

Manual

KASP genotyping on the BIO-RAD CFX-series instruments

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Manual

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Contents

1. Introduction	3
2. Tips and suggestions before you start	3
3. Overview of the procedure	3
4. KASP thermal cycling conditions	4
5. Step-by-step user guide	5
5.1 Create a new protocol in CFX Manger	5
5.2 Create a new plate layout file in CFX Manager	7
5.3 Run the thermal cycle with plate read	9
5.4 View the data from your plate	10
5.5 Recycling your assay plate	11
5.6 Future runs	12
5.7 Running the KASP Trial Kit on the BIO-RAD CFX	13
6. Further Support	13

Manual

KASP genotyping on the BIO-RAD CFX-series instruments

1. Introduction

This document is intended as a manual to running KASP™ genotyping reactions on the BIO-RAD CFX-series instrument.

[KASP chemistry](#) for allelic discrimination, from LGC Biosearch Technologies, performs well on a BIO-RAD CFX-series machine and this step-by-step protocol will enable users to successfully set-up, run and read plates on the CFX.

Please be aware that some of the default settings within the CFX Manager software are not suitable for reading KASP and must be changed prior to performing plate runs and analysis of data.

2. Tips and suggestions before you start

1. Optimal cycling conditions for KASP require a touchdown two-step PCR protocol. The cycling conditions for most assays will be as described in this manual (section 4), although you must check the cycling conditions included in your assay information pack to ensure that your assay does not have any specific cycling conditions.
2. KASP is an end-point chemistry and will require a final read once the PCR amplification steps have been completed. Completed KASP reactions must be read below 40 °C.
3. Data capture should only be performed once thermal cycling has ended – no useful data will be captured during the thermal cycling protocol.
4. The KASP recycling program will often improve results, especially for assays that are slow to group.
5. KASP is not a SYBR-Green based chemistry. The CFX Manager software defaults to presets that use this wavelength and these may produce strange/no results from your experiment. Ensure that you use FAM and HEX fluorophores only for your plate reads. You should include ROX as a third fluorophore if you plan to export data for analysis in alternative software e.g. KlusterCaller™. The CFX can read ROX but the CFX Manager software cannot use ROX signal for normalisation purposes.

3. Overview of the procedure

1. Create a new protocol for the KASP thermal cycle in CFX Manager – see section 5.1.
2. Create a plate layout file specific for your samples, ensuring that the correct fluorophores are defined – see section 5.2.
3. Run the thermal cycle and subsequent plate read step for your sample plate – see section 5.3.
4. View the data – see section 5.4.
5. Recycle the plate if necessary – see section 5.5.

Manual

KASP genotyping on the BIO-RAD CFX-series instruments

4. KASP thermal cycling conditions

4.1. KASP thermal cycling conditions

1. 94 °C for 15 minutes
2. 94 °C for 20 seconds
3. 61 °C for 60 seconds (drop - 0.6 °C per cycle)
Repeat steps 2-3 nine times (a total of 10 cycles) achieving a final annealing temperature of 55 °C
4. 94 °C for 20 seconds
5. 55 °C for 60 seconds
Repeat steps 4-5 25 times (a total of 26 cycles)
6. Add read step at 37 °C for 1 min*

4.2. KASP recycling conditions

1. 94 °C for 20 seconds
2. 57 °C for 60 seconds
Repeat steps 1-2 twice (a total of 3 cycles)
3. Add read step at 37 °C for 1 min*.

*KASP cannot be read at temperatures above 40 °C.

Manual

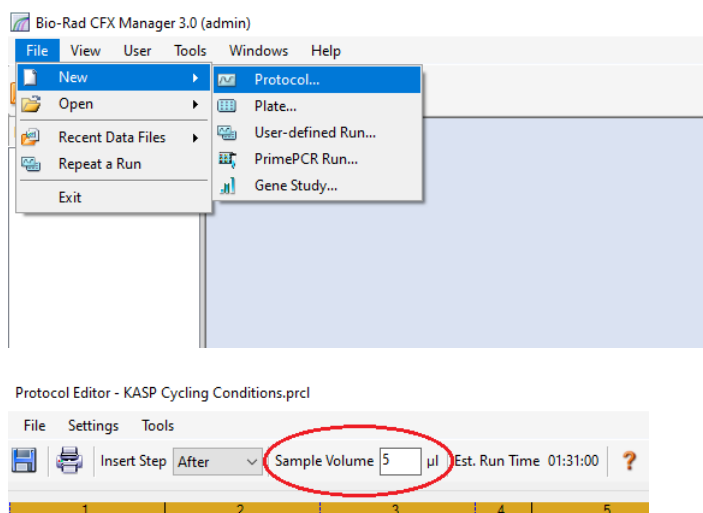
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5. Step-by-step user guide

5.1. Create a new protocol in CFX Manager

TIP: As the cycling conditions for KASP differ to standard PCR conditions, it is advisable to create a new protocol specifically for KASP thermal cycling that you can save and re-use each time that you run a new plate.

- Open the CFX Manager software
- Click on the 'File' menu and expand the menu options for 'New'.
- Click on 'Protocol...'
- This will open the 'Protocol Editor' window. N.B. The default thermal cycle has three steps so will require editing.
- Edit the thermal cycle programme according to the KASP cycling conditions detailed below.
- Check the default sample volume at the top of the screen and edit if necessary.



Cycling conditions

1. 94 °C for 15 minutes
2. 94 °C for 20 seconds
3. 61 °C for 60 seconds

Decrement: 0.6 °C per cycle. Go to step 2 for nine cycles (a total of 10 cycles) achieving the annealing temperature 55 °C

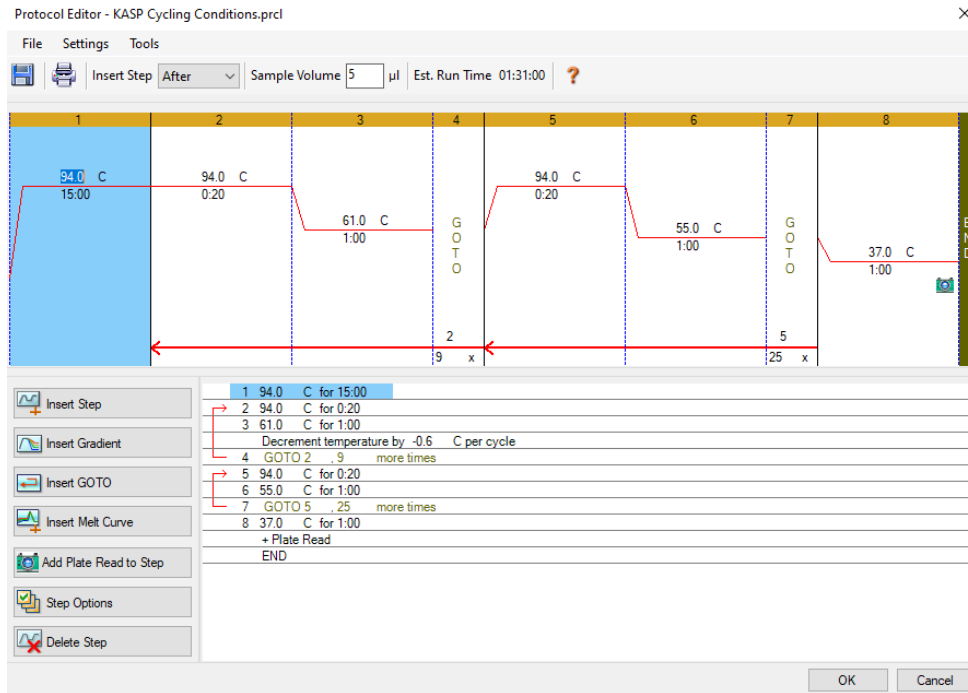
4. 94 °C for 20 seconds
5. 55 °C for 60 seconds

Repeat steps 5-6 25 times (a total of 26 cycles)

6. Add read step at 37 °C for 1 min.

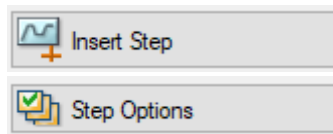
Manual

KASP genotyping on the BIO-RAD CFX-series instruments



Hints for editing the thermal cycle protocol

- Click on 'insert step' to add new cycling parameters after the currently selected step.
- To add a temperature decrement (°C per cycle), click on the step that you want to add it to (the step will be highlighted in blue). Click on the 'step options' button on the left hand side of the screen. The step parameters can be edited in this window. N.B. Step 3 of the standard KASP thermal cycle requires a decrement of -0.6 °C per cycle.



Step Options

Step 3

Plate Read

Temperature 61.0 °C

Gradient °C

Increment -0.6 °C/cycle

Ramp Rate °C/sec

Time 1:00 sec/cycle

Extend sec/cycle

Beep

Gradient

A

B

C

D

E

F

G

H

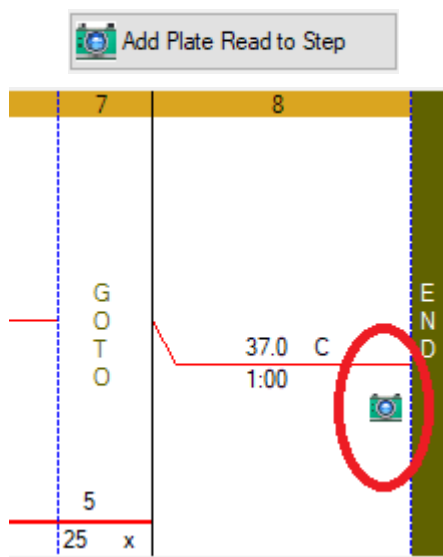
OK Cancel

Manual

KASP genotyping on the BIO-RAD CFX-series instruments

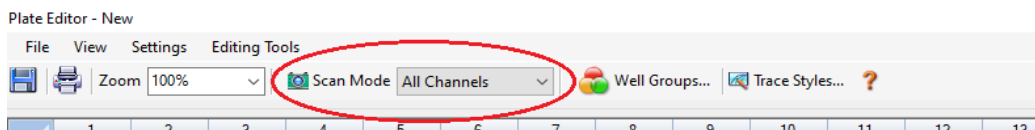
Hints for editing the thermal cycle protocol

- Add a plate read to the final 37 °C step of the program (Step 8). A plate read will be performed if the camera icon is shown on the step. Ensure that you remove all plate reads from earlier steps as they are redundant.
- Save the protocol – you can re-use this protocol each time that you run KASP.
- Click 'OK'.



5.2. Create a new plate layout file in CFX Manager

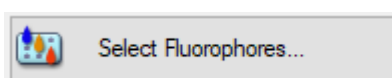
- After pressing 'OK' at the end of step 5.1, the 'Run Setup' window will open with your newly created protocol detailed on the 'Protocol' tab. Check that the protocol displayed is as expected.
- Click the 'Next' button. This will take you to the 'Plate Layout' tab.
- Click the 'Create New' button. This will open a 'Plate Editor' window.
- Select 'All Channels' for scan mode (top of window).



Manual

KASP genotyping on the BIO-RAD CFX-series instruments

- In the 'Settings' menu, choose the plate type you will be using e.g. 384-well white plate.
- In the plate layout grid, highlight the required wells and choose 'Sample type' from the drop down menu on the right hand side of the screen. You will need to define the location of your unknown samples and your no template controls (NTCs). The well colour will change based on the sample type selected.
- Click on the 'Select Fluorophores' button (right hand side of window). This will open the fluorophores window.



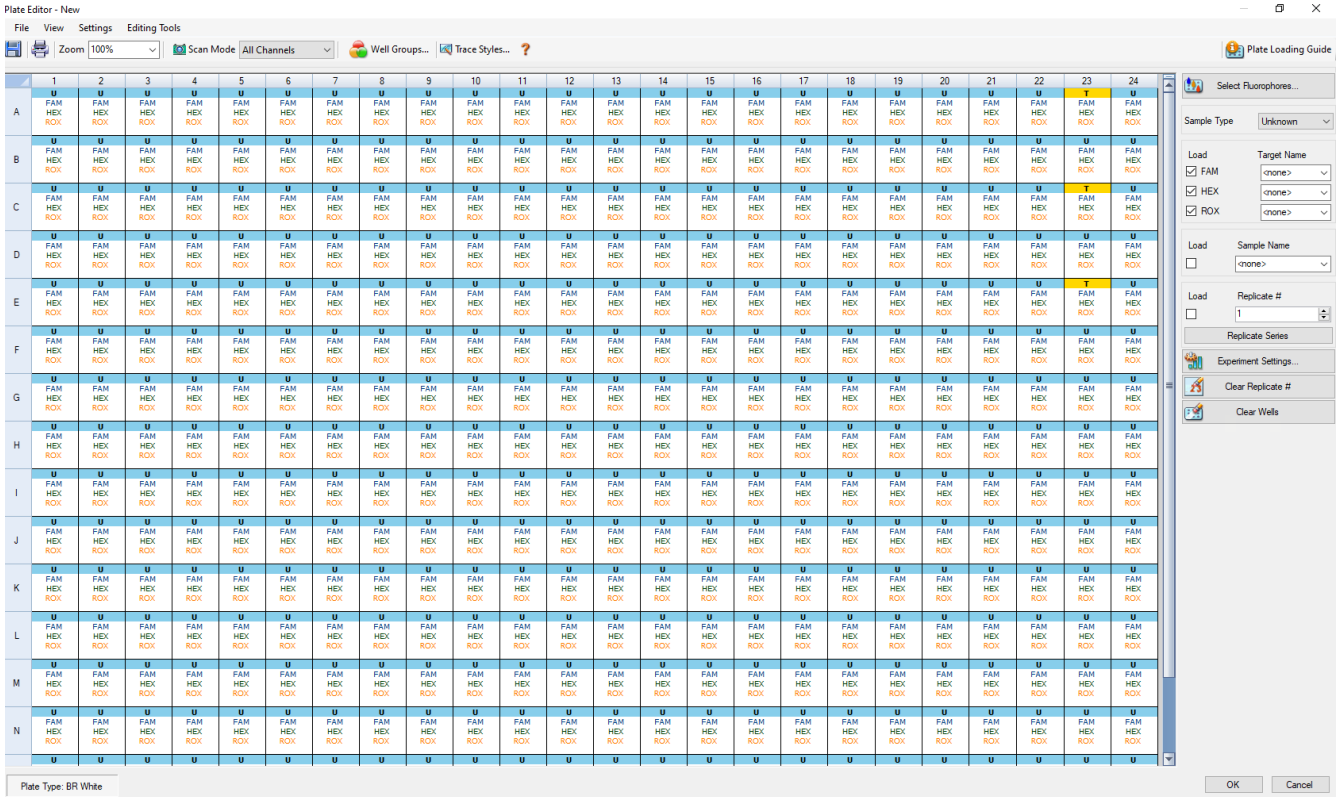
Select Fluorophores

	Channel	Fluorophore	Selected	Color
	1	FAM	<input checked="" type="checkbox"/>	Blue
		SYBR	<input type="checkbox"/>	Green
	2	HEX	<input checked="" type="checkbox"/>	Dark Green
		TET	<input type="checkbox"/>	Teal
		Cal Gold 540	<input type="checkbox"/>	Light Green
		VIC	<input type="checkbox"/>	Olive
	3	ROX	<input type="checkbox"/>	Orange

- Select fluorophores 'FAM' for channel 1 and 'HEX' for channel 2. Select ROX for channel 3 if you plan to export data for analysis in alternative software.
- FAM, HEX and ROX will now be visible on the right hand side of the window. Highlight all wells of the plate that contain sample/NTC and tick each of the three fluorophores. The fluorophore names will then be visible in the plate layout.

Manual

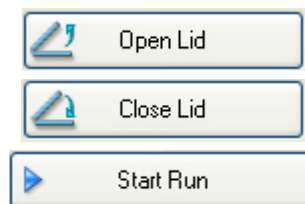
KASP genotyping on the BIO-RAD CFX-series instruments



- Save the plate format. You can re-use this plate layout file for subsequent genotyping plates if applicable.
- Click 'OK' to close the Plate Editor window and to return to the 'Run Setup' window.

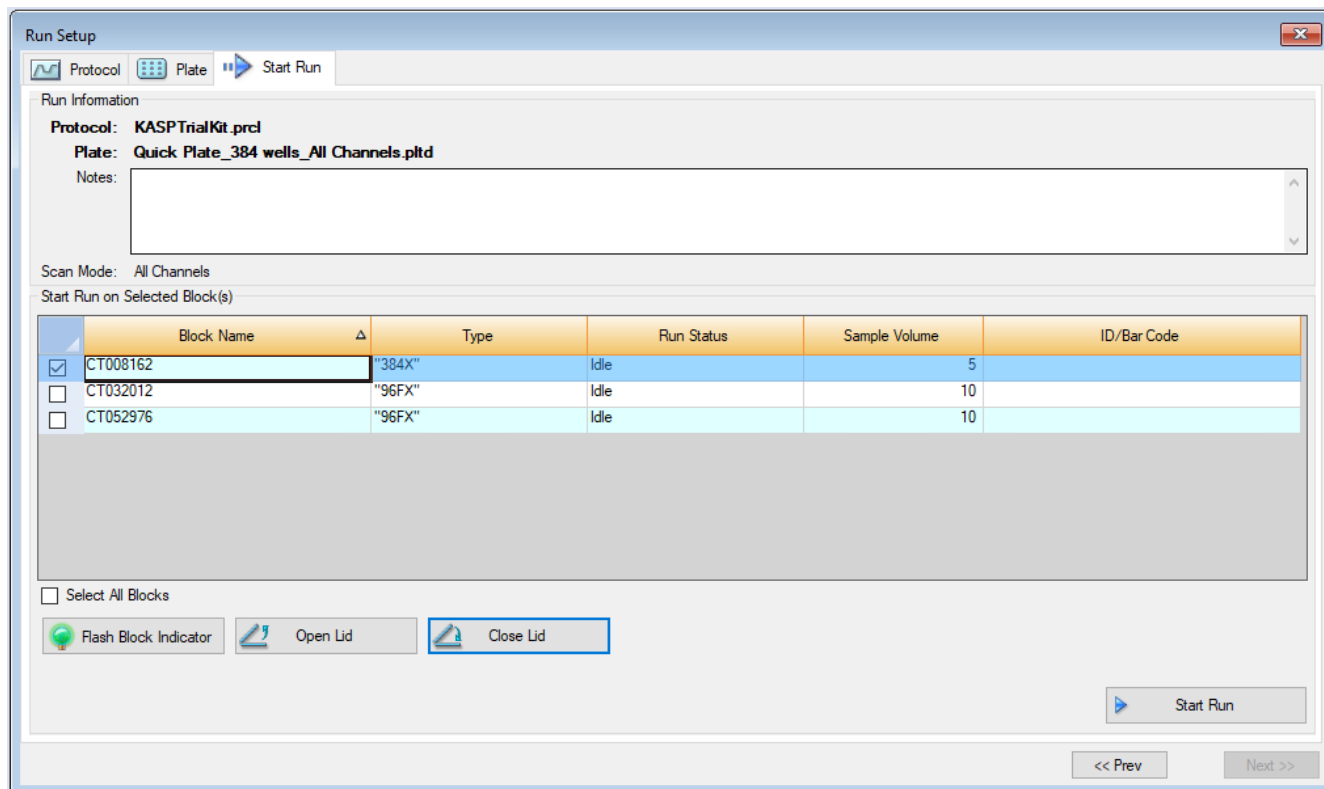
5.3. Run the thermal cycle with plate read

- In 'Run Setup' window, click 'Next'. This will take you to the 'Start Run' tab.
- The protocol and plate layout files that you have created will be detailed in bold.
- There is a 'Notes' box that you can use to fill in any relevant information about your experiment.
- Open the lid of the instrument and insert your reaction plate.
- Close the instrument lid.
- Click 'Start Run'.
- On a CFX, the standard KASP thermal cycle programme takes approximately 1.5 hours.



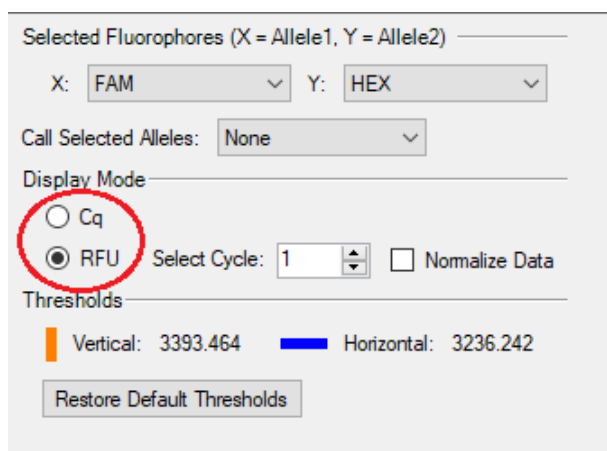
Manual

KASP genotyping on the BIO-RAD CFX-series instruments



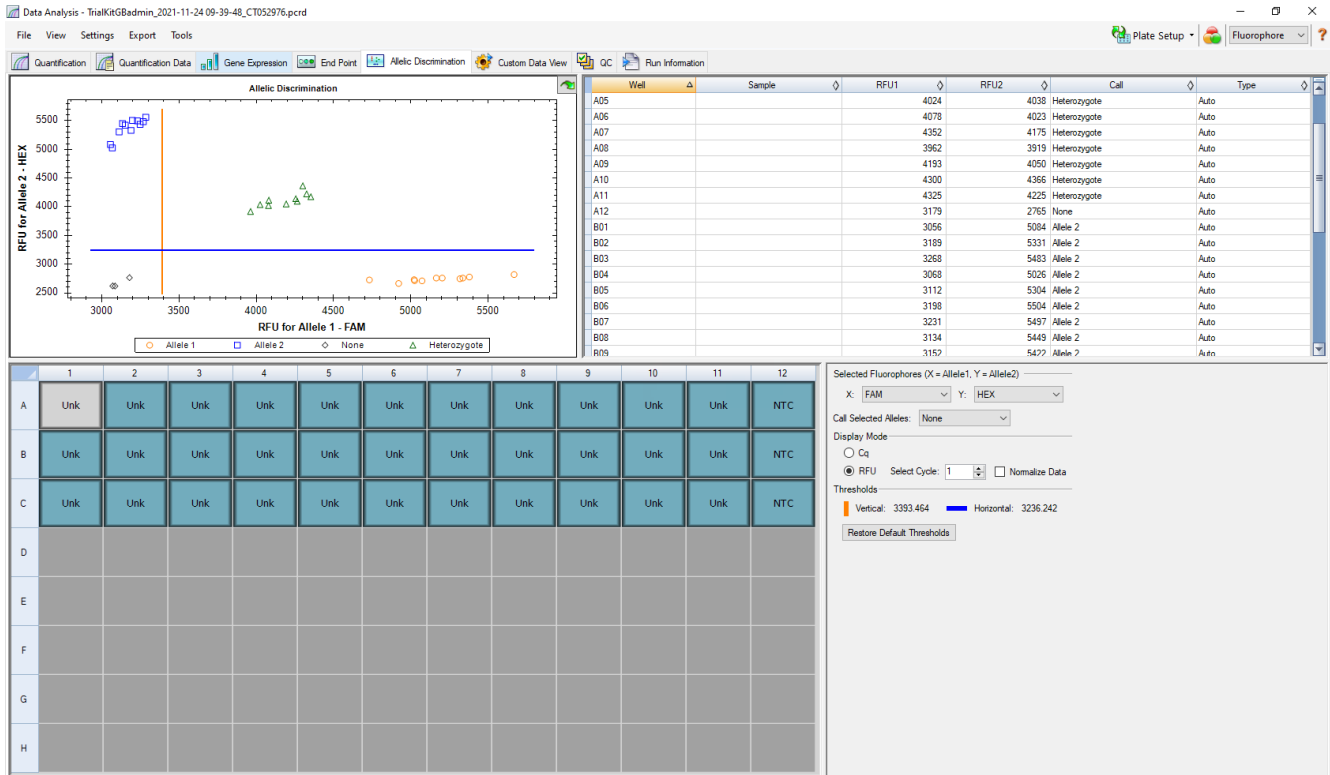
5.4. View the data from your plate

- At the end of the run, you can view your results in the BIO-RAD data analysis software.
- In the data analysis window, there are several different tabs. Click on the 'Allelic Discrimination' tab as this is the tab that is relevant for KASP genotyping results.
- In the display mode section (bottom right hand side of window), select 'RFU' (Cq is usually selected as default).
- The software will automatically call the alleles for your samples based on their position in the allelic discrimination plot. You can readjust the positions of the X (blue) and Y (orange) axis to obtain the best scoring for your assay.



Manual

KASP genotyping on the BIO-RAD CFX-series instruments



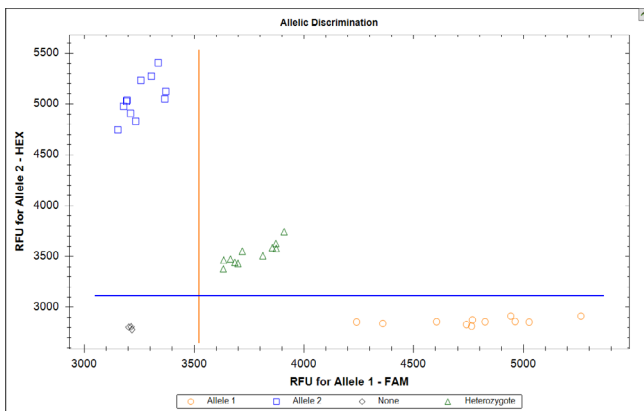
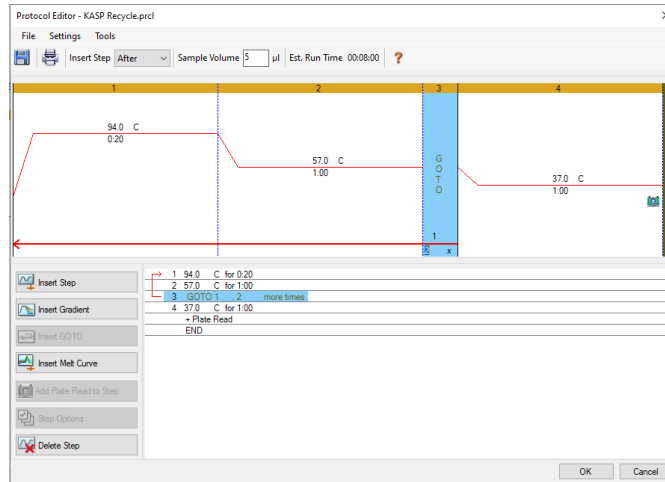
5.5. Recycling your assay plate

- If your data has not formed tight clusters after the initial thermal cycling protocol, you can recycle the plate in the CFX and re-read it.
- As before: Create a new protocol (see section 5.1). Use the following thermal cycling conditions:
 1. 94 °C for 20 seconds
 2. 57 °C for 1 minuteRepeat steps 1-2 twice (a total of three cycles)
 3. Add a read step at 37 °C for 1 minute
 - Select the plate layout file that you created when the original thermal cycle was run (see section 5.2)
 - Run the recycling step (this takes approximately 8 minutes on the CFX) and plate read (see section 5.3)
 - View the data from the recycled plate (see section 5.4). Continue to repeat the recycling step until tight clusters are formed.

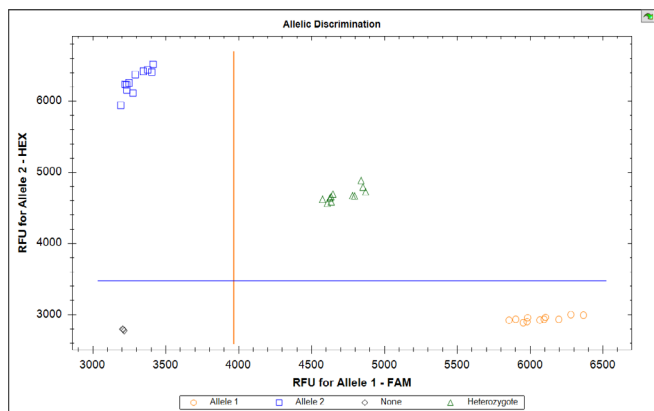
NOTE: Following the thermal cycle, KASP reactions are stable at room temperature for up to a week and can still be recycled at this stage.

Manual

KASP genotyping on the BIO-RAD CFX-series instruments



Allelic discrimination plot after completion of the initial KASP thermal cycle. Tight clusters have not formed.



Allelic discrimination plot after completion an additional cycle step (3 additional cycles). The data points have formed tighter clusters and can be more confidently assigned a genotype.

5.6. Future runs

- Once you have set up the protocols for the KASP thermal cycle and the recycle step, you can re-use these in all subsequent genotyping runs (unless a particular assay has specific thermal cycling conditions that differ from the standard).
- If you will be using the same plate layout for each reaction plate that you run, you can re-use the layout file created in section 5.2.

Special note for performing 'Plate read only' runs

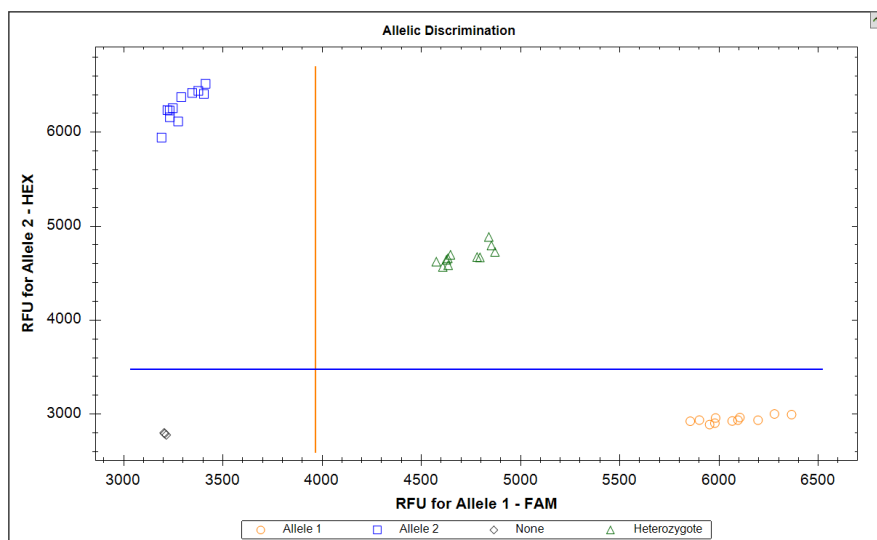
In the CFX Manager software, there is an option to perform an 'end point read'. Do not use this option as it does not enable the data to be viewed on the 'Allelic Discrimination' tab. If you perform the thermal cycle on a different instrument (e.g. a Peltier block) and just need to perform the plate read step on the CFX, always set up a protocol for this (as per section 5.1). The protocol will just require one step: 37 °C for 1 minute with a plate read. The plate layout will also need to be defined with the three fluorophores (FAM, HEX and ROX) – see section 5.2.

Manual

KASP genotyping on the BIO-RAD CFX-series instruments

5.7. Running the KASP Trial Kit on the BIO-RAD CFX

- If you have requested a KASP Trial Kit to run on your BIO-RAD CFX machine, please follow the protocol included with the kit to set up your reaction plate. This CFX manual can be used to help you to programme the instrument to run the Trial Kit reaction plate.
- After the initial KASP thermal cycle, the Trial Kit reaction plate should produce data similar to the figure below.



Results from the KASP Trial Kit reaction plate when run on the BIO-RAD CFX instrument using the standard KASP thermal cycle. This data was produced using a BIO-RAD white 96-well plate.

6. Further support

If you require any further support, please contact our technical support team at techsupport@lgcgroup.com or [submit a request](#) for support directly into our case system.



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