Echo650 Liquid Handler

Standard Operating Protocol

I. Preparation

1. Use Echo qualified source plate (PP-0200, LP-0200).

	PP plate (#PP-0200)	**LDV plate (#LP-0200)**
Vol range	15-65 uL	3-12 uL

***Depends on the liquid property, see Appendix Table 1.*

2. Prepare the sample and load adequate volume (include dead volume) to the Echo-qualified source plate.
3. Centrifuge the plate at 1500 *g* for 5 min to remove trapped air bubble.
4. Clean the bottom of the plate with Kimwipe.

II. Initialization

5. Log in PPMS tracker.
   Launch the software *Echo Plate Reformat v1.8.2*, select ***Connect***.
6. In Protocol setup, select appropriate options in (a) source plate, (b) destination plate and (c) mapping mode.

(a) <u>Source Plate</u>

Plate format: select ***384PP*** or ***384LDV***

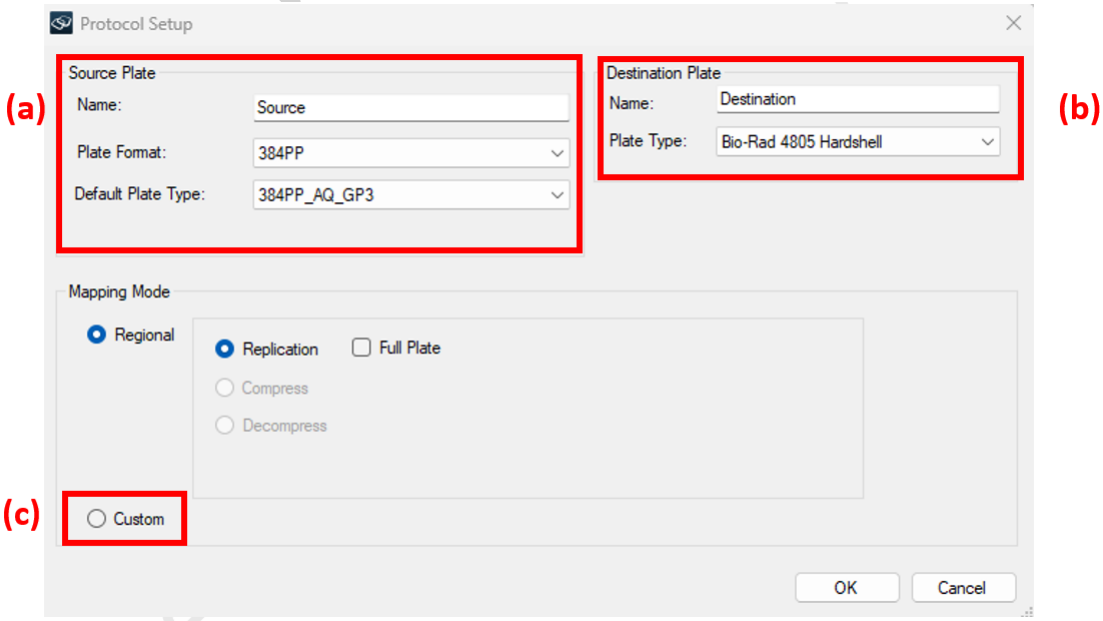
- Default Plate Type: select appropriate calibration protocol based on liquid properties. (Check ***Appendix, Table 1***)

(b) <u>Destination Plate</u>

- Plate type: select Biorad-4805 Hardshell

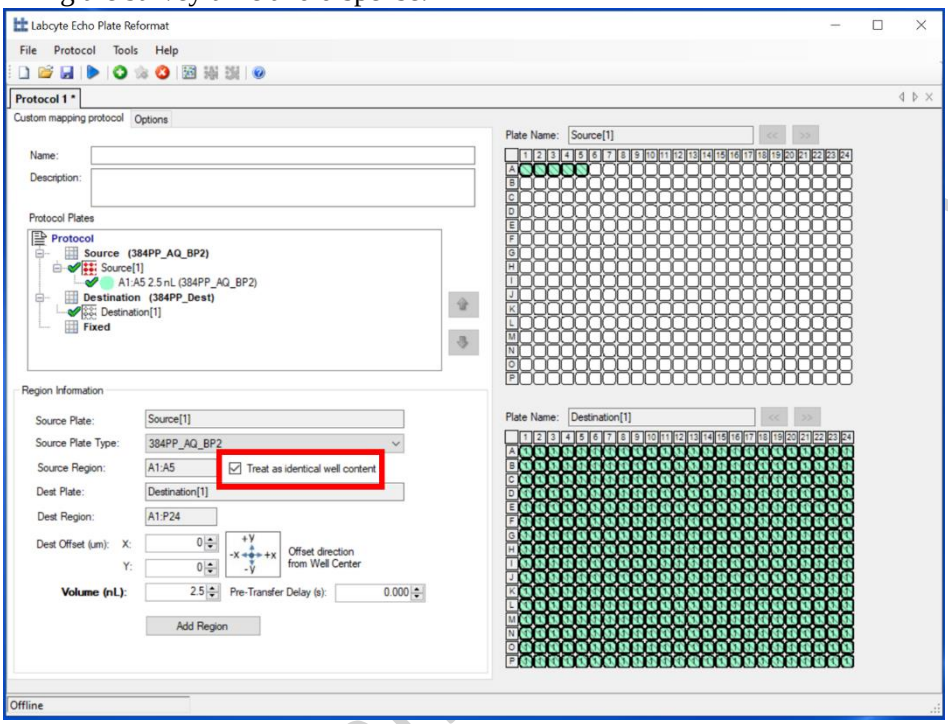
(c) <u>Mapping mode</u>

- select Custom

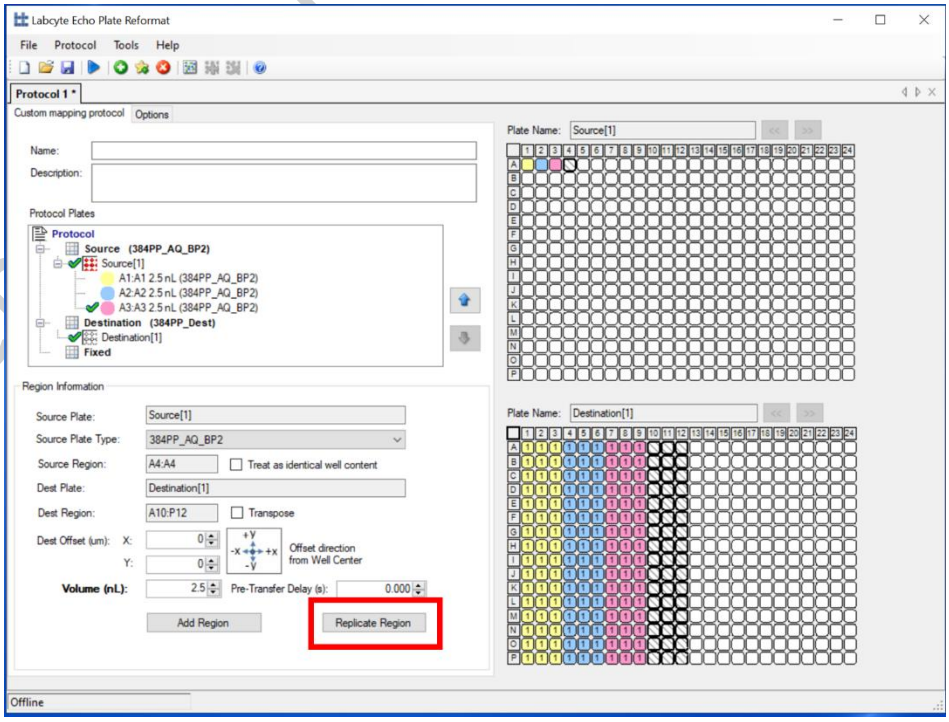


III. Transferring liquid

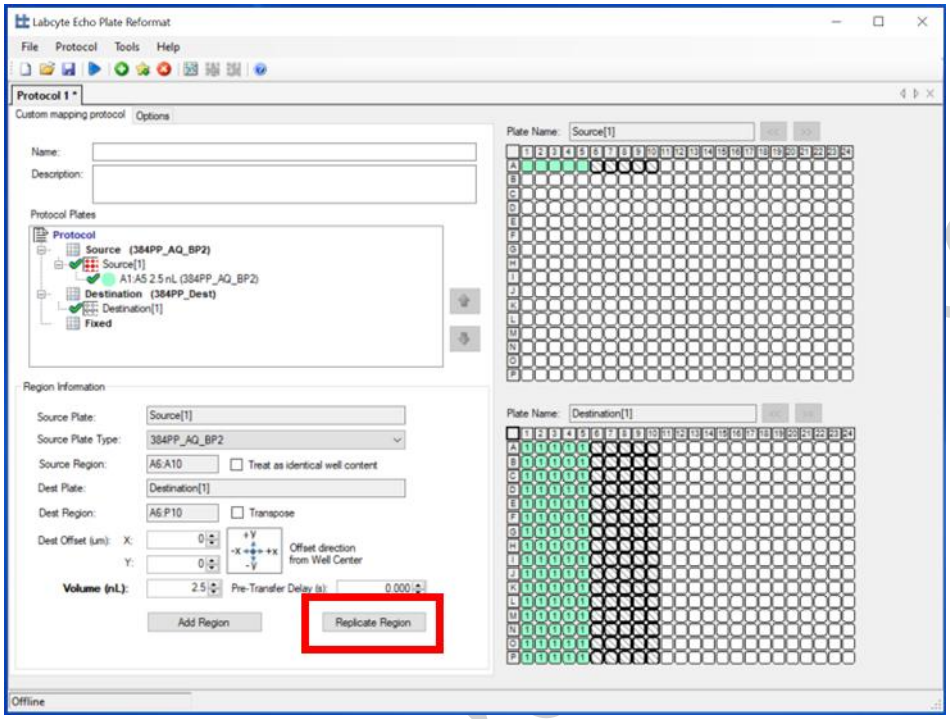
7. Create a new protocol.
8. Select the well(s) in the sources plate which contains the fluid to be transfer and select the well(s) in destination plate to receive the transfer volume.
    a. If multiple wells have same content (e.g. master mix), check the box ***Treat as identical well content*** for optimizing the survey time and dispense.



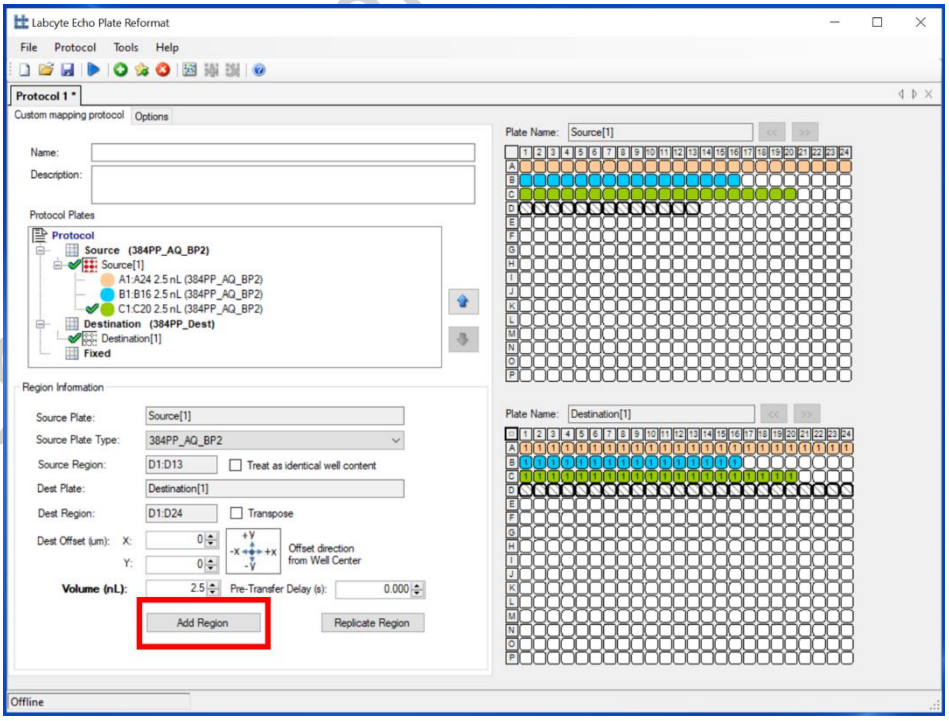
    b. If a sample from one well will be dispensed to multiple wells (e.g., cDNA), select ***Replicate Region***.



c. If multiple dispenses of samples from A1 to A5 for 16 times (i.e. across the whole column), each at A1 to P1 and A5 to P5 respectively, select ***Replicate Region***.

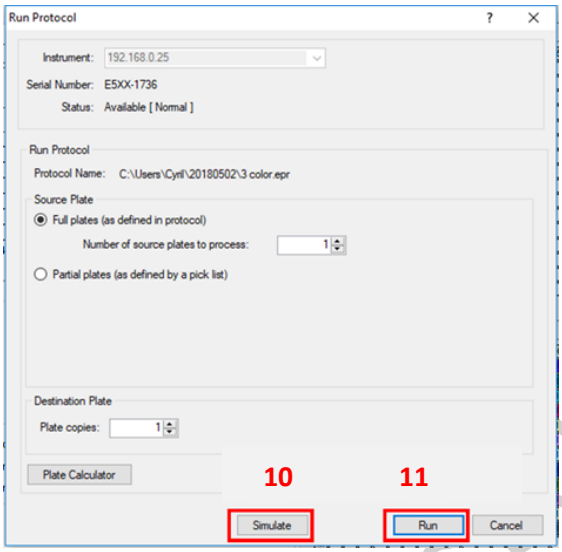


d. If samples will be replicated to the destination plate, select ***Add Region***.



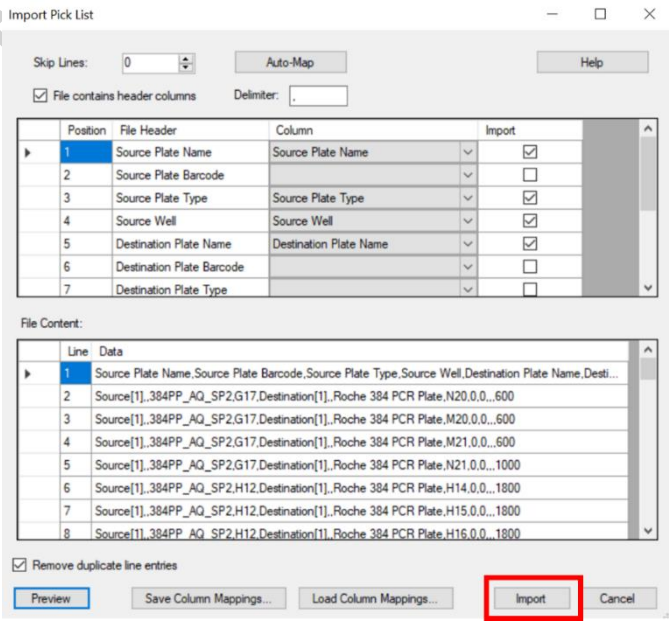
9. Input the volume to be transferred.
Save the Protocol in D:\Users\Department\PI's Name\User Name. Click on ***Start*** button.

10. **(Optional)** Use ***Simulate*** to verify the protocol was set up properly. Adjust the delays in Animation if necessary. Skip the animation if needed. Check whether the volume and event counts in source and destination wells are as expected.



Close the Simulation tap. Amend the protocol for any issues being identified and repeat the simulation again if needed.

11. Click Start in Echo Reformat and click ***Run***.
12. Put source plate on the reservoir plate gripper. Click ***OK***
13. Put the destination plate in the receiver plate gripper. Click ***OK*** to start the transfer.
    When the transfer finishes, remove the plates from Echo650 and put a cover on it or put it on ice if needed. Click ***OK*** after removal of each plate.
14. Upon transfer completion, if there are no exceptions, Click "OK", otherwise there is exceptions, Click "Show Exceptions Report"
    Go to "Echo Plate Reformat" folder and check the transfer record Make up for any transfer exception by importing a pick list (by editing the exception report) to transfer the makeup volume. To import excel file for transferring, click a new dispense file and click ***File > Import Region Definition***. Choose the amended excel file. Click ***Import***.



15. Close Echo Reformat and log out PPMS tracker.
16. Sign on the logbook.

IV. Appendix

Table 1. Calibration Protocol based on liquid properties.

LDV plate

Calibration	Summary	Example fluids	Vol (µL)
384LDV_DMSO	384-plate, DMSO	70-100% DMSO	2.5 - 12
1536LDV_DMSO	1536-plate, DMSO		1 – 5.5
384LDV_AQ_B2	384-plate, buffer	Buffer without surfactant E.g. Oligos, cDNA, RNA, Guide-RNA, gene fragments and libraries	3 – 12
384LDV_AQ_P2	384-plate, protein	Buffers without surfactant containing proteins E.g. Enzymes/antibodies	6 – 16

PP plate

Calibration	Summary	Example fluids	Vol µL)
384PP_DMSO2	DMSO	70-100% DMSO	15 – 65
384PP_AQ_SP2	surfactant / protein	Buffers containing surfactant E.g. PCR mixes, lysis buffer, serum and plasma	15 – 65
384PP_AQ_GP3	buffer / protein	Reagents without surfactant E.g. Small molecules in water or buffer DNA/ RNA/ Plasmid/ Primers in TE/ water Proteins in water, buffer or glycerol storage solution Cell culture medium	15 – 65
384PP_AQ_CP	crystallography reagents, salts, etc.	Reagents w/o surfactants. MPD/PEGS/Osmotic solutions.	20 – 50