Lab sheet #5

Designing PCR Primers using Primer3, UCSC in-Silico PCR and primer-BLAST

Objectives:

- To know how to design primers using primer 3and check its specificity using BLAST.
- 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, GC%, GC clamp, melting temperature, 3' end stability, product size, ... etc. In this lab exercise you will be asked to design a set of PCR primers that specifically anneal to human **factor IX gene** (**NG_007994.1**).

1. Retrieve the DNA sequence of the human factor IX gene.
2. Copy the full sequence in FASTA format.
3. Go to **Primer3Plus (http://primer3plus.com/cgi-bin/dev/primer3plus.cgi).**
4. Paste the desired DNA sequence in the sequence box.
5. Go to the general setting and adjust: the expected PCR product length **(300-500)** in **Product Size Ranges.**
6. Design a proper using the following guidelines:

Primer length	18-25 nucleotides – optimum is 20
GC%	40 -60% - optimum is 50%
Tm	50-60°C – maximum difference is 5°C
Sequence	3’ terminal position needs to be a G or C, but not more than 2 G or C on last 5 bases. Avoid complementary sequences within the primer. Avoid primer-dimer by complementarity sequence at 3’ ends of primer pairs. Avoid mismatch at 3’ end.

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: Primer-Blast:

1. Go to **primer-BLAST**.
2. In primer parameters section, paste your primer sequences.
3. Make sure that the selected database is **nr.**
4. Then click **Get primers**.
5. Check your primer specificity.

SECTION 3: in-Silico PCR:

1. Go to the webpage: (**http://genome.ucsc.edu/cgibin/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 **USCS In-Silico PCR** elsewhere.