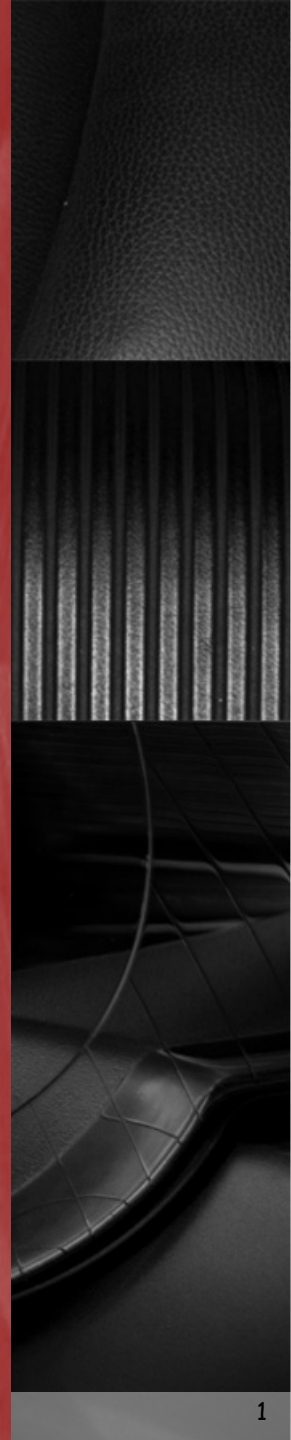
Primer design using Primer-BLAST and OligoAnalyzer



MOL BIOL 4XX3, McMaster University

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PCR

- Amplification of target DNA sequences
- Steps:
  1. Initial denaturation
  2. Denaturation
  3. Annealing
  4. Extension
  5. Final Extension

  } 30 cycles
- Reagents:
  - DNA template
  - Buffer (containing $MgCl_2$)
  - Primers
  - dNTPs
  - Taq polymerase

Primer design

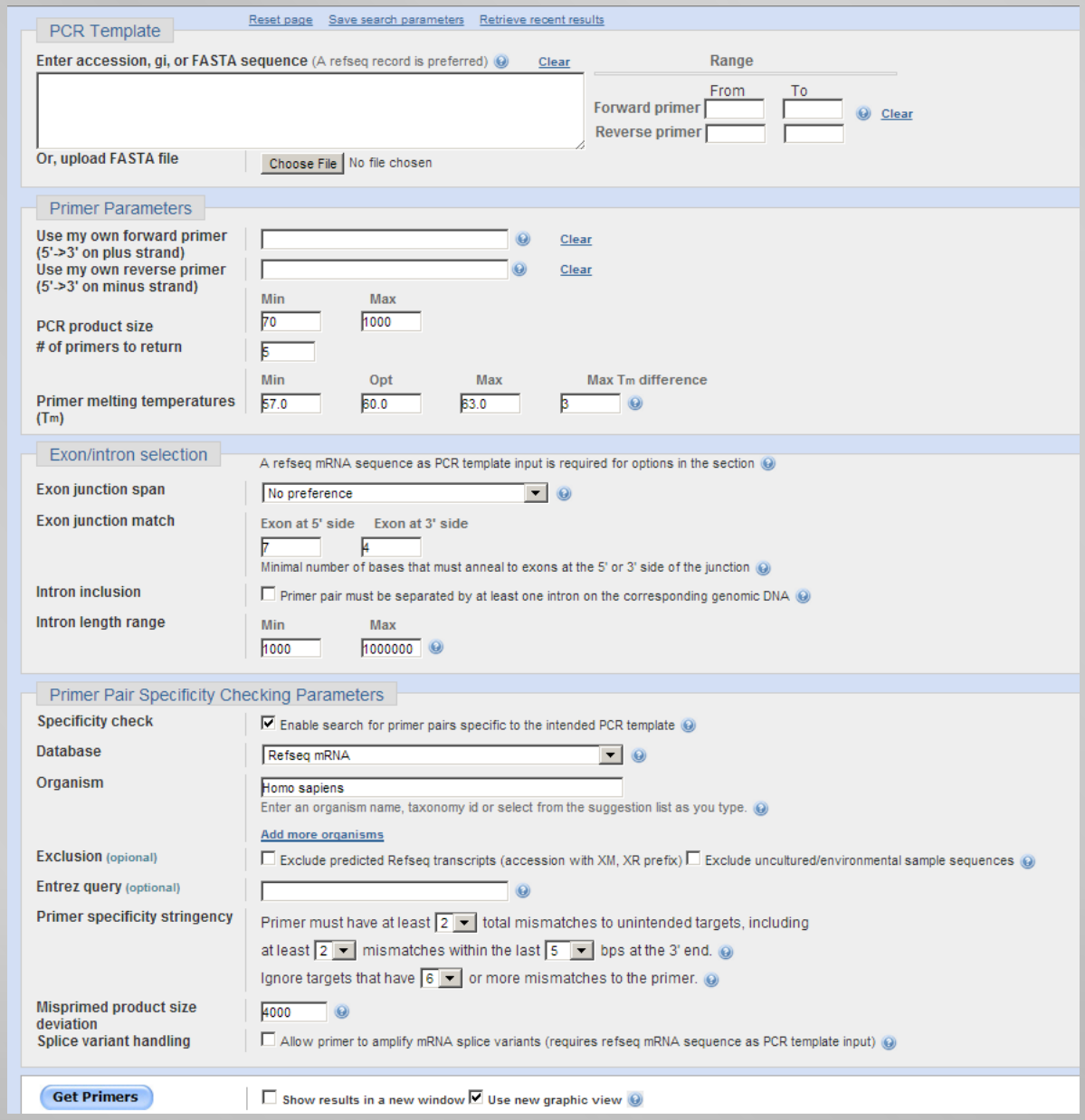
1. Primer length: 18-22 bases
2. Primer melting temperature: 52–58°C
3. Primer annealing temperature
4. GC content: 40–60%
5. GC clamp: GC at 3’
6. Primer secondary structures: try to avoid these
7. Repeats: maximum 4 repeats (e.g., ATATATAT)
8. Runs: maximum 4 bases (e.g., AAAA)
9. End stability: less stability at 3’, less false priming
10. Avoid template secondary structure
11. Avoid cross homology

Primer-BLAST

- Available through NCBI
  (URL: http://www.ncbi.nlm.nih.gov/tools/primer-blast)
- Used to construct primers
  - Paste a sequence or specify an NCBI accession number for a gene
- Adjustable parameters include:
  PCR product size, melting temperature, GC content, GC clamp, salt concentrations, etc.

- Can also be used to check specificity of primer pairs
  - Databases: Refseq mRNA, Genome, Refseq RNA, nr
  - Narrow search to organisms from certain taxonomic groups

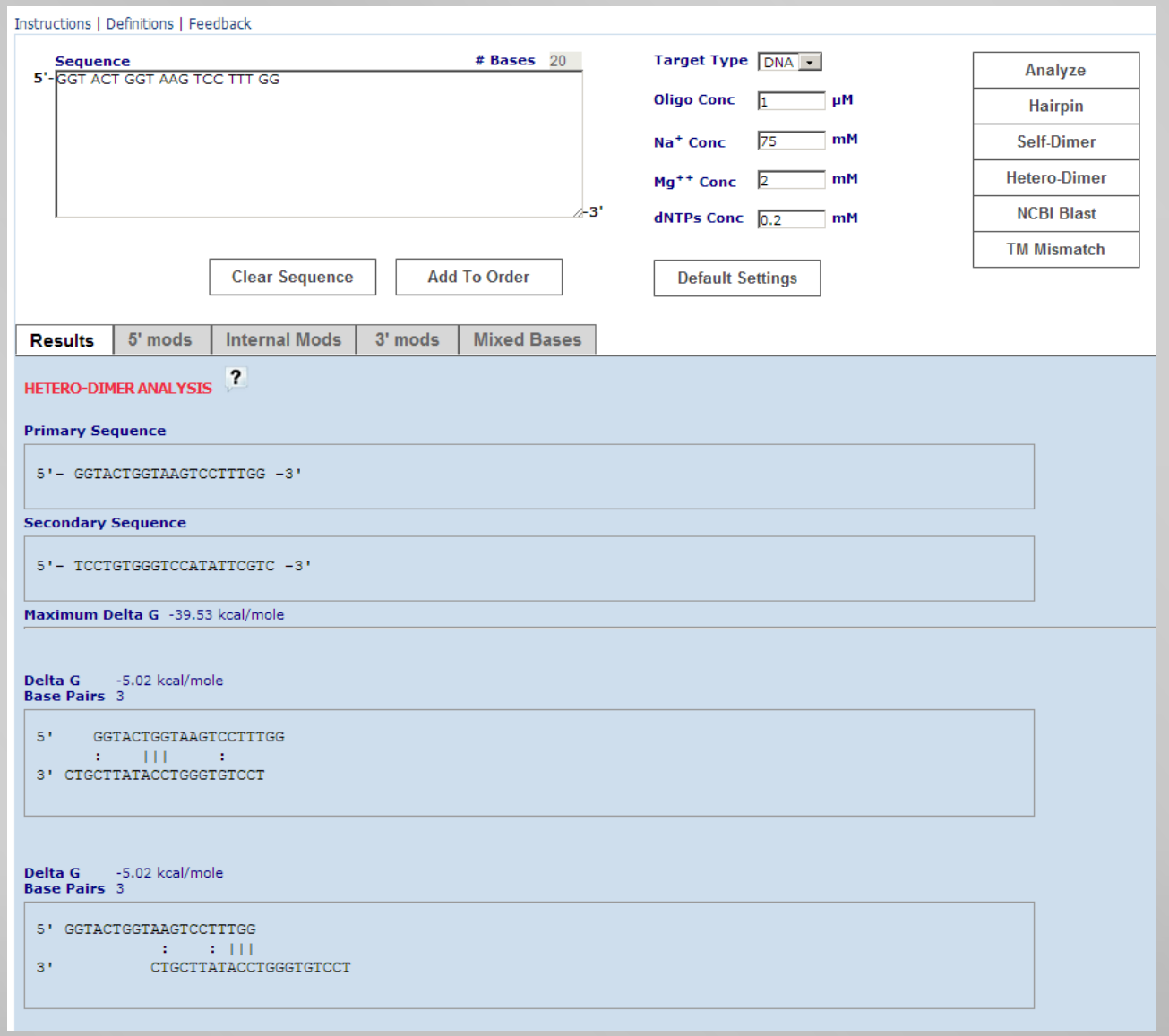
Primer-BLAST



OligoAnalyzer

- Available through IDT
  (URL: http://www.idtdna.com/analyzer/applications/oligoanalyzer)
- User can input desired concentrations of primer, Na^{+}, Mg^{2+} and dNTPs
  - Affect melting temperature
- Given a primer sequence, calculates:
  - Melting temperature
  - GC content
  - Hairpin formation
  - Self-dimer/hetero-dimer possibilities
- Useful for visualizing possible primer dimers

OligoAnalyzer



Primer construction

- Primers made using both Primer-BLAST and OligoAnalyzer
  - Primer-BLAST → picking primer pairs, specificity check
  - OligoAnalyzer → primer dimer analysis

Assignment

- Design a pair of primers to amplify the *CAT (chloramphenicol transacetylase)* gene of pCA24N
- Submit your candidate primers to Avenue to Learn.