

Single-cell RNA-Seq

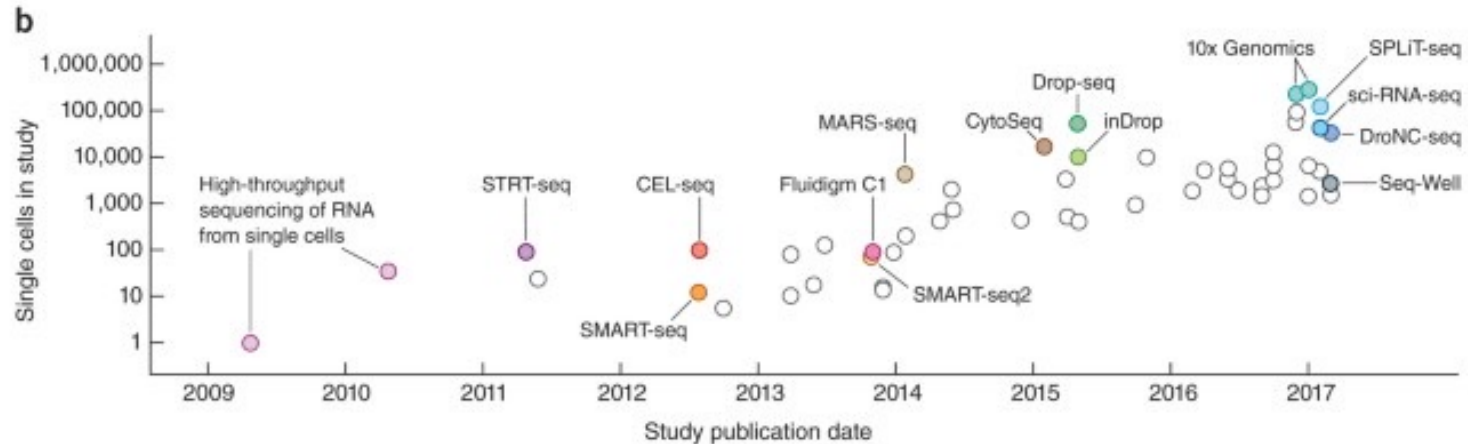
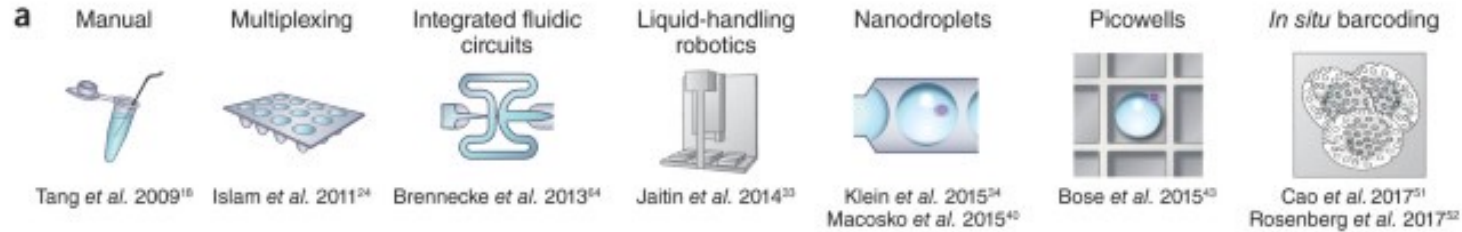


Bulk



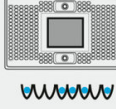
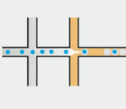
Single Cell



Single Cell RNA-Seq: a recent exploding technology



Isolating Single Cells is a big challenge

	Micro-manipulation / Automated Pipetting	FACS	Microwell encapsulation	Droplet encapsulation
				
Cell Stress	Low	Moderate	Moderate	Moderate
Selection	Yes	Yes	No* / Yes**	No*
Doublet	Low	Low	Low-High	Moderate
Throughput	Low	Moderate	Moderate	High
Capture efficiency	Low	Moderate	Moderate	Low-Moderate
Academic / Commercial scRNA workflow	- CellenONE (Cellenion) [†] - Smart-Seq2 (42)	- MARS-Seq (39) - Smart-Seq2 (42)	- C1 (Fluidigm) - ddSeq (Biorad / Illumina) - iCell8 (Clontech) ^{†*} - Rhapsody (BD)	- InDrop (1 CellBio) - DropSeq (Dolomite-bio) - 10X (Chromium)
Example of use	Fragile rare cells	Rare cells based on phenotype or marking	Large cell numbers	Large cell numbers

	FACS		Microwell encapsulation				Droplet encapsulation		
	Smart-Seq2	MARS-Seq	C1	ddSeq	iCell8	Rhapsody	InDrop	DropSeq	10X
Singlet Capture efficiency	82%	92%	39%	2.6%	37%++	Not reported	7%	Not reported	50%
Doublet rate	Not reported	2.27%	3-30%	5.8%	1.3-4%	0.6%	4%	0.36-11.3	1.6-3%
Reference	42	39	37 FWP	PB	PB	PB	36	37	26

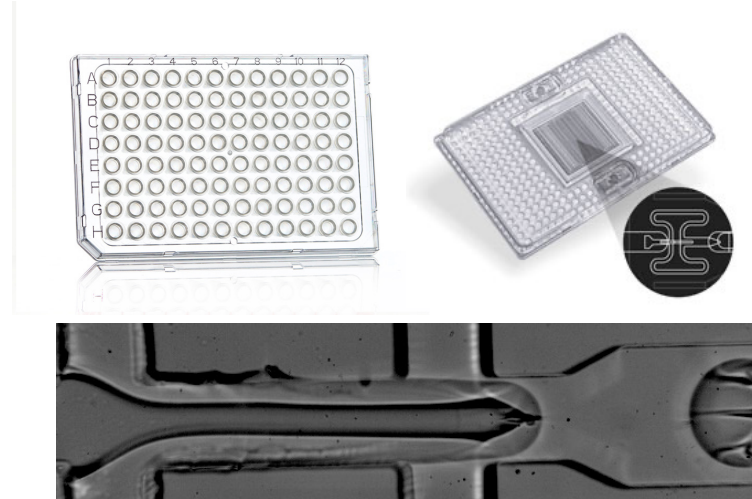
[†]Automated pipetting system

^{*}Preselection or enrichment can be performed prior

⁺⁺Only reagents added to wells containing singlets, determined by system

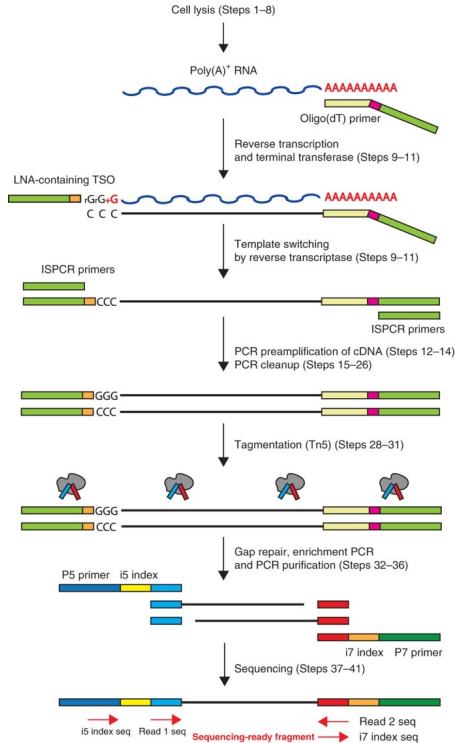
FWP: Fluidigm white paper

PB: Product brochure / manual



<https://www.frontiersin.org/articles/10.3389/fimmu.2018.01553/full>

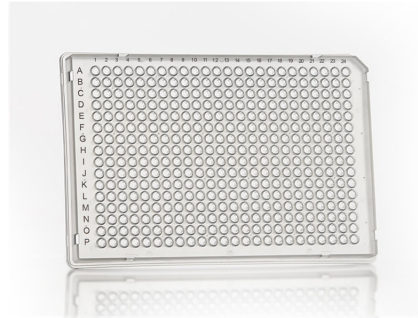
Smart-seq(2)



Smart-seq (current version V4, the most used/well known is V2) is used to sequence the **full-length transcriptome** of individual cells

Smaller throughput, usually < 1000 cells

Since fewer cells are sequenced, sequencing depth tends to be higher and thus **more genes are captured**



<https://www.nature.com/articles/nprot.2014.006>

Requires laborious lab work
Usually for detailed analysis of specific
hard to get cell populations (eg. pollen)

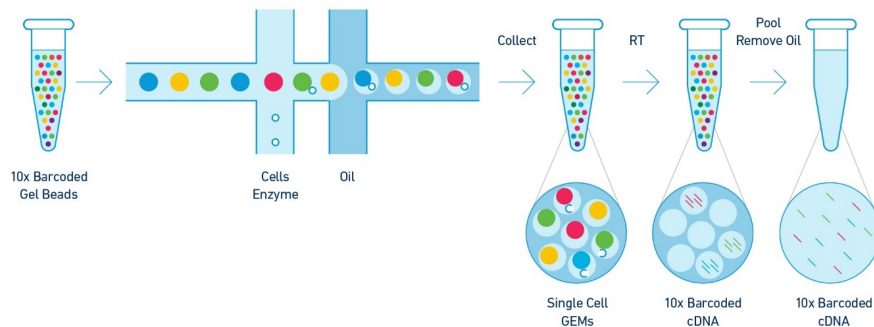
Chromium 10x Genomics

Uses microdroplet encapsulation for individual lysis and amplification

Current version is v3.1; has 3' and 5' sequencing variants (**not full-length transcripts**)

High throughput (5-10k cells/well) – good for organs or even whole small organisms

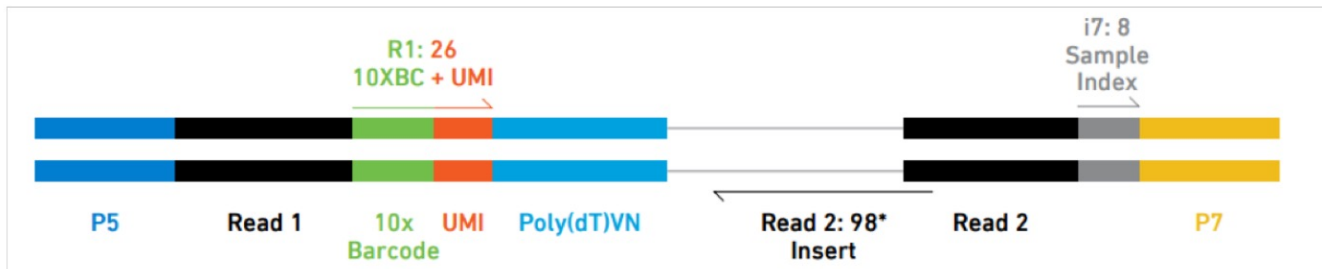
Tends to get smaller amount of RNA per cell / **noisier data**



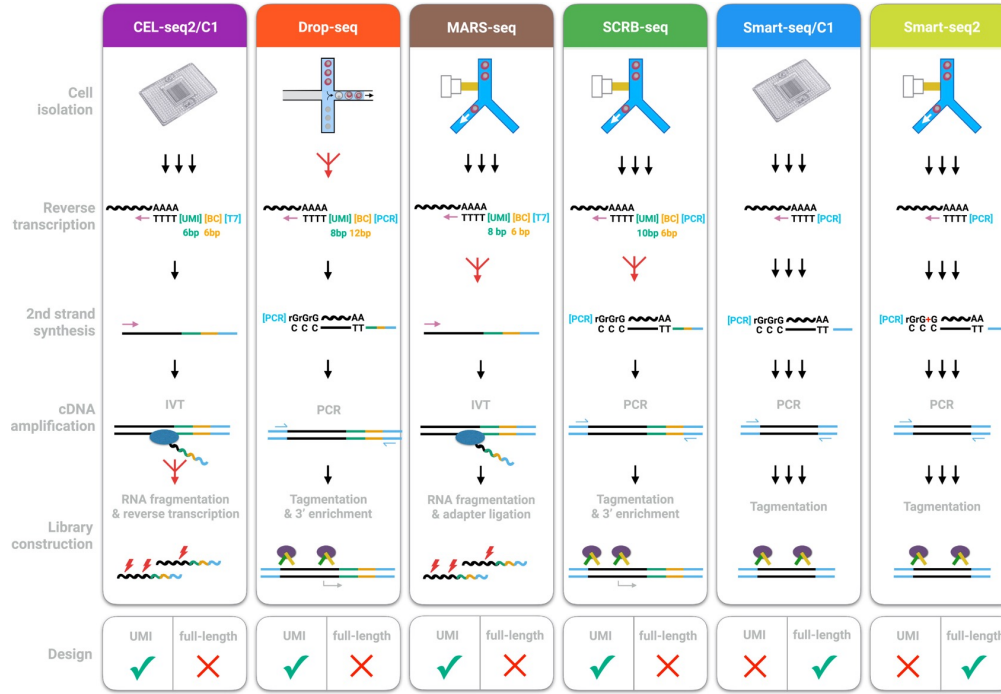
<https://www.nature.com/articles/ncomms14049>

A few extra notes

- Some applications add “spike-in” RNA
 - Used to control quality of samples, since we know the amounts of spike-in beforehand
 - Spikes often found identified as “ERCCxxx”
- The use of UMIs are becoming quite common
 - To avoid effect of amplification of small amounts of RNA (particularly for 10x genomics)
 - Each molecule is tagged with a UMI at library preparation
 - Counts with same UMI should be counted as only 1
 - Smart-Seq2 does not have it (smart-seq3 now includes)



Several factors need to be taken into account



In the end, you use what you have at your institute