NUM SAMPLES 24

Step	Name	Reagent	Stored at	Before	Input	ID	Machine	Operation	Accessory	Quantity
1	**Anneal RNA**	EPH3	-20	Thaw ambient						
					Sample plate					
					Reagent plate					
					New plate: CDNA1					
							OT-REAGENT	Distribute EPH3 to Reagent plate	P300 single	8,5 ul per sample
					REAGENT PLATE TRANSFER to OT-PREP					
						1	OT-PREP1	Distribute Reagent EPH3 to CDNA1	P20 multi	8,5 ul per sample
						2	OT-PREP1	Add samples to CDNA1 and mix	P20 multi	8,5 ul
						3	OT-PREP1	Thermal cycle	Thermal Cycler	
					REAGENT PLATE TRANSFER to OT-REAGENT					
	Synthesize Dirst			to mix then keep on						
2	**Strand cDNA**	FSM	-20	ice						
		RTV	-20	Invert to mix; keep on						
					New 1.7ml tube: mastermix					
							OT-REAGENT	Add FSM to mastermix tube	P300 single	9 ul per sample
							OT-REAGENT	Add RTV to mastermix tube	**P300 single/P20 single**	1 ul per sample
						1	OT-REAGENT	Mix and distribute mastermix to Reagent plate	P300 single	10 ul per sample
					REAGENT PLATE TRANSFER to OT-PREP					
						3	OT-PREP1	Transfer from Reagent plate to CDNA1	P20 multi	8 ul per sample
						4		Thermal cycle	Thermal Cycler	
					REAGENT PLATE TRANSFER to OT-REAGENT					
Safe stop										
3	**Amplify cDNA**	IPM	-20	Thaw ambient; invert						
		CPP1	-20	Thaw ambient; keep						
		CPP2	-20	Thaw ambient; keep						
		water			New PCR Plate (COV1-COV2)					
					New 2 15ml tubes (MM1, MM2)					
					>>1.7ml	1	OT-REAGENT	Add IPM to MM1 and MM2	P300 single	30 ul per sample
						2	OT-REAGENT	Add water to MM1 and MM2	P300 single	9,4 ul per sample
						3	OT-REAGENT	Add CPP1 to MM1 and mix	P300 single	4,3 ul per sample
							OT-REAGENT	Distribute MM1 to Reagent plate	P300 single	20 ul per sample
						4	OT-REAGENT	Add CPP2 to MM2 and mix	P300 single	4,3 ul per sample
							OT-REAGENT	Distribute MM2 to Reagent plate	P300 single	20 ul per sample
					REAGENT PLATE TRANSFER to OT-PREP					
						5	OT-PREP1	transfer from Reagent MM1 to COV1	P300 single	20 ul per sample
						6	OT-PREP1	transfer from Reagent MM2 to COV2	P300 single	20 ul per sample
						7	OT-PREP1	transfer from CDNA1 to COV1	P20 multi	5 ul per sample
						8	OT-PREP1	transfer from CDNA1 to COV2	P20 multi	5 ul per sample
					To be verified: plate seal needed					
						9		Thermal cycle	Thermal Cycler	3 h
					REAGENT PLATE TRANSFER to OT-REAGENT					
Safe stop										
	Tagment PCR									
4	**Amplicons**	EBLTS	4	Bring ambient; vortex						
		TB1	-20	Bring ambient; vortex						
					New PCR Plate (TAG1)					
					New 1.7ml tube (TM tagmentation mastermix)					
						1	OT-PREP1	Transfer from COV1 to TAG1	P20 multi	10 ul
						2	OT-PREP1	Transfer from COV2 to TAG1	P20 multi	10 ul
						3	OT-REAGENT	Add TB1 to TM	P300 single	12 ul per sample
						4	OT-REAGENT	Add EBLTS to TM	P300 single	4 ul per sample

						5	OT-REAGENT	Add water to TM	P300 single	20	ul per sample
							OT-REAGENT	Transfer TM to Reagent Plate	P300 single	90	ul per well
					REAGENT PLATE TRANSFER to OT-PREP						
						6	OT-PREP1	Transfer from Reagent Plate to TAG1 and mix	P300 multi	30	ul per sample
						7	OT-PREP1	Thermal cycle	Thermal Cycler		
					REAGENT PLATE TRANSFER to OT-REAGENT						
					TAG1 TRANSFER FROM THERMAL CYCLER TO MAGNETIC MODULE						
	Post tagmentation										
5	**clean up**	ST2	25	Vortex							
		TWB	4	Vortex							
					Reservioir for TWB						
						1	OT-REAGENT	Distribute ST2 to Reagent plate	P300 single	10	ul per sample
					REAGENT PLATE TRANSFER to OT-PREP						
						2	OT-PREP1	Trasfer from Reagent plate to TAG1 and mix	P20 multi	10	ul per sample
						3	OT-PREP1	Incubate		5	min
						4	OT-PREP1	Activate magnetic module			
						5	OT-PREP1	Wait for liquid to be clear		3	min
						6	OT-PREP1	Discard the supernatant	P300 multi	50	ul
						7	OT-PREP1	Disable magnetic stand			
					REAGENT PLATE TRANSFER to OT-PREP						
							OT-REAGENT	Distribute TWB to 12-well Reservoir	P1000/P300 single	200	ul per sample
					12-WELL RESERVOIR TRANSFER to OT-PREP						
						8		Add TWB to TAG1	P300 multi	100	ul per sample
						9		Mix	P300 multi	100	ul
						10		Engage magnetic module			
						11		Discard the supernatant	P300 multi	100	ul
						12		Add TWB to TAG1	P300 multi	100	ul per sample
						13		Mix	P300 multi	100	ul
	Amplify Tagmented										
6	**Amplicons**	EPM	-20	Invert to mix; keep on ice							
		Index adapters	-20	Thaw ambient; vortex;							
		water			New 15ml tube (MM)						
						1	OT-REAGENT	Add EPM to MM	P300 single	24	ul per sample
						2	OT-REAGENT	Add water to MM	P300 single	24	ul per sample
						3	OT-REAGENT	Mix MM	P300 single		
							OT-REAGENT	Transfer MM to Reagent Plate			
					REAGENT PLATE TRANSFER to OT-PREP						
						4	OT-PREP1	Remove TWB from TAG1	P300 multi	100	ul
							OT-PREP1	Remove TWB from TAG1	P20 multi		
						5	OT-PREP1	Disable magnetic stand			
						6	OT-PREP1	Transfer MM to TAG1	P300 multi	40	ul
						7	OT-PREP1	Puncture index adapter foil and transfer 10ul TAG	P20 multi	10	ul
						8	OT-PREP1	Resuspend beads	P300 multi	50	ul
					TAG1 TRANSFER FROM MAGNETIC MODULE TO THERMAL CYCLER						
						10	OT-PREP1	Thermal cycle	Thermal Cycler	22	min
	Pool and clean up										
7	**libraries**	ITB	25	Vortex before each use							
		RSB	4	Wait 30 min to bring at							
		EtOH	0	Prepare fresh; 80%							
					New 1.7ml tube (Pooled ITB)						
					New 200ul tube (Eluted)						
					EtOH Reservoir						
					TAG1 TRANSFER FROM THERMAL CYCLER TO MAGNETIC MODULE						
						2	OT-PREP1	Engage magnetic module	Magnetic module	3	min
						3	OT-PREP1	Transfer from TAG1 to Pool Strip	P20 multi	5	ul per sample

					POOL STRIP TRANSFER TO OT-PREP2 (Magnetic module)				
				4	OT-PREP2	Transfer from Pool Strip to Pooled ITB and mix	P300 single	15	ul per well
					QUANTIFICATION PLATE TRANSFER TO OT-PREP2				
				5	OT-PREP2	Mix ITB	P300 single		
				6	OT-PREP2	Add ITB to Pooled ITB and mix	P300 single	108	ul
				7	OT-PREP2	Incubate		5	min
				9	OT-PREP2	Activate Magnetic Module			
					OT-PREP2	Wait for liquid to be clear		5	min
				10	OT-PREP2	Discard supernatant	P300 single	400	ul
				12	OT-PREP2	Wash beads: add EtOH 80% to Pooled ITB	P300 single	1000	ul
				13	OT-PREP2	Wait		30	s
				14	OT-PREP2	Discard supernatant	P300 single	1000	ul
				15	OT-PREP2	Wash beads: add EtOH 80% to Pooled ITB	P300 single	1000	ul
				16	OT-PREP2	Wait		30	s
				17	OT-PREP2	Discard supernatant	P300 single	1000	ul
				18	OT-PREP2	Discard supernatant with 20ul pip	P20 single		
					OT-PREP2	Disengage magnetic module			
				19	OT-PREP2	Add RSB to Pooled ITB and mix	P300 single	55	ul
				20	OT-PREP2	Incubate		2	min
				21	OT-PREP2	Engage magnetic module			
				22		Wait for liquid to be clear		2	min
				23		Transfer to Eluted tube	P300 single	50	ul

Safe stop

8	**Quantify and Normalize Library**	dsDNA HS Reagent	4	Bring at ambient
		dsDNA HS Buffer	<=30	Bring at ambient
	See Qubit dsDNA HS Assay Kit User Guide	dsDNA HS Std1	4	Bring at ambient
		dsDNA HS Std2	4	Bring at ambient
		RSB	4	Wait 30 min to bring at room temperature; vortex and invert

New 3 Qubit Assay Tubes (STD1, STD2, SAMPLE)
New 1.7ml tube (working solution)
[New microcentrifuge tube (Diluted)]

				1	OT-REAGENT	Add dsDNA HS Buffer to working solution	P300 single	600	ul circa
				2	OT-REAGENT	Add dsDNA HS Reagent to working solution and mix	P20 single	1:200	rispetto a HS Bu
				3	OT-REAGENT	Transfer working solution to STD1, STD2	P300 single	190	ul
				4	OT-REAGENT	Transfer working solution to SAMPLE	P300 single	198	ul
				5	OT-REAGENT	Add dsDNA HS Std1 to STD1	P20 single	10	ul
				6	OT-REAGENT	Add dsDNA HS Std2 to STD2	P20 single	10	ul
					QUANTIFICATION PLATE TRANSFER TO OT-PREP2				
				7	OT-PREP2	Add Eluted to SAMPLE	P20 single	2	ul
				8	OT-PREP2	Mix STD1	P300 single		
				9	OT-PREP2	Mix STD2	P300 single		
				10	OT-PREP2	Mix SAMPLE	P300 single		
					USER INTERVENTION: Qubit quantification (using STD1, STD2, SAMPLE) Result: SAMPLE concentration in ng/ul				
				11		Calculate volume for dilution to 4nM			
				12	OT-PREP2	Add RSB to Eluted as per dilution	P300 single/P20 single	?	ul

9	**Dilute to final loading concentration**			

See Miniseq Denature and Dilute Libraries Guide			
	HT1	-20	Thaw ambient; then keep 4°C until use; vortex before use
	NaOH		Prepare fresh; 1ml 0.1N
	water		
	200mM Tris-HCl pH 7.0		
(Questions)	RSB ?	-20	Thaw ambient; then keep 4°C until use

New 3 1.7ml tube (NAOH, LIBRARY, LIBDILUTED)
New microcentrifuge tube (TUBE1)

-> Prepare NaOH 0.1N

1	OT-REAGENT	Add water to NAOH	P300 single	900	ul
2	OT-REAGENT	Add NaOH to NAOH	P300 single	100	ul
		DENATURE PLATE TRANSFER TO OT-PREP2			

-> Dilute library to 1nM

3	OT-PREP2	Transfer Library to TUBE1	P20 single	25	ul
4	OT-PREP2	Transfer RSB to TUBE1 and mix	P300 single	75	ul

-> Denature library

5		Transfer from TUBE1 to LIBRARY	P20 single	5	ul
6		Transfer from NAOH to LIBRARY and mix	P20 single	5	ul
7		Incubate at room temperature		5	min
8		Add 200mM Tris-HCl pH 7.0 to LIBRARY and mix	P20 single	5	ul

-> Dilute library to final loading concentration of 1.2pM

9		Add HT1 to LIBDILUTED	P300 single	985	ul
10		Transfer from HT1 to LIBDILUTED	P300 single	380	ul

-> MiniSeq loading volume is 500ul

11		Transfer from LIBRARY to LIBDILUTED and mix	P300 single	120	ul

Preparation finished -> Load to MiniSeq