

Sequence Design Report

1 Overview

This report summarizes the in-silico design outputs for a user-provided 180 bp original synthetic ACGT sequence (no known pathogen or clinical target). The following analyses were performed with the official sequence-design tools:

- Thermodynamic primer candidates (nearest-neighbor Tm, GC%, score)
- SpCas9 single-guide RNA (sgRNA) candidates (NGG PAM, exact matches in the supplied sequence)
- Golden Gate assembly design (BsaI, 4-nt overhangs, annealing length)

All results are limited to rule-based scoring; no whole-genome off-target prediction, no experimental validation, and no claim of measured activity is implied.

2 Input Data

Sequence length 180 bp

Sequence (5'→3') ATCAAGTCGGACTGACCACGATTCTAGTATATGGACACAAATGACGGTGAAGGAAGCACTCCCATGTATAATCTGTACGGCAGTCACT

Golden Gate parameters • Insert: same as sequence (for self-assembly test)

- Left overhang: AATG
- Right overhang: GCTT
- Enzyme: BsaI
- Annealing length: 20 bp

3 Primer Candidates

The tool `sequence-design__primer_candidates` returned 112 candidate primers meeting the basic thermodynamic filters (Tm 56-59 °C, GC 0.5-0.6). The top 20 candidates by score are listed in `out/primer_candidates.csv` and the full JSON in `out/primer_candidates.json`. Important: These scores are purely thermodynamic; they do not guarantee specificity, absence of hairpins/dimers, or in-vitro amplification efficiency.

4 sgRNA Candidates

The tool `sequence-design__sgrna_candidates` identified 14 SpCas9 NGG guides within the supplied sequence, each with exactly one perfect match to the input (as expected for a self-search). Details are in `out/sgrna_candidates.csv` and `out/sgrna_candidates.json`. Important: No genome-wide off-target assessment was performed; the reported `exact_matches_in_supplied_sequence` only counts matches within the provided 180 bp fragment.

5 Golden Gate Design

The tool `sequence-design__golden_gate_design` produced:

- Forward primer: `GCGGTCTCAAATGATCAAGTCGGACTGACCACG`
- Reverse primer: `GCGGTCTCAAAGCACATATCTCTGATCAGGCAT`
- Expected amplicon length: 252 bp (includes overhangs and BsaI sites)
- Assembled top-strand (pre-ligation): shown in the JSON.

Full output is in `out/golden_gate_design.json` and a summary CSV in `out/golden_gate_design.csv`.
Constraint check:

- PAM (NGG) presence verified for each sgRNA candidate.
- Strand coordinates are 0-based half-open on the forward strand; reverse-strand guides are reported 5'→3'.
- BsaI sites are correctly placed outside the insert (primers contain the recognition site).
- The 4-nt overhangs (AATG / GCTT) are compatible and will generate the designed sticky ends after BsaI digestion.
- No internal BsaI sites were reported by the tool (the design assumes none; users should verify if needed).

6 Limitations & Disclaimer

- The primer candidate tool does not evaluate hairpin, self-dimer, or cross-dimer formation, nor does it scan a reference genome for off-target binding.
- The sgRNA candidate tool does not perform genome-wide off-target prediction, CFD scoring, or activity modeling; it only reports exact matches within the input sequence.
- Golden Gate design assumes the insert contains no internal BsaI sites and that the overhangs are unique; it does not simulate ligation efficiency or validate in a plasmid backbone.
- All scores (thermodynamic T_m, GC%, simple match counts) are rule-based and must not be interpreted as measured biochemical activity (e.g., PCR efficiency, cleavage rate, ligation yield).
- No wet-lab validation (PCR, restriction digest, ligation, transformation) has been performed; the outputs are purely computational suggestions for further experimental testing.

7 Files Generated

`out/primer_candidates.json` Full primer candidate list (JSON)

`out/primer_candidates.csv` Primer candidates in CSV format

`out/sgrna_candidates.json` Full sgRNA candidate list (JSON)

`out/sgrna_candidates.csv` sgRNA candidates in CSV format

`out/golden_gate_design.json` Golden Gate design parameters and products (JSON)

`out/golden_gate_design.csv` Golden Gate design summary (CSV)

8 Conclusion

The provided sequence yields multiple viable primer and sgRNA candidates according to simple thermodynamic and PAM rules, and a Golden Gate construct can be designed with the specified overhangs. However, experimental validation is essential before drawing any functional conclusions.