Designing PCR Primers & In-Silico PCR

Objectives:

- To know how to design primers using primer 3.
- To test the primers in silico.

SECTION 1: Primer Design:

Several parameters should be taken in consideration when designing a pair of PCR primers, **these include:** primer length, product size, GC%, annealing temperature, 3' end stability, etc. In this lab exercise you will be asked to design a set of PCR primers that specifically anneal to human **factor IX gene** (**accession number A07407.1**).

1. Retrieve the DNA sequence of the human factor IX gene (**A07407.1**).
2. Copy the full FASTA format sequence.
3. Go to **Primer3Plus (http://primer3plus.com/cgi-bin/dev/primer3plus.cgi).**
4. Past the desired DNA sequence in the sequence box.
5. Go to the general setting and adjust: **Product Size Ranges 300-500.**
6. Adjust the primer size, primer Tm and primer GC%.
7. Then click **Pick Primers.**
8. The results will appear to you as the program nominates different pairs of primers.
9. Select the best pair that matches the criteria you had once entered into your search.

SECTION 2: In-Silico PCR:

1. Log on to the **USCS In-Silico PCR** webpage (**http://genome.ucsc.edu/cgi-bin/hgPcr?db=hg18**).
2. Configure the PCR tool by choosing the Genome and Assembly on which you are working.
3. The sequence for each primer must be at **least 15 bases long.**
4. The **Reverse Primer** must be on the opposite strand and pointing back toward the forward primer. If your reverse primer sequence is from the same strand, check the **Flip Reverse Primer** checkbox—this will reverse complement the sequence of your reverse primer.
5. Enter the **Max Product Size**. This is the maximum total genomic sequence length that the PCR tool should look for; primer hits that exceed this length will not be displayed in the output.
6. After entering your primers and configuring the tool, press the submit button.
7. If there is at least one match, the resulting page displays all hits in FASTA format. The FASTA body is capitalized in areas where the primer sequence matches the genomic sequence and in lowercase elsewhere.