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EGFR Pyro® Plug-in Quick-Start Guide

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

Sample to Insight

QIAGEN

About the EGFR Pyro Plug-in

The EGFR Pyro Plug-in package contains the following:

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

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

Installation of the EGFR Pyro Plug-in

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

1. Close 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 PyroMark Q24 Software 2.0. The EGFR Pyro Plug-in reports now appear under "AQ Add On Reports/EGFR" in the "Reports" menu in AQ mode.
6. Verify the Plug-in functionality (see "Verification of the EGFR Pyro Plug-in functionality" below).

Verification of the EGFR 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 EGFR Example run under "Shortcuts/Example Files/PyroMark Runs/EGFR" in the shortcut browser.
2. Perform an "EGFR Exon 19 Deletions" 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 EGRF Pyro Plug-in is confirmed.

Analysis of a PyroMark Q24 Run

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

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/EGFR" and "Exon 18 Codon 719", "Exon 20 Codon 768", "Exon 20 Codon 790", "Exon 21 Codons 858 to 861", or "Exon 19 Deletions" from "Reports" in the menu (Figure 1).

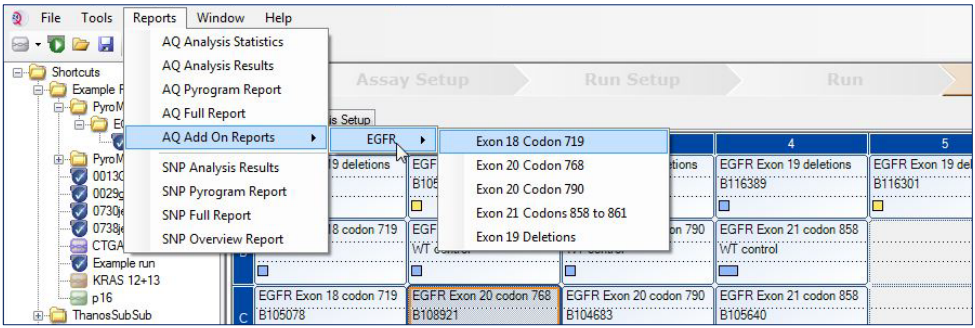

Figure 1. Mutation analysis of a finished EGFR run using the EGFR Pyro Plug-in.

5. The wells will automatically be analyzed for all mutations for which LOD is given in Table 1. The results will be presented in an overview table (see example in Figure 2 below), followed by detailed results comprising Pyrograms® and analysis quality.

Table 1. LOB and LOD determined for specific mutations with the EGFR Pyro Plug-in

Mutation	Amino acid substitution	LOB (% units)	LOD (% units)	COSMIC ID* (V70)
Exon 19 Deletions				
2233del15	K745_E749del	0.6	1.6	26038
2235_2248>AATTC	E746_A750>IP	0.8	1.6	13550
2235_2252>AAT	E746_T751>I	1.1	2.8	13551
2235del15	E746_A750del	0.9	1.8	6223
2236del15	E746_A750del	0.2	1.2	6225
2237_2252>T	E746_T751>V	0.8	2.4	12386
2237_2255>T	E746_S752>V	0.6	1.6	12384
2237del15	E746_T751>A	0.9	1.9	12678
2237del18	E746_S752>A	0.5	1.7	12367
2238_2248>GC	L747_A750>P	0.8	2.5	12422
2238_2252>GCA	L747_T751>Q	0.2	0.6	12419
2238del18	E746_S752>D	0.3	1.1	6220
2239_2248>C	L747_A750>P	1.8	2.4	12382
2239_2251>C	L747_T751>P	0.6	1.7	12383

Mutation	Amino acid substitution	LOB (% units)	LOD (% units)	COSMIC ID* (V70)
2239_2258>CA	L747_P753>Q	1.3	3.9	12387
2239del18	L747_S752del	0.6	1.5	6255
2239del9	L747_E749del	2.0	3.7	6218
2240del12	L747_T751>S	0.4	1.5	6210
2240del15	L747_T751del	0.9	1.9	12369
2240del18	L747_P753>S	0.9	1.9	12370
Exon 18 codon 719 (GGC)				
AGC	G719S	0.9	1.5	6252
TGC	G719C	1.0	1.6	6253
GCC	G719A	4.7	9.1	6239
Exon 20 Codon 768 (AGC)				
ATC	S768I	2.6	5.0	6241
Exon 20 Codon 790 (ACG)				
ATG	T790M	7.0	10.7	6240
Exon 21 Codon 858 (CTG)				
CGG	L858R	0.6	2.6 (5.5)†	6224
Exon 21 Codon 861 (CTG)				
CAG	L861Q	3.2	4.3	6213
CGG	L861R	1.9	4.2	12374

* 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	Sample ID	Result	Frequency [% units]	Nucleotide Substitution	Amino Acid Substitution	Info
A1	B104683	Mutation	34.0	2236del15	E746_A750del	
A2	B105072	Wildtype				
A3	B116390	Mutation	26.6	2240del18	L747_P753>S	
A4	B116389	Wildtype				
A5	B116301	Potential low level mutation	3.2	2233del15	K745_E749del	⚠
A6	B116392	Mutation	15.4	2235del15	E746_A750del	
A7	WT control	Wildtype				
A8	NTC	Failed Analysis				⚠

⚠ See detailed results for further explanation.

NOTE: For further information about data evaluation please refer to the handbook.

Figure 2. Example results summary from an EGFR 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 that is not analyzed by the Plug-in report. These samples should be analyzed manually using PyroMark Q24 Software and considering unexpected mutations. See the appropriate EGFR 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.

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