PCR Primer Design Bio 181 Spring 2006

DUE: ~~Wed. March 29th~~

5 points

Some guidelines to consider when choosing primer sequences:
{These are not unbreakable "rules". Lots of computer programs exist to assist with this kind of analysis.**}**

1. Primers should be about 18-30 bases in length
2. Base composition should be 50-60% (G+C)
3. Avoid long stretches of just Gs & Cs especially at 3' end
4. T_m (melting temperature) between 55-80ºC are preferred (**PCR annealing temperature should be about 5 ºC below T_m.**)
5. 3'-ends of the two primers should not be complementary (ie. base pair) to each other, as otherwise primer dimers will be synthesized preferentially to any other product
6. Primer self-complementarity (ability to form 2º structures such as hairpins) should be avoided.

Remember: The primers are *part of* each amplified DNA molecule; therefore the primer sequence is included in the DNA produced by PCR.

Assignment: You wish to use PCR to amplify the sequence underlined below.

- **List the sequences of an upstream and downstream primer pair** that will amplify the underlined region. ***IMPORTANT:*** Primer sequences should be written in standard format, that is, from 5' to 3'.
- **Determine the basic T_m** (melting temperature) for each oligonucleotide primer (go to www.promega.com/biomath)
- **Choose an appropriate temperature** for your PCR annealing step. (Think: if the primers have different T_m's, which should you use to determine annealing temp?)
- Please **tell me the # of the starting nucleotide** (from the whole sequence below) for each of your primers.

5′ cacaaggcta cttccctgat tagcagaact acacaccagg gccagggatc agatatccac

61 tgacctttgg atggtgctac aagctagtac cagttgagcc agagaagtta gaagaagcca

121 acaaaggaga gaacaccagc ttgttacacc ctgtgagcct gcatggaatg gatgacccgg

181 agagagaagt gttagagtgg aggtttgaca gccgcctagc atttcatcac atggcccgag

241 agctgcatcc ggagtacttc aagaactgct gacatcgagc ttgctacaag ggactttccg

301 ctggggactt tccagggagg cgtggcctgg gcgggactgg ggagtggcga gccctcagat

361 ttatataata cagtagcaac cctctattgt gtgcatcaaa ggatagagat aaaagacacc