Roche LC96 and KASP

Troubleshooting guide

This factsheet is intended to give users a detailed explanation of the issues with running KASP™ genotyping on an LC96 instrument, and to outline the current work-around solutions that are available.

Issues with running KASP on the LC96

The Roche LC96 instrument contains the required filters to read KASP genotyping reactions but, unfortunately, settings within the instrument software mean that it is not a straightforward procedure to run and analyse results from KASP genotyping reactions. The main reasons for this are as follows:

1. KASP is an endpoint genotyping chemistry and a plate read is required only at the end of the thermal cycle. The LC96 instrument does not enable endpoint reads of reaction plates. Instead the instrument requires a plate read to be performed during each PCR cycle as the software follows the progression of the data plots throughout the thermal cycle. Data collected from these 'real-time' reads on the LC96 instrument is not meaningful for KASP as the reads must be performed above 40°C (KASP can only be read below 40°C). Reducing the temperature to below 40°C during each PCR cycle will adversely affect progression of the PCR.

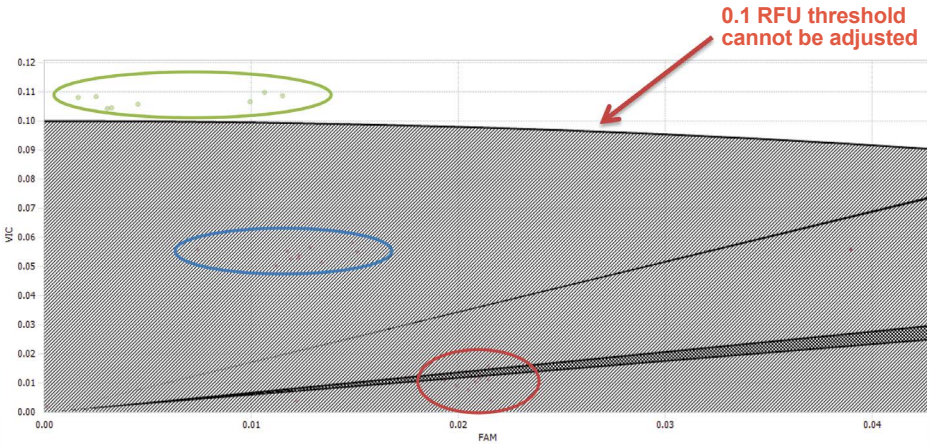


Figure 1. Data plot of KASP genotyping reactions run on the LC96 instrument. The threshold of 0.1 RFU cannot be adjusted and results in the majority of data points being plotted behind the greyed out area. Three clusters are visible, but data scoring options are not accessible within the LC96 software due to the set threshold.

2. The LC96 plotting software has a threshold set within it (0.1 RFU) that cannot be altered. At the end of the KASP thermal cycle on the LC96, several cycles of 30°C can be programmed with plate read steps to collect the required data to produce a genotyping plot within the LC96 software. Unfortunately, as it is not possible to adjust the set threshold, it is not possible to view genotyping data plots for KASP within the LC96 software.

Is there a solution to these issues?

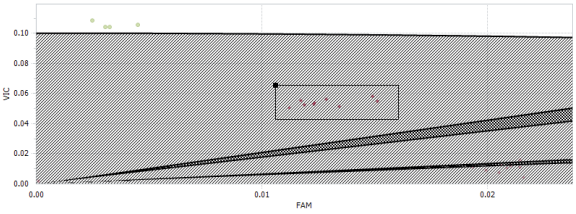
There are currently two options available to the user to enable results from KASP genotyping to be extracted from the LC96 software.

1. Identify the wells within each cluster

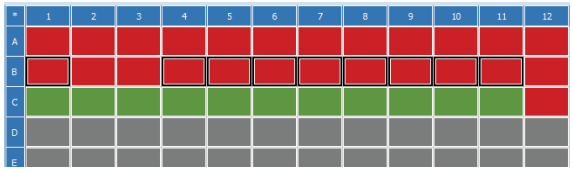
Within the LC96 software, use the 'selection tool' to draw a box around the samples in one of the genotyping clusters e.g. the heterozygous cluster. The selection tool will function even when the data points are in the shaded area (i.e. below the 0.1 RFU threshold). Once all data points within a cluster are selected, view the heat map. All the encased samples will be highlighted in the heat map, thus enabling the user to identify which samples are homozygous for the FAM allele. Repeat this procedure for the other two genotyping clusters where applicable.

1. Click on the selection tool.

2. Draw a box around one of the clusters.

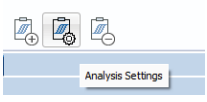


3. The relevant wells will be highlighted in the heat map, enabling the user to identify which samples have which genotype.

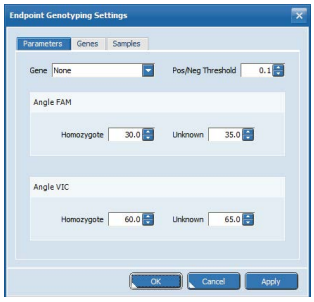


Please note: It is possible to change the angles for the FAM and VIC sections of the plot, even when they are positioned beneath the threshold line. To do this, click on the 'Analysis settings' button to open the 'Endpoint Genotyping Settings' box. Within this box you can manually edit both the FAM and VIC angles to maximise the number of data points that are easily visible on the plot.

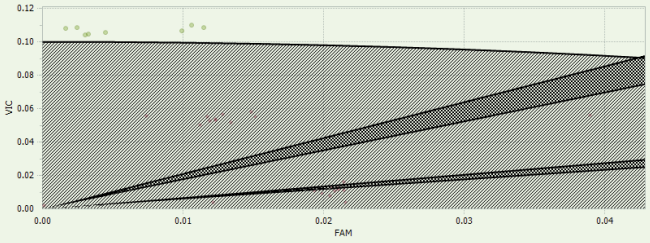
1. Click on the 'analysis settings'



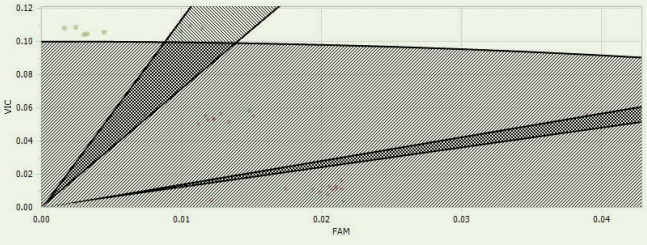
2. Adjust the angles for FAM and VIC



Before angle adjustment



After angle adjustment



For any queries on this manual or running KASP reactions in your laboratory please contact:
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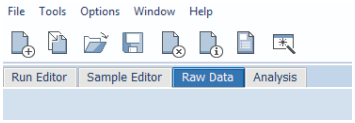
2. Export the genotyping data to alternative software

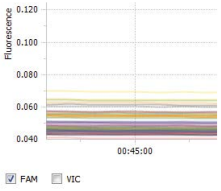
To view genotyping data collected using KASP chemistry on the LC96 instrument, the raw data values for FAM and HEX must be exported to alternative software for analysis. Values can be exported to spreadsheets, such as Microsoft Excel, and can then be plotted using a scatter plot. Alternatively, data can be exported to a cluster analysis software package such as KlusterCaller (LGC).

To export the endpoint reads (fluorescent data), do the following:

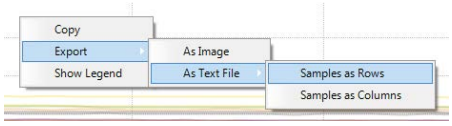
1. Click on the 'raw data' tab.



2. Ensure that only one of the two dyes is ticked at the bottom left of the 'fluorescence curves' plot.



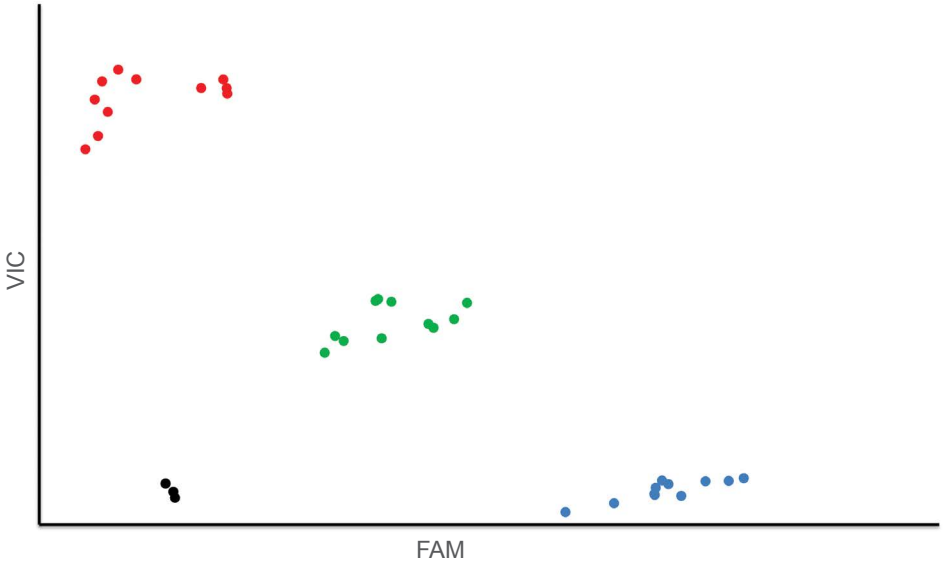
3. Right click on the 'fluorescence curves' plot. Click 'export' > 'as text file' > 'samples as rows'.



This exports a text file containing the raw data for FAM. Repeat the process for VIC and to obtain both sets of data. The text files can be opened in Excel. As this instrument requires a read every cycle, there are lots of columns of data for each well. You should use either the last or second to last columns (i.e. AB or AC) as these are from the reads performed at 30°C (all of the other reads will not give meaningful data).

KASP genotyping data collected on the LC96 instrument produces clear genotyping clusters, but to be able to plot these clusters, export of data and manual analysis is required. The user must decide if the additional data export step is workable within their laboratory workflow.

Figure 2. Data plot of KASP genotyping reactions run on the LC96 instrument. Raw data values for FAM and VIC (HEX) were exported to Excel and plotted using the scatter plot function. Data points were colour-coded individually.



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If you have any additional questions regarding the running of KASP genotyping on an LC96 instrument, please do not hesitate to contact our technical support team.

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