

	Standard Operating Procedures for the QuantStudio 12K Flex Real-Time PCR Setup
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Purpose:

This SOP is to provide information regarding the QuantStudio™ 12K Flex Real-Time PCR experiments.

1 Recommend Consumables (all can be ordered through the GRCF)

- 1.1 MicroAmp® Optical 384-Well Reaction Plate (catalog 4343370 or 4309849) or the equivalent
- 1.2 MicroAmp® Optical Adhesive Film (catalog 4311971) or the equivalent
- 1.3 Applied Biosystems Array Card (custom or off the shelf)
- 1.4 MicroAmp® Optical 96- Well Reaction Plate (N8010560) or the equivalent
- 1.5 Applied Biosystems TaqMan Reagents or the equivalent
- 1.6 Applied Biosystems SYBR Green Reagents or the equivalent

Procedures:

2 Making an Appointment

- 2.1 First come, first served, basis using iLab scheduling module.
https://johnshopkins.corefacilities.org/service_center/show_external/3774
- 2.2 Times:
 - 2.2.1 ****COVID-19 Density Restrictions****. Two time slots are available 6AM-9:AM; 10AM-1PM. Only one user may reserve per time slot.
 - 2.2.2 [Standard times: 7AM → 4PM in 1.5+ hour increments. 4PM → overnight (cannot collect data until the next morning at 6am).]
- 2.3 Detailed instructions for scheduling in iLab available at https://grcf.jhmi.edu/wp-content/uploads/2016/08/iLab_instructions_QS_080216-4.pdf

3 Fees

- 3.1 \$20/hour, you must schedule at least a half hour longer than you need to run your experiment
- 3.2 COVID density reduction special fees:
 - 3.2.1 \$20 flat cancellation fee
 - 3.2.2 \$40 flat fee if you extend past your reservation

4 Setting up an experiment

- 4.1 Open QuantStudio™ 12K Flex Software
 - 4.1.1 From Experiment Menu click the “Create” icon → Click “Experiment Properties”
- 4.2 Define Experiment name and type
 - 4.2.1 Enter your experiment name in the Experiment Name field. The default is a date and time stamp. Acceptable characters are limited to alpha-numeric, period (.), hyphen (-), underscore (_) and spaces ().
 - 4.2.2 Select the block type you are using to run the experiment, and the experiment type (Standard Curve, etc).
 - 4.2.3 Select the reagent you are using to detect your targets:
 - a TaqMan reagents
 - b SYBR Green reagents (if using the software will automatically include a melt curve)
 - c Other
 - 4.2.4 Save the experiment.

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- 4.3 Define targets, samples, and biological replicate groups
- 4.3.1 Defining targets
- a Click New to add targets and define them. Click a cell in the Target Name column to enter your target name(s). The default names are Target 1, 2 etc.
 - b Choose the correct **Reporter** and **Quencher** from the drop-down menu. Default reporters and quenchers are contingent on which reagent you selected in the experiment setup. For example, if TaqMan is the reagent you selected the default reporter is FAM and the default quencher NFQ-MGB.
 - c Select the target **Color** from the drop-down menu. This is arbitrary and simply the color you'd like to associate with each target.
 - d Click **Save to Library** to save your newly added targets to the target library. You can then use these targets for future runs and avoid having to re-enter the info.
 - 1 For future experiments to add targets previously saved in the target library, click **Import from Library**
- *Note: you can run multiple targets on one plate. The important thing is ensuring you have a standard curve as a reference point for each of your targets to facilitate absolute quantification of your unknowns.
- 4.3.2 Define samples
- a Click New to add samples. If desired you can name them.
 - b In the samples table click a cell in the Sample Name column for the sample to define and enter your sample name. Default is Sample 1, 2 etc.
 - c Select the sample **Color** from the drop-down menu. Again, the color is arbitrarily chosen.
 - d *Optionally* click **Save to Library** to save your samples to the sample library. This will allow you to avoid re-entering this info in the future.
 - 1 Similarly, to saved targets, click **Import from Library** to add samples saved to the sample library
- 4.3.3 Define biological replicates
- a In the Define Biological Replicates Groups table, click New to add a biological replicate group and name them. Limit is 100 characters.
 - b Select **Color** from the drop-down menu.
- 4.4 Assign targets, samples, and biological replicate groups to your reaction plate wells
You can start a run without designating these parameters, however there will be no real-time data in the amplification plots (it will only be visible AFTER you have setup the plate).
- 4.4.1 Click **Assign** to access your plate setup
- 4.4.2 Assign your targets.
- a Select wells using the plate layout or the well table on the Assign screen.
 - b Select a target and assign its task, in the plate, from the drop-down menu. The following are the options.

Experiment type	Legend	Tasks
Standard Curve	U	Unknown
	S	Standard
	N	Negative control

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Relative Standard Curve	U	Unknown
	S	Standard
	N	Negative control
Comparative CT	U	Unknown
	N	Negative control
Genotyping	U	Unknown
	1/1	+ control Allele 1/Allele 1
	2/2	+ control Allele 2/Allele 2
	1/2	+ control Allele 1/Allele 2
	N	No template control
Presence/Absence	U	Unknown
	I	Internal + control
	N	- control
	†	Blocked internal + control
Melt Curve	U	Unknown
	N	Negative control

- 4.4.3 Assign samples.
 - a Select wells on the plate layout or well table on the Assign screen
 - b Select the check box next to the sample to assign to the selected wells
 - 1 Only one sample is allowed to be added to each well
- 4.4.4 Assign biological replicate groups
 - a Select wells using the plate layout or well table on the Assign screen
 - b Select the check box next to the biological replicate group to assign the selected wells
- 4.4.5 Check your Assign screen and verify the plate layout and sample/target/group designation is correct.
- 4.5 Define the run method
 - 4.5.1 Click **Run Method** screen.
 - 4.5.2 Enter the reaction volume per well. The QuantStudio supports the following max reaction volumes
 - a 384-well = 30µL
 - b 96-well = 200µL
 - 4.5.3 In the **Graphical View** tab review the run method, and check the thermal profile compatibility with your reagents.
 - a Edit the ramp rate, PCR stage, cycle numbers etc
 - b Select **Enable AutoDelta** check box ot increase or decrease the temperatures and/or hold times for each subsequenc cycle, or to change the AutoDelta settings for the cycling stage in the AutoDelta Settings dialog box. Then click **Save Setting** to display the AutoDelta On icon.

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- c If SYBR Green is the reagent, the Melt Curve stage automatically appears in the Run Method screen. If you delete that section from the protocol the melt curve is active in the Add Stage drop-down menu.

5 Load the reaction plate or array card into the instrument

- 5.1 Ensure your samples are spun down and the plate is sealed properly with optical adhesive film.
- 5.2 Touch the Eject button (▲) on the instruments touch screen, or click **Open Door** in the Instrument Console screen of the software.
- 5.3 Carefully place the reaction plate on the plate adapter. Ensure the plate is properly aligned, with the A1 well positioned at the top-left of the tray and aligned with the A1 imprint on the plate holder.
- 5.4 Once your plate is properly placed, touch the Eject button (▲) again, or click **Close Door** in the instrument console screen. The plate should retract back into the instrument.

6 Run the experiment

You can run the experiment from the QuantStudio software or on the instrument touchscreen

Software

- 6.1 In the navigation pan in the software click **Start Run**.

Touchscreen

- 6.2 From the Main Menu screen (click the Home icon to get there) touch **Browse Experiments**
- 6.3 Touch the **Folders** icon, and touch the folder in which the experiment was saved
- 6.4 Select the desired experiment, then touch **View/Edit** to verify the experiment setup before running
- 6.5 In the **Start Run** screen, touch each field as needed to modify any of the parameters, then touch the **Start Run Now** button to start the experiment.
 - 6.5.1 If you choose not to create an experiment and define any of your wells you may simply choose **Start Run** without following the above steps.

7 Unload the instrument

- 7.1 When the run has completed the instrument displays the Main Menu screen.
- 7.2 Please unload your reaction plate from the instrument and properly dispose of your plate. Open the plate holder by either touching the Eject button (▲) on the instruments panel or by clicking **Open Door** in the software.
- 7.3 **Please do not leave your plate in the instrument for others to deal with, or leave it sitting on the counterspace for the instrument.** Proper disposal is your responsibility, and a biohazardous waste trash can is located directly behind the instruments.

8 Transfer experiment results

You can utilize either the instruments touchscreen or the software to save your data.

Software

- 8.1 On the home page under **Tools** select **Instrument Console** and select the instrument you just used to run the experiment
- 8.2 Click **Manage Instrument** from the upper tabs to open the Instrument Manager. Click **Manage Files**.
- 8.3 In the Experiments panel, select the experiment you want to download and click **Download**.
- 8.4 In the Save dialog box, select the folder to hold the experiment results and click **Save**.

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8.4.1 The experiments folder is: C:\AppliedBiosystems\QuantStudio12KFlex Software\experiments\

8.4.2 Copy the folder from the C:drive to the USB drive

Instrument

8.5 Insert your USB drive into the front panel of the instrument.

8.6 Touch the screen, from the main menu touch **Collect Results**.

8.7 Select the experiment(s) you want to save by touching them, then touch the **Save to USB**.

8.7.1 If the instrument has difficulty finding the USB drive remove it, then try again. If the instrument still doesn't recognize the drive please try another USB drive.

8.8 Touch the **Home** icon to return to the main menu. Remove the USB drive from the instrument, and connect it to your computer

9

Analyze data

9.1 To open and analyze your data you must first download the Expression Suite Software. Please go to <http://www.lifetechnologies.com/us/en/home/technical-resources/software-downloads/expressionsuite-software.html> to obtain the free software. No license key is required.