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GC

Rotor-Gene Q

Running KASP genotyping reactions on the QIAGEN Rotor-Gene Q instrument

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Running KASP genotyping reactions on the QIAGEN Rotor-Gene Q instrument

#### 1. Introduction

This document is intended as a guide to running <u>KASP™ genotyping reactions</u> on the QIAGEN Rotor-Gene<sup>®</sup> Q instrument.

KASP chemistry for allelic discrimination performs well on a QIAGEN Rotor-Gene Q instrument and this step-by- step protocol will enable users to successfully set-up, run and read plates on the QIAGEN Rotor-Gene Q.

#### 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 programme no useful data will be captured during the thermal cycling protocol.
- 4. The KASP recycling programme will often improve results, especially for assays that are slow to form clusters.

#### 3. Overview of the procedure

- 1. Create a new run for KASP genotyping see section 5.1.
- 2. Programme the thermal cycling conditions and read step see section 5.2.
- 3. Edit the samples included in the run see section 5.3.
- 4. View the data see section 5.4.
- 5. Recycle the reactions if required see section 5.5.

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#### 4. KASP thermal cycling conditions

#### 4.1 Standard KASP thermal cycling conditions

#### Stage 1

• 94 °C - 15 minutes

#### Stage 2

- 94 °C 20 seconds
- 61 °C 60 seconds<sup>1</sup>
- Repeat Stage 2 × 9 times (a total of 10 cycles) achieving a final annealing temperature of 55.6 °C.

#### <sup>1</sup>Drop -0.6 °C per cycle

#### Stage 3

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

#### Stage 4

Cool the reactions to 35 °C (suggested 2 minutes)\*

#### Stage 5

Add a read step at 35 °C for 30 sec\*

#### 4.2 KASP recycling conditions

#### Stage 1

- 94 °C 20 seconds
- 57 °C 60 seconds
- Repeat steps 1-2 twice (a total of 3 cycles)

#### Stage 2

Cool the reactions to 35 °C (suggested 2 minutes)\*

#### Stage 3

Add read step at 35 °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. Please contact the technical support team (details at the end of this document) for further information.

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#### 5. Step-by-step user guide

#### 5.1 Create a new run for KASP genotyping

- Open the Rotor-Gene Q software by double-clicking on the desktop icon.
- Click on the 'New' button in the top menu bar.



Ro	otor-G	ene Q	Series	Softwa	re - Run	2015-03	-31 (1)									
File	Analys	is Ru	n Gain	View	Window	Help										
Mew	Oper	) 📙	e Sta	rt Paus	e Stop	Help	View	Settings	Progress	Profile	JE Temp.	in Samples	) Analysis	Reports	Arrange	•
Char	nnels															

• This will open the 'New Run' window. (Please note that this window may open automatically when the software first launches depending upon your default settings).



• On the 'Advanced' tab, select 'Empty Run' and press 'New'.



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• You will then need to select the appropriate rotor type for the run that you plan to perform on your instrument.



• Before pressing 'Next', it is essential to tick the 'Locking Ring Attached' box. Once this is ticked, press 'Next' to progress through the wizard.



• The next window requires input of miscellaneous information regarding the run. It is essential to specify a reaction volume to proceed through the wizard. Press 'Next'.

This screen dis clicking Next v	ro splays miscellaneous options for the run. Complete the fields, when you are ready to move to the next page.	This box displays help on elements in the wizard. For help
Operator : Notes :	LGC Setting up the RotorGeneQ software for the standard KASP	on an item, hover your mouse over the item for help. You can also click on a
	memar openny prodocol	combo box to display help about its available settings.
Reaction Volume (µL): Sample Layou	10 <u>*</u>	
Skip Wiza	nd <u>«B</u> ack <u>N</u> ext>>	

Running KASP genotyping reactions on the QIAGEN Rotor-Gene Q instrument

• The next window contains an empty temperature profile box. Section 5.2 of this guide details how to programme the KASP thermal cycle within the Rotor-Gene Q software.

Edit Prof	ile				This box displays help on elements in the wizard. For help on an item, hover your mouse over the item for help. You can also click on a combo box to display help about its available settings.
hannel S Name	etup : Source	Detector	Gain	 Create New	
Green	470nm	510nm	5	E dit	
Yellow	530nm	555nm	5		
Diange Red	625nm	660pm	5	Edit <u>G</u> ain	
Crimson	680nm	710hp	7	<u>Remove</u>	
HBM	460nm	510nm	7	Reset Defaults	1
	misation	ſ			

#### 5.2 Programme the thermal cycling conditions and read step

• After completing the steps outlined in section 5.1 of this guide, you should have an open window of the 'New Run Wizard' containing a blank 'Temperature Profile' box. Click on the 'Edit Profile' button to open a new window within which the temperature profile can be programmed.

Temperature Profile :	Click this button to
	edit the profile shown in the box above.

Once programming is complete, the top box (outlined in green) will contain the thermal profile graph and the lower box (outlined in purple) will contain a list of the steps that have been programmed in the thermal cycle.

😥 Edit Profile	<b>x</b>
New Open Save As     Help     The run will be accorded to complete. The graph below represents the run to be performed :	
Citize a suda belan te an dita b	
Insert after	
Remove	
	<u>K</u>

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#### 5.2.1 Programme the activation stage (Stage 1): 94 °C; 15 minutes

• Click on the 'Insert after...' button and select 'New Hold at Temperature'. A stage called 'Hold' will be added to your thermal cycle protocol.

🛍 Edit Profile	×
🖉 🚬 📁 📕 🕘	
New Open Save As Help The run will take approximately 0 second(s) to complete The graph below represents the run to be performed.	
Click on a cycle below to modify it :	
Insert after. New Cycling	
Insert before New Melt	
Remove New HRM Step	
Copy of Current Step	
	<u>K</u>

• Click on the 'Hold Temperature' button, edit this to 94 °C and press 'OK'.



• Click on the 'Hold Time' button, edit this to 15 min and press 'OK'.

12 Edit Profile	×
New Open Save As Help	
The run will take approximately 2 minute(s) to complete. The graph below represents the run to be perf	omed :
Cick on a cycle below to modily it : Hot Hot Invest alle	
Hold Time Minutes	
fold Temperature : 94 fac	
fold Time: 1 mins 0 secs 15 0K Cancel	
	<u>K</u>

Running KASP genotyping reactions on the QIAGEN Rotor-Gene Q instrument

The correctly programmed stage (called 'Hold') should appear as shown below:



#### 5.2.2 Programme the touchdown stage (Stage 2) (10 cycles):

- 94 °C 20 seconds
- 61 °C 60 seconds (drop -0.6 °C per cycle)
- Click on the 'Insert after...' button and select 'New Cycling'. A default cycling stage in the Rotor-• Gene Q software contains 45 cycles of 3-step PCR – this requires editing for the KASP thermal cycling protocol.



Remove the third step of the default cycling stage by clicking on the step and subsequently clicking the minus (-) icon.

+ Remove



Running KASP genotyping reactions on the QIAGEN Rotor-Gene Q instrument

• Edit the temperature for the first step of the touchdown cycling stage to 94 °C. To do this, highlight the step in the image (it will appear grey) and press the temperature button (default is '95 °C'). In the window that opens, change the temperature to 94 °C and press 'OK'.



- Ensure that the time for the first step of the touchdown cycling stage is set to 20 seconds (this is the default).
- Edit the temperature for the second step of the touchdown cycling stage to 61 °C. To do this, highlight the step in the image (click on the step and it will appear grey) and press the temperature button (default is '60 °C'). In the window that opens, change the temperature to 61 °C and press 'OK'.



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• Edit the time for the second step of the touchdown cycling stage to 1 minute. Press the time button (default is 20 seconds) and, in the window that opens, change the time to 60 seconds and press 'OK'.



• With the second step of the touchdown cycling stage highlighted (grey, 61 °C for 20 seconds), click to put a tick in the 'Touchdown' box. This will open the 'Cycles to touch down' window.



• Edit the temperature decrease to 0.6 °C each cycle and ensure that the number of cycles is set to 10. Press 'OK'.



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• Reduce the total number of cycles for this cycling stage to 10 cycles.



• The correctly programmed stage (called 'Cycling') should appear as shown below:



- 5.2.3 Programme the amplification stage (Stage 3) (26 cycles):
  - 94 °C 20 seconds
  - 61 °C 60 seconds (drop -0.6 °C per cycle)

Running KASP genotyping reactions on the QIAGEN Rotor-Gene Q instrument

• Click on the 'Insert after...' button and select 'New Cycling'. A default cycling stage in the Rotor-Gene Q software contains 45 cycles of 3-step PCR – this requires editing for the KASP thermal cycling protocol.

🕅 Edit Profile	×
New Open Save As Help	
The run will take approximately 37 minute(s) to complete. The gra	aph below represents the run to be performed :
Cleck on ore of the steep below to modify it :	And Safe New Crycling New Krycking Remoy, New Hold at Temperature New Hold at Temperature New Hold Step Copy of Current Step and anothere temperature mulciplas
Tend Step  SetC SetC SetC SetC SetC SetC SetC SetC	51% for 60 eecs
	<u>Dk</u>

- A third stage called 'Cycling2' will be added to your protocol.
- Remove the third step of the default cycling stage by clicking on the step and subsequently clicking the minus (-) icon.

p Edit Profile	X
New Open Sare As Help	
The run will take approximately 122 minute(s) to complete. The graph below represent	its the run to be performed :
Click on a cycle below to modify it :	
Hold Inset after	
Remove	
This cycle repeats 45_time(s) Cick on one of the steps below to modify it, or press + or - to add and remove steps f	or this cucle.
Timed Step	Remove Last Cy
on Green	C for 20 secs
	QK

• Edit the temperature for the first step of the second cycling stage to 94 °C. To do this, highlight the step in the image (click on the step and it will appear grey) and press the temperature button (default is '95 °C'). In the window that opens, change the temperature to 94 °C and press 'OK'.



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- Ensure that the time for the first step of the Cycling2 stage is set to 20 seconds (this is the default).
- Edit the temperature for the second step of the Cycling2 stage to 55 °C. To do this, highlight the step in the image (it will appear grey) and press the temperature button (default is '60 °C'). In the window that opens, change the temperature to 55 °C and press 'OK'.



• Edit the time for the second step of the Cycling2 stage to 1 minute. Press the time button (default is 20 seconds) and, in the window that opens, change the time to 60 seconds.

17 totic Profile Cpen Save Ac Heb The run will take approximately 106 minute(s)	to complete. The graph below re	presents the run to be pe	ntomed :
Cisi or a rock ballow to modely J (Code Code Generation (Code Code Code (Code Code (Code Code (Co	Intel dia:	SO OK Carcel	95% for 20 secs

• Reduce the total number of cycles for this cycling stage to 26 cycles and press 'OK'.



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• The correctly programmed stage (called 'Cycling2') should appear as shown below:



#### 5.2.4 Programme the plate cooling stage (Stage 4) (1 cycle):

• Click on the 'Insert after...' button and select 'New Hold at Temperature'. A fourth stage called 'Hold2' will be added to your thermal cycle protocol.



• Click on the 'Hold Temperature' button, edit this 35 °C and press 'OK'. Please note: Completed KASP reactions must be cooled to below 40 °C as KASP chemistry cannot be read above 40 °C.



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• Click on the 'Hold Time' button, edit this to 2 minutes and press OK.



• The correctly programmed stage (called 'Hold2') should appear as shown below:



#### 5.2.5 Programme the plate read stage (Stage 5) (1 cycle):

• Click on the 'Insert after...' button and select 'New Cycling'. A fifth stage called 'Cycling3' will be added to your thermal cycle protocol.

i Edit Profile	×
Ver Voen Save As Help	
The run will take approximately 96 minute(s) to complete. The graph below represents the run to be performed :	
Cirk on a carda beirav lo modify it -	
Noti Carto 2     [Intert Macs     Texced bit       Carto 2     Intert Macs     New Net       Premo     New Net     New Net       New Net     Premo     New Net       New Net     New Net       Net <td></td>	
<u>DK</u>	

Running KASP genotyping reactions on the QIAGEN Rotor-Gene Q instrument

• Remove the third step of the default cycling stage by clicking on the step and subsequently clicking the minus (-) icon.

👖 Edit Profile	×
New Open Save As Help	
The run will take approximately 182 minute(s) to o	complete. The graph below represents the run to be performed :
Click on a cycle below to modify it :	
Hold Cycling Cycling 2	Inset alter
Cycling 3	Bemove
This cycle repeats 45 time(s). Click on one of the steps below to modify it, or pre-	sss + or - to add and remove steps for this cycle.
Timed Step  96%	C for 20 secs
20 seconds	Remove Last Cyc
Acquiring to Cycling A	724C for 20 secs
on Green	60MC for 20 secs
Touchdown	
,	OK

• Remove the second step of the default cycling stage by clicking on the step and subsequently clicking the minus (-) icon.

📲 I dit Profile			X
New Open :	imately 161 minute(s) to complete	The graph below represents the run to	be performed :
Click on a cycle below Hold Cycling 2 Hold 2 Cycling 2 Hold 2 Hol	o mođly it : : tranjc) zo belov to mođly it, or press + or 	Insert after. Insert after. Retrove to add and remove steps for this cycle.	Remove Last Cre BRC for 20 year
			QK

• Click on the temperature button, edit this 35 °C and press OK.



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• Click on the time button, edit this to 30 seconds and press OK.



• To define the parameters for reading the completed KASP reactions, click on the 'Not Acquiring' button. This will open the 'Acquisition' window.

Timed Step	Acquisitio Same as Pr	n evinus : [	New Acrui	sition
35ºC 30 seconds Not Acquiring	Acquisitio Available Crimson HRM Orange Red Yellow To acquir	n Configu Channels	ration :	Acquiring Channels :
Long Range Touchdown	Dye Charl	select it in	the right-ha	nd list and click <. To remove all acquisitions, click <<.
1000	Channel	Source	Detector	Dyes
	Green	470nm	510nm	FAM <sup>®</sup> , SYBR Green 1 <sup>®</sup> , Fluorescein, EvaGreen <sup>®</sup> , Alexa Fluor 488 <sup>®</sup>
	Yellow	530nm	555nm	JOE <sup>(3)</sup> , VIC <sup>(3)</sup> , HEX, TET <sup>(3)</sup> , CAL Fluor Gold 540 <sup>(3)</sup> , Yakima Yellow <sup>(3)</sup>
	Orange	585nm	610nm	$ROX^D$ , CAL Fluor Red 610 <sup>D</sup> , Cy3.5 <sup>D</sup> , Texas Red <sup>D</sup> , Alexa Fluor 568 <sup>D</sup>
	Pied	625nm	660nm	Cy5 <sup>1)</sup> , Quasar 670 <sup>1)</sup> , Alexa Fluor 633 <sup>1)</sup>
	Crimson	680nm	710hp	Quasar705 <sup>1)</sup> , Alexa Fluor 680 <sup>1)</sup>
	HRM	460nm	510nm	SYTO 9 <sup>1</sup> , EvaGreen <sup>1</sup>

- KASP uses the fluorophores FAM and HEX for distinguishing genotypes. The passive reference dye ROX is also used to allow normalisation of variations in signal caused by differences in well-to-well liquid volume.
- The 'Green' channel is included in the 'Acquiring Channels' box by default. This channel detects FAM.



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• To programme the instrument to read HEX, click on 'Yellow' in the available channels list, and then the right arrow button (>) to move it to the 'Acquiring Channels' box.

ame as P	evicus · [	(New Acqui	isition
		(rectrineda	
Acquisitio	on Configu	ration :	A service Channels .
wallable	Channels		Acquiring channels .
Name			> Name
HBM			< Yellow
Orange			
Red			
channel,	select it in	the right-ha	and list and click <. To remove all acquisitions, click <<.
Dye Char ye Char	select it in	the right-ha	Ind list and click <. To remove all acquisitons, click <
Dye Char ye Char Thannel	select it in	the right-ha	Ind list and click <. To remove all acquisitions, click <<. <table>          QK         Don't Acquire         Help           It         Dyss         Functionance         Alexa Flux 498.00</table>
Dye Char ye Char Channel	select it in t>> nel Sele 470nm 530nm	the right-ha	nd list and click <. To remove all acquisitions, click <
Dye Char Dye Char ye Char Channel Areen Yellow Drange	select it in t>> nel Sele Source 470nm 530nm 585nm	ction Cha Detector 510nm 555nm 610nm	Ind list and click <. To remove all acquisitions, click <           DK         Don't Acquire         Help           Int         Dess         FAM <sup>2</sup> , SYBR Green 1 <sup>(1)</sup> , Fluorescein, EvoSireen <sup>(2)</sup> , Alexa Fluor 488 <sup>(3)</sup> JDE <sup>2</sup> , MC <sup>3</sup> , HC <sup>3</sup> , FE <sup>1,3</sup> , CAJ, Fluor Gold 540 <sup>(3)</sup> , Yakina Yellow <sup>3</sup> Total Acquire 10 <sup>(2)</sup> , CQA, Fluor Gold 540 <sup>(3)</sup> , Yakina Yellow <sup>3</sup>
Dye Char ye Char Channel The Channel Areen Yellow Drange	select it in mel Sele Source 470nm 530nm 585nm 625nm	ction Cha Detector 510nm 555nm 610nm 660nm	Ind list and click <. To remove all acquisitions, click <<
Dye Char ye Char Channel Channel Green Yellow Drange	select it in select it in source 470nm 530nm 585nm 625nm 680nm	ction Cha Detector 510nm 555nm 610nm 660nm 710hp	Ind list and click < To remove all acquisitons, click << <u>DK</u> Don't Acquire Hep M Dyse FAM <sup>2</sup> , SYBR Green <sup>10</sup> , Fluorescein, EvaGreen <sup>10</sup> , Alexa Fluor 488 <sup>10</sup> JOE <sup>10</sup> , VIC <sup>10</sup> , HEX, TET <sup>10</sup> , CAL, Fluor Gold 540 <sup>10</sup> , Yakima Yellow <sup>10</sup> RCK <sup>20</sup> , CAL Fluor Red 510 <sup>10</sup> , Cyg 35 <sup>10</sup> , Texas Red <sup>10</sup> , Alexa Fluor 558 <sup>10</sup> Cy5 <sup>10</sup> , Quasar 750 <sup>10</sup> , Alexa Fluor 633 <sup>10</sup> Quasar 750 <sup>10</sup> , Alexa Fluor 633 <sup>10</sup>

• To programme the instrument to read ROX, click on 'Orange' in the available channels list, and then the right arrow button (>) to move it to the 'Acquiring Channels' box. Press 'OK'.

Acquisitio	n		
Same as P	revious : [	(New Acqui	sition)
Acquisitic Available	on Configu Channels	ration : :	Acquiring Channels :
Name			> Name
Crimson	0		Green
Bed			
1100			< <u></u>
Dye Char Dye Char	t>>   nnel Sele	ction Cha	Don't Acquire Help
Channel	Source	Detector	Dyes
Green	470nm	510nm	FAM <sup>®</sup> , SYBR Green 1 <sup>®</sup> , Fluorescein, EvaGreen <sup>®</sup> , Alexa Fluor 488 <sup>®</sup>
Yellow	530nm	555nm	JOE <sup>(1)</sup> , VIC <sup>(1)</sup> , HEX, TET <sup>(1)</sup> , CAL Fluor Gold 540 <sup>(1)</sup> , Yakima Yellow <sup>(1)</sup>
Orange	585nm	610nm	ROX <sup>1</sup> , CAL Fluor Red 610 <sup>1</sup> , Cy3.5 <sup>1</sup> , Texas Red <sup>1</sup> , Alexa Fluor 568 <sup>1</sup>
Red	625nm	660nm	Cy5 <sup>(1)</sup> , Quasar 670 <sup>(1)</sup> , Alexa Fluor 633 <sup>(1)</sup>
Crimson	680nm	710hp	Quasar705 <sup>1)</sup> , Alexa Fluor 680 <sup>1)</sup>
HRM	460nm	510nm	SYTO 9 <sup>(1)</sup> , EvaGreen <sup>(1)</sup>

• In the 'Edit Profile' window, the acquisition button now states 'Acquiring to Cycling A'. It also lists the dyes that have been selected below the button (i.e. Green, Orange and Yellow).



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• The 'Cycling3' stage is set to repeat 45 times by default. Click on the number of cycles button, reduce this to 1 cycle, and press 'OK'.

1	50	100
1		<u>0</u> K

• The correctly programmed stage (called 'Cycling3') should appear as shown below:

The run will take approximately 13	minute(s) to complete. The graph below represents the run to be performed :
Lick on a cycle below to modify it	
Hold	Inset after
Excline	
Cycling Cycling 2	Inset before
Cycling Cycling 2 Hold 2 Cycling 3	Inset before Remove
Cycling Cycling 2 Hold 2 Critery 3 This cycle repeats 1 ime(s). Click on one of the steps below to Timed Step v 35PC	Indiffy it, or press + or - to add and remove steps to this cycle.
Cycling 2 Cycling 2 Hold 2 Cycling 3 This cycle repeats 1 time(s). Click on one of the steps below to Timed Step v 36PC 30 seconds	Renove Renove modely & or press + or 10 add and remove ideps for this syste.
Cycling 2 Cycling 2 Cycling 2 Cycling 3 This cycle repeats 1 im(s). Cick on one of the steps below to Timed Step v 39°C 30 seconds Acquiring to Cycling A on Green, Orange, Yellow	nodly)z, or pres > or to add and memore steps to this cycle.

- After 'Cycling3' is programmed, press 'OK' at the bottom right of the 'Edit Profile' window.
- This will then enable you to see a summary of the temperature profile (graphical format). The gains settings for each channel are also shown these can be left as the default settings. Press 'Next'.

Pas Dat					<ul> <li>The box explays</li> <li>help on elements in the wizard. For help on an item, hover your mouse over the item for help. You can also click on a combo box to displa</li> <li>help about its available settings.</li> </ul>
Channel S	etup :				
Name	Source	Detector	Gain	Create New	
Green	470nm	510nm	5	Edit	
Yellow	530nm	555nm	5		
Orange	585nm	610nm	5	Edit <u>G</u> ain	
Red	625nm	660nm	5	Barren	
Unmson	68Unm	/10hp	4	Hemove	
HHM	46Unm	nunc	1	Reset Default	

Running KASP genotyping reactions on the QIAGEN Rotor-Gene Q instrument

• The final window of the 'New Run Wizard' provides a summary of the thermal profile, and the rotor and read settings.

Setting	Value	1	
Green Gain	5		
Drange Gain	5		
Yellow Gain	5		
Rotor	72-Well Rotor		
Sample Layout	1, 2, 3,		
Reaction Volume (in microliters)	10		
			<u>S</u> tart Run
nce you've confirmed that your r egin the run. Click Save Templat	un settings are cr e to save setting	orrect, click Start Run to s for future runs,	Save Template

• At this stage, it may be helpful to save the KASP thermal cycle as a template that can be used for future KASP genotyping experiments. To do this, click on the 'Save Template' button. You will then be prompted to give the template a suitable name. By saving it as a template, it will then be available in the 'New Run' window in the future.

Start Bun	Quick Start Advanced
Save Template	Perform Last Run Empty Run Three Step with Melt
	Two Step
	Uther Runs Instrument Maintenance HRM <sup>m</sup> Demo kit
	CASP themail cycle
	Show This Screen When Software Opens

• To start the run, press the 'Start Run' button.

10000		
Setting	Value	
Green Gain	5	
Orange Gain	5	
Yellow Gain	5	
Rotor	72-Well Rotor	
Sample Layout	1, 2, 3,	
Reaction Volume (in mic	roliters) 10	
		Start Run
nce you've confirmed th	at your run settings are or	rect click Start Bun to Qaue Tomolate

- You will be prompted to choose a suitable location to save the completed run file in.
- It is then possible to edit the sample information for the run, before the run commences. Alternatively, sample information can be edited whilst the run is in progress, as detailed in section 5.3.

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#### 5.3 Edit the samples included within the run

Whilst the KASP genotyping reactions are running on the Rotor-Gene Q, the samples can be defined within the software.

• In the main Rotor-Gene Q window, click on the 'Samples' button in the top menu bar.



- Alternatively, you can click on the 'Edit Samples' button at the bottom of the sample list (right hand side of Rotor-Gene Q window).
  - Edit Samples
     Image: Control Source

     Standard Room Source
     Vector

     Standard Room Source
     Vector

     Standard Room Source
     Vector

     Standard Room Notes
     Vector
- Both of these options will open the 'Edit Samples' window.

- Complete the 'Name' and 'Type' fields for each individual sample, according to the experiment that you are running.
- Sample names can be typed directly into the box next to the sample ID number.

ven Conc. Formet :			Unit : Copies	<ul> <li>More Option</li> </ul>
moles :				
nknown			- D 😜 🖡	
ID Name	Inc	Groups	Giteen Conc	Selected
1 Sample 1	Unknown			Yes
2 Sample 2	Unknown			Yes
3 Sample 3	Unknown			Yes
4 Sample 4	Unknown			Yes
5 Sample 5	Unknown			Yes
6 Sample 6	Unknown			Yes
7 Sample 7	Unknown			Yes
8 Sample 8	Unknown			Yes
9 Sample 9	Unknown			Yes
10 Sample 10	Unknown			Yes
11 Sample 11	Unknown			Yes
12 NTC	Unknown			Yes
13	Unknown			Yes
14	Unknown			Yes
15	Unknown			Yes
16	Unknown			Yes
17	Unknown			Yes

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• DNA samples that are to be genotyped should contain 'Unknown' in the 'Type' field. No template controls (NTC) should be defined as such within the 'Type' field. This can be done by clicking on the corresponding 'Type' box and selecting 'NTC' from the drop down 'Samples' menu above the table.

Edit Samples				2	
e Edit Format Security					
andard Rotor Style					
etings :					
irven Conc. Formet :	- U	a : [0	opico 💌	Moro Opl	tiono
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• It is also possible to view the sample information in a rotor format, with sample names corresponding to their position within the rotor.



Running KASP genotyping reactions on the QIAGEN Rotor-Gene Q instrument

#### 5.4 View and analyse the data

- It does not appear to be possible to directly analyse endpoint genotyping data within the QIAGEN Rotor-Gene Q software.
- At the end of the run, click on the 'File' menu at the top of the window. Select 'Save As...' and choose 'Excel Data Sheet'.



- You will then be prompted to choose an appropriate location to save the MS Excel file to.
- A message window will then open giving you the option to choose to transpose the raw data before exporting. LGC Biosearch Technologies suggest exporting the raw data rather than transposing it first (i.e. do not tick the 'Transpose raw data' box).

Parameters : This filter allows you to export raw/processed channel data to comma-delimited text files (suitable for Excel).	Parameters : This filter allows you to export raw/processed channel data to comma-delimited text files (suitable for Excel). Transpose raw data	Save As Excel Data Si	heet	
This filter allows you to export raw/processed channel data to comma-delimited text files (suitable for Excel).	This filter allows you to export raw/processed channel data to comma-delimited text files (suitable for Excel).	Parameters :		
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I ranspose raw data		🦵 Transpose raw data		

- Press 'OK'.
- The resulting MS Excel file will contain run information, and the raw fluorescent read values for each sample.
- The 'Green' channel data gives the FAM fluorescence values for each sample.
- The 'Yellow' channel data gives the HEX fluorescence values for each sample.
- The 'Orange' channel data gives the ROX fluorescence values for each sample.
- To normalise the data, the raw values for FAM and the raw values for HEX should be divided by the corresponding ROX values.

FAM	HEX	ROX	Normalised FAM	Normalised HEX
GREEN	YELLOW	ORANGE	FAM ÷ ROX	HEX ÷ ROX
3.886	1.505	1.514	2.566	0.994
3.616	1.436	1.276	2.833	1.125
4.173	1.628	1.785	2.337	0.912
3.922	1.483	1.533	2.557	0.967
3.951	1.598	1.471	2.685	1.086
4.280	1.695	1.857	2.305	0.913
4.457	1.729	1.944	2.292	0.889
4.275	1.648	1.759	2.431	0.937
3.029	5.004	2.233	1.356	2.241
2.962	4.901	2.065	1.434	2.374
2.884	4.739	2.116	1.363	2.239
3.144	5.375	2.221	1.415	2.420
2.568	4.032	1.404	1.829	2.872
2.728	4.342	1.709	1.596	2.541
2.627	4.275	1.623	1.619	2.634

Running KASP genotyping reactions on the QIAGEN Rotor-Gene Q instrument

• To view a genotyping cluster plot, highlight the 'Normalised FAM' and the 'Normalised HEX' data and select 'Scatter' from the 'Charts' section of the 'Insert' menu.

Insert	Pag	e Layout	Formu	ulas D	ata	Review	Vi	ew	Develop	er	
Picture	Clip Art	D Shapes	SmartArt	Column	Line	Pie *	Bar	Area	Scatter	Other Charts *	Q. Hyperlink
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- A cluster plot of the data will then be inserted into your MS Excel workbook and can be used to determine the genotypes of your samples. Ensure that the X and Y axes are scaled comparably to prevent misinterpretation of the data.
- A typical cluster plot, with three clear genotyping clusters and no template controls at the origin (no amplification), will look similar to the figure below:



### 5.5 Recycle the reactions if required

- If your data has not formed tight clusters after the initial thermal cycling protocol, you can recycle the reactions in the QIAGEN Rotor-Gene Q and perform a second post-read.
- Follow the instructions in section 5.1 to create a new empty run for KASP recycling.
- The KASP recycling conditions are:

#### Stage 1

- 94 °C 20 seconds
- 94 °C 20 seconds

Repeat steps 1-2 twice (a total of 3 cycles)

Running KASP genotyping reactions on the QIAGEN Rotor-Gene Q instrument

• To programme this, first add a 'New Cycling' stage to the thermal profile within the empty run. A stage called 'Cycling' will be added to the thermal profile.



• Remove the third step of the default cycling stage by clicking on the step and subsequently clicking the minus (-) icon.



- Edit the temperature for the first step of the second cycling stage to 94 °C. To do this, highlight the step in the image (click on it and it will appear grey) and press the temperature button (default is '95 °C'). In the window that opens, change the temperature to 94 °C and press 'OK'.
- Ensure that the time for the first step of the cycling stage is set to 20 seconds (this is the default).
- Edit the temperature for the second step of the touchdown cycling stage to 57 °C. To do this, highlight the step in the image (click on it and it will appear grey) and press the temperature button (default is '60 °C'). In the window that opens, change the temperature to 57 °C and press 'OK'.
- Edit the time for the second step of the touchdown cycling stage to 1 minute. Press the time button (default is 20 seconds) and, in the window that opens, change the time to 60 seconds and press 'OK'.
- Reduce the total number of cycles for this stage to 3.

Running KASP genotyping reactions on the QIAGEN Rotor-Gene Q instrument

• The edited 'Cycling' stage should then appear as shown below:

Click on a cycle below to modify it : Dick on a cycle below to modify it : Dick on a cycle below to modify it :	
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The spek repear. 3 [Intel]. Calc on one of the first block to modify L, or press + or - b add and ennove Tened Step	e shape for this cycle. 

- Click on the 'Insert after...' button and select 'New Hold'. A second stage called 'Hold' will be added to your thermal cycle protocol.
- Edit the 'Hold Temperature' of this stage to 35 °C and ensure that the 'Hold Time' is 2 minutes. This stage will ensure that the reactions are cooled to below 40 °C prior to performing the fluorescent read.

New Open Save As Help	to The such below concerns the up to be not	in mod :
e naminare approximately to minare protocomp	see. The graph become prevents the full to be per	onned.
ck on a cycle below to modify it : voling	Insett after	
old yolng 2	Inset before	
	Remove	
old Temperature : 35 nc		
old Time : 2 mins 0 secs		

- Click on the 'Insert after...' button and select 'New Cycling'. A third stage called 'Cycling2' will be added to your thermal cycle protocol.
- Remove the second and third steps of the default cycling stage by clicking on the each of the steps in turn and subsequently clicking the minus (-) icon.
- Click on the temperature button, edit this 35 °C and press OK
- Click on the time button, edit this to 30 seconds and press OK.

Running KASP genotyping reactions on the QIAGEN Rotor-Gene Q instrument

• Reduce the number of repeats for this cycle to 1.

The num Hi bide appointedly Smrutel() to complete. The graph below represents the num to be performed:	Investigi to complete. The graph below represents the an to be performed :	The run will take approximately 9 minute(s) to con	plete. The graph below represents the run to be performed :	
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SPL SPL 30 Records Not Acquiring Transformer	39°C (or 30 mer.	Timed Step 💌		
Net Acquing	39°C for 30 mers	30°L		
Long Range	39°C (or 30 secs	Net Acquiring		
Long Range	39C for 30 sees	intersection of		
	394C for 30 secs	Long Range		
Touchdown	39°C for 30 secs	Touchdown		
	35%C for 30 secs			
35AC for 30 secs			35%C for 30 secs	

• To define the parameters for reading the completed KASP reactions, click on the 'Not Acquiring' button. This will open the 'Acquisition' window.



• To programme the instrument to read FAM, HEX and ROX, ensure that 'Green', 'Yellow' and 'Orange' are all moved to the 'Acquiring Channels' box.

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To acqui channel, Dye Char Dye Char Dye Char Channel Green Yellow Orange Fred Cimson	e from a c select it in mel Sele 470nm 530nm 585nm 625nm 680nm	hannel, sele the right-ha ction Cha Detector 510nm 555nm 610nm 660nm 710hp	et, It from He list in the left and click >. To stop acquiring from a d list and click <. To remove all acquisitions, click <<. <u>DK</u> Don't Acquire <u>Help</u> t Dyse FAM <sup>1</sup> S YBR Green 1 <sup>10</sup> , FLuorescein, EvaStreen <sup>10</sup> , Alexa Fluor 488 <sup>10</sup> JDE <sup>10</sup> , VIC <sup>10</sup> , HEX, TET <sup>10</sup> , CAL, Fluor Gold 540 <sup>10</sup> , Yaloma Yellow <sup>10</sup> RDK <sup>10</sup> , CAL, Fluor Red 510 <sup>10</sup> , CyS 59 <sup>1</sup> , Texas Red <sup>10</sup> , Alexa Fluor 568 <sup>10</sup> CyS <sup>10</sup> , Quasar 767 <sup>10</sup> , Alexa Fluor 633 <sup>10</sup>

Running KASP genotyping reactions on the QIAGEN Rotor-Gene Q instrument

• The correctly programmed stage (called 'Cycling2') should appear as shown below:

New Open Save As Help		
The run will take approximately 9 minute(s) to	comprete. The graph below represents the run to be performe	1:
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Lick on a cycle below to modify it : Cycling	Inset after	
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This cycle repeats <u>1</u> time(s). Click on one of the steps below to modify it, Timed Step <u>35PC</u>	Remove  or press + or - to add and remove steps for this cycle.	<u>.</u>
This cycle repeats <u>1</u> time(s). Click on one of the steps below to modify it, Timed Step <u>v</u> <u>35%C</u> <u>30 reconds</u>	Remove $\label{eq:remove} \ensuremath{cr}\xspace$ or press + or - to add and remove steps for this cycle.	<u>.</u>
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- The recycling programme can be repeated until tight clusters have formed (re-read the reactions after each recycling programme).
- Once results have been read, completed KASP reactions are stable at room temperature for up to one week as long as the reaction tubes remain well sealed.
- If it is found that recycling is always necessary for a particular assay, extra cycles can be added to the PCR amplification stage (i.e. 29 cycles instead of 26).

#### 5.6 Running the KASP trial kit on the QIAGEN Rotor-Gene Q

- If you have requested a KASP trial kit to run on your QIAGEN Rotor-Gene Q instrument, please follow the protocol included with the kit to set up your reaction plate. This Rotor-Gene Q manual can be used to help you to programme the instrument to run the trial kit reactions.
- After running the KASP thermal cycle, the trial kit reactions should produce data similar to the figure below.



Results from the KASP trial kit reaction plate when run on the QIAGEN Rotor-Gene Q instrument using the standard KASP thermal cycle. Data has been exported and plotted using the scatter plot function in MS Excel.

#### **Further support**

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



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