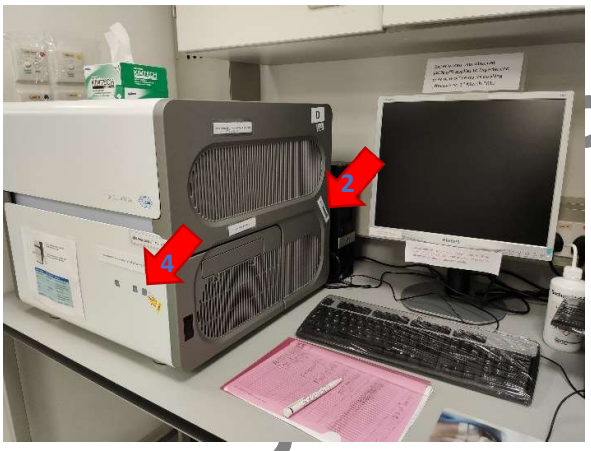
LightCycler480

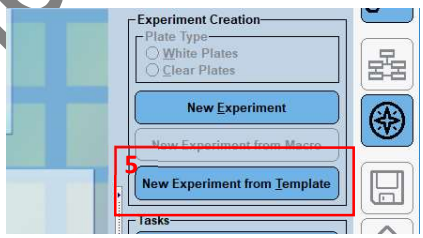
Standard Operating Protocol

I. Initialization of instrument

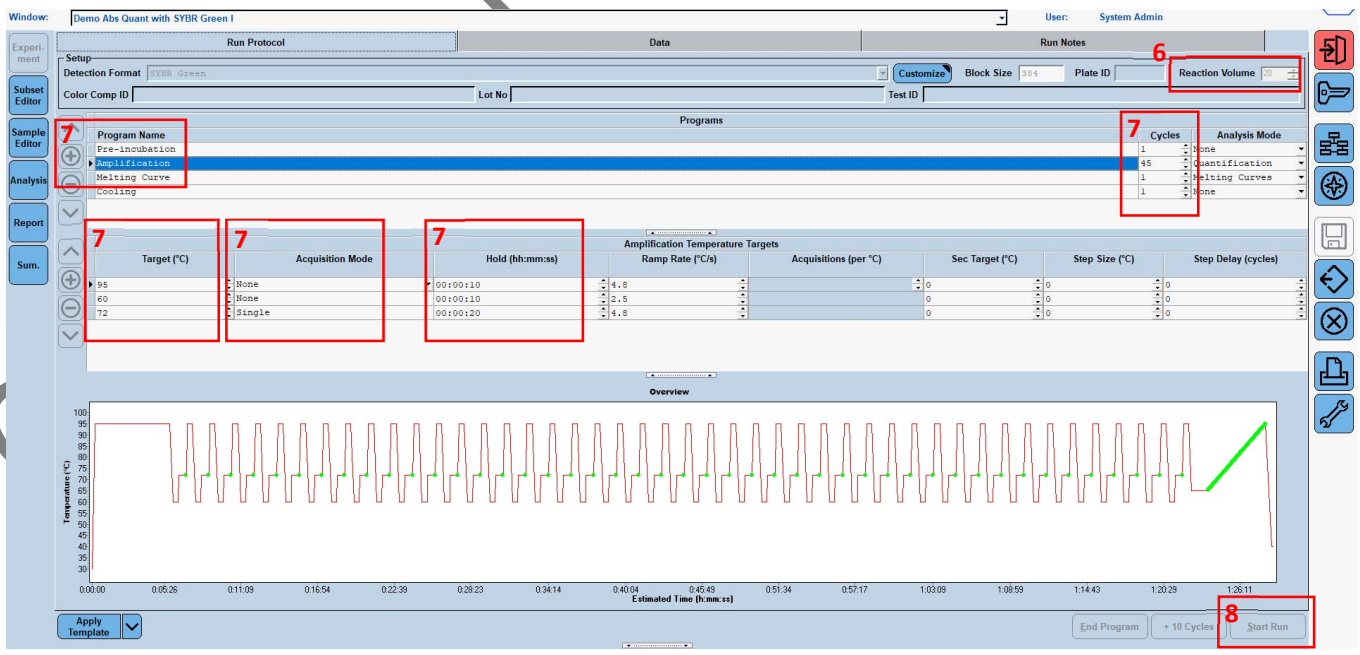


1. Sign in the Log book.
2. Power ON LC480 with the swtich at the right back and controller computer. Login Windows with the account information at the bottom of the monitor of the controller computer.
3. Spin the PCR plate briefly with plate microcentrifuge while the LC480 is initializing. Clean the film with kimwipe.
4. When the system is ready and the left LED turn green, load the PCR plate in the plate holder.

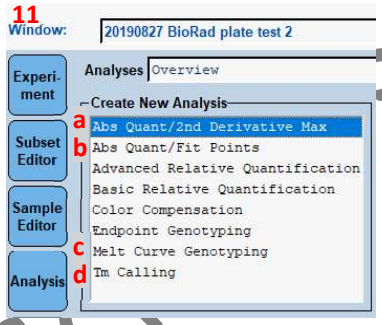
II. Setting of run protocol



5. Login in LightCycler480 software and create an experiment from template with appropriate probe chemistry and filter.
6. Check the filter set in “customize” and update Reaction volume in µl.
7. Input the Program, Cycle Number, Temperature, Acquisition Mode, Hold time.
   * Please make sure the protocol contains acquisition point as indicated in green in the temperature plot.
8. Save the experiment under your own directory and Start the Run.
9. Input Sample Subset and Sample Name if needed.
10. Remove the PCR plate when program completed.



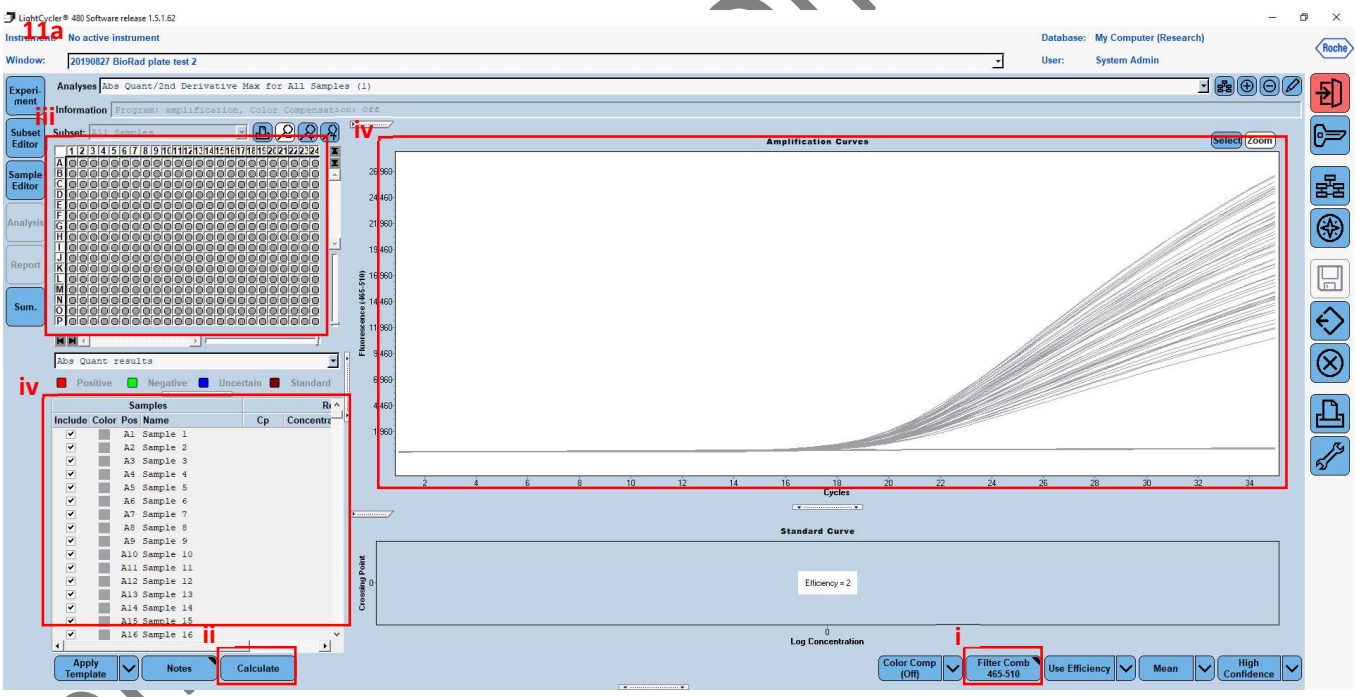
III. Software analysis



11. Click Analysis Tab and select the required mode of analysis.
    a. Determination of Cp
    b. Determination of Ct by threshold, method compatible with other qPCR instruments
    c. Identify genotype by melting curve
    d. Identify melting temperature by melting curve

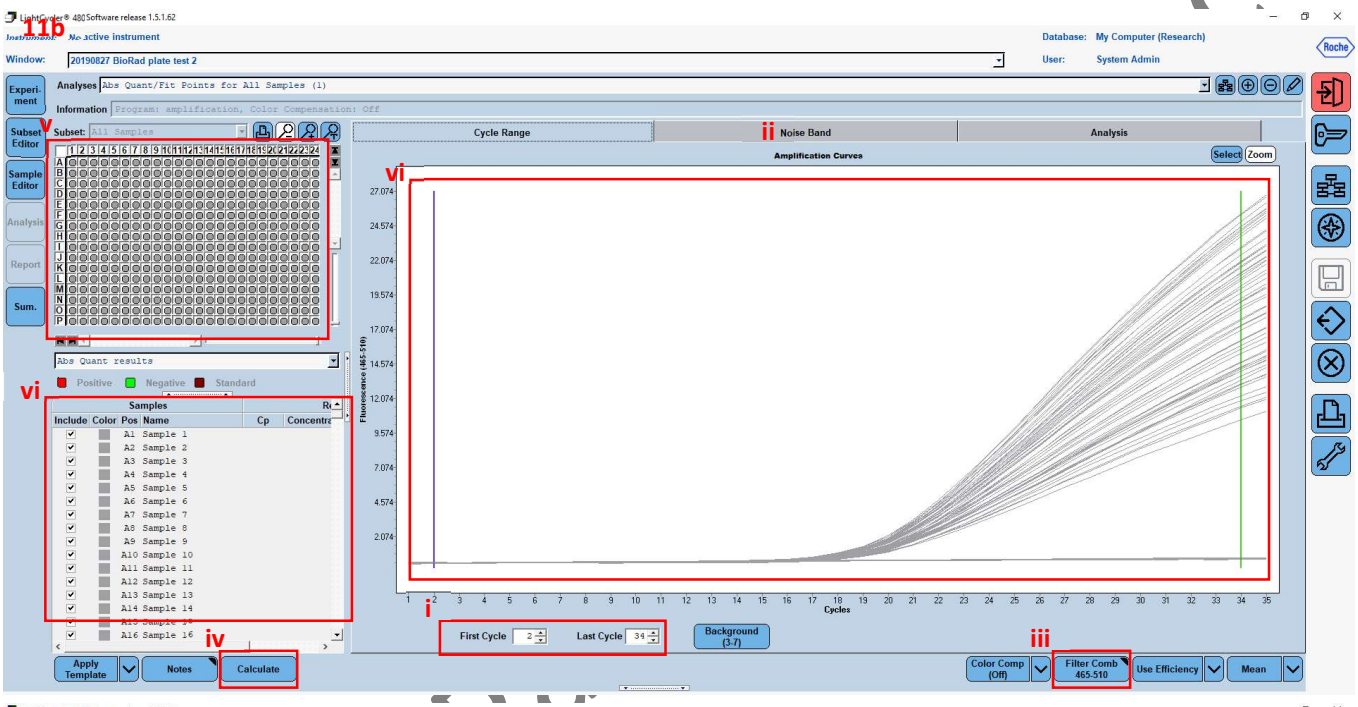
11a. Determination of Cp

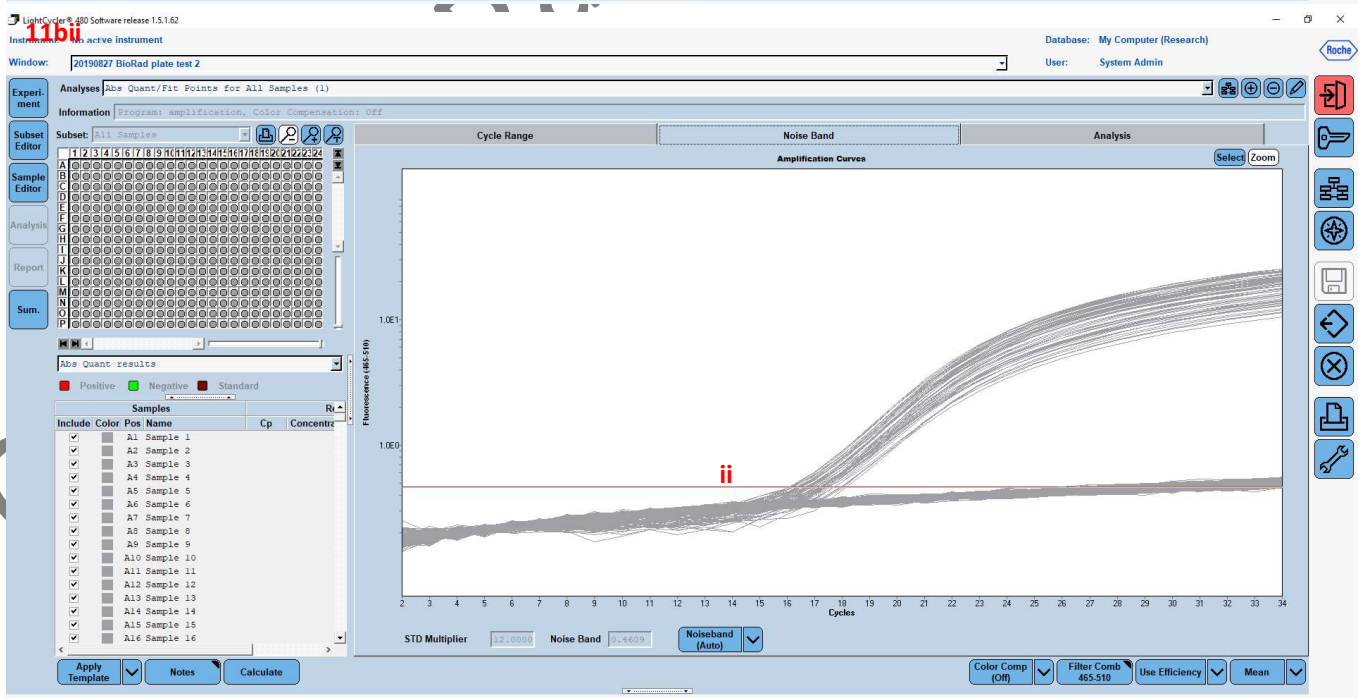
i. Select “Filter Comb” for the appropriate channel of analysis. “465-510” is green channel for SYBR Green experiment.
ii. Click “Calculate” to run the analysis.
iii. Highlight the plate area for the wells of interest to show the corresponding amplification curves.
iv. Right click on the table to export the data including Cp values; graph for raw fluorescent value.



11b. Determination of Ct by threshold

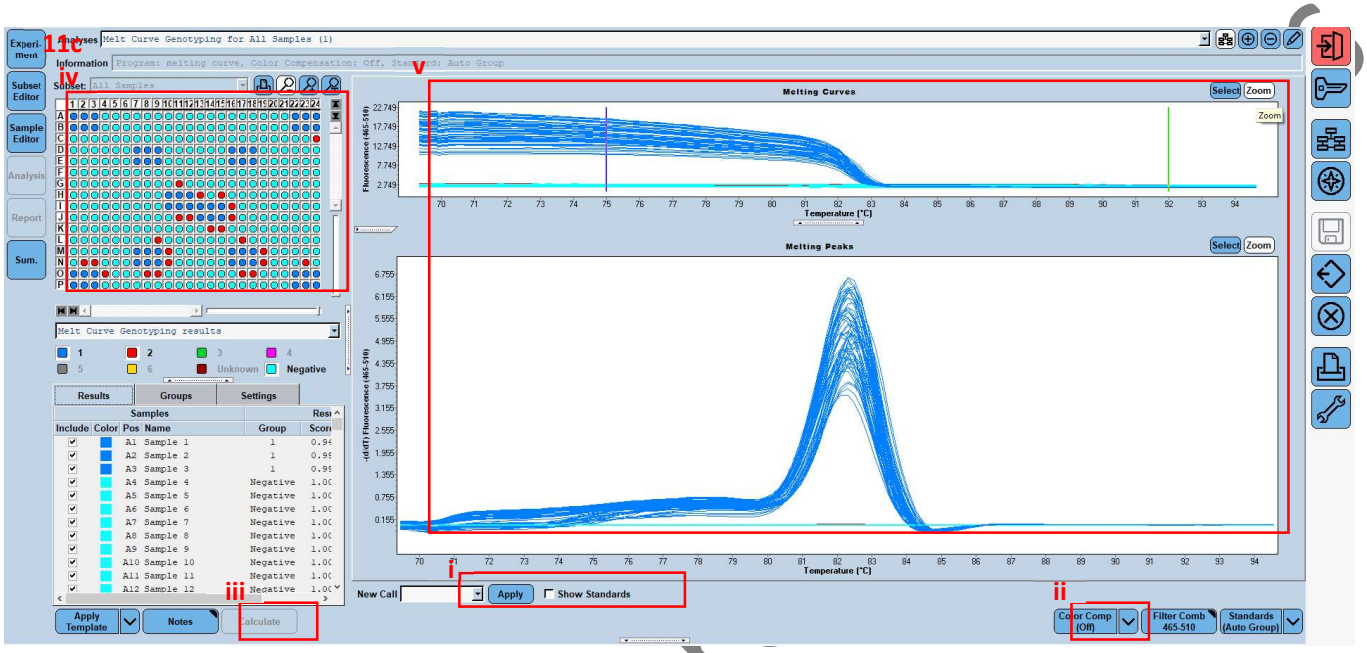
i. Select the “First Cycle” and “Last Cycle” number. (Optional)
ii. Select the Noise Band, set the threshold level by dragging the bar. (Optional)
iii. Select “Filter Comb” for the appropriate channel of analysis
“465-510” is green channel for SYBR Green experiment.
iv. Click “Calculate” to run the analysis.
v. Highlight the plate area for the wells of interest to show the corresponding amplification curves.
vi. Right click on the table to export the data including Ct values; graph for raw fluorescent values.





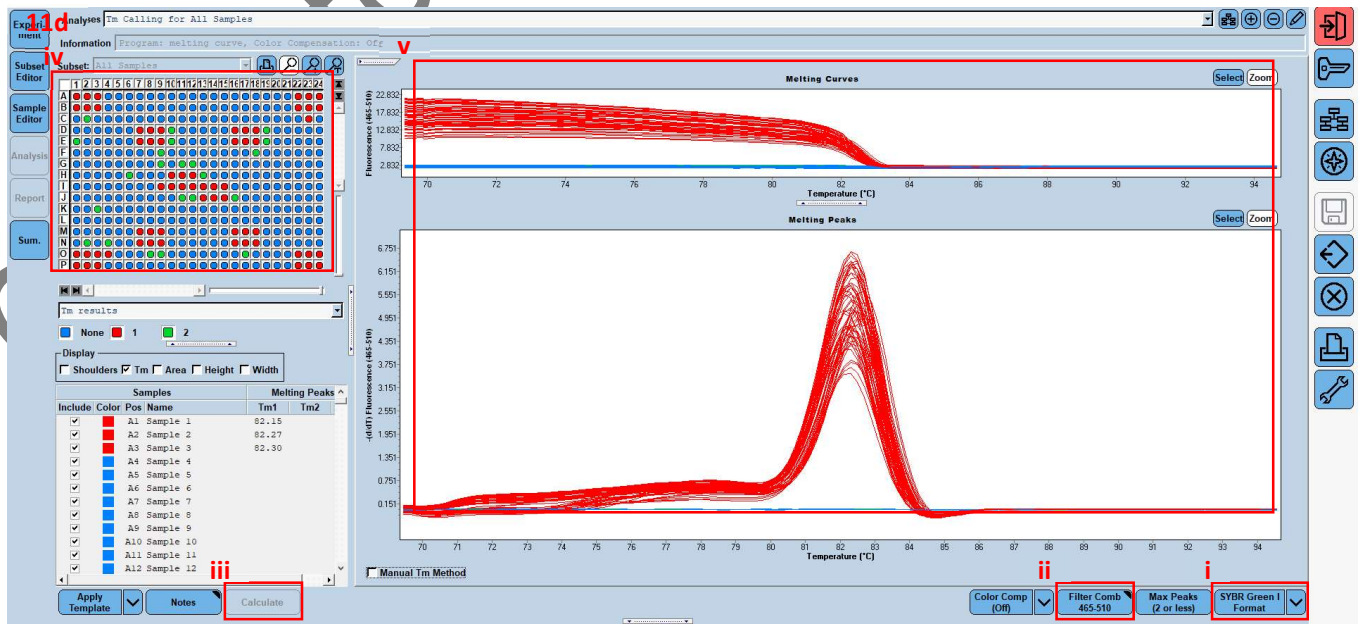
11c. Identify genotype by melting curve

i. Select the “First Cycle” and “Last Cycle” by dragging the bar. (Optional)
ii. Select “Filter Comb” for the appropriate channel of analysis.
“465-510” is green channel for SYBR Green experiment.
iii. Click “Calculate” to run the analysis.
iv. Highlight the plate area for the wells of interest to show the corresponding melting curves and peaks.
v. Right click on the table to export the sample group data; graphs for raw FU and dFU/dT values.



11d. Identify melting temperature by melting curve

i. Select the “SYBR Green I Format”.
ii. Select “Filter Comb” for the appropriate channel of analysis.
“465-510” is green channel for SYBR Green experiment.
iii. Click “Calculate” to run the analysis.
iv. Highlight the plate area for the wells of interest to show the corresponding melting curves and peaks.
v. Right click on the table to export the melting temperature data; graphs for raw FU and dFU/dT values.



12. Transfer the Data by Export  to ONLY the designed data transfer USB flash drive and upload via MEDVPN in the data transfer station.
13. Exit the software and turn off computer and the instrument.
14. Sign the Log book before leaving.

Appendix

3430-40S	SSIBio 384well PCR plate (white) for Biorad C1000/CFX384 Roche LightCycler LC480 qPCR 10pcs/pack
3455-40	SSIBio 96well PCR plate(white) for Roche 10pcs/pack
HSR4805	BioRad 384-Well PCR Plates, clear/white, for Biorad C1000/CFX384 Roche LightCycler LC480 qPCR 25pcs/pack
HSR9905	BioRad 96-well PCR Plates (clear/white) for Biorad C1000/CFX384 Roche LightCycler LC480 qPCR 25pcs/pack
1725122	BioRad iTaq™ Universal SYBR® Green Supermix 1000 x 20µl reactions 10 ml (10 x 1 ml) 2x qPCR mix can be used on any qPCR machine(Bio-Rad Life Tech Roche etc.) Roche LightCycler LC480
1725211	BioRad Sso Fast EvaGreen supermix (with ROX) 5ml (5x 1 mk) 2x qPCR supermix inhibitor tolerant & with better signal than SYBR green can be used on any qPCR machine (Bio-Rad Life Tech Roche etc.) Roche LightCycler LC480
4729749001	Roche 384well PCR plate (white) with sealing film for Roche LightCycler LC480 qPCR 10pcs/pack
MSB1001	Microseal® 'B' Adhesive Seals optical seals for 96-well qPCR plates Pkg of 100 Roche LightCycler LC480
MSC1001	Microseal® 'C' PCR optically clear adhesive Plate Sealing Film for PCR plates 100/pack Roche LightCycler LC480