

Quick-Start Guide

PyroMark[®] Assay Design 2.0

PyroMark Assay Design Software is a tool for designing PCR and sequencing primers for Pyrosequencing[®] assays. Four different assay types can be designed: "Genotyping (SNP)", "Allele Quantification (AQ)", "Sequencing Analysis (SQA)", and "Methylation Analysis (CpG)".

Set up an assay

1. Create a new assay file; select **File > New** and then the desired type in the menu bar.
2. Enter the sequence to base the design on in the **(Original) Sequence Editor** tab.
There are three ways of entering a sequence:
 - Copy and paste a sequence from a text editor, from an Internet browser, or from another assay (right-click the sequence and select **Copy Entered Sequence**).
 - Import the sequence; click .
 - Type the sequence as continuous text in the 5'–3' direction.
3. If the sequence was typed or pasted into the software, press **Enter** to parse it.
4. If creating a CpG assay, select whether bisulfite conversion should be performed on the upper or the lower strand by checking the box to the left of the desired strand.
5. Set the target region (only required for CpG assays):
 - a. Select the variable position(s) and the desired sequence length before and after.
 - b. Right-click and select **Target Region > Set Target Region**.The target region for AQ, SNP, and SQA assays is automatically set by the software but can, if desired, be modified.

Optional

If desired, enter a description for the assay in the "Description" field, assign names to the variable positions in the "Variable Positions" area, and set PCR primer regions.

Note: It is also possible to base a new assay on a previously saved assay design file; open the file (select **File > Open**) and then select **Assay > Clone Assay** in the menu bar.

Allowed characters and rules

The following rules apply when entering the DNA sequence in the software:

- Variable positions should be entered using either a forward slash “/” (e.g., A/T) or the corresponding IUPAC code.
- Unknown sequences should be entered as a number of N-nucleotides.
- The allowed characters for sequence input are A, C, G, and T as well as IUPAC codes.
- InDels and STRs (short tandem repeats) should be entered using square bracket notation “[]” (e.g., [AT]).
- The sequence should not include more than 10,000 characters.

If the entered sequence includes characters that are not allowed, the first one will be highlighted in **red**.

Run an automatic assay design

Run an automatic assay design to generate primer sets by clicking . During the design, a progress bar is shown in the lower left corner.

View results and select a final primer set

The primer sets generated by the software are listed in the “Primer Set” area. Every primer set has been assigned a score and quality, which reflects its suitability for Pyrosequencing analysis. By default, the primer set with the highest score is selected as the final primer set.

For a primer set with high quality (a blue left column), none of the performed analysis steps have identified any problems or concerns. Thus, the primer set can be used directly without further checks or analyses.

For a primer set with a lower quality, however, we recommend reviewing the primer set.

Color	Quality
Blue	High quality
Yellow	Medium quality
Orange	Low quality
Red	Poor quality

Review a primer set

Select the desired primer set in the “Primer Set” area and view the information available in the **Graphic View** tab. If any of the analysis steps identified a problem or potential concern, a warning triangle and an associated warning message will be displayed.

To view more detailed information about the highlighted primer set (e.g., the different analyses performed on each primer and primer combination), click  or double-click the primer set. A report is then opened.

Select the final primer set

To select a primer set as the final set, select it and click the ✓ button.

☐ Unique Seq. Primers		Primer Set Score			
#1	✓	→ F1	GTTGTAGGTAGGAGGTTTAG		
89		← R1	CCAACTCCAAAAAACCCCTATAT		
		→ S1	GTTAGGAGGTTTAGG		
#2		→ F1	GTTGTAGGTAGGAGGTTTAG		
89		← R2	ATCCAACTCCAAAAAACCCCTATAT		
		→ S1	GTTAGGAGGTTTAGG		
#3		→ F2	GTTGTAGGTAGGAGGTTTAG		
85		← R1	CCAACTCCAAAAAACCCCTATAT		

The selected primer set is highlighted in light blue, while the primer set that has been selected as the final set is surrounded by a gray box.

Save the assay

Save the assay (). The assay can now be imported and used in other Pyrosequencing software.

More information

The online help can be accessed by pressing the F1 key.

QIAGEN® offers pyrosequencing assay design free of charge via the application lab. For more information, please contact your local QIAGEN representative or reach out to our Technical Support Center at www.qiagen.com/support/technical-support

Document revision history

Revision	Description
January 2026	Updated the QIAGEN layout.

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