic metabolisms. Acid-loving thermophilic Archaea that live in the margins of volcanic solfataras obtain their energy from reduced sulphur compounds such as hydrogen sulphide (H₂S) and carbon disulphide (CS₂). The oxidation of these compounds into sulphuric acid creates the extremely acidic environment that characterizes solfataras. The hyperthermophilic *Acidithiobacillus* strain A1-3, which was isolated from the fumaric, ancient vents building at the Solfataras volcano (Naples, Italy), was shown to rapidly convert CS₂ into H₂S and carbon dioxide (CO₂), but nothing has been known about the modes of action and the evolution of the enzyme involved. Here we describe the structure, the proposed mechanism and evolution of a CS₂ hydrolase from *Acidithiobacillus* A1-3.

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LETTER

Lyn is a redox sensor that mediates leukocyte wound attraction *in vivo*

Xia Kan Yao¹, Taylor W. Starnes¹, Qing Deng¹ & Anna Huttenlocher^{1,2}

Tissue wounding induces the rapid recruitment of leukocytes. Wounds and tumours—a type of ‘oxidized wound’—generate hydrogen peroxide (H₂O₂) through an NADPH oxidase (NOX). This extracellular H₂O₂ mediates recruitment of leukocytes, particularly the first responders of innate immunity, neutrophils, to injured tissues¹. However, the sensor that neutrophils use to detect the redox state at wounds is unknown. Here we identify the Src family kinase (SFK) Lyn as a redox sensor that mediates initial neutrophil recruitment to wounds in zebrafish larvae. Lyn activation in neutrophils is dependent on wound-derived H₂O₂ after recruitment, and inhibition of Lyn attenuates neutrophil wound recruitment. Inhibition of SFKs also disrupted H₂O₂-mediated chemotaxis of primary human neutrophils. In vitro analysis identified a single cysteine residue, C666, as being responsible for direct oxidation-mediated activation of Lyn. Furthermore, transgenic

but not in neutrophils¹. Dark knockdown of Lyn in neutrophils (Fig. 1d, e), induced neutrophil recruitment to wounds¹. However, a rigorous understanding of the relation between a protein's structure, dynamics and function remains elusive. This is because many of the conformations on its energy landscape are only transiently formed and marginally populated (less than a few per cent of the total number of molecules), so that they cannot be individually characterized by most biophysical tools. Here we study a lysozyme mutant from phage T4 that binds hydrophobic molecules and populates an excited state transiently (about 1 ms) to about 3% at 25 °C (ref. 5). We show that such binding occurs only via the ground state, and present the atomic-level model of the ‘invisible’, excited state obtained using a combined strategy of relaxation-dispersion NMR (ref. 4) and CS₂ hydrolase activity assays. Our results show that the highly populated ground state is in a conformation that is not the same as the excited state.

LETTER

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

Gilles Bouvignies^{1,2,3,4}, Pramod Vellurugall^{1,2,3,4}, D. Flemming Hansen^{1,2,3,4}, Bruno E. Correia^{1,2}, Oliver Lange^{1,2}, Alaji Baki¹, Robert M. Vernon^{1,2}, Frederick W. Dahlquist¹, David Baker¹ & Lewis E. Kay^{1,2,3,4}

Proteins are inherently plastic molecules, whose function often critically depends on excursions between different molecular conformations (conformations¹). However, a rigorous understanding of the relation between a protein's structure, dynamics and function remains elusive. This is because many of the conformations on its energy landscape are only transiently formed and marginally populated (less than a few per cent of the total number of molecules), so that they cannot be individually characterized by most biophysical tools. Here we study a lysozyme mutant from phage T4 that binds hydrophobic molecules and populates an excited state transiently (about 1 ms) to about 3% at 25 °C (ref. 5). We show that such binding occurs only via the ground state, and present the atomic-level model of the ‘invisible’, excited state obtained using a combined strategy of relaxation-dispersion NMR (ref. 4) and CS₂ hydrolase activity assays. Our results show that the highly populated ground state is in a conformation that is not the same as the excited state.

通往分子生物学与蛋白质研究的捷径

❓ 基因难以克隆

主要因素：GC含量；重复序列；二级结构等

解决办法：基因合成

❓ 蛋白截断

主要因素：密码子偏好性；隐蔽剪切位点等

解决办法：密码子优化；调整蛋白纯化参数

❓ 蛋白低水平表达或不表达

主要因素：密码子偏好性；隐蔽剪切位点；翻译起始必需元件缺失；RNA或蛋白不稳定等

解决办法：密码子优化；选择合适的蛋白表达宿主及表达载体

❓ 蛋白非正确折叠或失去功能

主要因素：S-S键难以形成；蛋白表达太快或表达水平过高等

解决办法：密码子优化控制翻译速率；优化蛋白表达条件；改变宿主胞质内的还原环境；使用标签促进S-S键形成

客户评价.....

"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

OptimumGene™密码子优化效果展示

1. 运用OptimumGene™密码子优化技术，基因表达与蛋白可溶性水平迅速提升

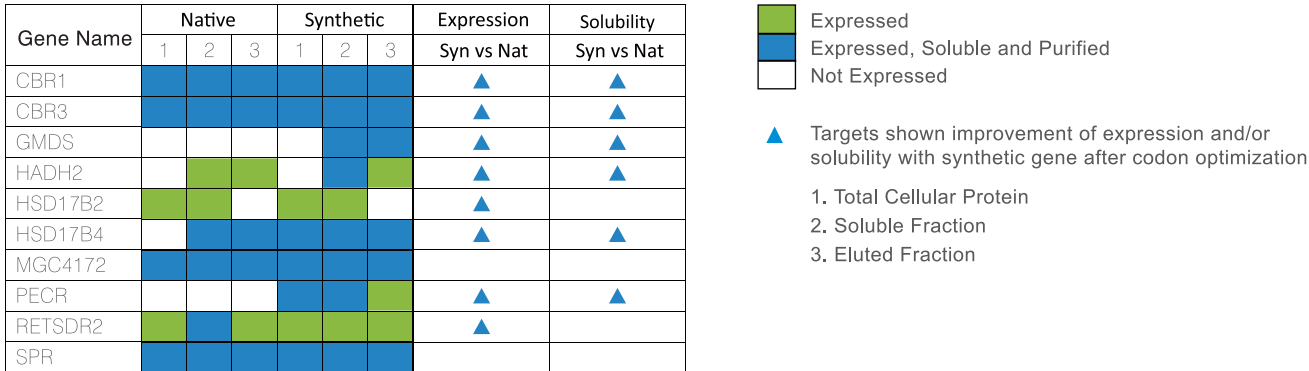


图2. 与未优化过的基因相比，金斯瑞 OptimumGene™密码子优化过的基因表达量 (8 out of 10 genes) 与蛋白可溶性均有提高 (6 out of 10 genes)

(参考文献: 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)

2. 经密码子优化后，表达的重组酶可进行功能折叠

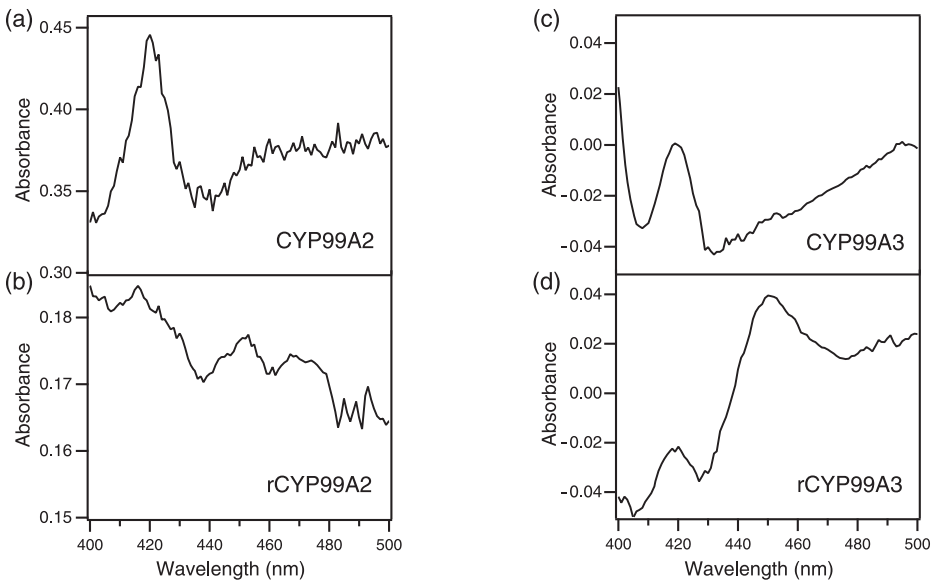
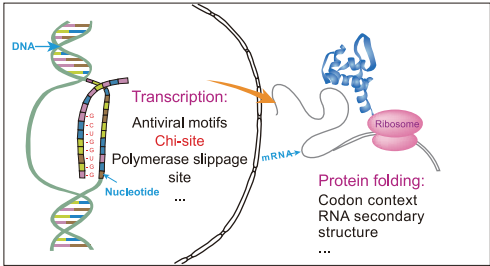


图3. 自然基因序列表达的重组酶 (CYP99A2, CYP99A3) 折叠错误且失去蛋白功能，如上 (a), (c) 所示; 而经密码子优化过的基因表达的重组酶能正确折叠且具正常的功能，如上 (b), (d) 所示。

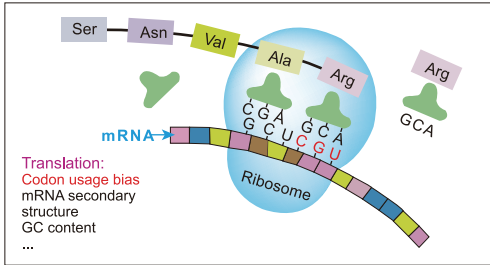
(参考文献: Q. Wang, *et al.* CYP99A3: functional identification of a diterpene oxidase from the momilactone biosynthetic gene cluster in rice. **Plant Journal**. 2011. 65: 87-95)

密码子优化考虑因素



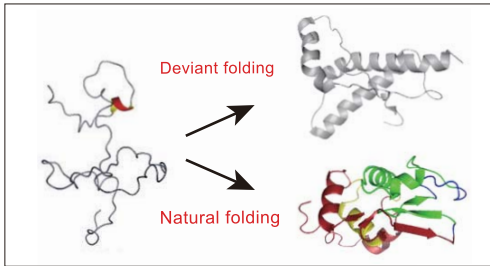
转录效率

- GC含量
- CpG二核苷酸含量
- 隐藏剪接位点
- 阴性CpG岛
- SD序列
- TATA框
- 终止信号



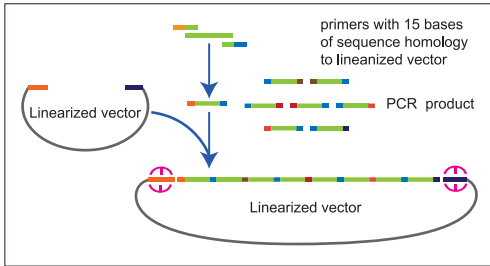
翻译效率

- 密码子偏爱性
- GC含量
- mRNA二级结构
- PolyA早期信号
- 抑制位点
- RNA不稳定性基序
- mRNA自由能稳定性
- 潜在的Chi序列和核糖体结合位点



蛋白折叠

- 密码子偏爱性
- RNA二级结构
- 密码子上下文关联
- 密码子与反密码子交互作用

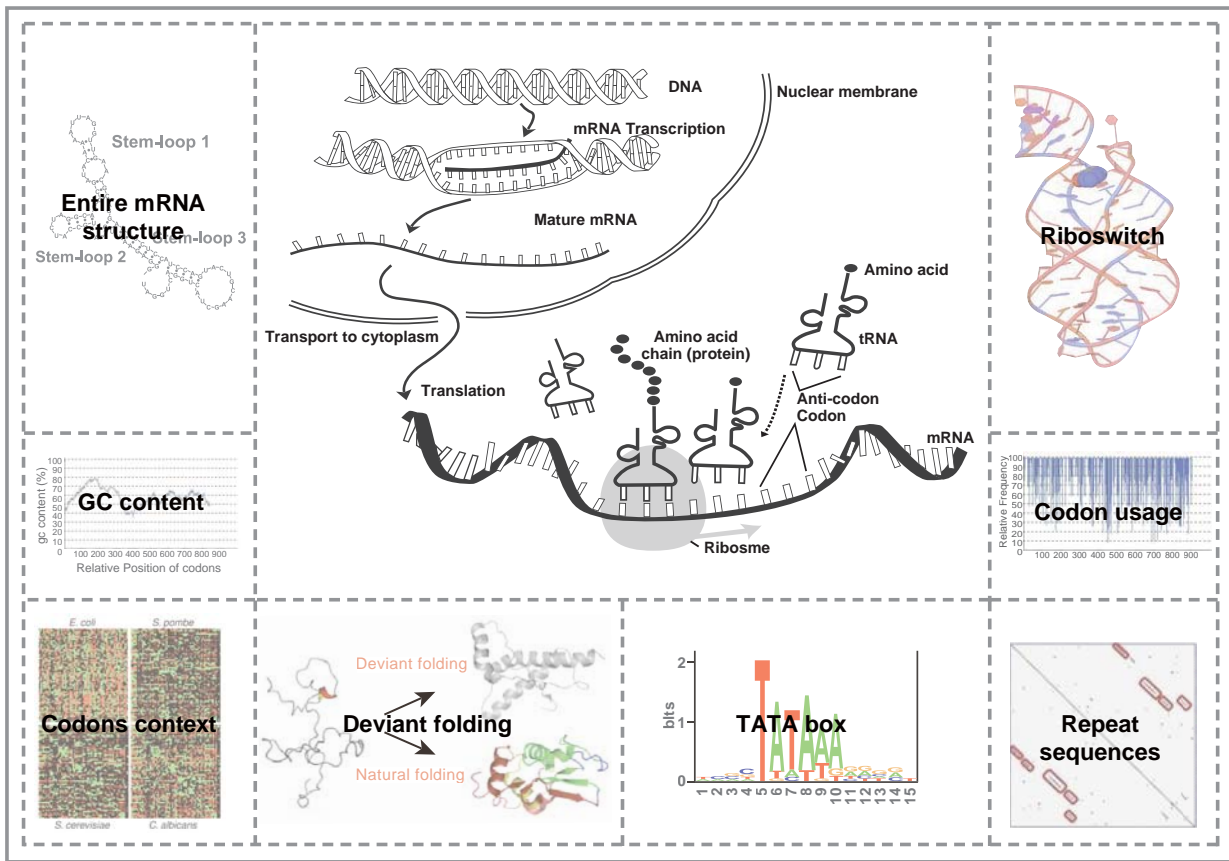


基因合成

- GC含量
- 二级结构
- 重复序列

OptimumGene™ 基因优化算法

多重参数优化

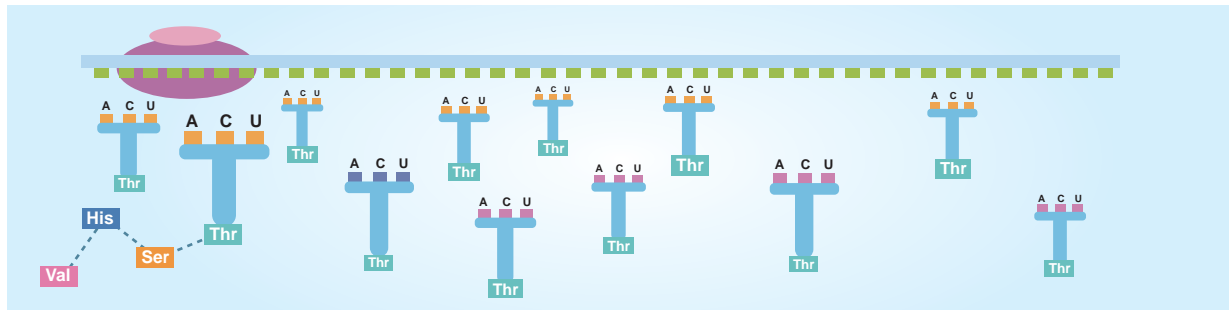


- Premature PolyA sites
- 阴性CpG岛
- 密码子与反密码子交互作用
- 终止信号
- 潜在的Chi序列和核糖体结合位点
- 更多.....

1. tRNA丰度

由于不同的tRNAs识别几种同义密码子，而这些同义tRNAs之间的含量也有不同，因此翻译的效率也取决于tRNA的数量。多数tRNA识别的密码子要比稀少tRNA识别的密码子使用频率高得多，在高表达的基因中尤其如此，这可能是在翻译过程中翻译与稀有tRNA所识别的密码子相互选择的结果。

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



2. 密码子偏爱性

在细菌 *Escherichia coli*, 酵母及一些高等生物表达系统中, 同义密码子的使用具有较强的偏爱性, 直接影响着翻译效率。表1列出了四种生物的部分密码子使用频率, 充分说明基因在异源系统中表达时, 需考虑密码子偏爱性这一因素的重要性。

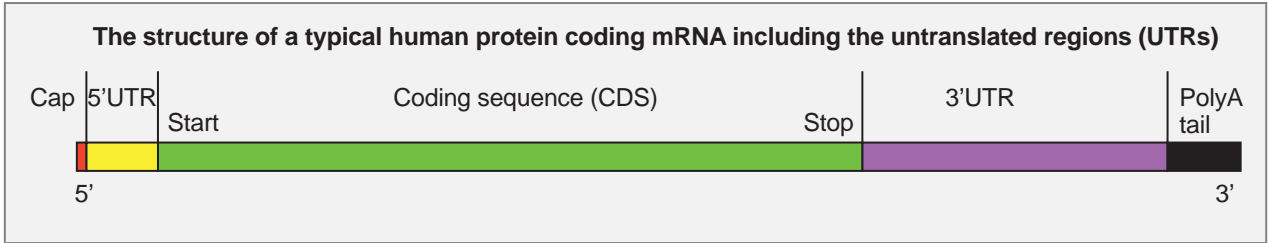
表1. 昆虫、酵母、*E.coli* 及哺乳动物细胞表达系统的部分密码子使用频率表



		<i>Sf9</i>	<i>Pichia pastoris</i>	<i>E.coli</i>	<i>Homo.sapiens</i>
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. 完整的mRNA结构

除5' UTR与3' UTR之外，完整的mRNA结构对蛋白翻译过程也有着一定影响。近来对蛋白表达主要影响因素的系统研究发现，mRNA折叠尤其是RBS附近的mRNA结构对蛋白表达起着尤为重要的作用：例如，通过减少起始密码子附近的二级结构，可以大大提高蛋白表达量。



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

4. 基因设计理论

密码子适应指数 (CAI)

CAI测量的是某个基因所用的密码子与高表达基因所用密码子的接近程度。此值为0~1.0，越接近1表示基因的表达水平越高，通常CAI>0.8 即认为基因表达水平较高。

(参考文献：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)

密码子上下文关联指数 (CCI)

大量研究表明特定宿主的密码子上下文关联极大地影响着异源基因的表达。密码子关联如密码子使用，双联密码子使用，翻译动力学及GC 含量，描述了一个种属密码子选择的综合进化环境特征。

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

OptimumGene™密码子优化为如下蛋白表达难题而设计：

- 不能在异源系统中表达
- 表达水平很低
- 不能正确折叠
- 失去功能活性

OptimumGene™密码子优化分析报告
——被众多研究文献所引用



最优的基因序列，高水平的蛋白表达，为您开启科研的成功之门！

金斯瑞为您提供免费的密码子优化服务。如需技术咨询，请拨打电话：025-58897288-5820，或将您的基因序列及需求发送至邮箱：gene@genscript.com.cn



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Technical Support
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金斯瑞生物科技
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