Distribute Reagent EPH3 to CDNA1 Add samples to CDNA1 and mix Thermal cycle Add FSM to mastermix tube Mix and distribute mastermix to Reagent plate Transfer from Reagent plate to CDNA1 Thermal cycle Thermal cycle Transfer from Reagent plate to CDNA1 Thermal cycle	gent EPH3 to CDNA1 o CDNA1 and mix astermix tube stermix tube oute mastermix to Reagent plate wth and MM2 MM1 and MM2 MM1 and mix It to Reagent plate IM2 and mix C to Reagent plate CDNA1 to COV1 CDNA1 to COV1 CDNA1 to COV2 CDNA1 to COV2 CDNA1 to COV2	gent EPH3 to CDNA1 O CDNA1 and mix Thermal Cycler Thermal Cycler Thermal Cycler Thermal Cycler P300 single P	gent EPH3 to CDNA1 P20 multi P300 single P300 single P300 single P300 single P20 multi P200 single P300 single
OT-REAGENT Add FSM to mastermix tube OT-REAGENT Add RTV to mastermix tube 1 OT-REAGENT Mix and distribute mastermix to Reagent plate REAGENT PLATE TRANSFER to OT-PREP 3 OT-PREP1 Transfer from Reagent plate to CDNA1 4 Thermal cycle AGENT PLATE TRANSFER to OT-REAGENT	OT-REAGENT Add FSM to mastermix tube OT-REAGENT Add RTV to mastermix tube OT-REAGENT Mix and distribute mastermix to Reagent plate 3 OT-PREP1 Thermal cycle Thermal cycle Thermal cycle Thermal cycle Thermal cycle Add water to MM1 and MM2 2 OT-REAGENT Add water to MM1 and mix OT-REAGENT Add water to MM2 and mix OT-REAGENT Add CPP1 to MM2 and mix OT-REAGENT Add CPP2 to MM2 and mix OT-REAGENT Distribute MM1 to Reagent plate 4 OT-REAGENT Add CPP2 to MM2 and mix OT-REAGENT Distribute MM2 to COV2 5 OT-REAGENT Transfer from Reagent MM1 to COV1 6 OT-PREP1 transfer from Reagent MM2 to COV2 7 OT-PREP1 transfer from CDNA1 to COV2 7 OT-PREP1 transfer from CDNA1 to COV2 8 OT-PREP1 transfer from CDNA1 to COV2 7 OT-PREP1 transfer from CDNA1 to COV2 8 OT-PREP1 transfer from CDNA1 to COV2 7 OT-PREP1 transfer from CDNA1 to COV2 8 OT-PREP1 transfer from CDNA1 to COV2 7 OT-PREP1 transfer from CDNA1 to COV2 8 OT-PREP1 transfer from CDNA1 to COV2 7 OT-PREP1 transfer from CDNA1 to COV2 8 OT-PREP1 transfer from CDNA1 to COV2 9 OT-PREP1 transfer from CDNA1 to COV2 1 To be verified: plate seal needed 1 Thermal cycle	OT-REAGENT Add FSM to mastermix tube OT-REAGENT Add RTV to mastermix tube OT-REAGENT Mix and distribute mastermix to Reagent plate 1 OT-REAGENT Mix and distribute mastermix to Reagent plate 3 OT-PREP1 Transfer from Reagent plate to CDNA1 4 Thermal cycle REAGENT PLATE TRANSFER to OT-REAGENT Thermal cycle REAGENT Add IPM to MM1 and MM2 2 OT-REAGENT Add Water to MM1 and mix OT-REAGENT Add CPP1 to MM2 and mix OT-REAGENT Add CPP1 to MM2 to Reagent plate 4 OT-REAGENT Add CPP2 to MM2 and mix OT-REAGENT Distribute MM2 to Reagent plate 5 OT-REAGENT DISTRIBUTE MM2 to COV1 6 OT-REP1 transfer from Reagent MM2 to COV2 7 OT-PREP1 transfer from CDNA1 to COV1 8 OT-PREP1 transfer from CDNA1 to COV1 7 OT-PREP1 transfer from CDNA1 to COV1 8 OT-PREP1 transfer from CDNA1 to COV1 7 OT-PREP1 transfer from CDNA1 to COV1 8 OT-PREP1 transfer from CDNA1 to COV1 8 OT-PREP1 transfer from CDNA1 to COV1 7 OT-PREP1 transfer from CDNA1 to COV1 8 OT-PREP1 transfer from CDNA1 to COV1 8 OT-PREP1 transfer from CDNA1 to COV1 7 OT-PREP1 transfer from CDNA1 to COV1 8 OT-PREP1 transfer from CDNA1 to COV1 8 OT-PREP1 transfer from CDNA1 to COV1 8 OT-PREP1 transfer from CDNA1 to COV1 10 OT-PREP1 transfer from CDNA1 to COV1 11 OT-PREP1 transfer from CDNA1 to COV1 12 OT-PREP1 transfer from CDNA1 to COV1 13 OT-PREP1 transfer from CDNA1 to COV1 14 OT-PREP1 transfer from CDNA1 to COV1 15 OT-PREP1 transfer from CDNA1 to COV1 16 OT-PREP1 transfer from CDNA1 to COV1 17 OT-PREP1 transfer from CDNA1 to COV1 18 OT-PREP1 transfer from CDNA1 to COV1	New 1.7ml tube: mastermix OT-REAGENT OT-REAGENT OT-REAGENT OT-REAGENT OT-REAGENT OT-REAGENT Add FWY to mastermix tube 1 OT-REAGENT And distribute mastermix to Reagent plate to CDNA1 ADT-PREP1 Transfer from Reagent plate to CDNA1 Add PM to MM1 and MM2 3 OT-REAGENT New PCR Plate (COV1-COV2) New PCR Plate (COV1-COV2) New PCR Plate (COV1-COV2) New PCR Plate (TAGENT Add PM to MM1 and MM2 3 OT-REAGENT Add OPP2 to MM1 and mix OT-REAGENT OT-REAGENT OT-REAGENT Transfer from CDN41 to COV2 To be verified: plate seal needed Deverified: plate seal needed Them I or OVI to TAGI New 1.7ml tube (TM tagmentation mastermix) 1 OT-REP1 Transfer from COVI to TAGI
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	to MM1 and MM2 I to MM1 and MM2 I to MM1 and mix E MM1 to Reagent plate E to MM2 and mix E MM2 to Reagent plate rom Reagent MM1 to COV1 rom Reagent MM2 to COV2 rom CDNA1 to COV2	to MM1 and MM2 I to MM1 and MM2 I to MM1 and mix E MM1 to Reagent plate I to MM2 and mix E MM2 to Reagent plate I to MM2 to COV1 I com Reagent MM1 to COV1 I com CDNA1 to COV1 I com CDNA1 to COV2	1 OT-REAGENT Add IPM to MM1 and MM2 2 OT-REAGENT Add water to MM1 and MM2 3 OT-REAGENT Add CPP1 to MM1 and MM2 OT-REAGENT Add CPP1 to MM1 to Reagent plate 4 OT-REAGENT Distribute MM1 to Reagent plate 5 OT-REAGENT Distribute MM2 to Reagent plate GENT PLATE TRANSFER to OT-PREP 5 OT-PREP1 transfer from Reagent MM1 to COV1 6 OT-PREP1 transfer from CDNA1 to COV2 7 OT-PREP1 transfer from CDNA1 to COV2 8 OT-PREP1 transfer from CDNA1 to COV2 9 Thermal cycle 9 Thermal cycle 1 OT-PREP1 1 OT-PREP1 1 Transfer from COV1 to TAG1
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OT-REAGENT Add water to MM1 and MM2	P300 single PMM2 to Reagent plate P300 single P20 multi	to MM2 and mix MM2 to Reagent plate P300 single P300 single P300 single P300 single P300 single P300 single P20 multi P20 m	4 OT-REAGENT Add CPP2 to MM2 and mix OT-REAGENT Distribute MM2 to Reagent plate OT-REAGENT Distribute MM2 to Reagent plate GENT PLATE TRANSFER to OT-PREP 5 OT-PREP1 transfer from Reagent MM1 to COV1 7 OT-PREP1 transfer from Reagent MM2 to COV2 7 OT-PREP1 transfer from CDNA1 to COV2 8 OT-PREP1 Thermal cycle 9 Thermal cycle OF DEMIT! 1 OT-PREP1 Transfer from COV1 to TAG1 P20 multi
OT-REAGENT Add water to MM1 and MM2 OT-REAGENT Add CPP1 to MM1 and mix OT-REAGENT Distribute MM1 to Reagent plate	rom Reagent MM1 to COV1 P300 single 20 rom Reagent MM2 to COV2 P300 single 20 P20 multi 5 P20 multi 5 P20 multi 7 P20 multi 8	rom Reagent MM1 to COV1 P300 single Tom Reagent MM2 to COV2 P300 single Tom CDNA1 to COV1 P20 multi S P20 multi S P20 multi S P20 multi S Sycle Thermal Cycler 3	GENT PLATE TRANSFER to OT-PREP 5 OT-PREP1 4 transfer from Reagent MM1 to COV1 6 OT-PREP1 4 transfer from Reagent MM2 to COV2 7 OT-PREP1 4 transfer from COV1 to TAG1 8 OT-PREP1 5 Thermal Cycler 9 1 OT-PREP1 Transfer from COV1 to TAG1
OT-REAGENT Add water to MM1 and MM2 OT-REAGENT Add CPP1 to MM1 and mix OT-REAGENT Distribute MM1 to Reagent plate OT-REAGENT Add CPP2 to MM2 and mix OT-REAGENT Distribute MM2 to Reagent plate	rom Reagent MM1 to COV1 P300 single rom Reagent MM2 to COV2 P300 single P20 multi rom CDNA1 to COV2 P20 multi P20 multi P20 multi Thermal Cycler	rom Reagent MM1 to COV1 P300 single rom Reagent MM2 to COV2 P300 single rom CDNA1 to COV1 P20 multi P20 multi Thermal Cycler	5 OT-PREP1 transfer from Reagent MM1 to COV1 6 OT-PREP1 transfer from Reagent MM2 to COV2 7 OT-PREP1 transfer from CDNA1 to COV1 8 OT-PREP1 transfer from CDNA1 to COV1 1 Obe verified: plate seal needed 9 Thermal cycle 9 Thermal cycle 1 OT-PREP1 Transfer from COV1 to TAG1
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ter to MM1 and MM2 P1 to MM1 and mix ute MM1 to Reagent plate P2 to MM2 and mix ute MM2 to Reagent plate rfrom Reagent MM1 to COV1 rfrom Reagent MM2 to COV2 rfrom CDNA1 to COV2	ycle Thermal Cycler 3	ycle Thermal Cycler 3	ENT PLATE TRANSFER to OT-REAGENT 1 OT-PREP1 Transfer from COV1 to TAG1 P20 multi
ter to MM1 and MM2 P1 to MM1 and mix Ite MM1 to Reagent plate P2 to MM2 and mix Ite MM2 to Reagent plate From Reagent MM1 to COV1 From CDNA1 to COV1 From CDNA1 to COV2	KTALTIN DI ALT HANNITER TO LIERTALITIN	REAGENI PLAIE I KANSTEK TO OI-KEAGENI	1 OT-PREP1 Transfer from COV1 to TAG1 P20 multi
er to MM1 and MM2 I to MM1 and mix E MM1 to Reagent plate I to MM2 and mix E MM2 to Reagent plate From Reagent MM1 to COV1 From CDNA1 to COV1 From CDNA1 to COV2 From CDNA1 to COV2			1 OT-PREP1 Transfer from COV1 to TAG1 P20 multi

					5 OT-REAGENT OT-REAGENT	Add water to TM Transfer TM to Reagent Plate	P300 single P300 single	20 ul per sample 90 ul per well
					REAGENT PLATE TRANSFER to OT-PREP	to OT-PREP)	-
					6 OT-PREP1 7 OT-PREP1	Transfer from Reagent Plate to TAG1 and mix Thermal cycle	P300 multi Thermal Ovcler	30 ul per sample
					REAGENT PLATE TRANSFER to OT-REAGENT	OT-REAGENT		
				TAG1 TF	TAG1 TRANSFER FROM THERMAL CYCLER TO MAGNETIC MODULE	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	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
						Activate magnetic module		
					5 OI-PREPI 6 OT-PRFP1	Wait for liquid to be clear Discard the supernatant	P300 multi	3 min 50 ul
					7 OT-PREP1	Disable magnetic stand		;
					REAGENT PLATE TRANSFER to OT-PREP	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	R to OT-PREP		
					∞	Add TWB to TAG1	P300 multi	100 ul per sample
					თ	Mix	P300 multi	100 ul
					10	Engage magnetic module	3	-
					11	Discard the supernatant	P300 multi	100 ul
					12	Add IWB to IAGI	P300 multi	100 ul per sample
Amplify Tagmented		;	Invert to mix; keep on					
6 Amplicons	EPM Indox adaptors	-20	ice					
	water	07.	וומע מוווטפוור, עסו נפא,	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		
					REAGEN PLATE KANSFER to OT-PREP	to OI-PREP	:41::	
					4 OI-PREPI OT-PREP1	Remove TWB from TAG1	P20 multi	TOO OIL
					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		10 ul
				+	8 OI-PREP1	Kesuspend beads	P300 multi	50 ul
				IAG1 IF	IAGI IKANSFEK FKOM MAGNEIIC MODULE IO IHEKMAL CYCLEK	JLE IO I HERMAL CYCLER		
					10 OT-PREP1	Thermal cycle	Thermal Cycler	22 min
Pool and clean up								
7 libraries	ITB	25	Vortex before each use					
	KSB FtOH	4 C	Wait 30 min to bring at Prepare fresh: 80%					
		ò		New 1.7ml tube (Pooled ITB)				
				New 200ul tube (Eluted)				
				EtOH Reservoir				
				TAG1 TF	TAG1 TRANSFER FROM THERMAL CYCLER TO MAGNETIC MODULE 2 OT DBED1	TO MAGNETIC MODULE	Name of the second	
					2 OI-PREPI 3 OT-PREP1	Engage magnetic module Transfer from TAG1 to Pool Strin	Magnetic module P20 multi	5 til ner samnle
					i : : : :)	L.;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;	5	3.2.5

				4 OT-PREP2 Transfer from F	Transfer from Pool Strip to Pooled ITB and mix	P300 single	15 ul per well
			QUANT	QUANTIFICATION PLATE TRANSFER TO OT-PREP2	ER 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
dsDNA HS Reagent	4	Bring at ambient					
dsDNA HS Buffer	<=30	Bring at ambient					
S dsDNA HS Std1	4	Bring at ambient					
dsDNA HS Std2	4	Bring at ambient					
			1				
		Wait 30 min to bring at	; at				
	•	room temperature;					
KSB	4	vortex and invert		í			
			New 3 Qubit Assay Tubes (STD1, STD2, SAMPLE) New 1.7ml tube (working solution)	LE)			
			[New microcentrifuge tube (Diluted)]				
				1 OT-REAGENT	Add dsDNA HS Buffer to working solution	P300 single	600 ul circa
					Add dsDNA HS Reagent to working solution and		
				2 OT-REAGENT	mix		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	
				6 OT-REAGENT	Add dsDNA HS Std2 to STD2	P20 single	10 ul
			QUANT	QUANTIFICATION PLATE TRANSFER TO OT-PREP2	ER TO OT-PREP2		
				7 OT-PREP2	Add Eluted to SAMPLE	P20 single	2 ul
				0 OI-PREP2	INIX SIDI	rsoo single	
				9 OI-PREP2 10 OT-PREP2	Mix St D2 Mix SAMPLE	P300 Single	
				10 01-1 1/1-1 2	WILK JAINT EL		
				USER INTERVENTI	USER INTERVENTION: Qubit quantification (using STD1, STD2, SAMPLE)	(;	
					Result: SAMPLE concentration in ng/ui		
				11 12 OT PPED2	Calculate volume for dilution to 4nM	واحداد الدهاد المحاد المحادة	
				12 OI-PREP2	Add RSB to Eluted as per dilution	rsou single/rzu single	5

See Qubit dsDNA HS Assay Kit User Guide

Quantify and 8 Normalize Library

Safe stop

(Questions) RSB ?	-20	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	In 006
			2 OT-REAGENT	Add NaOH to NAOH	P300 single	100 ul
			DENATURE PLATE TRANSFER TO OT-PREP2	3 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
			9	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	ix P20 single	S ul
-> Dilute library to final loading concentration of 1.2pM	2pM		6	Add HT1 to LIBDILUTED	P300 single	ln 586
			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

Thaw ambient; then keep 4°C until use; vortex before use Prepare fresh; 1ml 0.1N

-20

HT1

See Miniseq Denature and Dilute Libraries Guide NaOH water 200mM Tris-HCl pH 7.0

Thaw ambient; then

Preparation finished -> Load to MiniSeq