SMARTseq2 day 1

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Abstract

The RT and amplification steps for SMARTseq2, as modified for previously extracted RNA slices.

Citation: Peter Combs SMARTseq2 day 1. **protocols.io**
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Before start

Thaw RNA samples on ice, as well as:

* First Strand Buffer (-20º)
* DTT (-20º)
* dNTPs (-20º)
* oligo dT30VN primer (-20º)
* Betaine (4º)
* TSO (-80º)

Protocol

Step 1.
Wipe down bench with RNase zap or similar

Step 2.
Dilute oligo-dT30VN primer by adding 37µL of 100µM primer to 63µL of nuclease free H20

Step 3.
Prepare RT mix

Reagent	Stock Conc	Amt/1	Amt/N	Final Conc
Nuclease Free H20	---	0.29		
FS Buffer	5x	2		1x
Betaine	5M	2		1M
MgCl2	1M	0.06		6mM
TSO	100µM	0.1		1µM
DTT	100mM	0.5		5mM
Superscript II	200U/µL	0.5		100U
RNase OUT	40U/µL			10U
Sample	-----	----- (4.3)	----	-----

Step 4.

Incubate at 72º for 3m, return to ice

DURATION

00:03:00

Step 5.

Take 4.3µL of hybridized oligo-dT+RNA and put in strip tubes.

Step 6.

Add 5.7µL RT mix

Step 7.

RT

Num Cycle	Group	Temp	Time
1x	A	42	90m
10x	B	50	2m
	B	42	2m
1x	C	70	15m
hold	D	4	hold

DURATION

02:15:00

Step 8.

Dilute ISPCR primers 1:100

Step 9.

Prepare PCR preamp mix

Component	Stock	Amt/1	Amt/N	Final Conc
(First Strand Rxn)	--	10µL	---	----
KAPA HiFi Hot Start	2x	12.5		1x
ISPCR primers	1µM	2.5µL		0.1µM
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Final Volume		25µL		

Step 10.

Add 15µL PCR preamp mix to 10µL RT

Step 11.

Preamp PCR

15 cycles is the current recommendation, but there's enough that I'll try 13

Step 12.

Ampure Cleanup. Use 0.6 volumes of beads, so 15µL of beads for the 25µL reaction.

Step 13.

Resuspend in 15.5µL EB, then measure concentrations using qubit.