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Part 2: Custom QXT

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This protocol details the procedure of Custom QXT.

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SmartSeq

Custom QXT, Fragmentation, Adaptor-tagging, Ampure bead purification 1, Amplification, Ampure bead purification 2, ASAPCRN

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Part of collection
SmartSeq

Materials:

- cDNA
- 5x TD Buffer
- QXTEnzyme
- StorageSoln
- 80% EtOH
- Ampure beads/sample

A: Fragmentation and Adaptor-tagging 1m

1 **FIRST, IF POSSIBLE:** Normalize cDNA samples to 2x the input mass (for **1 ng** cDNA input will be **2 ng** / **10.13 µL** , for the **5 ng** input will be **10 ng** / **10.13 µL**). Run quants (picogreen) to assess concentration. If normalization was successful, add one volume of water to the samples to bring it back to 1x concentration and continue with **10.16 µL** .

A	B	C	D	E	F
1ng cDNA input:			**5ng cDNA input:**		
☐ INPUT: 1ng cDNA in 10.16uL water			☐ INPUT: 5ng cDNA in 10.16uL water		
☐ QXT DILUTION: 1:20 dilution of QXT enzyme:Storage Soln., at least 2uL of dilution/sample			☐ QXT DILUTION: 1:6 dilution of QXT enzyme:Storage Soln., at least 2uL of dilution/sample		
☐ MASTER MIX:			☐ MASTER MIX:		
Reagent	**1 rxn (uL)**	**____rxn**	**Reagent**	**1 rxn (uL)**	**____rxn**
cDNA	10.16	N/A	cDNA	10.16	N/A
5x TD Buffer	8.84		5x TD Buffer	8.84	
QXTEnzyme	0.1		QXTEnzyme	0.33	
StorageSoln	1.9		StorageSoln	1.67	
Total:	21		Total:	21	

2 Place samples in thermocycler on “DNA Fragmentation” program:

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Step	Temp(C)	Time(hh:mm:ss)
1	55	00:10:00
2	4	00:01:00
3	4	Hold

3 

Add **5 µL** 0.2%SDS and **24 µL** water/sample.

Reagent	1 rxn (uL)	____rxn (uL)
0.2%SDS	5	
H2O	24	
Totals:	29	

4  1m

Seal, vortex, incubate at **Room temperature** for **00:01:00** .

B: Ampure bead purification 1 1m

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A	B
Single cleanup	**Double SPRI cleanup (experimental)**
❑ Vortex beads and incubate @ RT for 30minutes	❑ Vortex beads and equilibrate @ RT for 30minutes
❑ Add 49uL bead mix/sample (1.0x)	❑ 0.5x cleanup (>600bp fragment removal): Add 25uL beads to each 50uL sample, vortex, briefly spin
❑ Seal samples, vortex, briefly spin down	❑ Incubate samples 5 mins @RT
❑ Incubate samples @RT 5 minutes	❑ Add samples to magnet rack, allow solution to clear, save supernatant to new clean tube (optional: save beads)
❑ Put samples on magnet rack, allow to clear, discard supernatant	❑ 0.2x cleanup (removal of <150bp fragments, rounding out total cleanup to 0.7x): Add 10uL bead mix/sample, seal, mix, incubate for 5 mins @RT
❑ Wash 1 with 200uL 80% EtOH	❑ Put samples on magnet rack, allow to clear, discard supernatant
❑ Wash 2 with 200uL 80% EtOH	❑ Wash 1 with 200uL 80% EtOH
❑ Remove last of the EtOH, allow samples to air dry for at least 10 minutes.	❑ Wash 2 with 200uL 80% EtOH
❑ Elute with 24uL H2O, incubate for 2 mins @RT	❑ Remove the last Ethanol and allow samples to air dry for at least 10 minutes
	❑ Elute with 24uL H2O, incubate for 2 mins @RT

C: Amplification

6

Add **24 µL** Kapa Hotstart Master Mix/sample.

7

Add **1 µL** i7 primer and **1 µL** i5 indexing primer, or **2 µL** from a multiplex plate.

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NOTE THE USE OF CUSTOM PRIMERS.

8

Seal, vortex, briefly spin down samples, put in thermocycler on “Pre-Capture PCR” protocol.

A	B	C	D
Step	**Temp(C)**	**Time(hh:mm:ss)**	
1	68	00:02:00	
2	98	00:02:00	
3	98	00:00:30	
4	57	00:00:30	
5	72	00:01:00	
6	Return to step 3	13x for 1ng cDNA inputs	11x for 5ng cDNA inputs
7	72	00:05:00	
8	4	hold	

Avoid S511 QXT i5 primer

D: Ampure bead purification 2 7m

9

Avoid S511 QXT i5 primer.

Make sure beads are equilibrated to **Room temperature** .

10

Add **32.5 µL** - **35 µL** Ampure beads/sample (0.65x-0.7x beads:sample ratio), seal, mix, brief spin.

11 5m

Incubate sample/bead mix @ **Room temperature** for **00:05:00** .

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12 Move to magnet stand, allow solution to clear, discard supernatant.

13 

Wash 1 with **200 µL** of 80% EtOH.

14 

Wash 2 with **200 µL** of 80% EtOH.

15  2m

Elute in **20 µL** water. Add water to samples, take off magnet, seal, mix, spin, incubate @ **Room temperature** for **00:02:00** .

16 Transfer samples back to magnet stand, allow to clear, save supernatant in separate, clean tubes.

17 REPEAT Ampure bead purification 2 for a total of 2x bead cleanups.

18 Evaluate on Tapestation.

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