July 2016

ipsogen® JAK2 Muta*Screen* RS Handbook

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For JAK2 V617F/G1849T semi-quantitative analysis.

For research use only. Not for use in diagnostic procedures.

For use with Rotor-Gene® Q, Applied Biosystems®, ABI PRISM®, and LightCycler® instruments

REF 673113

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- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

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Contents

Intended Use

The *ipsogen* JAK2 Muta*Screen* RS Kit is intended for research use only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

The test can report false negative results in case of additional mutations located in codons 615 to 619.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Principle of the Procedure

In an allelic discrimination assay, two TaqMan® probes are used in a multiplexed assay. One is a perfect match to the allele 2 sequence (e.g., the wild-type allele), the other one is a perfect match to the allele 1 sequence (e.g., the allele with a mutation). Each probe is labeled with a distinctive fluorescent dye at its 5' end, the Reporter, such as FAM™ or VIC®, and contains a non-fluorescent Quencher at the 3' end. The probes also contain a minor grove binder (MGB™) permitting the use of shorter probes with greater stability and thereby a more accurate allelic discrimination.

During the extension phase of the PCR, the perfectly matched probe is cleaved by the 5'→3' exonuclease activity of *Taq* polymerase, separating the Reporter dye from the Quencher and thus releasing detectable fluorescence. The probe not perfectly matched will be displaced rather than cleaved by the *Taq* polymerase and no reporter dye is released. The fluorescence signal (VIC or FAM) generated is collected at the end of the PCR (end-point) and immediately indicates the presence of the targeted sequence(s) in the sample (wild-type allele, mutated allele or both) without the requirement of long and laborious post-PCR steps, which also increase the contamination risk. The actual quantity of target sequence is not determined.

The *ipsogen* JAK2 Muta*Screen* RS Kit uses this technology as illustrated (Figure 1).

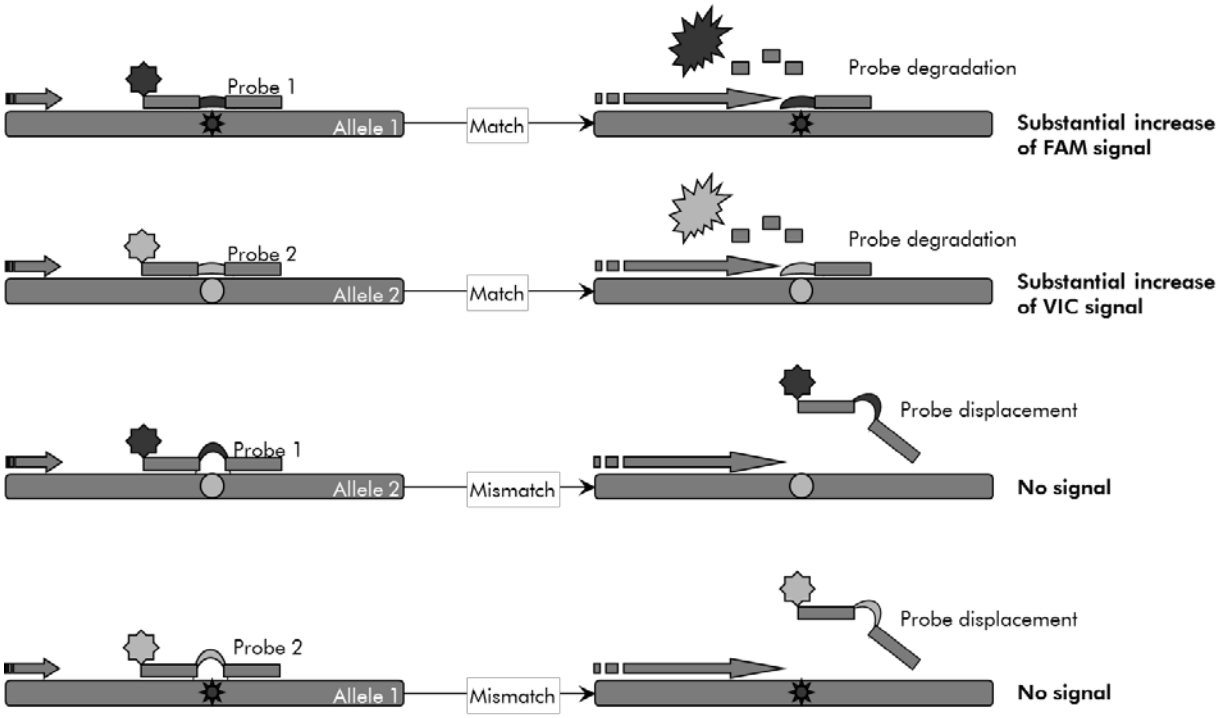

Figure 1. TaqMan probe multiplex assay. The *ipsogen* JAK2 Muta*Screen* RS Kit uses this technology for allelic discrimination.

Materials Provided

Kit contents

***ipsogen* JAK2 Muta*Screen* RS Kit**		**(19)**
Catalog no.		**673113**
Number of reactions		**19**
V617F Positive Control*	MP-VF	30 µl
V617F Negative Control†	MN-VF	30 µl
Reference Scale M1‡	M1-VF	30 µl
Reference Scale M2‡	M2-VF	30 µl
Reference Scale M3‡	M3-VF	30 µl
Reference Scale M4‡	M4-VF	30 µl
Reference Scale M5‡	M5-VF	30 µl
Reference Scale M6‡	M6-VF	30 µl
Primers and probes mix JAK2 V617F§	PPM-VF 10x	145 µl
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* Positive control: 100% V617F DNA.

† Negative control: 100% wild type DNA; 0% V617F DNA.

‡ Reference scale (genomic DNA dilutions).

§ Mix of specific reverse and forward primers for the *JAK2* gene, specific V617F FAM probe and wild type VIC probe.

Note: Briefly centrifuge tubes before use.

Note: Analyzing unknown samples with the *ipsogen* JAK2 Muta*Screen* RS Kit requires the extraction of genomic DNA. Reagents needed to perform DNA extraction (e.g., QIAGEN QIAamp® DNA Mini Kit, cat. no. 51304) are not provided and must be validated in combination with the kit.

Materials Required but Not Provided

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Reagents

- Nuclease-free PCR grade water
- Nuclease-free 1x TE buffer, pH 8.0 (e.g., Thermo Fisher Scientific Inc., cat. no. 12090-015)
- Buffer and *Taq* DNA polymerase: The recommended reagents are TaqMan Universal PCR Master Mix (Master Mix PCR 2x) (Thermo Fisher Scientific Inc., cat. no. 4304437) and LightCycler TaqMan Master (Master Mix PCR 5x) (Roche, cat. no. 04535286001)
- Reagents for 0.8–1% agarose gel in 0.5x TBE electrophoresis buffer

Consumables

- Nuclease-free aerosol-resistant sterile PCR pipet tips with hydrophobic filters
- 0.5 ml or 0.2 ml RNase- and DNase-free PCR tubes
- Ice

Equipment

- Microtiter pipets* dedicated for PCR (1–10 µl; 10–100 µl; 100–1000 µl)
- Benchtop centrifuge* with rotor for 0.2 ml/0.5 ml reaction tubes (capable of attaining 10,000 rpm)
- Spectrophotometer* for DNA quantitation
- Real-time PCR instrument:* Rotor-Gene Q 5plex HRM or other Rotor-Gene instrument; LightCycler 2.0, or 480; Applied Biosystems 7300 Real-Time PCR System, Applied Biosystems 7500 Real-Time PCR System, ABI PRISM 7000 SDS, ABI PRISM 7700 SDS, or ABI PRISM 7900HT SDS; and associated specific material
- Equipment* for pulsed-field gel electrophoresis

* Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

Warnings and Precautions

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Discard sample and assay waste according to your local safety regulations.

General precautions

qPCR tests require good laboratory practices, including equipment maintenance, that are dedicated to molecular biology and compliant with applicable regulations and relevant standards.

This kit is intended for research use. Reagents and instructions supplied in this kit have been tested for optimal performance. Further dilution of the reagents or alteration of incubation times and temperatures may result in erroneous or discordant data. PPM-VF reagent may be altered if exposed to light. All reagents are formulated specifically for use with this kit. For optimal performance of the kit, no substitutions should be made.

Use extreme caution to prevent:

- DNase contamination which might cause degradation of the template DNA
- DNA or PCR carryover contamination resulting in false positive signal

We therefore recommend the following.

- Use nuclease-free labware (e.g., pipets, pipet tips, reaction vials) and wear gloves when performing the assay.
- Use fresh aerosol-resistant pipet tips for all pipetting steps to avoid cross-contamination of the samples and reagents.
- Prepare pre-PCR master mix with dedicated material (pipets, tips, etc.) in a dedicated area where no DNA matrixes (DNA, plasmid) are introduced. Add template in a separate zone (preferably in a separate room) with specific material (pipets, tips, etc.).

Reagent Storage and Handling

The kits are shipped on dry ice and must be stored at –15°C to –30°C upon receipt.

- Minimize exposure to light of the primers and probe mixes (PPM-VF tube).
- Gently mix and centrifuge the tubes before opening.
- Store all kit components in original containers.

These storage conditions apply to both opened and unopened components. Components stored under conditions other than those stated on the labels may not perform properly and may adversely affect the assay results.

Expiration dates for each reagent are indicated on the individual component labels. Under correct storage conditions, the product will maintain performance until the expiration date printed on the label.

There are no obvious signs to indicate instability of this product. However, positive and negative controls should be run simultaneously with unknown specimens.

Procedure

Sample DNA preparation

Genomic DNA should be obtained either from whole blood, purified peripheral blood lymphocytes, polynuclear cells, or granulocytes. To be able to compare results, we recommend adopting the same cellular fraction and DNA extraction method. DNA extraction should be performed by any home brew or commercial method.

DNA quantity is determined by measuring optical density at 260 nm. DNA quality should be assessed by spectrophotometry or gel electrophoresis.

The A_{260}/A_{280} ratio should be 1.7–1.9. Smaller ratios usually indicate contamination by protein or organic chemicals. Electrophoretic analysis on a 0.8–1% agarose gel should allow visualization of the isolated DNA as a distinct band of about 20 kb. A slight smear is acceptable.

The resultant DNA is diluted to 5 ng/µl in TE buffer. The qPCR reaction is optimized for 25 ng of purified genomic DNA.

Storing nucleic acids

For short-term storage of up to 24 hours, we recommend storing purified nucleic acids at 2–8°C. For long-term storage of over 24 hours, we recommend storage at –20°C.

Protocol: qPCR on the Rotor-Gene Q instrument with 72-tube rotor

Using this instrument, we recommend performing all measurements in duplicate, as indicated in Table 1.

Table 1. Number of reactions for the Rotor-Gene Q 5plex HRM instrument with 72-tube rotor

Samples	Reactions
JAK2 V617F primers and probes mix (PPM-VF) (56 reactions)	
19 DNA samples	19 x 2 reactions
2 DNA controls	2 x 2 reactions (MP-VF, MN-VF, each one tested in duplicate)
Reference scale	6 x 2 reactions (M1 to M6, each one tested in duplicate)
Water control	2 reactions

Sample processing on the Rotor-Gene Q instrument with 72-tube rotor

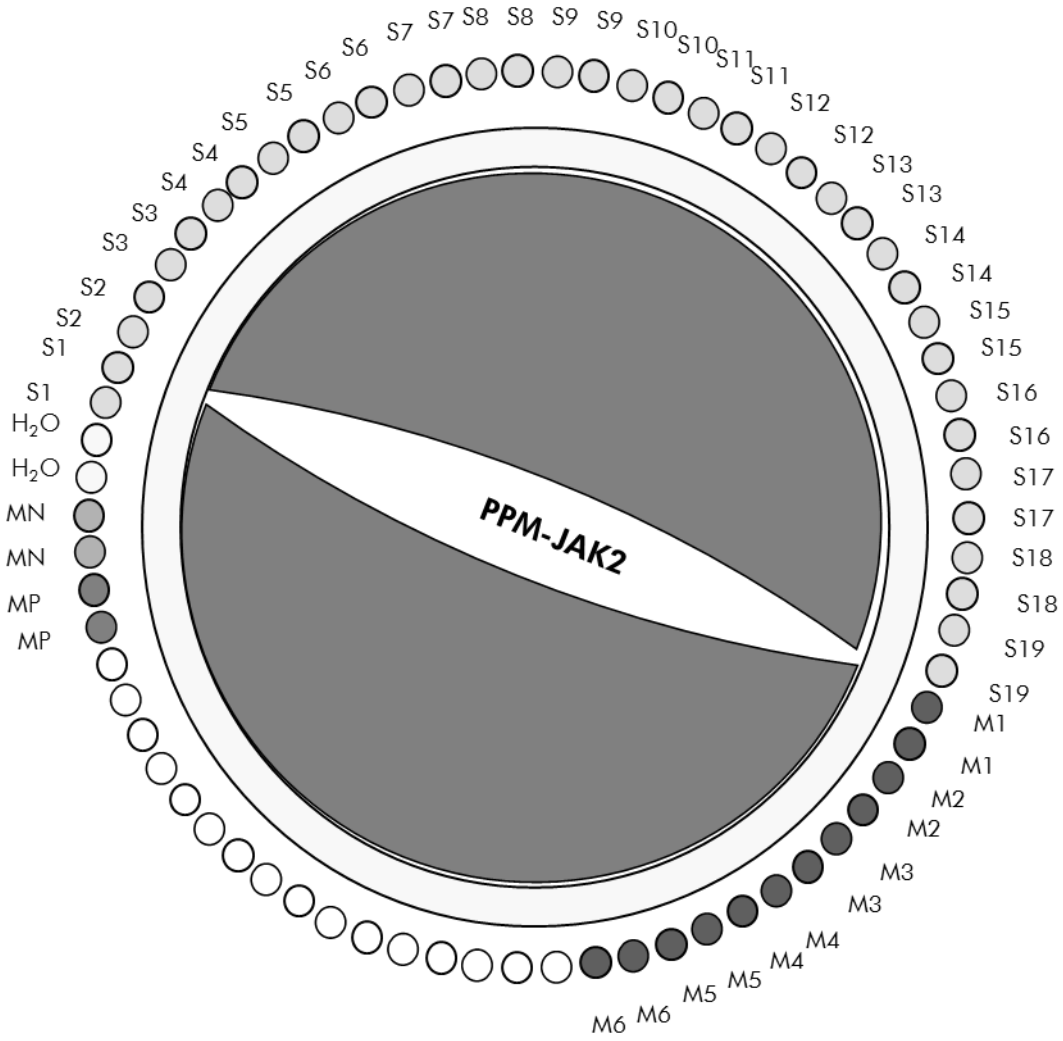


Figure 2. Suggested rotor setup for an experiment with the *ipsogen* JAK2 MutaScreen RS Kit. MP-VF: positive control; **MN-VF**: negative control; **M1 to M6**: reference scale; **S**: DNA sample; **H_2O**: water control.

Note: Take care to always place a sample to be tested in position 1 of the rotor. Otherwise, during the calibration step, the instrument will not perform calibration, and incorrect fluorescence data will be acquired.

Fill all other positions with empty tubes.

qPCR on the Rotor-Gene Q instrument with 72-tube rotor

Note: Perform all steps on ice.

Procedure

1. **Thaw all necessary components and place them on ice.**
   Components should be taken out of the freezer approximately 10 min before starting the procedure.

2. **Vortex and briefly centrifuge all the tubes (approximately 10 s, 10,000 rpm, to collect the liquid in the bottom of the tube).**
3. **Prepare the following qPCR mix according to the number of samples being processed.**

   All concentrations are for the final volume of the reaction.

   Table 2 describes the pipetting scheme for the preparation of one reagent mix, calculated to achieve a final reaction volume of 25 µl. A pre-mix can be prepared, according to the number of reactions, using the same primer and probe mix. Extra volumes are included to compensate for pipetting error.

   On Rotor-Gene instruments, the *ipsogen* JAK2 Muta*Screen* RS Kit can be used for analysis of 19 samples in duplicate in one experiment (Figure 2).

Table 2. Preparation of qPCR mix

Component	Number of reactions (µl)		Final concentration
	1	56+1*	
TaqMan Universal PCR Master Mix, 2x	12.5	712.5	1x
Primers and probes mix, 10x	2.5	142.5	1x
Nuclease-free PCR-grade water	5	285	–
Sample (to be added at step 4)	5	5 each	–
Total volume	25	25 each	–

* 19 samples; 1 experiment/kit.

4. **Vortex and briefly centrifuge the qPCR mix (approximately 10 s, 10,000 rpm, to collect the liquid in the bottom of the tube).**
5. **Dispense 20 µl of the qPCR pre-mix per tube.**
6. **Add 5 µl of the sample DNA material or controls in the corresponding tube (total volume 25 µl).**
7. **Mix gently, by pipetting up and down.**
8. **Close the PCR tubes. Place the tubes in the 72-tube rotor according to the manufacturer's recommendations. Fill all other positions with empty tubes.**

9. **Make sure that the locking ring (accessory of the Rotor-Gene instrument) is placed on top of the rotor to prevent accidental opening of the tubes during the run. Place the rotor in the Rotor-Gene Q instrument according to the manufacturer's recommendations.**
10. **For the detection of JAK2 DNA, create a temperature profile according to the following steps.**

Setting the general assay parameters	**Figures 3, 4**
Amplification of the DNA	**Figure 5**
Adjusting the fluorescence channel sensitivity	**Figure 6**

Please find further information on programming Rotor-Gene Instruments in the instrument user manual. In the illustrations, the software settings are framed in bold black. Illustrations are included for Rotor-Gene Q Instruments.

11. **Start the Rotor-Gene Software. In the "New Run" dialog box, click "New".**

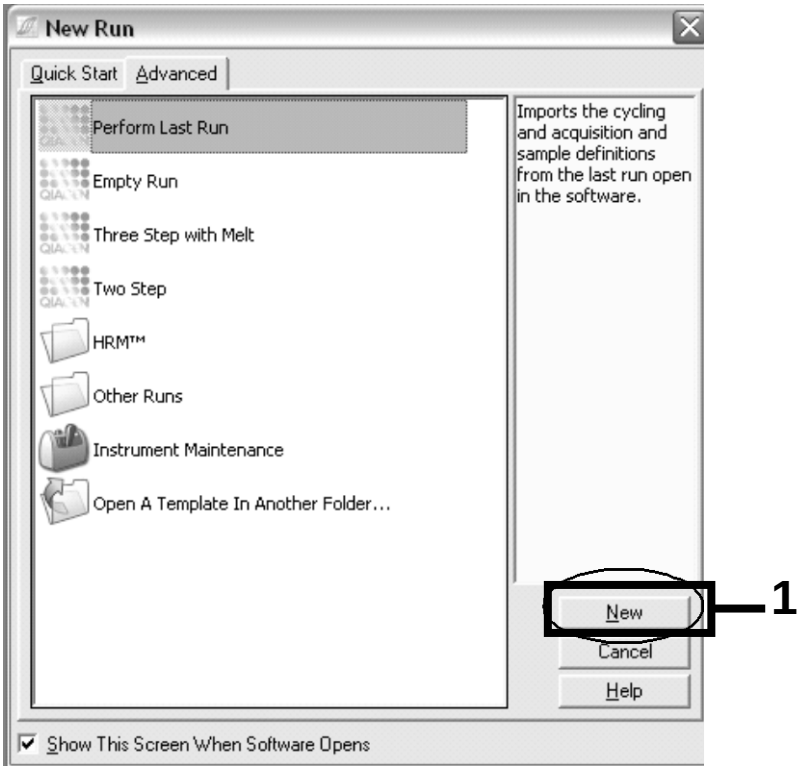


Figure 3. The "New Run" dialog box.

12. **In the "New Run Wizard", set the volume to 25 µl and click "Next".**

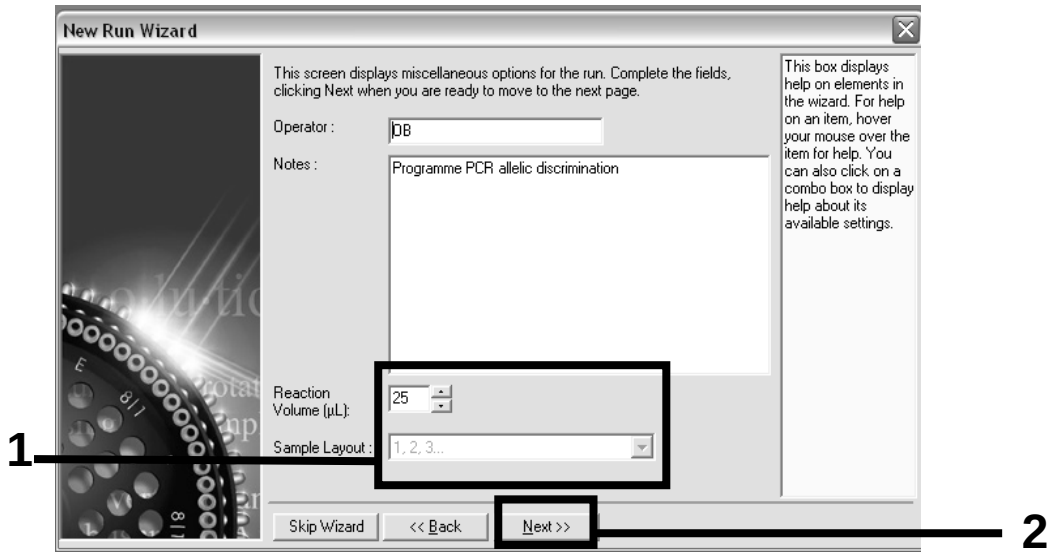


Figure 4. Setting the general assay parameters.

13. Click the "Edit Profile" button in the next "New Run Wizard" dialog box, and program the temperature profile as shown in Table 3 and Figure 5. Be sure to add the last acquiring step at 60°C, at each cycle, for both channels Green (FAM) and Yellow (VIC).

Table 3. Temperature profile

Hold	Temperature: 50 deg Time: 2 mins
Hold 2	Temperature: 95 deg Time: 10 mins
Cycling	50 times 92 deg for 15 secs 60 deg for 1 min; single Acquisition of FAM fluorescence in channel Cycling A Green Acquisition of VIC fluorescence in channel Cycling A Yellow

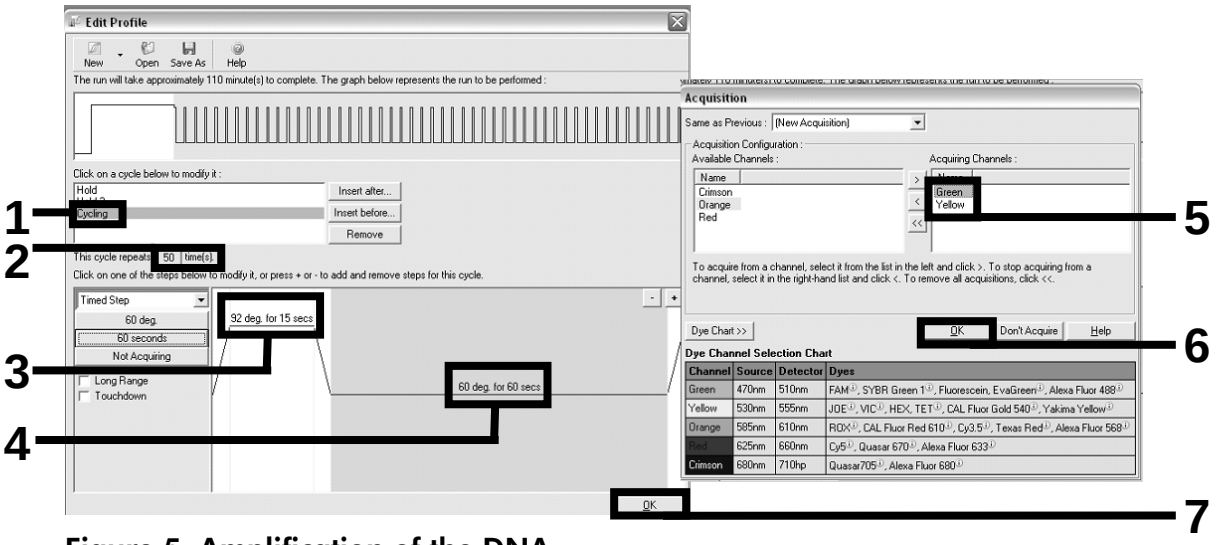


Figure 5. Amplification of the DNA.

14. The detection range of the fluorescence channels has to be determined according to the fluorescence intensities in the PCR tubes. Click "Gain Optimisation" in the "New Run Wizard" dialog box to open the "Auto-Gain Optimisation Setup" dialog box. Click "Optimise Acquiring" (Figure 6), and then click "OK" in the "Auto-Gain Optimisation Channel Settings" dialog boxes for each channel (Green and Yellow, Figure 6). Make sure that the "Perform Optimisation Before 1st Acquisition" box is checked for each channel (Figure 6).

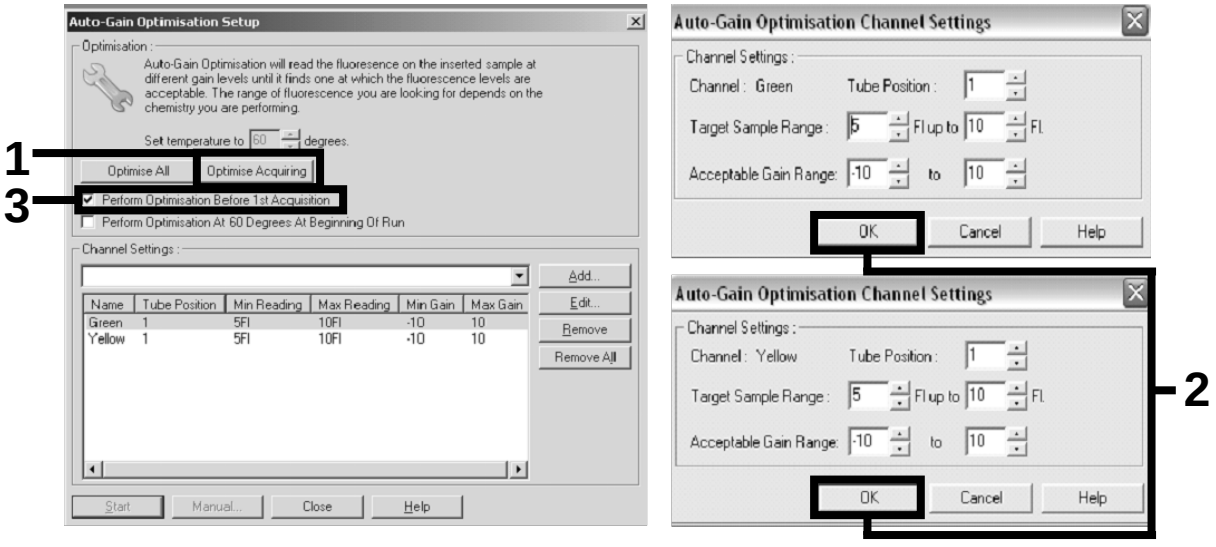


Figure 6. Adjusting the fluorescence channel sensitivity.

15. The gain values determined by the channel calibration are saved automatically and are listed in the last menu window of the programming procedure. Click "Start Run" to run the program.

16. Enter the rotor setup in the Rotor-Gene software (Figure 7).

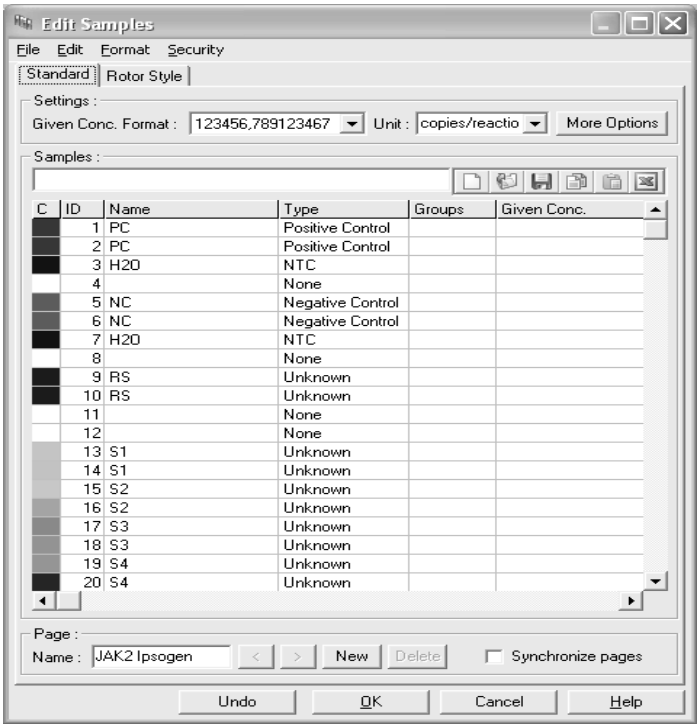


Figure 7. Rotor-Gene setup: "Edit Samples".

End point analysis procedure for Rotor-Gene Q 5plex HRM instrument setting

17. After the PCR program has ended, click "Analysis" in the toolbar (Figure 8).

Figure 8. Analysis.

18. In the "Analysis" dialog box (Figure 9), double-click "Cycling A Green", and then "OK". Repeat for Cycling A yellow.

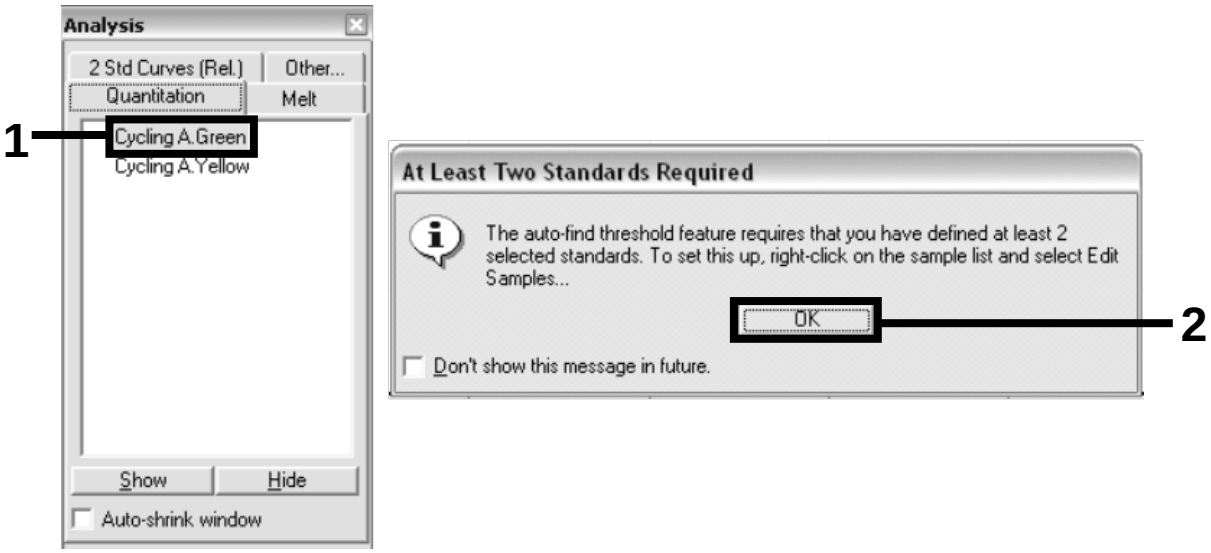


Figure 9. Quantitation: "Cycling A. Green".

19. A new window appears (Figure 10). Click "Slope Correct" in both panels, as shown in Figure 10.

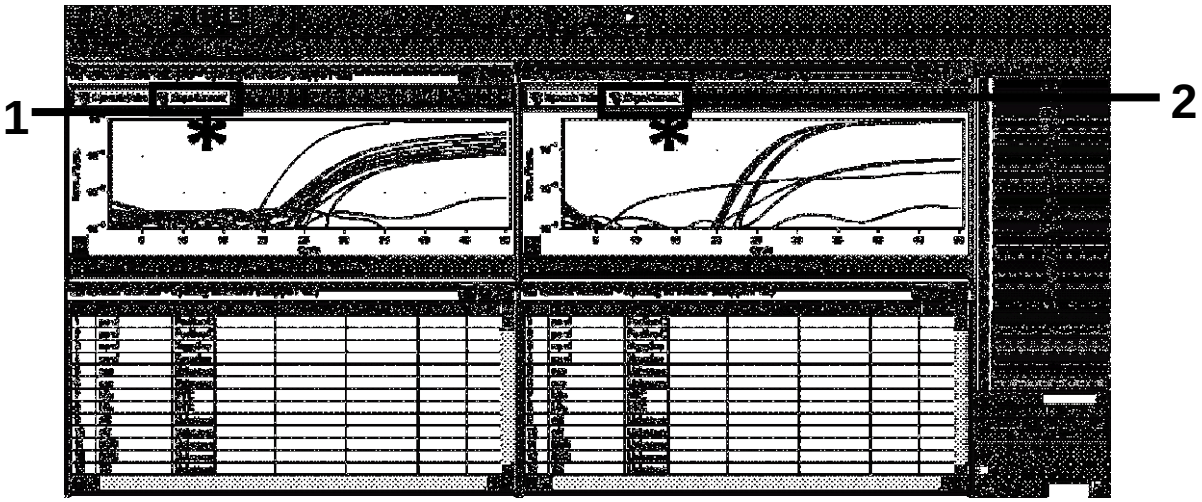


Figure 10. Setting "Slope Correct".

20. To export data, save as an Excel® data sheet. Click "OK", give a name to the export file, and save the text file (*.txt).

21. Open the text file in Excel and select column A. Click "Data", then "Convert", and "Next". Select "Comma" and then click "End". The results will appear as shown in Figure 11.

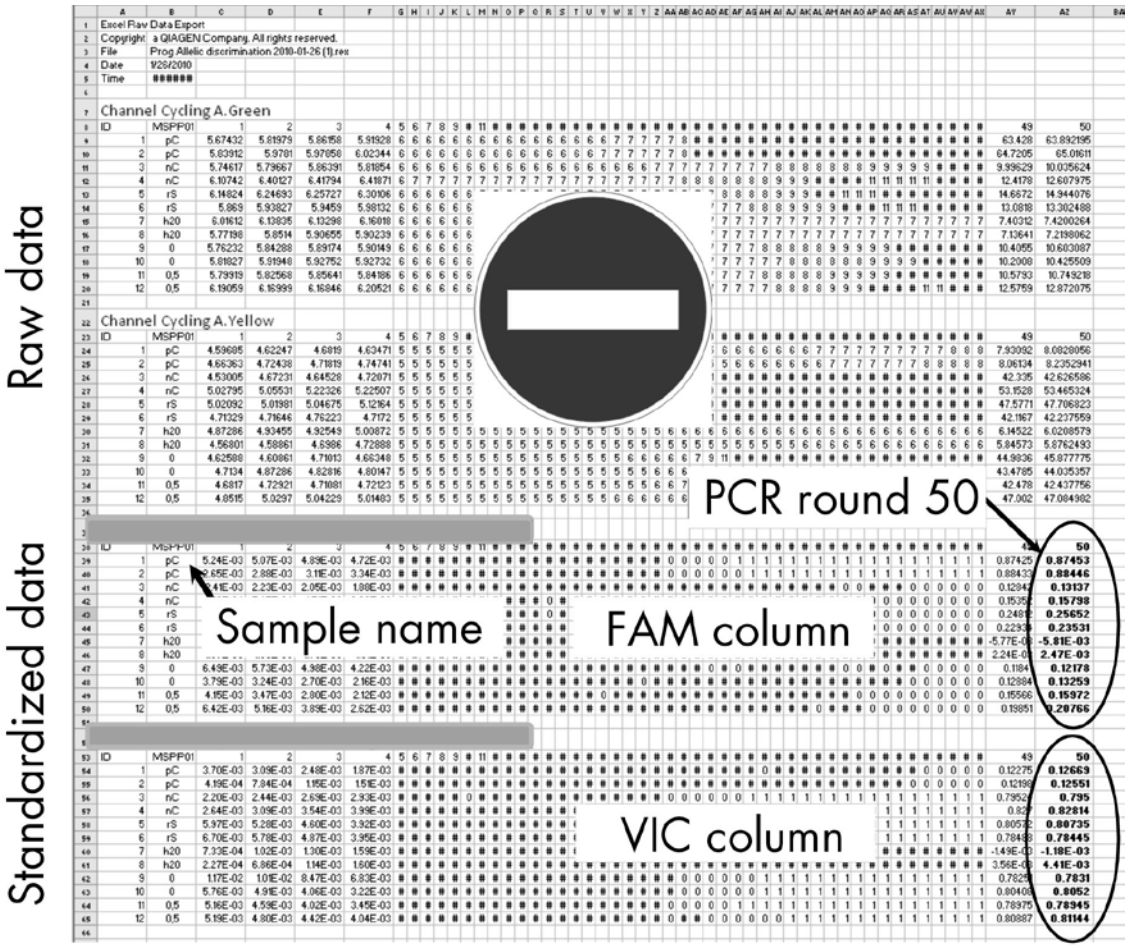


Figure 11. Example of results, shown in Excel file.

Note: The file contains both raw data and standardized data. Only standardized data must be considered.

These data are given in the Quantitative analysis of channel Cycling A Green and Quantitative analysis of channel Cycling A Yellow sections of the table. The data intended for interpretation are those acquired at PCR cycle 50 (in circles on the right).

Protocol: qPCR on Applied Biosystems and ABI PRISM instruments

Using 96 wells plate qPCR equipment, we recommend performing all measurements in duplicate as indicated in Table 4.

Table 4. Number of reactions for Applied Biosystems 7300 and 7500, ABI PRISM 7000, ABI PRISM 7700, or ABI PRISM 7900HT instruments

Samples	Reactions
JAK2 V617F primers and probes mix (PPM-VF) (56 reactions)	
19 DNA samples	19 x 2 reactions
2 DNA controls	2 x 2 reactions (MP-VF, MN-VF, each one tested in duplicate)
Reference scale	6 x 2 reactions (M1 to M6, each one tested in duplicate)
Water control	2 reactions

Sample processing on Applied Biosystems 7300 and 7500, ABI PRISM 7000 and 7700, or ABI PRISM 7900HT instruments

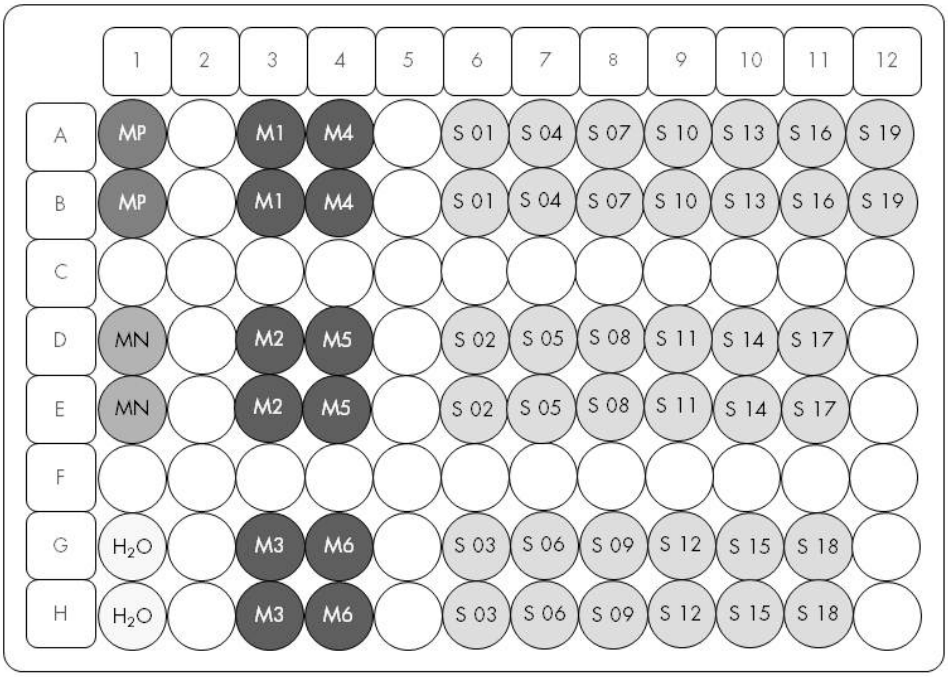


Figure 12. Suggested plate setup for an experiment with the *ipsogen* JAK2 MutaScreen RS Kit. MP: positive control; **MN**: negative control; **M1 to M6**: reference scale; **S**: DNA sample; **H_2O**: water control.

qPCR on Applied Biosystems 7300 and 7500, ABI PRISM 7000, ABI PRISM 7700, or ABI PRISM 7900HT instruments

Note: Perform all steps on ice.

Procedure

1. **Thaw all necessary components and place them on ice.**
   Components should be taken out of the freezer approximately 10 min before starting the procedure.
2. **Vortex and briefly centrifuge all the tubes (approximately 10 s, 10,000 rpm, to collect the liquid in the bottom of the tube).**
3. **Prepare the following qPCR mix according to the number of samples being processed.**
   All concentrations are for the final volume of the reaction.

   Table 5 describes the pipetting scheme for the preparation of one reagent mix, calculated to achieve a final reaction volume of 25 µl. A pre-mix can be prepared, according to the number of reactions, using the same primer and probe mix. Extra volumes are included to compensate for pipetting error.

   On the Applied Biosystems 7300 and 7500, ABI PRISM 7000, ABI PRISM 7700, or ABI PRISM 7900HT instruments, the *ipsogen* JAK2 Muta*Screen* RS Kit can be used for analysis of 19 samples in duplicate in one experiment (Figure 12).

Table 5. Preparation of qPCR mix

Component	Number of reactions (µl)		Final concentration
	1	56+1*	
TaqMan Universal PCR Master Mix, 2x	12.5	712.5	1x
Primers and probes mix, 10x	2.5	142.5	1x
Nuclease-free PCR grade water	5	285	–
Sample (to be added at step 4)	5	5 each	–
Total volume	25	25 each	–

* 19 samples; one experiment/kit.

4. **Vortex and briefly centrifuge the qPCR mix (approximately 10 s, 10,000 rpm, to collect the liquid in the bottom of the tube).**
5. **Dispense 20 µl of the qPCR pre-mix per well.**
6. **Add 5 µl of the sample DNA material or controls in the corresponding well (total volume 25 µl).**
7. **Mix gently, by pipetting up and down.**
8. **Close the plate and briefly centrifuge (300 x *g*, approximately 10 s).**
9. **Place the plate in the thermal cycler according to the manufacturer's recommendations.**
10. **Program the thermal cycler with the thermal cycling program as indicated in Table 6, and start the run.**

Table 6. Temperature profile for Applied Biosystems and ABI PRISM instruments

Hold	Temperature: 50°C Time: 2 minutes
Hold 2	Temperature: 95°C Time: 10 minutes
Cycling	50 times 92°C for 15 seconds 60°C for 1 minute

Post-read run analysis procedure for Applied Biosystems and ABI PRISM instruments

For programming details of the Applied Biosystems 7300 and 7500, ABI PRISM 7000, ABI PRISM 7700, or ABI PRISM 7900HT instruments, refer to the instrument user guide. For a better overview, the software settings are framed in bold black.

11. **After the run is finished, select "Start/Program", and then select "File/New".**
12. **In the "New Document Wizard" dialog box, click the "Assay" drop-down list, and select "Allelic Discrimination" (Figure 13).**
13. **Accept the default settings for the "Container" and "Template" fields ("96-Well Clear" and "Blank Document", Figure 13). In the "Plate Name" field, type *AD Post-read* (Figure 13), and then click "Next>" to access the "Select Markers" dialog box.**

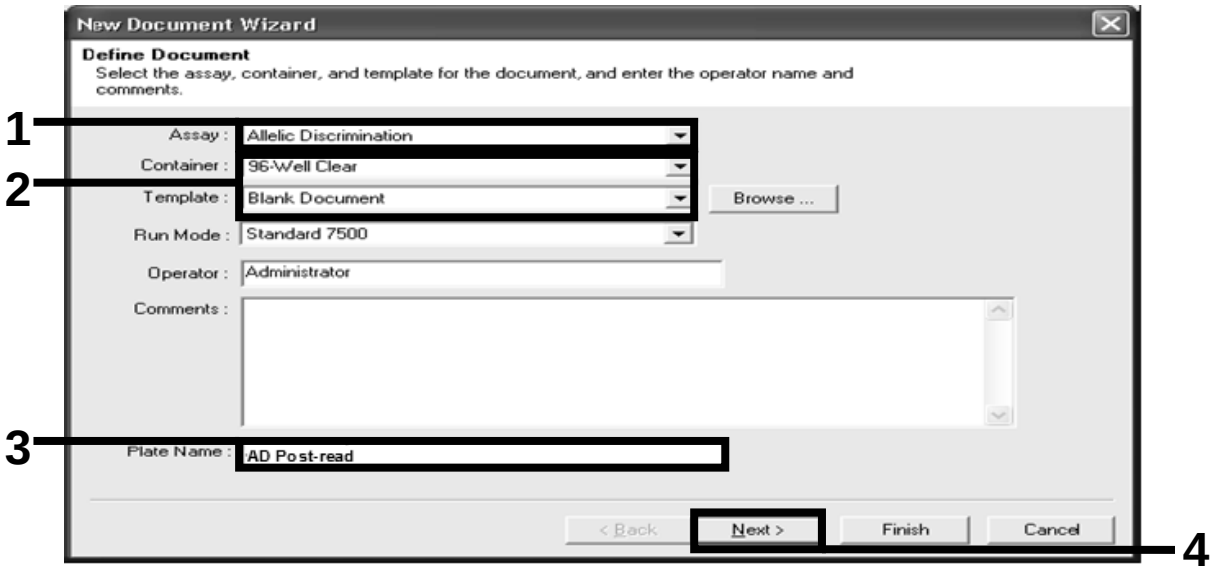


Figure 13. Pre-settings for creating a new post-read run (New Document Wizard).

14. **If the "Markers in Document" panel in the "Select Markers" dialog box contains a suitable marker for your application, proceed with step 18. If not, then continue with step 15.**
15. **Create detectors and markers as follows. Click "New Detector" (Figure 14).**

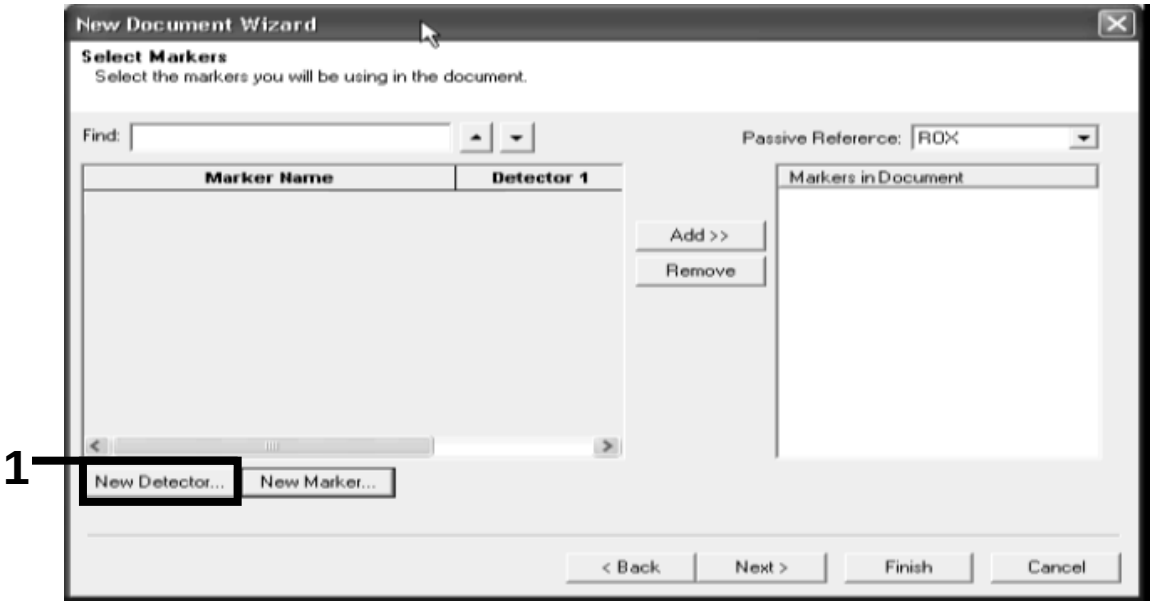


Figure 14. The "Markers in Document" panel does not contain a suitable marker for your application.

16. **In the "New Detector" dialog box, type *Allele A* in the "Name" field (Figure 15). Leave the "Reporter Dye" set to "FAM". Click the "Color" button, select a color, and then click "OK" (Figure 15). Click "Create Another" (Figure 15).**

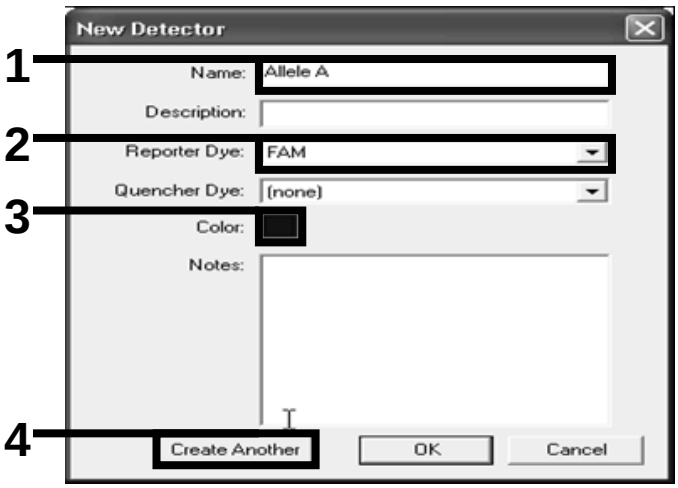


Figure 15. Creating detectors.

17. **In the next "New Detector" dialog box, type *Allele B* in the "Name" field. Select "VIC" in the "Reporter Dye" field. Click the "Color" button, select a color, and then click "OK".**
18. **Click "New Marker" in the "Select Markers" dialog box (see Figure 14).**
19. **In the "New Marker" dialog box, type *JAK2* in the "New Marker Name" field (Figure 16). Select the "Allele A" and "Allele B" detectors as created in steps 16 and 17 (or already defined), and click "OK" (Figure 16).**

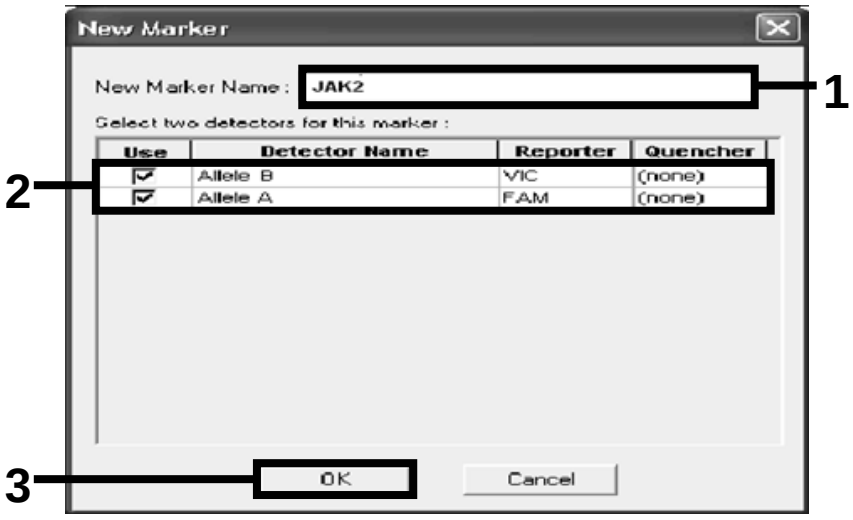


Figure 16. Creating markers.

20. **In the "Select Markers" dialog box, select "JAK2", as created above, or a suitable predefined marker, and then click "Add>>" (Figure 17).**

    Note: To remove a marker, select it and then click "Remove".

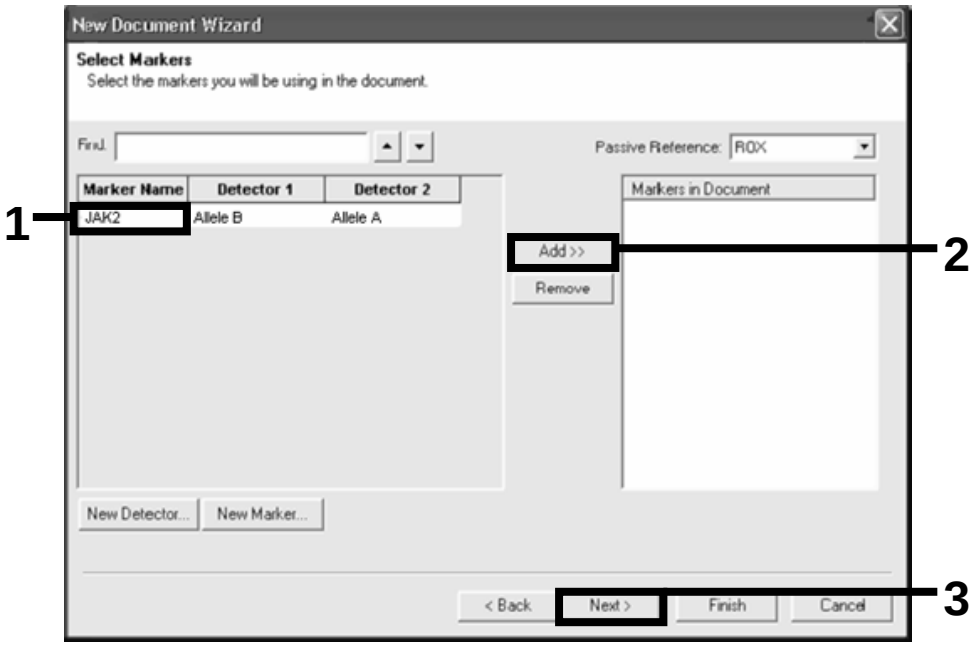


Figure 17. Selecting markers.

21. **Click "Next>".**
22. **In the "Setup Sample Plate" dialog box, click and drag to select the marker for wells that contain samples. Click "Finish".**
23. **Select the "Instrument" tab, and change the sample volume to *25 µl*.**
24. **Select "File/Save" and then click "Save" to retain the name you assigned when you created the plate.**
25. **Load the reaction plate into the instrument according to the manufacturer's recommendations**

26. Start the post-read run. Click "Post-Read".

The instrument will perform a run of 1 cycle for 60 s at 60°C. During this run, the instrument collects FAM and VIC fluorescence in each well (Figure 18).

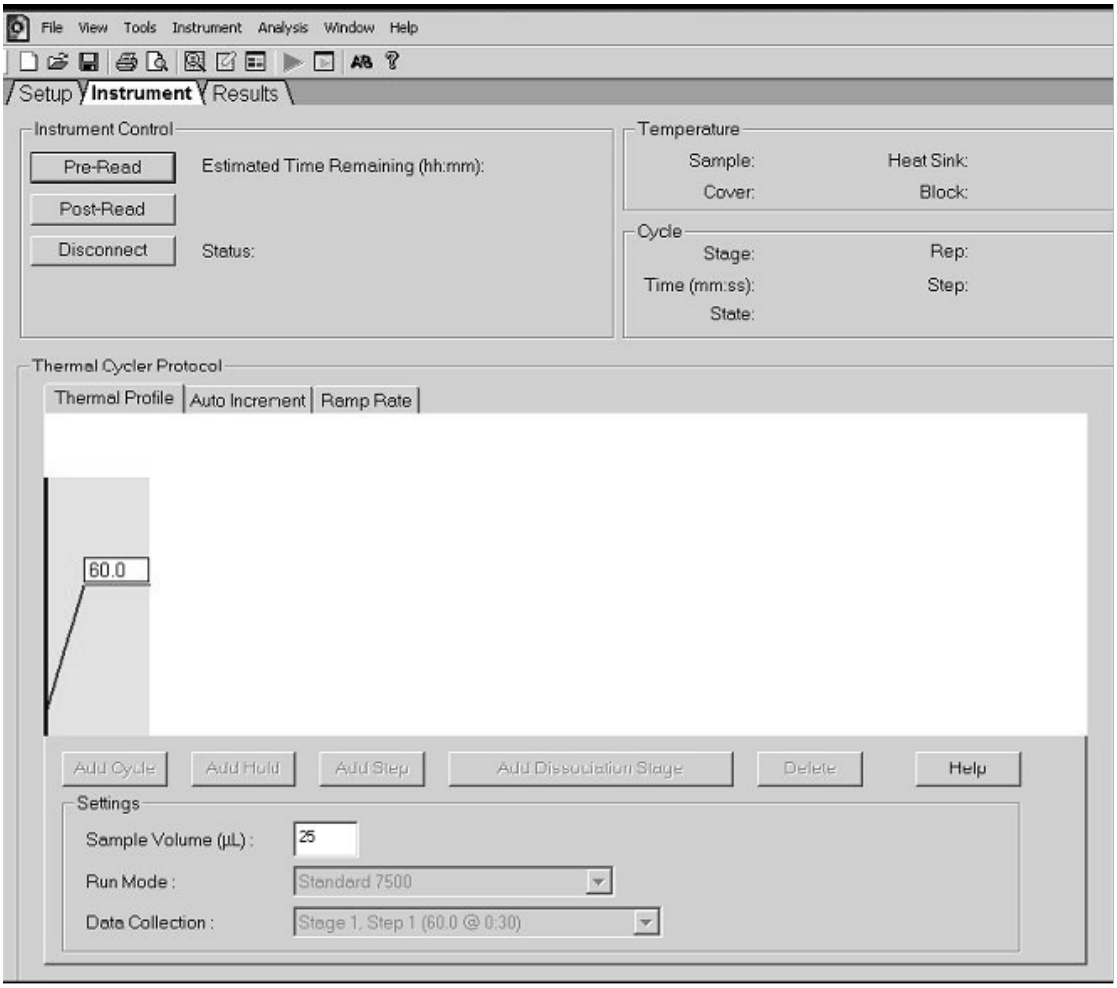


Figure 18. Post-read run.

27. Select "File/Export" and then click "Results" to export the results to an Excel file. The results will appear as shown in Figure 19.

12	Comments:										
13	SDS v1.2										
14											
15	Well	Sample Name	Marker	Task	Passive Ref	Allele X	Allele Y	Allele X Rn	Allele Y Rn	Call	Quality Value	Method
16	A1	sample 1	VIC	Unknown	247.897	JAK2-VIC	JAK2-FAM	2.184	6.221	Undetermined	100.00	Manual Call
17	A2	sample 1	VIC	Unknown	295.565	JAK2-VIC	JAK2-FAM	2.451	6.805	Undetermined	100.00	Manual Call
18	A3	sample 2	VIC	Unknown	351.338	JAK2-VIC	JAK2-FAM	2.595	6.2	Undetermined	100.00	Manual Call
19	A4	sample 2	VIC	Unknown	379.909	JAK2-VIC	JAK2-FAM	2.553	6.01	Undetermined	100.00	Manual Call
20	A5	sample 3	VIC	Unknown	372.895	JAK2-VIC	JAK2-FAM	2.913	5.329	Undetermined	100.00	Manual Call
21	A6	sample 3	VIC	Unknown	359.717	JAK2-VIC	JAK2-FAM	2.806	5.278	Undetermined	100.00	Manual Call
22	A7	sample wt	VIC	Unknown	343.536	JAK2-VIC	JAK2-FAM	2.569	1.948	Undetermined	100.00	Manual Call
23	A8	sample wt	VIC	Unknown	277.677	JAK2-VIC	JAK2-FAM	2.684	2.015	Undetermined	100.00	Manual Call
24	A9	C-	VIC	Unknown	330.943	JAK2-VIC	JAK2-FAM	2.623	1.967	Undetermined	100.00	Manual Call
25	A10	C-	VIC	Unknown	314.623	JAK2-VIC	JAK2-FAM	2.672	2.013	Undetermined	100.00	Manual Call
26	A11	C-	VIC	Unknown	269.500	JAK2-VIC	JAK2-FAM	2.82	1.892	Undetermined	100.00	Manual Call
27	A12	C+	VIC	Unknown	211.520	JAK2-VIC	JAK2-FAM	1.249	6.14	Undetermined	100.00	Manual Call
28	B1	C+	VIC	Unknown	270.623	JAK2-VIC	JAK2-FAM	1.346	6.894	Undetermined	100.00	Manual Call
29	B2	C+	VIC	Unknown	365.112	JAK2-VIC	JAK2-FAM	1.265	6.528	Undetermined	100.00	Manual Call
30	B3	ER	VIC	Unknown	372.150	JAK2-VIC	JAK2-FAM	2.214	2.03	Undetermined	100.00	Manual Call
31	B4	ER	VIC	Unknown	404.145	JAK2-VIC	JAK2-FAM	2.419	2.295	Undetermined	100.00	Manual Call
32	B5	ER	VIC	Unknown	410.977	JAK2-VIC	JAK2-FAM	2.681	2.52	Undetermined	100.00	Manual Call
33	B6	H2O	VIC	Unknown	395.431	JAK2-VIC	JAK2-FAM	0.655	1.346	Undetermined	100.00	Manual Call
34	B7	H2O	VIC	Unknown	415.223	JAK2-VIC	JAK2-FAM	0.727	1.241	Undetermined	100.00	Manual Call
35	B8	H2O	VIC	Unknown	366.885	JAK2-VIC	JAK2-FAM	0.606	1.277	Undetermined	100.00	Manual Call

Figure 19. Example of results, shown in an Excel file.

Protocol: qPCR on the LightCycler 480 instrument

Using 96-well plate qPCR equipment, we recommend performing all measurements in duplicate as indicated in Table 7.

Table 7. Number of reactions for the LightCycler 480 instrument

Samples	Reactions
JAK2 V617F primers and probes mix (PPM-VF) (56 reactions)	
19 DNA samples	19 x 2 reactions
2 DNA controls	2 x 2 reactions (MP-VF, MN-VF, each one tested in duplicate)
Reference scale	6 x 2 reactions (M1 to M6, each one tested in duplicate)
Water control	2 reactions

Sample processing on the LightCycler 480 instrument

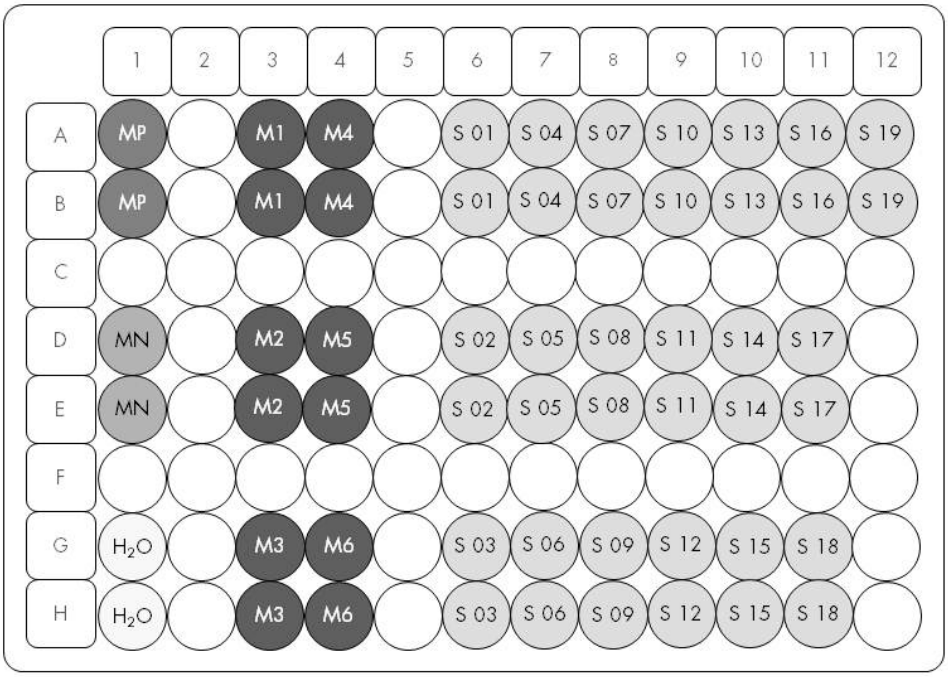


Figure 20. Suggested plate setup for an experiment with the *ipsogen* JAK2 *MutaScreen* RS Kit. MP: positive control; **MN**: negative control; **M1 to M6**: reference scale; **S**: DNA sample; **H_2O**: water control.

qPCR on the LightCycler 480 instrument

Note: Perform all steps on ice.

Procedure

1. **Thaw all necessary components and place them on ice.**
   Components should be taken out of the freezer approximately 10 min before starting the procedure.
2. **Vortex and briefly centrifuge all the tubes (approximately 10 s, 10,000 rpm, to collect the liquid in the bottom of the tube).**
3. **Prepare the following qPCR mix according to the number of samples being processed.**
   All concentrations are for the final volume of the reaction.

   Table 8 describes the pipetting scheme for the preparation of one reagent mix, calculated to achieve a final reaction volume of 25 µl. A pre-mix can be prepared, according to the number of reactions, using the same primer and probe mix. Extra volumes are included to compensate for pipetting error.

   On the Applied Biosystems 7300 and 7500, ABI PRISM 7000, ABI PRISM 7700, or ABI PRISM 7900HT instruments, the *ipsogen* JAK2 Muta*Screen* RS Kit can be used for analysis of 19 samples in duplicate in one experiment (Figure 20).

Table 8. Preparation of qPCR mix

Component	Number of reactions (µl)		Final concentration
	1	56+1*	
TaqMan Universal PCR Master Mix, 2x	12.5	712.5	1x
Primers and probes mix, 10x	2.5	142.5	1x
Nuclease-free PCR-grade water	5	285	–
Sample (to be added at step 4)	5	5 each	–
Total volume	25	25 each	–

* 19 samples; one experiment/kit.

4. **Vortex and briefly centrifuge the qPCR mix (approximately 10 s, 10,000 rpm, to collect the liquid in the bottom of the tube).**

5. **Dispense 20 µl of the qPCR pre-mix per well.**
6. **Add 5 µl of the sample DNA material or controls in the corresponding well (total volume 25 µl).**
7. **Mix gently, by pipetting up and down.**
8. **Close the plate and briefly centrifuge (300 x *g*, approximately 10 s).**
9. **Place the plate in the thermal cycler according to the manufacturer's recommendations.**
10. **On the home page, select "New Experiment".**
11. **For the LightCycler 480 I, follow step 11a. For the LightCycler 480 II, follow step 11b.**

    For programming details of the LightCycler 480 instrument, refer to the instrument user guide. For a better overview, the software settings are framed in bold black.

11a. LightCycler 480 I: Select "Multi Color Hydrolysis Probe", click "Customize", and then check that the channels "FAM (483–533)" and "Hex (533–568)" (i.e., VIC) are selected (Figure 21). Set the reaction volume to "25" µl (Figure 21) and proceed with step 12.

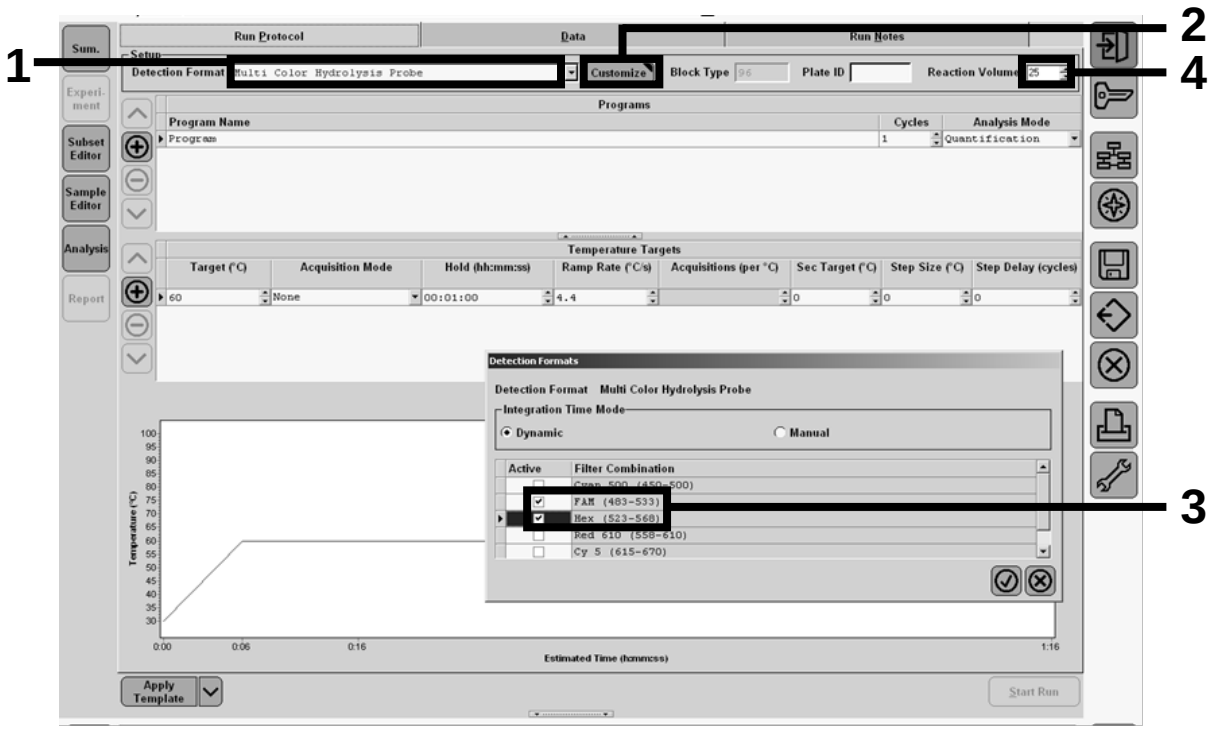


Figure 21. LightCycler 480 I: Setting the detection format.

11b. LightCycler 480 II: Select "Dual Color Hydrolysis Probe", click "Customize", and then check that the channels" FAM (465–510)" and "VIC / HEX / (533–580)" are selected (Figure 22). Set the reaction volume to "25" µl (Figure 22) and proceed with step 12.

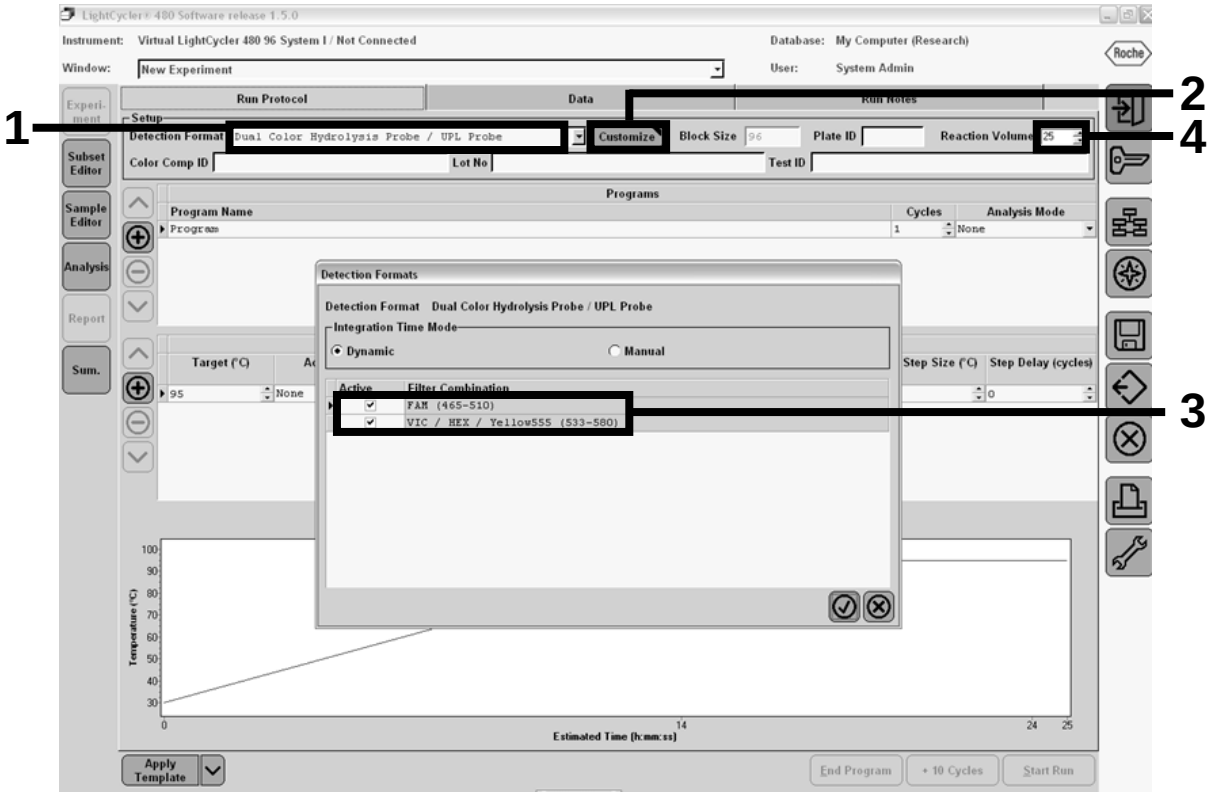


Figure 22. LightCycler 480 II: Setting the detection format.

12. Program the thermal cycler with the thermal cycling program as indicated in Table 9, and start the run.

Note: When describing the plate setup on the instrument, select "Endpt Geno" in the "Step 1: select workflow" section.

Table 9. Temperature profile for the LightCycler 480 instrument

Hold	Temperature: 50°C Time: 2 minutes
Hold 2	Temperature: 95°C Time: 10 minutes
Cycling	50 times 92°C for 15 seconds; single 60°C for 1 minute; single
Hold 3	60°C for 1 minute; single

End-point analysis procedure for the LightCycler 480 instrument

13. **After the run is finished, click "Analysis".**
14. **In the "Create New Analysis" dialog box, select "Endpoint Genotyping", and then select the subset to analyze in the "Subset" menu (Figure 23).**

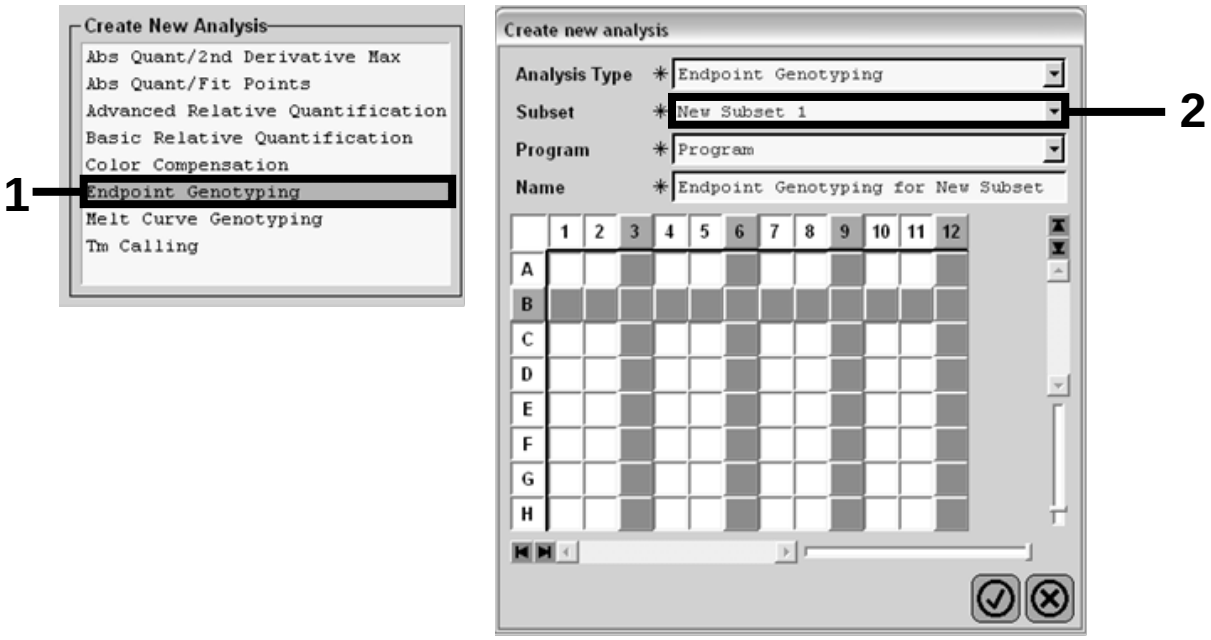


Figure 23. Selecting analysis type and subset to analyze.

15. **In the next window, select "Hex" (i.e., VIC) fluorescence for "Allele X" and "FAM" fluorescence for "Allele Y" (Figure 24).**

Figure 24. Selecting fluorescence for "Allele X" and "Allele Y".

16. **The next window (Figure 25) shows plate setup (1, upper left), fluorescence results for each sample (2, bottom left), and the scatter plot with allelic discrimination (3, right; FAM and VIC fluorescence measured at the 50th PCR cycle).**

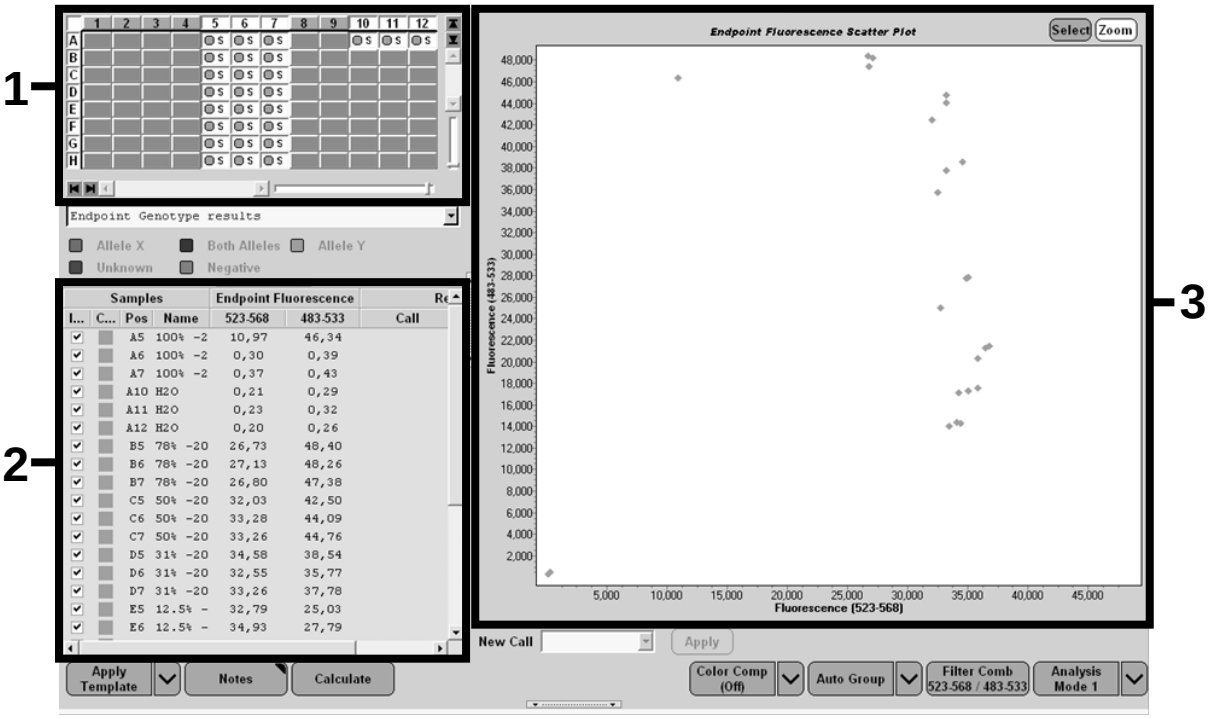
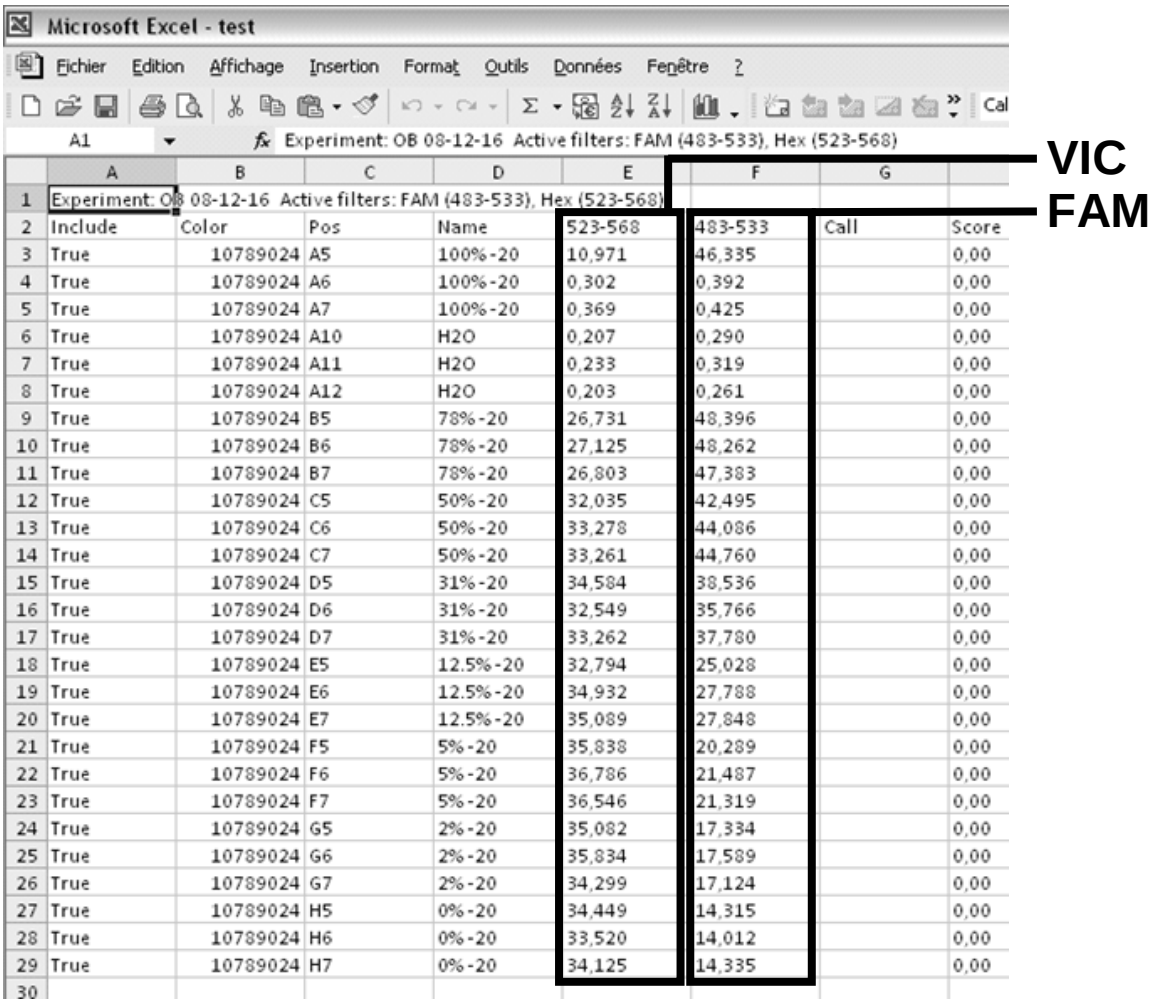


Figure 25. Data summary.

17. **To export data, right-click on the sample results template, and then select "Export Table". The file will be saved in a text (.txt) file format.**

18. **To view and analyze results, open the file using Excel. The results will appear as shown in Figure 26.**



	A	B	C	D	E	F	G	
1	Experiment: OB 08-12-16 Active filters: FAM (483-533), Hex (523-568)							
2	Include	Color	Pos	Name	523-568	483-533	Call	Score
3	True	10789024	A5	100%-20	10,971	46,335		0,00
4	True	10789024	A6	100%-20	0,302	0,392		0,00
5	True	10789024	A7	100%-20	0,369	0,425		0,00
6	True	10789024	A10	H2O	0,207	0,290		0,00
7	True	10789024	A11	H2O	0,233	0,319		0,00
8	True	10789024	A12	H2O	0,203	0,261		0,00
9	True	10789024	B5	78%-20	26,731	48,396		0,00
10	True	10789024	B6	78%-20	27,125	48,262		0,00
11	True	10789024	B7	78%-20	26,803	47,383		0,00
12	True	10789024	C5	50%-20	32,035	42,495		0,00
13	True	10789024	C6	50%-20	33,278	44,086		0,00
14	True	10789024	C7	50%-20	33,261	44,760		0,00
15	True	10789024	D5	31%-20	34,584	38,536		0,00
16	True	10789024	D6	31%-20	32,549	35,766		0,00
17	True	10789024	D7	31%-20	33,262	37,780		0,00
18	True	10789024	E5	12.5%-20	32,794	25,028		0,00
19	True	10789024	E6	12.5%-20	34,932	27,788		0,00
20	True	10789024	E7	12.5%-20	35,089	27,848		0,00
21	True	10789024	F5	5%-20	35,838	20,289		0,00
22	True	10789024	F6	5%-20	36,786	21,487		0,00
23	True	10789024	F7	5%-20	36,546	21,319		0,00
24	True	10789024	G5	2%-20	35,082	17,334		0,00
25	True	10789024	G6	2%-20	35,834	17,589		0,00
26	True	10789024	G7	2%-20	34,299	17,124		0,00
27	True	10789024	H5	0%-20	34,449	14,315		0,00
28	True	10789024	H6	0%-20	33,520	14,012		0,00
29	True	10789024	H7	0%-20	34,125	14,335		0,00
30								

Figure 26. Example of results, shown in an Excel file.

Protocol: qPCR on the LightCycler 2.0 instrument

Note: Because of particular technological requirements, LightCycler 2.0 experiments must be performed using specific reagents. We recommend the use of the LightCycler TaqMan Master. Follow the manufacturer's instructions to prepare the Master Mix 5x.

Using a 32-capillary rotor, we recommend performing all measurements in duplicate as indicated in Table 10.

Table 10. Number of reactions for the LightCycler 2.0 instrument

Samples	Reactions
JAK2 V617F primers and probes mix (PPM-VF) (32 reactions)	
7 DNA samples	7 x 2 reactions
2 DNA controls	2 x 2 reactions (MP-VF, MN-VF, each one tested in duplicate)
Reference scale	6 x 2 reactions (M1 to M6, each one tested in duplicate)
Water control	2 reactions

Sample processing on the LightCycler 2.0 instrument

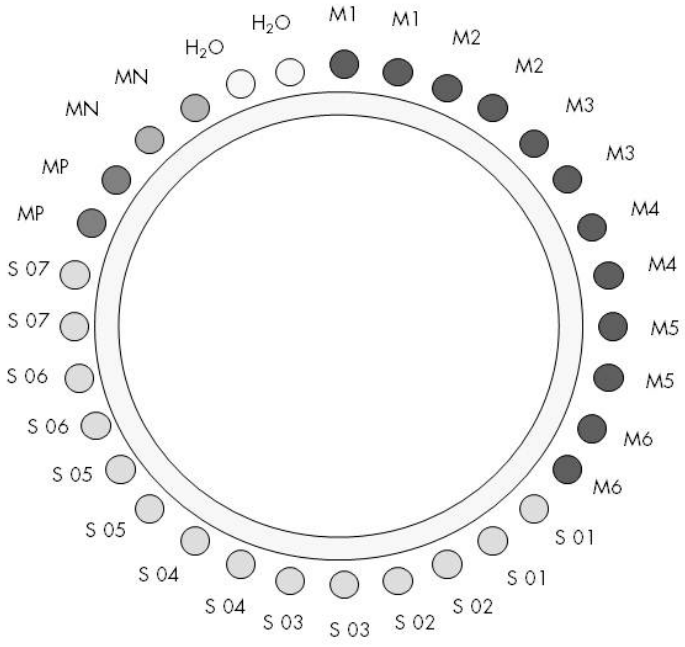


Figure 27. Suggested rotor setup for an experiment with the *ipsogen* JAK2 *MutaScreen* RS Kit. MP: positive control; **MN**: negative control; **M1 to M6**: reference scale; **S**: DNA sample; **H_2O**: water control.

qPCR on the LightCycler 2.0 instrument

Note: Perform all steps on ice.

Procedure

1. **Thaw all necessary components and place them on ice.**
   Components should be taken out of the freezer approximately 10 min before starting the procedure.
2. **Vortex and briefly centrifuge all the tubes (approximately 10 s, 10,000 rpm, to collect the liquid in the bottom of the tube).**
3. **Prepare the following qPCR mix according to the number of samples being processed.**
   All concentrations are for the final volume of the reaction.

   Table 11 describes the pipetting scheme for the preparation of one reagent mix, calculated to achieve a final reaction volume of 20 µl. A pre-mix can be prepared, according to the number of reactions, using the same primer and probe mix. Extra volumes are included to compensate for pipetting error.

   On the LightCycler 2.0 instrument, the *ipsogen* JAK2 Muta*Screen* RS Kit can be used for analysis of 7 samples in duplicate in one experiment (Figure 27).

Table 11. Preparation of qPCR mix for the LightCycler 2.0 instrument

	Number of reactions (µl)		
Component	**1**	**32+1***	**Final concentration**
LightCycler TaqMan Master Mix, 5x	4	132	1x
Primers and probes mix, 10x	2	66	1x
Nuclease-free PCR-grade water	9	297	–
Sample (to be added at step 4)	5	5 each	–
Total volume	20	20 each	–

* 14 samples; 2 experiments/kit.

4. **Vortex and briefly centrifuge the qPCR mix (approximately 10 s, 10,000 rpm, to collect the liquid in the bottom of the tube).**
5. **Dispense 15 µl of the qPCR pre-mix per capillary.**
6. **Add 5 µl of the sample DNA material or controls in the corresponding capillary (total volume 20 µl).**
7. **Mix gently, by pipetting up and down.**
8. **Place the capillaries in the adapter provided with the instrument and briefly centrifuge (700 x *g*, approximately 10 s).**
9. **Load the samples in the thermal cycler according to the manufacturer's recommendations.**
10. **Program the thermal cycler (Figure 28) with the program as indicated in Table 12.**

    For programming details of the LightCycler 2.0 instrument, refer to the instrument user guide. For a better overview, the software settings are framed in bold black.

    Note: Make sure that the setting is for Quantification and single acquisition of FAM fluorescence and single acquisition of VIC fluorescence in both the amplification/cycling step and the final hold at 60°C.

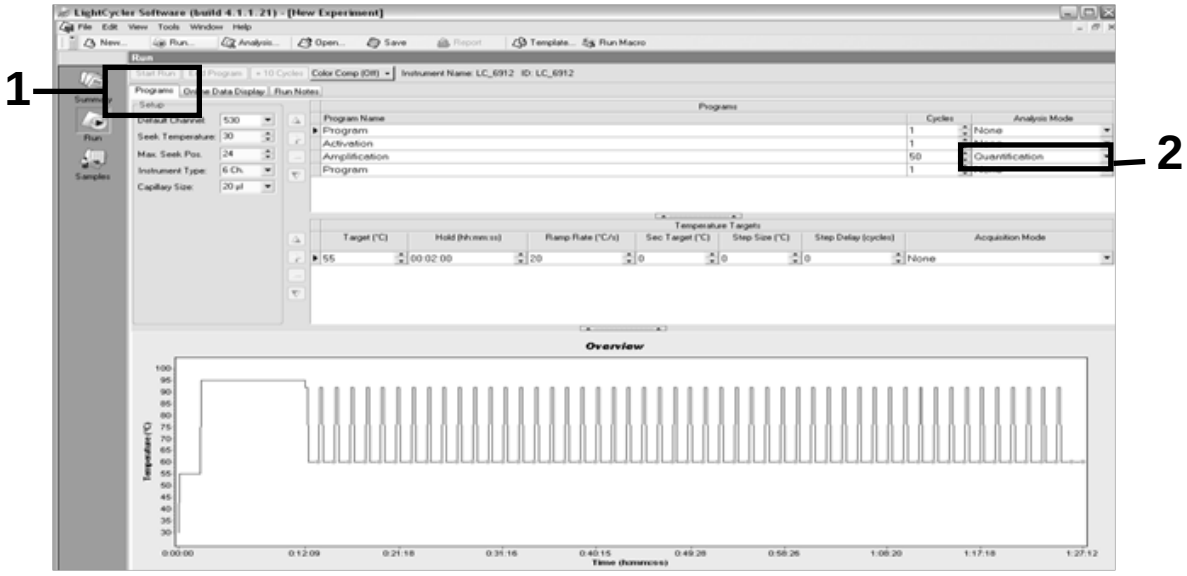


Figure 28. Programming screen for LightCycler 2.0.

Table 12. Temperature profile for the LightCycler 2.0 instrument

Hold	Temperature: 55°C Time: 2 minutes Ramp: 20
Hold 2	Temperature: 95°C Time: 10 minutes Ramp: 20
Cycling	50 times 92°C for 15 seconds; ramp: 20 60°C for 1 minute; ramp 20
Hold 3	60°C for 1 minute; ramp 20

End-point analysis procedure for the LightCycler 2.0 instrument

11. At the end of the amplification run, click the tab for "Online Data Display" (Figure 29). Open the display menu on the top left of the "Current Fluorescence" window, then write *51* in "Acquisition no.".

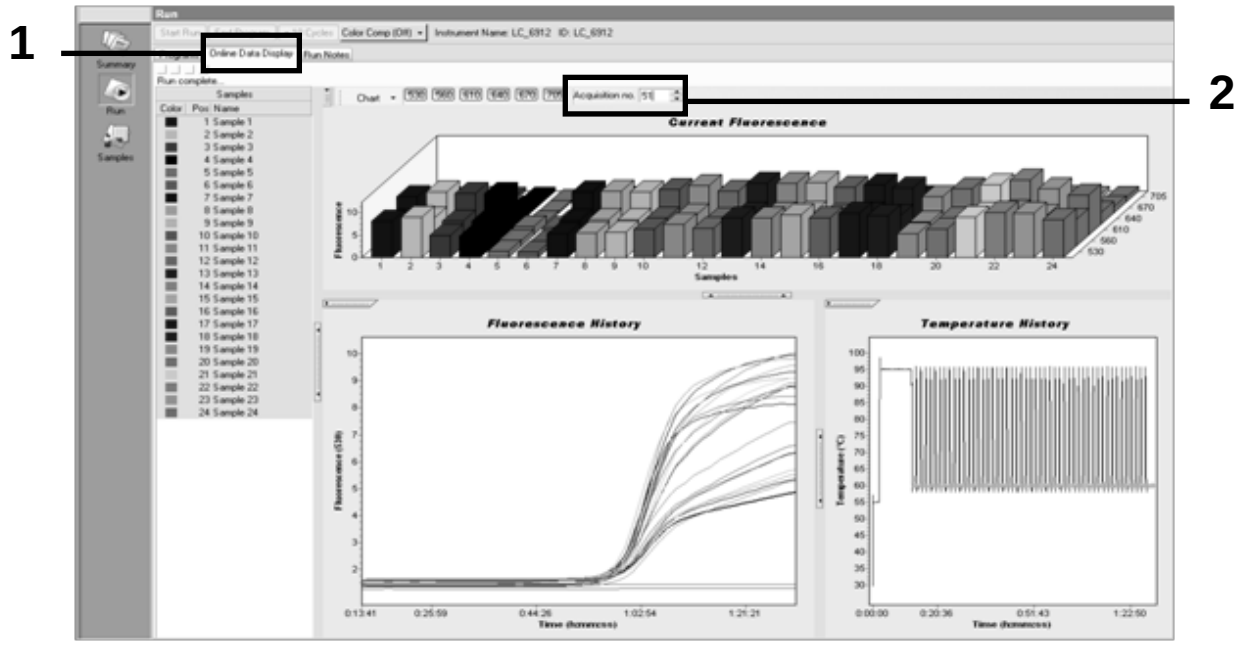


Figure 29. Results and history in Online Data Display.

12. Right-click near the "Current Fluorescence" graph and select "Export".

13. Click the "Excel" box on the "Export chart" dialog box (Figure 30). Enter a name in the "Filename" dialog field. Select an export destination for the result file with the ... button. Click "Export".

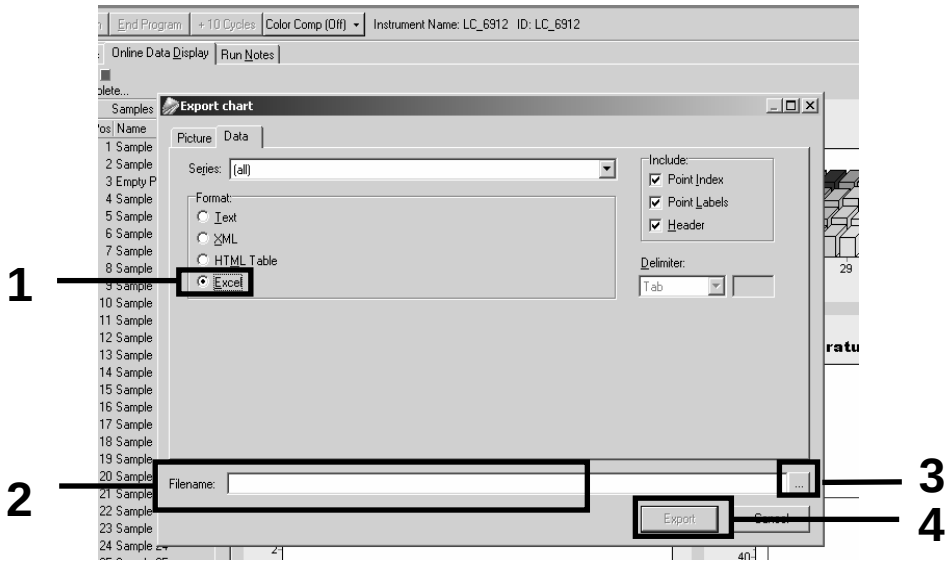


Figure 30. Selecting the export format and data file destination.

14. To view and analyze results, open the file in Excel. The results for LightCycler 2.0 will appear as shown.

	I	J	K	L	M	N	O	P	Q	R	S	T	U
	X	Bar	Text	X	Bar	Text	X	Bar	Text		Bar		
1	1	2,9709	1: Sample 1 (610)	1	8,2734	1: Sample 1 (560)	1	6,6361	1: Sample 1 (530)	1	4,9943		
2	2	3,0182	2: Sample 2 (610)	2	8,4428	2: Sample 2 (560)	2	6,7659	2: Sample 2 (530)	2	5,0767		
3	3	2,9496	3: Sample 3 (610)			3: Sample 3 (560)	3	6,5568	3: Sample 3 (530)	3	4,9699		
4	4	2,9526	4: Sample 4 (610)	4	8,2887	4: Sample 4 (560)	4	6,6163	4: Sample 4 (530)	4	4,9119		
5	5	2,9450	5: Sample 5 (610)	5	8,2689	5: Sample 5 (560)	5	6,6209	5: Sample 5 (530)	5	4,9638		
6	6	2,9969	6: Sample 6 (610)	6	8,4184	6: Sample 6 (560)	6	6,7674	6: Sample 6 (530)	6	5,1209		
7	7	3,0045	7: Sample 7 (610)	7	8,4520	7: Sample 7 (560)	7	6,7506	7: Sample 7 (530)	7	5,0507		
8	8	3,2822	8: Sample 8 (610)	8	9,1936	8: Sample 8 (560)	8	7,3960	8: Sample 8 (530)	8	5,5314		
9	9	3,0274	9: Sample 9 (610)	9	8,5557	9: Sample 9 (560)	9	6,8437	9: Sample 9 (530)	9	5,0843		
10	10	2,8336	10: Sample 10 (610)	10	7,9713	10: Sample 10 (560)	10	6,3905	10: Sample 10 (530)	10	4,7883		
11	11	2,8275	11: Sample 11 (610)	11	7,9774	11: Sample 11 (560)	11	6,3874	11: Sample 11 (530)	11	4,7669		
12	12	2,8351	12: Sample 12 (610)	12	8,0171	12: Sample 12 (560)	12	6,4118	12: Sample 12 (530)	12	4,7944		
13	13	2,9511	13: Sample 13 (610)	13	8,3726	13: Sample 13 (560)	13	6,6957	13: Sample 13 (530)	13	4,9699		
14	14	2,8367	14: Sample 14 (610)	14	8,0217	14: Sample 14 (560)	14	6,4439	14: Sample 14 (530)	14	4,7654		
15	15	2,9908	15: Sample 15 (610)	15	8,4337	15: Sample 15 (560)	15	6,7445	15: Sample 15 (530)	15	5,0523		
16	16	2,8885	16: Sample 16 (610)	16	8,1498	16: Sample 16 (560)	16	6,5568	16: Sample 16 (530)	16	4,9577		
17	17	3,0152	17: Sample 17 (610)	17	8,4901	17: Sample 17 (560)	17	6,8193	17: Sample 17 (530)	17	5,1225		

Position

VIC

FAM

Figure 31. Example of LightCycler 2.0 results, shown in an Excel file.

Results

Obtain a file suitable to extract exported data for all instruments: Rotor-Gene Q 5plex HRM or other Rotor-Gene instrument, LightCycler 2.0, or 480; Applied Biosystems 7300 or 7500 Real-Time PCR System, ABI PRISM 7000 SDS, 7700 SDS, or 7900HT SDS, and check the fluorescence levels (these must be consistent between duplicates).

Prepare a graphical representation (scatter plot) of fluorescence data. The x axis is VIC fluorescence; the y axis is FAM fluorescence.

Graphical representation and quality control criteria

An example of a scatter plot is shown in Figure 32.

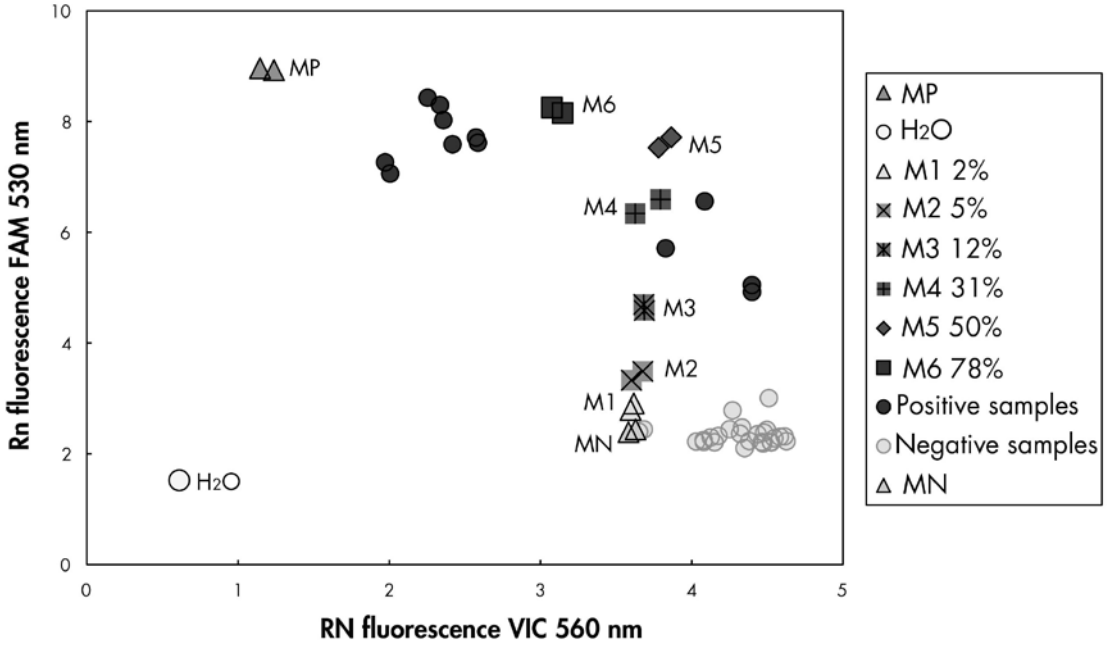


Figure 32. Scatter plot of a representative allelic discrimination experiment.
Instruments: Rotor-Gene Q, Applied Biosystems, ABI PRISM, and LightCycler 480.

Samples should be located on the arc connecting the negative controls (MN) to the positive controls (MP).

Improper positioning of any control may indicate an experimental error.

- Positive controls should be located in the upper left.
- Negative controls should be located in the bottom right.
  - Poor positioning of a negative control may indicate contamination.
- The cut-off sample (M1 of the reference scale) should appear above the negative controls.

- Water controls should be located at the bottom left.
  - Poor positioning of a water control (higher than MN for FAM measurement or higher than MP for VIC) may indicate contamination.

Note: Positioning of the controls may be different on analysis of LightCycler 2.0 instrument data (Figure 33). The water controls should still be located at the bottom left.

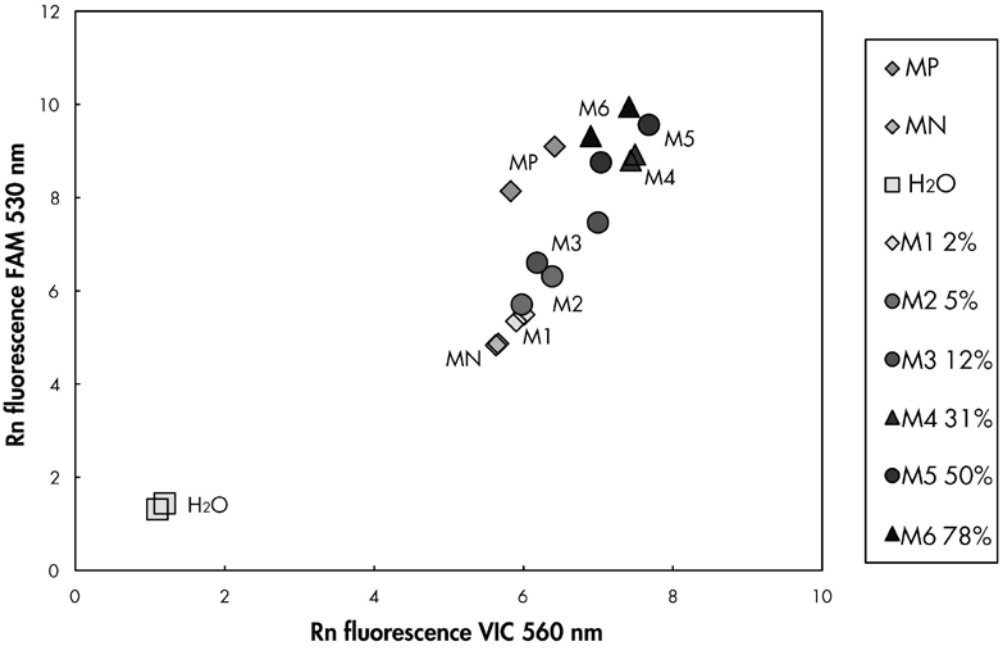


Figure 33. Scatter plot of a representative allelic discrimination experiment. Instrument: LightCycler 2.0.

Calculation of normalized FAM/VIC ratio and genotyping

Calculate the FAM/VIC ratios for all the samples. Calculate the FAM/VIC ratios for the positive control (MP), the cut-off sample (M1), the negative control (MN), and the reference scale (M2 to M6). The ratios must be consistent between duplicates. Calculate the average ratio of all duplicates.

Calculate the normalized ratio (NRatio) for the cut-off sample (M1) and for all the samples:

$$NRatio_{Sample} = \frac{Ratio_{Sample}}{Ratio_{MN}}$$

Note: The gray zone (GZ) of a test is defined as an area of values where the discriminatory performance is insufficiently accurate. A value in the gray zone indicates that the target marker cannot be scored as either present or absent. The gray zone must be calculated for each experiment.

Calculate the gray zone, or the incertitude area, around the normalized ratio of the cut-off sample (M1) ($NRatio_{M1}$):

$$GZ: [(NRatio_{M1} \times 0.94); (NRatio_{M1} \times 1.06)]$$

Compare the Normalized Ratio of each sample to the $NRatio_{M1}$ GZ. Interpretation of results is outlined in Table 14.

Table 14. Interpretation of genotyping results using normalized ratios

Results	Interpretation
$NRatio_{Sample} > NRatio_{M1} \times 1.06$	JAK2 V617F is detected
$NRatio_{Sample} < NRatio_{M1} \times 0.94$	JAK2 V617F is not detected
$NRatio_{Sample}$ within $NRatio_{M1}$ GZ	Result inconclusive

A semiquantitative result for the mutation burden can be obtained by comparing the value of each unknown sample ratio ($Ratio_{Sample}$) with the mean ratio values of the reference scale ($Ratio_{M1–6}$) (see Table 15).

Table 15. Semiquantitative values for the JAK2 V617F mutation burden using the reference scale

Results	Mutation burden
$Ratio_{M1} < Ratio_{Sample} < Ratio_{M2}$	2–5% JAK2 V617F
$Ratio_{M2} < Ratio_{Sample} < Ratio_{M3}$	5–12.5% JAK2 V617F
$Ratio_{M3} < Ratio_{Sample} < Ratio_{M4}$	12.5–31% JAK2 V617F
$Ratio_{M4} < Ratio_{Sample} < Ratio_{M5}$	31–50% JAK2 V617F
$Ratio_{M5} < Ratio_{Sample} < Ratio_{M6}$	50–78% JAK2 V617F
$Ratio_{M6} < Ratio_{Sample}$	78–100% JAK2 V617F

An example of data calculation and interpretation is given in Table 16.

Table 16. An example of fluorescence data calculation and interpretation using the reference scale

Sample	VIC	FAM	Ratio	Mean ratio	NRatio	Interpretation
MN	49.613	3.8	0.077	0.078	1.000	Mutation not detected
MN	49.797	3.976	0.08			
MP	12.516	37.037	2.959	2.951	37.722	Mutation detected
MP	12.958	38.121	2.942			
M1	54.394	6.39	0.117	0.119	1.516	Cut-off sample
M1	58.266	6.973	0.12			
M2	61.798	10.882	0.176	0.172	2.202	Mutation detected
M2	54.814	9.231	0.168			
M3	57.364	16.604	0.289	0.297	3.797	Mutation detected
M3	59.742	18.192	0.305			
M4	56.965	28.99	0.509	0.505	6.462	Mutation detected
M4	58.077	29.158	0.502			
M5	54.251	37.221	0.686	0.672	8.586	Mutation detected
M5	54.979	36.125	0.657			
M6	46.185	44.498	0.963	0.954	12.2	Mutation detected
M6	45.077	42.598	0.945			
S 1	13.47	37.409	2.777	2.852	36.464	Mutation (78–100%)
S 1	14.559	42.616	2.927			
S 2	50.432	24.958	0.495	0.505	6.46	Mutation (12.5–31%)
S 2	53.797	27.746	0.516			
S 3	52.038	5.995	0.115	0.117	1.49	Inconclusive result
S 3	54.01	6.364	0.118			
S 4	50.811	4.842	0.095	0.094	0.791	Inconclusive result
S 4	51.112	4.753	0.093			
GZ	**1.425**	**1.607**				

Troubleshooting guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see "Contact Information", page 46).

	Comments and suggestions
Positive control signal negative	
a) Pipetting error	Check pipetting scheme and the setup of the reaction. Repeat the PCR run.
b) Inappropriate storage of kit components	Store the *ipsogen* JAK2 Muta*Screen* RS Kit at –30 to –15°C and keep primers and probes mix (PPM) protected from light. See "Reagent Storage and Handling", page 9. Avoid repeated freezing and thawing. Aliquot reagents for storage.
Negative controls are positive	
Cross-contamination	Replace all critical reagents. Repeat the experiment with new aliquots of all reagents. Always handle samples, kit components, and consumables in accordance with commonly accepted practices to prevent carry-over contamination.
No signal, even in positive controls	
a) Pipetting error or omitted reagents	Check pipetting scheme and the setup of the reaction. Repeat the PCR run.
b) Inhibitory effects of the sample material, caused by insufficient purification	Repeat the DNA preparation.

	Comments and suggestions
c) LightCycler: Incorrect detection channel chosen	Set Channel Setting to F1/F2 or 530 nm/640 nm.
d) LightCycler: No data acquisition programmed	Check the cycle programs. Select acquisition mode "single" at the end of each annealing segment of the PCR program.
Absent or low signal in samples but positive controls okay	
Poor DNA quality or low concentration	Always check the DNA quality and concentration before starting.
LightCycler: Fluorescence intensity too low	
a) Inappropriate storage of kit components	Store the *ipsogen* JAK2 Muta*Screen* RS Kit at –30 to –15°C and keep primers and probes mix (PPM) protected from light. See "Reagent Storage and Handling", page 9. Avoid repeated freezing and thawing. Aliquot reagents for storage.
b) Very low initial amount of target DNA	Increase the amount of sample DNA. **Note**: Depending of the chosen method of DNA preparation, inhibitory effects may occur.
LightCycler: Fluorescence intensity varies	
a) Pipetting error	Variability caused by so-called "pipetting error" can be reduced by analyzing data in the F1/F2 or 530 nm/640 nm mode.
b) Insufficient centrifugation of the capillaries	The prepared PCR mix may still be in the upper vessel of the capillary, or an air bubble could be trapped in the capillary tip. Always centrifuge capillaries loaded with the reaction mix as described in the specific operating manual of the apparatus.
c) Outer surface of the capillary tip dirty	Always wear gloves when handling the capillaries.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of *ipsogen* JAK2 MutaScreen RS Kit is tested against predetermined specifications to ensure consistent product quality. Certificates of Analysis are available upon request at **www.qiagen.com/support/**.

Symbols

The following symbols may appear on the packaging and labeling:

 <N>	Contains reagents sufficient for <N> reactions
	Use by
	Catalog number
	Lot number
	Material number
	Global Trade Item Number
	Temperature limitation
	Manufacturer
	Consult instructions for use

Contact Information

For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/Support**, call 00800-22-44-6000, or contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit **www.qiagen.com**).

Ordering Information

Product	Contents	Cat. no.
ipsogen JAK2 MutaScreen RS Kit (19)	For 19 reactions: V617F Positive Control, V617F Negative Control, Reference Scale, Primers and Probes Mix JAK2 wild-type and JAK2 V617F	673113
Rotor-Gene Q – for outstanding performance in real-time PCR		
Rotor-Gene Q 5plex HRM Platform	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training not included	9001580
Rotor-Gene Q 5plex HRM System	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9001650

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Notes

Notes

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