

Manual



Running KASP genotyping reactions on the ABI StepOne and StepOnePlus instruments

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1. Introduction

This document is intended as a guide to running KASP™ genotyping reactions on the (Applied Biosystems™) ABI StepOne™ and StepOnePlus™ instruments.

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

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). However 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 StepOne and StepOnePlus software, a pre-read (before the thermal cycle) and a post-read (after the thermal cycle) should be included in the thermal cycle programme.
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 programme 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 StepOne and StepOnePlus 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. Define the experimental properties – see section 5.1.
2. Programme the plate set up including assay information and sample names – see section 5.2.
3. Programme 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.

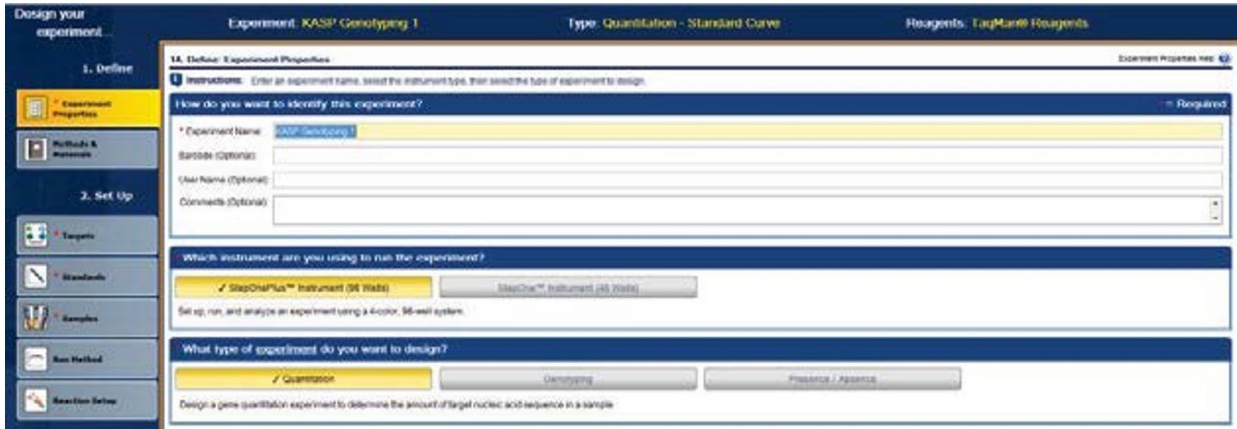
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5. Step-by-step user guide

5.1 Define the experimental properties

- Open the ABI StepOne and StepOnePlus software



- The 'Design Wizard' will open and will guide you through all stages of experimental setup.
- In the Define menu, the first stage is to define the 'Experiment Properties'.

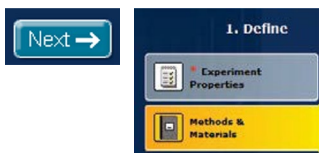
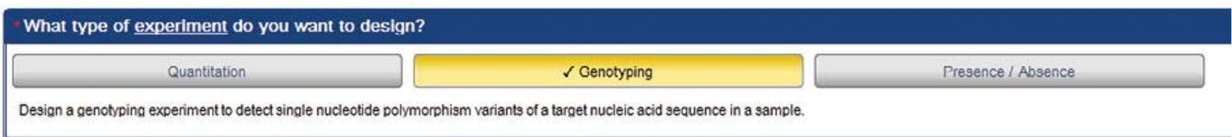
- Step 1: Give your experiment a name

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

- Step 2: Select the instrument that you are using i.e. StepOnePlus (96 wells) or StepOne (48 wells).



- Step 3: Select the experiment type – for KASP, this will be 'Genotyping'.



Press the 'Next' button at the bottom of the window, or click on 'Methods and Materials' under the 'Define' menu (left hand side).

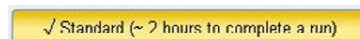
- Step 4: Select 'Other' for the reagents type.



- Step 5: Select either 'Wet DNA' or 'Dry DNA' depending upon your experimental setup.



- Step 6: Select 'Standard' for the ramp speed.



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- Step 7: 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).

Which stages do you want to include in the instrument run?

Pre-PCR Read Amplification Post-PCR Read

Press the 'Next' button or click on 'SNP Assays' under the 'Set Up' menu (left hand side).

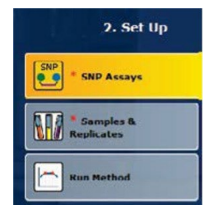


5.2 Programme the plate setup

- Once the experimental properties are defined, click on 'SNP Assays' within the 'Set Up' menu.
- Define the number of SNP assays that you intend to run in this experiment
- Select the button 'No (Set Up SNP Assay Properties Manually)'

How many SNP assays are you studying in this experiment?

Have you ordered and received the assay and the assay information file (AIF) for each SNP assay?



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

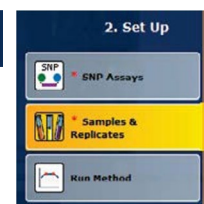
SNP Assay 1

SNP Assay Name: Color: Assay ID:

Allele 1 Name: Color: Reporter: Quencher:

Allele 2 Name: Color: Reporter: Quencher:

- Press the 'Next' button or click on 'Samples and Replicates' under the 'Set Up' menu (left hand side).
- In the 'Samples & Replicates' menu, define the numbers of samples that you intend to run, and the number of replicates for each sample. *Optional:* Edit the sample names for the experiment.



Set Up Samples

How many samples do you want to test in the reaction plate?

How many replicates do you need?

For each sample in the reaction plate, enter a sample name and select a sample color.

* Enter Sample Name	Color
<input type="text" value="Sample 1"/>	<input type="text" value="Blue"/>
<input type="text" value="Sample 2"/>	<input type="text" value="Green"/>

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- Define the number of negative controls and the number of (optional) positive controls that will be included on your reaction plate.

Set Up Controls
• How many negative controls do you need for each SNP assay?
(Optional) How many positive controls do you need for each SNP assay?

- The software will automatically define the layout of these samples on the plate layout tab.

Please note: The automatic layout that the software defines may not match the layout of your reaction plate. You can edit the plate layout in 'Advanced Setup' once you have worked through the wizard and have pressed the 'Finish Designing the Experiment' button.

	1	2	3	4	5	6	7	8	9	10	11
A	Sampl... KAS	Sampl... KAS	Sampl... KAS	Sampl... KAS	Sampl... KAS	Sampl... KAS	Sampl... KAS	Sampl... KAS	Sampl... KAS	Sampl... KAS	Sampl... KAS
B	Sampl... KAS	Sampl... KAS	Sampl... KAS	Sampl... KAS	Sampl... KAS	Sampl... KAS	Sampl... KAS	Sampl... KAS	Sampl... KAS	Sampl... KAS	Sampl... KAS
C	Sampl... KAS	Sampl... KAS	Sampl... KAS	Sampl... KAS	Sampl... KAS	Sampl... KAS	Sampl... KAS	Sampl... KAS	Sampl... KAS	Sampl... KAS	Sampl... KAS

- Press the 'Next' button or click on 'Run Method' under the 'Set Up' menu (left hand side).



5.3 Programme 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: Pre-read stage

- 30 °C 1 min. (Data capture on)

Stage 2: Hold stage

- 94 °C 15 minutes

Stage 3: PCR stage (touchdown)

- 94 °C 20 seconds
- 61 °C 60 seconds.

****Decrement -0.6 °C per cycle****

Set the decrement up using the 'Enable AutoDelta' option.

Repeat stage 2 × 9 times (a total of 10 cycles) achieving a final annealing temperature of 55 °C

Stage 4: PCR stage

- 94 °C 20 seconds
 - 55 °C 60 seconds
- Repeat stage 3 × 25 times (a total of 26 cycles)

Stage 5: Post-read stage

- 30 °C for 1 minute.

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

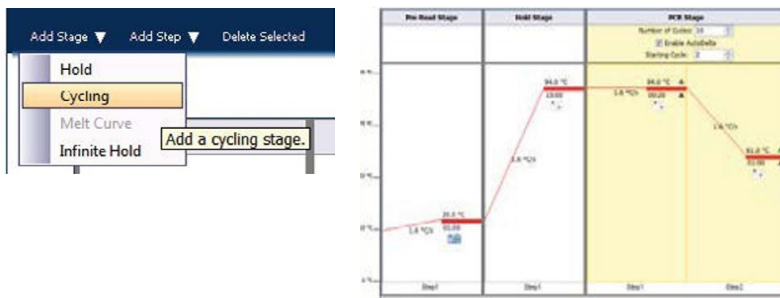
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- Ensure that the reaction volume is correct for your experiment (LGC Biosearch Technologies recommend 10 μ L reaction volume for 96-well plates).

Reaction Volume Per Well: μ L

- The default thermal cycle will consist of the following four stages: Pre-PCR read, Holding stage, Cycling stage and Post-PCR 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 programme 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 100% by default, and these do not need to be adjusted for KASP chemistry.
- To programme the touchdown stage, you need to click in the 'Enable AutoDelta' box of the relevant cycling stage so that the green tick shows. 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 $^{\circ}$ C). This will open up the 'AutoDelta Settings' window.

Enable AutoDelta
Starting Cycle: 61.0 $^{\circ}$ C ▲

- Select the minus sign (for a decrement) and 0.6 for the temperature. This will ensure that the temperature decreases by 0.6 $^{\circ}$ 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.

61.0 $^{\circ}$ C ▲

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- 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-PCR read is performed, and for the last stage (30 °C for 1 min) so that the post-PCR read 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.



ABI StepOne/StepOnePlus software programmed with the standard KASP thermal cycling conditions.

- Click on the 'Finish Designing Experiment' button at the bottom centre of the window.
- This will open up a new window within which you can review the experimental setup, access advanced plate layout options, and start the thermal cycle run on the instrument.



I Review the plate layout, then select what you want to do next.

Save Experiment	Start Run for This Experiment	Edit Plate Layout	Create Another Experiment Using the Design Wizard	Return to the Wizard
Save and close this experiment.	Save this experiment, then start the run. Make sure the reaction plate is loaded into the instrument.	Use Advanced Setup to edit the plate layout.	Save and close this experiment, then create another experiment using the Design Wizard.	Continue designing this experiment using the Design Wizard.

- Click on 'Edit Plate Layout' to access 'Advanced Setup' options.
- 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.

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- 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'.

<input checked="" type="checkbox"/>	KASP Assay	FAM/MIC	Unknown
			Unknown
			Negative Control
			Positive Control Allele 1/Allele 1

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

Select the dye to use as the passive reference.

ROX

- 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 U KASP.	Sample 2 U KASP.	Sample 3 U KASP.	Sample 4 U KASP.	Sample 5 U KASP.	Sample 6 U KASP.	Sample 7 U KASP.	Sample 8 U KASP.	Sample 9 U KASP.	Sample 10 U KASP.	Sample 11 U KASP.	Sample 12 N KASP.
B	Sample 13 U KASP.	Sample 14 U KASP.	Sample 15 U KASP.	Sample 16 U KASP.	Sample 17 U KASP.	Sample 18 U KASP.	Sample 19 U KASP.	Sample 20 U KASP.	Sample 21 U KASP.	Sample 22 U KASP.	Sample 23 U KASP.	Sample 24 N KASP.
C	Sample 25 U KASP.	Sample 26 U KASP.	Sample 27 U KASP.	Sample 28 U KASP.	Sample 29 U KASP.	Sample 30 U KASP.	Sample 31 U KASP.	Sample 32 U KASP.	Sample 33 U KASP.	Sample 34 U KASP.	Sample 35 U KASP.	Sample 36 N KASP.
D												

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 green 'Start Run' button at the top right of the window.



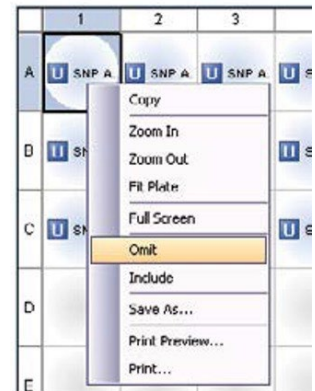
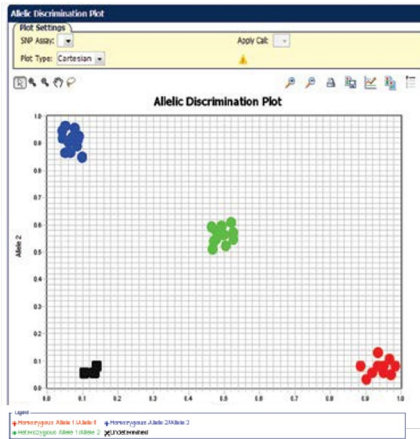
- Fluorescent data will be collected during the pre-read and post-read stages.

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5.5 Analysis of results

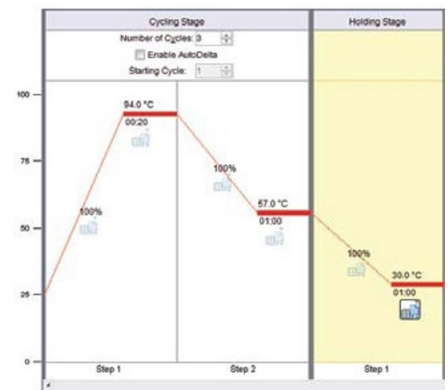
- Once the run has completed, click on the Analysis section of the experiment menu.



- 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 StepOne and StepOnePlus (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 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 programme. The reactions will have already been thermally cycled and will therefore already be emitting FAM/VIC fluorescent signal.
- The data can then be viewed from the 'Analysis' section of the experiment menu, using the 'Allelic Discrimination Plot'.

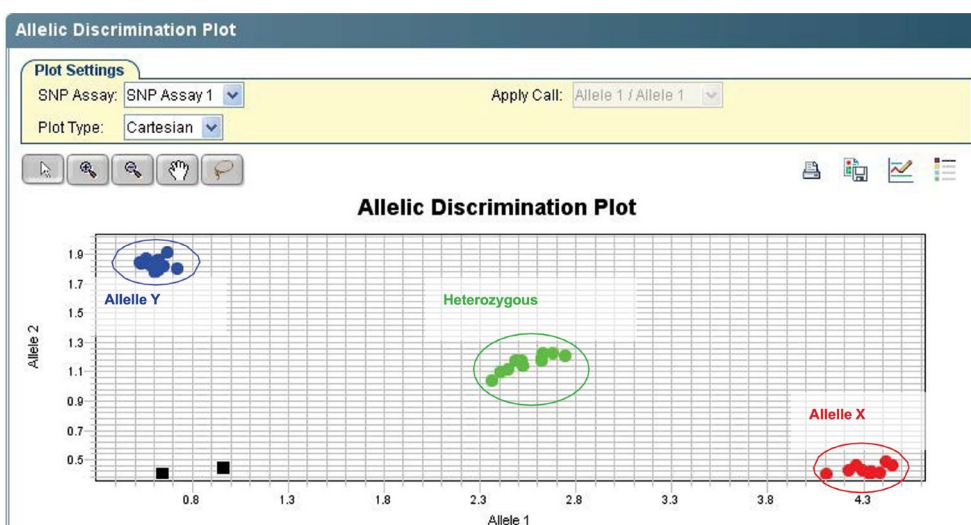


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5.7 Running the KASP trial kit on the ABI StepOne and StepOnePlus

- If you have requested a KASP trial kit to run on your ABI StepOne and StepOnePlus instrument, please follow the protocol included with the kit to set up your reaction plate. This ABI StepOne and StepOnePlus 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 StepOnePlus instrument using the standard KASP thermal cycle and one recycle programme.

6. Further support

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



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