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Part 1: SmartSeq

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This protocol details the procedure of SmartSeq.

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SmartSeq

SmartSeq, Anneal primers, ASAPCRN

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Part of collection

SmartSeq

Materials:

oligo-dT primer ([M] **10 Micromolar (µM)**)

dNTP mix ([M] **10 Milimolar (mM)**)

SuperScriptII reverse transcriptase (**200 U/µL**)

RNAse inhibitor (**40 U/µL**)

Superscriptll first-strand buffer (5x)

DTT ([M] **100 Milimolar (mM)**)

Betaine([M] **5 Molarity (M)**)

Nuclease-free H2O

TSO([M] **100 Micromolar (µM)**)

First-strand rxn (previous step)

Kapa HiFi HotStart ReadyMix (2x)

IS PCR primers ([M] **10 Micromolar (µM)**)

H2O

INPUT:

1 Use total RNA, ranging between **10 pg** - **30 pg** up to **10 ng** . **2.6 µL** will be used per sample.

ANNEAL PRIMERS: 8m

2 **On ice** , add each sample (**2.6 µL** total RNA) to a thin-walled **0.2 mL** PCR tube and add:

A	B	C	D
Item	**Volume (uL)**	**_____xMM**	**Lot#**
oligo-dT primer (10uM)	1		
dNTP mix (10mM)	1		

3 3m 10s

Centrifuge at **700 x g** for **00:00:10** at **Room temperature** . **Incubate @ 72 °C for 00:03:00** , spin down, put **On ice** .

4 REVERSE TRANSCRIPTION: add the following **On ice** :

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A	B	C	D
Item	**Volume(uL)**	**_____xMM**	**Lot#**
SuperScriptII reverse transcriptase (200U/uL)	0.50		
RNAse inhibitor (40U/uL)	0.25		
SuperscriptII first-strand buffer (5x)	2		
DTT (100mM)	0.50		
Betaine(5M)*	2		
Nuclease-free H2O	0.06		
TSO(100uM)**	0.10		
Total volume:	5.41		N/A

*stored at 4^{O}C.
**template-switching oligos, stored in -80^{O}C.

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Mix, spin down, and incubate in thermocycler on following settings:

A	B	C
Step	**Temp(C)**	**Time(hh:mm:ss)**
1	42	01:30:00
2	50	00:02:00
3	42	00:02:00
4	Return to step 2, 5x	
5	70	00:15:00
6	4	hold

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PCR PRE-AMPLIFICATION: Prepare Master Mix during Reverse Transcription rxn.

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A	B	C	D
Item	**Volume(uL)**	**_____xMM**	**Lot#**
First-strand rxn (previous step)	10		N/A
Kapa HiFi HotStart ReadyMix (2x)	12.5		
IS PCR primers (10uM)	0.25		
H2O	2.25		
Total volume:	25		N/A

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Add **15 µL** PreAmp Master Mix to each sample, seal, mix, spin down, and incubate in thermocycler on following settings:

A	B	C
Step	**Temp(C)**	**Time(hh:mm:ss)**
1	98	00:03:00
2	98	00:00:20
3	67	00:00:15
4	72	00:06:00
5	Return to step 2	17x (18 cycles total)
6	72	00:05:00
7	4	hold

8 Do a standard Ampure or equivalent 1X (1:1 ratio bead to sample) Bead cleanup (add **25 µL** beads to each sample).

8.1  5m

Allow beads to incubate with sample for **00:05:00** at **Room temperature** . After 5 minutes, place samples on magnet until the supernatant is clear and all beads are on the wall of the tube. Carefully remove the supernatant and immediately move to wash steps.

8.2  30s

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Carefully pipette **150 µL** - **200 µL** of freshly prepared 80% EtOH to the supernatant and incubate for **00:00:30** . Remove EtOH and immediately repeat this step.

8.3

80% EtOH wash 2. Once second EtOH wash is completed and EtOH has been removed from the beads, allow the beads to dry until they look glossy.

> Do not overdry, but do not allow excess EtOH to remain on beads. This usually takes about 2 minutes, depending on ambient environment.

8.4 7m

Elute with **17.5 µL** H2O, keep **15 µL** . Once beads are dry, resuspend in eluate and incubate for **00:02:00** - **00:05:00** . Place slurry on magnet and allow supernatant to clear. Once all beads are on the magnet, carefully remove **15 µL** of the clear eluate.

9 Quant the cleaned cDNA on Tapestation, using D5000 reagents or HSD5000, depending on expected yield and according to manufacturers suggestions.

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