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Primer Design Activity

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Primer Design Activity

Tips for primer design:

1. Primers should be 17-28 bases in length.

2. Base composition should be 50-60% (G+C).

3. Primers should end (3') in a G or C, or CG or GC: this prevents "breathing" of ends and increases efficiency of priming.

4. Tms between 55-80oC are preferred.

5. Primers should not form hairpins or dimers with each other, as this will decrease the availability of primers for annealing.

6. 3'-ends of primers should not be complementary (i.e. base pair), as otherwise primer dimers will be synthesized preferentially to any other product.

7. Primer self-complementarity (ability to form 2o structures such as hairpins) should be avoided.

8. Runs of three or more Cs or Gs at the 3'-ends of primers may promote mispriming at G or C-rich sequences (because of stability of annealing), and should be avoided.

Adapted from:

Gurr, S. J. (1991), PCR protocols-a guide to methods and applications: Edited by M A Innis, D H Gelfand, J J Sninsky and T J White. pp 482. Academic Press, London 1990. $39.95 ISBN 0-12-372181-4. Biochemical Education, 19: 45. doi: 10.1016/0307-4412(91)90165-5

See primer creation directions here for more info on thresholds, values tolerated for hairpins, etc. as you critique your primers.

http://www.premierbiosoft.com/tech_notes/PCR_Primer_Design.html

Activity:

1. Find the sequence for the first exon of the c-myc gene in humans (or a gene of your choice, just chose a relatively small target section).

2. Using the tips and directions indicated, choose two primer sequence, a forward and a reverse primer.

3. Use http://www.idtdna.com/calc/analyzer to analyze both of your primers. For each primer, take a screen-shot of each analysis and include a caption that demonstrates why the analysis supports the use of this primer.

4. When you are done, write up a paragraph or two discussing why the criteria that go into primer design are important, and describing the process that you went through in designing your primer, including failed attempts (primers that did not meet criteria) and how you overcame them, what was the most challenging criteria to meet and why, etc.