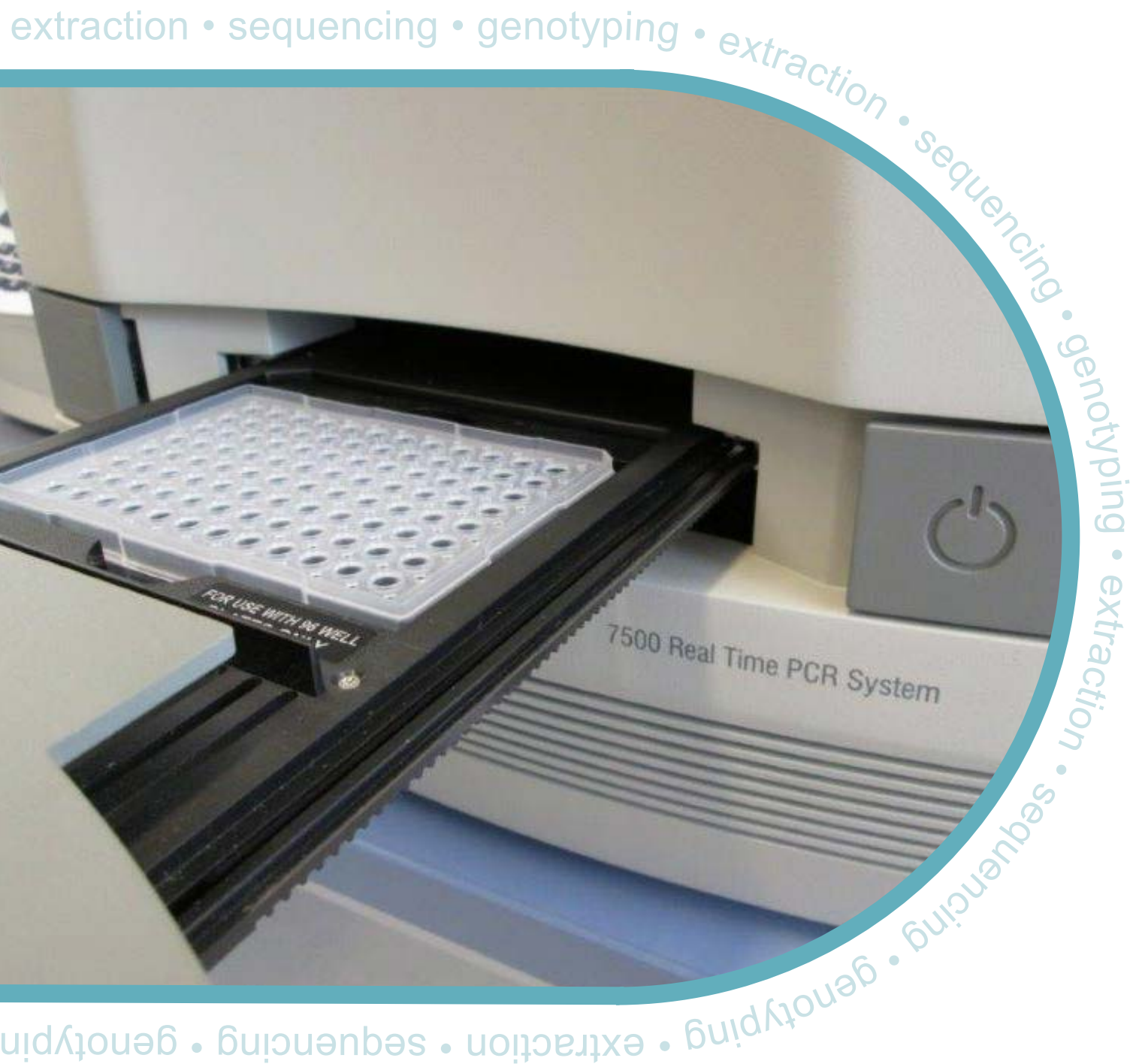




Guide to running KASP genotyping on the ABI 7500 instrument



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1. Introduction

This document is intended as a guide to running KASP™ genotyping reactions on the ABI 7500 instrument.

KASP chemistry for allelic discrimination performs well on an ABI 7500 machine and this step-by-step protocol will enable users to successfully set-up, run and read plates on the 7500.

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 7500 software, a pre-read (before the thermal cycle) and a post-read (after the thermal cycle) should be included in the thermal cycle program.
4. Data capture should not be performed during the thermal cycle program – 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 program will often will 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 ABI 7500 software only has an option for programing with VIC so ensure that you use this for the HEX allele.

3. Overview of the procedure

1. Define the experimental properties – see section 5.1.
2. Program the plate set up including assay information and sample names – see section 5.2.
3. Program 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.

4. KASP thermal cycling conditions

4.1 KASP thermal cycling conditions*

1. 94°C 15 minutes
2. 94°C 20 seconds
3. 61°C 60 seconds (drop by - 0.6°C/per cycle)
Repeat steps 2-3 9 times (a total of 10 cycles) achieving a final annealing temperature of 55°C
4. 94°C 20 seconds
5. 55°C 60 seconds
Repeat steps 4-5 for 25 times (a total of 26 cycles)
6. Read plate at less than 40°C.

4.2 KASP recycling conditions

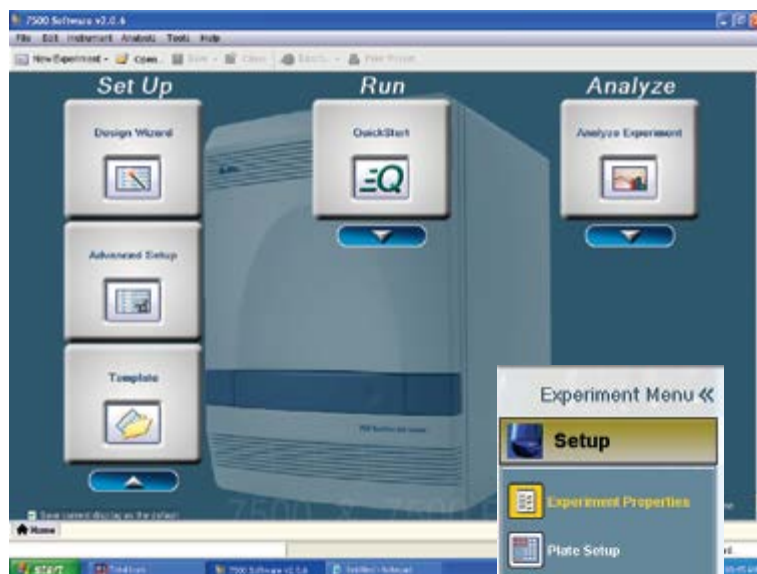
1. 94°C 20 seconds
2. 57°C 60 seconds
Repeat steps 1-2 twice (a total of 3 cycles)
3. Read plate at less than 40°C.

*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 ABI 7500 software
- On the front screen, click on the 'Design Wizard' button. This will open up a new experiment. The wizard will guide you through all stages of experimental setup.
- In the Setup menu, the first stage is to define the 'Experimental Properties'.



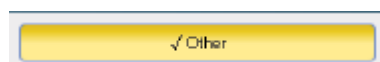
- **Step 1:**

Give your experiment a name
Optional – add in plate barcode information, your user name and any comments relevant to the experiment.

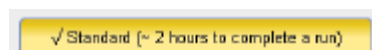
- **Step 2:** Select the instrument that you are using (i.e. Standard or Fast)
- **Step 3:** Select the experiment type – for KASP, this will be 'Genotyping'



- **Step 4:** Select 'Other' for the reagents type



- **Step 5:** Select 'Standard' for the ramp speed



- **Step 6:** 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).

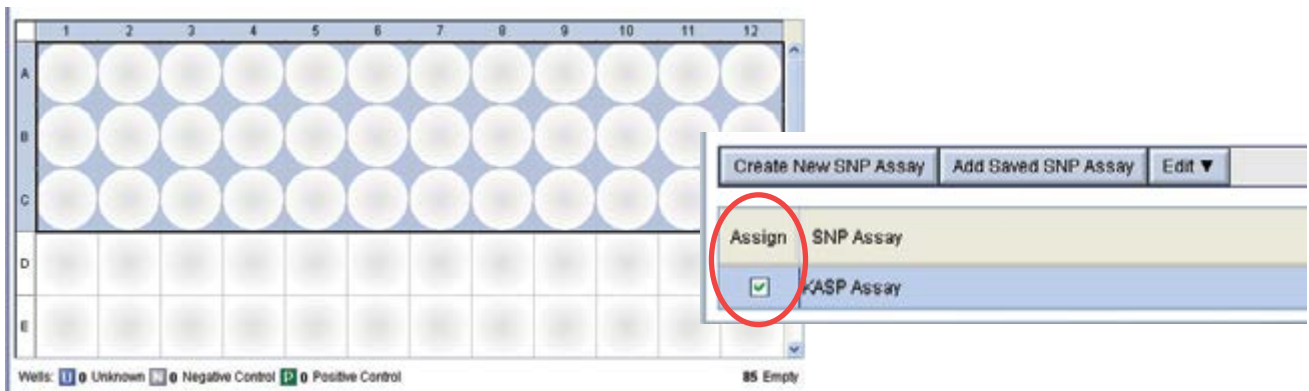


5.2 Programme the plate setup

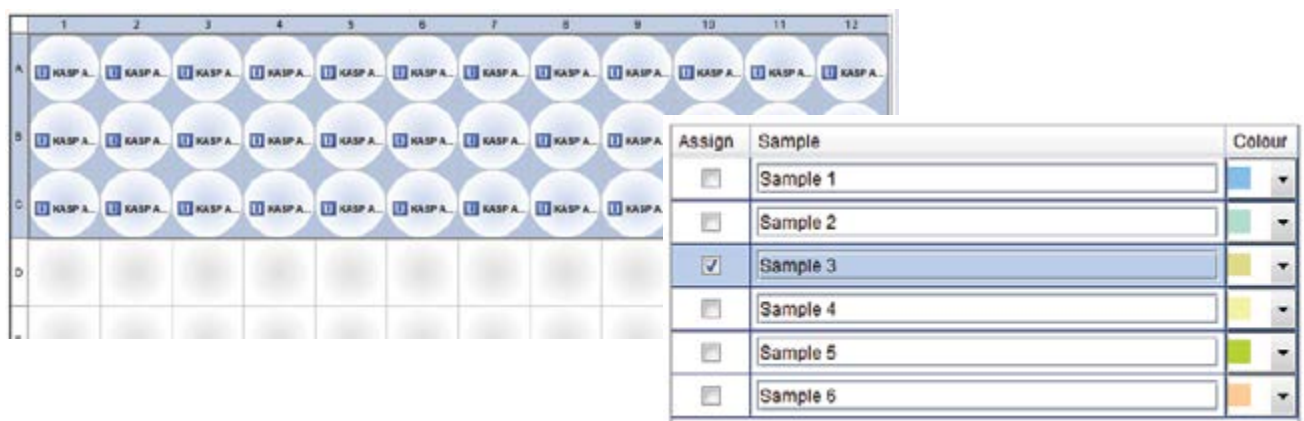
- Once the experimental properties are defined, click on the 'Plate Setup' menu.
- Click on the 'Create New SNP Assay' button to open up the 'New SNP Assay' dialogue box. Fill in all the fields including a name for your assay. One allele should have FAM as the reporter and the other should have VIC as the reporter. (HEX is the second fluorophore used in KASP, but this is not an option in the 7500 software. VIC and HEX can be read at the same wavelength).



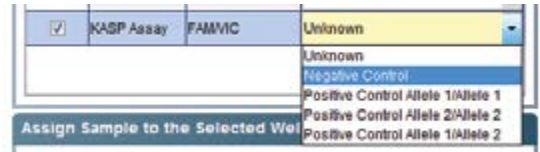
- Once you have created all the assays that you need for your experiment, assign these to the relevant wells of the plate. This is done by highlighting the wells, and then adding a tick to the 'Assign' column in the assay table.



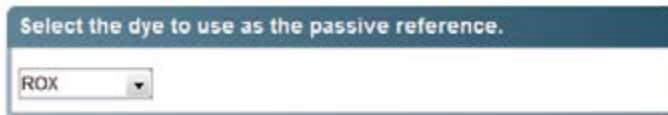
- Edit the sample names for the experiment. You can add new samples and display samples that have been defined in previous experiments.
- Assign the sample names to the relevant wells of the plate. This is done by highlighting the wells, and then adding a tick to the 'Assign' column in the sample table.



- Once the SNP assays and sample names have been assigned to the wells, 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' and No Template Controls should be defined as 'Negative Control'.



- Ensure that ROX™ is selected as the passive reference dye for the experiment.



- The completed plate setup can be viewed from the 'View Plate Layout' tab.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 1 KASP A...	Sample 1 KASP A...	Sample 2 KASP A...	Sample 2 KASP A...	Sample 3 KASP A...	Sample 3 KASP A...	Sample 4 KASP A...	Sample 4 KASP A...	Sample 5 KASP A...	Sample 5 KASP A...	Negative con... KASP A...	Negative con... KASP A...
B	Sample 1 KASP A...	Sample 1 KASP A...	Sample 2 KASP A...	Sample 2 KASP A...	Sample 3 KASP A...	Sample 3 KASP A...	Sample 4 KASP A...	Sample 4 KASP A...	Sample 5 KASP A...	Sample 5 KASP A...	Negative con... KASP A...	Negative con... KASP A...
C	Sample 1 KASP A...	Sample 1 KASP A...	Sample 2 KASP A...	Sample 2 KASP A...	Sample 3 KASP A...	Sample 3 KASP A...	Sample 4 KASP A...	Sample 4 KASP A...	Sample 5 KASP A...	Sample 5 KASP A...	Negative con... KASP A...	Negative con... KASP A...
D												
E												

5.3 Program the run method

- Once the plate layout has been defined, move to the 'Run Method' 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

- 94°C 15 minutes

Stage 2

- 94°C 20 seconds
- 61°C 60 seconds. ****Decrement -0.6°C/per cycle****
Set the decrement cycle using the 'Auto Increment' tab.
Repeat Stage 2 x9 times (a total of 10 cycles) achieving a final annealing temperature of 55°C.

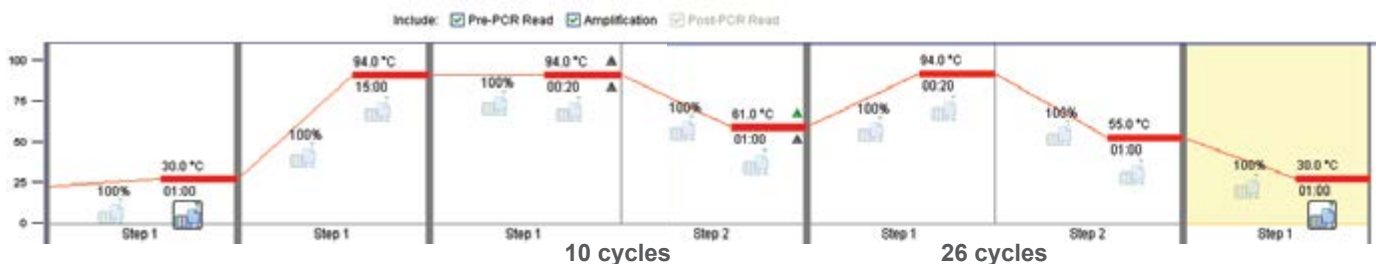
Stage 3

- 94°C 20 seconds
- 55°C 60 seconds
Repeat Stage 3 x25 times (a total of 26 cycles)

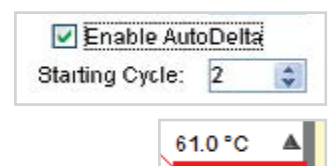
Stage 4

- 37°C for 1 minute. This will bring the reaction plate to the correct temperature for reading the fluorescence.

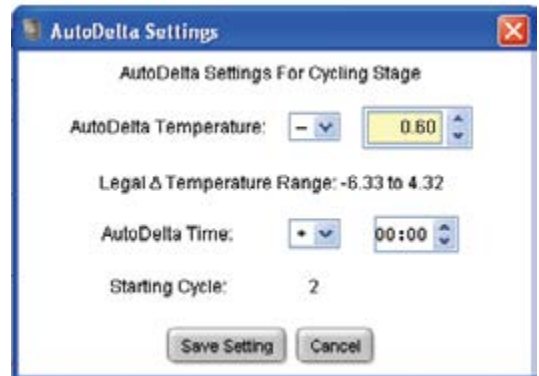
- Ensure that the data capture symbol is on (i.e. in colour) for the first stage (30°C for 1 min) so that the pre-read is performed, and for the last stage (30°C for 1 min) so that the post-read is performed.



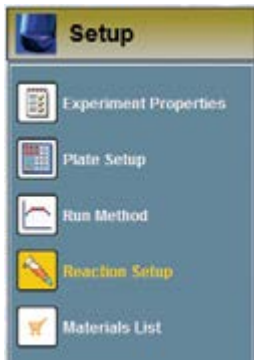
- To programme the touchdown stage, you need to click in the 'Enable AutoDelta' box of the relevant cycling stage. Then click on the grey triangle next to the temperature (61°C). This will open up the 'AutoDelta Settings' window.



- 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.



- The remaining two setup menus are not required for KASP ('Reaction Setup' and 'Materials List').

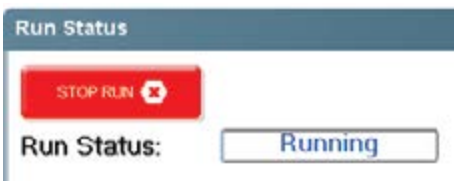


5.4 Run the thermal cycle including pre- and post-PCR reads

- Once the experimental properties, plate setup and run method have all been programmed, you can begin the run.
- Click on the 'Run' section of the experiment menu.
- Ensure that the software is connected to the instrument, and then press the green 'Start Run' button.



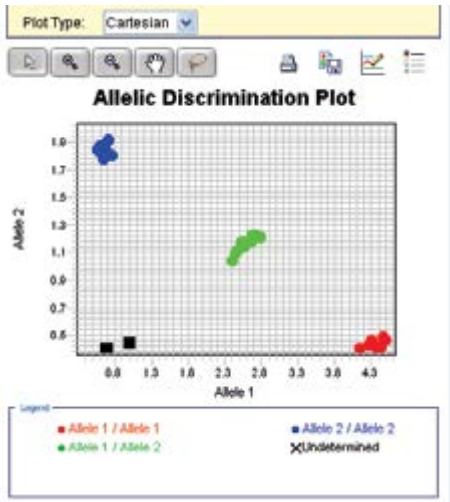
- The 'Run Status' will then change to 'Running' and the thermal cycle will begin.



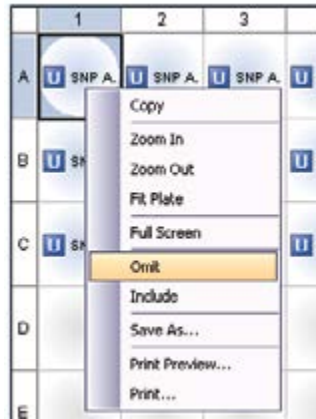
- Fluorescent data will be collected during the pre-read and post-read stages.

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'.

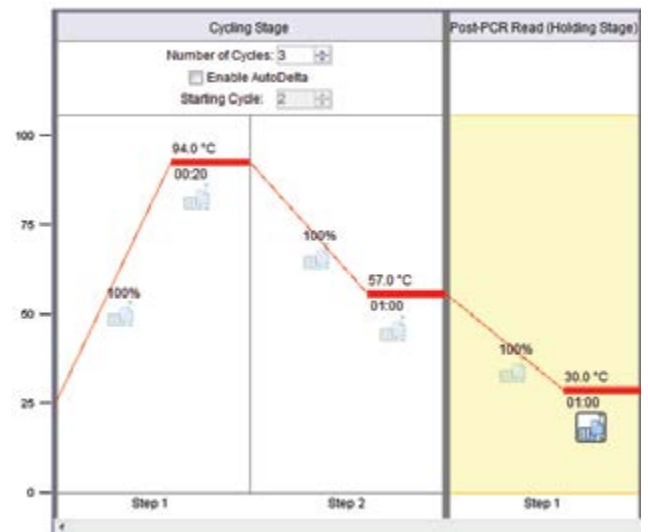


- If there are any anomalous wells (e.g. due to pipetting error), these can be omitted from the analysis. Right click on the well in the plate layout view, and select 'Omit'.



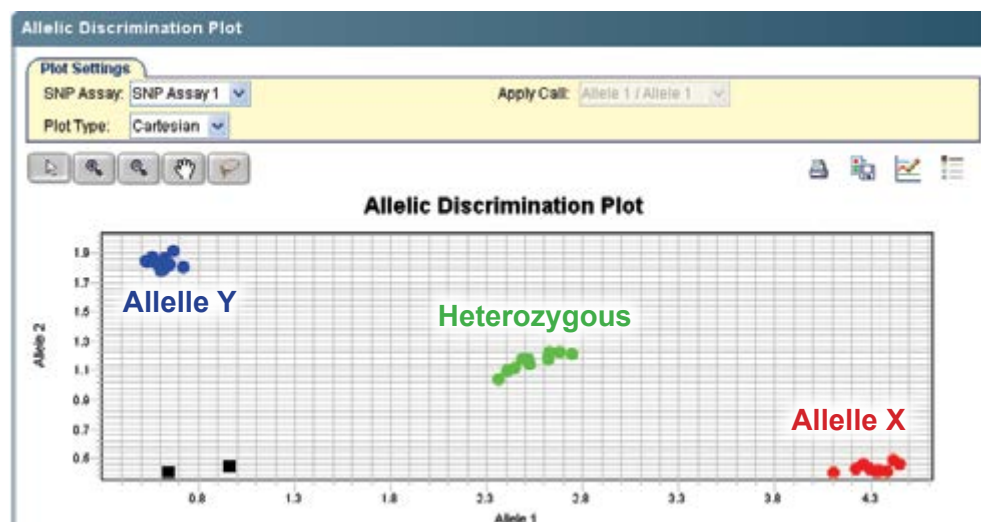
5.6 Recycle the plate if necessary

- If your data has not formed tight clusters after the initial thermal cycling protocol, you can recycle the plate in the ABI 7500 (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:
 - 94°C for 20 seconds
 - 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 that the data collection symbol is on.
- The data can then be viewed from the 'Analysis' section of the experiment menu, using the 'Allelic Discrimination Plot'.



5.7 Running the KASP trial kit on the ABI 7500

- If you have requested a KASP trial kit to run on your ABI 7500 instrument, please follow the protocol included with the kit to set up your reaction plate. This ABI 7500 manual can be used to help you to programme the instrument to run the 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 when run on the ABI 7500 instrument using the standard KASP thermal cycle and one recycle program.

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