

Manual



Running KASP genotyping on the Agilent Technologies Mx3000P/Mx3005P instruments

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1. Introduction

This document is intended as a guide to running KASP™ genotyping reactions on the Agilent Technologies Mx3000P/3005P instruments.

KASP chemistry for allelic discrimination performs well on an Mx3000P/3005P machine and this step-by-step protocol will enable users to successfully set-up, run and read plates on the Mx3000P/3005P.

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 MxPro 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 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 MxPro software only has an option for programming with VIC so ensure that you use this for the HEX allele.

3. Overview of the procedure

1. Select the correct program type for genotyping – see section 5.1.
2. Program the plate setup including SNP 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.

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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 × 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.

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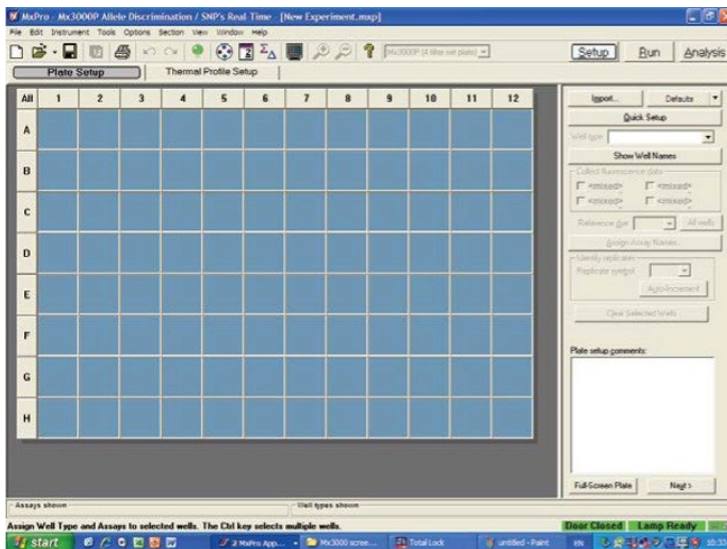
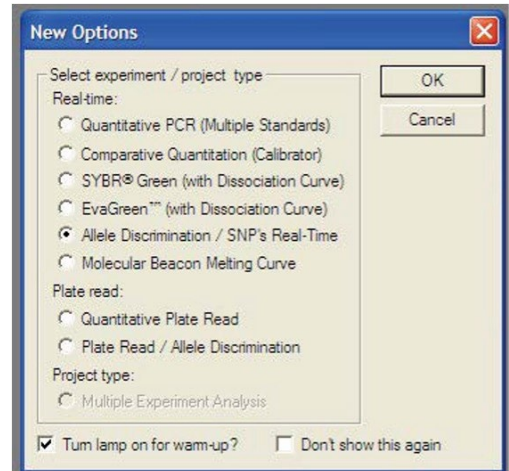
5. Step-by-step user guide

5.1 Select the correct experiment type for SNP genotyping

- Open the MxPro software.



- Select 'Allele Discrimination/SNP's Real-Time' from the new experiment options box.
N.B. If you plan to perform the thermal cycle on a Peltier PCR block rather than on the Mx3000P/Mx3005P, select 'Plate Read/Allele Discrimination' instead.
- A new experiment will open in the software containing a blank plate setup and a default thermal cycle.

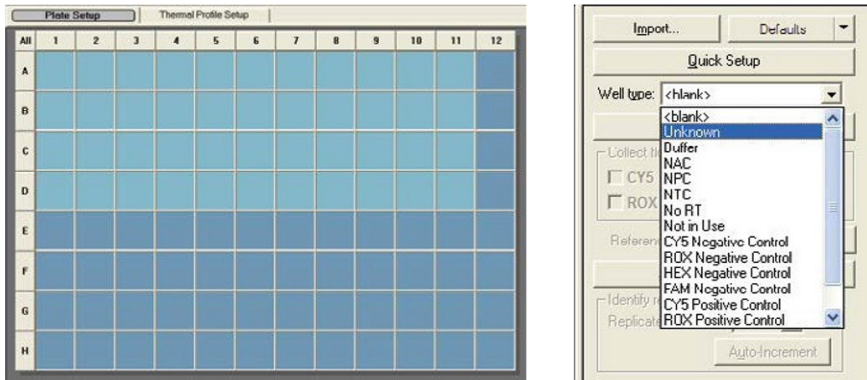


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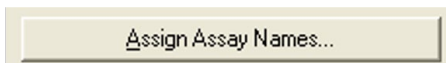
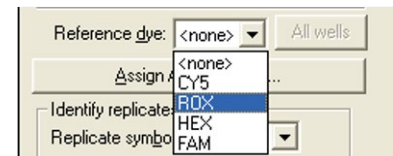
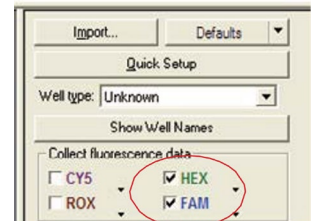
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5.2 Define the plate setup

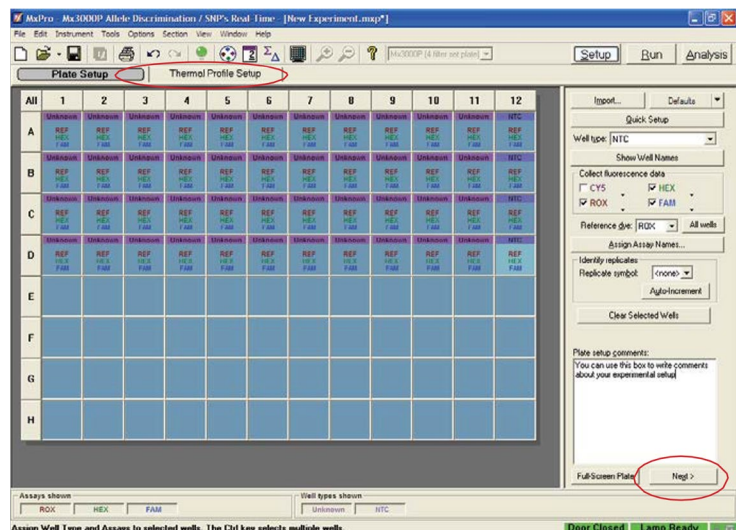
- On the 'Plate Setup' tab, define the well types for your experiment. To do this, highlight the relevant wells and select the correct well type from the drop down menu on the right hand side of the plate setup tab. For your DNA samples, select 'Unknown'. For the no template controls, select 'NTC'.



- Select the relevant fluorophores to be read in the experiment and assign these to the wells that contain samples and NTCs. To do this, highlight the relevant wells and tick both HEX and FAM™ from the 'Collect fluorescence data' section on the right hand side of the plate setup tab. N.B. The dyes must be assigned to the different well types individually. You cannot highlight 'Unknowns' and 'NTCs' and assign dyes to both at the same time.
- Highlight the wells containing your samples and select ROX™ as the passive reference dye from the drop down menu. If desired, assign assay names to the wells by clicking on the 'Assign Assay Names..' button.



- When the plate setup is complete, press 'Next' or click on the 'Thermal Profile Setup' tab.



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5.3 Define the thermal profile setup for KASP

- On the 'Thermal Profile Setup' tab, edit the default thermal profile to the conditions required for KASP genotyping.
- Please note: on the Mx3000P/3005P it is advisable to include pre-read at the start of the thermal cycle (30 °C for 1 minute).
- Please see the 'Hints for programming the thermal profile' for advice on how to set up the thermal profile in MxPro (below).

Cycling conditions

Stage 1

1. 30 °C 1 minute (Pre-read).

Stage 2

1. 94 °C 15 minutes.

Stage 3

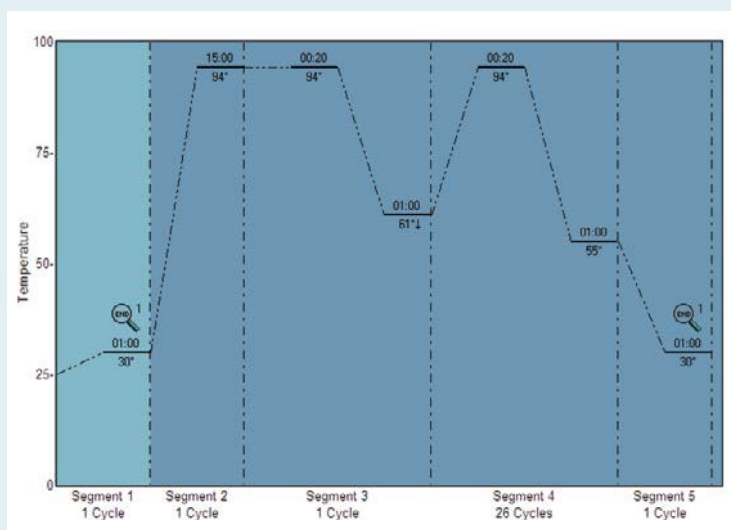
1. 94 °C 20 seconds
2. 61 °C 60 seconds
Decrement -0.6 °C/per cycle
Program the decrement by editing the plateau properties.
Repeat stage 3 × 9 times (a total of 10 cycles) achieving a final annealing temperature of 55 °C.

Stage 4

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

Stage 5

1. 37 °C for 1 minute.
N.B. KASP cannot be read at temperatures above 40 °C.



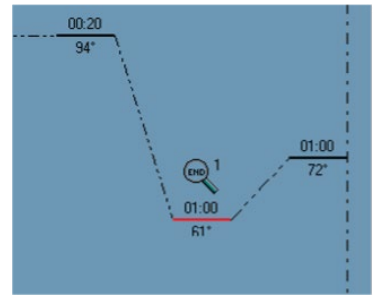
Hints for programming the thermal profile in the MxPro software

- Before editing the default segments of the thermal cycle in MxPro, make sure that you add sufficient segments for the entire KASP thermal cycle. There should be a total of 5 segments, including the pre- and post-PCR reads. The MxPro software always adds new segments at the beginning of the thermal profile (rather than at the end) hence adding them as you work through the thermal profile can be frustrating.
- Including a pre-read segment in the thermal profile enables the FAM, VIC (HEX) and ROX levels to be read before the PCR begins.

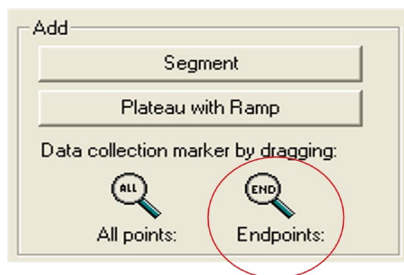
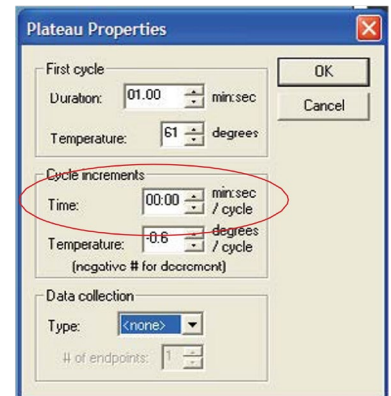
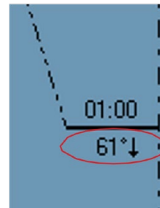
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- To program a temperature decrement:
 - Highlight the relevant temperature plateau within the segment (it will be red when selected).
 - Click on the 'Edit Properties' button in the 'Selection' box on the right hand side of the 'Thermal Profile Setup' tab.

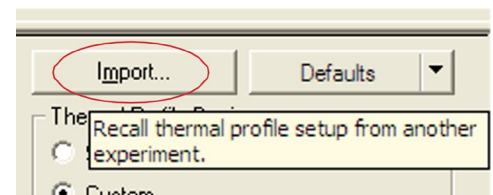
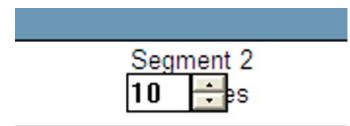


- In the 'Plateau Properties' window, program the required decrement for KASP in the temperature box. This will be -0.6 °C. Ensure that you type in a minus (-) symbol before the number to program a decrement. The data collection for this stage should be changed to '<none>'.
- A segment that has had a decrement programmed will be identifiable by the arrow symbol next to the temperature.



- To add a plate read to a step, click on the 'Endpoints' magnifier symbol and drag it to the required segment of the thermal profile.
- To edit the number of cycles for a particular segment, click on the number of cycles at the bottom of the thermal profile. This will open up an input box; you can then increase or decrease the number of cycles using the arrow buttons.

- Once the thermal profile has been correctly programmed, it can be used for all future KASP genotyping runs. When you create a new experiment, click on the 'Import...' button in the 'Thermal Profile Setup' tab. This will enable you to select a previously run experiment from which to import a thermal profile.



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

- Allow the instrument lamp to complete its warm up. The lamp status should change from 'Lamp Warm-up' in yellow, to 'Lamp Ready' in green.



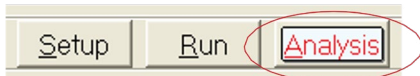
- Place your prepared reaction plate into the instrument and shut the door.
- Ensure that the experiment setup file is connected to the instrument.
- Click on the 'Start Run' button.



- A standard KASP thermal cycle with a pre- and post-PCR read should take approximately 1 hour and 20 minutes on the Mx3000P/Mx3005P instruments.

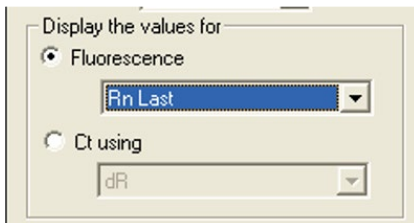
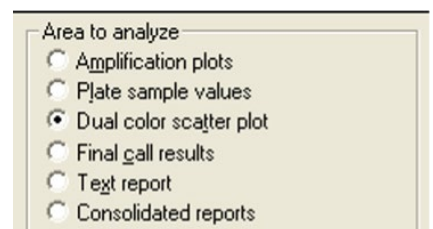
5.5 Analysis of results

- Once the thermal cycle run has completed, you can analyse the read data.
- On the 'Analysis Selection/Setup' tab, highlight the wells that you wish to analyse and click on the 'Analysis' button at the top right of the experiment window.



Analysis Selection/Setup					
All	1	2	3	4	5
A	Unknown	Unknown	Unknown	Unknown	Unknown
	REF HEX FAM	REF HEX FAM	REF HEX FAM	REF HEX FAM	REF HEX FAM
B	Unknown	Unknown	Unknown	Unknown	Unknown
	REF HEX FAM	REF HEX FAM	REF HEX FAM	REF HEX FAM	REF HEX FAM
	Unknown	Unknown	Unknown	Unknown	Unknown

- Click on the 'Results' tab. At the top right hand side, there is an 'Area to analyze' box that gives several options for viewing the data.
- Select 'Dual color scatter plot' to view the genotyping plot.
- In the 'Display the values for' box, select 'Fluorescence' and then 'Rn Last' from the drop down menu.



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- You can choose which allele you would prefer to plot on the X-axis and the Y-axis using the 'Allele associations' box. Allele A is plotted on the X axis and Allele B is plotted on the Y axis. **PLEASE NOTE:** the genotyping results shown in the KASP trial kit manual have the FAM allele on the X axis and the HEX allele on the Y axis.

Allele associations

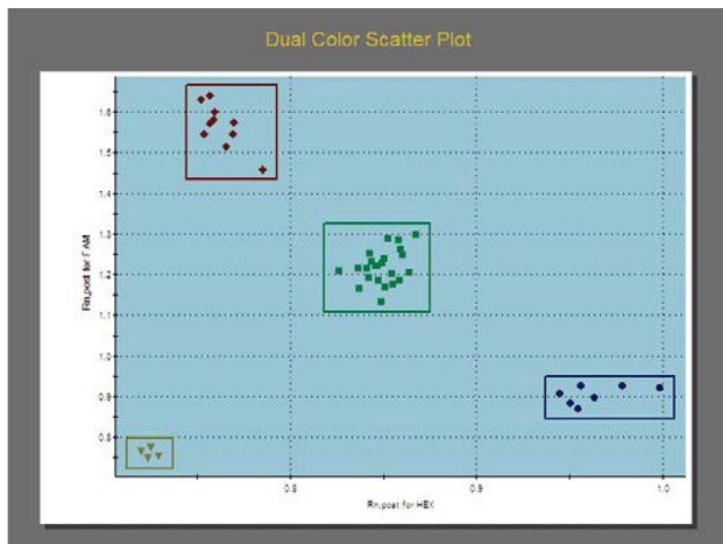
Allele A:

Allele B:

Both: HEX and FAM

- To manually call your genotyping results, click on the 'Show All Genotypes' button. This will bring up three boxes on the genotyping plot, one for Allele A, one for Allele B, and one for Both.

- Re-size these boxes to incorporate all samples within a cluster. Data points within a box will change to the appropriate colour for that genotype.
- The NTC samples should be in the bottom left hand corner of the plot.

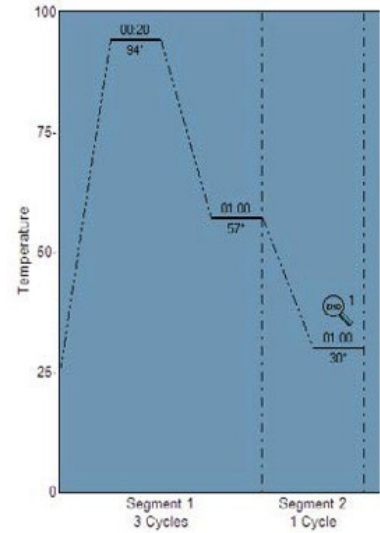


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5.6 Recycle your reaction plate if required

- If your data has not formed tight clusters after the initial thermal cycling protocol, you can recycle the plate in the Mx3000P/ Mx3005P (or on a Peltier block) and perform a second read.
- You will need to create a new experiment and program the required thermal profile conditions on the 'Thermal Profile Setup' tab.
- The KASP recycling conditions are:
 1. 94 °C for 20 seconds
 2. 57 °C for 60 secondsRepeat steps 1-2 twice (a total of 3 cycles).
The second segment should be 30 °C for 1 minute to enable the plate to be read.
- Analysis of the new read data can then be carried out as described in section 5.5.

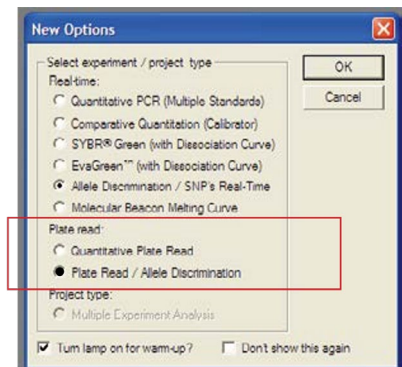
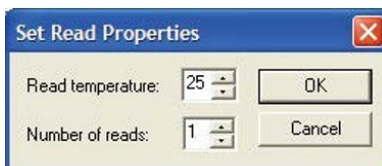


5.7 Using the Mx3000P/Mx30005P as a plate reader

- If you prefer to run your genotyping PCR reactions on a Peltier block, the Mx3000P/Mx3005P instruments can be used to read the plate after reaction completion.
- After opening MxPro, select 'Plate Read/Allele Discrimination' from the 'Plate read' section of the options box.
- Define the plate setup as detailed in section 5.2.
- Click on the 'Set Read Properties' button.



- In the 'Set Read Properties' window, ensure that the read temperature is set below 40 °C as KASP can only be read below 40 °C.

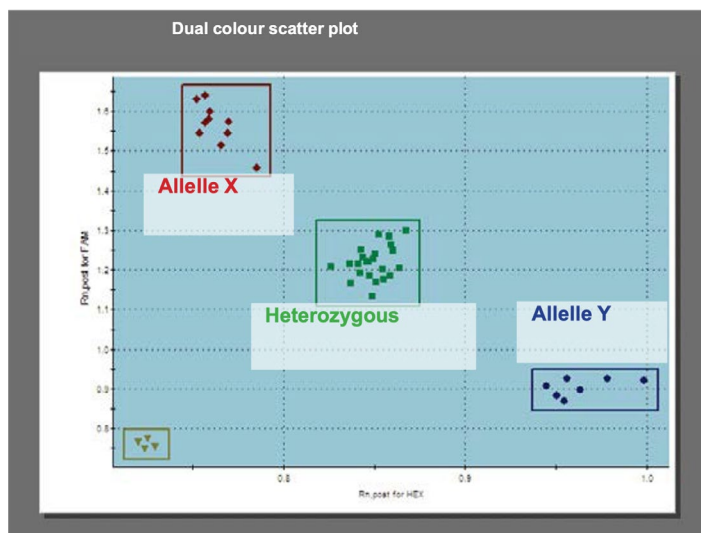


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5.8 Running the KASP trial kit on the Mx3000P/Mx3005P

- If you have requested a KASP trial kit to run on your Mx3000P/3005P instrument, please follow the protocol included with the kit to set up your reaction plate. This Mx3000P/3005P 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 to the right.



Results from the KASP trial kit reaction plate when run on the Mx3000P instrument using the standard KASP thermal cycle

6. Further support

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



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