7.13 Experimental Microbial Genetics

Fall 2008

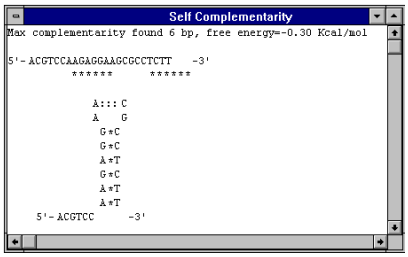
Primer Design Guidelines for PCR Reactions

General types of PCR reactions you may perform:

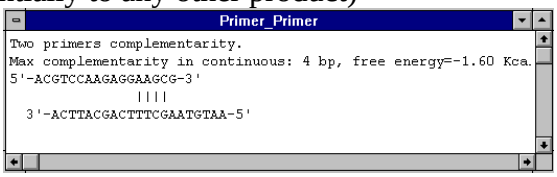
- Gene amplification (with introduction of restriction sites for downstream cloning)
- Point mutations
- Deletions

General considerations for primer design:

- Primers should be roughly 17-35 bases in length
- Tm (primer melting temperature) ~ 55-70°C
  - most simply calculated as Tm = [4(G+C) + 2(A+T)]°C
  - or use a web-based calculator (e. g. http://www.idtdna.com/SciTools/SciTools.aspx)
- Primers should end (3') in a G or C, or CG or GC
  - improves efficiency of priming
- Avoid runs of three or more Cs or Gs at the 3'-ends of primers
  - may result in mis-priming at G or C-rich sequences
- Avoid primer *self*-complementarity (within a single primer such that 2° structures like hairpins form)



- Avoid primer *pair* complementarities (primer dimers will form and be synthesised preferentially to any other product)



- Avoid use of 4 or more consecutive Guanine nucleotides in primer (forms a significantly stable "cruciform" of "guanine tetraplex" 2° structure)

Specific considerations for primer design:

- Remember to maintain plasmid elements required for protein expression:
  - In-frame fusions
  - Stop codons (*reverse primer*)
  - Shine Dalgarno sequence (*forward primer*)
- Always consider restriction sites available in the MCS (multiple cloning site)
- When engineering restriction sites at ends of an amplified PCR product, always include a few additional bases after the restriction site (http://www.neb.com/nebecomm/tech_reference/restriction_enzymes/cleavage_oligonucleotides.asp)

Reference where more details can be found: http://www.mcb.uct.ac.za/pcroptim.htm