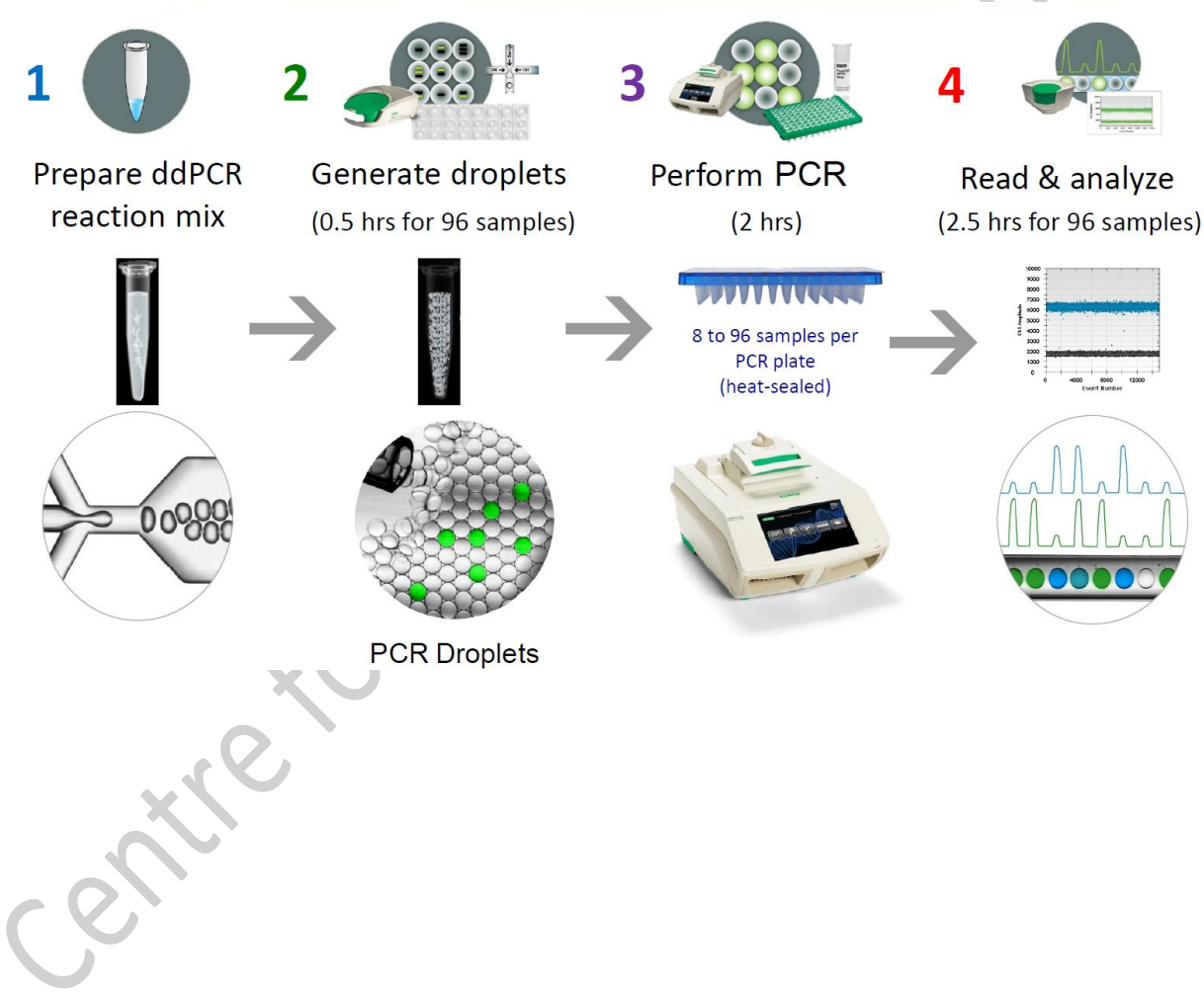
Bio-Rad QX200 Droplet Digital PCR

Standard Operating Protocol

I. Overview of QX200™ Droplet Digital™ PCR System

- QX200 Droplet Reader process one 96-well PCR plate at a time
- Starting sample details: 20 µL reaction mixture per well, containing up to 330 ng DNA in a reaction
- Droplets per 20 µL sample: ~13000 - 20000
- 4-5.5 hours for complete workflow for 96 samples



II. Preparation of reaction mixture

Time required:

- 30-60 minutes for thawing and mixing of reagents, preparation of master mix and sample dilution. Time required is highly variable with the complexity of experiment and user experience.

Procedure:

1. Thaw all components to room temperature. Mix by ≥ 2 cycles of vortexing and centrifugation.
2. (Optional: for gDNA samples, perform restriction enzyme digestion with 10-20 enzyme units per microgram of gDNA.)
   a. Perform the restriction enzyme digestion for 1 hour in the recommended conditions for the restriction enzyme.
   b. Dilute the sample for at least 10-fold after restriction enzyme digestion to reduce salt content.
3. Dilute samples at the desired concentration before setting up the reaction mix. Mix well.
4. Prepare master mix reaction(s) as referring to the following tables:

*Component for **EvaGreen** experiment*	*Volume per reaction, µL*	*Final concentration*
*2x QX200 ddPCR **EvaGreen** Supermix*	10	1x
Forward primer	Variable	250 nM
Reverse primer	Variable	250 nM
*Sample**	Variable	Up to 100 ng**
RNase-/DNase-free water	Variable	--
Total Volume	20	--

*Suggested amplicon length: 80-250 bp.
**For gene expression analysis, starting with 1-30 ng cDNA input is recommended.

*Component for **Probe** experiment*	*Volume per reaction, µL*	*Final concentration*
*2x QX200 ddPCR **Probe** Supermix*	10	1x
20uM target primers mix	0.9	900 nM
5uM target probe (FAM)	1	250 nM
20uM reference primers mix	0.9	900 nM
5uM reference probe (HEX/VIC)	1	250 nM
*Sample**	Variable	Up to 330 ng**
*Restriction enzyme**	Variable	2-5 UI/20uL reaction
RNase-/DNase-free water	Variable	--
Total Volume	20	--

*For gDNA sample, sample concentration >66 ng per reaction and certain applications may require restriction digestion for optimal target detection. HaeIII, MseI, AluI, HindIII and CviQI works well in the mix.
**For CNV analysis, 1-50 ng gDNA input is recommended.

Avoid bead-based extraction/purification before droplet generation.
Any particulate would block the microfluidic channels. Quick spin reagents and pipette from the surface!

5. Mix thoroughly by ≥ 2 cycles of vortexing and centrifugation. Allow the reaction tubes to equilibrate at room temperature for ~3 minutes before loading into the DG8 cartridge.

III. QX200 Droplet Generator Protocol

Time required:

- Process 1 cartilage of at most 8 samples at a time
- Each round (8 samples) takes about 3 minutes

Materials needed:

- 20 µL PCR reaction mix in vials/ 96-well PCR plates
- 20 µL and 200 µL barrier tips
- 1X Buffer control (provided by FCF)
- Droplet generation (DG) oil (70ul/well)
- DG8 cartridge and gasket

Workflow (QX200 Droplet Generator)

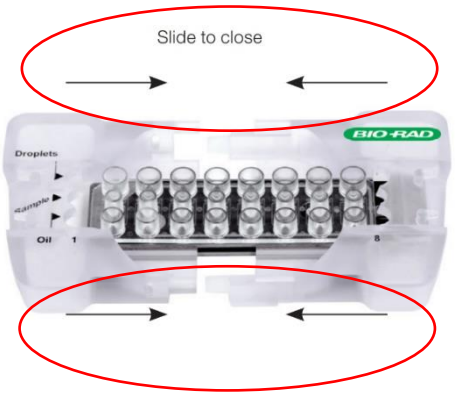
1. Equilibrate the ddPCR reaction mix to room temperature (~3 mins) before loading into the DG8 cartridge.

2. Sign on the log book.

3. Press the middle latches to slide open the cartridge holder.

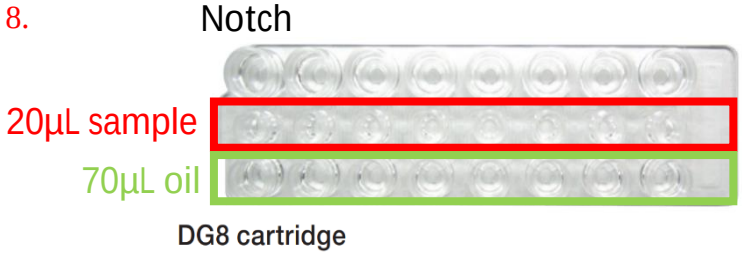
4. With the notch on the upper left corner, slide the DG8 cartridge into the right half of the holder, then press the left side down.

5. Press the two sides of the holder to the center until a ‘click’ sound to close.



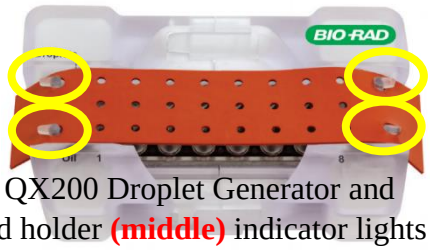
6. Transfer 20 µL of each sample along the bottom ridge to the sample wells **(middle row)** of the cartridge.
   * Avoid air bubbles at the bottom.

7. Fill **ALL** unused sample wells with 20 µL 1X Buffer control if needed.
   * All wells must be filled before start of step 8.



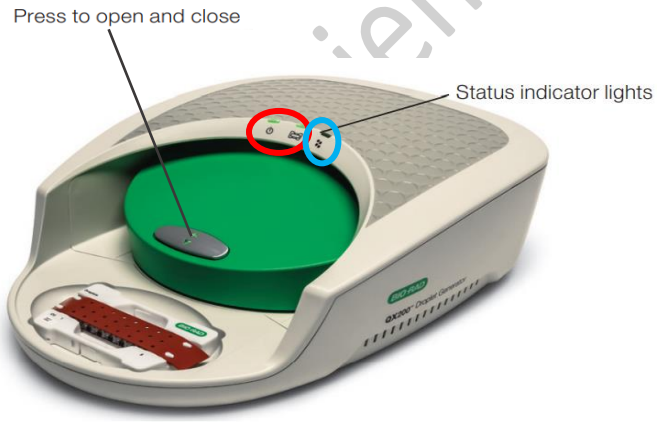
8. Only after completion of step 7, fill 70 µL Droplet generation oil into all of the oil wells **(bottom row).** (Use of single channel pipette and reverse pipetting is recommended.)

9. **Securely** hook the gasket over the holder using the holes on both sides.



10. Switch ON the wall socket switch. Press button on green cover to open QX200 Droplet Generator and place the cartridge holder into the instrument which the power **(left)** and holder **(middle)** indicator lights turn **green**.

    (Flashing amber light indicates problem during applying pressure due to no gasket or empty well.)



11. Press the button to close the door and initiate droplet generation. Droplet indicator light **(right)** will start flashing in **green** after 10 sec indicating droplet generation is in progress. There are two cartridge holders to allow for the loading of oil and samples into the second cartridge when the machine is running.

12. Do not press the button and ***Wait*** until finished when all three lights turn **solid green**. Press the button to open the door.

13. Remove the holder with DG8 cartridge from the Droplet Generator.

14. Discard the disposable gasket from the holder. Keep the DG8 cartridge in the holder. (Milky fluid in the wells of the top "Droplets" row indicates completed droplet generation.)
    * Avoid pipette up and down, centrifugation and any procedure creating shearing or coalescing force after droplet generation until the end of the whole experiment.

15. Proceed to the PCR Reaction Plate Preparation. (Recommended: within 1.5 hours)

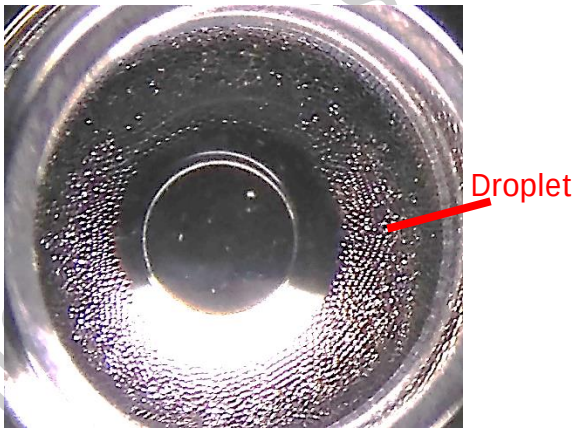
IV. PCR Reaction Plate Preparation

Time required:

- Process 1 cartilage of at most 8 samples at a time
- Each round (8 samples) takes about 10 minutes

Materials required:

- Generated ddPCR reaction droplet in DG8 cartridge
- 96-well PCR plate (Biorad 12001925 / Eppendorf 951020362) Only
- 200 µL barrier tips
- Multichannel pipette (if necessary)



1. Pipette **40 µL** of the oil droplets **SLOWLY** in **slanted** position from the side of the well.
   (~5 µL air is expected)
   * Droplets tend to float on the surface.
   * Pipette slowly to prevent shearing or coalescing the droplets.
2. Remove excessive DG oil in the pipette tip if any.
3. Dispense droplets **SLOWLY** along the side of the well into a single column of 96-well PCR plate.
4. Cover the plate after each round with a sheet of parafilm to avoid evaporation.
5. Press the latches on the DG8 cartridge holder to open.
6. Remove the empty DG8 cartridge and discard.
7. Repeat oil generation process for all the samples and add into PCR plate.
8. Switch off the Droplet Generator and return the holder to FCF ddPCR accessories tray after use.
9. Sign out on the log book.
10. Seal the plate with foil (compatible with the PX1 PCR plate sealer & needles in QX200 Droplet Reader) immediately with *PX1 PCR Plate Sealer* to avoid evaporation.

V. PX1 PCR PLATE Sealer Prot

Time required:

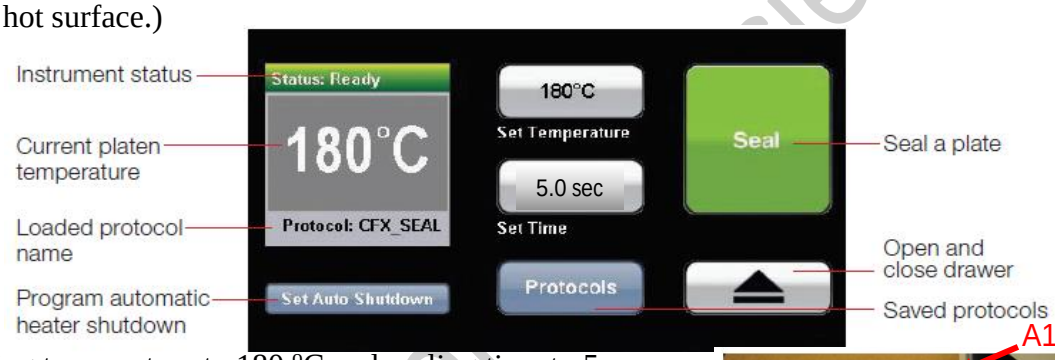
- 3-5 minutes for a 96-well plate

Materials needed:

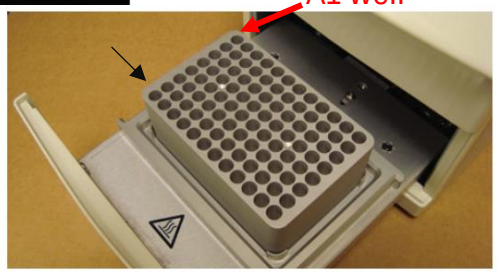
- Pierceable foil plate seals (Bio-Rad 181-4040) ONLY
- 96-well PCR plate (Biorad 12001925 / Eppendorf 951020362) Only

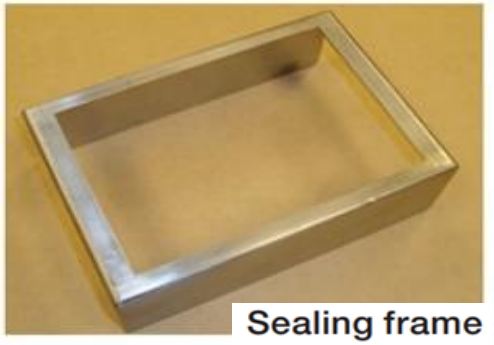
Workflow (PX1 PCR PLATE Sealer)

1. Power ON the PX1 PCR PLATE Sealer with the switch at the back.
2. Open the drawer by pressing ⏏ to remove the support block and sealing frame. (Caution of hot surface.)



3. Set the sealing temperature to 180 °C and sealing time to 5 sec.
4. Let the platen to heat to the set temperature.
5. Position the support block on the tray with 96-well side facing up.
6. Align the 96-well plate onto the support block.
7. Cover the plate with foil seal (The side with red line face up).
8. Add sealing frame if necessary.
9. When the set temperature is reached, the “Seal” button will turn green. Touch ”Seal” to close the tray and initiate heat sealing.
10. PX1 door will opens automatically when completed.
11. Remove plate from the block for thermal cycling.
12. Remove the block from the PX1.
13. Check if all wells are sealed with visible depression on the foil.
14. Proceed to thermal cycling within 30 minutes or store the plate at 4°C for up to 4 hours after sealing.
15. Switch off the PX1 PCR PLATE Sealer.





VI. C1000 Touch Thermal cycler

Time required:

- ~2 hours for one 96-well plate

Materials needed:

- Sealed 96-well PCR plates with PCR reaction mix

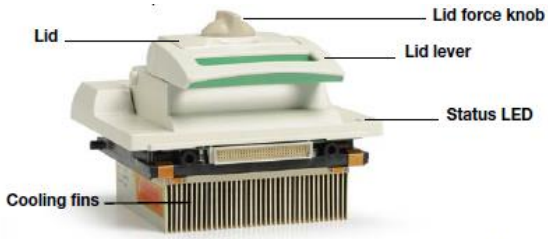
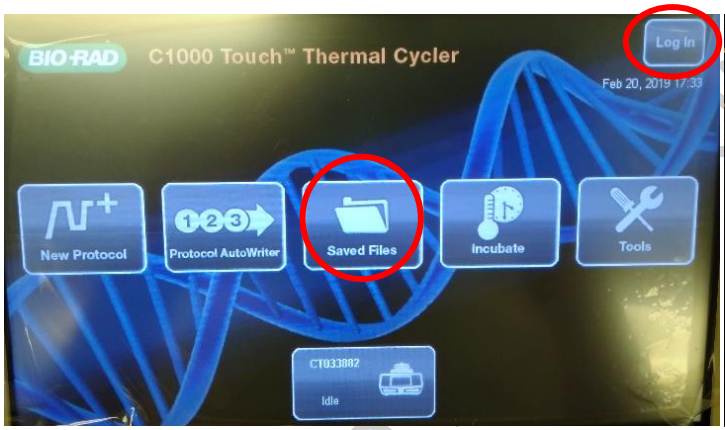


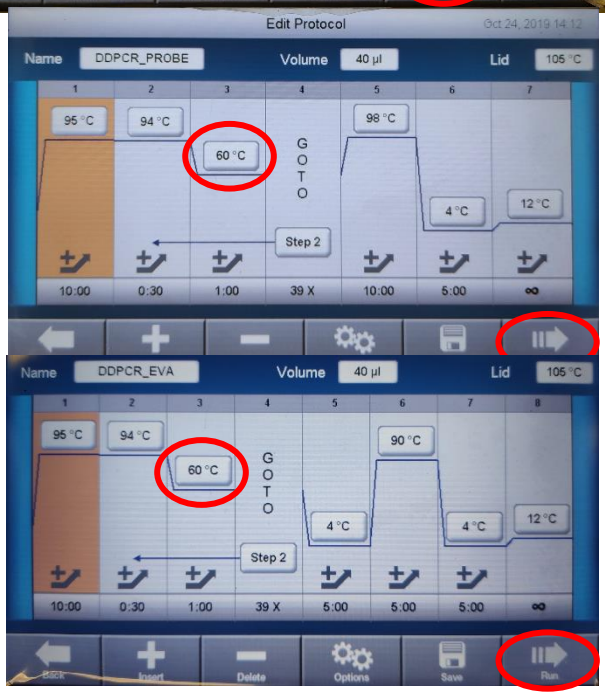
Figure 3. Components of a reaction module.

Workflow (C1000 Touch Thermal cycler)

1. Sign in on the log book and power ON C1000 with the switch at the back.
2. Turn the knob counterclockwise to raise the inner lid.
3. Raise the handle up to the vertical position.
4. Place the foil-sealed 96-well plate into the plate holder.
5. Lower the handle to horizontal position.
6. Turn the knob clockwise until the inner lid is tight. Stop turning when a “click” sound is heard.
7. Click “Saved files” -> “Root” -> “DDPCR TEMP” to select the protocol.



8. Touch “Edit” on the screen. Change the annealing temperature according to your primer conditions.
   Do not Save / overwrite protocol
9. Touch “RUN” on the screen.
10. Stop the program and ***return to the main menu after use.***
11. Power OFF the machine. Sign out on the log book.
    * No overnight run is allowed.



VII. QX200 Droplet Reader

Offline software available in:
https://drive.google.com/drive/folders/1cmH5tZ-gqxZ2T9UT89tB5YblTExWVvrk
User is recommended to set and save template file (.qlt) according to Step 5a before the booking of QX200 Droplet Reader to facilitate the use of equipment.

Time required:

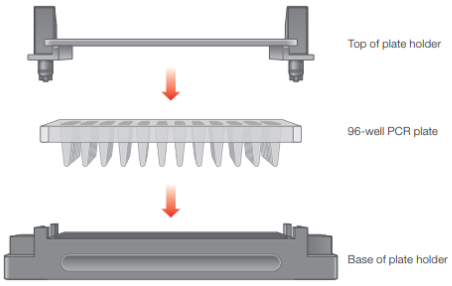
- ~2.5 hours for a 96-well plate (~12.5 mins for 1 column (8 samples))

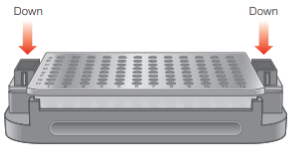
Materials needed:

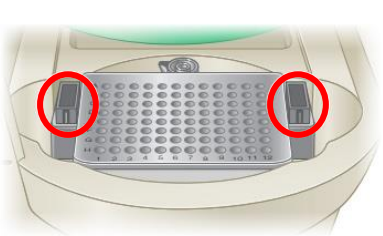
- 96-well PCR plate with PCR products (Sealed plate can be stored at 4ºC for 24-hour before reading.)

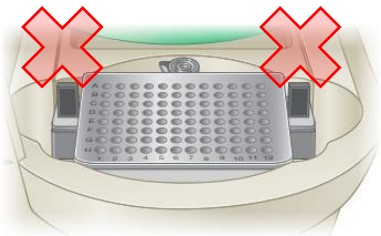
Workflow (QX200 Droplet Reader)

1. Sign on the log book. Power ON the reader with the switch at its back. Log in PPMS tracker.
2. Place the 96-well plate into the plate holder. (Match plate coordinates labeled on the holder.)
3. Press the button on the green lid and place the holder into the Droplet Reader.
4. Check the correct placement of the plate holder and confirm the first three indicator lights are **green**.
   *Plate holder lid must be in the "down" position, or otherwise the sampling needle would be damaged.





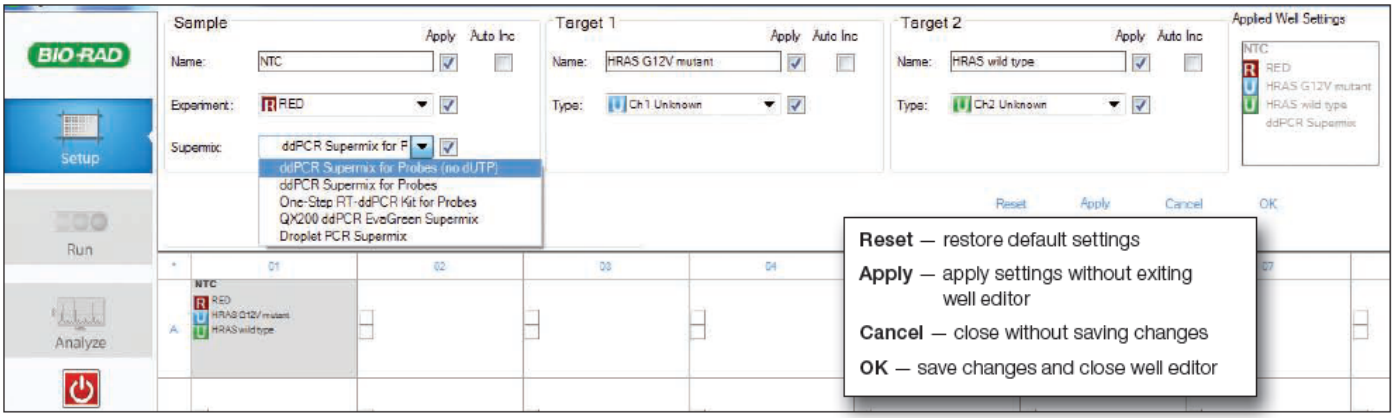




Correct (Down position) Incorrect

5. In the QuantaSoft Software,
    a. Click “Setup” to enter information about samples, assays, and experiments. (e.g. sample names, supermix used, FAM/VIC/HEX channel*) for assigned samples.

    *Target 1: FAM channel (For EvaGreen & probe); Target 2: VIC/HEX channel (For probe).



    b. Click “Run” to select the dye set. Then, the reader will start to run on the assigned samples.

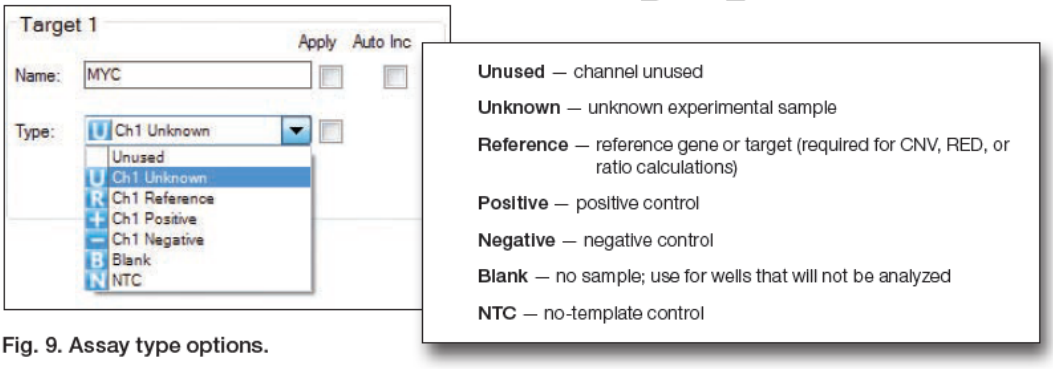
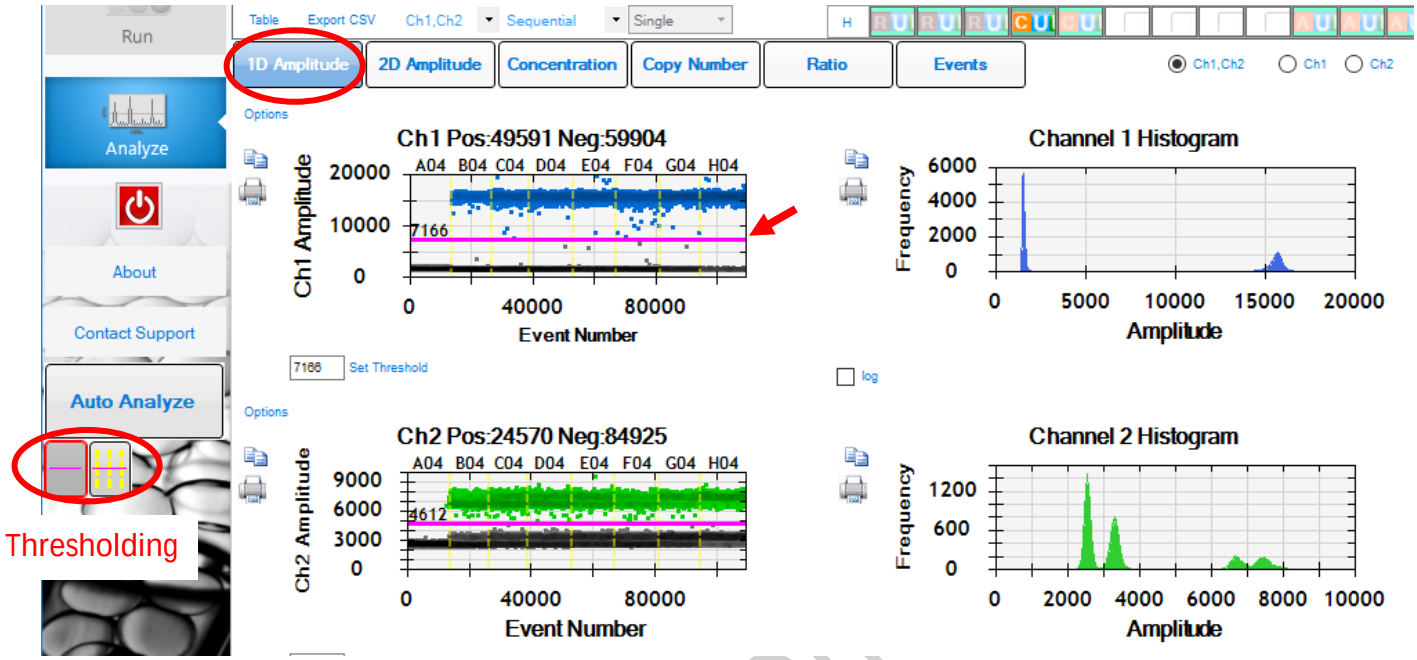


Fig. 9. Assay type options.

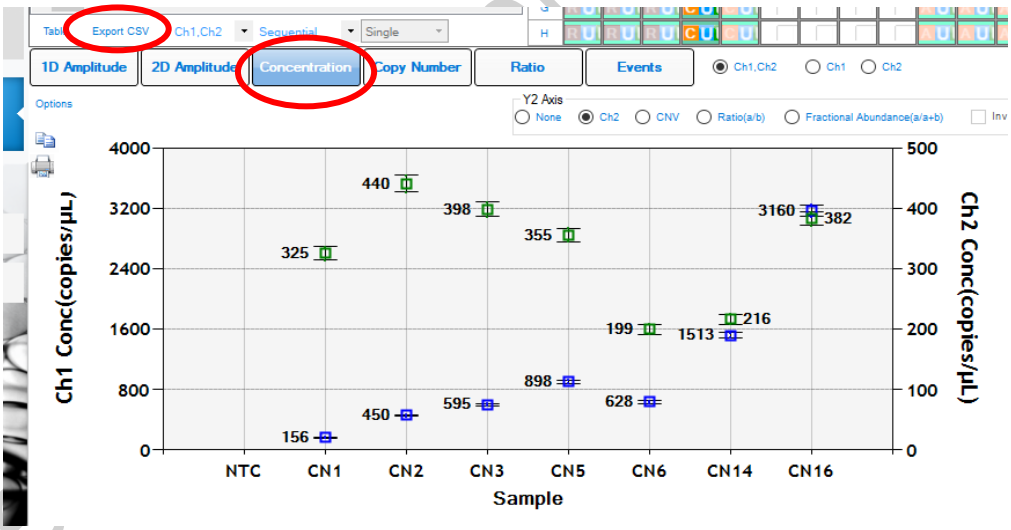
6. After the reading, remove your sample plate with the holder from the reader. Power OFF the reader. Return the plate holder to the tray beside the reader. Sign out on the logbook. Log out PPMS tracker.
7. For analysis using QuantaSoft, select all the samples and check the total number of droplet read by clicking the “Event” tab and check the “total”.

*Total droplet number in a well less than 8000 counts might suggest faults during the experiment, such as clogging of microfluidic channels.

8. Go to “1D Amplitude” to set threshold for positive droplet detection by clicking the thresholding button as indicated below. Drag the line representing the threshold above the negative population. Graphs can be copied to image editing software via the button.



9. Copy concentration would be calculated after the setting of threshold. It can be viewed under “Concentration” tab. The numerical data can be exported by clicking “Export CSV”.



10. Data can be transferred via MEDVPN2.

*Transfer and remove your own data from the computer asap if you concern about data confidentiality. Users bear the risk of data leakage storing data in the local computer.