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Primer design using NIH's Primer-BLAST tool.

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol demonstrates the basic usage of the **Primer-BLAST** tool in order to design PCR primers from a given FASTA sequence.
The results should show the sequence of at least one pair of primers (forward and reverse) within the chosen parameters.

Materials

Internet access.

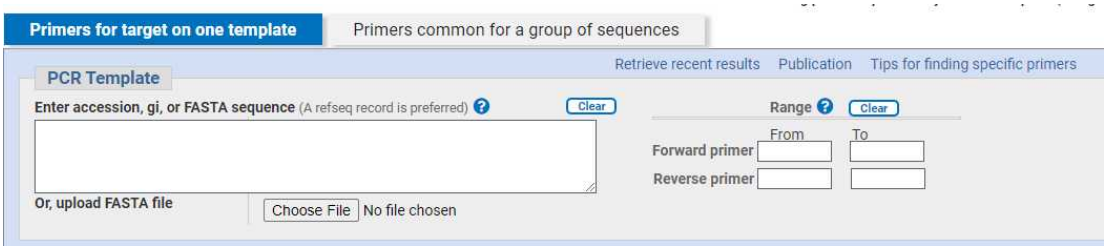
Go to NIH's Primer-BLAST tool

1 Open your internet browser (Google Chrome recommended).

2 Go to **https://www.ncbi.nlm.nih.gov/tools/primer-blast/**

Enter FASTA sequence

3



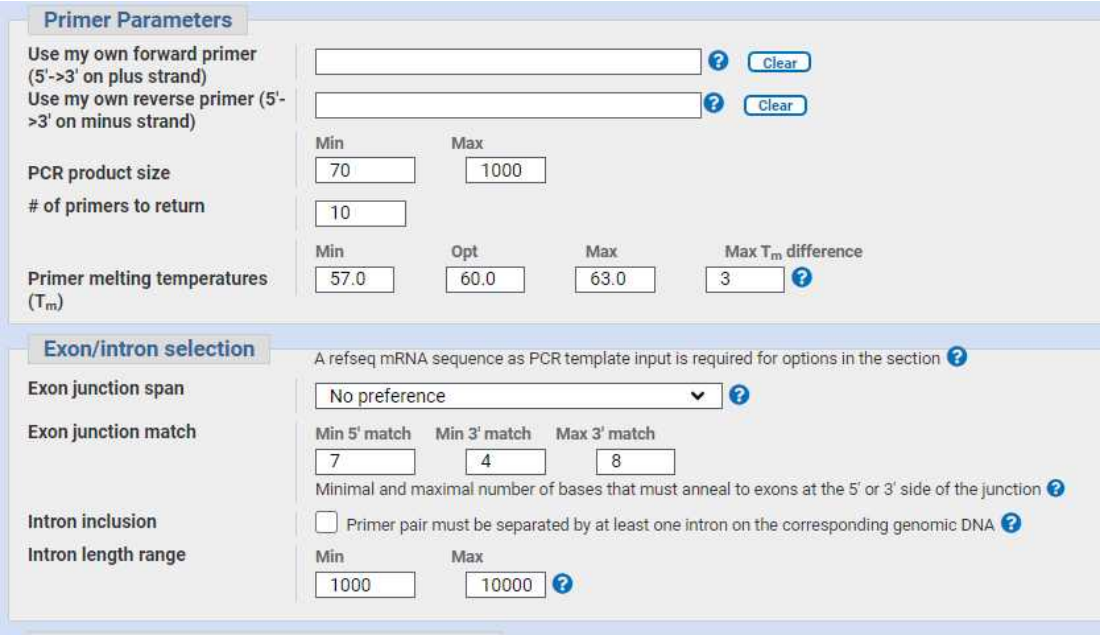
Screenshot of the **PCR Template section** with the corresponding text box.

Copy and paste your FASTA sequence in the text box or upload a FASTA file using the ¨Choose File¨ button.

4 You can select a custom range (i.e. the positional value of the first and last nucleotide of your ROI) for the forward and reverse primers.

Enter primer parameters

5



Screenshot of the **Primer Parameters** and **Exon/intron selection** sections.

Enter the optimal parameters for your primers. Depending on your application, the PCR product size and the primer Melting temperatures are extremely important values!

Analyze

6

Screenshot of the **Get Primers** button.

Click on the ¨Get Primers¨ button.