

Primer3

The *Primer3* plugin is a port of the [Primer3 tool](#), v. 2.6.1. It is intended to pick primers from a DNA sequence.

Usage

To use the *Primer3*, open a DNA sequence and select the *Analyze Primer3* context menu item. The dialog will appear:

Primer Designer

Choose preset:

Select the Task for primer selection

☒ Pick left primer
or use left primer below

☐ Pick internal oligo
or use oligo below

☒ Pick right primer
or use right primer below (5' to 3' on opposite strand)

5' Overhang:

Targets

Overlap Junction List

Excluded Regions

Pair OK Region List

Included region

Start Codon Position

Start Codon Sequence

Force Left Primer Start Force Right Primer Start

Force Left Primer End Force Right Primer End

Five Matches on Primer's 5' Five Matches on Primer's 3'

Five Matches on Internal Oligo's 5' Five Matches on Internal Oligo's 3'

Product Size Ranges Mispriming/Repeat library

Number to return Max 3' Stability

Max Library Mispriming Pair Max Library Mispriming

Region -

All available parameters are the same as the original Primer3 has.

NOTE: The "Primer Designer" dialog has a detailed description of each parameter - just move the mouse cursor to the parameter you're interested about (or its name) and the tooltip appears. Due to this fact, there is no detailed description of each parameter in this file.

Report

Then calculation is finished, the notification appeared in the bottom right corner. Click on it to open the report file.

[16:31:01] Report for task: 'Search primers to annotations'

No active tasks

The report looks as follow and contains the information about how many primers were considered during the searching process and how many of them were filtered due to one or another reason.

Task report [Search primers to annotations]

Status Finished
Time 0h 00m 00.147s

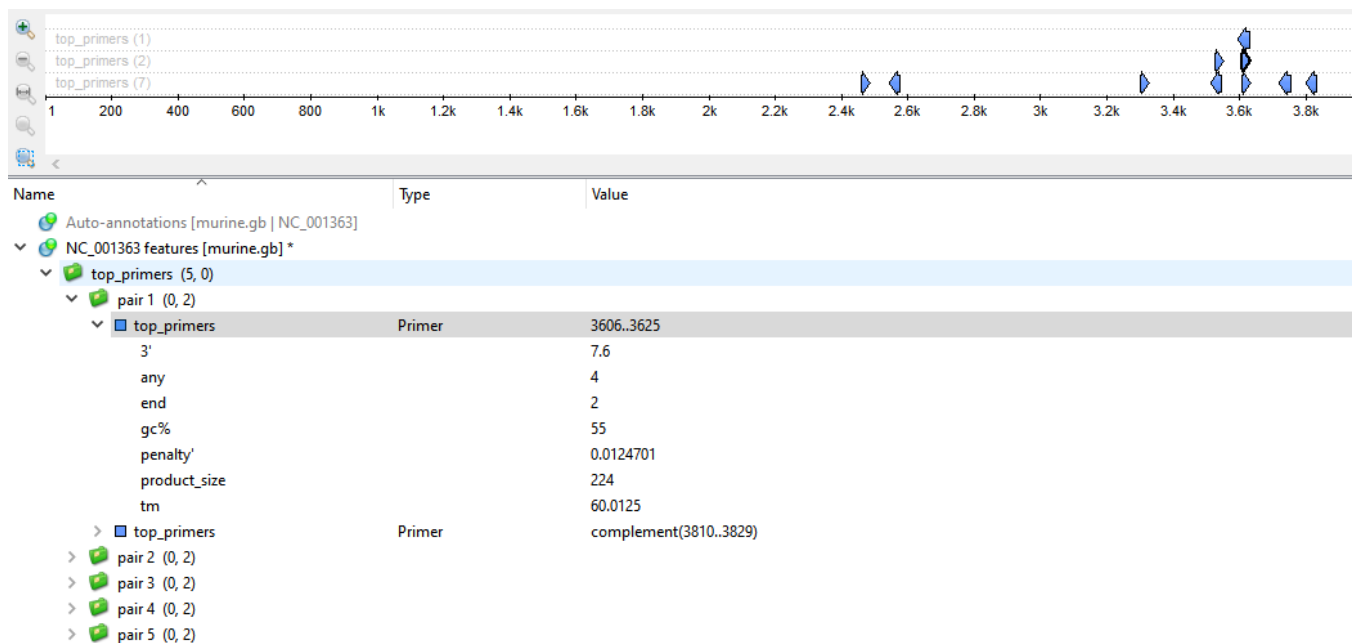
Statistics

	con	too	in	in		no	tm	tm	high	high	high		
	sid	many	tar	excl	bad	GC	too	too	any	3'	poly	end	
	ered	Ns	get	reg	GC%	clamp	low	high	compl	compl	X	stab	ok
Left	55795	0	0	0	564	0	10621	32510	0	2	224	0	11874
Right	55800	0	0	0	569	0	10403	32730	0	3	222	0	11873

Pair stats:
considered 621, unacceptable product size 613, high end compl 0, ok 8.

Results

All found primers will be available as annotations and marked on the target sequence:



The result annotations could have a set of the following qualifiers:

- 3' - free Gibbs energy of 5 bases on 3' end.

- any - value, which shows tendency of this primer to bind it's reverse-complement chain. This value calculates as follows: primer aligns on its reverse-complement representation, each match counts +1 point, each mismatch -1 point, each gap -2 points, each N -0.25 points.
- end - the same as any, but only about 5 bases on 3' end.
- gc - GC-content.
- penalty - penalty weight (see [this article](#) for details).
- hairpin - a hairpin loop melting temperature (if this kind of loop exists in the primer).
- product_size - size of the product.
- tm - primer melting temperature.

However there is one additional feature available which is not originally a part of Primer3 tool. It allows user design primers for RT-PCR experiments by choosing which exons/introns to span with the primer product. This feature is described in detailed below. When you select the parameters you can save and load settings with a help of the corresponding buttons in the right corner of the dialog.

- [Primer3 \(no target sequence\)](#)
- [Posterior Actions](#)
- [RTPCR Primer Design](#)