

In Silico PCR

In Silico PCR Overview

In silico PCR is used to calculate theoretical polymerase chain reaction (PCR) results using a given set of primers (probes) to amplify DNA sequences.

UGENE provides the In silico PCR feature only for nucleic sequences. To use it in UGENE open a DNA sequence and go to the *In silico PCR* tab of the Options Panel:

The screenshot shows the 'In Silico PCR' panel in the UGENE software. It contains the following elements:

- Forward primer:** A text input field containing the sequence 'ACGTACGTACGATAAAAGCGCGCGCG'. Below it, the melting temperature is calculated as 'Tm = 62.69°C, 26-mer'. There is a 'Mismatches' dropdown menu set to '0 bp'.
- Reverse primer:** A text input field containing the sequence 'AAAAAAAAAAGCGCGCGCGCGCGC'. Below it, the melting temperature is calculated as 'Tm = 65.54°C, 102-mer'. There is a 'Mismatches' dropdown menu set to '0 bp'.
- Settings:** A section with a 'Maximum product' dropdown menu set to '5000 bp'.
- Warnings:** A red warning message states: 'Warning: Self-dimer can be formed: Delta G: -23.7 kcal/mole Base Pairs: 8'.
- Action:** A button at the bottom labeled 'Find product(s) anyway'.
- Tools:** A vertical toolbar on the right side of the panel includes icons for sequence editing (ACG), alignment, visualization, and other functions.

There are the following parameters:

Forward primer - forward primer.

Reverse primer - on the opposite strand from the forward primer.

Mismatches - mismatches limit.

Maximum product - maximum size of amplified sequence.

Choosing primers

Type two primers for running In Silico PCR. If the primers pair is invalid for running the PCR process then the warning is shown. Also, primers for the running In silico PCR can be chosen from a [primer library](#). Click the following button to choose a primer from the primers library:

▼ Forward primer

ACACACGTACTGACAGTCAGCATACG

Tm = 61.39°C, 28-mer

Mismatches 7 bp

The following dialog will appear:

Choose Primer

Name	GC-content (%)	Tm (°C)	Length (bp)	Sequence
Primer	22.22	22	9	AAAAAACGT

Choose Cancel Help

The table consists of the following columns: name, GC-content (%), Tm, Length (bp) and sequence. Select primer in the table and click the *Choose* button.

Click the *Reverse-complement* button for making a primer sequence reverse-complement:

In Silico PCR

▼ Forward primer

ACACACGTACTGACAGTCAGCATACG

Tm = 61.39°C, 28-mer

Click *Show primers details* for seeing [statistic details](#) about primers.

When you run the process, the predicted PCR products appear in the products table.

Products table

There are three columns in the table:

- region of product in the sequence
- product length
- preferred annealing temperature

Click the product for navigating to its region in the sequence.

Click the *Extract product(s)* button for exporting a product(s) in a file or use double click for that.

Region	Length	Ta
60822 - 63999	3178	58.56

◀

||||

▶

Extract product(s)

A diagram with two red arrows. One arrow starts from the right side of the table and points diagonally up and to the left towards the 'Length' column of the first data row. The second arrow starts from the 'Extract product(s)' button and points diagonally up and to the left towards the 'Length' column of the first data row.

- [Primers Details](#)
- [Primer Library](#)