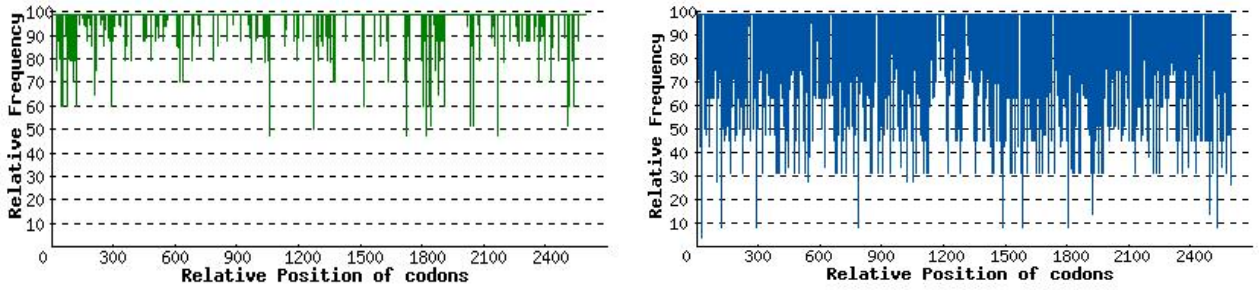
Results *Synechococcus sp. PCC 7002*

1. Codon usage bias adjustment

Codon Adaptation Index (CAI)

After OptimumGene™ Optimization



CAI: 0.96 0.66

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 > 0.8 is regarded as good, in terms of high gene expression level.

Frequency of Optimal Codons (FOP)

After OptimumGene™ Optimization

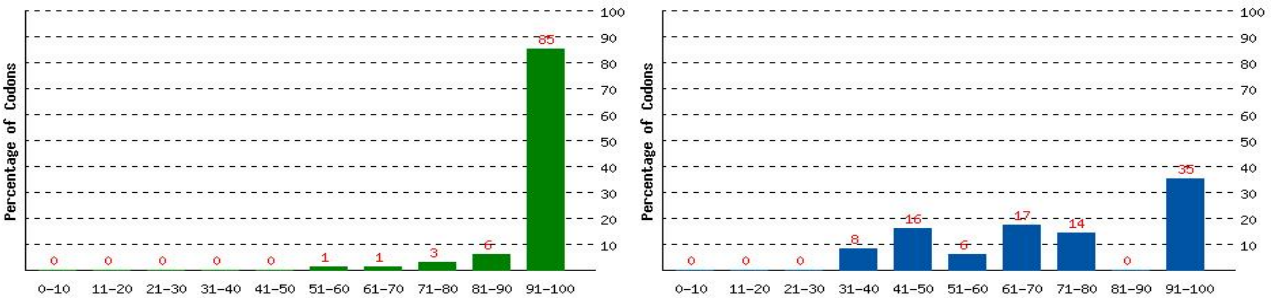
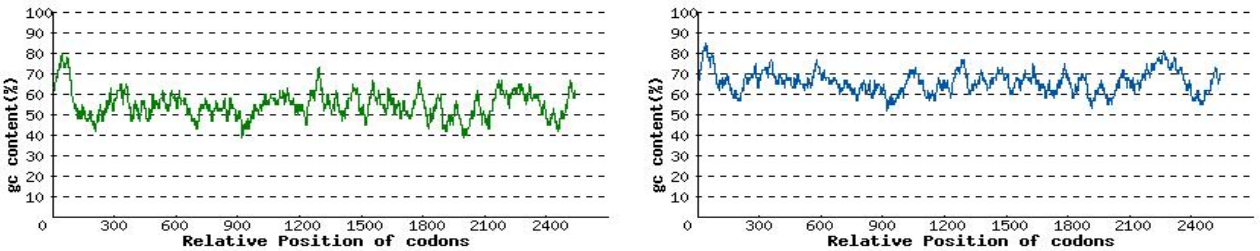


Figure 1b. The percentage distribution of codons in computed codon quality groups. The value of 100 is set for the codon with the highest usage frequency for a given amino acid in the desired expression organism.

2. GC Content Adjustment

GC Content Adjustment

After OptimumGene™ Optimization



Average GC content: 54.72 65.94

Figure 2. The ideal percentage range of GC content is between 30-70 %. Peaks of %GC content in a 60 bp window have been removed.

3. Restriction Enzymes and CIS-Acting Elements

Restriction Enzymes	Optimized	Original
* Green: filtered sites; Blue: checked sites (not filtered); Red: kept sites.		
EcoRI(GAATTC)	1(1)	2(1,23)
XbaI(TCTAGA)	1(16)	1(16)
SpeI(ACTAGT)	1(2614)	1(2614)
PstI(CTGCAG)	1(2628)	4(750,1464,1476,2628)
BamHI(GGATCC)	0	1(2594)
BglII(AGATCT)	0	0
MfeI(CAATTG)	0	0
Polymerase slippage site 1	0	2
Polymerase slippage site 2	0	2
Ribosome binding site	0	5(412,1549,1561,1846,2233)

CIS-Acting Elements	Optimized	Original
PolyT(TTTTTT)	0	0
PolyA(AAAAAAA)	0	0

4. Remove Repeat Sequences

After Optimization

Max Direct Repeat: Size:12 Distance:1599 Frequency:2
Max Inverted Repeat: Size: 12 Tm: 45.4 Start Positions: 2334, 846
Max Dyad Repeat: None

Before Optimization

Max Direct Repeat: Size:14 Distance:1438 Frequency:2
Max Inverted Repeat: Size: 13 Tm: 69.5 Start Positions: 86, 1523
Max Dyad Repeat: Size: 14 Tm: 71.0 Start Positions: 1523, 84

5. Optimized Sequence(Optimized Sequence Length:2633, GC%:54.72)

GAATTCGCGGCCGCTTCTAG
ATGAATAGTGCCGTGACCATCAGCCATCCCGGTGCCTTTCGTCCCGGTGCCCGTGCCCTCAAAAGTGCCCGGGCC
CGTGTGGCCCGGGGCTCCGCCCGGAAAGCCTTGCGGGTGCGTGCCTTTGCCCTCACCTCCAAAAAACCCAGTAAA
TCCGTGGATGAAGATGGTCGCAGTCTCATGTCCGATCCCCTCTCTGCCATGATGAACTTTATCAGTGAAATGAAT
CCCCTCGCCTCCGTGTTTAAAAGCCCCTCTGATAAACTCCACGTGCATTTTGGTGCCGGTCGTTTGGGTTTTGGT
CTCGTGATTCCCGCCCTCGAAAGTTCCGGCAGCCCCTATATTGTGATGAATCGGCCCTCTGCCGTGTGGGCCCCC
GTGGTGGAACGGGAAAAAGAAGCCCAACAACACGTGGCCGTGAAAATTAATGGCGAATGTACCTGTGGCGGTCGC
GGTCTCCATGTGATTACCGAAGATGATATTCGGGCCCACGAAGAAGGCATTCTCGGTATTGTGCATGATGCCCTC
GAAAATGGCGCCCGCGGTTTTCTCCTCCTCAGCATGGATGTGAATCTCTGTGCCCCCGTGATTGCCCAAGCCACC
AGTTTTTCTTGTGCCTTGGGTCCCGCCGTGCAAGTGTTGGCCGATCACCTCATGAATCTCCCCGATGTGGAAGAT
GAAACCCGGCCCGTGCTCTATGCCTGTGAAAATGATCATAGCTCTGTGGAAAAACTCCAATTTGCCCTCCGCACC
AAAGTGGAAGCCGTGCCCTGTATGGTGGATCGGATTTGTGTGGATCTCACCGTGTCTGATGATGCCAAAACCGTG
AATGTGACCGCCGAAGGCCACGAAGGTAGTATTGTGGTGCTCAATCAACCCGAATCCGCCGATGGCAAAAAAGAA
CAAGGCCCCCTCGCCGGTGATTTTGTGCAAAATCCCGAAAATGAAGCCGATAGTTCCTATCTCTACCGCAAAAAA
CTCCTCACCGTGAATGGTATGCATACCGTGATTGCCTTTCGCACCCTCTGTAGCTACGCCTCTACCCAACGGAAT
TTTAGTCCGCCCGAAAGTTGTCTCGCCCTCCCCCTCCTCAGTGATGAAACCGTGACCGATGAACAACGCGCCGAA
ATTTGGTCTTGGGGCGTGGCCCAAGTGCTCGTGCTCATGTGGGAACACGGTATTCCCACCATGATGCGGGTGCAT
GGCCAAGATACCACCGAAGGTCTCGTGAATATTCTCCTCGATCAACTCCGCACCACCCTCGATCGCTTTTTCACC
ATTGAAGATTCTACCGCCCGGGTGCTCGGCGGTGGCGTGTCTCTCCGCTATGAAGGCCGGCTCCTCCCCACCTAC
GAAACCATTACCAATGAATTGTTGACCGCCGGCTGGACCCCCGATTGTGCCCAAAATGTGCTCCTCAAACAAGCC
GGTCTCGAACTCGAAAGTCTCCAAACCGATCTCCAAATGCTCGTGGATGAAGCCCGCCCCTTTGCCGCCGTGGAT
AAACGGGCCCGTGCCATGAAAGAACTCGAAGAAGCCATGAAAGAAGAACAAGCCGCCGTGCAAATTCGGGAACAA
CAACTCCGCACCGCCGCCGCCTCTGAAGTGGCCGTGCTCTTTGATTTTGATGGTACCTTGGCCGATACCGAAGTG
CCCGCCATGGAAGTGGCCTTTTGGGAACTCGCCCCCTATTTTCCCAATGTGCAACCCGAAGATTTGACCCCGCAA
CGCATGCGGGAATTTATTCGCGTGAATGCCGGCAAAGCCTTTGATATTATGCTCGCCAGTGTGGAACGGGATCGT
GAAGCCGTGGGTCTCCCGCCCGTGGAAGAAGTGCGTGCCAAGTTTCAAGAATCCTTTGAAATCATCCAAGTGTGT
AACACCGCCCGGGCCGGTTTTGGTCTCCGTCCCATTGAACTCTGTCGCGAAGAACACAAAACCCTCAAAGAAAAA
CAAAAAGCCGAAACCCTCGAAGCCCTCGCCAATCTCGCCAAAACCAATGATGGCGTGGTGAAAAGCCTCAATTTT
CTCAAGGTGTCTGGTTTTAAGTTTGCCATTAGTACCACCTCCCCCAAAAGCCGGGTGATTGCCTGTCTCGATACC
ATGCGCCTCGCCGATGATTTTCCCCAAGAAAAAATTCATAGCGGCAGCTCTGATTTTGATCCGCCCCGCTTTAAA
CCCGCCCCCGATGTGTACCTCAAAGCCGCCGCCGCCGAAGAAGTGGGTGTGGAAGCCTGTATTGCCGTGGAAGAT
AGCCTCAGTGGTGTGGGTTCTGCCGCCGATGCCGGTGTGGCCCTCATTGTGGGTTATGTGGGTGGCGGTCACATT
ACCGCCGAAGCCCTCGATGGTTATGCCGAAGCCCTCTTGGCCGGCGGTAAAAGCAAAAATGGCCGGGGTGCCGAT
GTGGTGATTCGCGATATGGAAGATTTGCCCTCTGTGGTGAATACCTTTCTCGATATGCGGATGGATGATCCCACC
ACCTGTGATCGCGATCATTTTGATTTTGAAAAGATTCGTCCCGCCCTCCGGGATCGTTGTTGGACCACCGCCGGC
AAAACCGGTGTGGTGGAAGTGGAAGATCCCAATCCCCGCTAA
TACTAGTAGCGGCCGCTGCAG

6. DNA Alignment (Optimized Region)

Optimized	21	ATGAATAGTGCCGTGACCATCAGCCATCCCGGTGCCTTTCGTCCCGGTGCCCGTGCCCTC
Original	21	ATGAATTCCGCTGTCACGATATCCCACCCCGGCGCGTTCCGCCCGGGCGCGCGCGCTTTA
Optimized	81	AAAAGTGCCCGGGCCCGTGTGGCCCGGGGCTCCGCCCGGAAAGCCTTGCGGGTGCGTGCC
Original	81	AAGAGCGCGCGCGCGCGCGTCGCGCGCGGGAGCGCCCGAAAAGCGCTGCGGGTGAGGGCT
Optimized	141	TTTGCCCTCACCTCCAAAAAACCCAGTAAATCCGTGGATGAAGATGGTCGCAGTCTCATG
Original	141	TTCGCGCTGACGTCGAAGAAGCCGAGCAAGTCCGTGGACGAGGACGGGCGCTCGCTGATG
Optimized	201	TCCGATCCCCTCTCTGCCATGATGAACTTTATCAGTGAAATGAATCCCCTCGCCTCCGTG
Original	201	TCCGACCCGCTCAGCGCGATGATGAACTTCATCTCGGAGATGAACCCGCTCGCGAGCGTG
Optimized	261	TTTAAAAGCCCCTCTGATAAACTCCACGTGCATTTTGGTGCCGGTCGTTTGGGTTTTGGT
Original	261	TTCAAGTCCCCCAGCGACAAGCTCCACGTGCACTTCGGCGCGGGCAGGCTCGGTTTCGGT
Optimized	321	CTCGTGATTCCCGCCCTCGAAAGTTCCGGCAGCCCCTATATTGTGATGAATCGGCCCTCT
Original	321	CTGGTCATCCCCGCGCTGGAGTCGTCGGGGAGCCCGTACATCGTGATGAACCGCCCGAGC
Optimized	381	GCCGTGTGGGCCCCCGTGGTGGAACGGGAAAAAGAAGCCCAACAACACGTGGCCGTGAAA
Original	381	GCGGTGTGGGCGCCGGTCGTCGAGCGCGAGAAGGAGGCGCAGCAGCACGTCGCGGTCAAG
Optimized	441	ATTAATGGCGAATGTACCTGTGGCGGTCGCGGTCTCCATGTGATTACCGAAGATGATATT
Original	441	ATCAACGGCGAGTGCACGTGTGGCGGACGCGGGCTCCACGTCATCACCGAGGACGACATT
Optimized	501	CGGGCCCACGAAGAAGGCATTCTCGGTATTGTGCATGATGCCCTCGAAAATGGCGCCCGC
Original	501	CGCGCGCACGAAGAGGGCATCCTCGGCATCGTCCACGACGCGCTCGAGAATGGCGCGCGA
Optimized	561	GGTTTTCTCCTCCTCAGCATGGATGTGAATCTCTGTGCCCCCGTGATTGCCCAAGCCACC
Original	561	GGCTTCCTTCTCCTCTCCATGGACGTCAACCTCTGCGCGCCCGTGATCGCGCAGGCGACC
Optimized	621	AGTTTTTCTTGTGCCTTGGGTCCCGCCGTGCAAGTGTTGGCCGATCACCTCATGAATCTC
Original	621	TCCTTCTCCTGCGCGCTCGGACCCGCGGTGCAGGTGCTCGCGGATCACCTCATGAACCTC
Optimized	681	CCCGATGTGGAAGATGAAACCCGGCCCGTGCTCTATGCCTGTGAAAATGATCATAGCTCT
Original	681	CCGGACGTCGAGGACGAGACGCGCCCGGTGTTGTACGCGTGCGAGAACGACCACAGCTCG
Optimized	741	GTGGAAAAACTCCAATTTGCCCTCCGCACCAAAGTGGAAGCCGTGCCCTGTATGGTGGAT
Original	741	GTGGAGAAGCTGCAGTTCGCGTTGCGCACCAAGGTGGAGGCGGTGCCGTGCATGGTGGAC

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Optimized   801   CGGATTTGTGTGGATCTCACCGTGTCTGATGATGCCAAAACCGTGAATGTGACCGCCGAA
Original    801   AGGATCTGCGTGGATTTGACCGTTTCGGACGACGCCAAGACGGTCAACGTCACCGCGGAG

Optimized   861   GGCCACGAAGGTAGTATTGTGGTGCTCAATCAACCCGAATCCGCCGATGGCAAAAAAGAA
Original    861   GGGCACGAGGGATCGATCGTCGTGTTGAATCAACCCGAGAGCGCGGACGGTAAGAAGGAG

Optimized   921   CAAGGCCCCCTCGCCGGTGATTTTGTGCAAAATCCCGAAAATGAAGCCGATAGTTCCTAT
Original    921   CAGGGGCCGCTCGCGGGTGATTTCGTGCAAAACCCCGAGAACGAAGCCGACAGCAGTTAC

Optimized   981   CTCTACCGCAAAAAACTCCTCACCGTGAATGGTATGCATACCGTGATTGCCTTTCGCACC
Original    981   CTGTACCGCAAGAAGCTGCTCACGGTGAACGGAATGCACACGGTGATCGCGTTCCGAACG

Optimized  1041   CTCTGTAGCTACGCCTCTACCCAACGGAATTTTAGTCCGCCCGAAAGTTGTCTCGCCCTC
Original   1041   CTGTGCTCCTACGCGTCGACGCAGCGAAACTTCTCCCCCCCGGAGAGCTGCCTCGCGCTC

Optimized  1101   CCCCTCCTCAGTGATGAAACCGTGACCGATGAACAACGCGCCGAAATTTGGTCTTGGGGC
Original   1101   CCGCTCCTGTCGGACGAGACGGTGACGGATGAGCAACGCGCCGAGATTTGGTCGTGGGGC

Optimized  1161   GTGGCCCAAGTGCTCGTGCTCATGTGGGAACACGGTATTCCCACCATGATGCGGGTGCAT
Original   1161   GTCGCGCAAGTTTTGGTGTTGATGTGGGAACACGGCATCCCCACCATGATGCGCGTGCAC

Optimized  1221   GGCCAAGATACCACCGAAGGTCTCGTGAATATTCTCCTCGATCAACTCCGCACCACCCTC
Original   1221   GGCCAAGACACGACGGAGGGCCTCGTGAACATCCTCCTCGATCAGCTCCGCACGACGCTC

Optimized  1281   GATCGCTTTTTCACCATTGAAGATTCTACCGCCCGGGTGCTCGGCGGTGGCGTGTCTCTC
Original   1281   GACCGGTTCTTCACGATCGAGGACTCCACCGCGCGCGTCCTCGGCGGCGGCGTGAGCCTG

Optimized  1341   CGCTATGAAGGCCGGCTCCTCCCCACCTACGAAACCATTACCAATGAATTGTTGACCGCC
Original   1341   CGGTACGAAGGGCGGCTGTTGCCGACGTACGAGACCATCACGAACGAATTGCTCACCGCC

Optimized  1401   GGCTGGACCCCCGATTGTGCCCAAAATGTGCTCCTCAAACAAGCCGGTCTCGAACTCGAA
Original   1401   GGGTGGACGCCGGACTGCGCGCAGAACGTGCTGCTCAAGCAGGCTGGGCTAGAGCTCGAG

Optimized  1461   AGTCTCCAAACCGATCTCCAAATGCTCGTGGATGAAGCCCGCCCCTTTGCCGCCGTGGAT
Original   1461   TCGCTGCAGACGGACCTGCAGATGCTCGTGGACGAGGCGAGGCCGTTCGCTGCGGTGGAC

Optimized  1521   AAACGGGCCCGTGCCATGAAAGAACTCGAAGAAGCCATGAAAGAAGAACAAGCCGCCGTG
Original   1521   AAGCGCGCGCGCGCGATGAAGGAGCTCGAGGAGGCGATGAAGGAGGAGCAGGCGGCTGTG
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Optimized  1581   CAAATTCGGGAACAACAACTCCGCACCGCCGCCGCCTCTGAAGTGGCCGTGCTCTTTGAT
Original   1581   CAAATTCGCGAGCAGCAACTGAGGACGGCGGCGGCGAGCGAGGTTGCGGTGCTGTTCGAT

Optimized  1641   TTTGATGGTACCTTGGCCGATACCGAAGTGCCCGCCATGGAAGTGGCCTTTTGGGAACTC
Original   1641   TTCGACGGCACGCTCGCGGACACGGAGGTGCCCGCGATGGAGGTGGCGTTCTGGGAGCTC

Optimized  1701   GCCCCCTATTTCCCAATGTGCAACCCGAAGATTTGACCCCGCAACGCATGCGGGAATTT
Original   1701   GCGCCGTATTTCCCGAACGTTCAGCCCGAGGATCTCACGCCGCAACGCATGCGGGAGTTT

Optimized  1761   ATTCGCGTGAATGCCGGCAAAGCCTTTGATATTATGCTCGCCAGTGTGGAACGGGATCGT
Original   1761   ATCCGCGTCAACGCGGGGAAGGCTTTCGACATCATGCTGGCGTCGGTGGAACGCGACAGG

Optimized  1821   GAAGCCGTGGGTCTCCCGCCCGTGGAAGAAGTGCGTGCCAAGTTTCAAGAATCCTTTGAA
Original   1821   GAAGCCGTCGGGCTGCCTCCGGTGGAGGAGGTGCGCGCGAAGTTTCAGGAGAGCTTCGAG

Optimized  1881   ATCATCCAAGTGTGTAACACCGCCCGGGCCGGTTTTGGTCTCCGTCCCATTGAACTCTGT
Original   1881   ATCATCCAGGTGTGCAACACCGCGCGCGCGGGGTTCGGTCTGCGGCCGATCGAGCTGTGC

Optimized  1941   CGCGAAGAACACAAAACCCTCAAAGAAAAACAAAAAGCCGAAACCCTCGAAGCCCTCGCC
Original   1941   AGAGAGGAACACAAGACGCTGAAGGAGAAGCAAAAGGCGGAGACGTTGGAGGCGCTGGCG

Optimized  2001   AATCTCGCCAAAACCAATGATGGCGTGGTGAAAAGCCTCAATTTTCTCAAGGTGTCTGGT
Original   2001   AACCTCGCCAAGACCAACGACGGCGTCGTGAAGAGCCTGAACTTCCTCAAGGTGTCCGGG

Optimized  2061   TTTAAGTTTGCCATTAGTACCACCTCCCCCAAAAGCCGGGTGATTGCCTGTCTCGATACC
Original   2061   TTCAAGTTCGCCATCTCGACGACGTCGCCGAAGTCGCGCGTCATCGCGTGCCTGGACACG

Optimized  2121   ATGCGCCTCGCCGATGATTTTCCCCAAGAAAAAATTCATAGCGGCAGCTCTGATTTTGAT
Original   2121   ATGCGCCTCGCGGACGACTTCCCCCAGGAGAAGATCCACTCGGGCTCGTCGGATTTCGAC

Optimized  2181   CCGCCCCGCTTTAAACCCGCCCCCGATGTGTACCTCAAAGCCGCCGCCGCCGAAGAAGTG
Original   2181   CCGCCGCGGTTTAAACCCGCGCCGGACGTCTACCTTAAAGCCGCGGCGGCGGAGGAGGTG

Optimized  2241   GGTGTGGAAGCCTGTATTGCCGTGGAAGATAGCCTCAGTGGTGTGGGTTCTGCCGCCGAT
Original   2241   GGCGTCGAGGCGTGCATCGCCGTGGAGGATTCGCTCTCGGGCGTCGGCAGCGCCGCGGAC

Optimized  2301   GCCGGTGTGGCCCTCATTGTGGGTTATGTGGGTGGCGGTCACATTACCGCCGAAGCCCTC
Original   2301   GCGGGCGTCGCGCTCATCGTCGGCTACGTCGGCGGCGGGCACATCACGGCGGAGGCGTTG

Optimized  2361   GATGGTTATGCCGAAGCCCTCTTGGCCGGCGGTAAAAGCAAAAATGGCCGGGGTGCCGAT
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Original    2361    GACGGGTACGCGGAGGCGCTGCTGGCGGGGGGGAAGAGCAAAAACGGACGCGGCGCGGAC

Optimized   2421    GTGGTGATTCGCGATATGGAAGATTTGCCCTCTGTGGTGAATACCTTTCTCGATATGCGG
Original    2421    GTCGTCATTCGCGACATGGAGGATCTCCCGTCGGTGGTGAACACGTTCTTGGACATGCGC

Optimized   2481    ATGGATGATCCCACCACCTGTGATCGCGATCATTTTGATTTTGAAAAGATTCGTCCCGCC
Original    2481    ATGGACGATCCGACGACGTGCGACAGAGATCACTTCGACTTCGAGAAGATCCGGCCCGCG

Optimized   2541    CTCCGGGATCGTTGTTGGACCACCGCCGGCAAAACCGGTGTGGTGGAAGTGGAAGATCCC
Original    2541    CTGAGGGATCGGTGCTGGACGACGGCGGGAAAGACGGGGGTGGTGGAGGTGGAGGATCCG

Optimized   2601    AATCCCCGCTAA
Original    2601    AATCCGCGGTGA
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