Henrik Gezelius
2019-02-04
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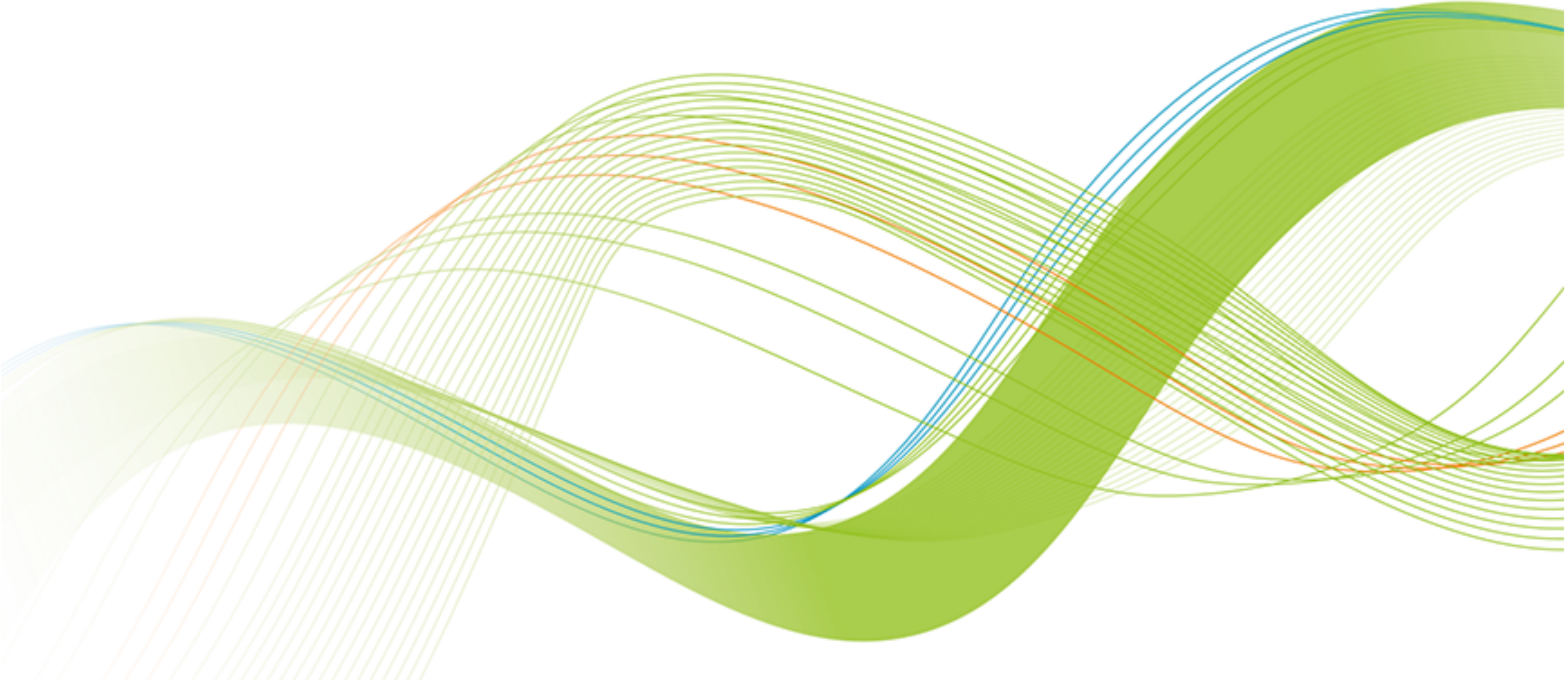
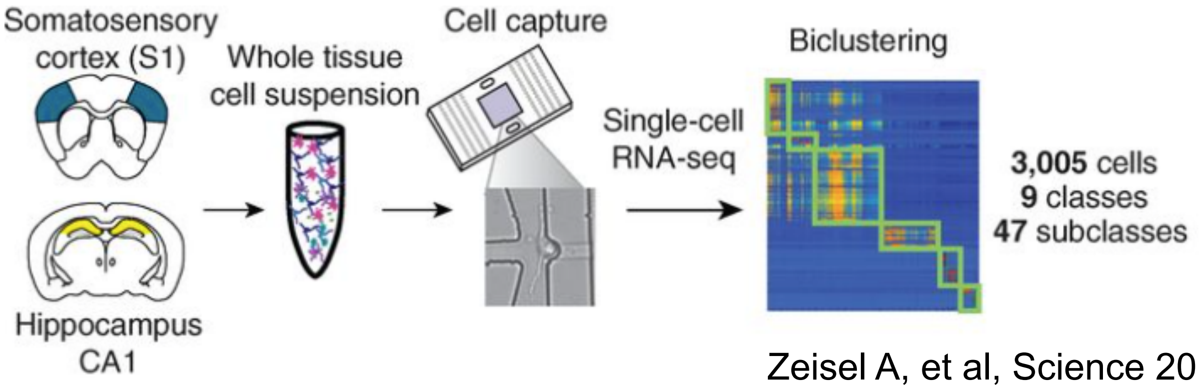
Single-cell transcriptomics (scRNA-seq)
Eukaryotic Single Cell Genomics facility



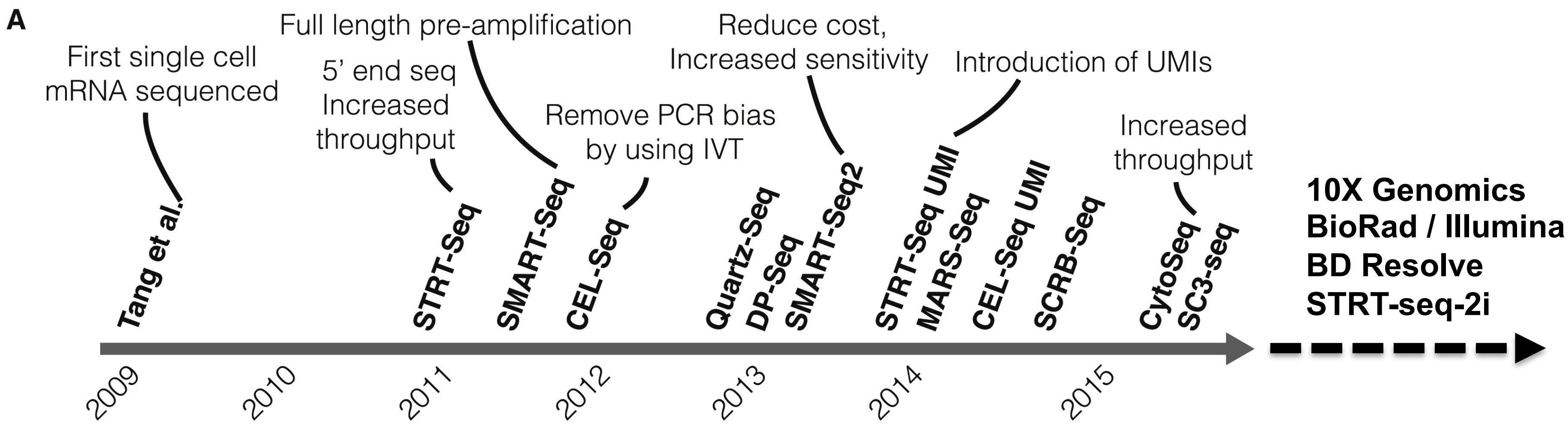
Applications for scRNA-sequencing

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



Zeisel A, et al, Science 2015

Short history of scRNA-seq



A

First single cell mRNA sequenced

Tang et al.

5’ end seq
Increased throughput

STRT-Seq

Full length pre-amplification

SMART-Seq

Remove PCR bias by using IVT

CEL-Seq

Quartz-Seq
DP-Seq

Reduce cost, Increased sensitivity

SMART-Seq2

Introduction of UMIs

STRT-Seq UMI
MARS-Seq
CEL-Seq UMI
SCRB-Seq

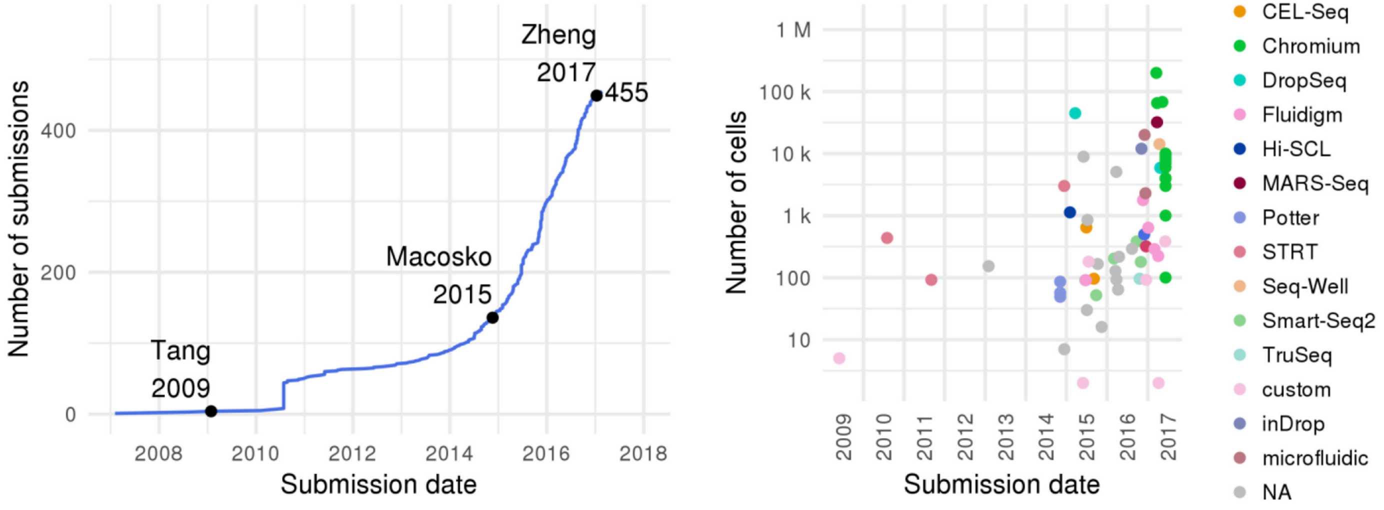
Increased throughput

CytoSeq
SC3-seq

10X Genomics
BioRad / Illumina
BD Resolve
STRT-seq-2i

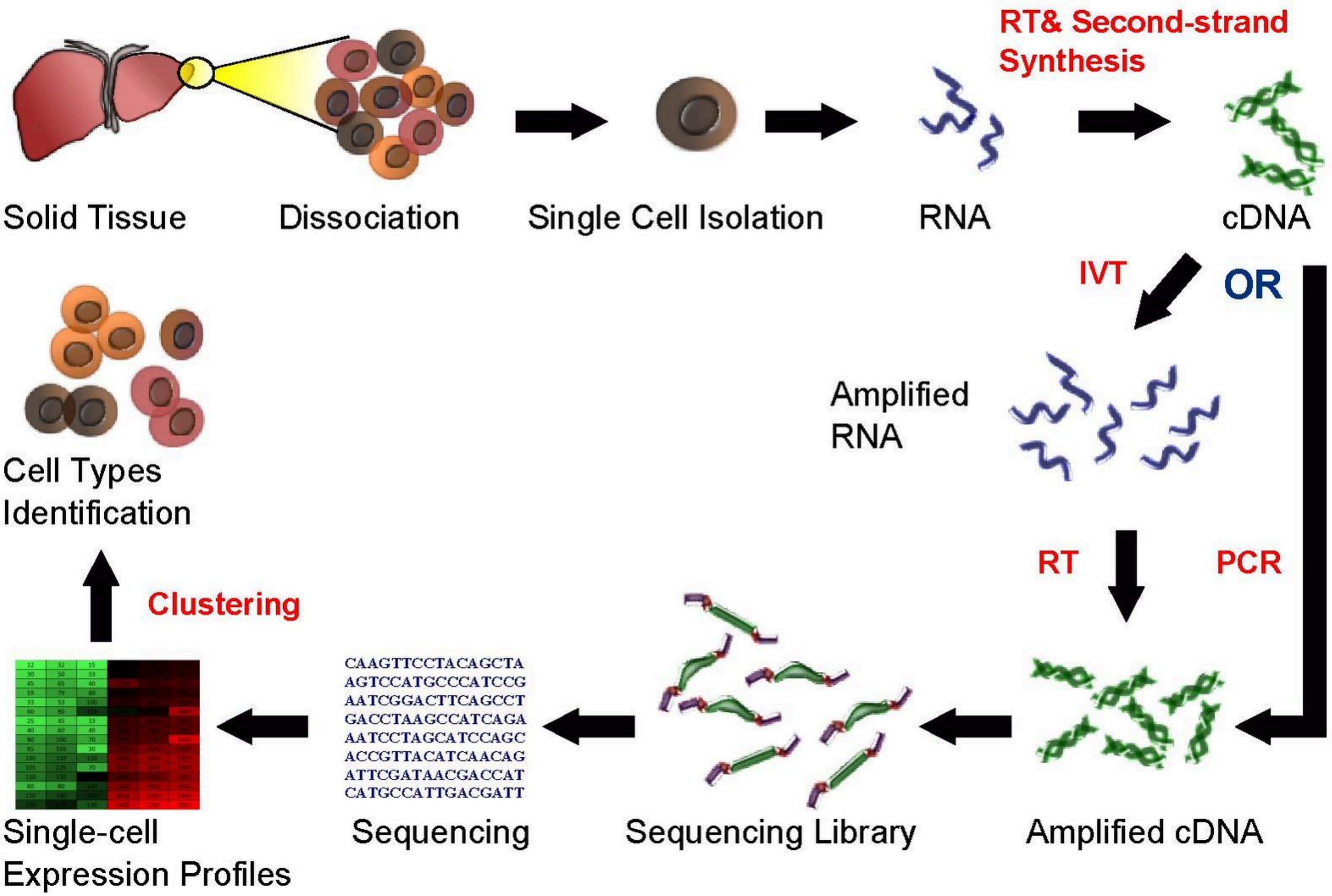
2009
2010
2011
2012
2013
2014
2015

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



Angerer et al, Curr Opin Sys Biol 2017

Single cell RNA seq workflow



RT& Second-strand Synthesis

Solid Tissue

Dissociation

Single Cell Isolation

RNA

cDNA

IVT

OR

Amplified RNA

RT

PCR

Amplified cDNA

Sequencing Library

CAAGTTCCTACAGCTA
AGTCCATGCCCATCCG
AATCGGACTTCAGCCT
GACCTAAGCCATCAGA
AATCCTAGCATCCAGC
ACCGTTACATCAACAG
ATTCGATAACGACCAT
CATGCCATTGACGATT

Sequencing

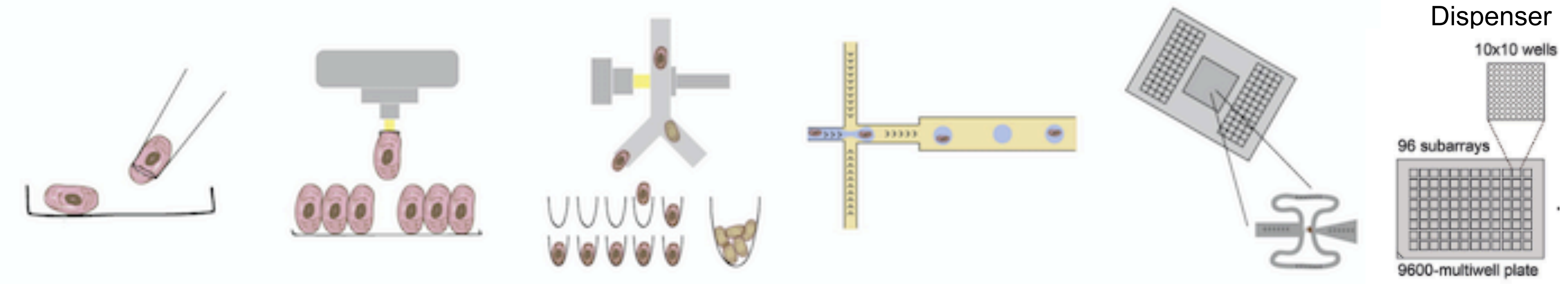
Single-cell Expression Profiles

Clustering

Cell Types Identification

From Wikipedia

Single-cell isolation or capture

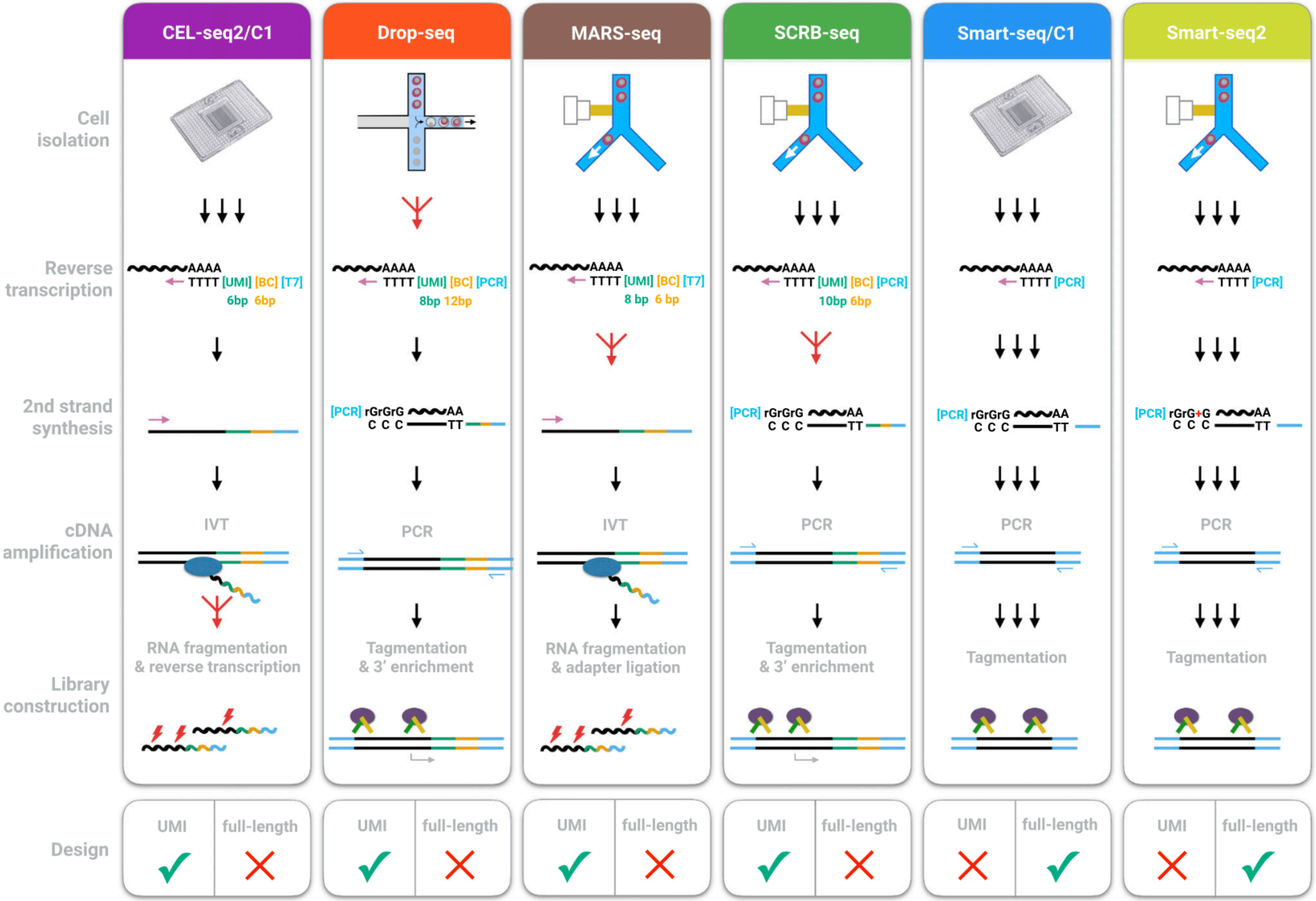


MICROPIPETTING MICROMANIPULATION	LASER CAPTURE MICRODISSECTION	FACS	MICRODROPLETS	MICROFLUIDICS e.g. FLUIDIGM C1	Multi-Sample Nano-Dispenser
low number of cells	low number of cells	hundreds of cells	large number of cells	hundreds of cells	large number of cells
any tissue	any tissue	dissociated cells	dissociated cells	dissociated cells	dissociated cells
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)	no selection
visualisation of cells	visualisation of cells	fluorescence and light scattering measurements	fluorescence detection	visualisation of cells	visualisation of cells
time consuming	time consuming	fast	fast	fast	fast
reaction in microliter volumes	reaction in microliter volumes	reaction in microliter volumes	reaction in nanoliter volumes	reaction in nanoliter volumes	reaction in nanoliter volumes

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

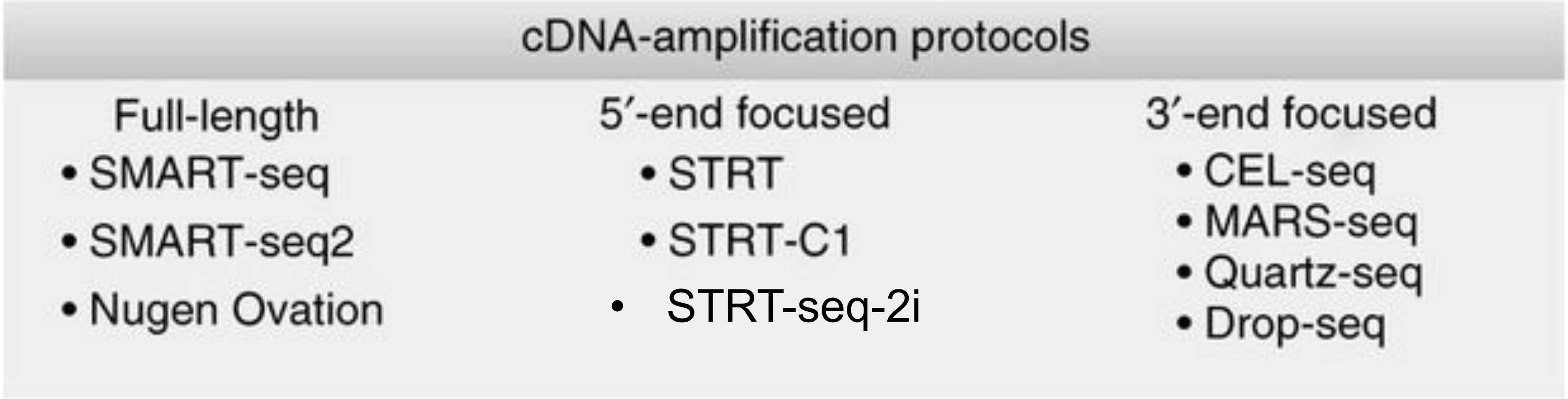
- **Cytoplasmic aspiration**
- **Patch-seq**

scRNA-sequencing protocol examples



Zieghain et al. Mol Cell 2017

scRNA-sequencing protocols



cDNA-amplification protocols

Full-length	5′-end focused	3′-end focused
• SMART-seq	• STRT	• CEL-seq
• SMART-seq2	• STRT-C1	• MARS-seq
• Nugen Ovation	• STRT-seq-2i	• Quartz-seq
		• Drop-seq

Adapted from Poulin JF et al, Nature Neuroscience, 2016

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

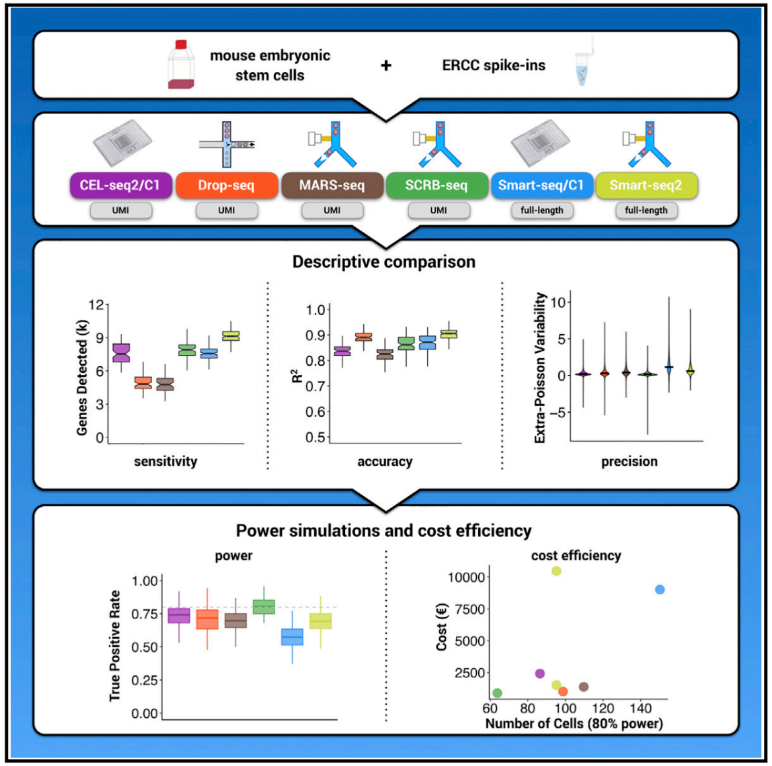
Single-cell RNA-sequencing protocols

-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

Comparison between methods

Comparative Analysis of Single-Cell RNA Sequencing Methods



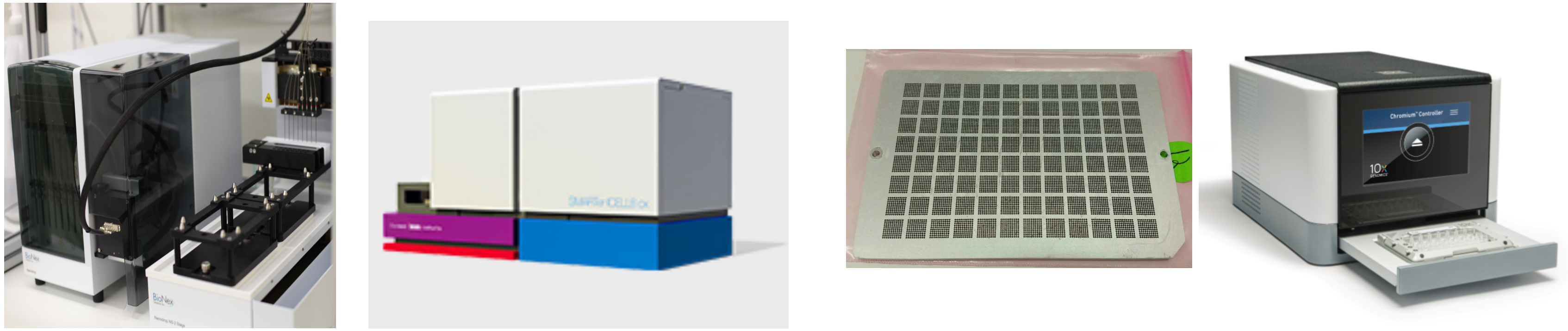
Zieghain et al. Mol Cell 2017

- Drop-seq is preferable when quantifying transcriptomes of large numbers of cells with low sequencing depth.
- (SCRB-seq and MARS-seq is preferable when quantifying transcriptomes of fewer cells.)
- Smart-seq2 is preferable when annotating and/or quantifying transcriptomes of fewer cells.
- STRT-seq / STRT-seq-2i not included in comparison.

ESCG facility platform

- Started in 2015
- Sten Linnarsson (STRT-seq, STRT/C1, STRT-seq-2i), Rickard Sandberg (Smart-seq2)

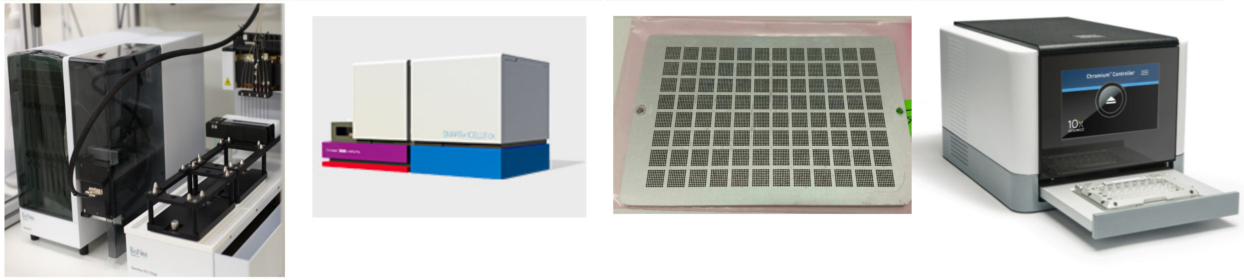
- High throughput single-cell RNA-sequencing, ATAC-seq and immune profiling
- Over 320,000 single cells sequenced (in March 2018)



ESCG facility scRNA-seq services

- From single cell suspension or FACSed cells
- cDNA generation and QC (no QC for Smart-seq ICELL8)
- Library preparation
- Sequencing
- Data de-multiplexing and alignment to ref genome (human and mouse)

	Full-length		Quantitative	
Method	**Smart-seq2**	**Smart-Seq ICELL8**	**STRT-seq-2i**	**10xGenomics**
Format	384-well plate	Microwell chip	Microwell chip	Chromium microfluidics chip
Input	FACS-sorted cells	Suspension	Suspension / FACS	Suspension
Transcript coverage	Full-length	Full-length	5’	3’

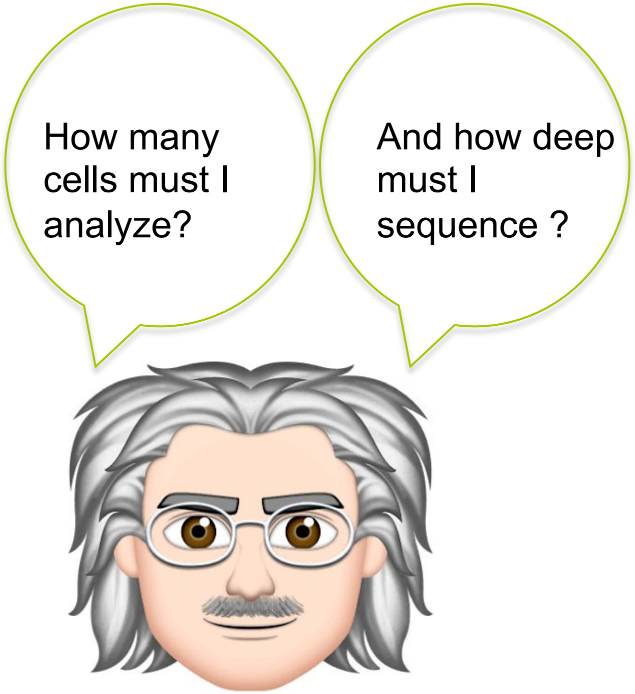


How do you get started?

SciLifeLab

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



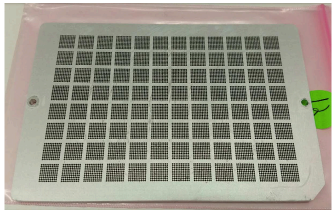
Single cell submission guidelines

- 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 before
  - Single cell suspension quality control

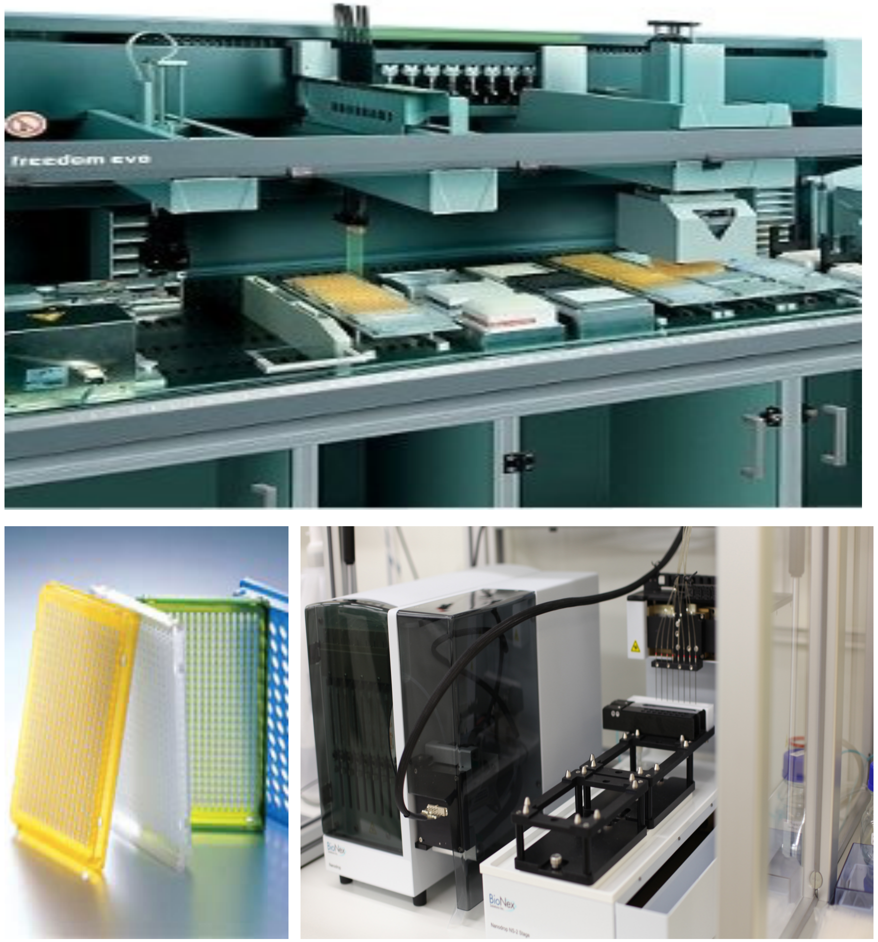
ESCG facility RNA-seq services

SciLifeLab

	FULL-LENGTH		QUANTITATIVE	
Method	Smart-seq2	Smart-Seq ICELL8	STRT-seq-2i (WaferGen)	Drop-seq (10XGenomics)
Format	384-well plate	Microwell chip (up to 8 samples)	Microwell chip (up to 8 samples)	Chromium microfluidics chip
Cells per run	384	Up to 1800	Up to 3000	8 x 500-10,000
Sample format	FACS dispensed cell/ nuclei	Fresh cells or nuclei suspensions	Fresh cell suspensions Nuclei suspensions	
Cell selection	Yes (FACS)	Yes (imaging)	Yes (imaging/FACS)	No
Transcript coverage	Full-length	Full-length	5’	3’
Reads per cell	~500k		~50k-100k	~50k-100k

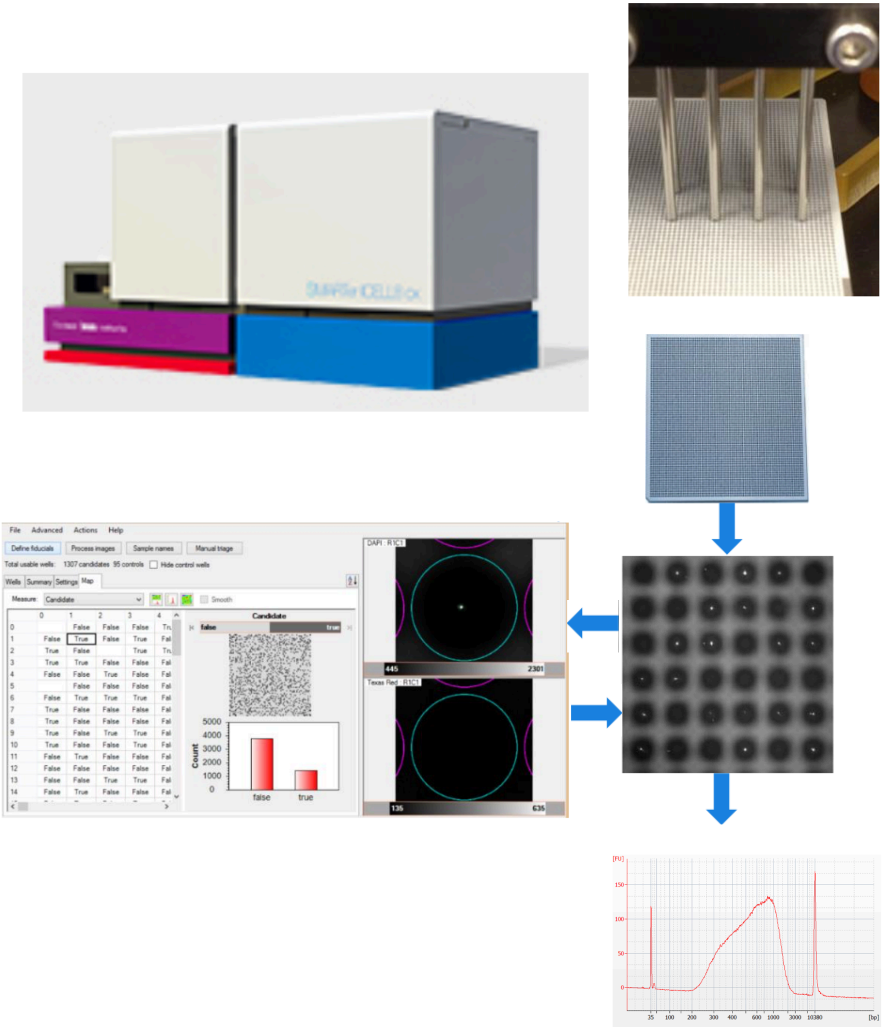


Smart-seq2 at ESCG



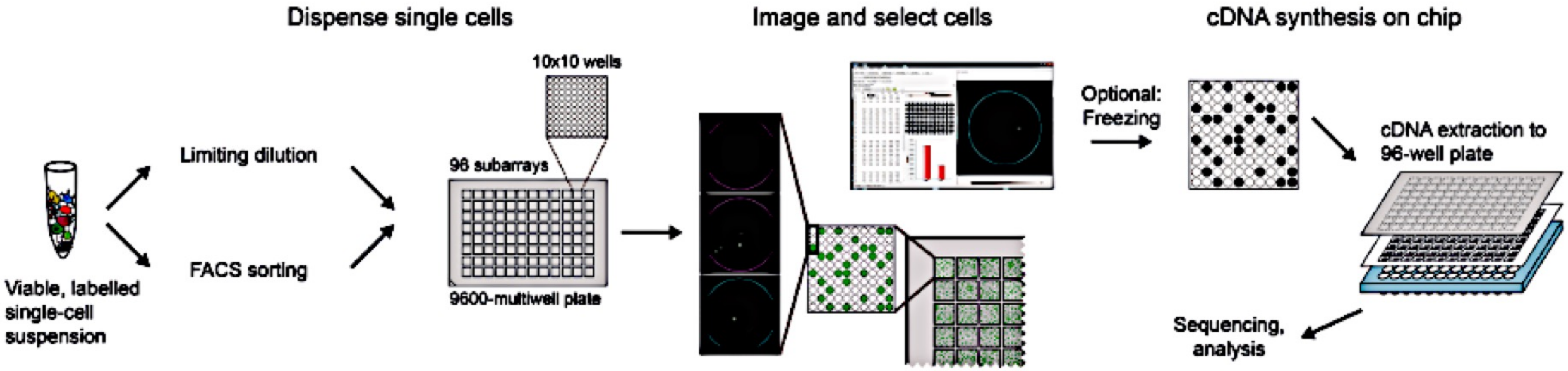
- 384 well plates
- Input: cells / nuclei
- Isolation: FACS
- Full-length
- Sequencing: 50bp single-read
- ERCC spike-ins
- Flexible delivery (shipment)

ICELL8 cx Smart-seq

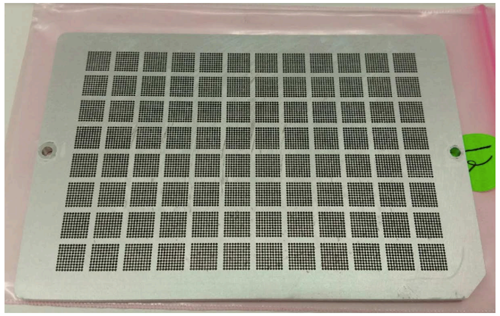


- On-chip, low volume reactions
- Input: cells / nuclei
- Isolation: Limiting dilution dispense
- Image-based cell selection
  - 1-3 fluorescent channels
  - Std. Hoechst (live) / PI (dead)
- Scale: 5184 wells (~1500 cells)
- Up to 8 samples per chip
- Full-length
- Sequencing:
- No size limitation

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

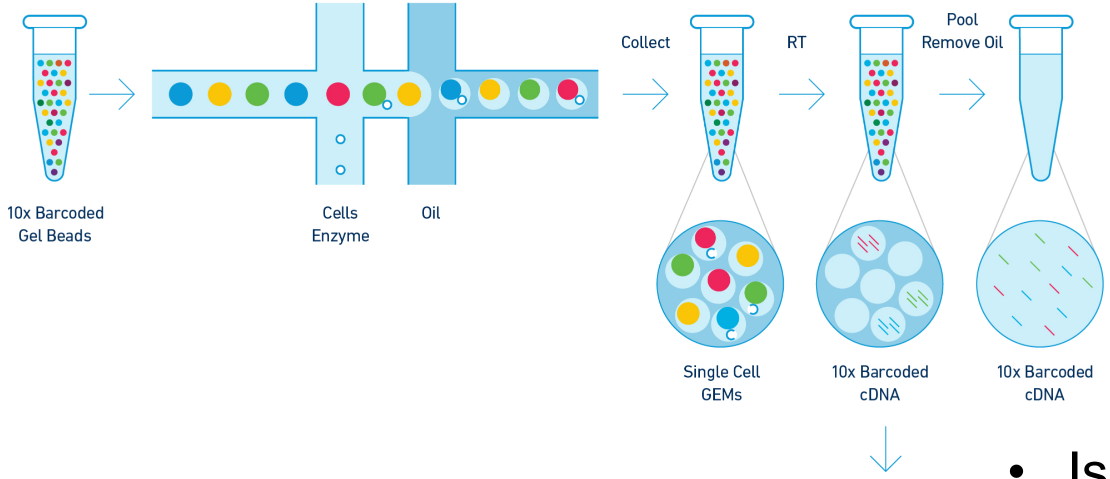


Adapted from: Hochgerner H, et al, SciRep, 2017

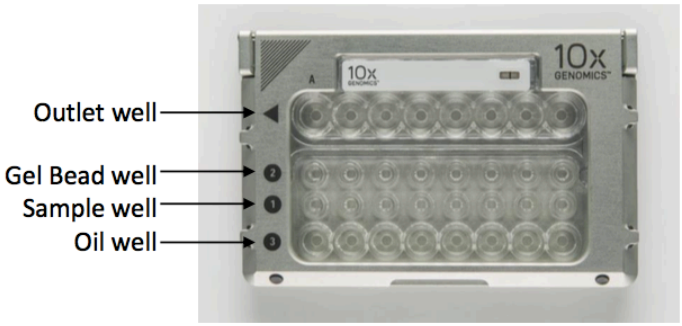


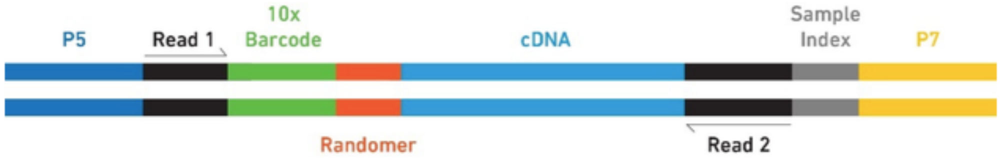
- Isolation: FACS/dispensing
- Input: cells / nuclei
- Scale: 9600 wells (~2500 cells)
- Up to 8 samples per chip
- Sequencing: 5’-tag (50 bp single read)
- No size limitation
- UMI:s

10X Genomics Chromium -Drop-seq technology



Single-use microfluidics chip





- Isolation: Droplets
- Input: cells / nuclei
- Scale: 500-10,000 x 8
- Sequencing: 3’-tag (HiSeq2500/NovaSeq)
- Up to 8 samples in parallel
- Size: up to 30µm (channels 50µm)
- Now with v3 kit, improved
- UMI, cell and sample barcodes
- CellRanger

Comparing our scRNA-seq services

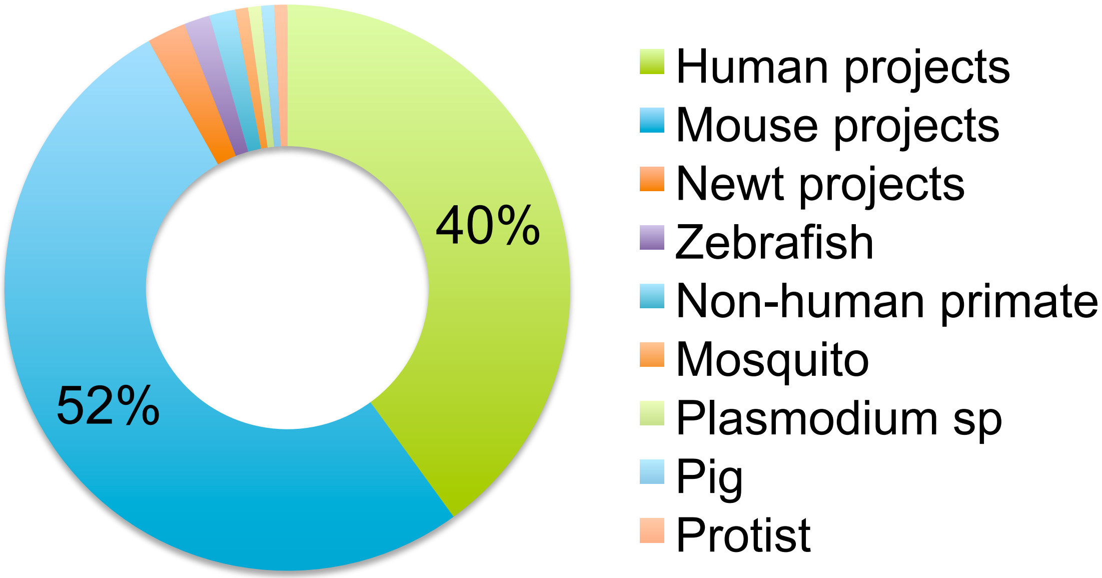
	Full-length		Quantitative	
	Smart-seq2	**Smart-Seq ICELL8**	**STRT-seq-2i**	**10xGenomics**
Format	384-well plate	Microwell chip	Microwell chip	Chromium microfluidics chip
Cell number	384	~1500	~2500	8 x 500-10,000
Input	FACS-sorted cells or nuclei	Suspension cells or nuclei	Suspension cells or nuclei	Suspension cells or nuclei
Transcript coverage	Full-length	Full-length	5’	3’
Features	•Flexible delivery •Isoforms, SNPs, mutations •ERCC spike-ins	•Limiting dilution •Cell selection •No size limit •8 samples parallel	•Limiting dilution/ FACS •Cell selection •No size limit •8 samples parallel	•High throughput •8 samples parallel •Sample pooling

Data delivery

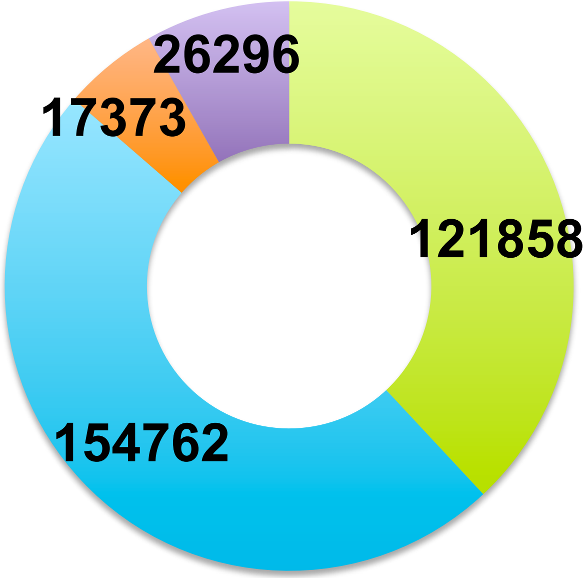
- Sequencing at NGI, HiSeq2500, NovaSeq
- Analysis pipelines for mouse and human
  - In-house: smart-seq2, STRT-seq-2i
  - Takarabio *mappa*: Smart-seq ICELL8
  - *Cell ranger*: 10xGenomics
- UPPMAX, Bioinformatics compute and storage
  - Users apply individually for projects
  - We deliver: de-multiplexed, annotated gene expression data, QC-files, Fastq
- Bioinformatics
  - Done by user
  - Support from BILS and WABI
  - Collaborations

ESCG in numbers

137 projects on 9 species



Over 320 000 cells sequenced



March 2018

Cell types analyzed at ESCG

SciLifeLab

Brain

Oligodendrocyte
Ependymal cells
Motor Neurons
All cell types: Nuclei-Frozen
Spinal cord
Neurons (sensory ganglia)
Neurons/glia
Primany neurons
Spinal cord injuries
Smaller and Large DRG neurons
Interneurons
Embryonic neural crest cells
Pericytes
Sensory Neurons
Glioblastoma (GBM) cells
Microglia
Retina/Spinal Cord
Enteric cells (neuron, glia)
OPCs
Schwann cells
NES cells
Astrocytes
Human Dopaminergic Neurons

Immune system

B cells
T cells
Tumor macrophages
B-cells from RA patients
CD4 T-cells
inactive T-cells
All immune cells

Skin

Keratinocytes
Endothelial cells
Skin: All cell types

Heart

Cardiomyocytes
Mouse Embryonic Progenitor Heart Cells
All cell types

Breast

Fibroblasts from mammary tumor
Breast cancer cells
Mammary gland epithelial cells

Cancer / tumor

CLL tumor cells
CAFs from colon tumors
Leukemia cells
Cancer cell lines co-cultured with Immune cells
myeloid cells from solid tumors
Patient Tongue tumor cells

Pancreas

pancreatic islets or islets of Langerhans

Bladder

Bladder normal epithelium
Bladder cancer cell line

Endometrium

Stromal Progenitor - Epithelium

Cell lines

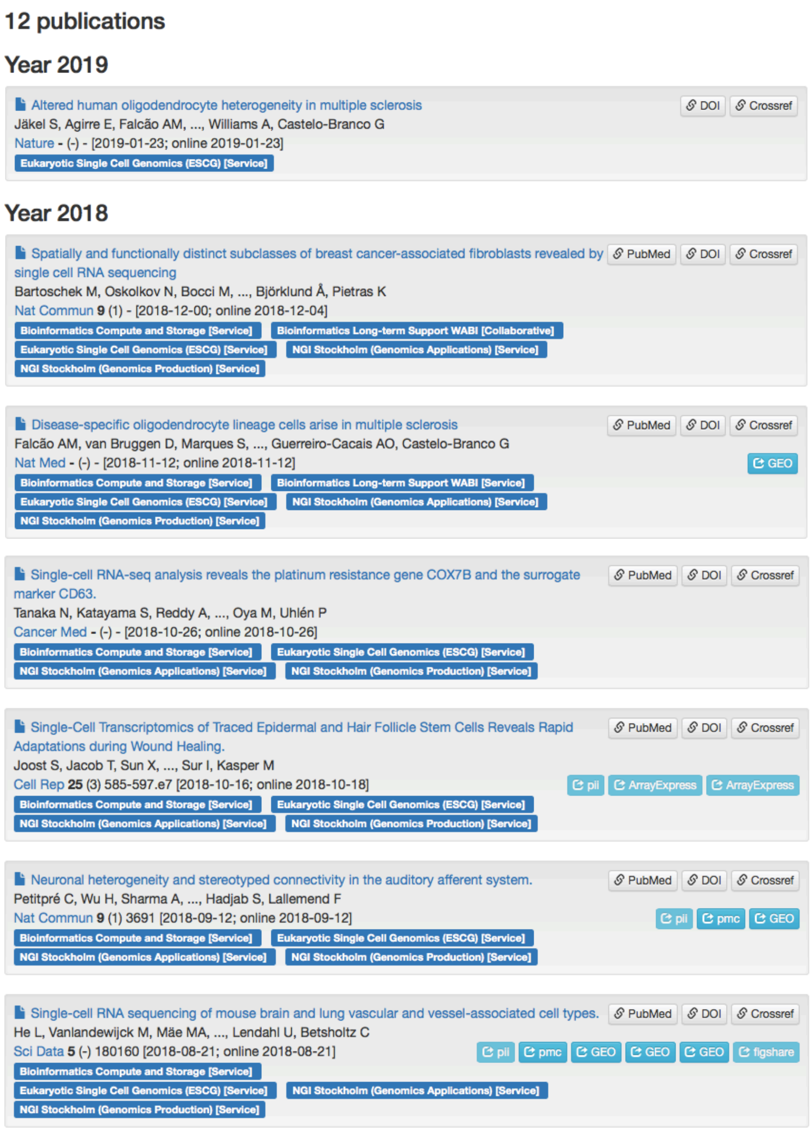
HCT116 - instestinal epithelial cell line
Human HeLa cells
HEK293
C2C12 cells

Other

Embryonic stem cells
Hematopoietic stem cell (HSC, mouse)
iPS cell lines
Pluripotent stem cells
Human Neuronal Stem cells
trophectoderm
Neural Crest Cells
Mesenchymal progenitors
ILCs
Primary bone marrow (BM, human)
Fibroblasts from POMPE patients
vascular smooth muscle cells
Artery cells
Thymus cells
Thymic epithelial cells
Kidney cells
Kidney pericytes
Liver cells
Spermatids & spermatogonia
vascular smooth muscle cells
Intestinal ILC
Blastema
Mosquito hemocytes
Plasmodium (MALARIA) eukaryotic cells
Protists

March 2018

Publications with data from ESCG



12 publications

Year 2019

Altered human oligodendrocyte heterogeneity in multiple sclerosis
Jäkel S, Agirre E, Falcão AM, ..., Williams A, Castelo-Branco G
Nature - (-) - [2019-01-23; online 2019-01-23]
Eukaryotic Single Cell Genomics (ESCG) [Service]

Year 2018

Spatially and functionally distinct subclasses of breast cancer-associated fibroblasts revealed by single cell RNA sequencing
Bartoschek M, Oskolkov N, Bocci M, ..., Björklund Å, Pietras K
Nat Commun **9** (1) - [2018-12-00; online 2018-12-04]
Bioinformatics Compute and Storage [Service]
Bioinformatics Long-term Support WABI [Collaborative]
Eukaryotic Single Cell Genomics (ESCG) [Service]
NGI Stockholm (Genomics Applications) [Service]
NGI Stockholm (Genomics Production) [Service]

Disease-specific oligodendrocyte lineage cells arise in multiple sclerosis
Falcão AM, van Bruggen D, Marques S, ..., Guerreiro-Cacais AO, Castelo-Branco G
Nat Med - (-) - [2018-11-12; online 2018-11-12]
Bioinformatics Compute and Storage [Service]
Bioinformatics Long-term Support WABI [Service]
Eukaryotic Single Cell Genomics (ESCG) [Service]
NGI Stockholm (Genomics Applications) [Service]
NGI Stockholm (Genomics Production) [Service]

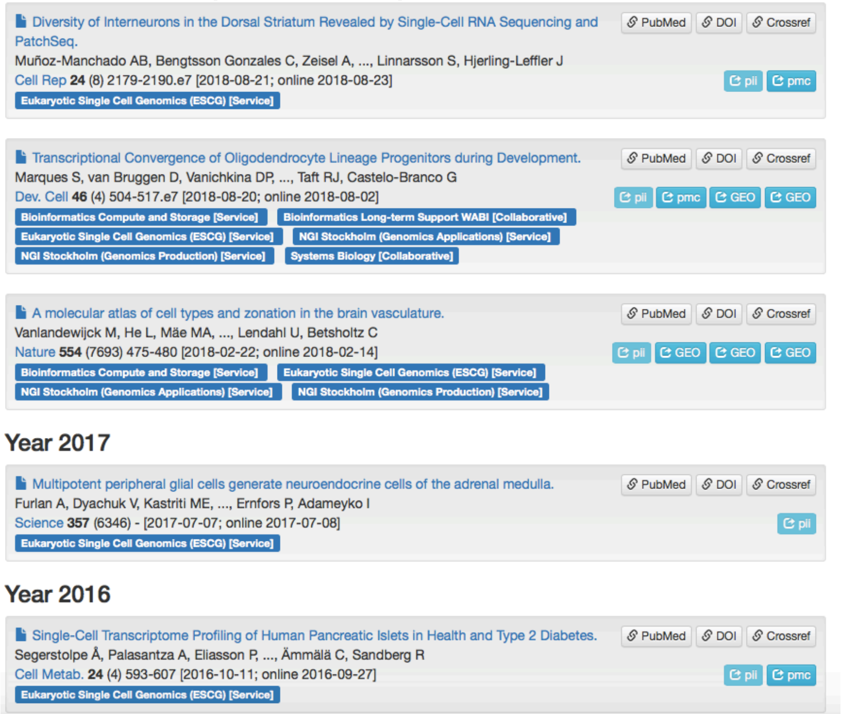
Single-cell RNA-seq analysis reveals the platinum resistance gene COX7B and the surrogate marker CD63.
Tanaka N, Katayama S, Reddy A, ..., Oya M, Uhlén P
Cancer Med - (-) - [2018-10-26; online 2018-10-26]
Bioinformatics Compute and Storage [Service]
Eukaryotic Single Cell Genomics (ESCG) [Service]
NGI Stockholm (Genomics Applications) [Service]
NGI Stockholm (Genomics Production) [Service]

Single-Cell Transcriptomics of Traced Epidermal and Hair Follicle Stem Cells Reveals Rapid Adaptations during Wound Healing.
Joost S, Jacob T, Sun X, ..., Sur I, Kasper M
Cell Rep **25** (3) 585-597.e7 [2018-10-16; online 2018-10-18]
Bioinformatics Compute and Storage [Service]
Eukaryotic Single Cell Genomics (ESCG) [Service]
NGI Stockholm (Genomics Applications) [Service]
NGI Stockholm (Genomics Production) [Service]

Neuronal heterogeneity and stereotyped connectivity in the auditory afferent system.
Petitpré C, Wu H, Sharma A, ..., Hadjab S, Lallemend F
Nat Commun **9** (1) 3691 [2018-09-12; online 2018-09-12]
Bioinformatics Compute and Storage [Service]
Eukaryotic Single Cell Genomics (ESCG) [Service]
NGI Stockholm (Genomics Applications) [Service]
NGI Stockholm (Genomics Production) [Service]

Single-cell RNA sequencing of mouse brain and lung vascular and vessel-associated cell types.
He L, Vanlandewijck M, Mäe MA, ..., Lendahl U, Betsholtz C
Sci Data **5** (-) 180160 [2018-08-21; online 2018-08-21]
Bioinformatics Compute and Storage [Service]
Eukaryotic Single Cell Genomics (ESCG) [Service]
NGI Stockholm (Genomics Applications) [Service]
NGI Stockholm (Genomics Production) [Service]

Year 2018 (continued)



Diversity of Interneurons in the Dorsal Striatum Revealed by Single-Cell RNA Sequencing and PatchSeq.
Muñoz-Manchado AB, Bengtsson Gonzales C, Zeisel A, ..., Linnarsson S, Hjerling-Leffler J
Cell Rep **24** (8) 2179-2190.e7 [2018-08-21; online 2018-08-23]
Eukaryotic Single Cell Genomics (ESCG) [Service]

Transcriptional Convergence of Oligodendrocyte Lineage Progenitors during Development.
Marques S, van Bruggen D, Vanichkina DP, ..., Taft RJ, Castelo-Branco G
Dev. Cell **46** (4) 504-517.e7 [2018-08-20; online 2018-08-02]
Bioinformatics Compute and Storage [Service]
Bioinformatics Long-term Support WABI [Collaborative]
Eukaryotic Single Cell Genomics (ESCG) [Service]
NGI Stockholm (Genomics Applications) [Service]
NGI Stockholm (Genomics Production) [Service]
Systems Biology [Collaborative]

A molecular atlas of cell types and zonation in the brain vasculature.
Vanlandewijck M, He L, Mäe MA, ..., Lendahl U, Betsholtz C
Nature **554** (7693) 475-480 [2018-02-22; online 2018-02-14]
Bioinformatics Compute and Storage [Service]
Eukaryotic Single Cell Genomics (ESCG) [Service]
NGI Stockholm (Genomics Applications) [Service]
NGI Stockholm (Genomics Production) [Service]

Year 2017

Multipotent peripheral glial cells generate neuroendocrine cells of the adrenal medulla.
Furlan A, Dyachuk V, Kastriti ME, ..., Ernfors P, Adameyko I
Science **357** (6346) - [2017-07-07; online 2017-07-08]
Eukaryotic Single Cell Genomics (ESCG) [Service]

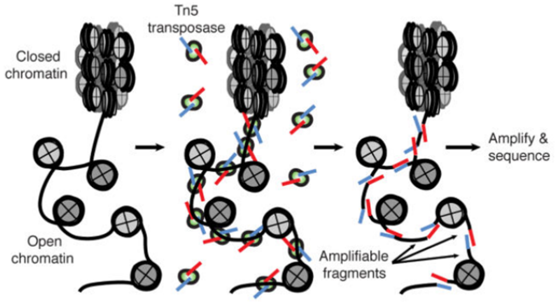
Year 2016

Single-Cell Transcriptome Profiling of Human Pancreatic Islets in Health and Type 2 Diabetes.
Segerstolpe Å, Palasantza A, Eliasson P, ..., Ämmälä C, Sandberg R
Cell Metab. **24** (4) 593-607 [2016-10-11; online 2016-09-27]
Eukaryotic Single Cell Genomics (ESCG) [Service]

https://publications.scilifelab.se, search on label

Remember to acknowledge us in your papers

New single-cell services offered



(Buenrostro, 2013)

- scATAC-seq (Chromatin accessibility)
  - 10x, *now available!*
    - Commercial kit
    - From isolated nuclei
  - ICELL8, *will be established*
    - From Greenleaf lab publication (Mezger, A. et al. 2018).
    - Can be run directly from cell suspension
- Immune profiling
  - 10x, *now available!*
    - Commercial V(D)J kits
      - T-/B-cell receptor identification
      - Can be combined with transcript profiling
- Transcriptome combined with surface protein expression
  - 10x, *available on request*

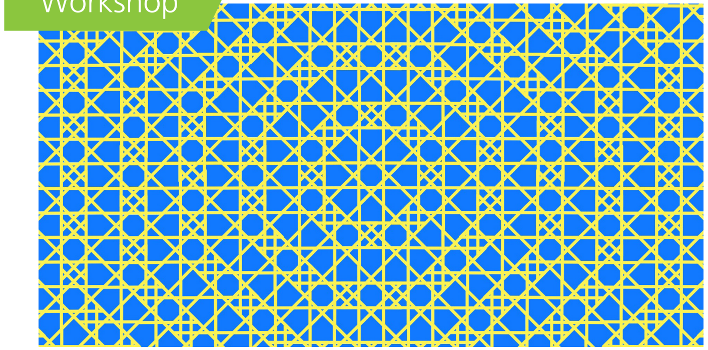
What lays ahead?

- Emerging techniques
  - Smart-seq3 (under development by R. Sandberg lab)
  - Transcriptome + Proteome
  - Transcriptome + Epigenome
  - CRISPR-Cas9 + Transcriptome
  - ‘split-pooling’ scRNA-seq
  - non-coding RNA-seq

- *In situ* sequencing
  - Facility service will be available

- We constantly update with available techniques
  - If you have requests, let us now

Workshop



Single cell biology meets diagnostics

12th International workshop on approaches to single cell analysis

March 4 –5th, 2019

A meeting focused on the future application of single cell analysis to diagnostics

Speaker highlights include:

- Christopher Walsh, Harvard Medical School
- Jun Kunisawa, NIBIOHN
- Nikolaus Rajewsky, Max Delbrück Center for Molecular Medicine
- Barbara Treutlein, MPI Leipzig
- Itaru Hamachi, Kyoto University

Meeting sponsors

SciLifeLab l Uppsala University l Waseda University l Osaka University l Japan Science and Technology Agency (JST) l 10X Genomics l Olink Proteomics l Fluidigm l Illumina

Venue: University Main Building,
Hall X, Uppsala

Poster abstract and registration deadline:
February 8th

More information and registration: www.scilifelab.se/events

SciLifeLab

Karolinska Institutet

KTH VETENSKAP OCH KONST

Stockholms universitet

UPPSALA UNIVERSITET

Deadline for registration
February 8th

SciLifeLab

Karolinska Institutet

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Eukaryotic Single Cell Genomics facility

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