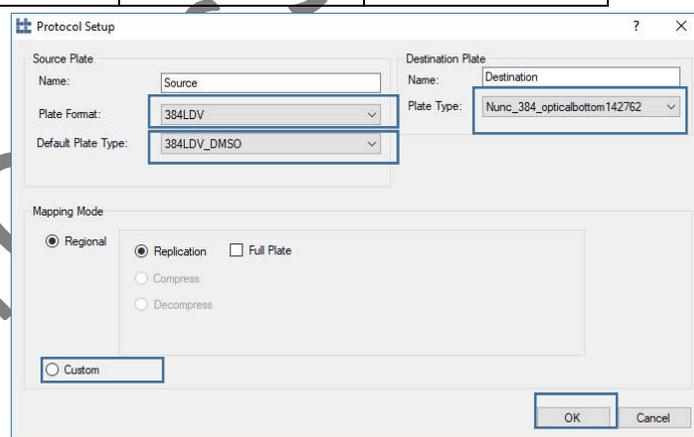


Preparing Reaction plate with Echo550

- Purchase Echo sources plate (PP-0200, LP-0200) and seal with film. Purchase Roche 384 PCR plate. PP-0200 (for mastermix, max. 65 μ l, dead volume of 15 μ l (~8 x 2 μ l reactions) LP-0200 (Low dead volume, for probe/primer or DNA/RNA template max. 14 μ l, dead volume of 3 μ l)
- Prepare the master mix in microcentrifuge tube with (n+8)*1.1 reactions according to below table. Mix well, spin and put on ice/dark.

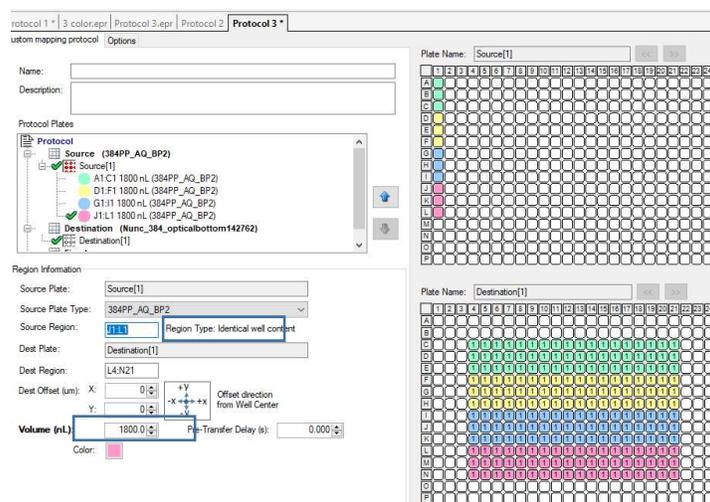
(μ l)	1 well	27 wells	54wells	54wells (pooling residual)	81well (pooling residual)
No. of reactions		(27+8)*1.1=38.5	(54+16)*1.1=77	(54+8)*1.1=68	(81+8)*1.1=98
2x SybrGreen MasterMix	1	38.5	77	68	98
20x Primermix (F/R)	0.1	3.85	7.7	6.8	9.8
H2O	0.7	26.95	53.9	47.6	68.6
Total	1.8	69.3	138.6	122.4	176.4
DNA/RNA template	0.2	0.2	0.2	0.2	0.2

- Use LP-0200 LDV plate for transferring primer mix separately if many primer to be used.
- Launch Echo Plate Reformat v1.6, select "Connect"
- Protocol Setup for Master mix
- For Source Plate, select 384PP or 384LDV in Plate Format. Select in type for **AQ SP2** fluid calibration. For Destination Plate, select the receiver plate type as Roche 384 PCR plate. In mapping mode, select Custom



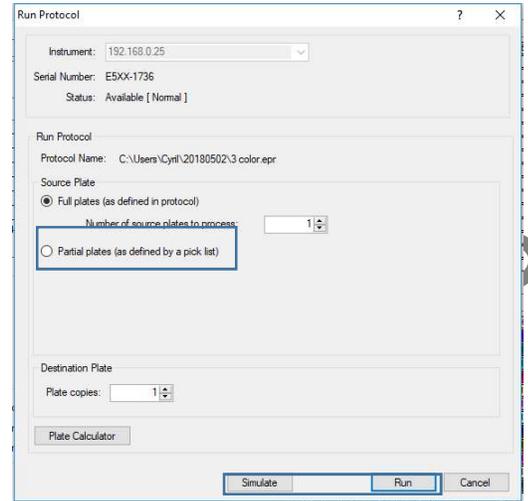
Transferring mix of master mix/primer/H₂O

- Create a new protocol.
- Select the well(s) in the sources plate (top) which contains the fluid to be transfer. Check the box for "Treat as identical well content" if more than one sources well containing the same fluid can be used.
- Select the well(s) in destination plate to receive the transfer volume.
- Input the volume (multiples of 2.5nl, e.g.1800nl) to be transferred and click Add Region for one destination well or Replicate Region for multiples wells.
- Repeat the procedure to add other transfer step.
- Save the Protocol in C:\Users\PI's Name\User Name. Click on Start button.
- Use Simulate to verify the protocol was set up properly. Adjust the delays in Animation if necessary. Check whether the volume and event counts (by moving mouse pointer over) in source and destination wells are as expected.



Delays: Animation: 15 ms Plate Prompt: 5.0 s

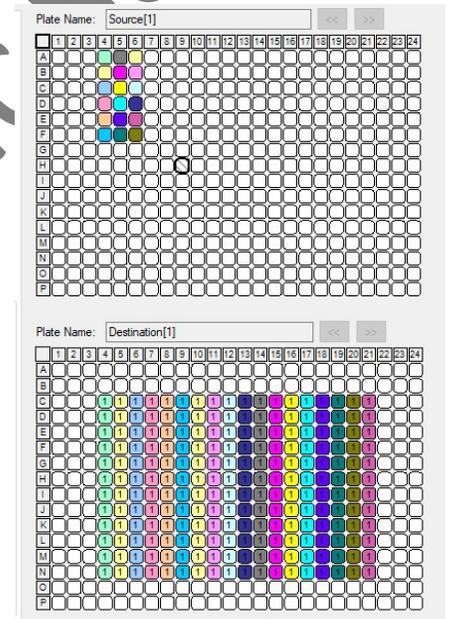
14. Close the Simulation tap. Amend the protocol for any issues being identified and repeat the simulation again if needed.
15. Add master mix into Echo plate and centrifuge at 4000rpm, 30s-20mins to remove trapped air bubble.
16. Clean the bottom of the plate with Kimwipe and click Start in Echo software.



17. Run the protocol again. Click Run and Start in subsequent page.
18. Change the adapter if required by the alert message. Put Echo plate on the reservoir plate gripper Click Okay and put PCR plate in the receiver plate gripper. Click Okay to start the transfer.
19. During the transfer, prepare the protocol for transfer of DNA/RNA template
20. Remove the plates from Echo550 and put a cover on plate or put the plate on ice if needed.
21. Go to Report folder to check the transfer record and make up for any transfer exception.
22. Repeat the procedure and Import a pick list if numerous different primers/probes (not mixed with master mix) are to be transferred (select **AQ BP** fluid calibration).

Transferring mix of DNA/RNA template

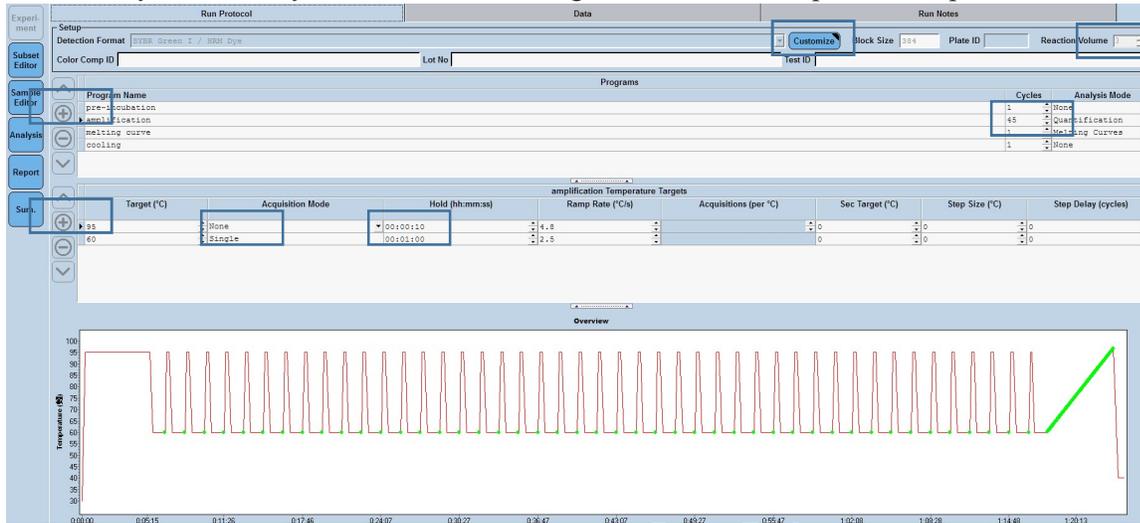
23. Create a new protocol.
24. Change Type to **AQ BP** fluid calibration.
25. Select the well(s) in the sources plate (top) which contains the fluid to be transfer.
26. Select the well(s) in destination plate to receive the transfer volume.
27. Input the volume (multiple of 2.5nl, e.g.200nl) to be transferred and click Add Region or Replicate Region. Use transpose if needed.
28. Repeat the procedure to add other transfer step.
29. Save the Protocol in C:\Users\PI's Name\User Name. Click on Start button.
30. Import a pick list is numerous different DNA/RNA samples (not mixed with master mix) are to be transferred in complicated layout.
31. Check the Simulation and Start the transfer as last session.
32. Go to Report folder to check the transfer record and fix or make up for any transfer exception.
33. Seal the plate with optical film immediately. Keep the plate in ice if needed.



34. Labcyte Echo Transfer Report
35. Sign on the logbook before proceed to qPCR. Sign on the logbook before proceed to qPCR.

qPCR in LightCycler480

36. Power ON LC480 with the switch at the right back and controller computer. Login with “operator” and “LC480”
37. Spin the PCR plate briefly with plate microcentrifuge while the LC480 is initializing. Clean the film with kimwipe.
38. When the system is ready and the left LED turn green, load the PCR plate in the plate holder.



39. Login in LightCycler480 software and create an experiment from template with appropriate probe chemistry and filter.
40. Check the filter set in “customize” and update Reaction volume to 3µl.
41. Input the Program, Cycle Number, Temperature, Acquisition Mode, Hold time.
42. Save the experiment under your own directory and Start the Run.
43. Input Sample Subset and Sample Name if needed.
44. Remove the PCR plate when program completed. Transfer the Data by Export to ONLY the designed data transfer USB flash drive.
45. Exit the software and turn of computer and LC480.
46. Sign in Log book before leave.
47. Return the USB flash drive after data transfer.