

Manual

Running KASP genotyping reactions on the Applied Biosystems Viia 7

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Guide

Running KASP genotyping reactions on the Applied Biosystems ViiA 7

Contents

1. [Introduction](#)
2. [Tips and suggestions before you start](#)
3. [Overview of the procedure](#)
4. [KASP thermal cycling conditions](#)
 - 4.1 [KASP thermal cycling conditions](#)
 - 4.2 [KASP recycling conditions](#)
5. [Step-by-step user guide](#)
 - 5.1 [Define the experimental properties](#)
 - 5.2 [Program the plate set up including assay information and sample names](#)
 - 5.3 [Program the run method for KASP](#)
 - 5.4 [Run the thermal cycle including pre- and post-read steps](#)
 - 5.5 [Analysis of results \(Allelic Discrimination Plot\)](#)
 - 5.6 [Recycle your reaction plate if required](#)
 - 5.7 [Running the KASP trial kit on the ViiA 7](#)
6. [Further support](#)

Guide

Running KASP genotyping reactions on the Applied Biosystems Viia 7

1. Introduction

This document is intended as a guide to running KASP™ genotyping reactions from LGC, Biosearch Technologies, on the Applied Biosystems™ Viia 7 instrument. KASP chemistry for allelic discrimination performs well on a Viia 7 machine and this step-by-step protocol will enable users to successfully set-up, run and read plates on the Viia 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 Viia7 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 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 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 Viia 7 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. 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.

Guide

Running KASP genotyping reactions on the Applied Biosystems ViiA 7

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 x 9 times (a total of 10 cycles) achieving a final annealing temperature of 55 °C
4. 94 °C for 20 seconds
5. 55 °C for 60 seconds
Repeat steps 4-5 x 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.

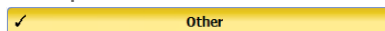
5. Step-by-step user guide

5.1 Define the experimental properties

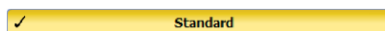
- Open the ViiA 7 software
- On the front screen, in the Set Up box, click on 'Experiment Setup'. This will open up a new experiment.
- In the Setup menu, the first stage is to define the 'Experiment Properties'.
- Step 1: Give your experiment a name
Optional – add in plate barcode information, your user name and any comments relevant to the experiment.
- Step 2: Select the block that you are using (i.e. 384-well or Array Card)
- 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).

Include: Pre-PCR Read Amplification Post-PCR Read



Guide

Running KASP genotyping reactions on the Applied Biosystems Via 7

5.2 Program the plate setup

- 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 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 Via7 software. VIC and HEX can be read at the same wavelength).



SNP Assay Name	NCBI SNP Reference	Context Sequence	Allele 1	Reporter	Quencher	Allele 2	Reporter	Quencher	Color
SNP Assay 1			Allele 1	VIC	NFQ-MGB	Allele 2	FAM	NFQ-MGB	

A dialog box titled 'Edit SNP Assay' with a close button. It contains the following fields: 'SNP Assay Name' (KASP SNP Assay 1), 'Color' (red), 'Assay ID' (empty), 'Gene Symbol' (empty), 'Gene Name' (empty), 'NCBI SNP Reference' (empty), 'Context Sequence' (empty), 'Allele 1 Name or Base(s)' (Allele 1), 'Color' (blue), 'Reporter' (VIC), 'Quencher' (NFQ-MGB), 'Allele 2 Name or Base(s)' (Allele 2), 'Color' (green), 'Reporter' (FAM), 'Quencher' (NFQ-MGB), and 'Comments' (empty). At the bottom are 'Reset Fields', 'OK', and 'Cancel' buttons.

- 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.



Sample Name
Sample 1
Sample 2
Sample 3

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

A dropdown menu titled 'Passive Reference' with a red asterisk icon. The selected option is 'ROX'.

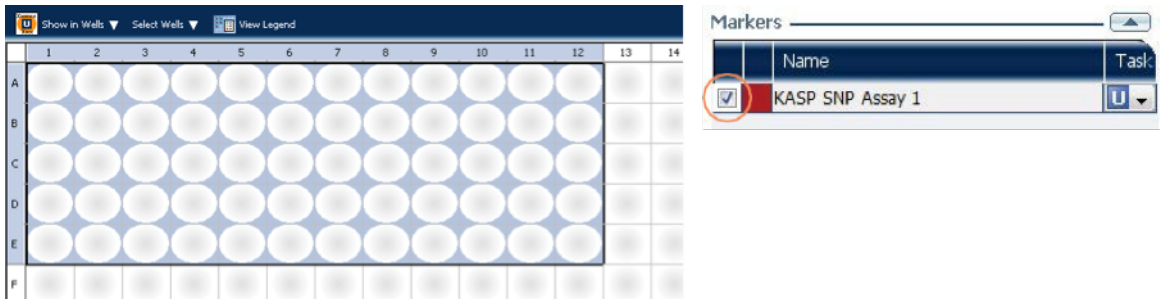
- Once the SNPs and samples have been defined, click on 'Assign' within the Setup menu.



Guide

Running KASP genotyping reactions on the Applied Biosystems ViiA 7

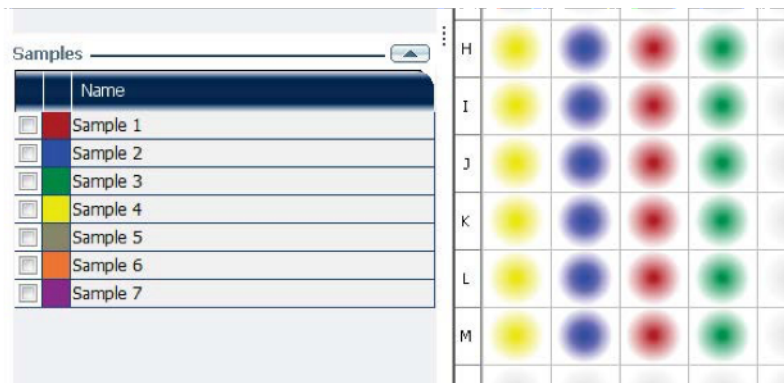
- Assign the markers (assays) that you have defined to the relevant wells of the plate. This is done by highlighting the wells, and then adding a tick to the 'Assign' column in the marker table.



- 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).



- 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.



Guide

Running KASP genotyping reactions on the Applied Biosystems Via 7

5.3 Program the run method

- 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 x 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

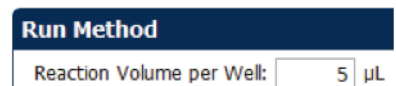
Repeat stage 4 x 25 times (a total of 26 cycles)

Stage 5: Post-read stage

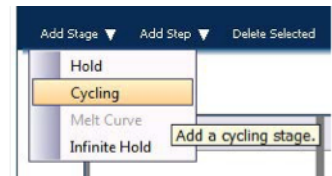
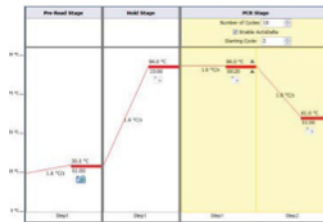
1. 30 °C for 1 minute.

This will bring the reaction plate to the correct temperature for reading the fluorescence.

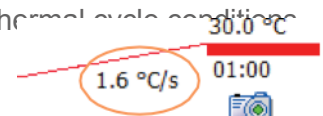
- Ensure that the reaction volume is correct for your experiment (Biosearch Technologies recommend 5 µL reaction volume for 384-well plates).



- 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'.



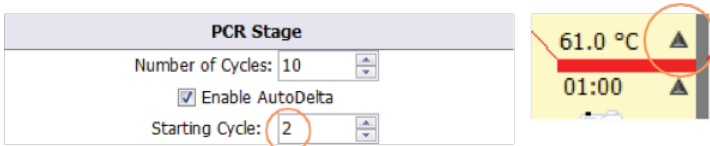
- The first PCR stage should be used to program the 10 cycles of touchdown PCR, and the second PCR stage should be used for the remaining 26 PCR cycles.
- Edit the thermal cycle parameters for all five stages according to the KASP thermal cycle conditions.
- The ramp rates will be set to 1.6 °C/s by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



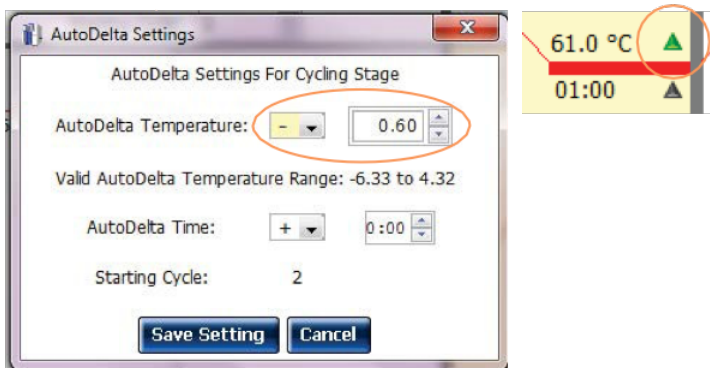
Guide

Running KASP genotyping reactions on the Applied Biosystems Via 7

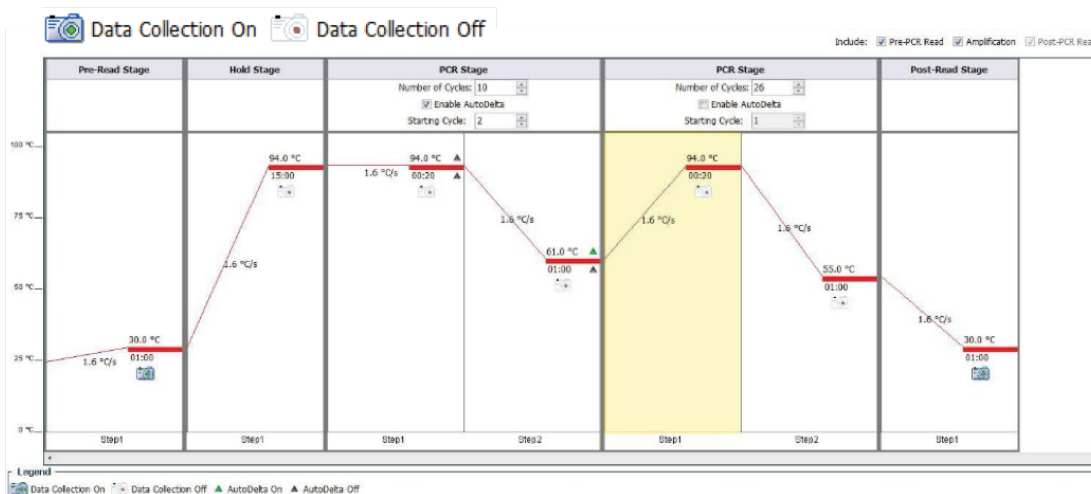
- To program the touchdown stage, you need to click in the 'Enable AutoDelta' box of the relevant cycling stage. Change the 'Starting Cycle' to 2 as the decrement should start from the second cycle. Then click on the grey triangle next to the temperature (61 °C) that requires a decrement. This will open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



- 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 'Material List' option is not required during the setup of KASP genotyping on the Via7.
- 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 postread 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.



Guide

Running KASP genotyping reactions on the Applied Biosystems ViiA 7

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' 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.

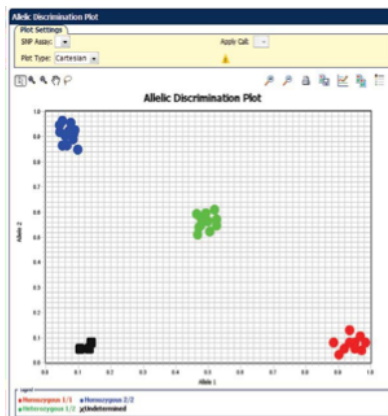
Run Status

- 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'.

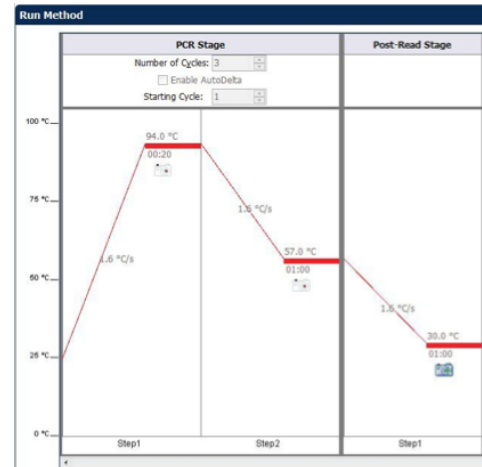


Guide

Running KASP genotyping reactions on the Applied Biosystems ViiA 7

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 ViiA 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 secondsRepeat 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.
- Please note: you should not perform a pre-read when running a recycle program. 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'.

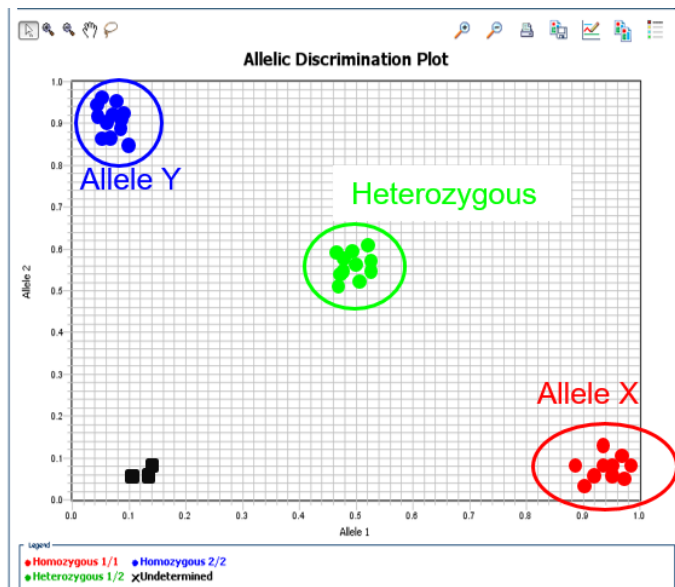


5.7 Running the KASP trial kit on the Applied Biosystems ViiA 7

- If you have requested a [KASP trial kit](#) to run on your ViiA 7 instrument, please follow the protocol included with the kit to set up your reaction plate. This ViiA 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.

6. Further support




If you require any further support, please do not hesitate to contact our Technical Support Team:
techsupport@lgcgroup.com



Results from the KASP trial kit reaction plate when run on the ViiA 7 instrument using the standard KASP thermal cycle and one recycle program.

The background features two large, overlapping organic shapes. The top shape is a gradient from purple to red, and the bottom shape is a teal color. They overlap in the center of the page.

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