

Apr 23, 2024

Primer design using NIH's Primer-BLAST tool.

DOI

dx.doi.org/10.17504/protocols.io.rm7vzj8j5lx1/v1



Pedro C. Díaz¹, Francisco Franco García Calderon¹

¹U.A.N.L.



Francisco Franco García Calderon

Uanl

OPEN  ACCESS



DOI: dx.doi.org/10.17504/protocols.io.rm7vzj8j5lx1/v1

Protocol Citation: Pedro C. Díaz, Francisco Franco García Calderon 2024. Primer design using NIH's Primer-BLAST tool..
[protocols.io https://dx.doi.org/10.17504/protocols.io.rm7vzj8j5lx1/v1](https://dx.doi.org/10.17504/protocols.io.rm7vzj8j5lx1/v1)

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: April 12, 2024

Last Modified: April 23, 2024

Protocol Integer ID: 98098

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



Primer Parameters

Use my own forward primer (5'→3' on plus strand) ?

Use my own reverse primer (5'→3' on minus strand) ?

PCR product size Min Max

of primers to return

Primer melting temperatures (T_m) Min Opt Max Max T_m difference ?

Exon/intron selection

A refseq mRNA sequence as PCR template input is required for options in the section ?

Exon junction span ?

Exon junction match Min 5' match Min 3' match Max 3' match

Minimal and maximal number of bases that must anneal to exons at the 5' or 3' side of the junction ?

Intron inclusion ☐ Primer pair must be separated by at least one intron on the corresponding genomic DNA ?

Intron length range Min Max ?

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

☐ Show results in a new window ☒ Use new graphic view ?

Screenshot of the **Get Primers** button.

Click on the "Get Primers" button.