BRAF Pyro® Plug-in Quick-Start Guide

For installation and use with PyroMark® Q24 Instruments and PyroMark Q24 Software version 2.0

QIAGEN

About the BRAF Pyro Plug-in

The BRAF Pyro Plug-in package contains the following:

- *BRAF Pyro Plug-in Quick-Start Guide*
- Two installation files
- Reference report for BRAF Pyro Plug-in functionality verification

Note: The BRAF Pyro Plug-in is intended to be used only in combination with the dedicated BRAF Pyro Kits indicated for applications described in the respective BRAF Pyro Kit handbooks.

Installation of the BRAF Pyro Plug-in

Important: The BRAF Pyro Plug-in must be installed on **PyroMark Q24 instruments with PyroMark Q24 Software version 2.0**.

1. Close the PyroMark Q24 Software 2.0 if it is open.
2. Open the installation *.zip file and extract the files.
3. Double-click the setup.exe file.
4. Follow the instructions in the dialog boxes that appear.
5. Start the PyroMark Q24 Software 2.0. The BRAF Pyro Plug-in Report now appears under "AQ Add On Reports/BRAF" in the "Reports" menu in AQ mode.
6. Verify the Plug-in functionality (see "Verification of the Plug-in functionality" below).

Verification of the BRAF Pyro Plug-in functionality

Important: The verification should be performed each time new software is installed or upgraded on the computer.

The following steps describe how to verify that the software is working correctly and has not been affected by any changes to the computer.

1. Open the BRAF Example run under "Shortcuts/Example Files/PyroMark Runs/BRAF" in the shortcut browser.
2. Perform a "BRAF" analysis for all wells as described in "Analysis of a PyroMark Q24 Run" below.
3. Compare the results with the reference report. If the results are identical, correct function of the BRAF Plug-in is confirmed.

Analysis of a PyroMark Q24 Run

Important: The Plug-in will report the mutation (Table 1) that best matches the observed Pyrogram.

Important: Some addressed mutations in codon 600 as well as codons 469–469 might not be precisely distinguished at mutation levels below 10%.

The following steps describe the mutation analysis of a finished BRAF run using the BRAF Pyro Plug-in.

1. Insert the USB stick containing the processed run file into the computer's USB port.

2. Move the run file from the USB stick to the desired location on the computer using Windows® Explorer.

3. Open the run file in the AQ mode of PyroMark Q24 Software either by selecting "Open" in the "File" menu or by double-clicking the file (⬤) in the shortcut browser.

4. Select "AQ Add On Reports/BRAF" from "Reports" in the menu (Figure 1).
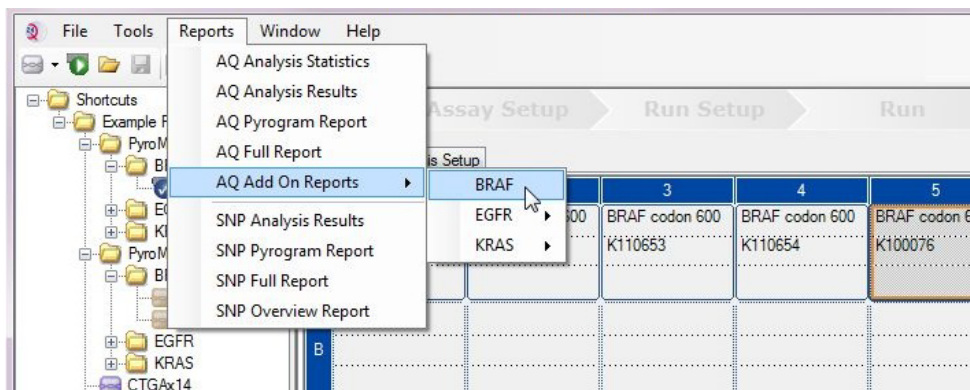

Figure 1. Mutation analysis of a finished BRAF run using the BRAF Pyro Plug-in.

5. The wells will automatically be analyzed for all mutations listed in Table 1. The results for both the BRAF codon 600 and BRAF codon 464–469 assay will be presented in an overview table (Figure 2), followed by detailed results comprising Pyrograms® and analysis quality.

Table 1. Mutations analyzed by the BRAF Pyro Plug-in

Nucleic acid substitution	Amino acid substitution	LOB (% units)	LOD (% units)	COSMIC ID* (V70)
BRAF codon 600				
1799T>A	V600E	0.4	2.4	476
1799T>G	V600G	0.1	2.1 (5)†	6137
1799T>C	V600A	0.2	2.2 (7)†	18443
1798G>A	V600M	0.4	2.4	1130
1799_1800TG>AA	V600Ecomplex	0.4	2.4	475
1799_1800TG>AT	V600D	2.3	4.3	477
1798_1799GT>AA	V600K	0.1	2.1	473
1798_1799GT>AG	V600R	0.2	2.2	474
BRAF codons 464–469				
1406G>A	G469E	1.1	3.1	461
1406G>C	G469A	1.2	3.8	460
1406G>T	G469V	1.1	3.1	459
1405_1406GG>TC	G469S	1.5	3.5	458
1397G>A	G466E	4.1	8.6	453
1397G>T	G466V	1.3	3.3	451
1391G>A	G464E	1.3	3.4	449
1391G>T	G464V	0.3	2.3	450

* From the Catalogue of Somatic Mutations in Cancer, available online at the Sanger Institute at **www.sanger.ac.uk/genetics/CGP/cosmic/**.

† Lowest mutation level in a sample resulting in a measured frequency ≥LOD.

Summary

Well	Assay Name	Sample ID	Result	Frequency [% units]	Nucleotide Substitution	Amino Acid Substitution	Info
A1	Codon 600	WT control	No mutation detected				
A2	Codon 600	K110652	Potential low level mutation	4.8	1799T>A	V600E	⚠
A3	Codon 600	K110653	No mutation detected				
A4	Codon 600	K110654	Mutation	34.6	1798_1799GT>AG	V600R	
A5	Codon 600	K100076	Mutation	26.4	1798_1799GT>AA	V600K	
A6	Codon 600	K110282	No mutation detected				
A8	Codon 600	NTC	Failed Analysis				⚠
C1	Codons 464 to 469	WT control	No mutation detected				
C2	Codons 464 to 469	K110652	No mutation detected				
C3	Codons 464 to 469	K110653	Mutation	29.0	1406G>T	G469V	
C4	Codons 464 to 469	K110654	No mutation detected				
C5	Codons 464 to 469	K100076	No mutation detected				
C6	Codons 464 to 469	K110282	Mutation	27.8	1391G>A	G464E	
C8	Codons 464 to 469	NTC	Failed Analysis				⚠

⚠ See detailed results below.

NOTE: The result must be validated by comparing the observed peaks with the expected peak heights displayed as grey bars. For further information about data evaluation and result interpretation please refer to the handbook.

Figure 2. Example results summary from a BRAF Pyro Plug-in analysis.

Interpretation of results and detection of low-level mutations

It is strongly recommended that a wild-type sample is included in every run for comparison and as a control for background levels.

Important: A "Check" or "Failed" quality assessment can be caused by an unexpected pattern of peaks. This may indicate an unexpected mutation which is not analyzed by the Plug-in Report. These samples should be analyzed manually using the PyroMark Q24 Software with the consideration that they may contain unexpected mutations. See the appropriate BRAF Pyro Kit handbook for details.

Important: The Pyrogram should always be compared to the histogram, which is shown in the detailed results of the Plug-in Report and can be displayed in the PyroMark Q24 software by right-clicking in the Pyrogram window. The Pyrogram should be examined for the appearance of unexpected peaks. In case the measured peaks do not match the height of the histogram bars and cannot be explained by rare or unexpected mutations, the result is not a basis for judgment of mutational status. It is recommended to rerun the sample.

Important: Samples with a reported potential low-level mutation (frequency in the range from LOD to LOD+ 3% units) should be rerun in duplicate together with a sample with unmethylated control DNA. A warning will be issued in this case. The sample should only be considered positive for the mutation if both duplicates confirm the result of the original analysis and are visibly different from the normal control. Otherwise, the sample should be judged as wild-type.

Important: For closer examination of samples with a reported potential low-level mutation, we recommend to additionally analyze the sample manually in the PyroMark Q24 Software, e.g., for comparison to the mutational frequency in the control sample (see the corresponding protocol for detailed instructions). A measured frequency above LOB in the control sample indicates a higher than usual level of background in the corresponding run, which may impact allele quantification especially for low mutational levels. In this case, reported potential low-level mutations are not a basis for judgment of mutational status and it is recommended to rerun samples with a potential low-level mutation.

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