Hands-On – Primers design for PCR

Objective. Design PCR primers and check them for specificity

Exercise 1.

Sequence 1

TCATAGCTCCGATCCGTTCCGTCAATCAATGTACCTGGGACATCCGCTCAACGGACGTCCAACCGGGG
AATATCGACGAGTGTTCTGCTGGCGGTAGGAAGGAGATCGGCGTTCACCTCTGCGATGCTCGACC
GCACTCGAAACCCGCCTCAAATGCTAGCCTACCTGGCTTTGGCAATATCTCTCCTAACGGTACCCGATG
GTGCCGAACTGTTCCCAAAGGCAATGGGAACCCAAGCTCCGCAAAAGTGGGAGTCAAAAACCGACCGAG
TGAGGTATGGCCTCCAGATCGGGCCGCACCGGTATAAGCCCAGTTCAGCCACAAAGATCAG
TTCACCAACCTTAATGAAACGCGCAACGCCCATGTGCTGCTATCCGACCCGAGTCGTAAGCAT
TGCGCCCCGCTTCCGCTCGCTCCGCGCCGCACTGCCGCCACCCCTAATGCTGCTGCAAGAGCTCTCCT
GTCGGCCCATAGGATTTTCGAAAAAATCTGAGACGTCCCTGAGTGTGCTGCTGACGTCAGGTATTTCC
AGCATAGCTCCACTGCACCTTTAAGGACAT

Procedure:

- Use the given sequence to design a couple of primers for PCR.
- Go to the primer3 program: [http://frodo.wi.mit.edu/](http://frodo.wi.mit.edu/)
- Paste your sequence in the window
- In the options, Pick the left and right primers
- 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
Rabat, January, 2013

° Optimum of GC content: 50%

Next step. Check the primers for their specificity
We will perform an *In silico* PCR amplification

° Use the following link ([http://insilico.ehu.es/PCR/](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 some 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

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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