



Dec 01, 2021

Part 2: Custom QXT



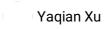
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This protocol details the procedure of Custom QXT.

d2bybg4xf.docx

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SmartSeq

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

_____ protocol,

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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
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1 FIRST, IF POSSIBLE: Normalize cDNA samples to 2x the input mass (for $\square 1$ ng cDNA input will be $\square 2$ ng / $\square 10.13$ μL), for the $\square 5$ ng input will be $\square 10$ ng / $\square 10.13$ 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 $\square 10.16$ μL .

Α	В	С	D	E	F
1ng cDNA input:		5ng cDNA			
		input:			
INPUT: 1ng cDNA in			INPUT: 5ng cDNA in		
10.16uL water 10.16uL water					
QXT DILUTION: 1:20 dilution of QXT			QXT DILUTION: 1:6 dilution of QXT		
enzyme:Storage			enzyme:Storage		
Soln., at least 2uL of dilution/sample		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	8.84	
			Buffer		
QXTEnzyme	0.1		QXTEnzyme	0.33	
StorageSoln	1.9		StorageSoln	1.67	
Total:	21		Total:	21	

Place samples in thermocycler on "DNA Fragmentation" program:

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

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Add $\Box 5 \mu L$ 0.2%SDS and $\Box 24 \mu L$ water/sample.

Reagent	1	rxn
	rxn (uL)	(uL)
0.2%SDS	5	
H20	24	
Totals:	29	

4



1m

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

B: Ampure bead purification 1

1m

5

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

C: Amplification

6

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

7 / 📜

Add $\Box 1 \mu L$ i7 primer and $\Box 1 \mu L$ i5 indexing primer, or $\Box 2 \mu L$ from a multiplex plate.

NOTE THE USE OF CUSTOM PRIMERS.



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

Α	В	С	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	11x for 5ng cDNA	
		inputs	inputs	
7	72	00:05:00		
8	4	hold		

7m

Avoid S511 QXT i5 primer

D: Ampure bead purification 2

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Avoid S511 QXT i5 primer.

Make sure beads are equilibrated to & Room temperature.

Add $\blacksquare 32.5 \,\mu\text{L} - \blacksquare 35 \,\mu\text{L}$ Ampure beads/sample (0.65x-0.7x beads:sample ratio), seal, mix, brief spin.

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



5

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



Wash 1 with 200μ L of 80% EtOH.

14

Wash 2 with **200 µL** of 80% EtOH.

Elute in $\ \Box 20\ \mu L$ water. Add water to samples, take off magnet, seal, mix, spin, incubate @ 8 Room temperature for $\ \odot 00:02:00$.

- 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.