Lab sheet 4

Designing PCR Primers using Primer3 and 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 **5'-aminolevulinate synthase 2 (ALAS2) transcript** (**accession number NM_000032.5**).

1. Retrieve the mRNA sequence of the gene.
2. Copy the full sequence.
3. Go to **Primer3Plus (** **http://primer3plus.com/cgi-bin/dev/primer3plus.cgi** **).**
4. Past the desired sequence in the sequence box.
5. To set the range of the expected PCR product length, go to **Product Size Ranges** and type **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: Checking Primer Specificity:

a) Primer-BLAST

1. Go to primer-BLAST.
2. In primer parameters section, paste your primer sequences.
3. Make sure that the selected database is **Refseq mRNA.** *(since the primer is designed for a mRNA sequence. In case of DNA sequence, the selected database should be* ***nr****).*
4. Restrict the search by **organism**.
5. Adjust Max target amplicon size to **500** bp
6. Then click Get primers.
7. Check your primer specificity.

b) UCSC in-Silico PCR:

1. Go to the webpage: (**http://genome.ucsc.edu/cgi-bin/hgPcr**).
2. Configure the PCR tool by choosing the Target and Assembly on which you are working.
    - Target: Genome assembly for DNA sequences, GENECODE genes for RNA sequences.
    - Assembly: Pick the latest assembly (GRCh38/hg38)
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.

Note that primers designed by primer3plus are already flipped

All primers in Primer3plus are shown in 5' to 3' direction.

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.

Exercise:

In this lab exercise design a set of PCR primers that specifically anneal to human oxytocin gene (accession number M11186.1) and check your primer specificity.