

A Step-by-Step Guide to Setting up sparQ Fast Library Quant Run Files Without a Template

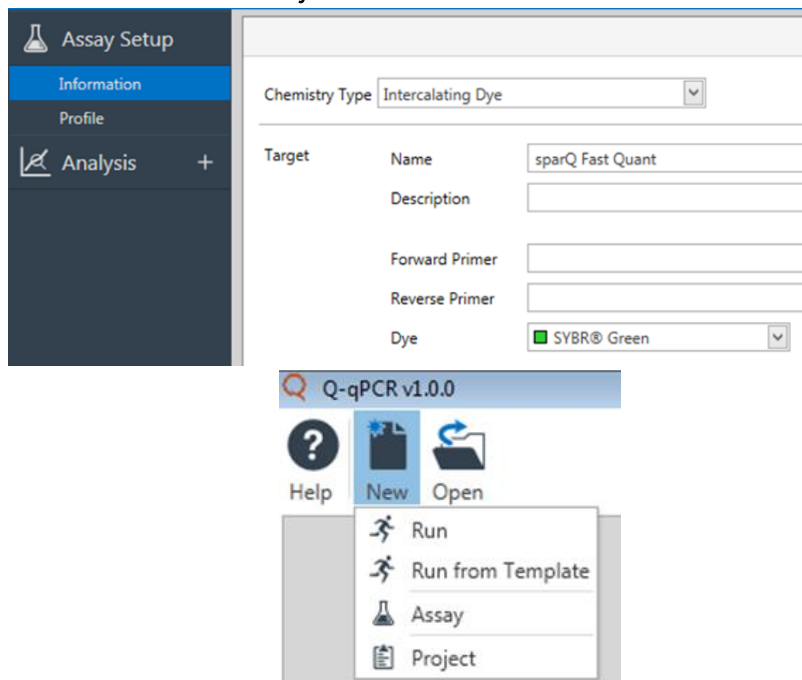
Introduction

Template files provide the Q software information about assay and run parameters. Two template files for use with the sparQ Fast Library Quant Kit, for 10 µl or 20 µl reactions, are available for download from the Quantabio [website](#). These are pre-configured with the cycling protocol and information about locations of the six sparQ DNA standards included as triplicate reactions in the run.

The purpose of this document is to provide guidance on how to manually set up the assay and run files for customers who require more flexibility regarding the number of replicates, the positions of the sparQ DNA standards, or the cycling parameters.

Assay Setup

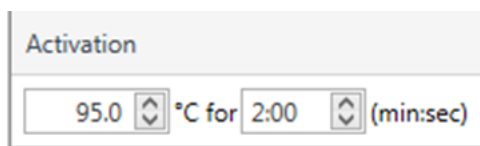
1. Open the Q software.
2. Select the **New** icon and then select **Assay**.



3. Under the **Information** tab, fill in the box titled **Name** with 'sparQ Fast Quant'. The box titled **Description** is optional and does not require any input but can be useful to provide further information about the target and experiment. Make sure the **Chemistry Type** is set to Intercalating Dye and **Dye** is 'SYBR® Green'.

4. Under the **Profile** tab, set the following parameters for our recommended cycling conditions*:
(* for maximum flexibility, customers can choose to adjust any of these settings)

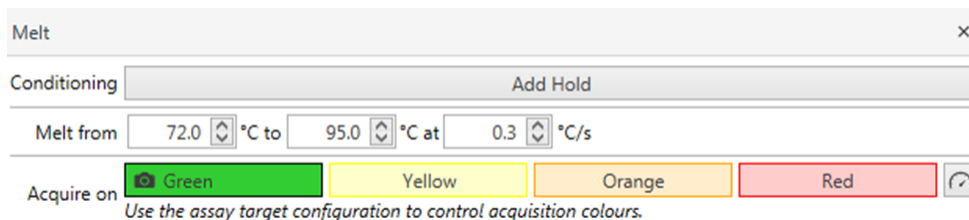
a) In the **Activation** panel, set Activation to 95°C for 2:00 (min:sec).



b) In the **Cycling** panel, delete the 72°C step to make it a 2-step reaction. Set the number of cycles to 35, configure step 1 as 95°C for 5 sec (s), and configure step 2 as 65°C for 25 s. Make sure the camera icon is highlighted in Step 2 to acquire data at this temperature from the green channel.



c) *Optional melt step:* In the **Melt** panel, ensure the parameters are set as below:

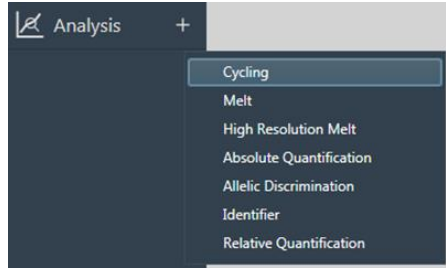


d) In the **Profile** panel, set the **Temperature Control** pull down to 'Fast TAQ (v3)' and set the **Volume** to the desired volume.

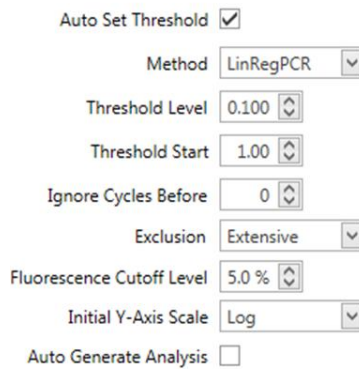


5. Defining the **Analysis** settings.

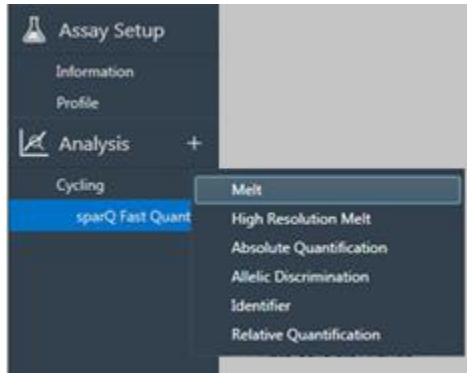
- a) Left-click the '+' icon next to the **Analysis** title and from the dropdown menu select **Cycling**.



- b) Once the **Cycling** analysis is created, select 'sparQ Fast Quant' and ensure the parameters are set as in the next figure.



- c) If the optional melt step is included in the run profile, create the **Melt** analysis by left-clicking the '+' icon next to the Analysis title and from the dropdown menu select **Melt**.



- d) Once the **Melt** analysis is created, select 'sparQ Fast Quant' and ensure the parameters are set as in the figure below.

Threshold

Threshold Start (°C)

Invert Data

Filtering

Auto Generate Analysis

Genotypes

Genotype	+	+	
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- e) Create the **Absolute Quantification** analysis by left-clicking the '+' icon next to the Analysis title and from the dropdown menu select **Absolute Quantification**. Ensure the parameters are as below:

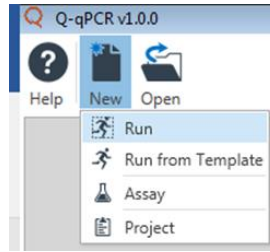
Standard Curve: *Calculated*

Auto Generate Analysis

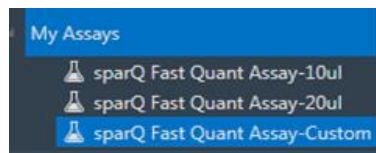
6. Save the assay by clicking the **Save** icon at the top of the screen and provide a descriptive name to the file such as 'sparQ Fast Quant Assay – Custom'. The software will automatically save the assay to the default location that is created during software installation C: Libraries/Documents/Quantabio/Q-qPCR/Assays.

Run Setup

1. Select the **New** icon then select **Run**.

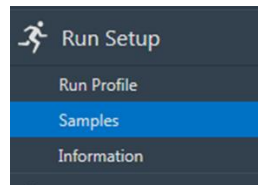


2. Add the assay to the run by selecting the '+' icon beside the **Assays** tab. A dropdown menu will appear with available assays saved to the default location. Select the newly-created assay, in our example 'sparQ Fast Quant Assay – Custom'.



Sample Editor

1. The next step is to complete the sample editor setup. Left-click on the **Samples** option in the navigation bar.



2. Enter the names of the unknown NGS libraries to be measured or the sparQ DNA standards in the **[Name]** column. The sample rows must match the positions of the reactions in the Q rack and corresponding rotor positions. Row indicators can be toggled between numeric only or alpha-numeric using the icons at the top of the sample editor.

Numeric			Alpha-Numeric		
<div style="display: flex; justify-content: space-around;"> 1.. A1.. </div> <div style="display: flex; justify-content: space-around;"> 48 H6 </div>			<div style="display: flex; justify-content: space-around;"> 1.. A1.. </div> <div style="display: flex; justify-content: space-around;"> 48 H6 </div>		
↓			↓		
Colour	Name		Colour	Name	
1	sparQ DNA Standard1		A1	sparQ DNA Standard1	
2	sparQ DNA Standard1		B1	sparQ DNA Standard1	
3	sparQ DNA Standard1		C1	sparQ DNA Standard1	
4	sparQ DNA Standard2		D1	sparQ DNA Standard2	
5	sparQ DNA Standard2		E1	sparQ DNA Standard2	
6	sparQ DNA Standard2		F1	sparQ DNA Standard2	

- All sparQ DNA Standards should be assigned as 'Standard' by clicking in the [Type] column and choosing from the dropdown menu. Library samples to be measured should be kept as 'Unknown'. Any no template control reactions included in the run should be assigned as 'NTC'.

sparQ DNA Standard1	Standard	Library1	Unknown	No template control	NTC
sparQ DNA Standard1	Unknown	Library1	Unknown	No template control	Unknown
sparQ DNA Standard1	Standard	Library1	Standard	No template control	Standard
sparQ DNA Standard2	Positive Control	Library2	Positive Control		Positive Control
sparQ DNA Standard2	Negative Control	Library2	Negative Control		Negative Control
sparQ DNA Standard2	NTC	Library2	NTC		NTC
sparQ DNA Standard2	NRT	Library3	NRT		NRT
sparQ DNA Standard3		Library3	Unknown		
		Library3	Unknown		

- All sparQ DNA Standards must have the concentrations defined in the sample editor. In the [Standards Concentration] column, click on the copies/μL and replace by typing 'picomolar' or 'pM'.



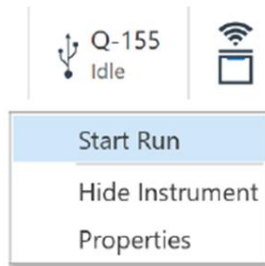
- Enter the concentrations for each of the sparQ DNA Standards according to the values in the chart below.

Name	Type	Groups	Assay	Standards Concentration
sparQ DNA Standard1	Standard		sparQ Fast Quant Assay-Custom	20
sparQ DNA Standard2	Standard		sparQ Fast Quant Assay-Custom	2
sparQ DNA Standard3	Standard		sparQ Fast Quant Assay-Custom	0.2
sparQ DNA Standard4	Standard		sparQ Fast Quant Assay-Custom	0.02
sparQ DNA Standard5	Standard		sparQ Fast Quant Assay-Custom	0.002
sparQ DNA Standard6	Standard		sparQ Fast Quant Assay-Custom	0.0002

Save the Run File and Start the Run

- Once all settings and samples are entered, click the [Save] icon at the top of the screen. This will trigger a pop-up window where the run file is assigned a name and designated folder location.
- Load the capped sample tubes into the appropriate positions in the Q rotor by matching the markings on the loading rack with the markings on the rotor. Place the tube clamp on top of the rotor and close the lid of the Q.

3. Click on the instrument icon near the top right corner of the Q qPCR software window and choose **[Start Run]**.



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For guidelines on data analysis, please refer to our related document – **A Step-by-step Guide to Analysis of sparQ Fast Library Quantification Data**.