

Primer3

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

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

The image shows a screenshot of the 'Primer Designer' dialog box. It has a title bar with a question mark and a close button. Below the title bar is a tabbed interface with tabs for 'Main', 'General Settings', 'Internal Oligo', 'Penalty Weights', 'RT-PCR', 'Sequence Quality', and 'Result Settings'. The 'Main' tab is active. It contains several input fields and checkboxes. At the top, there are 'Excluded regions' and 'Targets' text boxes. Below these is a 'Product size ranges' section with a list of ranges (150-250, 100-300, 301-400, 401-500, 501-600, 601-700, 701-850, 851-1000) and a 'Mispriming/Repeat library' dropdown set to 'NONE'. The next section has six input fields with up/down arrows: 'Number to return' (5), 'Max 3' stability' (9.00), 'Max repeat mispriming' (12.00), 'Pair max repeat mispriming' (24.00), 'Max template mispriming' (12.00), and 'Pair max template mispriming' (24.00). There is also a 'Start codon position' text box. Below these are three checkboxes: 'Pick left primer' (checked), 'Pick hybridization probe (internal oligo)' (unchecked), and 'Pick right primer' (checked). Each checkbox has a corresponding text box below it: 'or use left primer below', 'or use oligo below', and 'or use right primer below (5' to 3' on opposite strand)'. At the bottom, there is a 'Region' dropdown set to 'Whole sequence', followed by a range '1 - 199950'. On the far left is a 'Help' button, and on the far right are 'Save settings', 'Load settings', 'Reset form', and 'Pick primers' buttons.

Primer Designer

Main General Settings Internal Oligo Penalty Weights RT-PCR Sequence Quality Result Settings

Excluded regions

Targets

Product size ranges 150-250 100-300 301-400 401-500 501-600 601-700 701-850 851-1000 Mispriming/Repeat library NONE

Number to return 5 Max 3' stability 9.00

Max repeat mispriming 12.00 Pair max repeat mispriming 24.00

Max template mispriming 12.00 Pair max template mispriming 24.00

Start codon position

☒ Pick left primer ☐ Pick hybridization probe (internal oligo) ☒ Pick right primer

or use left primer below or use oligo below or use right primer below (5' to 3' on opposite strand)

Region Whole sequence 1 - 199950

Help Save settings Load settings Reset form Pick primers

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

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.

- [RT-PCR Primer Design](#)