theranos	Standard Operating Procedure	Document Number: CL SOP-~~16104~~ 17008 Revision: A
	CLIA Laboratory	Effective Date: 3/18/15
Roche LightCycler® Simplified Protocol		

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The Laboratory Director or designee will review this procedure at least annually including revisions.

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Contents

1. Principle

The Polymerase Chain Reaction (PCR) technique invented in 1985 by Kary B. Mullis, allowed scientists to make millions of copies of a scarce sample of DNA. The technique has revolutionized many aspects of current research, including the diagnosis of genetic defects and the detection of the AIDS virus in human cells. The technique is also used by criminologists to link specific persons to samples of blood or hair via DNA comparison. PCR also affected evolutionary studies because large quantities of DNA can be manufactured from fossils containing but trace amounts.

The LightCycler® 480 Instrument is intended for performing rapid, accurate polymerase chain reaction (PCR) in combination with real-time, online detection enabling Absolute or Relative Quantification of a target nucleic acid, as well as post-PCR analysis of the amplified nucleic acid by Melting Curve analysis.

The LightCycler® 480 Instrument is intended for general laboratory use and must be used exclusively by laboratory professionals trained in laboratory techniques and having studied the instructions for use of this instrument.

This SOP will give a basic overview of how the Theranos CLIA Molecular Diagnostic department should use the instrument. Further details should be found the official Roche user manual.

2. Scope

This SOP applies to all personnel performing real time PCR in the Theranos CLIA lab.

3. Definitions and Abbreviations

PCR or Polymerase chain reaction is a laboratory technique used to make multiple copies of a segment of DNA. **PCR** is very precise and can be used to amplify, or copy, a specific DNA target from a mixture of DNA molecules.

qPCR is the technique that makes the positive values of PCR quantitative by assigning it a value based on copy number present in the sample.

4. Responsibilities

4.1 It is the responsibility of the Laboratory Director or designee thereof (i.e. technical or general supervisor) to ensure testing personnel are adequately trained in these methods.

4.2 It is the responsibility of designated testing personnel to maintain competency, supplies, and prompt specimen processing.

4.3 It is the responsibility of the CLS and designated testing personnel to use universal precautions when working with cultures.

5. Materials and Reagents

5.1 LightCycler® 480 plates and seals

5.2 Mastermix and samples of interest

5.3 Roche LightCycler®

6. Procedure

1) Prepare your plate using master mix and sample according to your protocol, taking care to avoid contamination. Seal the plate tightly using a clear adhesive cover from Roche. Note: you should only use the seals provided by Roche because they have been validated for proper optical readings.

2) After sealing the plate, spin it down to pop any bubbles that may have formed.

3) Carefully take the sealed plate to the room with the LightCycler®. Turn on the machine by switching the button found on the back right of the instrument.

4) Log in to windows and open the LightCycler® 480 program. Log in using:

User name: admin **password: Roche480**

5) Allow the machine to warm up. Pay attention to the lights on the front of the machine: they should both be solid green or one green and one orange when the instrument it ready to use.

Color of Left LED	Color of Right LED	Indication
Orange (flashing)	Orange (flashing)	Instrument is initializing.
Green	Orange	Instrument is turned on. Instrument status is ready. No plate loaded.
Green	Orange (flashing)	Plate is loading.
Green	Green	Instrument is turned on. Instrument status is ready. Plate is loaded.
Green (flashing)	Green (flashing)	Instrument is running.

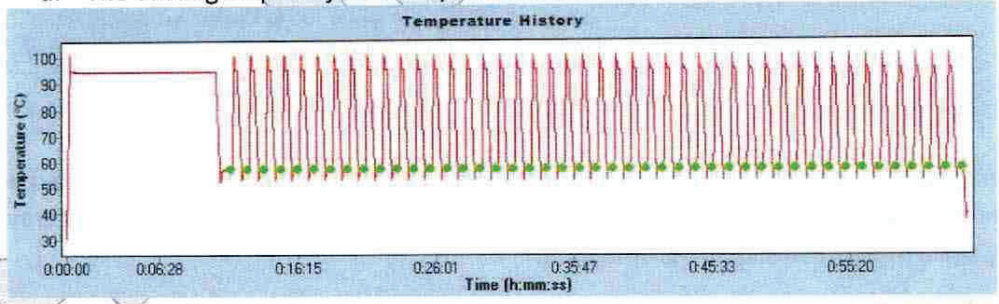
6) When the machine is ready, open the plate tray by pressing the square grey button located on the front of the machine. Load the plate so that the A1 position is in the back left corner of the tray. Close the tray by again pressing the square grey button.

7) In the LC480 program, click 'New Experiment From Template' button, highlight the appropriate protocol and then choose it by clicking the check mark button.

Note: To create a new LC480 cycling protocol please do the following:

1) After loading the plate, click 'New Experiment' from the Overview menu.
2) In the *Programs* and *Temperature Targets* section, click to add as many additional programs or temperature targets as needed for the protocol (the first program is always provided by default). For each program row, specify the *Program Name*, *Cycles*, *Analysis Mode*, etc).

a. Generally speaking, we have 3 programs: denature, amplification, and cooling.
b. The denature step usually has one temperature for one cycle for around 10m.
c. The amplification step has two or more temperatures that cycles 45 times with a single acquisition at around 55C.
d. The cooling step has just one cycle at 37C for 10s to bring the block temperature down.



8) Name your plate according to the lab's naming convention. Make sure it includes the date and assay type at a minimum. Click the check mark to begin the protocol. Front lights should flash green when running.

9) When the run is complete, the front lights should be solid green.

10) Once the run is complete, it is safe to re-open the plate tray to remove the plate. Seal plate in a zip lock bag and throw it in the biohazardous waste to reduce the chance of amplicon contamination throughout the lab.

11) To choose a subset:

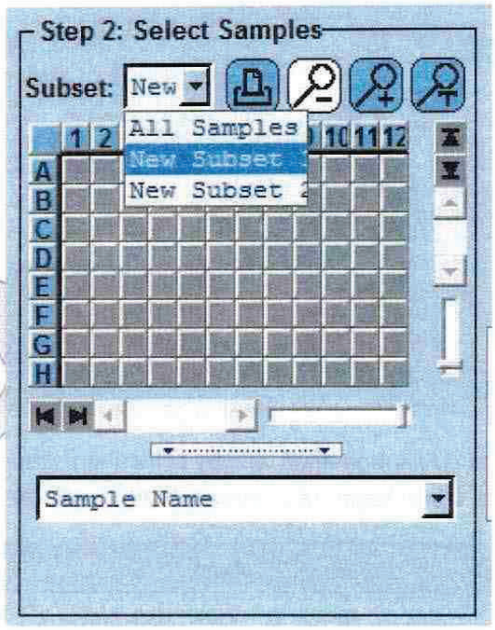
1. Click the blue 'subset editor' box on the left hand column of the LC480 program.

2. Click the blue '+' button near the bottom left. Highlight the cells you would like to analyze. You can use the ctrl button to select wells that are not adjacent to one another. When all desired cells are chosen, click the blue 'apply' button near the bottom right.

12) To name samples:

1. Click the blue 'sample editor' box on the left hand column of the LC480 program.

2. If you previously made a subset, you can isolate these boxes by selecting 'new subset' in the drop down box near the word 'Subset.'

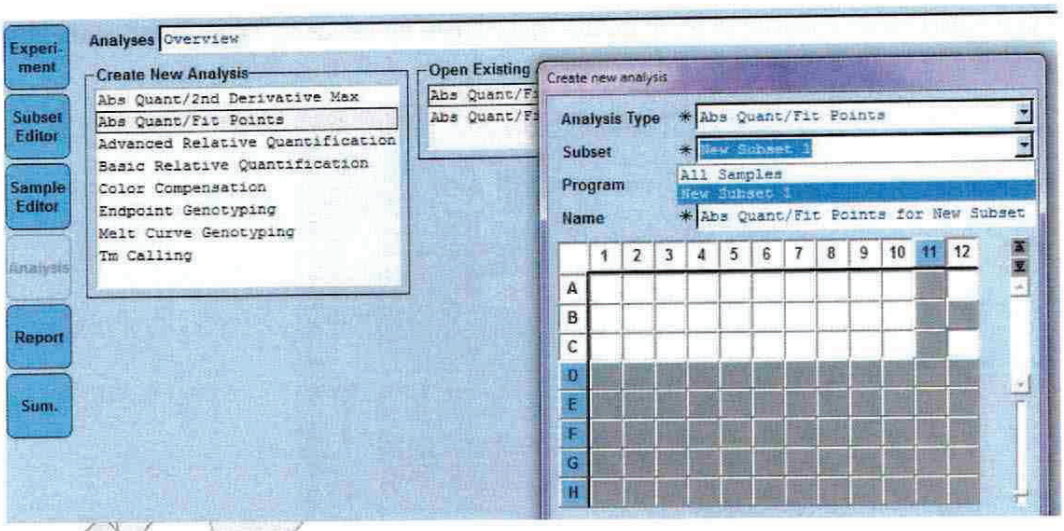


3. Highlight the cell(s) you wish to name, then click the pre-named 'Sample1, Sample 2, etc.' and type to change the name. Note: if you highlight multiple boxes, you can change the name to the same thing all at once.

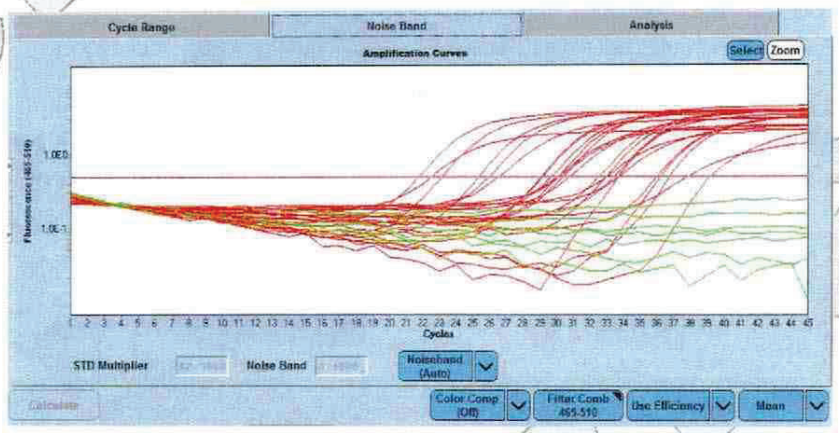
12) To analyze the run:

1) Click the square blue button named 'analysis' on the left hand column of the LC480 program.

2) Highlight 'Abs Quant/Fit Points' then choose 'New Subset' from the drop down box that appears. Hit the check mark to open the run.



3) Click the blue 'Calculate' box and examine each well by selecting them. Note: You can adjust the noise band if needed by going in to the 'noise band' tab and moving the red line to where needed.



13) To import the data set for email:

1) Click the blue 'export' button located on the right-hand tool bar.

2) Save the data in the proper folder. This can now be attached and sent via email in .ixo format.

7. Quality Control and Maintenance

1) The master mix you use should have positive and negative controls built in to it so that you can tell if the run was successful and/or contaminated.

2) The area around the LightCycler® 480 Instrument should be checked regularly, to ensure that the air flow is unrestricted and that books, papers, or other supplies are not interfering with the air flow.

3) For general cleaning:
Regular cleaning of the LightCycler® 480 Instruments and accessories is not obligatory. If necessary, clean the housing of the LightCycler® 480 Instrument, the thermal block cycler, and the block cycler cover with a mild commercial detergent. If necessary, use 70% ethanol for disinfecting the instrument housing, the thermal block cycler, and the block cycler cover.

Cleaning of the LightCycler® 480 block cycler unit: pipette into all wells 20µl (384-wellblock) respectively 125µl (96 well block) of 70% Ethanol or Isopropanol. After waiting 15 minutes pipette up and down several times. Remove the liquid and let the block cycler unit dry before using again. Take care not to destroy the block coating.

4) When necessary, you may have to change the xenon lamp, dust filters, or fuses inside the instrument. For instructions on how to do this, please see the official Roche user's manual.

8. Limitations

8.1 The Roche LightCycler® 480 has limitations regarding probes it can successfully read as well the temperatures it can reach.

4.3.2 Filter Set of the LightCycler® 480 Instrument II

	Bandpass	Half Band Width (HBW)
Excitation wavelengths (nm)	440 nm	35 nm
	465 nm	25 nm
	498 nm	40 nm
	533 nm	25 nm
	618 nm	35 nm
Detection wavelengths (nm)	488 nm	20 nm
	510 nm	20 nm
	580 nm	20 nm
	610 nm	20 nm
	640 nm	20 nm
	660 nm	95 nm (low pass)

Temperature control	Peltier-based heating and cooling
Temperature range	37 – 95°C 20°C starting temperature to perform specific Melting Curve analysis if required
Heating rate	96-well block: 4.4°C/s 384-well block: 4.8°C/s
Cooling rate	96-well block: 2.2°C/s 384-well block: 2.5°C/s

8.2 When running multiplexed reactions, signal bleed through may become an issue. To help resolve the problem, Color Compensation should be run. See SOP for Color Compensation for further instructions.

9. Safety

9.1 Make sure the working area is clean and kept clear.

9.2 Follow local guidelines for disposal of waste material according to federal, state and local laws.

9.3 Follow universal precautions and wear appropriate PPEs.

10. Records

10.1 Results will be retained for a minimum period of three years.

11. Competency

11.1 Competency should be performed by a supervisor biannually.

12. Proficiency Testing

12.1 Surveys should be run a minimum of 2 times per year from an appropriate vendor.

13. References

http://siarchives.si.edu/research/videohistory_catalog9577.html

http://icob.sinica.edu.tw/pubweb/bio-chem/Core%20Facilities/Data/R401-core/LightCycler480%20II_Manual_V1.5.pdf

14. Revision History

REVISION HISTORY			
Revision Level	**Effective Date**	**Initiator**	**DCO Number**
A		B Bivens	
Section Number	**Description and Justification of Changes**		
All	Initial Release		