SMART-Seq2

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Library preparation

1. Plate-based (96-, 384-well plates)
2. No UMIs
3. Post-amplification cell barcoding with primers
4. Recovery of full length mRNAs
5. Biases: standard biases as all PCR techniques
6. 2 days till library prep

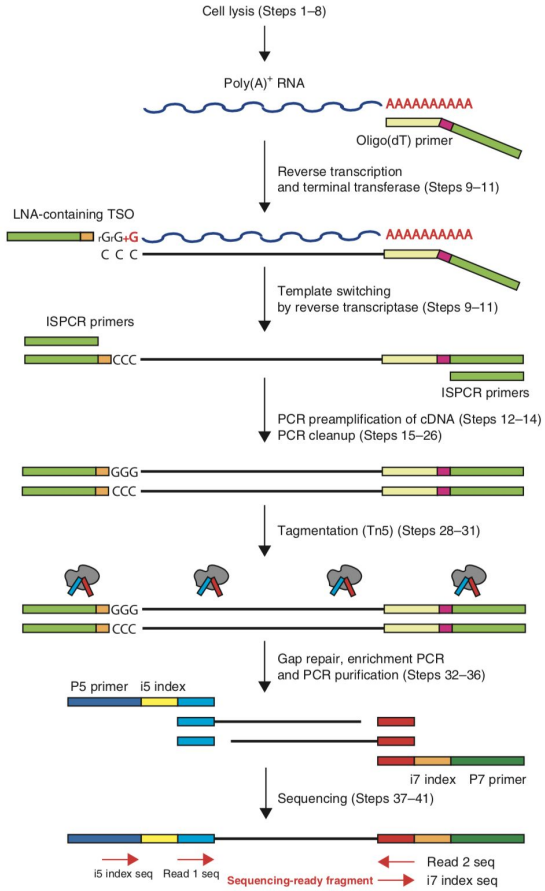
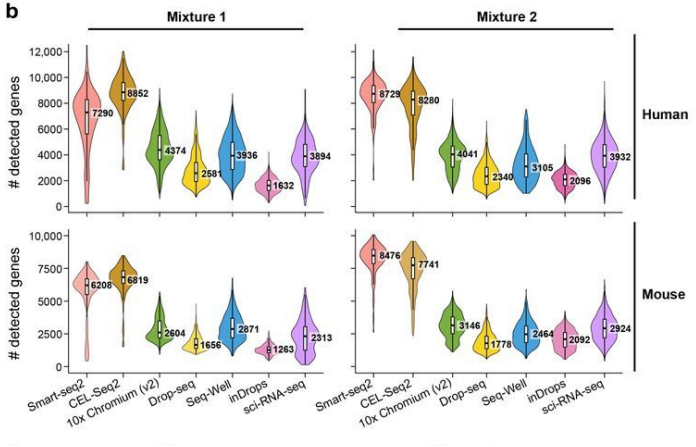
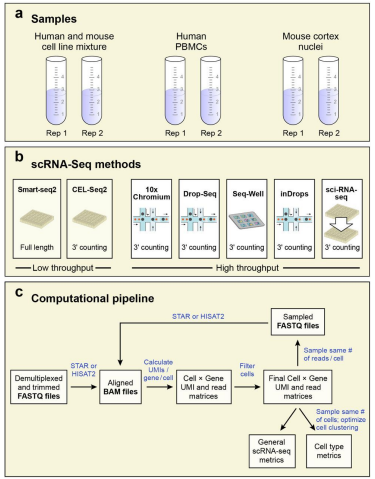


Figure 1 | Flowchart for Smart-seq2 library preparation. Outline of the protocol and the corresponding procedure steps. The oligo-dT primer, TSO and ISPCR primer are described in the main text, whereas tagmentation uses primers that are included in the Nextera XT sample preparation and index kits.

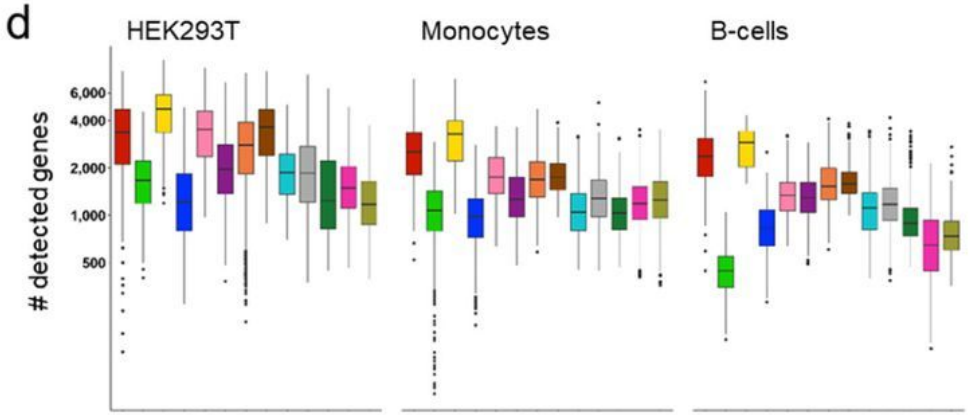
Ding,... Regev, Levin, 2019, BioRxiv
https://www.biorxiv.org/content/10.1101/632216v1.full

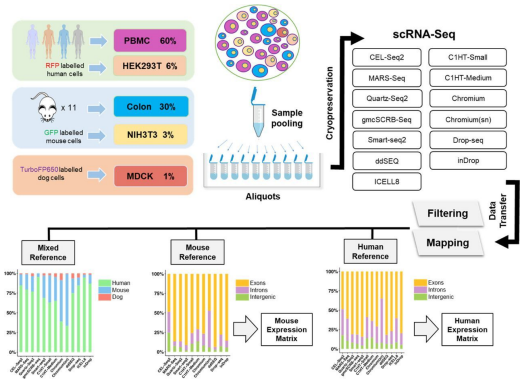




How many genes per cell can you detect on average?

Mereu,... Stegle, Heyn, 2019, BioRxiv
https://www.biorxiv.org/content/10.1101/630087v1.full





Advantages/disadvantages compared to other techniques?

Pros

Recovery of full length transcripts seq

(e.g. SNP analysis)

High coverage of the transcriptome

Immune phenotype, T/B cell receptors

Index sort

Cons

Less cell count

Experimental Design

5 donors, 2 time points → 10 blood samples

B and T cells will be sorted by FACS from each sample with B/T cell markers

We would like use 384-well plate/sample/cell type

Day 1/7→ 5 samples → cell isolation, FACS sorting (avoid batch bias by randomization of donors/plate) to plates → 1st step of library prep→ freeze

Drawback: Even though SMART-seq2 is not suitable for this type of experiment
Restricted cell number, diff. Cell states (low frequency?)

Suggestion: 10x sc-Seq first to see the bigger picture

Cost/time estimation

Process	1 x sample price	Total price
Low input RNA-seq with SMARTer kit	$460	$9200
Agilent Bioanalyzer (up to 12 samples, pool the plate use is as 1 sample)	$160	$320
Illumina HiSeq 2500 Sequencing 2 x 100bp Paired-end Read Sequencing (1lane/plate, 1plate/sample, ~0.5mio reads/cell)	$3300	$66000

Total cost **$ 75520** with time estimation:

- Sample collection → < 1 month
- Library preparation → 2 days/person/plate
- Sequencing → 2 months
- Analysis → 2-3 months

Thank you :)

Which parts of the mRNA are covered by the sequencing reads?

How many cells can you measure in one experiment?

	SMART-seq2	CEL-seq2	STRT-seq	Quartz-seq2	MARS-seq	Drop-seq	inDrop	Chromium	Seq-Well	sci-RNA-seq	SPLiT-seq
Single-cell isolation	FACS, microfluidics	FACS, microfluidics	FACS, microfluidics, nanowells	FACS	FACS	Droplet	Droplet	Droplet	Nanowells	Not needed	Not needed
Second strand synthesis	TSO	RNase H and DNA pol I	TSO	PolyA tailing and primer ligation	RNase H and DNA pol I	TSO	RNase H and DNA pol I	TSO	TSO	RNase H and DNA pol I	TSO
Full-length cDNA synthesis?	Yes	No	Yes	Yes	No	Yes	No	Yes	Yes	No	Yes
Barcode addition	Library PCR with barcoded primers	Barcoded RT primers	Barcoded TSOs	Barcoded RT primers	Barcoded RT primers	Barcoded RT primers	Barcoded RT primers	Barcoded RT primers	Barcoded RT primers	Barcoded RT primers and library PCR with barcoded primers	Ligation of barcoded RT primers
Pooling before library?	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Library amplification	PCR	In vitro transcription	PCR	PCR	In vitro transcription	PCR	In vitro transcription	PCR	PCR	PCR	PCR
Gene coverage	Full-length	3′	5′	3′	3′	3′	3′	3′	3′	3′	3′
Number of cells per assay											

	Single cell isolation/capture	2nd strand synthesis	Full-length cDNA synthesis	Barcode addition	Pooling before library	Library amplification	Gene coverage
SMART-seq/ SMART-seq2	FACS or Fluidigm C1	TSO	Yes	Library PCR with barcoded primers	No	PCR	full-length