<u>Echo550 Liquid Handler</u>

Standard Operating Protocol

I. Preparation

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

	PP plate (#PP-0200)	LDV plate (#LP-0200)	1
Vol range	15-65 uL	3-12 uL	
**Depends or	ı the liquid property, see Appendix Ta	ble 1.	

**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 4000rpm for 1 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 v1.6*, select *Connect*. Make sure the software *Echo Liquid Handler* is on. If not, please contact staff for assistance.

6. In Protocol setup, select appropriate options in (a) source plate, (b) destination plate and (c) mapping mode.

(a) Source Plate

- Plate format: select 384PP or 384LDV.
- > Default Plate Type: select appropriate calibration protocol based on liquid properties. (Check *Appendix, Table 1*)
- (b) **Destination Plate**
- Plate type: select appropriate destination plate type (for SSIBio, Bio-rad and Roche plate, choose *Roche 384 PCR plate*).
- (c) <u>Mapping mode</u>
- select Custom.

oouroe i late			Destination Pla	ate
Name:	Source		Name:	Destination
Plate Format:	384LDV	~	Plate Type:	Nunc_384_opticalbottom1427
Default Plate Typ	e: 384LDV_DMSO	~		
Regional	Replication Full Plate			
	○ Compress			
	O Decompress			

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. mastermix), 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*.

	Labcyte Echo Plate Reformat	-		×		
	File Protocol Tools Help					
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	ratacal 1 *			4 Þ ×		
4	ustom mapping protocol Options					
	Plate Name: Source[1] <<<					
	Name:	1222324				
	Description:					
	Protocol Plates	-HHH				
		1000				
	B→ B Source (384PP_AQ_BP2) B→ Source (384PP_AQ_BP2) B→ Source (384PP_AQ_BP2) B→ Source (384PP_AQ_BP2) B→ Source (384PP_AQ_BP2) B→ Source (384PP_AQ_BP2)					
	A1:A1 2.5 nL (384PP_AQ_BP2)	2000				
	A2A2 25nL (384PP A0, BP2)	-666				
	Destination (384PP_Dest)	2000				
		5000				
		2000				
	Region Information					
	Source Plate: Destination[1]	25				
	Source Plate Type: 384PP_AQ_BP2	122 23 24				
	Source Region: A4:A4 Treat as identical well content.	2000				
	Dest Plate: Destination[1]	5000				
	Dest Region: A10:P12 Transpose	1000				
		5000				
	V Ofeet direction	-999				
		1000				
	Volume (nL): 2.5 Pre-Transfer Delay (s): 0.000 K111(1)(11111(1)) L111(1)(1111(1)) L111(1)(1111(1)) L111(1)(1)(1)(1)(1)(1)(1) L111(1)(1)(1)(1)(1)(1)(1)(1)(1)(1)(1)(1)(-666				
	Add Region Replicate Region	1000				
		1000				
0	fline					

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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*.

otocol 1 *				4 Þ ×
International and a second sec	Options b84PP_AQ_BP2) 11 A5 25 nL (384PP_AQ_BP2) 13 14 15 17 18 19 19 10 11 384PP_AQ_BP2 A6A10 10 Treat as identical well content	9	Pate Name Source[1] 1000000000000000000000000000000000000	4 P ×
Dest Plate: Dest Region: Dest Offset (um): X. Y: Volume (nL):	Destination[1] A6:P10 0:0:1 Transpose]		

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

Labcyte Echo Plate Re	tormat	-
File Protocol Tool	s Help	
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Protocol 1 *		
Custom mapping protocol	Options	7 · · · · · · · · · · · · · · · · · · ·
Name		Plate Name: Source[1]
Description:		
Development.		
Protocol Plates		
Protocol		
Source (184PP_AQ_BP2) 1]	
- A1:	A24 2.5 nL (384PP_AQ_BP2)	
C1	C20 2.5 nL (384PP_AQ_BP2)	
Destinatio	n (384PP_Dest)	
- Fixed		
Region Information		
	Courses[1]	Plate Name: Destination[1]
Source Plate:		
Source Plate Type.	D1D12	
Dest Plate:	Destination[1]	
Dest Pagien		
Dest negion.		
Dest Offset (um): X:	-x ++x Offset direction	
Y:		
Volume (nL):	2.5 Pre-Transfer Delay (s): 0.000	
	Add Region Replicate Region	

9. Input the volume to be transferred.

Save the Protocol in C:\Users\PI's Name\User Name. Click on *Start* button.

10. 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.

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un Protocol	?	×	
Instrument: 192.168.0.25 Senal Number: ESXX-1736 Status: Available [Normal]			
Pun Protocol Protocol Name: C-\Usera\Cyrt\20180502\3 color.epr			
			~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
Destination Plate Plate Copies: 10 Plate Calculator 12. 14. Simulate Pun	Canc	:el	ence

- 11. Close the Simulation tap. Amend the protocol for any issues being identified and repeat the simulation again if needed.
- 12. Click Start in Echo software and click *Run*.
- 13. Change the adapter if required by the alert message.
- 14. Put source plate on the reservoir plate gripper. Click **OK**
- 15. Put the destination plate in the receiver plate gripper. Click *OK* to start the transfer. When the transfer finishes, remove the plates from Echo550 and put a cover on it or put it on ice if needed. Click *OK* after removal of each plate.
- 16. Go to "Labcyte Transfer Report" 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 Defination*. Choose the amended excel file. Click *Import*.

	File contair	ns header columns Delir	miter:		
	Position	File Header	Column		Import
•	1	Source Plate Name	Source Plate Name	~	$\checkmark$
	2	Source Plate Barcode		~	
	3	Source Plate Type	Source Plate Type	~	
	4	Source Well	Source Well	~	$\checkmark$
	5	Destination Plate Name	Destination Plate Name	~	$\checkmark$
	6	Destination Plate Barcode		~	
	-				
File C	7 ontent:	Destination Plate Type		~	
File C	7 ontent: Line D 1 Sc	Destination Plate Type lata purce Plate Name,Source Plate	e Barcode,Source Plate Type,Sour	ce Well,De	stination Plate I
File C	7     ontent:     Line     1     2	Destination Plate Type lata surce Plate Name.Source Plate nurce[1]384PP_AQ_SP2.G17	e Barcode, Source Plate Type, Sour 7.Destination[1], Roche 384 PCR P	ce Well,De	stination Plate
File C	7 ontent: Line D 1 Sc 2 Sc 3 Sc	Destination Plate Type lata surce Plate Name,Source Plate surce[1]384PP_AQ_SP2,G17 surce[1]384PP_AQ_SP2,G17	e Barcode, Source Plate Type, Sour (Destination[1], Roche 384 PCR P (Destination[1], Roche 384 PCR P	ce Well,De late,N20,0	stination Plate
File C	7 ontent: Line D 2 Sc 3 Sc 4 Sc	Destination Plate Type lata nurce Plate Name,Source Plate nurce[1]384PP_AQ_SP2,G17 nurce[1]384PP_AQ_SP2,G17 nurce[1]384PP_AQ_SP2,G17	a Barcode, Source Plate Type, Sour ,Destination[1], Roche 384 PCR P ,Destination[1], Roche 384 PCR P ,Destination[1], Roche 384 PCR P	ce Well,De late,N20,0 late,M20,0 late,M21,0	stination Plate   0,600 .0,600 .0,600
File C	7 Dontent: Line D 2 So 3 So 4 So 5 So	Destination Plate Type lata nurce Plate Name,Source Plate nurce[1]384PP_AQ_SP2,G17 nurce[1]384PP_AQ_SP2,G17 nurce[1]384PP_AQ_SP2,G17 nurce[1]384PP_AQ_SP2,G17	a Barcode, Source Plate Type, Sour ,Destination[1], Roche 384 PCR P ,Destination[1], Roche 384 PCR P ,Destination[1], Roche 384 PCR P ,Destination[1], Roche 384 PCR P	ce Well,De late,N20,0 late,M20,0 late,M21,0 late,N21,0	stination Plate 1 0600 .0600 .0600 .0600 01000
File C	7 Line D 2 Sc 3 Sc 4 Sc 5 Sc 6 Sc	Destination Plate Type           lata           nurce Plate Name,Source Plate           nurce[1]384PP_AQ_SP2,G17           nurce[1]384PP_AQ_SP2,G17           nurce[1]384PP_AQ_SP2,G17           nurce[1]384PP_AQ_SP2,G17           nurce[1]384PP_AQ_SP2,G17           nurce[1]384PP_AQ_SP2,G17	a Barcode, Source Plate Type, Sour , Destination[1], Roche 384 PCR P , Destination[1], Roche 384 PCR P , Destination[1], Roche 384 PCR P , Destination[1], Roche 384 PCR P 2, Destination[1], Roche 384 PCR P	ce Well,De late,N20,0 late,M21,0 late,N21,0 late,N21,0 late,H14,0	stination Plate   0600 .0600 .0600 01000 01800
File C	Image: 7           Line         D           1         Sc           2         Sc           3         Sc           4         Sc           5         Sc           6         Sc           7         Sc	Destination Plate Type lata nurce Plate Name,Source Plate nurce[1],.384PP_AQ_SP2,G17 nurce[1],.384PP_AQ_SP2,G17 nurce[1],.384PP_AQ_SP2,G17 nurce[1],.384PP_AQ_SP2,H12 nurce[1],.384PP_AQ_SP2,H12	a Barcode, Source Plate Type, Sour , Destination[1], Roche 384 PCR P , Destination[1], Roche 384 PCR P , Destination[1], Roche 384 PCR P , Destination[1], Roche 384 PCR P 2, Destination[1], Roche 384 PCR P 2, Destination[1], Roche 384 PCR P	ce Well,De late,N20,0 late,M20,0 late,M21,0 late,N21,0 late,H14,0 late,H15,0	stination Plate 1 0600 .0600 .0600 01000 01800 01800

17. Apart from the software "*Liquid Handler*", close all other software and log out PPMS tracker.18. Sign on the logbook.

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## IV. Appendix

Table 1. Calibration Protocol based on liquid properties.

### LDV plate

Calibration	Summary	Example fluids	Vol (uL)
384LDV_DMSO	384plate, DMSO	Solution reported in % DMSO hydrate (70% 100%)	2.5 - 12
1536LDV_DMSO	1536 plate, DMSO	Solution reported in %DWSO hydrate (70%-100%)	1 – 5.5
384LDV_AQ_B	384 plate, buffer	PBS, cell culture media w/o protein, cDNA, primers/probes	3 – 12
384LDV_AQ_P	384 plate, protein	Buffers, reagents containing protein (no surfactants), e.g. Enzymes, serum, antibodies	6 – 14

#### PP plate

Calibration	Summary	Example fluids	Vol (uL)
384PP_DMSO2	DMSO	Solution reported in %DMSO hydrate (70%-100%)	15 - 65
384PP_AQ_SP2	surfactant/ protein	Buffers, reagents with surfactant, e.g. PCR mastermix, lysis buffer, Triton X-100, Tween-20, SDS, NP-40, with or w/o plasma or serum	15 - 65
384PP_AQ_BP2	buffer / protein	Reagents w/o surfatants. Can contain DNA/protein/serum/plasma. Solutions reported in % Glycerol (<30%)	15 – 65
384PP_AQ_CP	crystallography reagents, salts, etc.	Reagents w/o surfatants. MPD/PEGS/Osmotic solutions. Solutions reported in Mrayl.	25 - 50
384PP_AQ_GP	glycerol + buffer/protein	Enzymes/Antibodies/Proteins stored in glycerol. Solutions reported in % Glycerol (15%-55%)	15 – 65
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