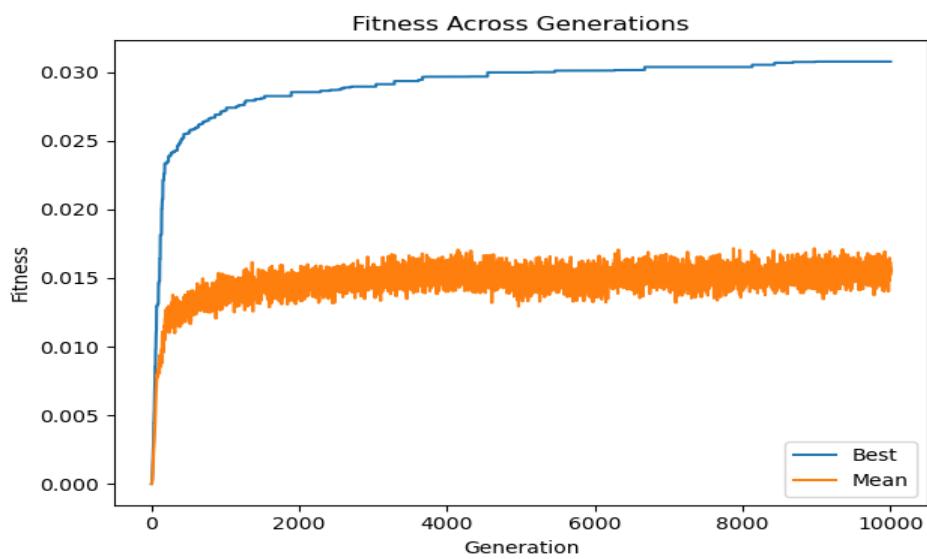


Codon Optimization Report

1. Genetic Algorithm Optimization Summary

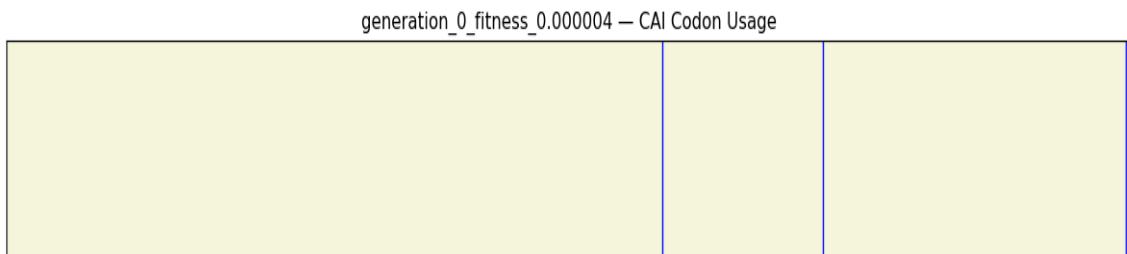
Total generations: 9999
Best composite fitness achieved: 0.030773

1.1 Fitness Progression

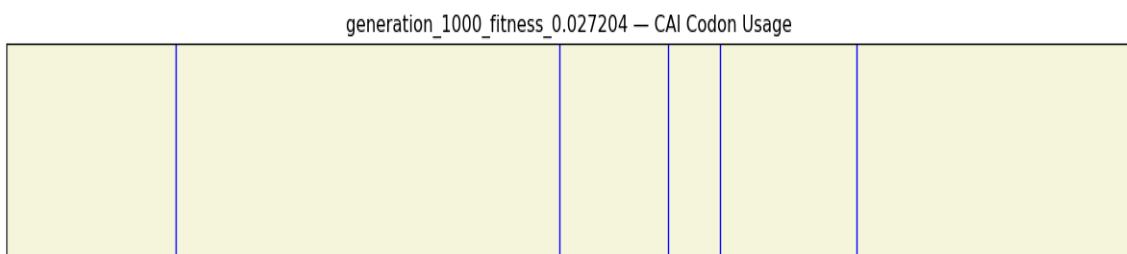


1.2 CAI Positional Plots Across Generations

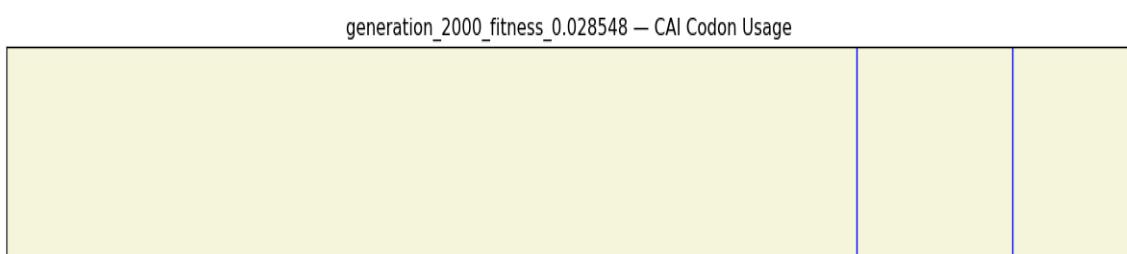
Generation 0



Generation 1000

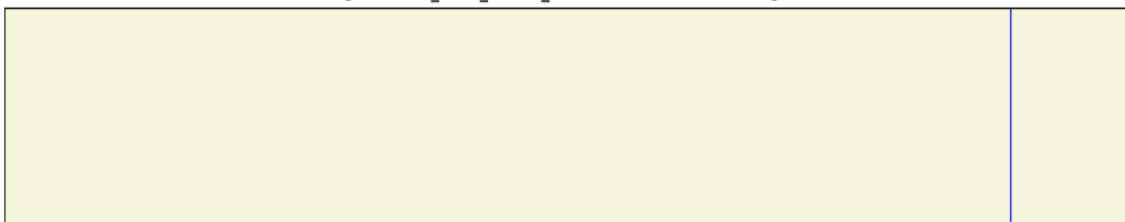


Generation 2000



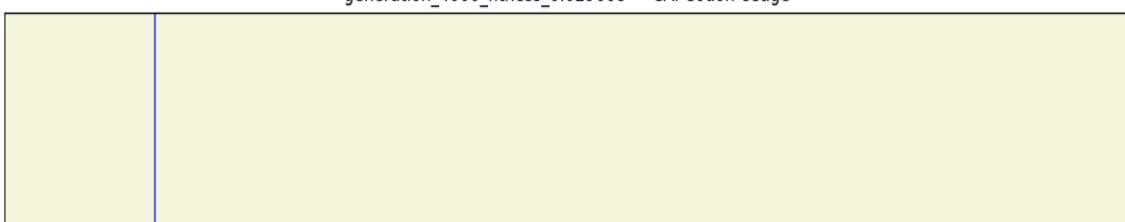
Generation 3000

generation_3000_fitness_0.028951 – CAI Codon Usage



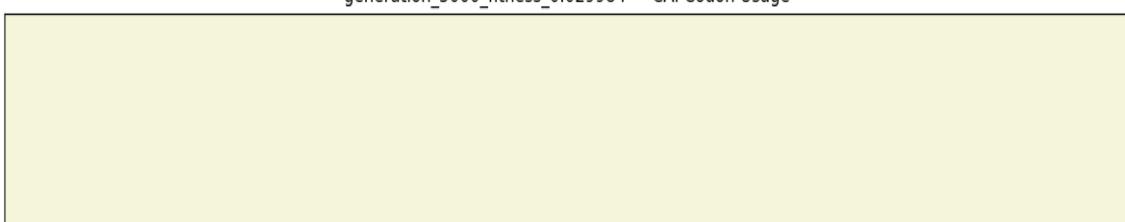
Generation 4000

generation_4000_fitness_0.029668 – CAI Codon Usage



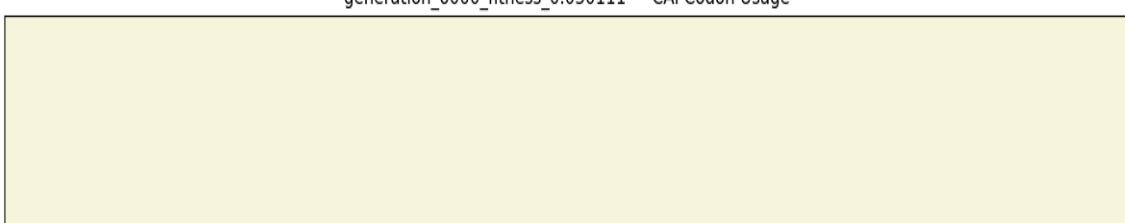
Generation 5000

generation_5000_fitness_0.029984 – CAI Codon Usage



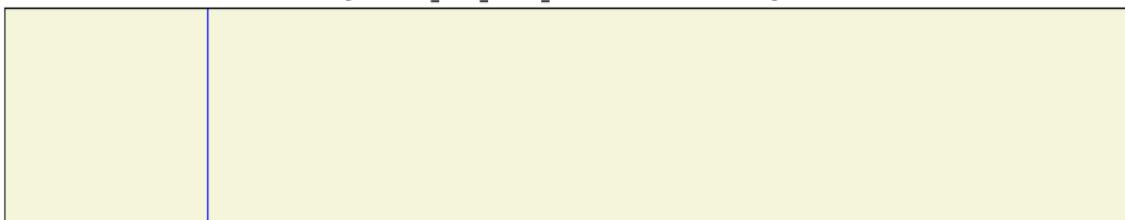
Generation 6000

generation_6000_fitness_0.030111 – CAI Codon Usage



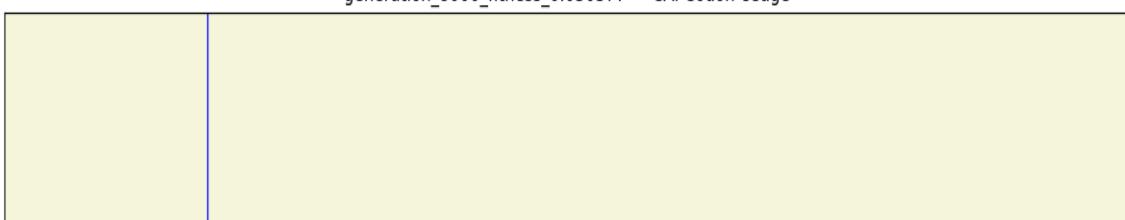
Generation 7000

generation_7000_fitness_0.030377 – CAI Codon Usage



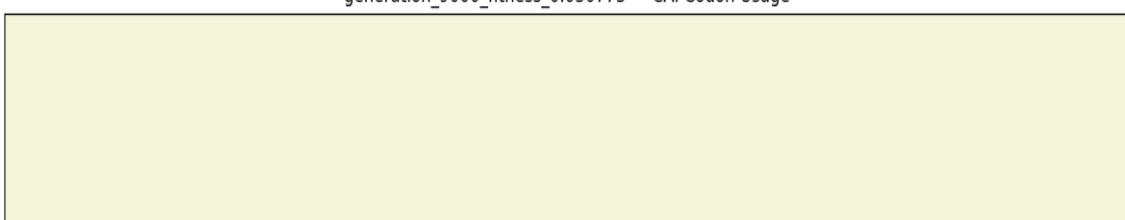
Generation 8000

generation_8000_fitness_0.030377 – CAI Codon Usage



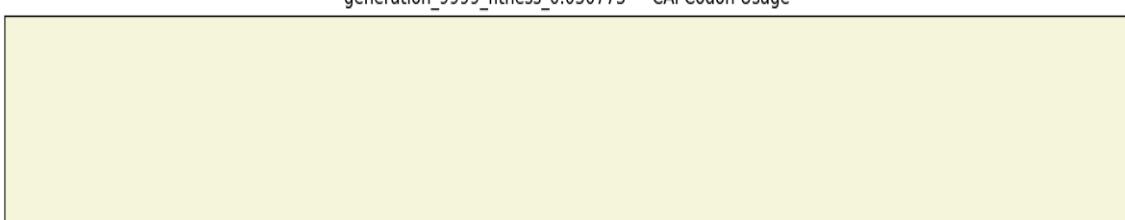
Generation 9000

generation_9000_fitness_0.030773 – CAI Codon Usage



Generation 9999

generation_9999_fitness_0.030773 – CAI Codon Usage



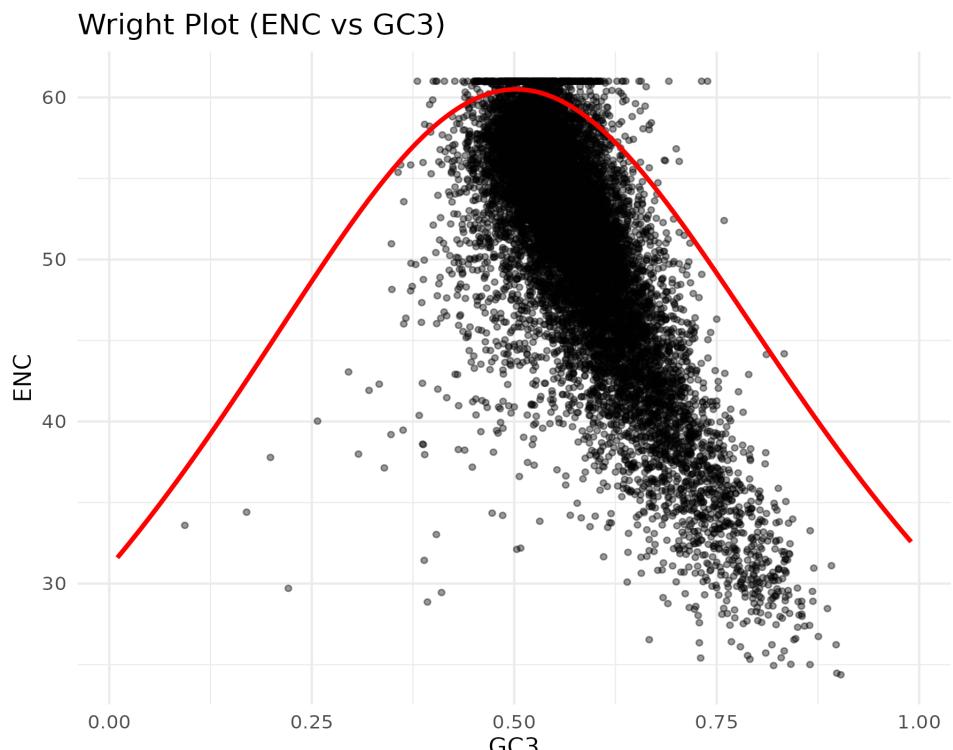
2. Final Optimized Sequence Metrics

Sequence length: 1215 nt
GC content: 0.5259
GC3 content: 0.5654

2.1 Final Optimized Sequence

```
>alfa_nb_linker_mScarlet_optimized_final
ATGGTCTCCAAGGGCGAAGCTGTCAAGGAATTGAGATTCAAAGTCCACATGGAG
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GGCACCCAGACAGCAAAGCTGAAAGTCACAAGGGCGGCCCTTGCCTATTTCATGGAT
ATTCTTAGCCCACAATTGATGTACGGCTCTAGAGCCTTATTAGCACCCTGCCGATATT
CCAGATTACTACAAGCAATCTTCTGAAGGCTCAAATGGGAGCGCGTTATGAATTTC
GAAGACGGAGGAGCAGTCAGTGACTCAAGACACTAGCTTGGAAAGATGGAACCCCTGATC
TACAAAGTTAAATTGAGAGGAACTAACCTCCCACCGATGGCCTGTTATGCAGAAGAAG
ACTATGGGCTGGGAAGCCTCAACCGAGCGCTCACCCCTGAAGATGGCGTCCTCAAGGGA
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GACAACCTGAAGCCTGAAGACACCGCTGTTACTACTGCCACGTCTTGAGGAACAGAGTT
GATTCAATTCCATGATTACTGGGTCAAGGTACCCAGTTACCGTTCTTCGAAACTCGAA
CGCCCTCACCGTGAC
```

3. Genome Codon Bias (Wright Plot)



4. Interpretation Notes

- Optimization balances translational efficiency and genome-wide codon conformity.
- GC content is constrained using a Gaussian penalty centered on genome average.
- Cryptic splice-site penalties were applied during genetic algorithm optimization.
- Final sequence should be validated experimentally before synthesis.