

GENEius Optimises Your Sequence.

The acquisition of Entelechon and its gene optimization software Leto presented Eurofins Genomics with a unique opportunity to combine the strengths of two outstanding software platforms in one.

We integrated all the key beneficial features of Leto into our advanced gene optimisation software GENEius. GENEius, now powered by Leto, provides a comprehensive optimisation solution for your synthetic genes.

GENEius optimisation process

- 20 amino acids are coded by 61 triplets
- Triplets do not occur in all organisms to the same extent
- GENEius optimises your sequence by employing those triplets which are naturally used by your expression system
- GENEius not only uses the best triplet but a combination of triplets which are adjusted to the heterologous expression system

Prevent secondary structures

- Avoid direct or inverted repeats
- Remove hairpins from the final sequence

Exclude bad motifs

- Avoid specific restriction sites e.g. making your 5' and 3' cloning sites unique
- Omit customised "bad motifs" like transcription factor binding sites or splice donor and acceptor sites

Distribution of GC

- GC content is harmonised for the entire genes
- Optimisation of GC content in accordance with codon usage table

Insert good motifs

- Add customised "good motifs"
- Facilitate further subcloning of single protein domains by inserting unique restriction sites

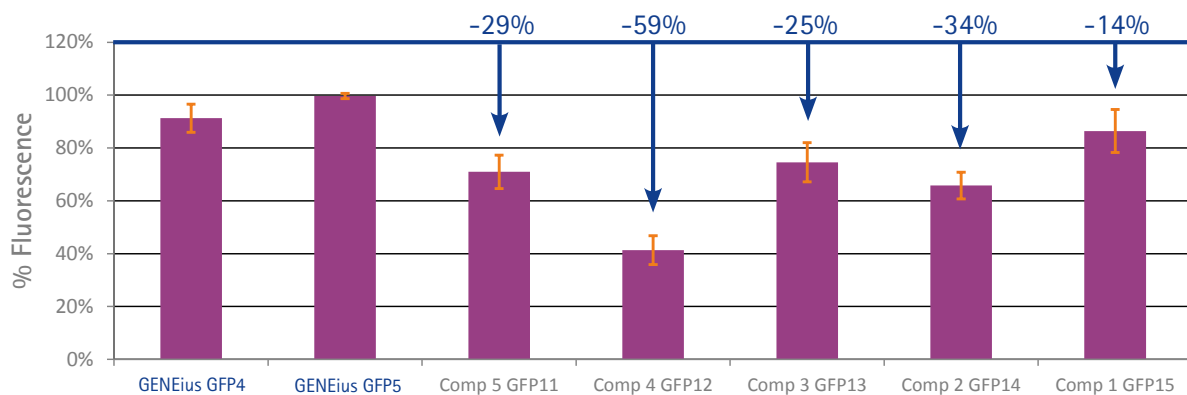


Fig. 1 GENEius outperforms the competition - We tested the optimisation performance of GENEius in comparison to five other software packages from five main competitors. The sequence of the jellyfish *Aequorea victoria* wild-type GFP was optimised using all six software packages for best expression results in *E.coli*. After optimisation, sequences were synthesised and expression studies in *E.coli* were performed. The results clearly show higher expression for GFP sequences optimised by GENEius compared to the GFP sequences from the competitors. Read the GENEius application note for a detailed analysis of the experiment.

Performance Of GENEius.

Synthesised genes are often employed to achieve higher quantities of proteins in heterologous expression systems, for example expression of a human gene in *E.coli*. We demonstrated the superior performance of GENEius by adapting a human gene for the expression in Sf9 cells.

	Best conditions for Sf9 cell expression	Before GENEius optimisation	After GENEius optimisation
GC content	50%	36.66%	45.48%
Direct repeats	none	9	none
Bad motifs (e.g. PolyA or artificial splice sites)	none	1 PolyA signal present	none
Bad motifs (cloning sites)	irrelevant	internal BamHI site	none
Good motifs	irrelevant		EcoRI at 5' end + BamHI at 3' end

Achieved Codon Usage
(Format: <optimal frequency> | <pre-optimisation frequency> | <optimised frequency>)

D(10)	E(11)	F(9)	G(24)
ggt: 0.40 0.50 0.40	gaa: 0.45 0.45 0.45	ttt: 0.27 0.44 0.22	ggt: 0.34 0.17 0.33
gac: 0.60 0.50 0.60	gag: 0.55 0.55 0.55	ttc: 0.73 0.56 0.78	ggc: 0.31 0.33 0.29
			gga: 0.28 0.25 0.29
			ggg: 0.07 0.25 0.08
A(20)	C(7)	* (1)	L(24)
gct: 0.35 0.25 0.35	tgt: 0.39 0.43 0.43	taa: 0.63 0.00 1.00	tta: 0.10 0.08 0.08
gcc: 0.29 0.40 0.30	tgc: 0.61 0.57 0.57	tag: 0.18 0.00 0.00	ttg: 0.20 0.12 0.21
gca: 0.18 0.25 0.20		tga: 0.18 1.00 0.00	ctt: 0.12 0.12 0.12
gcg: 0.18 0.10 0.15			ctc: 0.21 0.21 0.21
			cta: 0.09 0.08 0.08
			ctg: 0.30 0.38 0.29
M(11)	N(16)	H(4)	I(12)
atg: 1.00 1.00 1.00	aat: 0.32 0.50 0.31	cat: 0.36 0.50 0.25	att: 0.30 0.33 0.33
	aac: 0.68 0.50 0.69	cac: 0.64 0.50 0.75	atc: 0.54 0.50 0.50
			ata: 0.16 0.17 0.17
K(23)	T(18)	W(7)	V(25)
aaa: 0.35 0.43 0.35	act: 0.27 0.28 0.28	tgg: 1.00 1.00 1.00	gtt: 0.20 0.16 0.20
aag: 0.65 0.57 0.65	acc: 0.32 0.33 0.33		gtc: 0.29 0.24 0.28
	aca: 0.23 0.28 0.22		gtg: 0.17 0.12 0.16
	acg: 0.18 0.11 0.17		gta: 0.34 0.48 0.36
Q(6)	P(10)	S(15)	R(22)
caa: 0.43 0.33 0.50	cct: 0.29 0.30 0.30	tct: 0.17 0.20 0.20	cgt: 0.24 0.09 0.23
cag: 0.57 0.67 0.50	ccc: 0.28 0.30 0.30	tcc: 0.21 0.20 0.20	cgc: 0.30 0.18 0.32
	cca: 0.28 0.30 0.30	tca: 0.17 0.13 0.13	cge: 0.08 0.09 0.09
	ccg: 0.16 0.10 0.10	tcg: 0.13 0.07 0.13	cgg: 0.00 0.18 0.00
		ctg: 0.14 0.13 0.13	agg: 0.19 0.23 0.18
		ctc: 0.18 0.27 0.20	arg: 0.19 0.23 0.18
Y(6)			
tat: 0.29 0.50 0.33			
tac: 0.71 0.50 0.67			

Fig. 2 GENEius optimised a human gene by comparing it to the input codon usage table for Sf9 cells found in the Kazusa Codon Usage Database. For example, the glycine triplet ggg was adjusted by GENEius from a frequency of 0.25 to 0.08 resulting in the optimum frequency for Sf9 cells (0.07).

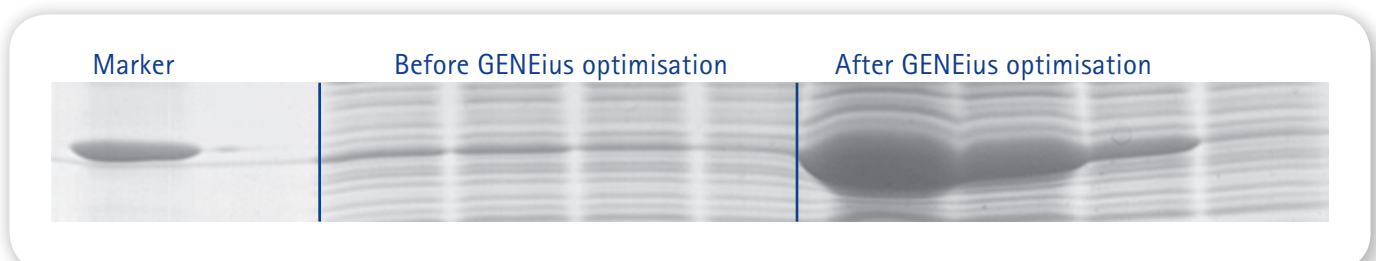


Fig. 3 Expression levels for a GENEius optimised gene in a heterologous system.

Making GENEius Work For You.

- GENEius optimises all protein coding sequences from short DNA fragments of 100 bases to long genes with > 12,000 bases
- GENEius adapts either DNA or amino acid sequences
- Access GENEius via our Ecom ordering system <https://ecom.mwgdna.com>
- Enter your order by choosing "order or request synthetic genes" in our Gene Synthesis menu
- Check the option for "codon usage adaption required"
- Select the species from the dropdown menu for codon usage adaption
- Choose or generate your own "bad motifs" like your cloning sites that will be excluded during adaption
- Click "adapt and optimise sequence" to start the sequence optimisation by GENEius
- Our Ecom system will show you the optimised sequence from GENEius

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GENEius FOR FREE
ON OUR WEBSITE!**

www.geneius.de

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