Heterologous gene expression in E.coli: Avoiding the hazard of rare codons.

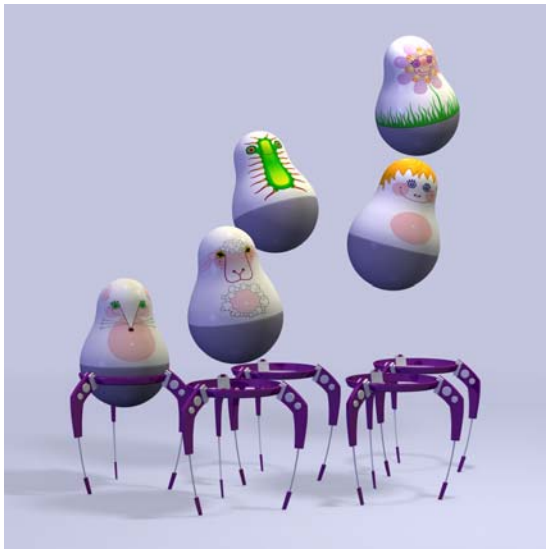
Delphi Genetics Inc.

Sharpened Tools for Lifescience Discoveries

Technical Note

Technical notes provide unique applications, innovative methods, and clear protocols designed specifically for Delphi Genetics products.

INTRODUCTION



In all organisms, most amino acids are encoded by more than one codon: 61 codons are available for 20 amino acids. But each organism is characterized by a specific "codon bias" i.e. it preferentially uses some codons over others. In practice, when a heterologous gene is expressed in *E. coli*, this gene might exhibit some codons that are common in the original host but are rarely used in *E. coli*. Whereas, the presence of only a small number of rare codons might not severely depress target protein synthesis, the presence of clusters of and/or numerous rare codons generates a demand for one or more rare tRNAs. In turn, the rarity of some tRNAs leads to very low expression of the target protein due to premature translation termination, translation frameshifting, amino acid misincorporation, growth inhibition and plasmid instability (1). The Staby™Codon technology was developed by Delphi Genetics to avoid these problems.

The StabyCodon™ technology has been extensively tested and validated in the Laboratory of Molecular Parasitology of Prof. E. Pays at the Université Libre de Bruxelles. In this laboratory, StabyCodon has consistently outperformed protein expression kits from other leading suppliers. One of the main research interests of the aforementioned laboratory is the human gene ApoL1. This gene has been implicated in human trypanosoma resistance as well as different pathological conditions including schizophrenia (2, 3). The Expression of human ApoL1 (refseq: NM_003661) in E.coli has been attempted. This gene is highly enriched in rare codons (for E. coli). Following a detailed analysis of the gene of interest (GOI) using the StabySoft™ analysis software it was established that 34 out of 430 were rare codons, there were 3 groups of several rare codons. The use of the StabyCodon™ T7 expression system was thus an appropriate choice for the expression of the ApoL1 gene. In parallel, the same experiments have been implemented using Novagen's Rosetta system and the C41(DE3) strain from Lucigen.

PROTOCOL:

The experiments using the Rosetta or the C41(DE3) bacteria have been realized following the manufacturers instructions.

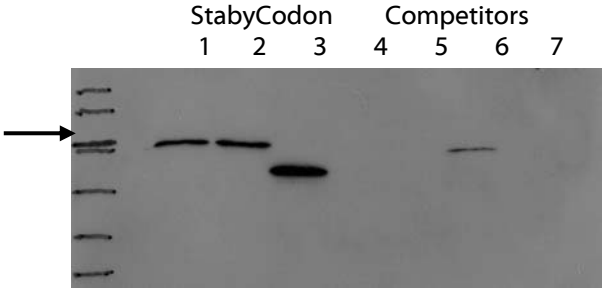
For the StabyCodon system:

- The gene of interest was cloned in the expression plasmid pSCodon1.2 using restriction digestion and ligation.
- The recombinant plasmid was transformed in the cloning host CYS21 and the desired construction was selected.
- Positive recombinant plasmids were transformed in the expression host SE1.
- Cells were grown to an OD_{600} of 0.8.
- Expression was induced by adding IPTG (1mM).

- Total cellular proteic extracts were obtained.
- The expression of the desired protein was assessed using a Western Blot. The presence of ApoL1 was evaluated using an ApoL1 antibody.

RESULTS

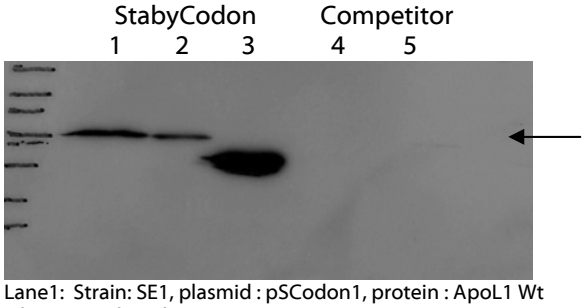
The first figure illustrates the results obtained when expressing the ApoL1 protein at the relatively low temperature of 18° during 20 hours. The arrow indicates the expected size for Wt ApoL1.



Lane1: Strain: SE1, plasmid : pSCodon1, protein : ApoL1 Wt (clone 1), induced
Lane 2: Strain: SE1, plasmid : pSCodon1, protein : ApoL1 Wt (clone 2), induced
Lane 3: Strain: SE1, plasmid : pSCodon1, protein : ApoL1 delta*, induced
Lane 4 : Strain: C41(DE3)pLysS, plasmid: pET-21d, protein: ApoL1 Wt, not induced
Lane 5 : Strain: C41(DE3)pLysS, plasmid: pET-21d, protein: ApoL1 Wt, induced
Lane 6 : Strain: Rosetta2(DE3)pLysS, plasmid: pET-21d, protein: ApoL1 Wt, induced
Lane 7: Strain: Rosetta2(DE3)pLysS, plasmid: pET-21d, protein: ApoL1 delta*, induced

*:ApoL1 delta is a C-terminally truncated version of the ApoL1 gene.

The second figure illustrates the results obtained when expressing the ApoL1 protein at the standard temperature of 37°C during 3 hours. The arrow indicates the expected size for Wt ApoL1.



Lane1: Strain: SE1, plasmid : pSCodon1, protein : ApoL1 Wt (clone 1), induced
Lane 2: Strain: SE1, plasmid : pSCodon1, protein : ApoL1 Wt (clone 2), induced
Lane 3: Strain: SE1, plasmid : pSCodon1, protein : ApoL1 delta*, induced
Lane 4 : Strain: Rosetta2(DE3)pLysS, plasmid: pET-21d, protein: ApoL1 Wt, induced
Lane 5: Strain: Rosetta2(DE3)pLysS, plasmid: pET-21d, protein: ApoL1 delta*, induced

*:ApoL1 delta is a C-terminally truncated version of the ApoL1 gene.

These results clearly indicate that Staby™Codon is better suited to express the ApoL1 protein than the other kits tested by the Laboratory of Molecular Parasitology. A visible band is obtained when using the Staby™Codon kit whereas only a week expression is observed using Novagen's Rosetta system. When using a system without codon correction, no expression is observed indicating that the codon bias indisputably hinders protein production in this case (even when using strain specifically isolated for toxic proteins as the C41 strain). The superiority of the Staby™Codon kit may be due to several of the unique features of the Staby™ technology. The greatest enhancement over the competition is the fact that the tRNAs are located directly on the expression plasmid thus avoiding an additional replicative burden to the expression host. The overall recombinant protein yields are higher. This is presumably due to the fact that (i) not all the bacteria transformed with both of Novagen's vectors are able to retain the Rosetta vector and (ii) the additional burden of a second plasmid hinders the effective replication of the Rosetta2(DE3) cells. Overall yields are consistently higher when using our proprietary Staby™ technology for plasmid stabilization. This system ensures that only plasmid-bearing clones can grow whereas every clone losing the plasmid will inevitably die. This unique feature guarantees the highest possible yields from a given amount of bacteria.

BENEFITS OF THE Staby™Codon SYSTEM:

- High yield of heterologous protein expression, even when rare codons are abundant in the transcript
- The necessity to mutate each codon is avoided
- The plasmid is perfectly stabilized
- No additional plasmid in bacteria, thus reducing the replicative burden
- Reduced background

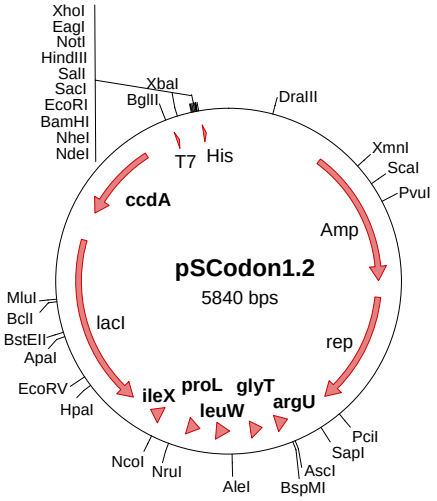
ABOUT THE Staby™Codon TECHNOLOGY:

Six rare codons can cause problems in *E. coli* B (*e.g.*; BL21(DE3) or SE1): AGG and AGA (both encoding arginine using the *argU* tRNA), AUA (isoleucine, *ileX* tRNA), CUA (leucine, *leuW* tRNA), GGA (glycine, *glyT* tRNA), and CCC (proline, *proL* tRNA) (see table).

Amino acid	Codon	Frequency in E. coli B (SE1) (%)	Frequency in Homo sapiens (%)	Frequency in Arabidopsis thaliana (%)	Frequency in Saccharomyces cerevisiae (%)
Arginine	CGT	35	8	17	14
	CGC	40	19	7	6
	CGA	5*	11	12	7
	CGG	11	20	9	4
	AGA	5	21	35	48
	AGG	4	21	20	21
Glycine	GGT	30	16	34	47
	GGC	41	34	14	19
	GGA	10	25	37	22
	GGG	18	25	15	12
Isoleucine	ATT	48	36	41	46
	ATC	44	47	35	27
	ATA	7	17	24	27
Leucine	TTA	14	8	13	28
	TTG	15	13	22	28
	CTT	11	13	26	13
	CTC	12	20	17	6
	CTA	3	7	11	14
	CTG	45	40	11	11
Proline	CCT	14	28	38	31
	CCC	6	33	11	16
	CCA	18	27	33	41
	CCG	61	11	18	12

* CGA codon does not cause problem because large amounts of the corresponding tRNA are present

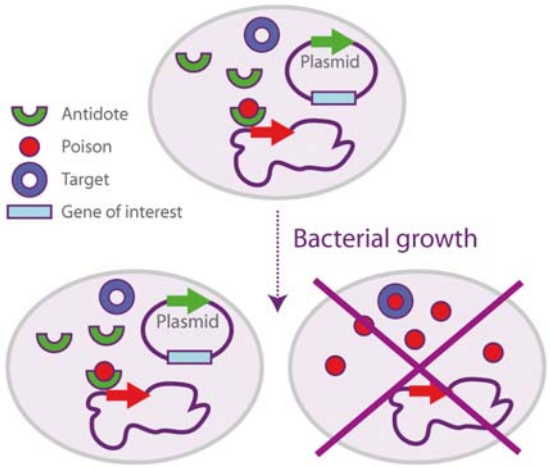
When expressing heterologous proteins, it is of paramount importance to check the gene of interest (GOI) for the presence of rare codons. Several systems have been devised to avoid the hazard of rare codons when expressing heterologous genes. The basic idea behind these systems is to supplement the expression host with the missing tRNAs. Two main options have been implemented to date: (i) using a distinct plasmid supplementing the rare tRNAs (e.g. the Rosetta plasmids from Novagen) or (ii) the use of a special expression vector encoding the six missing tRNAs on the same DNA molecule (e.g. StabyCodon from Delphi Genetics). The pSCodon plasmid from the StabyCodon kit allows protein expression under the control of the T7 promoter, efficient plasmid stabilization even without antibiotics and production of the missing t-RNAs.



ABOUT THE STABY™ SYSTEM:

The stabilization system is based on the use of bacterial antidote/poison genes naturally found in the F plasmid of E.coli (ccdA and ccdB). In the stabyCodon system, the antidote gene ccdA is introduced in the plasmid DNA under the control of a constitutive promoter. The bacterial toxin gene ccdB is introduced in the chromosome of the cloning or expression bacteria (CYS21 and SE1 respectively) (See figure below).

Expression of the poison gene is under the control of a strongly repressed promoter in the presence of the plasmid. When the plasmid is lost, the antidote protein is degraded and the production of the toxin is induced causing cell death. This unique system allows for the perfect stabilization of the plasmid without the use of antibiotics. Furthermore this system guarantees that during protein expression every bacterium is carrying the expression plasmid thus enhancing the overall yield of any given protein expression experiment. If some bacteria lose the vector, they will not obtain a selective (growth speed) advantage but will die. In practice, this additional stabilization technology solves the problem of plasmid instability and insures that during bacterial growth, 100% of the bacteria will carry the vector. Thus, the production of the protein of interest is higher and purer (lower amount of non-target proteins).



KIT COMPONENTS

- pSCodon1.2 DNA
- CYS21 bacteria (for cloning)
- SE1 bacteria (for expression)
- Regeneration medium
- Fw and Rv sequencing primer
- Expression control

The Staby™ Range of Products is available with either electro or chemically – competent cells.

ORDER REFERENCES

- SCT7-0505: electro-competent cells, 5 reactions
- SCT7-0707: chemically-competent cells, 5 reactions
- SCT7-1010: electro-competent cells, 10 reactions
- SCT7-1212: chemically-competent cells, 10 reactions
- SET7-0020: additional bacteria for expression (SE1), electrocompetent cells, 20 reactions
- SET7-0022: additional bacteria for expression (SE1), chemically-competent cells, 20 reactions

RELATED PRODUCTS

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(2) Vanhollebeke B and Pays E. The function of apolipoproteins, 2006, L. Cell Mol Life Sci. 63 (17): 1937-44

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LEGAL

StabyCodon™ is a trademark of Delphi Genetics.

ACKNOLOWLODGEMENTS

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