

PyroMark[®] Q24 Advanced Software quick-start guide

This Technical Information describes standard functions of the PyroMark Q24 Advanced Software. More detailed information is available in the *PyroMark Q24 Advanced Software User Guide*, which can be accessed at anytime by pressing the “F1” key.

Setting up an assay

1. In the shortcut browser, right-click the folder in which you want to save the assay file and select “New Assay”. Select the desired assay type (AQ, SNP, CpG, or SEQ) from the context menu. If necessary, you can add a shortcut to an existing folder or drive by clicking “Add Folder Shortcut”.
2. Enter the name of the assay file and press “Enter”.
3. If creating an AQ or SNP assay, enter the “Sequence to Analyze” and then click the “Generate Dispensation Order” button. If creating a CpG assay, enter the “Sequence Before Bisulfite Treatment” and then click the “Generate Dispensation Order” button. If creating an SEQ assay, type in the “Dispensation Order”.

Note: For analysis of methylation at CpN sites, check the box next to “CpN mode enable”, and select which CpN sites to investigate.

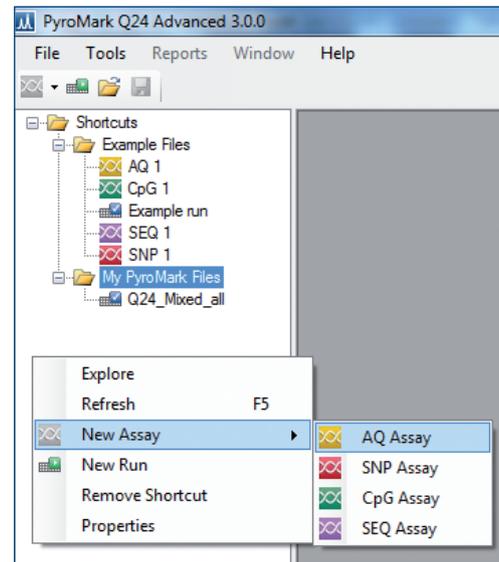
4. Optional: Enter information about the assay in the “Note” textbox and, if applicable, set up the variable positions in the “Variable Positions” tab.
5. Click the “Save” icon in the toolbar to save the assay setup.

Note: Before running your samples, make sure to validate your assay. See the *PyroMark Q24 Advanced User Manual* for details.

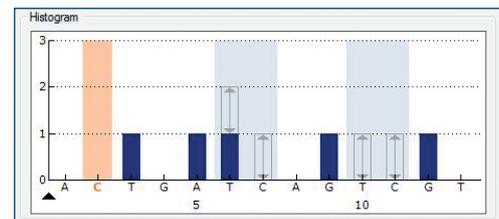
Note: If using QIAGEN[®] assay kits, use the settings outlined in the kit handbook.

Bisulfite treatment controls

We recommend that CpG assays contain one bisulfite treatment control, preferably at the beginning of the sequence. This is automatically selected for most assays with a “Sequence Before Bisulfite Treatment”. If the software could not select a suitable control, one can be added manually by adding a **C** before or after a **T** in a forward assay, or by adding a **G** before or after an **A** in a reverse assay, in the dispensation order.

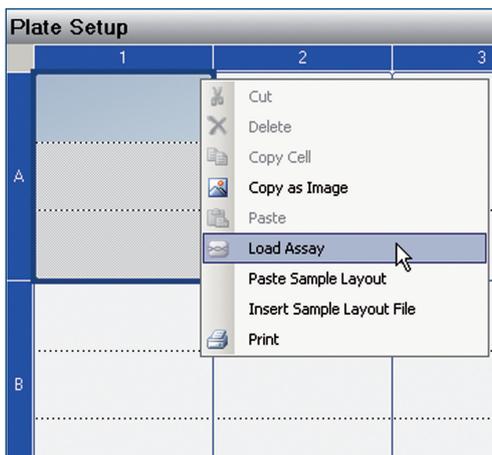


Adding a new assay.

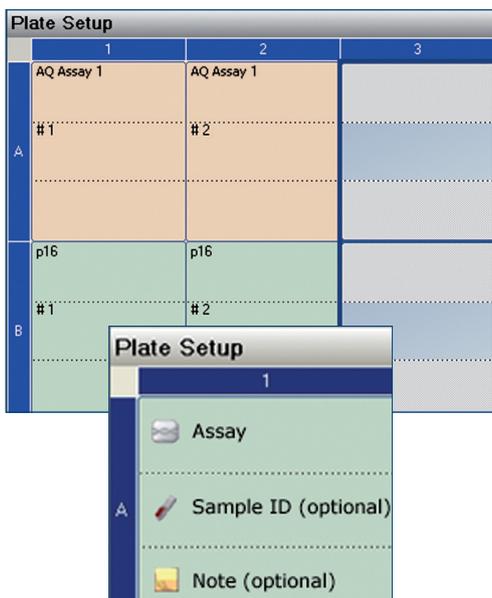


The bisulfite treatment control is displayed in orange and is highlighted light orange.





Loading an assay to a selected well.



Entering optional sample ID and note for a well. Note that the well coloration depends on the loaded assay type.

Setting up a run

1. In the shortcut browser, right-click the folder in which you want to save the run file and select "New Run".
2. Enter the name of the run file and press "Enter".
3. Select "Instrument Method" according to instructions supplied with the reagent kit and cartridge.
4. Optional: Enter the "Reagent Lot ID", "Plate ID", plate "Bar code", or a "Run Note".
5. To add the desired assay to each well used, right-click a well or selection of wells and select "Load Assay". Alternatively, drag an assay to a well or selection of wells. A well is colored according to the assay loaded to the well.
6. Optional: Select a cell to enter the sample ID or a note. The selected cell is highlighted with a blue background color.
7. Click the "Save" icon in the toolbar to save the run setup.
8. Print the plate setup and a list of required reagent volumes: select "Pre Run Information" from the "Tools" menu and click the "Print" icon in the toolbar.
9. Close the run file and copy it to the USB stick supplied with the PyroMark Q24 Advanced. The run file can now be processed by inserting the USB stick into the USB port at the front of the PyroMark Q24 Advanced Instrument (see the *PyroMark Q24 Advanced User Manual*).

Analyzing the run

1. Transfer the processed run file from the USB stick to the desired location on the computer running PyroMark Q24 Advanced Software.
2. Double-click the run file in the shortcut browser. If several assay types are included in the run, select the analysis mode in the dialog box. All wells on the plate with an Assay Setup corresponding to the selected analysis mode will be automatically analyzed.

Analysis modes

PyroMark Q24 Advanced Software has 4 analysis modes: AQ, SNP, CpG, and SEQ. To toggle between these modes, select "AQ", "SNP", "CpG", or "SEQ" in the toolbar.

Viewing the analysis results

By selecting an analyzed well in the "Overview" tab, the corresponding Pyrogram® is displayed in the "Pyrogram" area and the well information (including analysis warnings) is listed in the "Well Information" area.

Note: Reports of analysis results, statistics, and Pyrograms can be generated by selecting the desired report from the "Reports" menu.

Quality assessments

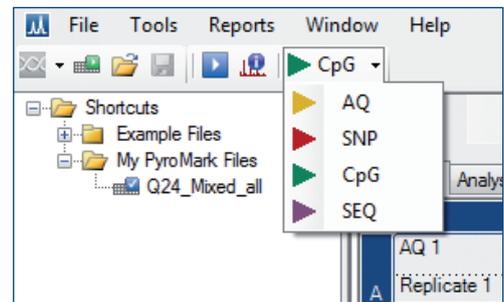
The plate overview in the "Overview" tab summarizes quality assessments. Select the "Quality" option in the upper right corner to display the quality assessment of all variable positions in a selected well or of all bases in the base-called sequence. SEQ assays also include quality assessment at the end of the quality control window. Quality levels are indicated with different colors:

- Blue: passed
- Yellow: check
- Red: failed

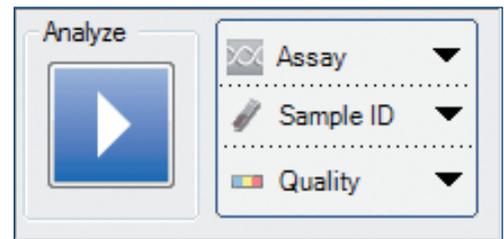
Wells that are not analyzed, either because analysis is not supported by the software (e.g., analysis of single nucleotide polymorphisms in CpG mode) or because the variable position has been deselected by the user (AQ, SNP, and CpG assays only), are not assigned a quality assessment level and are indicated in off-white.

AQ, SNP, and CpG analysis results

Allele frequencies (AQ analysis), genotypes (SNP analysis), and methylation percentages (CpG analysis) are displayed at the top of the Pyrogram. The quality assessment is indicated by the background color of the result. Variable positions in both analysis modes are highlighted with a blue-gray background color.



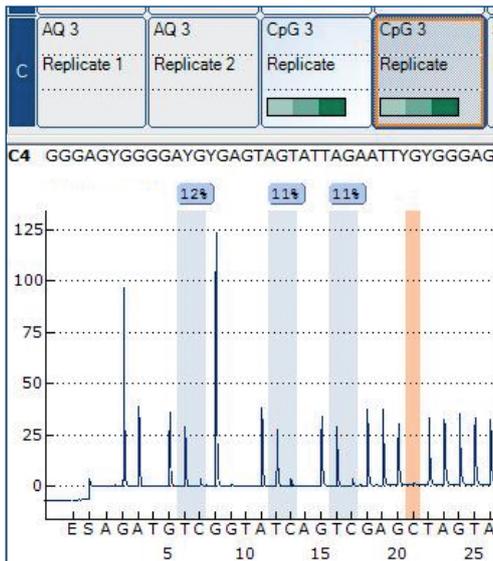
Selecting analysis mode.



"Quality" option in the upper right corner of the "Overview" tab.

	1	2
A	AQ Assay 1 # 1 ■	AQ Assay 1 # 2 ■ ■ ■
B	AQ assay 1 # 4 ■	AQ assay 1 # 5 ■
C	CTGAx14 # 7	CTGAx14 # 8

Quality assessment indicators in the "Overview" tab.



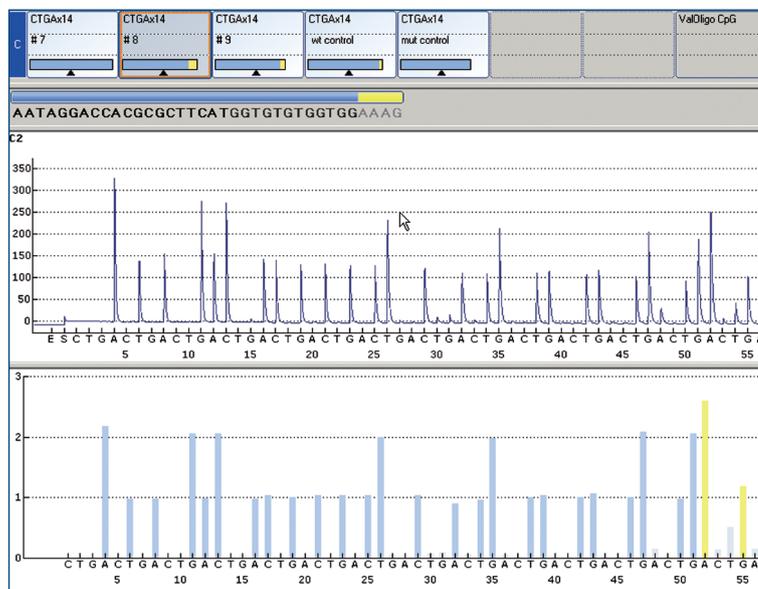
Pyrogram of a well analyzed in CpG mode and methylation bars displayed in selected wells.

In CpG analysis, bisulfite treatment controls are highlighted in the Pyrogram with a light orange background color. Select the "Ranges" option in the upper right corner of the "Overview" tab to show methylation bars in the plate overview. These bars indicate the methylation level for each CpG site in a selected well, as compared to the expected level. Expected ranges can be set under the "Analysis Setup" tab.

- Light green: below the expected range
- Green: within the expected range
- Dark green: above the expected range

SEQ analysis results

The base-called sequence is displayed in the "Overview" tab above the Pyrogram of the selected well. The bases in the base-called sequence and the peaks in the compensated Pyrogram at the bottom of the window are colored according to their quality assessments.



Overview tab of SEQ analysis results, including plate overview, base-called sequence, Pyrogram, and compensated Pyrogram.

For up-to-date licensing information and product-specific disclaimers, see the respective *PyroMark Q24 Advanced User Manual*. The *PyroMark Q24 Advanced User Manual* is available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

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