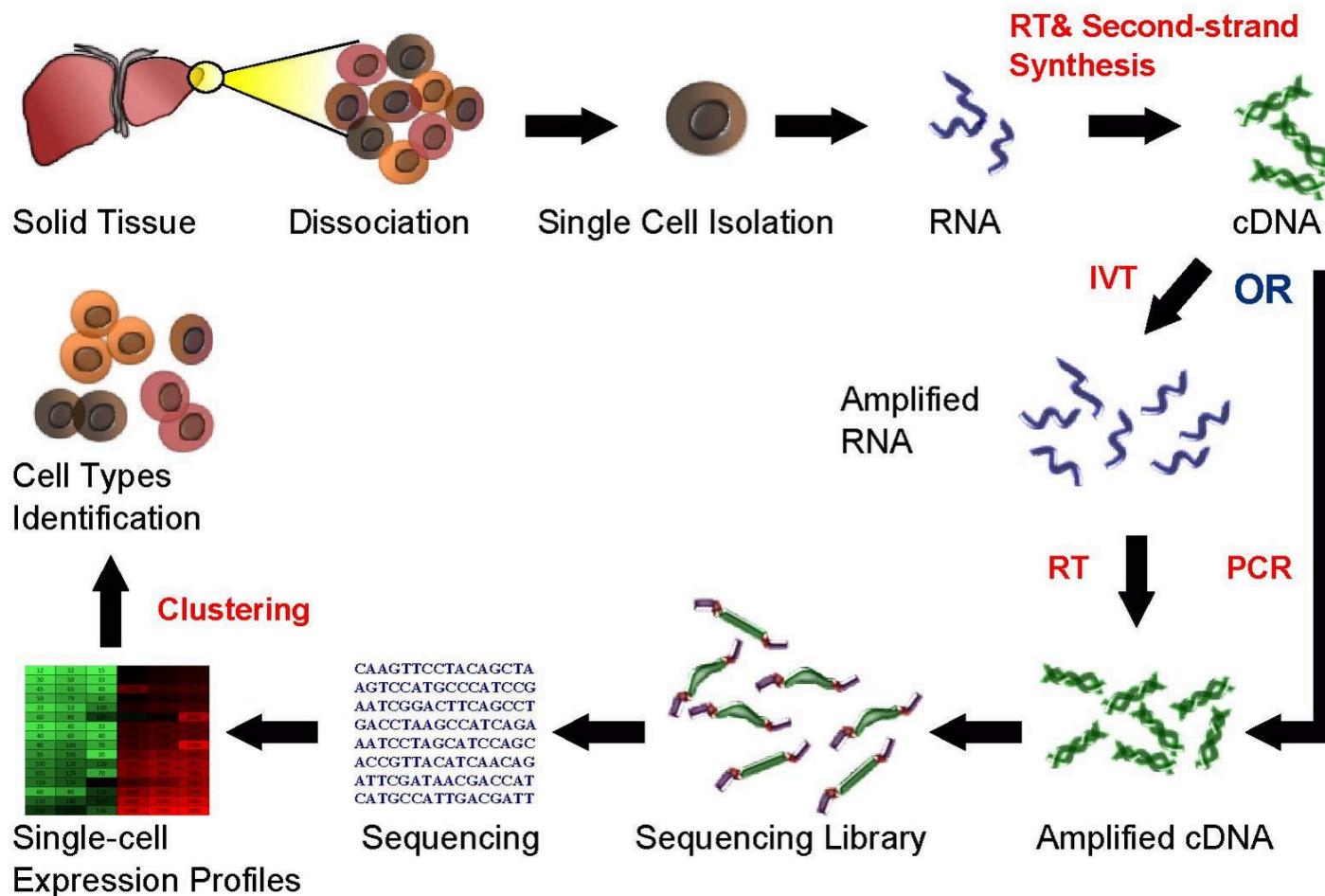


Single-cell RNA sequencing methodologies and ESCG platform

Karolina Wallenborg

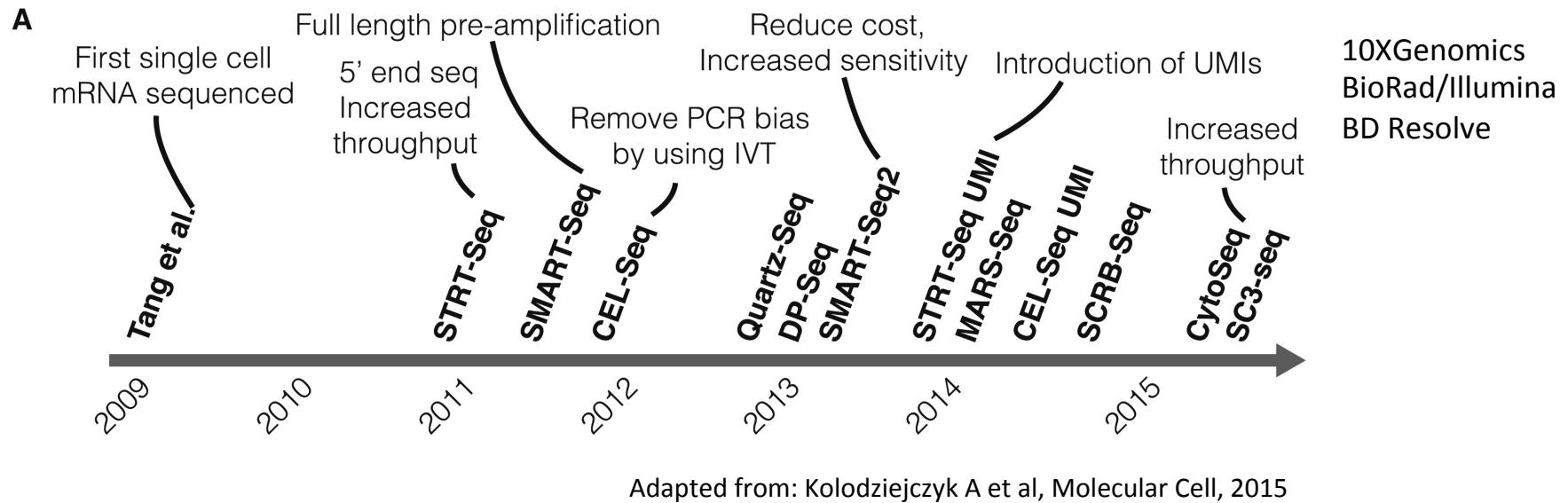
October 2, 2017

Single Cell RNA Sequencing Workflow



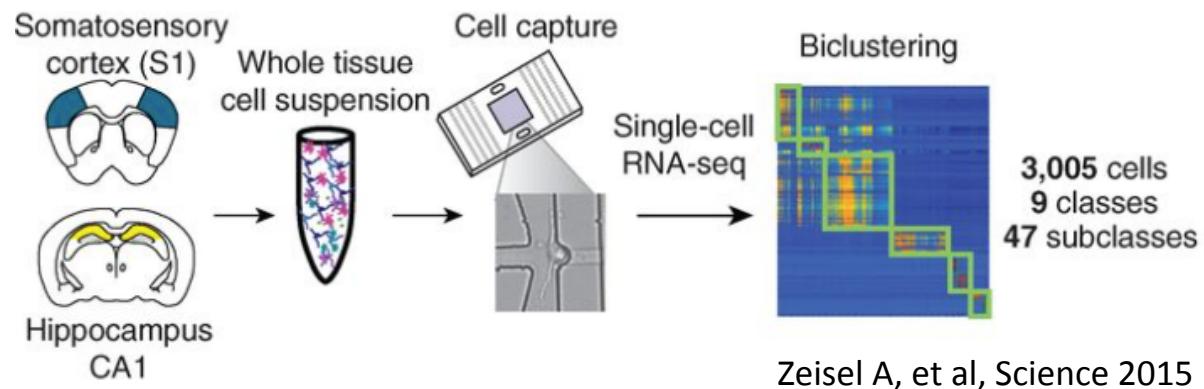
From Wikipedia

Short history of scRNA-seq

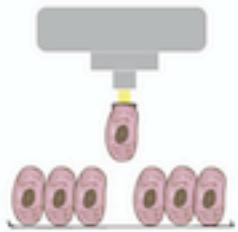
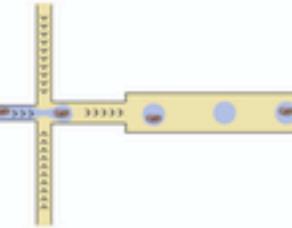
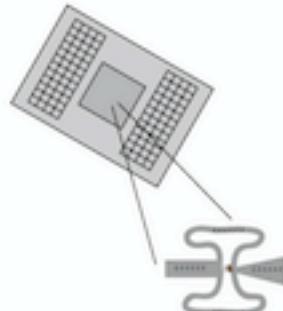


Applications for scRNA-sequencing

- Heterogeneity analysis
- Cell type identification
- Lineage tracing, cellular states in differentiation and development
- Monoallelic gene expression, splicing patterns



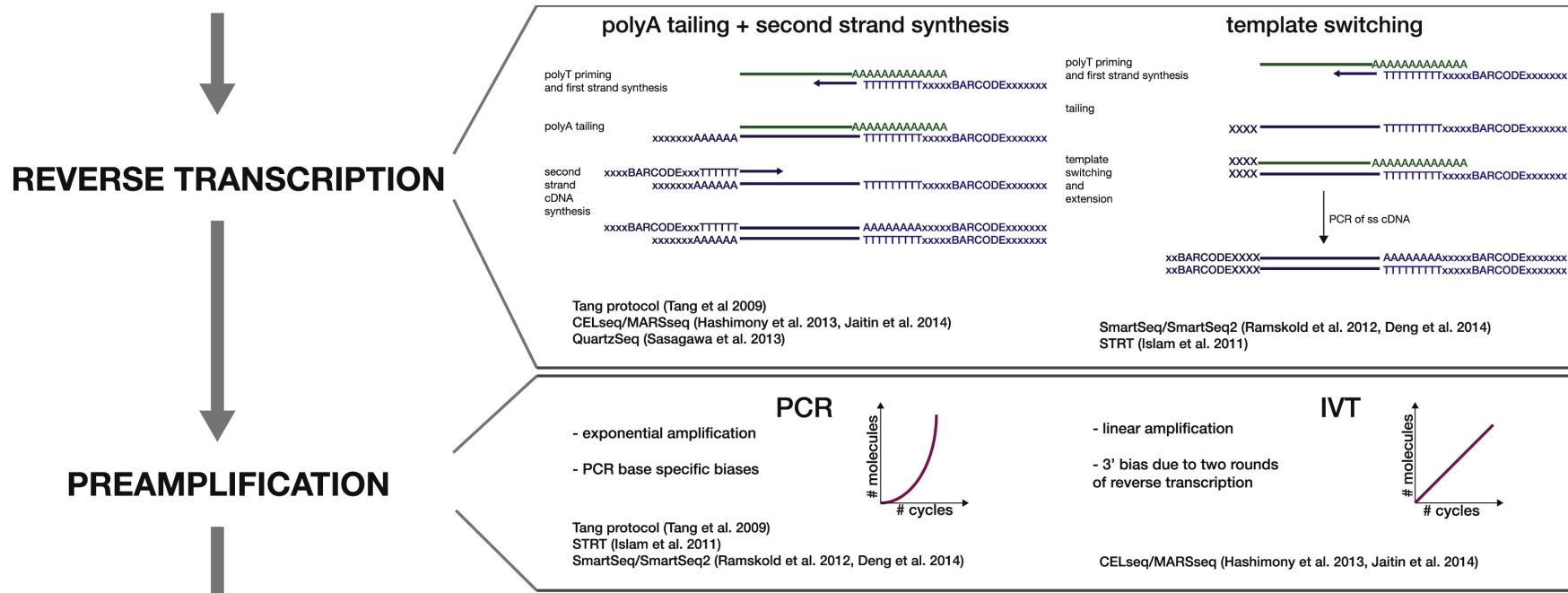
Single-cell isolation or capture

MICROPIPETTING MICROMANIPULATION	LASER CAPTURE MICRODISSECTION	FACS	MICRODROPLETS	MICROFLUIDICS e.g. FLUIDIGM C1	Multi-Sample Nano- Dispenser
					
low number of cells	low number of cells	hundreds of cells	large number of cells	hundreds of cells	Multi-Sample Nano-Dispenser
any tissue	any tissue	dissociated cells	dissociated cells	dissociated cells	Micro-well chip
enables selection of cells based on morphology or fluorescent markers	enables selection of cells based on morphology or fluorescent markers	enables selection of cells based on size or fluorescent markers	no selection of cells (can presort with FACS)	no selection of cells (can presort with FACS)	
visualisation of cells	visualisation of cells	fluorescence and light scattering measurements	fluorescence detection	visualisation of cells	
time consuming	time consuming	fast	fast	fast	
reaction in microliter volumes	reaction in microliter volumes	reaction in microliter volumes	reaction in nanoliter volumes	reaction in nanoliter volumes	

Cytoplasmic aspiration

Adapted from: Kolodziejczyk A et al, Molecular Cell, 2015

Reverse transcription and amplification



Adapted from: Kolodziejczyk A et al, Molecular Cell, 2015

- Poly(T) primer
- Single cell contain ~10 pg total RNA
- 1-5% is mRNA
- 10-20% of the transcripts get reverse transcribed

Current scRNA-sequencing protocols

cDNA-amplification protocols		
Full-length	5'-end focused	3'-end focused
<ul style="list-style-type: none">• SMART-seq• SMART-seq2• Nugen Ovation	<ul style="list-style-type: none">• STRT• STRT-C1	<ul style="list-style-type: none">• CEL-seq• MARS-seq• Quartz-seq• Drop-seq

Adapted from Poulin JF et al, Nature Neuroscience, 2016

Single-cell RNA-sequencing protocols

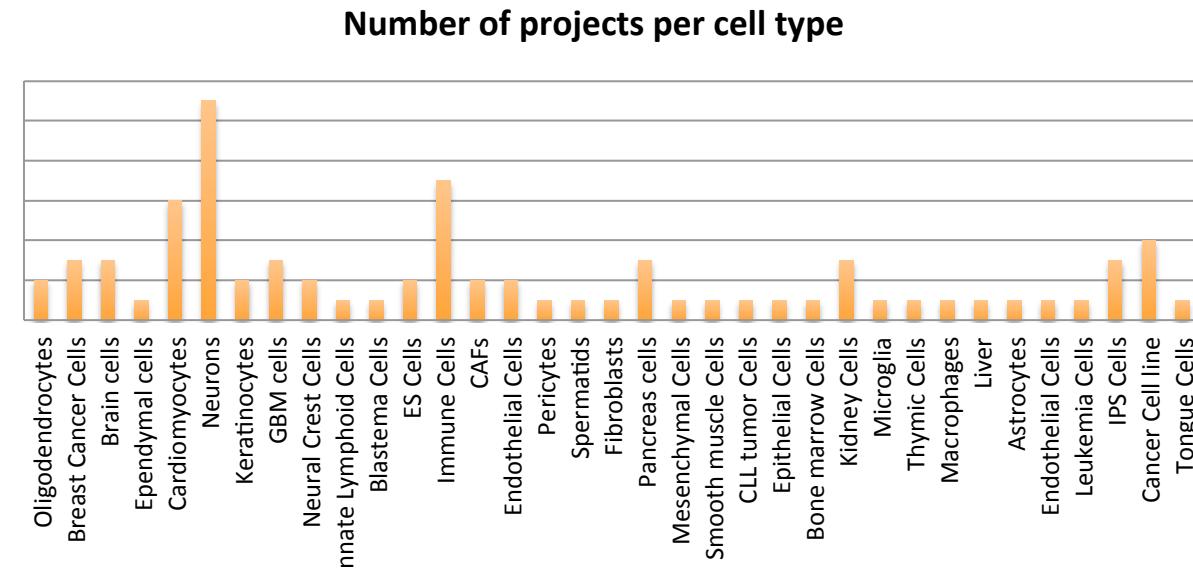
-Which method suits you?

- **Full-length**
 - Whole transcript information
 - Gene expression quantification
 - Isoform, SNP and mutations
- **Tag-based methods (5' or 3')**
 - Estimate of transcript abundance
 - Early multiplexing
 - Combined with molecular counting
 - Retain DNA strand information



ESCG platform

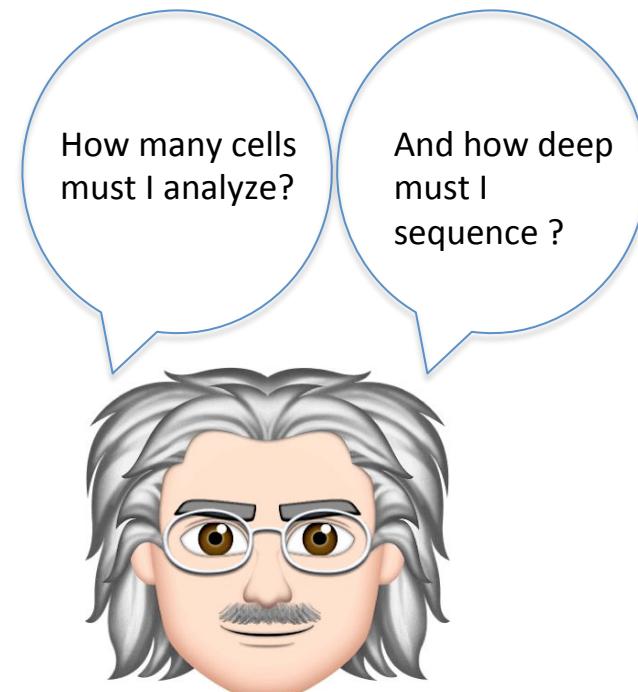
- Started in 2015
- Sten Linnarsson (STRT-seq, STRT/C1), Rickard Sandberg (Smart-seq2)
- High throughput single-cell RNA-sequencing
- 280,000 single cells sequenced
- 77 projects



How do you get started?

User meeting

- Project discussion
 - Feasibility
 - Tissue, cells
 - Project size
 - Time line
- Choice of method
 - Data output
 - Number of cells to be analyzed
 - Location, cell delivery
- Bioinformatics
 - Early contact
 - National Bioinformatics Infrastructure Sweden (NBIS)
- Data delivery
- User fees



Single cell submission guidelines

Optimize your cell isolation protocol

- Limit time of isolation
- Be gentle

Single cell suspension criteria

- High viability (>80%)
- No cell clumps or debris
- Cell strain and wash

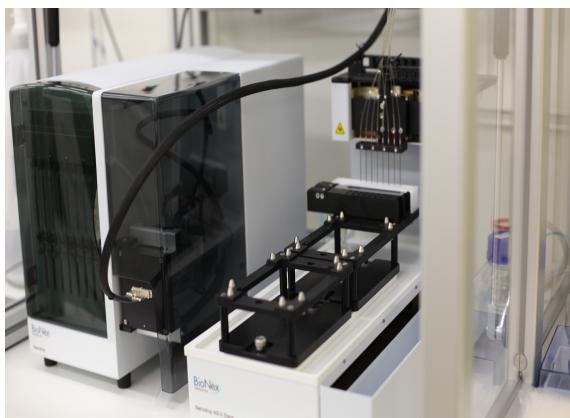
FACS facility

- Cell viability stain

Visit us

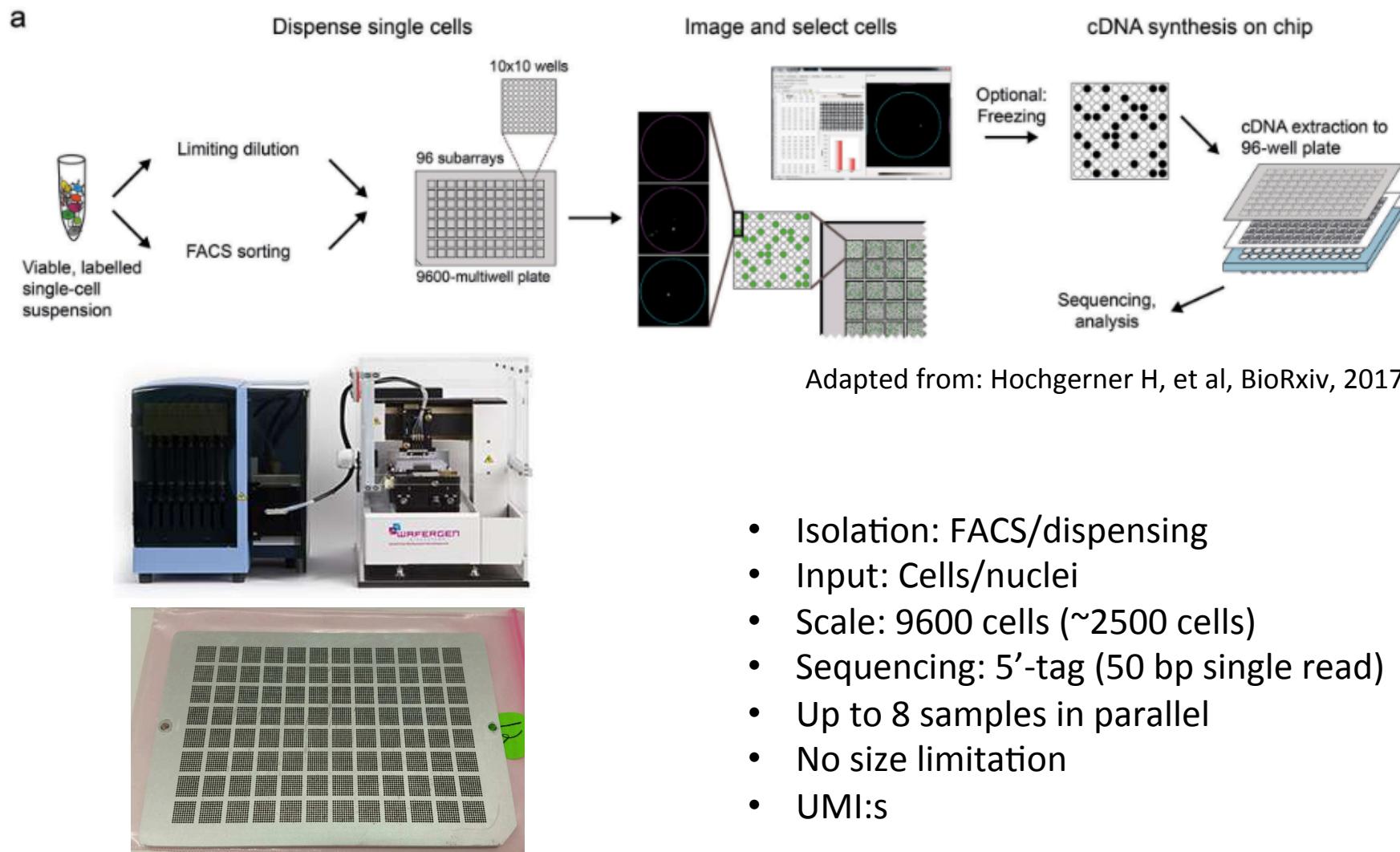
- Single cell suspension quality control

Smart-seq2 at ESCG



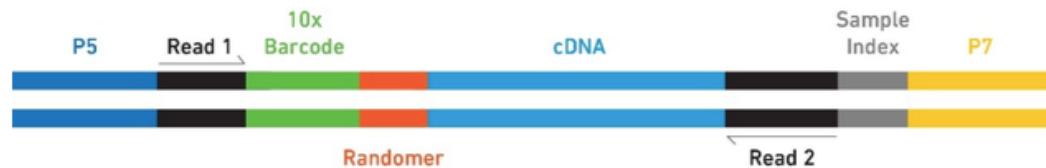
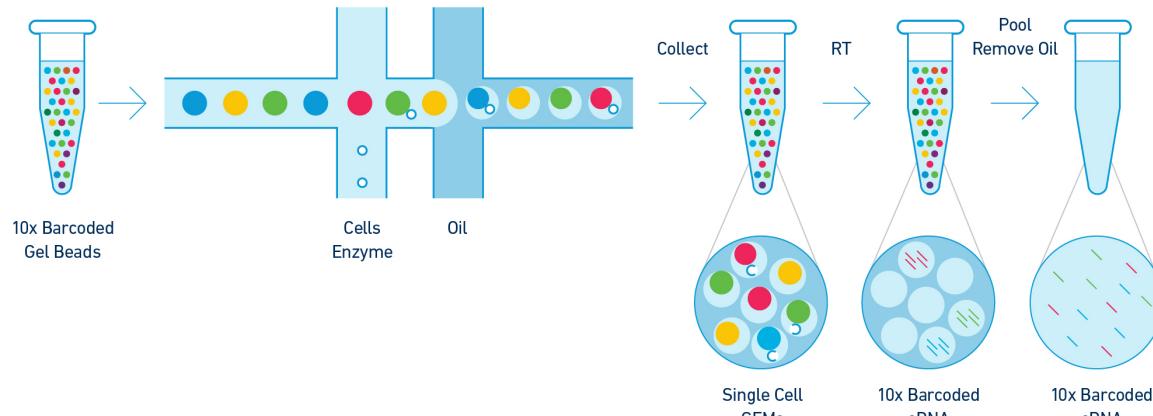
- 384 well plates
- Isolation: FACS
- Input: cells/nuclei
- Full-length
- Sequencing: 50bp single-read
- ERCC spike-ins
 - Two different dilutions
- Flexible delivery

STRT-seq-2i: dual-index 5' single-cell RNA-sequencing



10X Genomics

-Drop-seq technology



- Isolation: Droplets
- Input: Cells/nuclei
- Scale: 500-10,000 x 8
- Sequencing: 3'-tag (HiSeq2500/NovaSeq)
- Up to 8 samples in parallel
- Validated up to 30 μ m (channels 50 μ m)
- UMI, cell barcode, sample barcode
- CellRanger, Loupe, R-package

Comparing our services

	Full-length	Quantitative	
	Smart-seq2	STRT-Wafergen	10xGenomics
Format	Eppendorf Twin-tek	Microwell chip	Chromium microfluidics chip
Cell number	384	9,600 (~2,500)	8 x 500-10,000
Input	FACS-sorted cells	Suspension	Suspension
Transcript coverage	Full-length	5'	3'
Features	<ul style="list-style-type: none"> • Flexible delivery • Isoforms, SNPs, mutations • Nuclei • ERCC spike-ins 	<ul style="list-style-type: none"> • Limiting dilution/ FACS • Cell selection • Unbiased • 8 samples parallel • Nuclei 	<ul style="list-style-type: none"> • High throughput • 8 samples parallel • Nuclei • Sample pooling

Data delivery

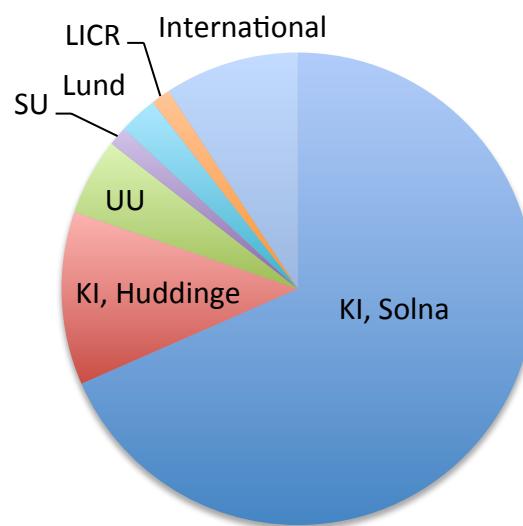
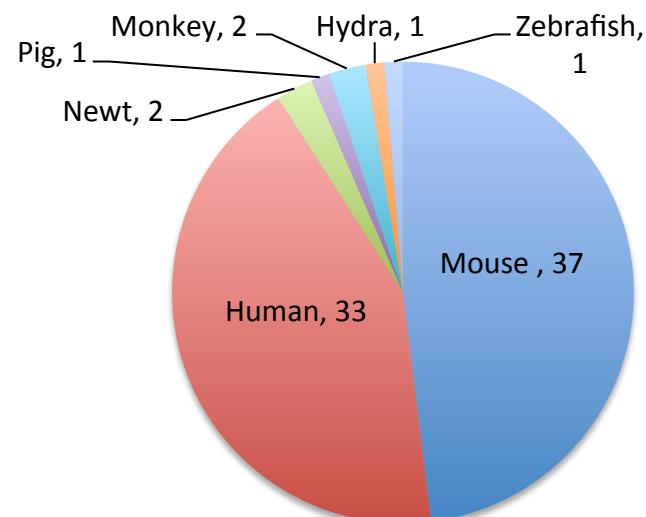
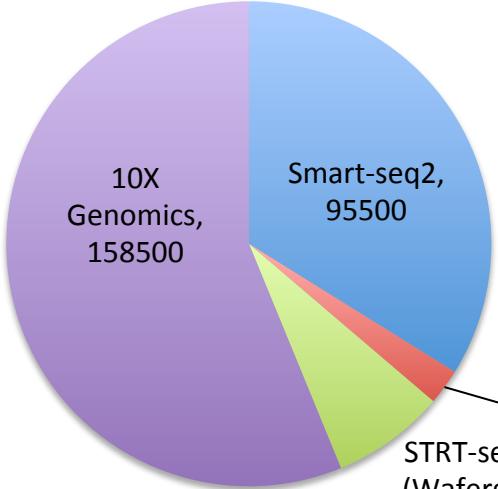
- Sequencing at NGI, HiSeq2500, NovaSeq
- Analysis pipelines for mouse and human
 - In-house: STRT, smart-seq2
 - Cell ranger: 10xGenomics
- UPPMAX, Bioinformatics compute and storage
 - Users apply individually for projects
 - Annotated gene expression data, QC-files, Fastq
- Bioinformatics
 - Done by user
 - Support from BILS and WABI
 - Collaborations

User fees

Smart-seq2	STRT-Wafergen	10XGenomics
384 well plate	9600 wells chip (~2,500 cells)	1 sample (~3,000 cells)
<ul style="list-style-type: none">• Validation• Smart-seq2 library• Sequencing• (50 bp, single-read)	<ul style="list-style-type: none">• Validation• STRT library (dual index)• Sequencing (50 bp single-read)	<ul style="list-style-type: none">• Validation• Illumina library• Sequencing (paired-end, dual index)
~40,000 SEK	~50,000 SEK	~42,000 SEK

Costs include: Reagents, consumables, instrument depreciation, instrument service, personnel. Overhead is not included.

ESCG Statistics



Cell Metab. 2016 Oct 11;24(4):593-607. doi: 10.1016/j.cmet.2016.08.020. Epub 2016 Sep 22.

Single-Cell Transcriptome Profiling of Human Pancreatic Islets in Health and Type 2 Diabetes.

Segerstolpe Å¹, Palasantza A², Eliasson P³, Andersson EM³, Andréasson AC³, Sun X⁴, Picelli S⁵, Sabirsh A³, Clausen M⁶, Bjursell MK⁷, Smith DM⁸, Kasper M⁴, Ämmälä C³, Sandberg R⁹.

Science. 2017 Jul 7;357(6346). pii: eaal3753. doi: 10.1126/science.aal3753.

Multipotent peripheral glial cells generate neuroendocrine cells of the adrenal medulla.

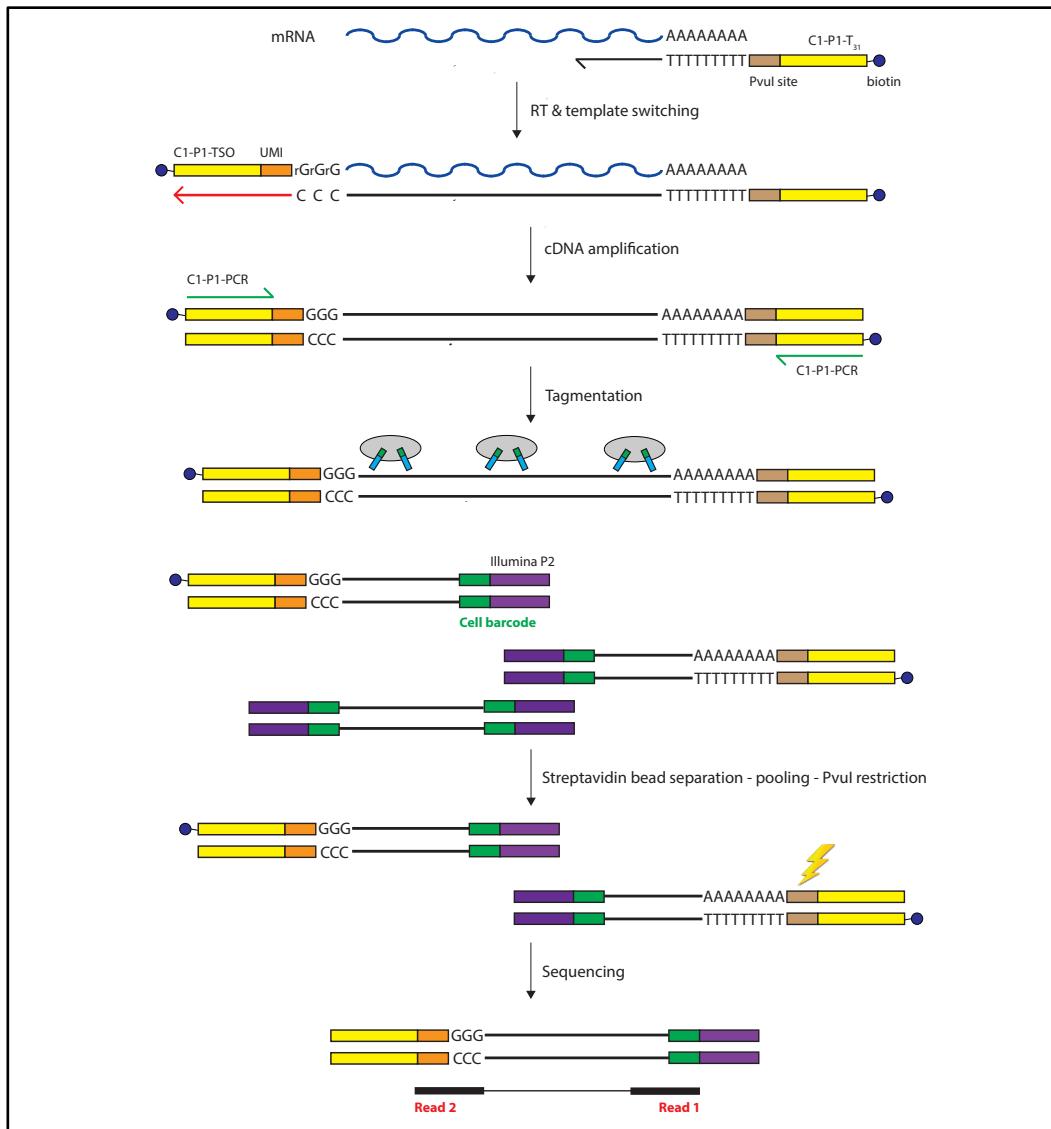
Furlan A¹, Dyachuk V^{2,3,4}, Kastriti ME⁵, Calvo-Enrique L¹, Abdo H¹, Hadjab S², Chontorotzea T⁵, Akkuratova N^{6,7}, Usoskin D¹, Kamenev D², Petersen J^{5,8}, Sunadome K⁵, Memic F¹, Marklund U¹, Fried K², Topilko P⁹, Lallemand F², Kharchenko PV¹⁰, Ernfors P¹¹, Adameyko I^{12,8}.

What lays ahead?

- Emerging techniques
 - Single nuclei RNA-sequencing
 - Single cell ATAC-seq
 - Transcriptome + Epigenome
 - Transcriptome + Proteome
 - CRISPR-Cas9 + Transcriptome
 - ‘split-pooling’ scRNA-seq
- Validation
 - Small molecule FISH
- Human Cell Atlas

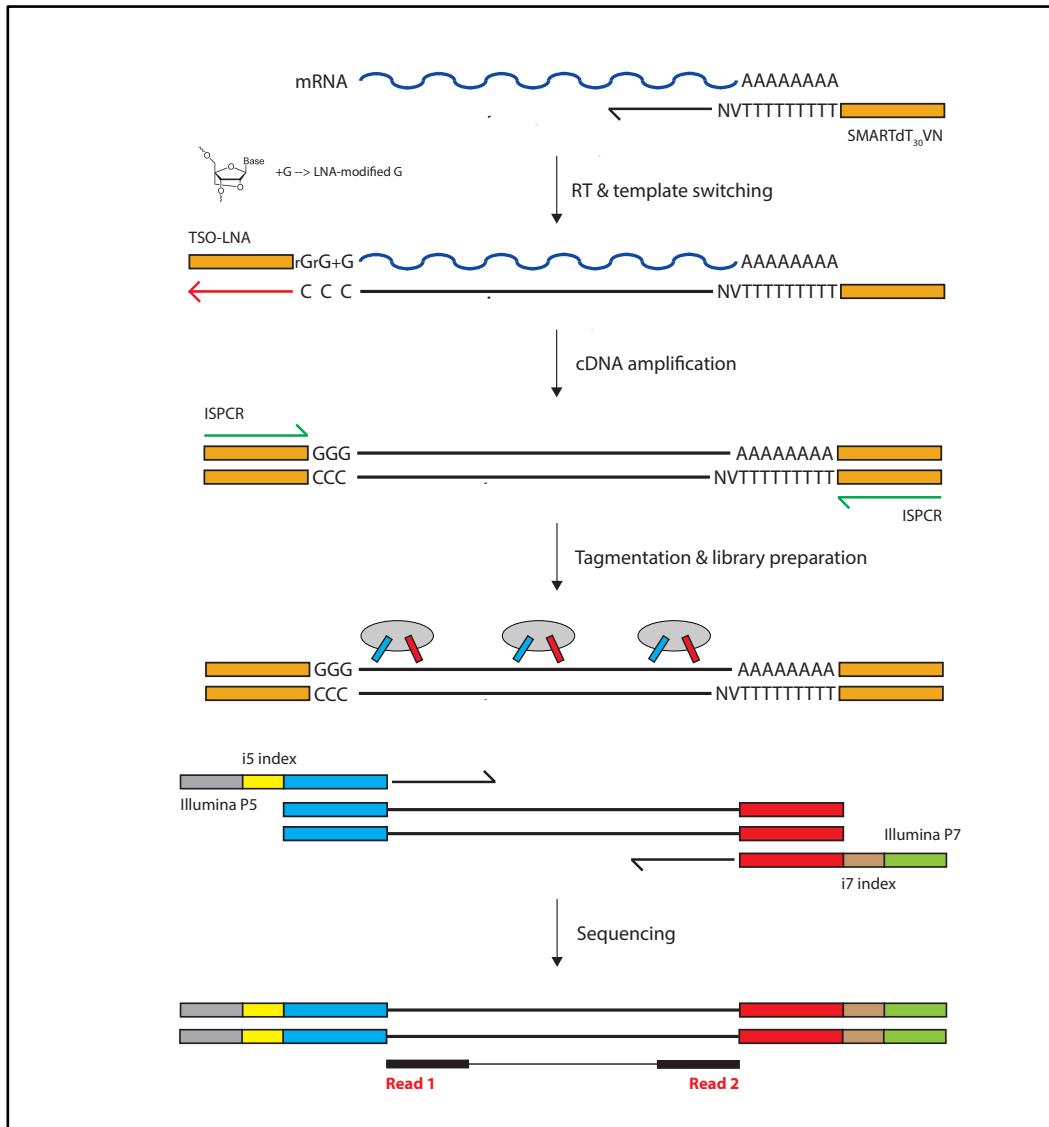


The *STRT/C1* method



Modified from: Picelli (2016), RNA Biology, July 21: 1-14

The Smart-seq2 method



Modified from: Picelli (2016), RNA Biology, July 21: 1-14