Low Input RNAseq libraries - SMART-Seq v4 cDNA (½ reaction)

SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing (Clontech Cat.# from 634888 to 634894)
SeqAmp™ DNA Polymerase (Clontech Cat.#638504)

- Prepare a 10X Reaction Buffer by mixing 19 ul of 10X Lysis Buffer and 1 ul of RNase Inhibitor
- Dilute the samples in Reaction Buffer:

			¼ reaction	___rxns
10X Reaction Buffer	0.5	ul	0.3 ul	_____
3'SMART-Seq CDS P. IIA	1	ul	0.5 ul	_____
	1.5	ul	0.8 ul	
Sample (Cells or RNA)	4.75	ul	2.4 ul	
Total volume	6.25	ul	3.2 ul	

hot lid (80°C)

72°C	- 3 min
4°C	- forever

- Make master mix with 10% extra rxn:

				¼ reaction	
5X First-Strand Buffer	2	ul		1 ul	_____
SMART-Seq v4 Oligo	0.5	ul		0.3 ul	_____
RNase Inhibitor	0.25	ul		0.2 ul	_____
SMARTScribe enzyme	1	ul		0.5 ul	_____
Total volume per rxn	3.75	ul	add to 6.25 ul - Total 10ul	2 ul	

hot lid (80°C)

42°C	- 90 min
70°C	- 10 min
4°C	- forever

SAFE STOP OVERNIGHT

- Make master mix with 10% extra rxn:

				¼ reaction	
2X SeqAmp Buffer	12.5	ul		6.3 ul	_____
PCR Primer IIA	0.5	ul		0.3 ul	_____
SeqAmp Polymerase	0.5	ul		0.3 ul	_____
Nuclease Free Water	1.5	ul		0.8 ul	_____
Total volume per rxn	15	ul	add to 10 ul - Total 25ul	7.7 ul	

95°C	- 1 min
cycles	X
|98°C	- 10 sec
|65°C	- 30 sec
|68°C	- 3 min
72°C	- 10 min
4°C	- forever

SAFE STOP OVERNIGHT

RNA	Cells	cycles
5000 pg	500	9
500 pg	50	12
50 pg	5	16
5 pg	1	19

- Add 25 ul of Ampure XP beads (1.0x), wash, dry and elute in 17 ul of Elution Buffer. ¼ reaction (13 ul)
- Quantify with BioA DNA HS chip - range 200bp to 9000bp.

Low Input RNAseq libraries - NEXTERA XT DNA Library (½ reaction)

Nextera XT DNA Library Preparation Kit (Illumina Cat.# 24 samples FC-131-1024, 96 samples FC-131-1096)
Nextera XT Index Kit v2 Set A (96 indices, 384 samples FC-131-2001)

- Make master mix with 10% extra rxn:

			¼ reaction	___rxns
TD	5	ul	2.5 ul	_____
ATM	2.5	.ul	1.3 ul	_____
	7.5	ul	3.8 ul	
add DNA (30pg/ul) (75pg)	2.5	.ul	(40pg) 1.3 ul	
Total volume	10	ul	5.1 ul	

hot lid (60°C)

55°C	- 5 min
10°C	- forever

¼ reaction

- Add 2.5 ul of NT and incubate for 5 min at room temperature. 1.3 ul

- Add a different combination of:
  2.5 ul of Index 1 (i7 - orange cap - 12) and 2.5 ul of Index 2 (i5 - white cap - 8) 1.3 ul + 1.3 ul

- Add 7.5 ul of NPM. Total volume per rxn is 25 ul. 3.8 ul. Tot. 12.8

72°C	- 3 min
95°C	- 30 sec
cycles	12 cyc.
|95°C	- 10 sec
|55°C	- 30 sec
|72°C	- 30 sec
72°C	- 5 min
10°C	- forever

SAFE STOP OVERNIGHT

- Add 45 ul of Ampure XP beads (1.8x), wash, dry and elute in 17 ul of Elution Buffer. ¼ reaction (23 ul)

	N701	N702	N703	N704	N705	N706	N707	N708	N709	N710	N711	N712
N502												
N503												
N504												
N505												
N506												
N507												
N508												
N517												