OptimumGene™ Codon Optimization Examples

A wide variety of factors regulate and influence gene expression levels, and our OptimumGene™ algorithm takes into consideration as many of them as possible, producing the single gene that can reach the highest possible level of expression. In this case, the native gene employs rare codons that can reduce the efficiency of translation or even disengage the translational machinery. We removed the codon usage bias by upgrading the CAI.

Low GC content and unfavorable peaks can cause overly quick mRNA turnover. We have adjusted GC content of your gene to prolong the half-life of its mRNA. In addition, we found several negatively cis-acting motifs that can hamper expression in your designated host organism. Our optimization process successfully removed these negative cis-acting sites. The additional motifs as listed in the introduction were also screened and modified accordingly.

Improve the host-specific codon usage

1. **Codon usage bias adjustment**

   a. **Codon Adaptation Index (CAI)**

After OptimumGene™ Optimization | **Before Optimization**

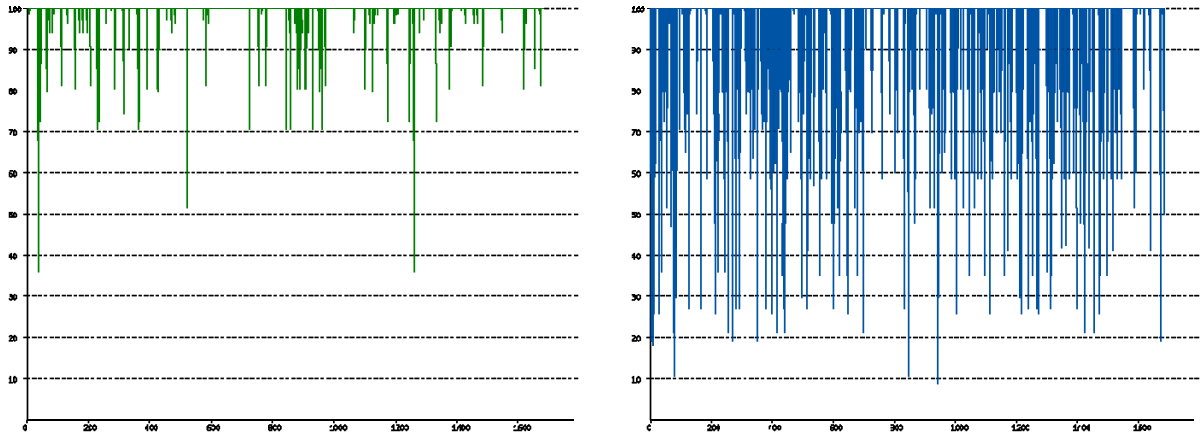

Figure 1a. The distribution of codon usage frequency along the length of the gene sequence. A CAI of 1.0 is considered to be perfect in the desired expression organism, and a CAI of >0.9 is regarded as very good, in terms of high gene expression level.

b. **Frequency of Optimal Codons (FOP)**

After OptimumGene™ Optimization **Before Optimization**

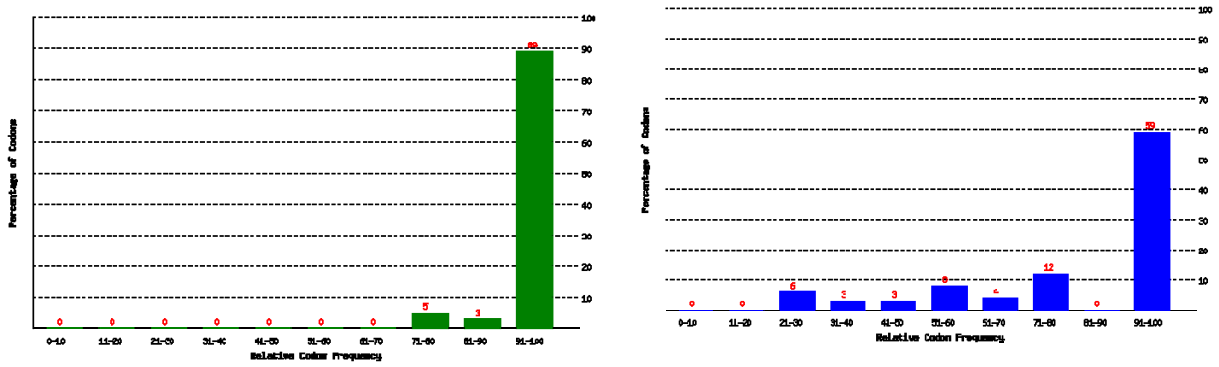

Figure 1b. The percentage distribution of codons in computed codon quality groups. The value of 100 is set for the codon with the highest usage frequency for a given amino acid in the desired expression organism.

2. **GC Content Adjustment**

After OptimumGene™ Optimization **Before Optimization**

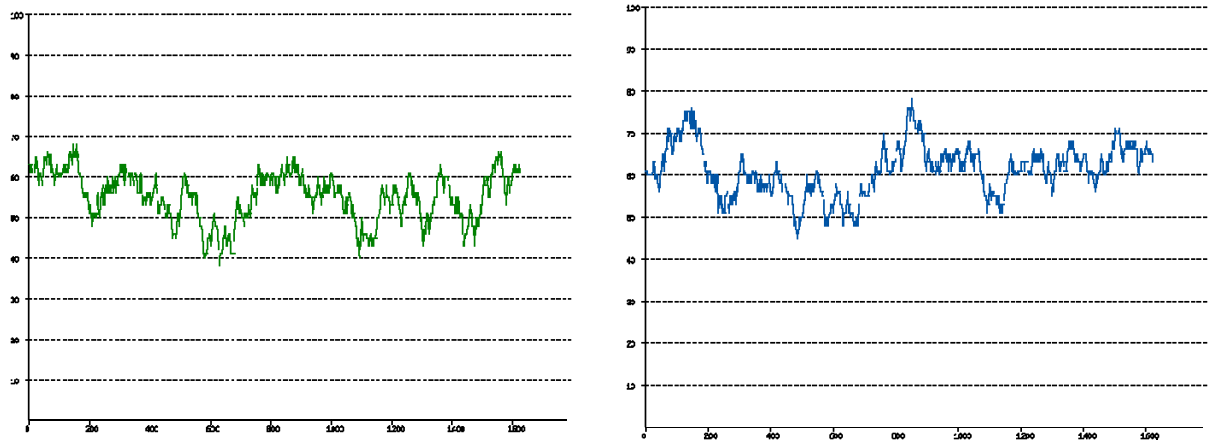

Figure 2. In human, the ideal percentage range of GC content is between 40-70%. Peaks of %GC content in a 60 bp window have been removed.

3. Restriction Enzymes and CIS-Acting Elements

Restriction Enzymes*	Optimized	Original
EcoRI(GAATTC)	0	2
XhoI(CTCGAG)	0	1
HindIII(AAGCTT)	0	2
SalI(GTCGAC)	1	1
EcoRV(GATATC)	1	1
BssHII(GCGCGC)	1	1
PstI(CTGCAG)	1	1
Bpu10I(CCTNAGC)	1	1

CIS-Acting Elements	Optimized	Original
Procaryotic inhibitor motifs	0	2
Procaryotic inhibitor motifs	0	4
consensus (cryptic) splice donor site	0	3
RNA instability motifs	0	2

Antiviral motifs	Optimized	Original
Antiviral motifs	0	1

*EcoRI(GAATTC), XhoI(CTCGAG), HindIII(AAGCTT) are the avoiding restriction enzyme sites, SalI(GTCGAC), EcoRV (GATATC), BssHII(GCGCGC), PstI(CTGCAG) and Bpu10I(CCTNAGC) are the keeping restriction enzyme sites.