Hands-On – Primers design for PCR

Objective: Design PCR primers and check them for specificity

Exercise 1:

Sequence1

TCATAGCTCCGATCCGTTCCGTTCAATCAATGTACCTGGCGGCCATTCGTTCAACGGACGTCCAACCGGG
AATATCACGACGAGTGGGTCCTCGGTGCGCGTGGAAGGGAGATCGGCGTTCACCTCTGCGATGCTCGACC
GCACTCGAACCCCCGTCAAAATGCTAGCCATACCGTTTTGGCAATATCTCTCAACGTGGTTCCGCATGGC
GTGCCGAACTGTCCCAAAGGCGAATGGGACACCAAGCTCCCGCAAAGTTGGAGTCAAAGCACCGACCGAG
TGAGCGATGCCCATTCGCTCCAGATCGGGCCGCACCGCGTATAAGCCCAGTTCAGCCACAAGGAGATCAG
TCTCACCAACCTTAATGAAACGGCGCAACACGCCCATGTGGCTTGCTATCCCGACCGAGTCGTAAGCAAT
TGCGCGGCGTTCCGGTCTCGCGCCGGCCCAACTGCGGCCACCCTCAAATGGTTTCGCGTGGAACGCTCCT
GTGGGCCCATAGGATTTTCGAAAAAATTCTGAGAGCTCCTGGTGGTCTGCACGTTCCAGCTGATTTTCCC
AGCATAGCTTCCACTGCACTTTTAAGGACAT

Procedure:

- Use the given sequence to design a couple of primers for PCR.
- Go to the primer3 program: http://frodo.wi.mit.edu/
- Paste your sequence in the window
- In the options: Pick the left and right primers
- And you have to choose some options:
  - ° The product size ranges: 100-300 (you can give different ranges)
  - ° Specify the number of primers to return (you can choose as many as you want)
  - ° Optimum of Tm : 60°C

° Optimum of GC content: 50%

Next step: Check the primers for their specificity

We will perform an *Insilico* PCR amplification

° Use the following link (http://insilico.ehu.es/PCR/)

° Paste the picked primers in the corresponding window

° Check if the primers can amplify all the species of the *sinorhizobium*

° Specify the maximum length of bands

° Click the amplify button

° Interpret the result

° Get the Fasta format of the amplicons

° Check if the sequences of the amplicons are the same

Restriction mapping

In order to check if come sequences can be identified based on their restriction profile; we are going to perform an *insilico* restriction digest of complete genome:

- Will try the *sinorhizobium* genome
- Specify the "Only restriction enzymes with known bases (no N,R,Y...)"
- Click the restriction enzyme digest of genome
- Interpret the result

- Click the link in the right of the table for the PFGE simulation

Exercise 2:

The accession number FJ236988.1 correspond to eukaryotic gene.

We would like to design primers to amplify only mRNA, but have to flank the gene intron.

° Why is it interesting to have primers flanking intron?

° Use the primer3 program