



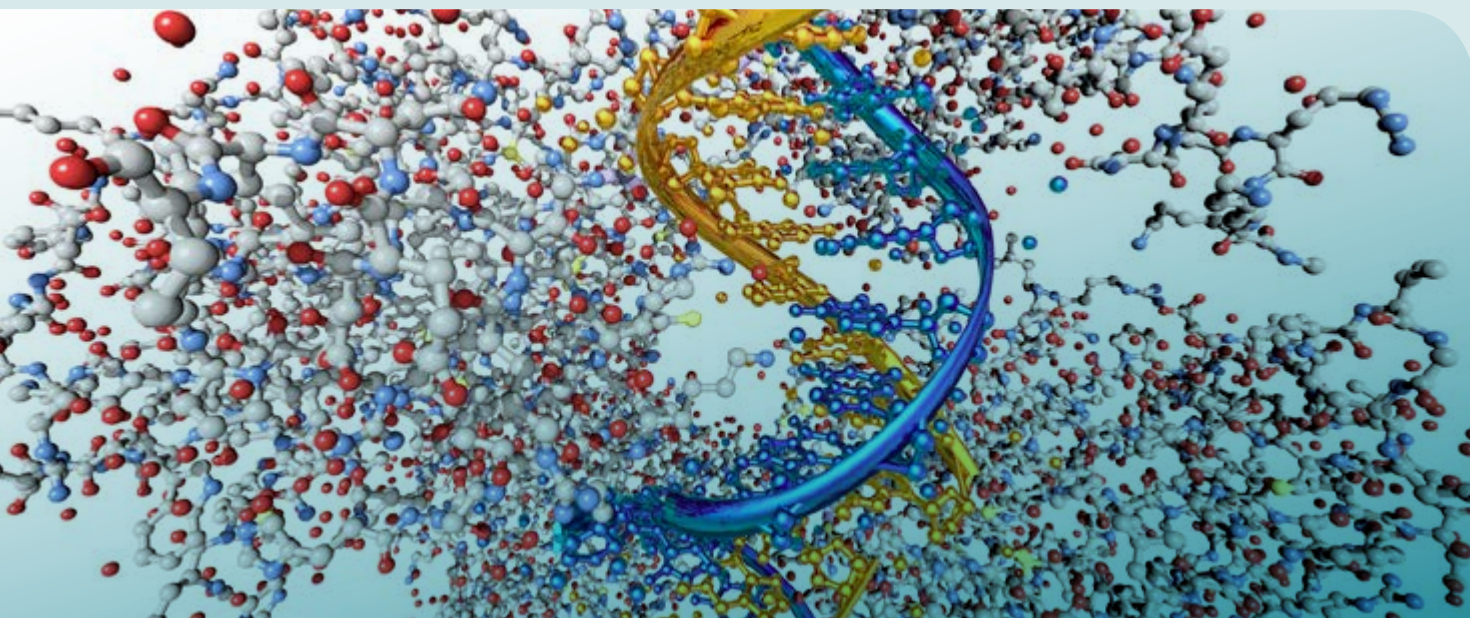
Guide to running KASP genotyping reactions on the Illumina Eco RT instrument



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Cover iustrument image courtesy of Illumina, Inc.



1. Introduction

This document is intended as a guide to running KASP™ genotyping reactions on the Illumina Eco RT instrument.

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

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 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 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. Data capture should only be performed at the end of the thermal cycle program – 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.

3. Overview of the procedure

1. Create a new protocol for the KASP thermal cycle – see section 5.1.
2. Program the plate set-up including assay information and sample names – see section 5.2.
3. View the data – see section 5.3.
4. Recycle the plate if necessary – see section 5.4.



Courtesy of Illumina, Inc.

4. KASP thermal cycling conditions

4.1 KASP thermal cycling conditions

Stage 1

1. 94°C 15 minutes

Stage 2

1. 94°C 20 seconds
2. 61°C 60 seconds
3. Drop -0.6°C per cycle

Repeat Stage 2 x9 times (a total of 10 cycles) achieving a final annealing temperature of 55°C.

Stage 3

1. 94°C 20 seconds
2. 55°C 60 seconds

Repeat Stage 3 x25 times (a total of 26 cycles)

Stage 4

1. Add read step at 40°C for 1 min*

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. Add read step at 40°C for 1 min*

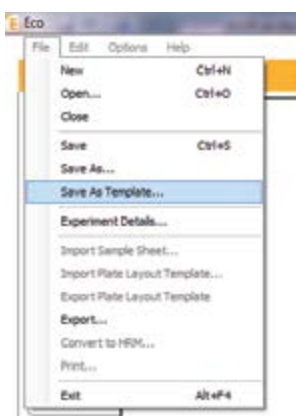
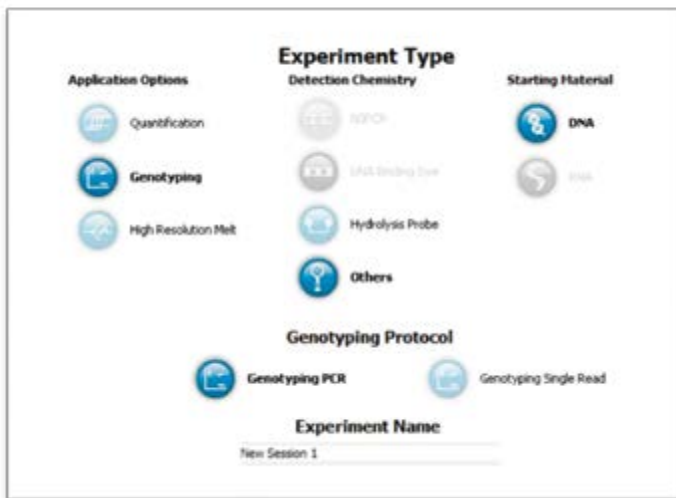
*KASP cannot be read at temperatures above 40°C.

Please note: We can provide a setup file for this machine for standard KASP thermal cycling.

5. Step by step user guide

5.1 Create a new protocol for KASP genotyping

- Open the Eco control software.
- Select 'Genotyping' as the application, and 'Genotyping PCR' as the protocol type.
- Click the 'tick' symbol in the bottom right hand corner to open the new experiment.



- In the 'File' menu, select 'Save As Template...' so that you can use the setup file for all future runs.
- For each new experiment, rename the file with a unique name.

In the 'Setup' tab, edit the thermal cycle to the conditions required for KASP genotyping.



- Program the activation stage: 94°C 15 minutes.
- Program the touchdown stage:
 - 94°C 20 seconds
 - 61°C 60 seconds (drop -0.6°C /per cycle)


Repeat these steps x9 times (a total of 10 cycles) achieving the final annealing temperature of 55°C.

Please note: A separate step will need to be programmed for each of the touchdown cycles as a touchdown program is not available with the Illumina Eco RT software.



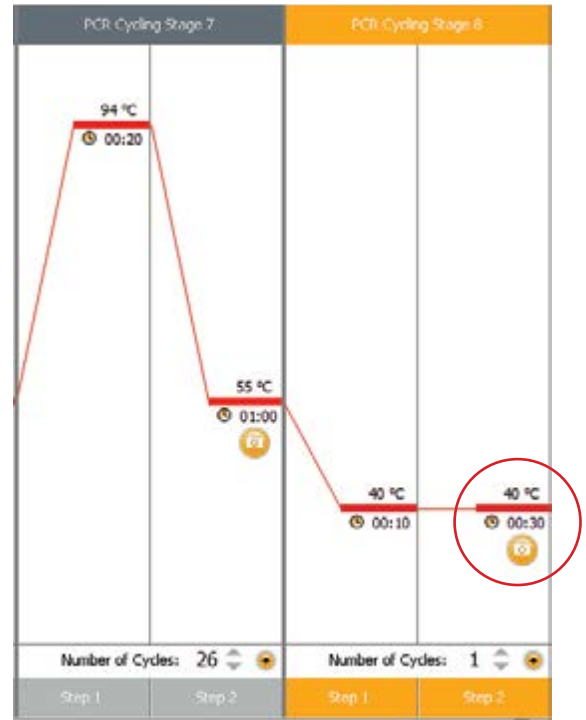
- Program the amplification stage:
 - 94°C 20 seconds
 - 55°C 60 seconds

Repeat these steps x25 times (a total of 26 cycles).

The orange symbol indicates a data acquisition step. It is not possible to switch off the data acquisition at this stage, however any data acquired is not useable as KASP is not a real time chemistry. 

- Add 2 read steps:
 - 40°C for 10 seconds to cool the plate
 - 40°C for 30 seconds to read the plate*
(ensure that the orange camera symbol is on this step)

* Please note that KASP cannot be read above 40°C.

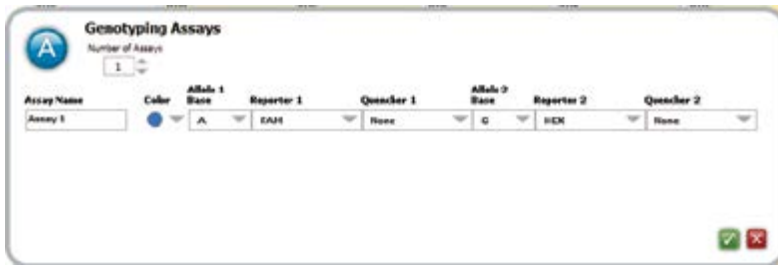


5.2 Program the plate setup

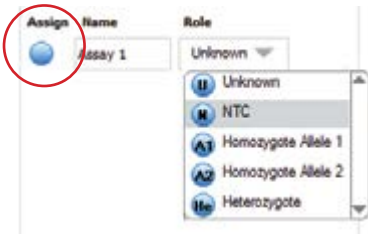
- Once the thermal profile is defined, click on the 'Plate Layout' tab.
- Click on the 'Set Up Samples' button. In the dialogue box, input the number of different sample types that you have. It is also possible to name these individually if required. Click on the tick symbol to return to the plate layout.
- Highlight the relevant wells on the plate layout and designate them as the samples that you have just created. The colour linked with the sample name will then be displayed in the appropriate wells.



- Click on the 'Set Up Assays' button. In the dialogue box, complete the parameters for your KASP assay. You can name the assay if required. Choose FAM for 'Reporter 1' and HEX for 'Reporter 2', and designate the relevant allele call for these. The quenchers should be set to 'None'.

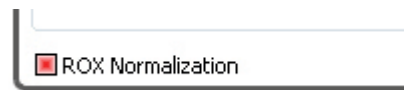


- Highlight all the wells containing your samples. In the 'Set Up Assays' box, click on 'Assign' next to the assay name to link this assay to all relevant wells.



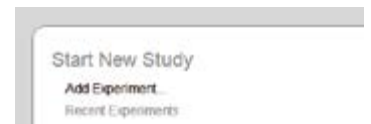
- Highlight the wells that contain your No-template controls (NTC) and designate them using the drop down menu. Positive control samples can also be designated using this menu.

- The 'ROX normalisation' box at the bottom left hand corner should be selected / checked as KASP Master mix includes ROX as a passive reference dye.

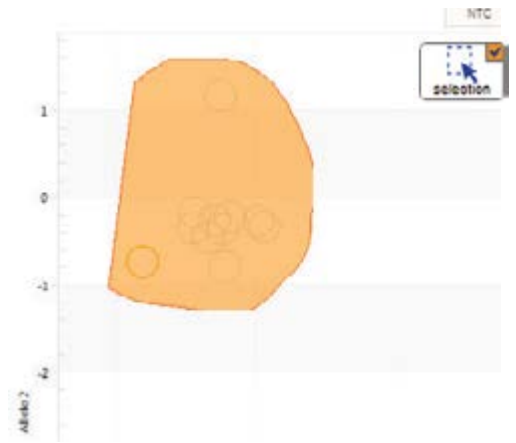
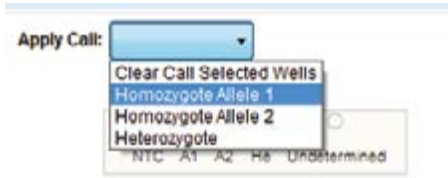


5.3 Analysis of results using Eco Study software

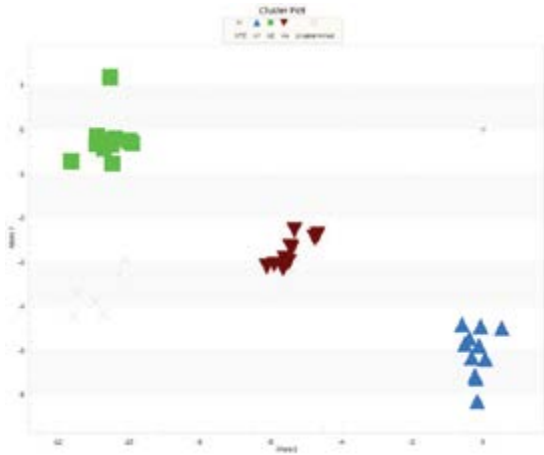
- Once the PCR program and plate read have completed, open the Eco Study software to perform your analysis.
- On the start up screen, click on 'Add Experiment' under the 'Start New Study' section.
- Navigate to where your recently completed experiment is stored and select the file. It will then open within a new study. (All the plate setup parameters that you programmed during setup will be imported with the file).
- Click on the 'Results' tab at the top right hand side of the screen to navigate to the data.
- The data will be plotted on a cluster plot on the left hand side of the window, and in table form on the right hand side of the window.



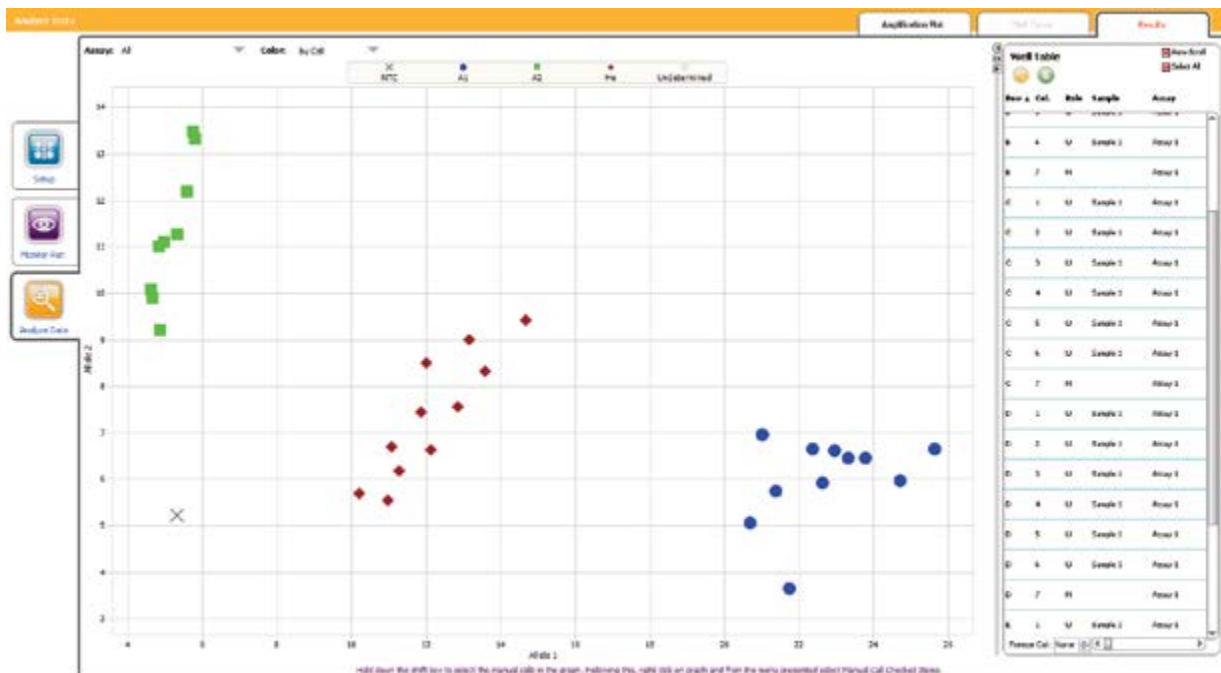
- Based on the clustering of the data points on the plot, they can be manually called as the appropriate genotypes.
- Use the 'selection' tool to highlight all the samples within a cluster (orange bubble) and apply the appropriate call to each selection from the drop down menu.



- Click the 'select all' button and then choose colour 'by call' from the drop down menu.



Data output - scoring



For any queries on this manual or running KASP reactions in your laboratory please contact:
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5.4 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 Eco RT (or on a Peltier block) and perform a second post-read.
- Follow the instructions in section 5.1 to create a new protocol for KASP recycling.
- The KASP recycling conditions are:
 1. 94°C 20 seconds
 2. 57°C 60 secondsRepeat steps 1-2 twice (a total of 3 cycles)
- Add 2 read steps:
 - 40°C for 10 seconds to cool the plate
 - 40°C for 30 seconds to read the plate
- The recycling program can be repeated until tight clusters have formed (re-read the plate after each recycling program).
- Once results have been read, completed KASP reactions are stable at room temperature for up to one week as long as the plates remain well sealed.
- For other cycling conditions, the file can be adapted by altering the temperatures.
- If it is found that recycling is always necessary, extra cycles can be added to the PCR amplification stage (i.e. 29 cycles instead of 26).

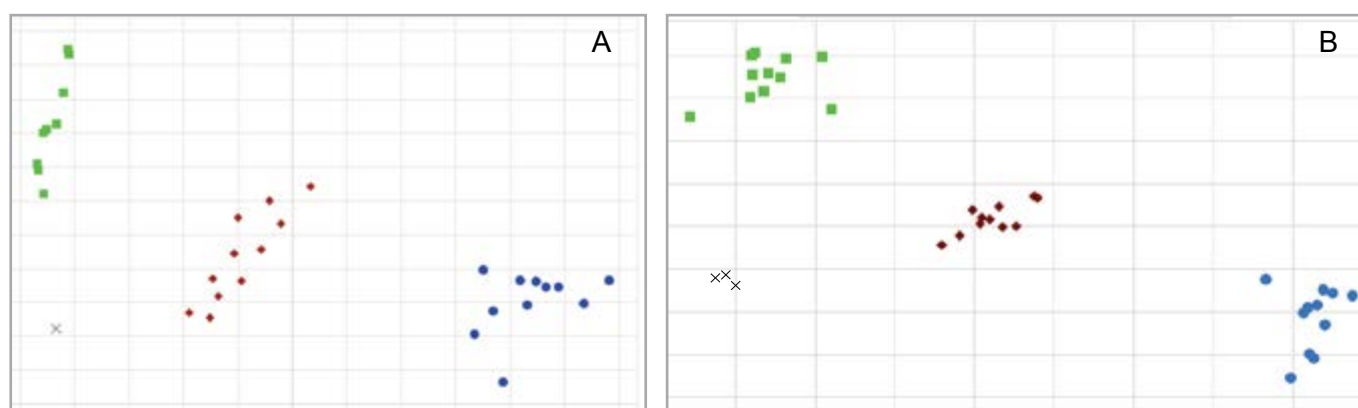
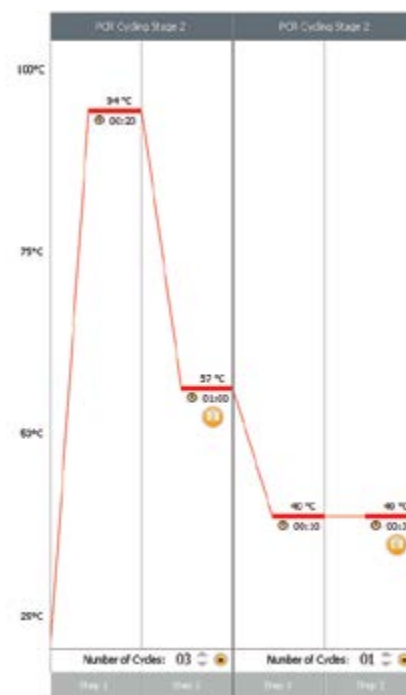


Figure A shows KASP genotyping data after the initial thermal cycle. The reaction plate was then recycled once (three PCR cycles) to produce tighter genotyping clusters (Figure B).

5.5 Running the KASP trial kit on the Eco RT

- If you have requested a KASP trial kit to run on your Illumina Eco RT instrument, please follow the protocol included with the kit to set up your reaction plate. This Eco RT 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 Illumina Eco RT instrument using the standard KASP thermal cycle.

For any queries about this guide or running KASP reactions in your laboratory please contact the technical support team:

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