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GIST RapidScreen 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 GIST RapidScreen Pyro Plug-in

The GIST RapidScreen Pyro Plug-in package contains the following:

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

Note: The GIST RapidScreen Pyro Plug-in is intended to be used only in combination with the dedicated *therascreen*® GIST RapidScreen Pyro Kit (cat. no. 971510) indicated for applications described in the therascreen *GIST RapidScreen Pyro Kit Handbook*.

Installation of the GIST RapidScreen Pyro Plug-in

Important: The GIST RapidScreen Pyro Plug-in must be installed on either the **PyroMark Q24 Instrument with PyroMark Q24 Software version 2.0** or the **PyroMark Q24 MDx Instrument with PyroMark Q24 MDx Software version 2.0**

Close the PyroMark Q24 Software 2.0 if it is open.

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

Verification of GIST RapidScreen 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.

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

Analysis of a PyroMark Q24 Run

The following steps describe the mutation analysis of a finished GIST run using the GIST RapidScreen 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/GIST" from "Reports" in the menu (Figure 1).

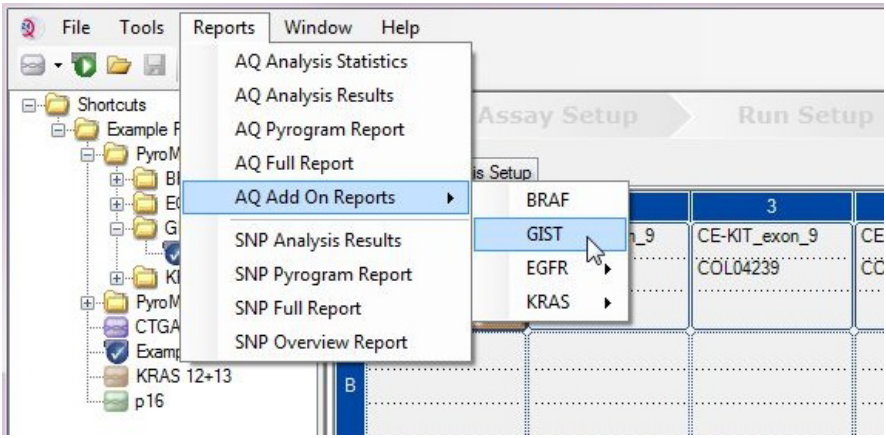

Figure 1. Mutation analysis of a finished GIST RapidScreen run using the GIST RapidScreen Pyro Plug-in

5. The wells will automatically be analyzed for all mutations listed in Table 1. The results for both the KIT exon 9 and PDGFRA exon 18 assay will be presented in an overview table (Figure 2), followed by detailed results comprising Pyrograms® and analysis quality.

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

Table 1. Mutations analyzed by the GIST RapidScreen Pyro Plug-in

Nucleic acid substitution	Amino acid substitution	LOB (% units)	LOD (% units)	COSMIC ID* (V70)
KIT exon 9				
1509_1510insGCCTAT	Y503_F504insAY	1.9	4.9	1326
PDGFRA exon 18				
2525A>T	D842V	0.6	3.6	736
2524G>T	D842Y§	0.6	3.6	12396
2524_2535 del12 or† 2526_2537	del12 D842_H845del or† I843_D846del§	2.2	5.2	737 or† 96892
2527_2538 del12	I843_D846del§	3.0	6.0	12400
2528_2539 del12	I843_S847>T	4.2	7.2	12407
2530_2541 del12	M844_S847del	3.2	6.2	12402
2524_2532 del9	D842_M844del	1.5	4.5	12401
2524_2526 delGAC	D842del	0.9	3.9	12406
2526_2538 >G‡	D842_D846>E	0.3	3.3	12408
2524_2526 GAC>TAT	D842Y§	0.9	3.9	12397

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

† The mutations 2524_2535del12 and 2526_2537del12 result in the same nucleic acid change.

‡ The mutations 2526_2538 >G and 2524_2526GAC>TAT cannot be analyzed in the AQ mode of the PyroMark Q24 Software.

§ The mutations 2524G>T and 2524_2526 GAC>TAT, and 2526_2537 del12 and 2527_2538 del12 result in the same amino acid change, respectively.

Summary

Well	Assay Name	Sample ID	Result	Frequency [% units]	Nucleotide Substitution	Amino Acid Substitution	Info
A1	cKIT Exon 9	COL04237	No mutation detected				
A2	cKIT Exon 9	COL04238	Mutation	51.6	1509_1510insGCCTAT	Y503_F504insAY	
A3	cKIT Exon 9	COL04239	Mutation	29.6	1509_1510insGCCTAT	Y503_F504insAY	
A4	cKIT Exon 9	COL04240	No mutation detected				
A5	cKIT Exon 9	wt control DNA	No mutation detected				
A8	cKIT Exon 9		Failed Analysis				⚠
C1	PDGFRA Exon 18	COL04237	No mutation detected				
C2	PDGFRA Exon 18	COL04238	Potential low level mutation	4.5	2525A>T	D842V	⚠
C3	PDGFRA Exon 18	COL04239	No mutation detected				
C4	PDGFRA Exon 18	COL04240	Mutation	52.2	2524_2535del12 or 2526_2537del12	D842_H845del or I843_D846del	
C5	PDGFRA Exon 18	wt control DNA	No mutation detected				
C8	PDGFRA Exon 18		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 GIST RapidScreen 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 therascreen *GIST RapidScreen 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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