PyroMark® Q48 Autoprep Software

Setting up an assay

1. Click ⌧ in the toolbar and select the required assay. A new assay file is created. For analyzing methylation at CpN sites, select **New CpG Assay** and then enable the CpN mode by checking the box next to CpN mode enabled.

   Alternatively, you can create a new assay file in the shortcut browser by right-clicking the folder you wish to place it in and selecting **New Assay** followed by the desired assay type from the context menu.

2. Enter the sequence to analyze.

   Note: If creating a CpG assay, we recommend entering the **Sequence Before Bisulfite Treatment**. This enables the software to automatically generate the sequence to analyze and select the most appropriate bisulfite treatment control.

3. Click the **Generate Dispensation Order** button. If creating an SEQ assay, enter the Dispensation Order.

4. Click 💾 in the toolbar to save the assay.

   Note: Before running your samples, validate your assay using a reference DNA sample; see the *PyroMark Q48 Autoprep User Manual*.

5. **Optional**: If desired, enter an assay note in the **Assay Note** box. Set up variable positions in the **Variable Positions** tab (AQ, SNP and CpG assays only).

   Lock the assay for editing by clicking the **Lock Assay** button at the bottom of the assay setup window.

   For CpG assays, check that the software has automatically selected a bisulfite treatment control. If not, add one manually, preferably at the beginning of the sequence. A bisulfite treatment control can be added by adding a **C** before or after a **T** that was a **C** before bisulfite treatment in a forward assay, or by adding a **G** before or after an **A** that was a **G** before bisulfite treatment in a reverse assay, in the dispensation order.
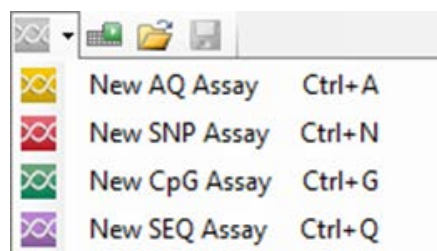
Adding a new assay.
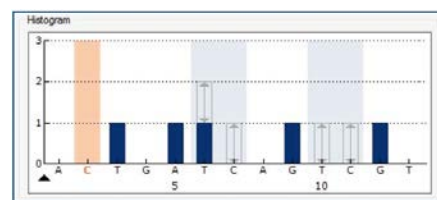
The bisulfite treatment control is displayed in orange and is highlighted in light orange.

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Setting up a run

1. Create a new **Run Setup** by clicking ⊞ in the toolbar. Alternatively, right-click a folder in the shortcut browser and select **New Run**. Enter a run name and press **Enter**.

   To base your run on a previous run, right-click the processed run file in the shortcut browser and select **Copy and Rerun**. Only the run setup, not the run and analysis data, will be copied.

2. If the new run has not yet been saved, click 🖫 to enter a run name and save the file in the desired folder.

3. Set up the disc in the disc layout of the run file by adding assays to wells and, if desired, entering the sample ID and note for each used well.

4. Add an assay to each well used by right-clicking a well and selecting **Load Assay**. Alternatively, drag an assay from the shortcut browser to a well or a selection of wells.

   A well is colored according to the assay loaded into the well. Wells with AQ assays are yellow; wells with SNP assays are red; wells with CpG assays are green; wells with SEQ assays are purple.

   It is also possible to import and paste a sample layout defined in a text file. For details, see the *PyroMark Q48 Autoprep User Manual*.

5. To enter a sample ID or note, select the cell and enter the text. A selected cell is highlighted with a blue background color.

6. In the protocol drop-down list, select the desired protocol for the run. There are two protocols available for the PyroMark Q48 Autoprep:

   - **Standard protocol**: the default protocol.

   - **P4 Extra protocol**: enables the use of the fourth sequence primer injector (P4). When using this protocol, the binding buffer must be loaded manually by the user, because the BB/P4 injector will be used to dispense sequence primer instead of binding buffer.

7. Sequencing primers can be added manually or automatically. Select "manual" or "automatic" primer loading in the **Run Setup**. For automatic primer loading, one injector can be chosen for each assay.

8. Click 🖫 in the toolbar.

9. Print the disc setup by selecting **Pre Run Information** from the **Tools** menu. When the report opens, click 🖨.

10. Close the run file and copy it to the USB stick. Process the run file by inserting the USB stick into the port on the front of the instrument or by saving to a network drive and processing on the instrument directly.

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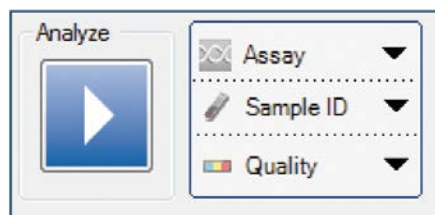

Selecting manual or automatic primer loading.

Analyzing the run

1. Transfer the processed run file from the USB stick to a computer running PyroMark Q48 Autoprep Software. Alternatively, the run file can be transferred via a network drive.

2. Double-click the run file in the shortcut browser or in Windows Explorer. If several assay types are included in the run, select analysis mode in the dialog box. The analysis modes displayed in the dialog box are limited to the assay types on the disc.

   Note: To update the contents of a folder in the shortcut browser, right-click it and select **Refresh**. Alternatively, press the **F5** key.

3. In the **Overview** tab, analyze selected wells with a valid analysis setup for the current analysis mode.

Analysis modes

PyroMark Q48 Autoprep Software has four analysis modes: AQ, SNP, CpG and SEQ. To toggle between the modes, select **AQ**, **SNP**, **CpG** or **SEQ** in the toolbar.

Note: How the analysis is performed can be modified using the **Analysis Setup** tab.

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.

Quality assessments

The disc overview in the **Overview** tab gives a quick overview of the quality assessments.

The color bar (▭) shows the quality assessment of all variable positions in the well or of all the bases in the base-called sequence using the following quality colors:

▯	Blue: Passed	▮	Red: Failed
▯	Yellow: Check	▯	White: Not analyzed*

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

Quality assessment indicators in the "Overview" tab.

* Either analysis is not supported by the software (e.g., analysis of SNP when in the CpG mode) or the variable position was deselected by the user (AQ, SNP and CpG assays only).

AQ analysis results

The allele frequencies are displayed in the Pyrogram, for example `A: 96% G: 4%` and `--: 56% AT: 44%` (InDel). The quality assessment is displayed by the background color of the result.

CpG analysis results

The methylation percentage `96%`, or average methylation percentage `Average: 25%`, are displayed in the Pyrogram. The quality assessment is displayed by the background color of the result.

A methylation bar in the disc overview shows the methylation level for each CpG site in the well:

- Light green: Below the expected range
- Green: Within the expected range
- Dark green: Above the expected range

Example Pyrogram for a CpG assay. Variable positions in AQ and CpG assays are highlighted in a blue-gray background color, and bisulfite treatment controls in CpG assays with a light orange background color.
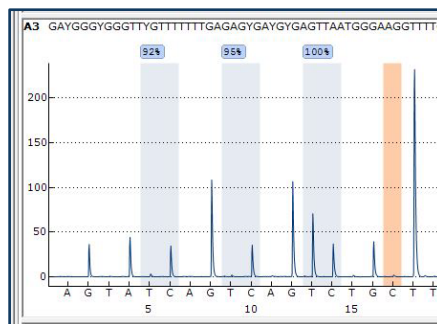
SEQ analysis results

The base-called sequence is displayed in the **Overview** tab. The bases in the base-called sequence and the peaks in the compensated Pyrogram are colored according to their quality assessments.

SNP analysis results

The SNP is displayed in the Pyrogram, for example `C/G` for heterozygote SNP and `A/A` for a homozygote SNP. The quality assessment is displayed by the background color of the result.

Example Pyrogram for a SNP assay.

Analysis reports

To generate a report, select the desired report from the Reports menu. For more information about the reports, see the *PyroMark Q48 Autoprep User Manual.*