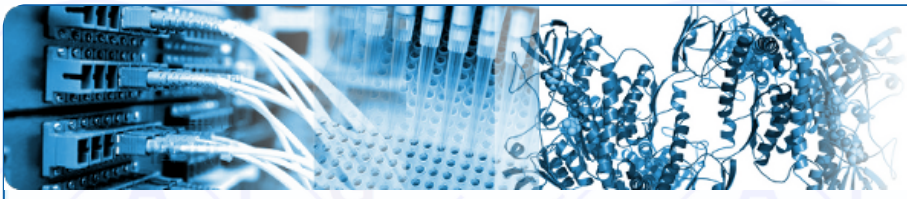


ATG:biosynthetics
Solutions
in biosynthetics

CODONEWS
Issue62

08 July 2014

Synthetic Gene Sequences -
Modulation - Optimization - Function

Dear customers,

ATG supports you in design & construction of your synthetic genes.

Many sequence parameters once at a time can be processes by our proprietary for formal-functional gene, gene clusters and expression cassettes optimization.

If you like to get a **Gene Check** combined with a specific codon table of your choice you can easily upload your gene sequences **here**.

Just select the "***Free Gene Check***" box under the inquiry form.

The first STEP for performing the assessment of predicting gene activity is a GeneCheck for a functional relevant sequence parameter visualization. (Please see an example below in Figure-1)

After the weak functional relevant formal parameters are identified - ATG supports you in all aspects of getting your synthetic genes, gene clusters and expression cassettes to work properly.

In case you agree with the design proposals we make - ATG will realize the sequence optimization and the synthesis of your genes.

Services:

Synthetic Genes
expression analysis and gene synthesis

***Pep*ID- bioPeptides**
the most convenient way to create complex non-random bio-peptideCDS libraries for affinity screenings

Products:

***Flex*TEC- Expression Cassettes**
enables the creation of modular genetic assemblies to systems

evoMAG
GeneIntelligence software for expression optimization

ATG: Bioinformatics services:

Pricing Table for Sequence Analyses, DNA- Modulations and Gene Optimizations:

Sequence - Modulation - Optimization - Function				
Sequence calculation level	**Elongation**	**Initiation**	**Termination**	**Cost/ Gene in €**
Expert-Level-V	5[1)]	5[1)]	5[1)]	5[1)]
Expert-Level-IV	1,2,3,4	1,2,3,4	1,2,3,4	**200**

Expert-Level-III	1,2,3	1,2,3	1,2,3	**150**
Expert-Level-II	1	1,2	-	**100**
Expert Level-I	1	-	-	**50**
Basic Level Gene Optimization	1[2)]	-	-	**for free**
Basic GeneCheck	yes[3)]	yes[3)]	yes[3)]	**for free**

1) expert-Level-V includes levels I..IV applied for functionally arranged multiple genes project mode and multiple gene systems arranged in clusters only
2) standard algorithm applied to genes according to http://bioinformatics.org/sms2/
3) providing on line support by visualization of sequence parameters - no reporting or consulting in depth

1) **Basic GeneCheck – on request – free of charge**: online presentation of results: coding sequence - CDS integrity without reporting and consulting, **-ΔG** - Gibbs Energy calculation of translational initiation sites; restriction sites, codon use assessment, local RNA - secondary extensive structures, repeats, direct repeats, inverted repeats.

   Sequence motives: Internal Shine-Dalgarno-sequences, internal splicing-sites, STOP - codons in non-used frames, cryptic restriction sites for **in**- and **out** calculations, internal promoter sequences, internal terminator/ pausing structures, poly-homo-nucleotide-stretches

2) **Basic Optimization Level – free of charge**: formal computational sequence calculation by applying a computer algorithm no further hands on by co-workers

   1.1Formal Translational Elongation: custom codon frequency adaption to a specific table1.1Formal Translational Elongation: custom codon frequency adaption to a specific table

   Protein sequence → back translation → DNA-Sequence

   a. Species specific table – codon use of codons random sampling or max. **CAI**

   b. **Simple codon conversion** of heterologous expression from **one species to another**

3) **Expert Level-I Analytical GeneCheck (see above like Basic GeneCheck but with consulting and individual reporting)**

   a. **Expert level** GeneCheck with **on-line** presentation of results and consulting by an

   b.additional expert's evaluation report and support - via e-mail or a phone call

4) **Expert Level-II**

   a. Expert level GeneCheck (Elongation, Initiation)

   b. Expert level translational Elongation: Treshold analysis (codon frequency adaption to the table by removing rare codons)

5) **Expert Level-III**

   a. Expert level GeneCheck (Elongation, Initiation, Termination)

   b. Expert level translational elongation: Treshold Analysis and codon frequency adaption to the table by removing rare codons

   c. Expert level translational initiation: Detailed Analyses of the initiation regions (SD, Kozak) and generation of variants with reasonable **–ΔG**-Values and relaxed structures on the RNA level

6) **Expert Level-IV**[1)]

   (Promotor, Leader, Initiation, Elongation, Termination) Integrated holistic analyses of all relevant parameters known for formal-functional relevant optimal sequence parameters

   a. Expert level GeneCheck (Leader, Initiation, Elongation, Termination)

   b. Expert level translational elongation: Treshold analysis and codon frequency adaption to the table by removing rare codons, Production specific tables according to codon bias analyses

   c. Expert level translational initiation: Detailed Analyses of the initiation regions (SD, Kozak) and generation of variants with reasonable **–ΔG**-Values and relaxed structures on the RNA level

   d. Analyses of all expression relevant sequence parameters

7) Expert Level-V[1)]

Project mode(Promotor, Leader, Initiation, Elongation, Termination) like expert level-IV but with customer requirement specifications)

*only in the context of gene cluster designs in the project mode including production specific codon tables, iterative **experimental design** processes and algorithms etc., Pareto optimized variant libraries

a. **Genomic Analyses** for all purposes in advanced genomics. Comparative Ortholog, Paralog analyses, Operon screenings, screening for natural compound candidate gene clusters, Comparative codon bias analyses, comparative regulators.

b. **RNAseq** – and **Proteomics** data analyses and condensation for extracting and gaining information out of it.

c. **Gene Cluster Designs** according to the customer's requirements including all structural and sequence handling work and redesigns

d. **GeneClusterCheck*** (Promotors, Leaders, Initiation, Re-Initiation, Elongation, Termination, professional genomic codon bias analyses)

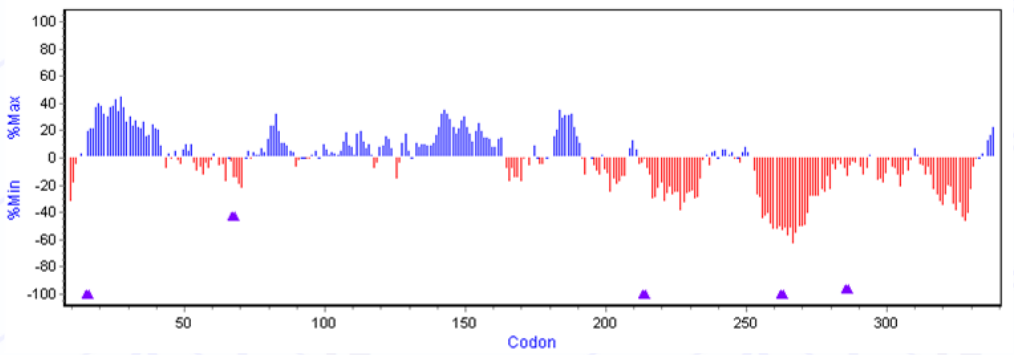
1.Operon check for formal-functional molecular parameters

2.Expression box check - in multiple gene expression

e.**Production tables**: professional analyses of genomic parameters for the assessment of the specific codon bias in a given organism. Generation of higly specific codon tables

f.**Support in RNA**seq data evaluation - condensation of results

A



B

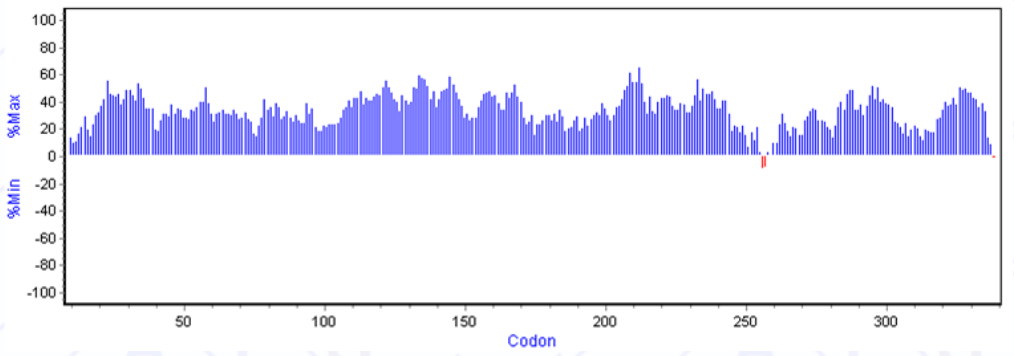


Figure-1 Pre- after comparison of a gene which one of our customers at first tried to optimize.

A This diagram shows the relative frequence of codon fractions along the message separating codons in over average frequency (blue) low frequency (red) and slow codons (violet)"slow codons cluster" - graph according to Clarke et. al.(3,4). The red regions are depicting stretches of more ore less rare (or slow) codons. These are in general in highly expressed genes suppressed but not completely avoided. Strategically placed, for example at domain boundaries, they could make sense to support protein folding. The violet triangles indicate direct repeats of codons of very below average frequency of codon usage, which are especially harmful in the case of the rare Arginine codons (ribosome stalling).

B Example of an translational elongation optimization of a customer project. The gene was not working in that no gene product and no activity of the gene products could be detected. All formal-functional features with a negative impact on gene expression were avoided. The TIR had a -DG value of -17,2 kcal/ mol another reason for malfunction which was eliminated subsequently.

References:

(1) Plotkin, J. B., & Kudla, G. (2010). Synonymous but not the same: the causes and consequences of codon bias. Nature Reviews Genetics, 12(1), 32-42.

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(3) Clarke IV, T. F., & Clark, P. L. (2008). Rare codons cluster. PLoS One, 3(10), e3412. (4) Clarke, T. F., & Clark, P. L. (2010). Increased incidence of rare codon clusters at 5'and 3'gene termini: implications for function. BMC genomics, 11(1), 118.

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