Roche Lightcycler 480 II

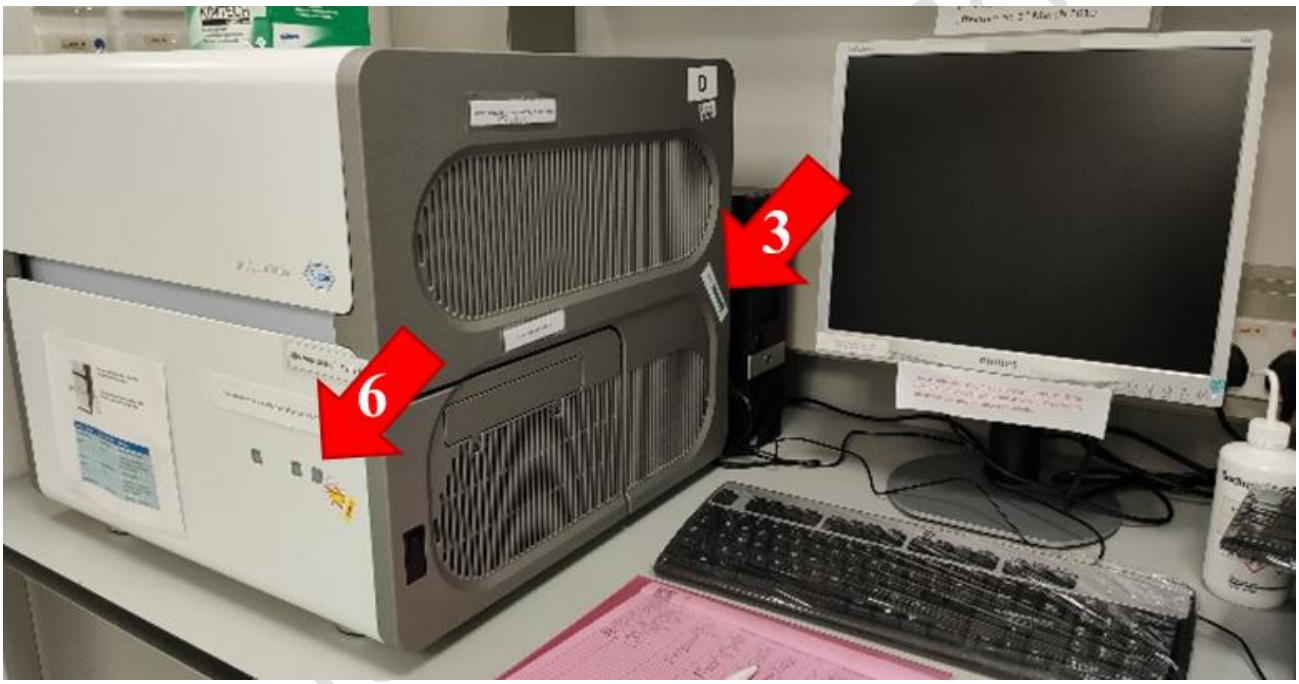
STANDARD OPERATION PROTOCOL

LightCycler480II

Standard Operating Protocol

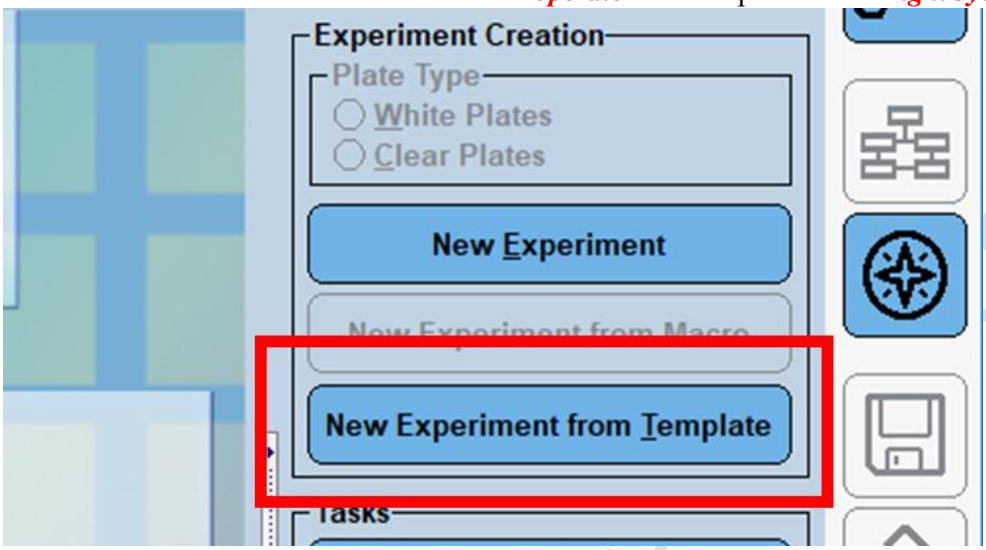
I. Initialization of instrument

1. Prepare the reaction mix in the appropriate plates (*Appendix Table 1*).
2. Sign in the Logbook.
3. 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. Login PPMS tracker.
4. Make sure the Data Sever "Exor 4" is on
5. Spin the PCR plate briefly with plate microcentrifuge while the LC480 is initializing. Clean the film with kimwipe.
6. When the system is ready and the left LED turn green, load the PCR plate in the plate holder.



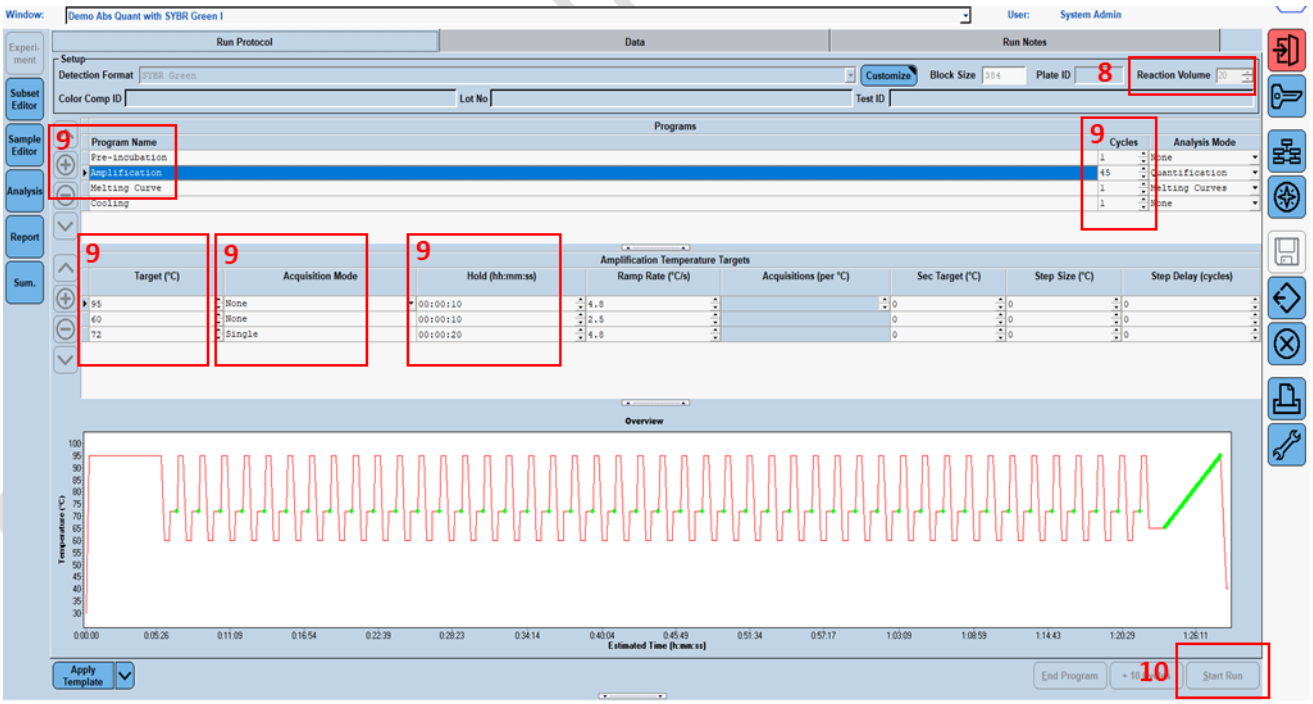
II. Setting of run protocol

7. Login in LightCycler480 software and create an experiment from template with appropriate probe chemistry and filter. The username of universal account is ***operator*** and the password is ***LightCycler480***.



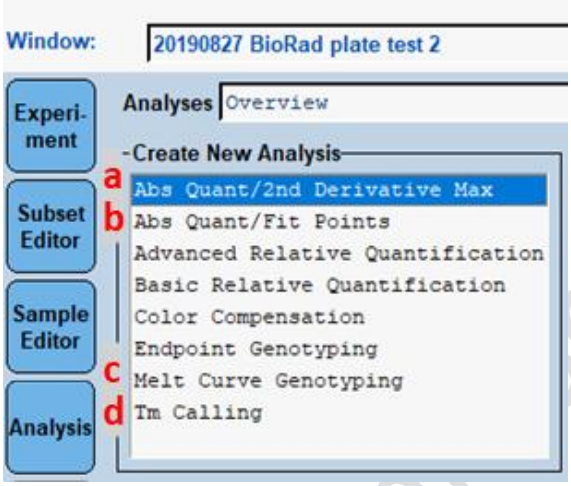
8. Check the filter set in "customize" and update Reaction volume in µl.
9. Input the Program, Cycle Number, Temperature, Acquisition Mode, Hold time.
   * Please make sure the protocol contains acquisition point as indicated in GREEN in the temperature plot. Save the Protocol as template if needed.
10. Save the experiment under your own directory and Start the Run.
11. Input Sample Subset and Sample Name if needed.
12. Remove the PCR plate when program completed. Don't leave the plate more than 15 minute.

**NEVER click "About Run" during the experiment.

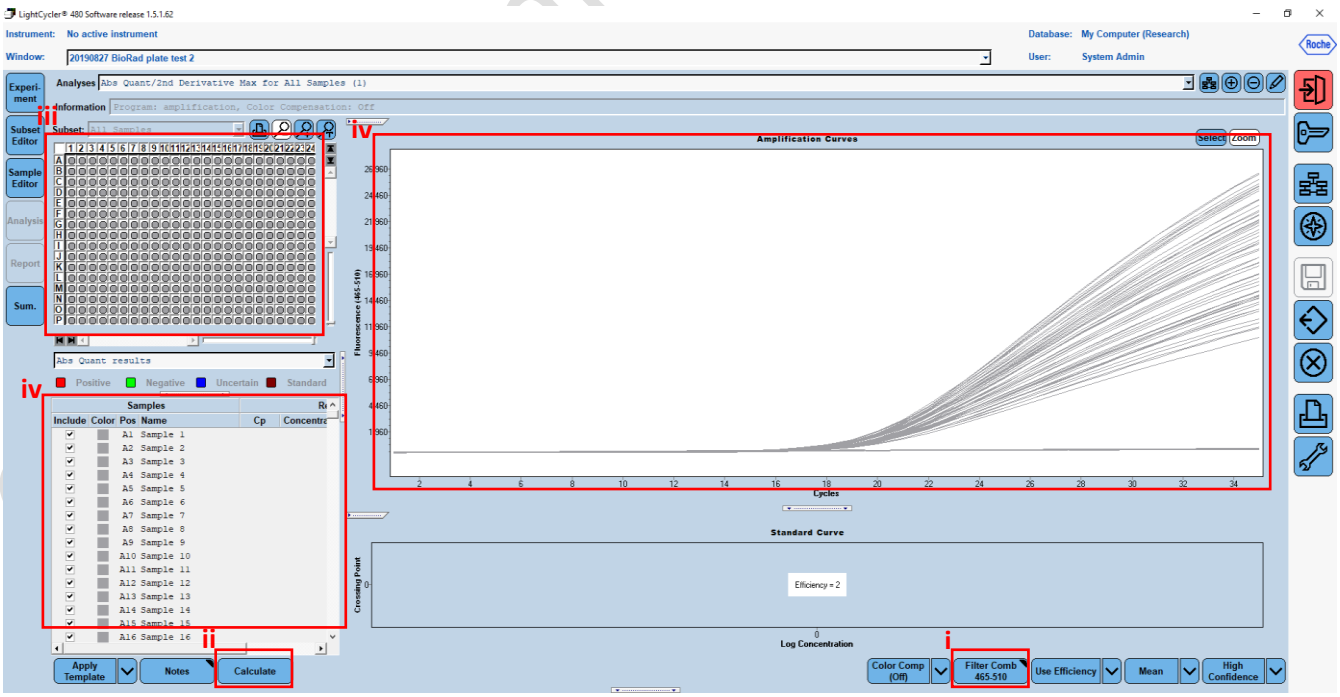


III. Software analysis

13. Click Analysis Tab and select the required mode of analysis.
    a. Determination of Ct by Second Derivative method (step 13a)
    b. Determination of Ct by Fit Point Method (step 13b)
    c. Identify genotype by melting curve (step 13c)
    d. Identify melting temperature by melting curve (For SYBR Green/ Hybridization Probe/Single-labeled Probe) (step 13d)

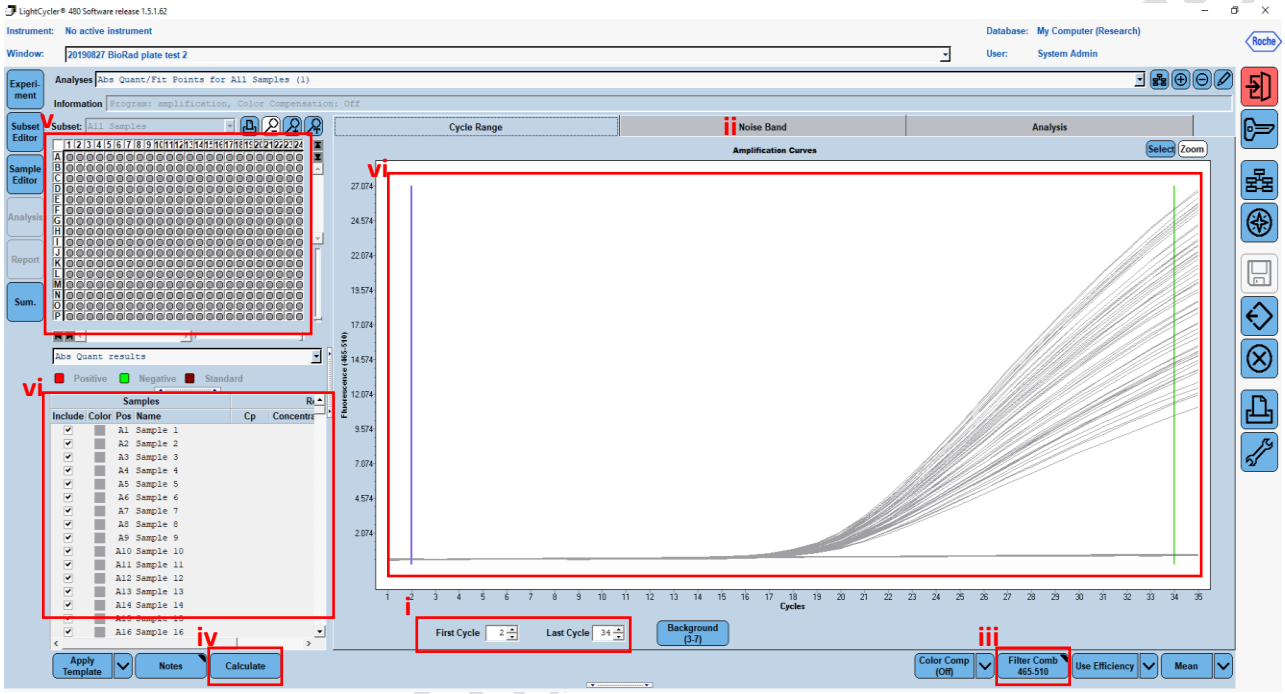


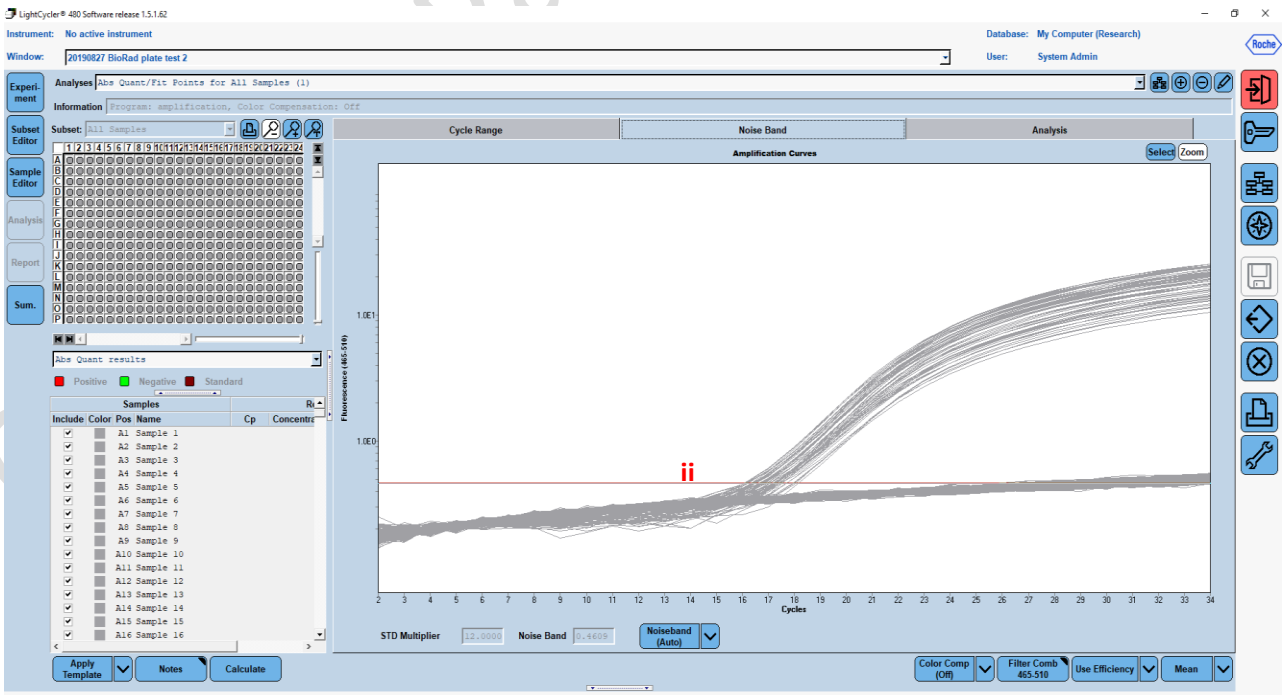
13a. Determination of Ct by Second Derivative Method
    i. Select "Filter Comb" for the appropriate channel of analysis. (*Appendix Table 2*) "465-510" is green channel for SYBR Green experiment.
    ii. Click "Calculate" to run the analysis.
    iii. Highlight the plate area for the wells of interest to show the corresponding amplification curves.
    iv. Right click on the table to export the data including Cp values (in .txt format); graph for raw fluorescent value.



13b. Determination of Ct by Fit Point Method

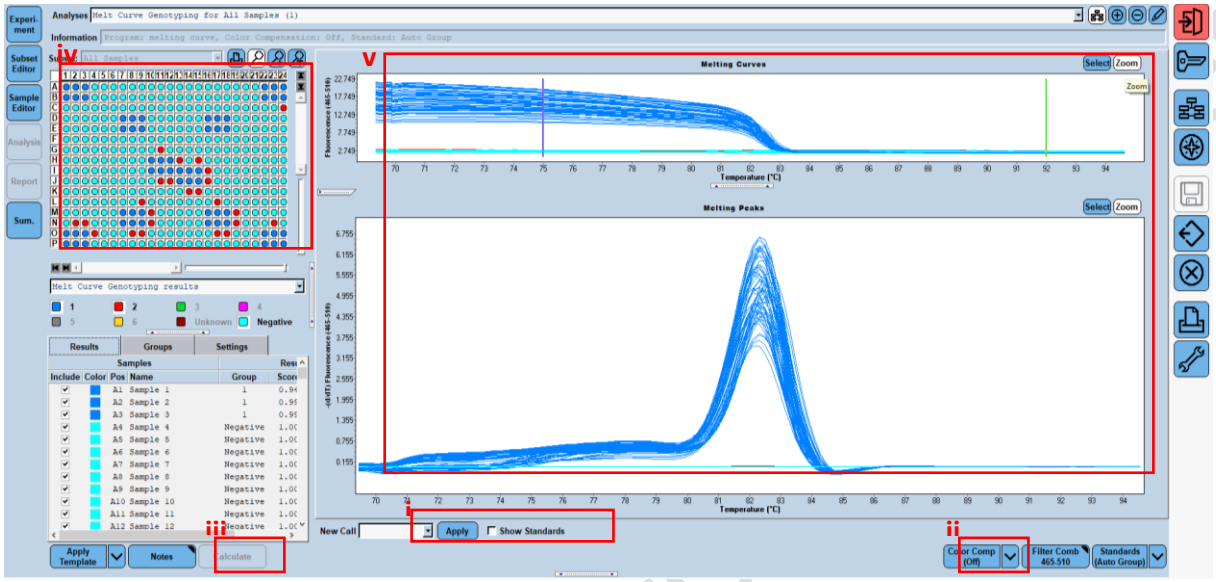
i. Select the "First Cycle" and "Last Cycle" number. (Optional)
ii. Select the Noise Band, set the noise band by dragging the bar. (Optional)
iii. Select the Analysis, set the threshold level by dragging the bar.
iv. Select "Filter Comb" for the appropriate channel of analysis. (*Appendix Table 2*)
"465-510" is green channel for SYBR Green experiment.
v. Click "Calculate" to run the analysis.
vi. Highlight the plate area for the wells of interest to show the corresponding amplification curves.
vii. Right click on the table to export the data including Ct values; graph for raw fluorescent values.





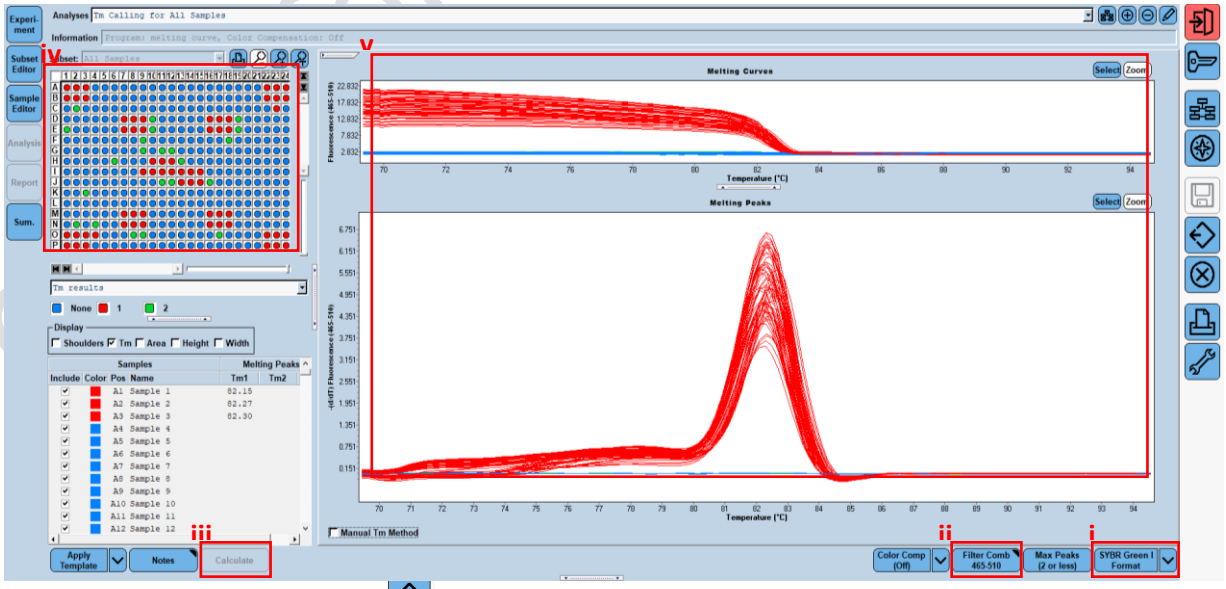
13c. Identify genotype by melting curve

i. Select the "First Cycle" and "Last Cycle" by dragging the bar. (Optional)
ii. Select "Filter Comb" for the appropriate channel of analysis. (*Appendix Table 2*) "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.



13d. Identify melting temperature by melting curve

i. Select the appropriate format for analysis.
ii. Select "Filter Comb" for the appropriate channel of analysis. (*Appendix Table 2*) "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.



14. Transfer the Data by Export to data transfer sever.
15. Exit the software and log out PPMS tracker.
16. Turn off the instrument.

17. Sign the Log book before leaving.

Appendix

Table 1. Plates, films and reagent that is compatible to LC480

Type	Catalog Number	Brand	Item Description
Plate	3430-40S	SSIBio	384well PCR plate (white)
	HSR4805-25	BioRad	384well PCR Plates (clear/white)
	HSR9905-25	BioRad	96well PCR Plates (clear/white)
Film	MSB1001	BioRad	Microseal® 'B' Adhesive Seals optical seals for 96-well qPCR plates
	MSC1001	BioRad	Microseal® 'C' PCR optically clear Pressure-Activated Adhesive Plate Sealing Film
	PESTSTXL	Plate Seal	Clear Polyester film
	PPO-100	Plate Seal	Pressure-Activated Adhesive film
Reagent	RR036A	TaKaRa	PrimeScript™ RT Master Mix (Perfect Real Time)
	RR037A	TaKaRa	PrimeScript™ RT reagent Kit (Perfect Real Time)
	RR047A	TaKaRa	PrimeScript™ RT reagent Kit With gDNA Eraser (Perfect Real Time)
	RR086A	TaKaRa	One Step SYBR® PrimeScript™ RT- PCR Kit II (Perfect Real Time)
	RR390A	TaKaRa	Premix Ex Taq™ (Probe qPCR)
	RR420A	TaKaRa	TB Green™ Premix Ex Taq™ (Til RNaseH Plus)
	RR820A	TaKaRa	TB Green™ Premix Ex Taq™ II (Til RNaseH Plus)
	1725122	BioRad	iTaq™ Universal SYBR® Green Supermix
	1725211	BioRad	Sso Fast EvaGreen supermix (with ROX)

Table 2. Filter setting for different fluorophores

Fluorophore	Excitation Filter	Emission Filter	Detection Format
LightCycler® Cyan 500	440	488	Hydrolysis Probes (Reporter)
SYBR Green I	465	510	SYBR Green I
Fluorescein (Fluos / FAM)	465	510	Hydrolysis Probes (Reporter) HybProbe Probes (Donor)
	498	580	SimpleProbe Probes Hydrolysis Probes (Reporter, only in combination with Cyan 500)
VIC / HEX / Yellow555 / Joe	533	580	Hydrolysis Probes (Reporter)
LightCycler® Red 610	533 498	610 610	Hydrolysis Probes (Reporter) HybProbe Probes (Acceptor)
LightCycler® Red 640	498	640	HybProbe Probes (Acceptor)
Cy5 / Cy 5.5 / LightCycler® Red 705	618 498	660 660	Hydrolysis Probes (Reporter) HybProbe Probes (Acceptor)