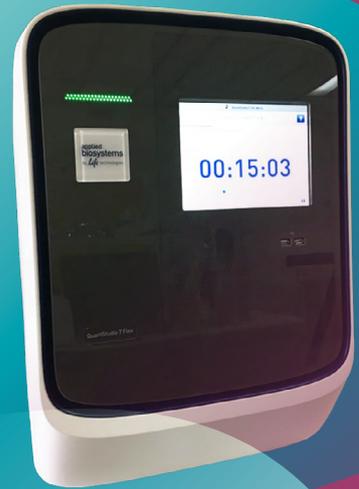


Manual



Running KASP genotyping reactions on the Applied Biosystems QuantStudio 6 and 7 Flex Systems

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Running KASP genotyping reactions on the Applied Biosystems QuantStudio 6 and 7 Flex Systems

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Running KASP genotyping reactions on the Applied Biosystems QuantStudio 6 and 7 Flex Systems

1. Introduction to the Applied Biosystems™ QuantStudio 6 and 7

This document is intended as a guide to running [KASP™ genotyping reactions](#) on the Applied Biosystems QuantStudio 6 and 7 instruments.

KASP chemistry for allelic discrimination performs well on QuantStudio instruments and this step-by-step protocol will enable you to successfully set-up, run and read plates on the QuantStudio 6 and 7.

2. Tips and suggestions before you start

1. Optimal cycling conditions for KASP require a touchdown 2-step PCR protocol. The cycling conditions for most SNP assays will be as described in this manual (section 4), although you must check the cycling conditions included in your SNP assay information pack to ensure that your assay does not have any specific cycling conditions.
2. KASP is an endpoint 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. In the QuantStudio 6 and 7 software, a pre-read (before the thermal cycle) and a post-read (after the thermal cycle) should be included in the thermal cycle programme.
4. Data capture should not be performed during the thermal cycle programme – no useful data will be captured as KASP chemistry cannot be read at these temperatures. Reading the plate throughout the thermal cycle will unnecessarily reduce the lifespan of your instrument.
5. The KASP recycling* programme will often improve results, especially for assays that are slow to group.
6. KASP is supplied with the HEX fluorophore but this has the same excitation and emission spectra as VIC; they are therefore interchangeable. The QuantStudio software only has an option for programming with VIC so ensure that you use this for the HEX allele.

* The efficiency of KASP genotyping reactions are dependent on a number of factors including concentration of sample DNA and composition of the DNA sequence surrounding the SNP site. DNA sequence composition will impact the efficiency of primer binding and hence affect the rate of the PCR reaction. For this reason, different KASP assays will reach completion at different rates and may require additional PCR cycles to produce clear genotyping clusters.

3. Overview of the procedure

1. Define the experimental properties – see section 5.1.
2. Programme the plate set up including assay information and sample names – see section 5.2.
3. Programme the run method for KASP (including pre- and post-read steps) – see section 5.3.
4. Run the thermal cycle for your sample plate – see section 5.4.
5. Analyse the data – see section 5.5.
6. Recycle the plate if necessary – see section 5.6.

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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 by -0.6 °C/per cycle)
Repeat steps 2-3 × 9 times (a total of 10 cycles) achieving the final annealing temperature 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. Read plate at less than 40 °C

4.2 KASP recycling conditions**

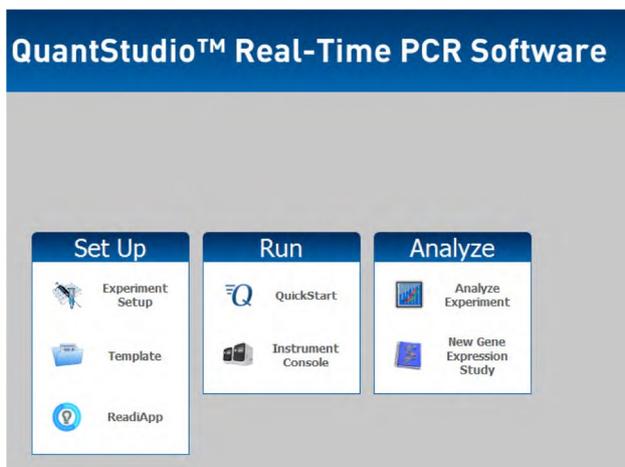
1. 94 °C for 20 seconds
2. 57 °C for 60 seconds
Repeat steps 1-2 twice (a total of 3 cycles)
3. Read plate at less than 40 °C

** KASP cannot be read at temperatures above 40 °C. Turn off data capture for each of the cycling steps as this is not necessary.

5. Step-by-step user guide

5.1 Define the experimental properties

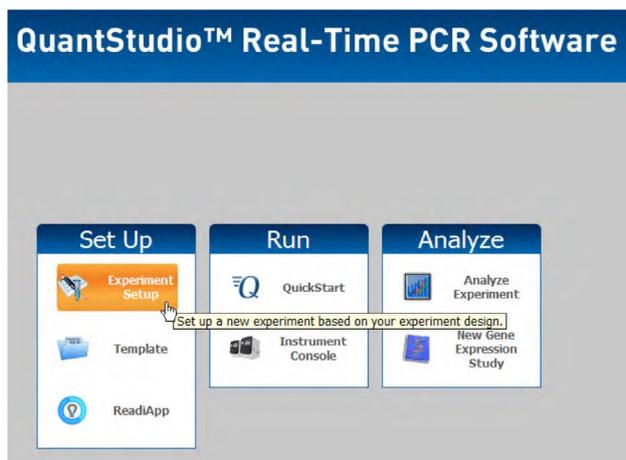
- Open the QuantStudio 6 and 7 software.



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- On the front screen, in the Set Up box, click on 'Experiment Setup'. This will open up a new experiment.



- In the Setup menu, the first stage is to define the 'Experiment Properties'.



- Step 1: Give your experiment a name
Optional: add in plate barcode information, your user name and any comments relevant to the experiment.
- Step 2: Select which instrument you are using



- Step 3: Select the block that you are using (i.e. 384-Well, Array Card or 96-Well (0.2 mL))



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- Step 4: Select the experiment type – for KASP, this will be ‘Genotyping’

What type of experiment do you want to set up?

Standard Curve	Relative Standard Curve	Comparative Cr (ΔΔCr)	Melt Curve
<input checked="" type="checkbox"/> Genotyping	Presence/Absence		

- Step 5: Select ‘Other’ for the reagents type

* Which reagents do you want to use to detect the target sequence?

TaqMan® Reagents	<input checked="" type="checkbox"/> Other
------------------	---

- Step 6: Select ‘Standard’ for the ramp speed

* What properties do you want for the instrument run?

<input checked="" type="checkbox"/> Standard	Fast
--	------

Include: Pre-PCR Read Amplification Post-PCR Read

- Step 7: Tick the boxes to include a ‘Pre-PCR Read’ and an ‘Amplification’ stage in the experimental run. **Please note:** Post-PCR Read is selected by default for genotyping experiments.
- Step 8: The experimental properties page should now be defined as shown in the example below if using the QuantStudio 7 and a 96-well plate.

Experiment: **KASP Trial Kit** Type: **Genotyping** Reagents: **Other**

How do you want to identify this experiment?

* Experiment Name: KASP Trial Kit Comments:

Barcode:

User Name:

Which instrument type are you using to run the experiment?

QuantStudio™ 6 Flex System	<input checked="" type="checkbox"/> QuantStudio™ 7 Flex System	VIIA™ 7 System
----------------------------	--	----------------

Which block are you using to run the experiment?

384-Well	Array Card	<input checked="" type="checkbox"/> 96-Well (0.2mL)	Fast 96-Well (0.1mL)
----------	------------	---	----------------------

What type of experiment do you want to set up?

Standard Curve	Relative Standard Curve	Comparative Cr (ΔΔCr)	Melt Curve
<input checked="" type="checkbox"/> Genotyping	Presence/Absence		

Which reagents do you want to use to detect the target sequence?

TaqMan® Reagents	<input checked="" type="checkbox"/> Other
------------------	---

What properties do you want for the instrument run?

<input checked="" type="checkbox"/> Standard	Fast
--	------

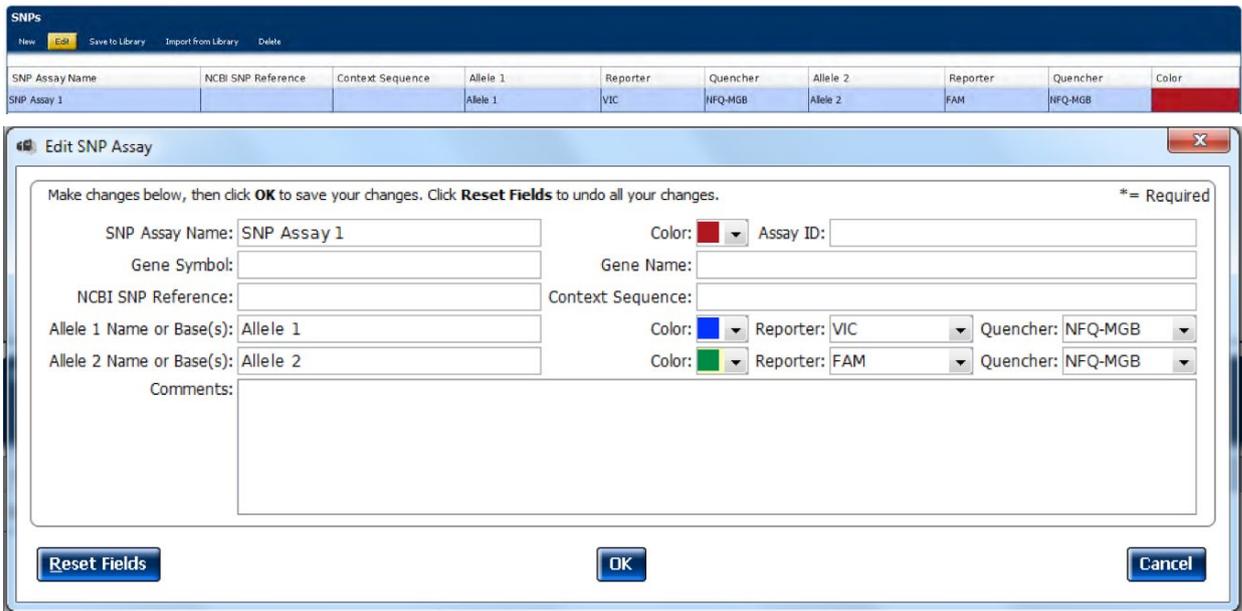
Include: Pre-PCR Read Amplification Post-PCR Read

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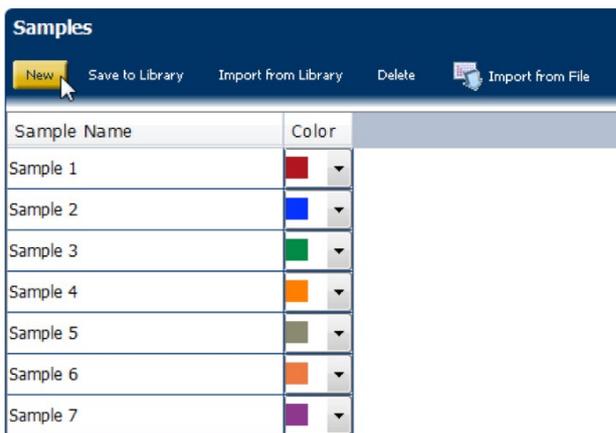
Running KASP genotyping reactions on the Applied Biosystems QuantStudio 6 and 7 Flex Systems

5.2 Programme the plate set up

- Step 1: Once the experimental properties are defined, click on 'Define' within the Setup menu.
 - The 'SNPs' box can be used to define the individual SNP assays that you plan to run. 'SNP Assay 1' is listed by default. Click on 'Edit' and fill in all the fields within the 'Edit SNP Assay' box including a name for your assay. One allele should have FAM as the reporter and the other should have VIC as the reporter. (HEX is the second fluorophore used in KASP, but this is not an option in the QuantStudio 6 and 7 software. VIC and HEX can be read at the same wavelength.)



- The 'Samples' box can be used to define the individual sample names of the DNA samples within the experiment.
- You can add new samples and display samples that have been defined in previous experiments.



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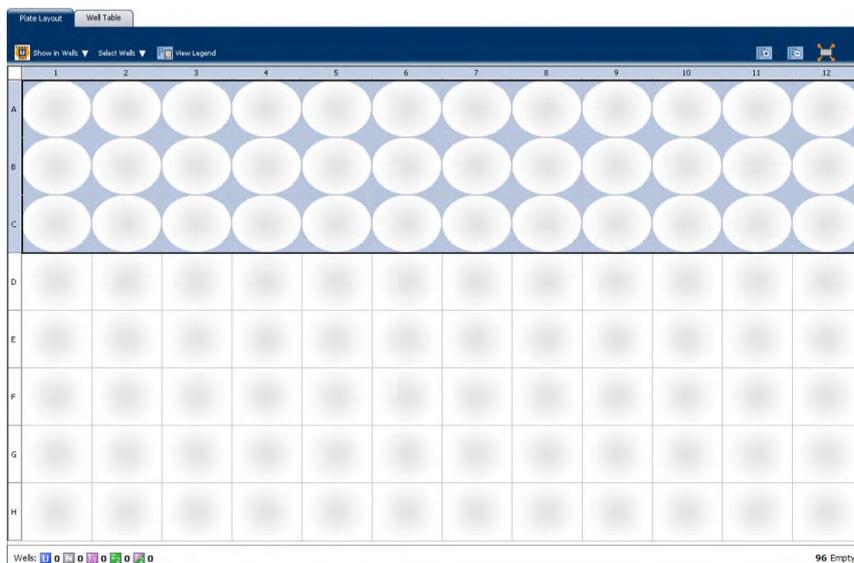
- Step 2: Ensure that ROX is selected as the passive reference dye for the experiment.



- Step 3: Once the SNPs and samples have been defined, click on 'Assign' within the Setup menu.



- Step 4: Assign the markers (assays) that you have defined to the relevant wells of the plate. This is done by highlighting the wells, and then adding a tick to the 'Assign' column in the marker table.



SNPs		
	Name	Task
<input checked="" type="checkbox"/>	SNP Assay 1	U

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- Step 5: Ensure that the task for each well is correct. Highlight the relevant well(s) and edit the task in the drop down menu in the assay box. Unknown DNA samples should be defined as unknown (U) and 'No Template Controls' should be defined as negative controls (N).

The screenshot shows the 'Well Table' tab of the software. The plate layout is a 96-well plate with columns 1-12 and rows A-H. Wells A1-A12 contain assay boxes with a task dropdown menu open, showing options U, N, 1, 2, 3. The 'Task' column in the assay box is currently set to 'U'. The 'SNPs' dropdown menu is also visible, showing 'SNP Assay 1'.

- Step 6: Assign the sample names to the relevant wells of the plate. This is done by highlighting the wells, and then adding a tick to the 'Assign' column in the sample table. The colour of the wells will indicate which DNA sample has been assigned to them.

The screenshot shows the 'Well Table' tab with sample names assigned to wells. Wells A1-A5 are assigned 'Sample 1' (red), B1-B5 are assigned 'Sample 2' (blue), and C1-C5 are assigned 'Sample 3' (green). The 'Samples' dropdown menu is open, showing a list of samples with checkboxes in the 'Assign' column. The 'Assign' column for Sample 1, Sample 2, and Sample 3 is checked.

- Alternatively it may be possible to import setup data from a Plate Setup file by clicking on 'Import', and then 'Import Plate Setup'.

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5.3 Programme the run method for KASP

- Step 1: Once the plate layout has been defined, click on 'Run Method' within the Setup menu.



- The 'Run Method' menu is where you can edit the thermal cycling conditions.
- Edit the default thermal cycle to the conditions required for KASP genotyping.

Cycling conditions

Stage 1: Pre-Read Stage

1. 30 °C for 1 min. (Data capture on)

Stage 2: Hold Stage

1. 94 °C for 15 minutes

Stage 3: PCR Stage (touchdown)

1. 94 °C for 20 seconds
 2. 61 °C for 60 seconds (decrement -0.6 °C per cycle)
- Set the decrement up using the 'Enable AutoDelta' option.

Repeat Stage 3 × 9 times (a total of 10 cycles) achieving a final annealing temperature of 55 °C

Stage 4: PCR Stage

1. 94 °C for 20 seconds
 2. 55 °C for 60 seconds
- Repeat Stage 4 × 25 times (a total of 26 cycles)

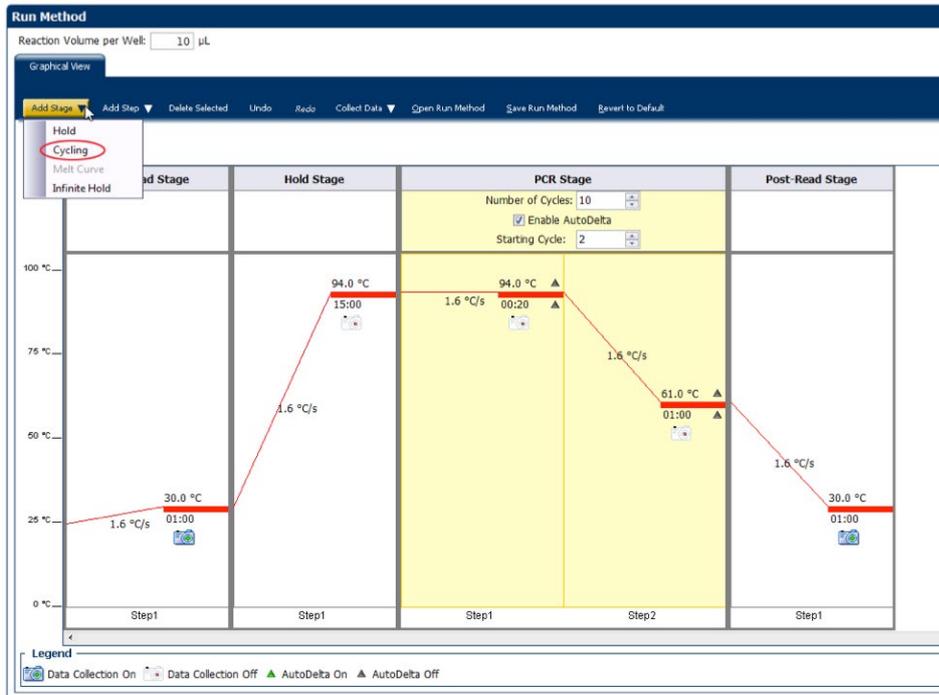
Stage 5: Post-Read Stage

1. 30 °C for 1 minute. (Data capture on)
- This will bring the reaction plate to the correct temperature for reading the fluorescence.

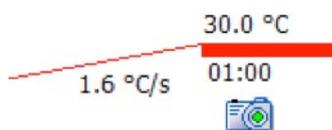
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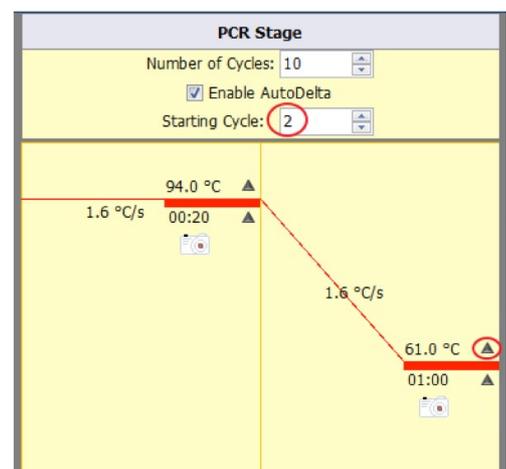
- The default thermal cycle will consist of the following four stages: Pre-Read, Hold Stage, PCR Stage and Post-Read Stage. Add an additional PCR stage to the thermal cycle. To do this, highlight the default PCR stage (yellow), click on the 'Add Stage' menu and select 'Cycling'.



- The first PCR stage should be used to programme the 10 cycles of touchdown PCR, and the second PCR stage should be used for the remaining 26 PCR cycles.
- Edit the thermal cycle parameters for all five stages according to the KASP thermal cycle conditions.
- The ramp rates will be set to 1.6 °C/s by default, and these do not need to be adjusted for KASP chemistry.



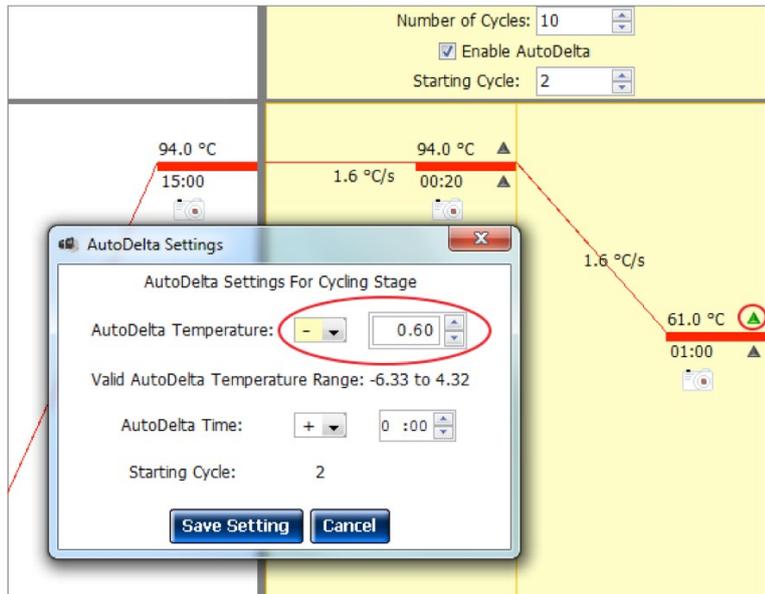
- To programme the touchdown stage, you need to click in the 'Enable AutoDelta' box of the relevant cycling stage. Change the 'Starting Cycle' to 2 as the decrement should start from the second cycle. Then click on the grey triangle next to the temperature (61 °C) that requires a decrement. This will open up the 'AutoDelta Settings' window.



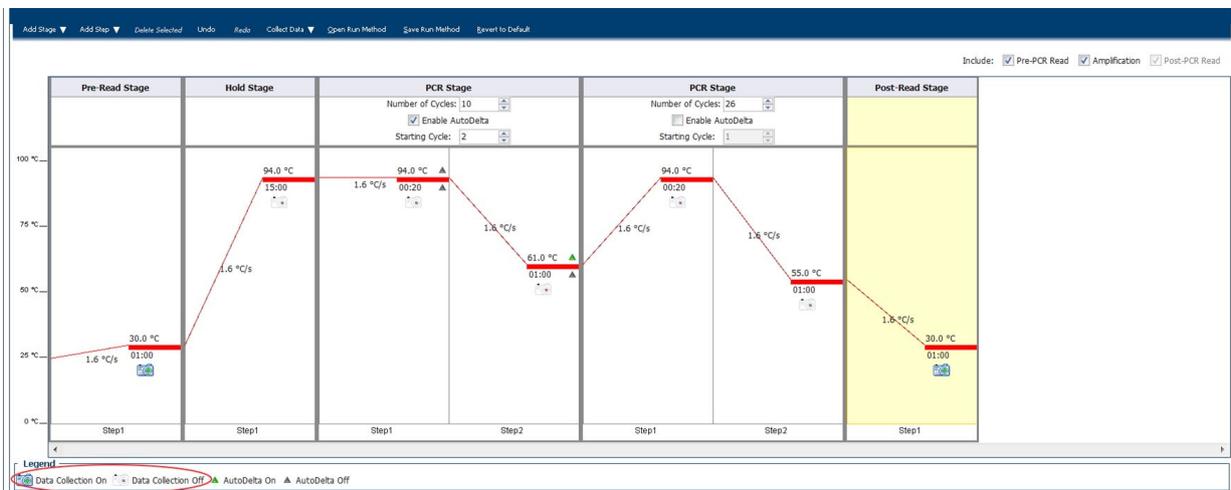
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- Select the minus sign (for a decrement) and 0.6 for the temperature. This will ensure that the temperature decreases by 0.6 °C each cycle in this cycling stage (10 cycles). Click 'Save Setting' to return to the run method window. The triangle will now be green to indicate that AutoDelta settings have been programmed.



- Ensure that the data capture symbol is on (i.e. in colour) for the first Pre-Read Stage (30 °C for 1 min) so that the pre-read is performed, and for the last Post-Read Stage (30 °C for 1 min) so that the post-read is performed. Data capture should be turned off during all of the PCR stages: KASP chemistry cannot be read at these temperatures and no useful data will be captured.

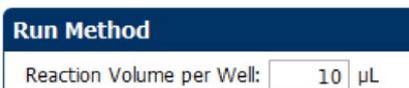


QuantStudio 6 and 7 software programmed with the standard KASP thermal cycling conditions.

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Running KASP genotyping reactions on the Applied Biosystems QuantStudio 6 and 7 Flex Systems

- Step 2: Ensure that the reaction volume is correct for your experiment (LGC Biosearch Technologies recommends 10 μ L reaction volume for 96-well plates and 5 μ L reaction volume for 384-well plates).



5.4 Run the thermal cycle including pre- and post-read steps

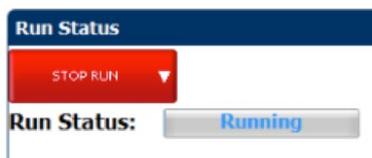
- Once the experimental properties, plate setup and run method have all been programmed, you can begin the run.
- Click on the 'Run' menu.



- Ensure that the software is connected to the instrument, and then press the green 'Start Run' button.



- The 'Run Status' will then change to 'Running' and the thermal cycle will begin.



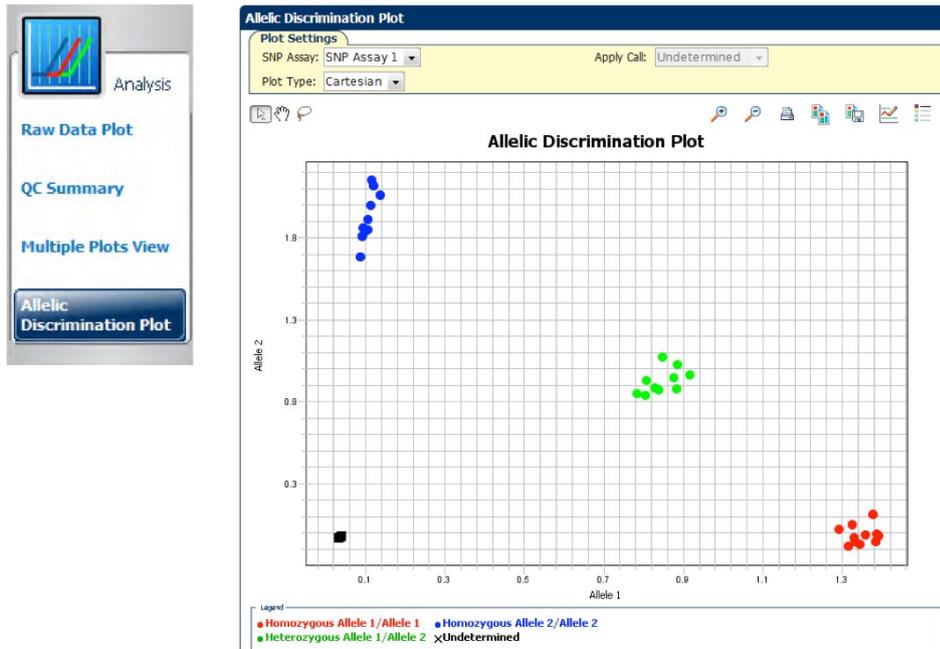
- Fluorescent data will be collected during the pre-read and post-read stages.

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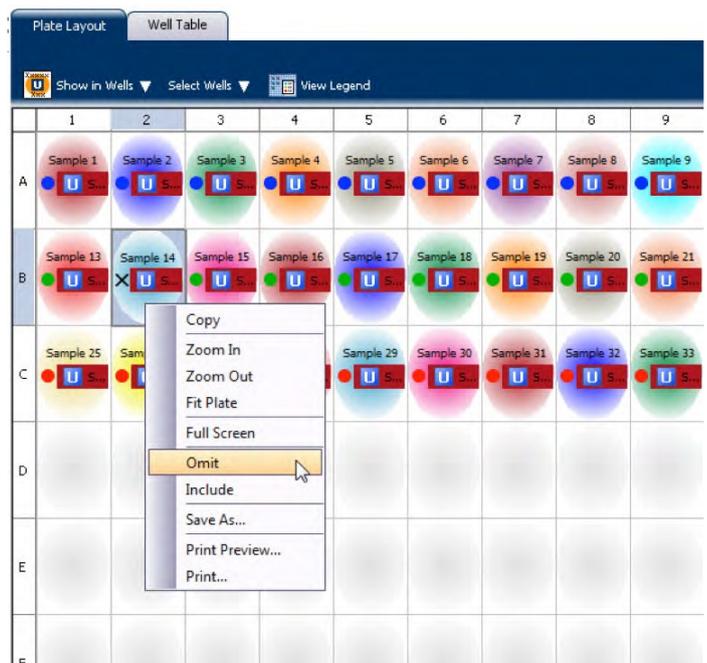
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5.5 Analysis of results

- Once the run has completed, click on the Analysis section of the experiment menu.
- Your genotyping data can be viewed by clicking on 'Allelic Discrimination Plot'.



- If there are any anomalous wells (e.g. due to pipetting error), these can be omitted from the analysis. Right click on the well in the plate layout view, and select 'Omit'. You can then click on 'Analyse' to view the genotyping data.

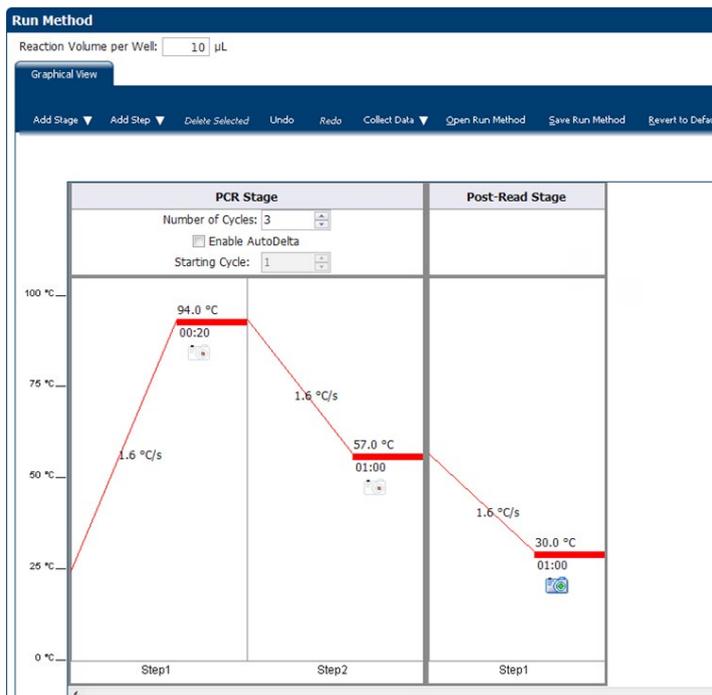


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5.6 Recycle your reaction plate if necessary

- If your data has not formed tight clusters after the initial thermal cycling protocol, you can recycle the plate in the QuantStudio 6 and 7 (or on a Peltier block) and perform a second post-read.
- You will need to create an experiment file with the thermal cycling conditions for KASP recycling conditions (see section 4.2).
- The KASP recycling conditions are:
 1. 94 °C for 20 seconds
 2. 57 °C for 60 seconds
 - Repeat steps 1-2 twice (a total of 3 cycles).
 - Add a second stage (post-PCR Read) at 30 °C for 1 minute to bring the plate to the correct temperature for reading, and ensure data capture is on.



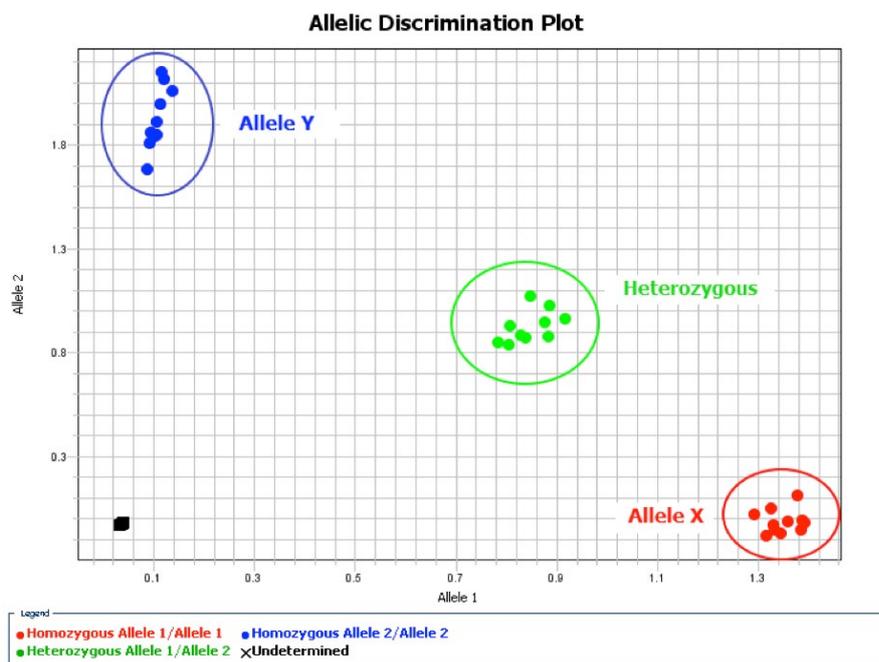
- **Please note:** you should not perform a pre-read when running a recycle programme. The reactions will have already been thermally cycled and will therefore already be emitting FAM/VIC fluorescent signal.
- The post-read data following recycling can be viewed from the 'Analysis' menu, using the 'Allelic Discrimination Plot'.

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5.7 Running the KASP trial kit

- If you have requested a KASP trial kit to run on your QuantStudio 6 and 7 instrument, please follow the protocol included with the kit to set up your reaction plate. This QuantStudio 6 and 7 manual can be used to help you to programme the instrument to run the KASP trial kit reaction plate.
- After running the KASP thermal cycle, the trial kit reaction plate should produce data similar to the figure below.



Results from the KASP trial kit reaction plate. The reaction plate was run on the QuantStudio 7 instrument using the standard KASP thermal cycle programme.

Further support

For any queries about this manual, please contact techsupport@lgcgroup.com.



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