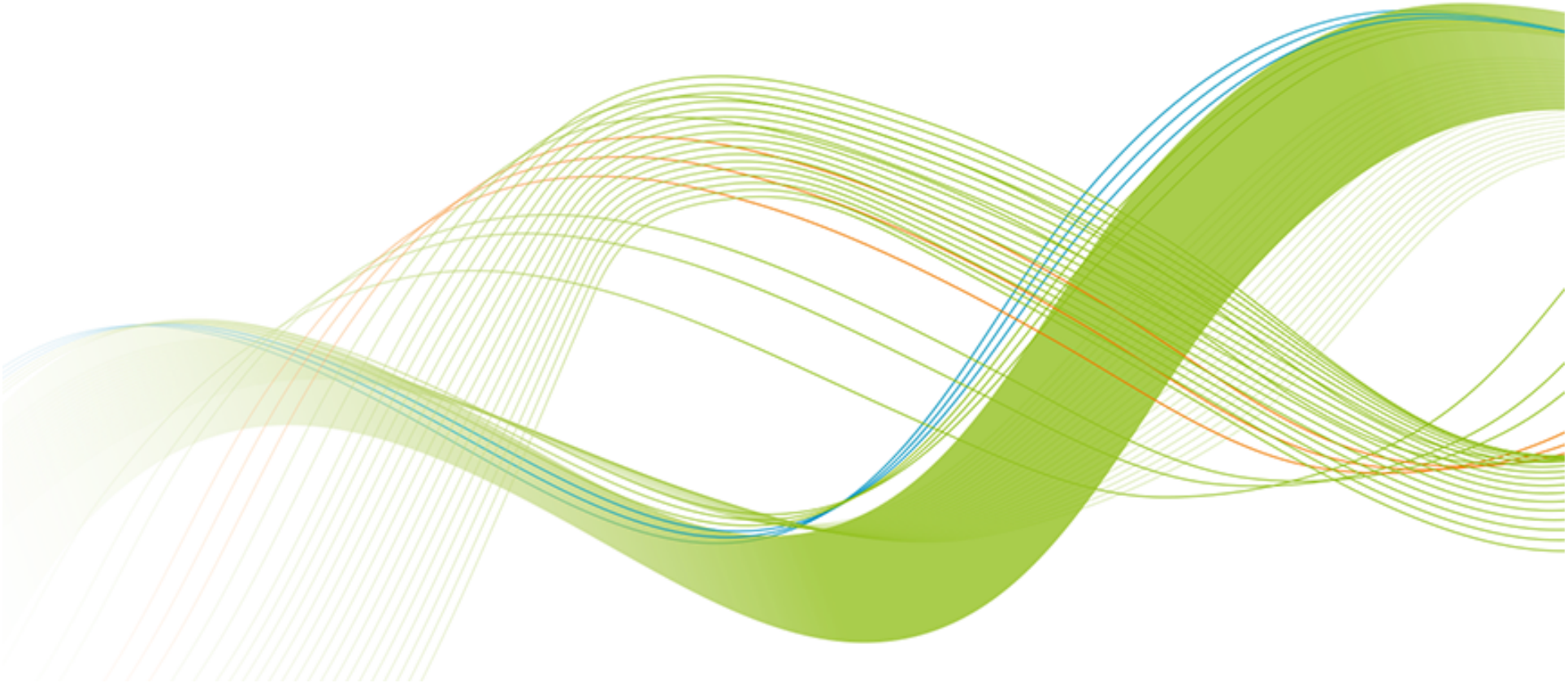
SciLifeLab

Henrik Gezelius
May 21, 2018

Single-cell transcriptomics (scRNA-seq)

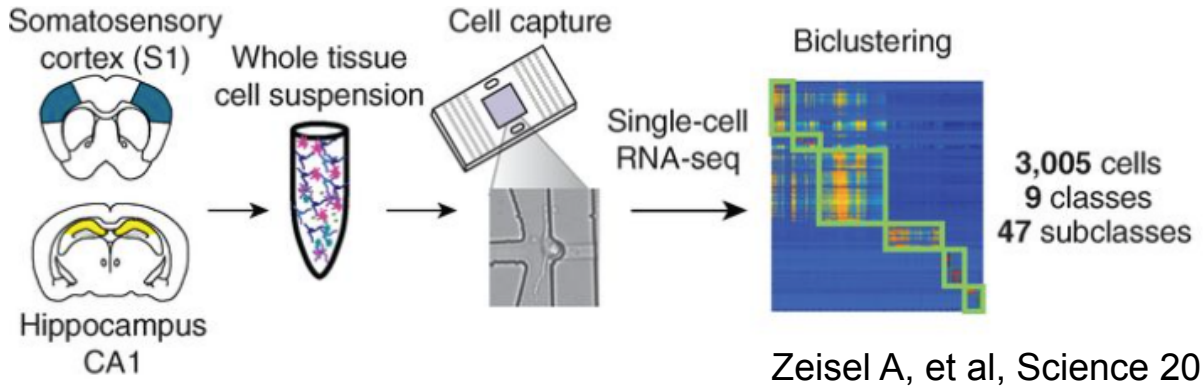
Eukaryotic Single Cell Genomics facility



Applications for scRNA-sequencing

SciLifeLab

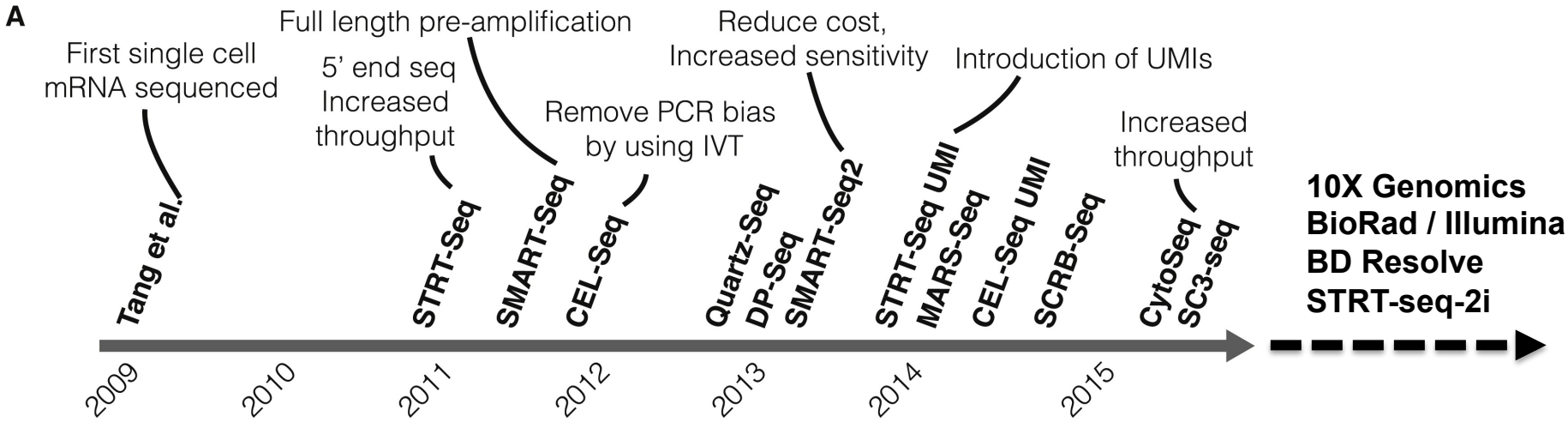
- Heterogeneity analysis
- Cell type identification
- Lineage tracing, cellular states in differentiation and development
- Monoallelic gene expression, splicing patterns
- More…



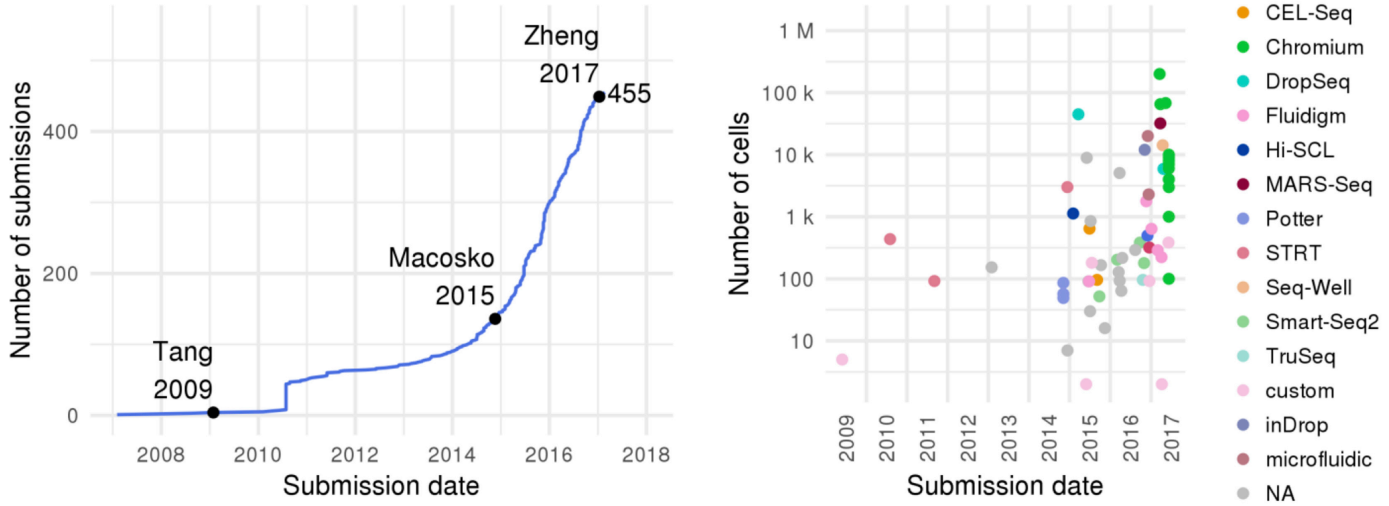
Zeisel A, et al, Science 2015

Short history of scRNA-seq

SciLifeLab

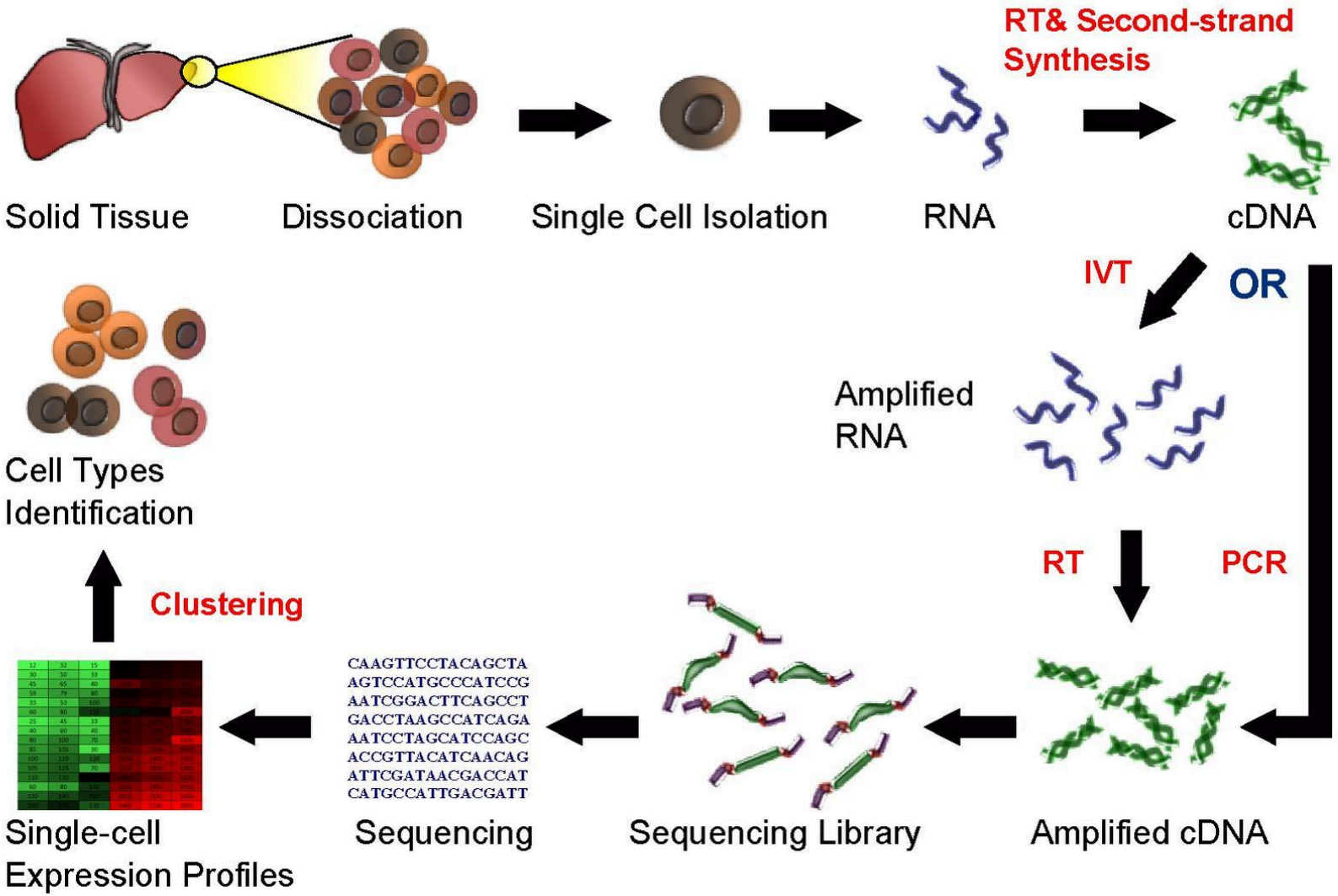


Adapted from: Kolodziejczyk A et al, Molecular Cell, 2015



Angerer et al, Curr Opin Sys Biol 2017

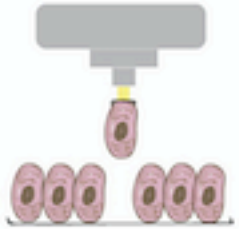
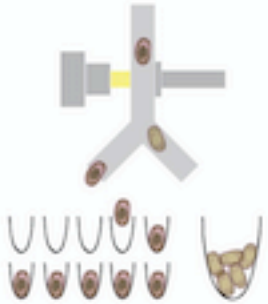
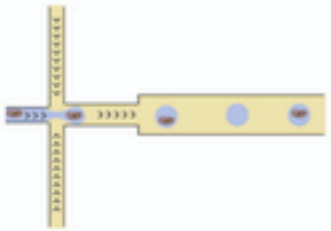
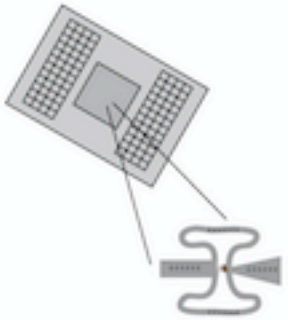
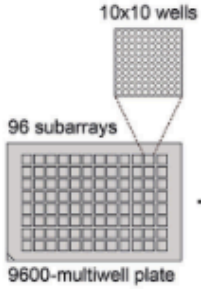
Single cell RNA seq workflow



From Wikipedia

Single-cell isolation or capture

SciLifeLab

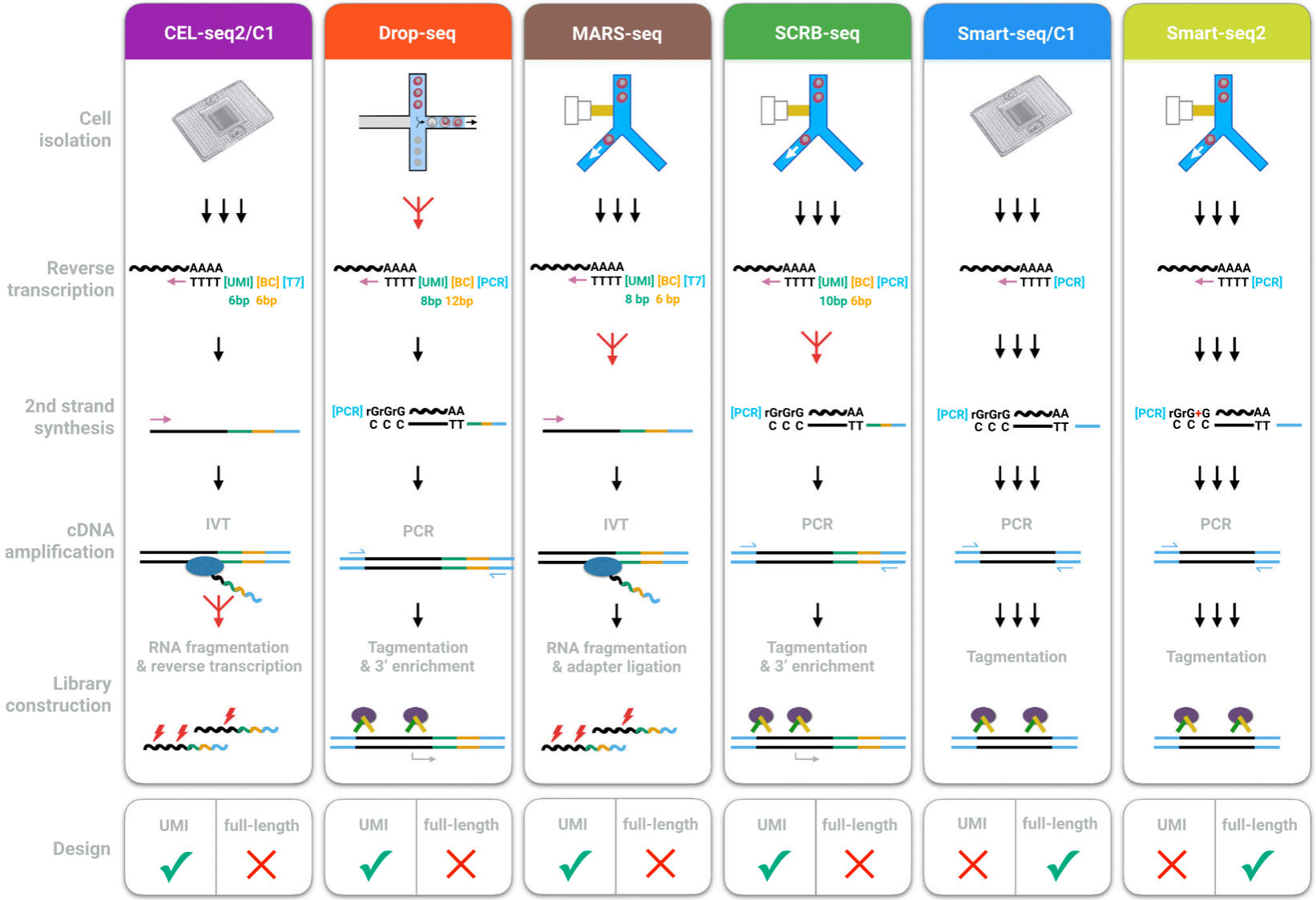
MICROPIPETTING MICROMANIPULATION	LASER CAPTURE MICRODISSECTION	FACS	MICRODROPLETS	MICROFLUIDICS e.g. FLUIDIGM C1	Multi-Sample Nano-Dispenser
					
low number of cells	low number of cells	hundreds of cells	large number of cells	hundreds of cells	large number of cells
any tissue	any tissue	dissociated 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

SciLifeLab



Zieghain et al. Mol Cell 2017

scRNA-sequencing protocols

SciLifeLab

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

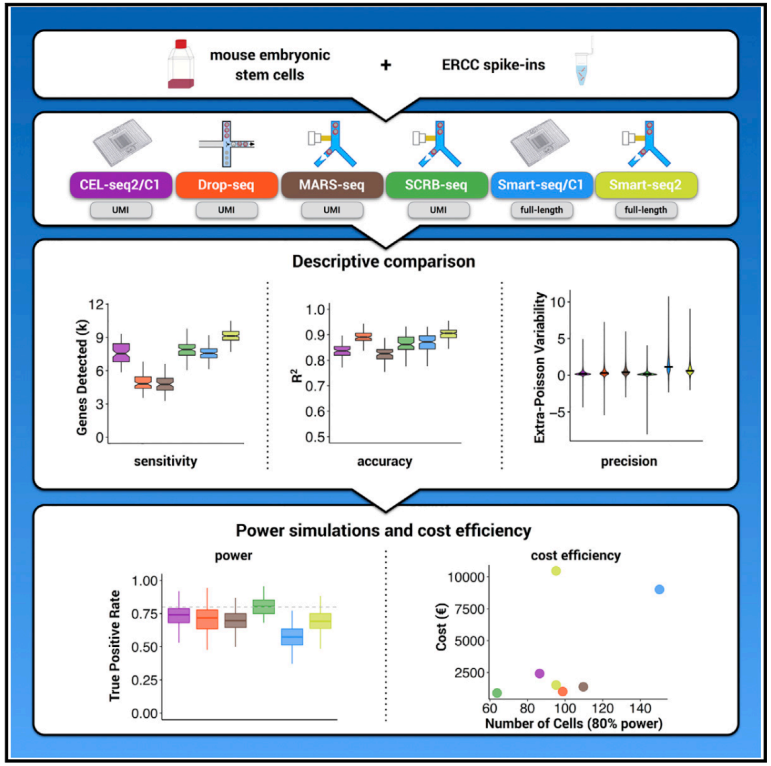
SciLifeLab

-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

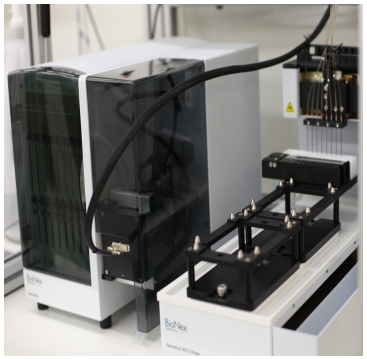
- Drop-seq is preferable when quantifying transcriptomes of large numbers of cells with low sequencing depth.
- (SCRB-seq and MARS-seq is preferable when quantifying transcriptomes of fewer cells.)
- Smart-seq2 is preferable when annotating and/or quantifying transcriptomes of fewer cells.
- STRT-seq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

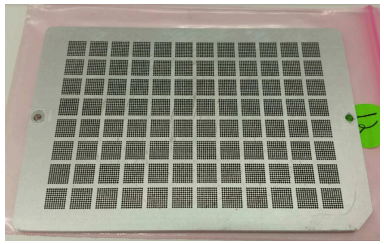
ESCG facility platform

SciLifeLab

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

- High throughput single-cell RNA-sequencing
- Over 320,000 single cells sequenced (in March 2018)

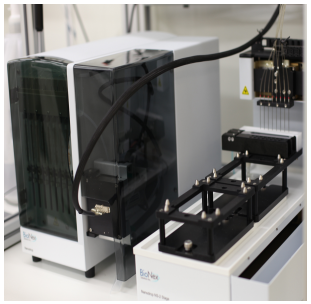


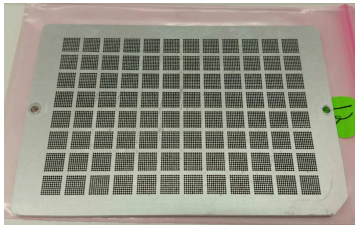


ESCG facility services

- From single cell suspension or FACSed cells
- cDNA generation and QC
- Library preparation
- Sequencing
- Data de-multiplexing and alignment to ref genome (human and mouse)

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



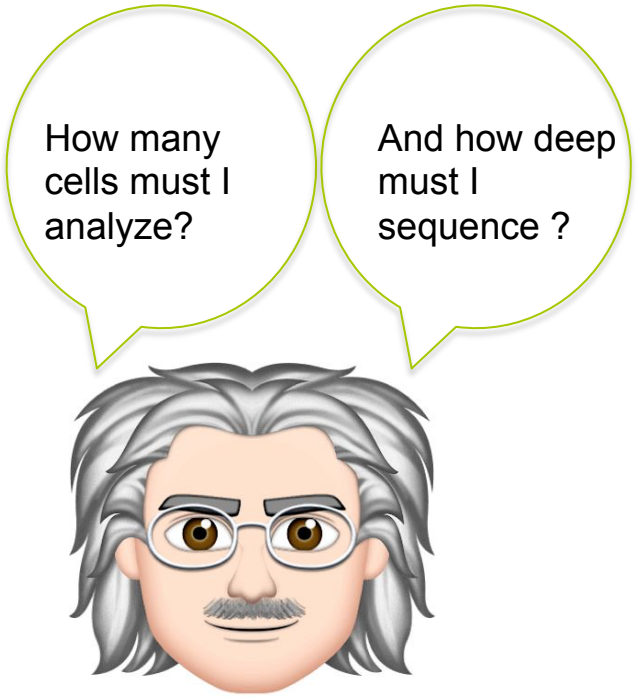


How do you get started?

SciLifeLab

User meeting

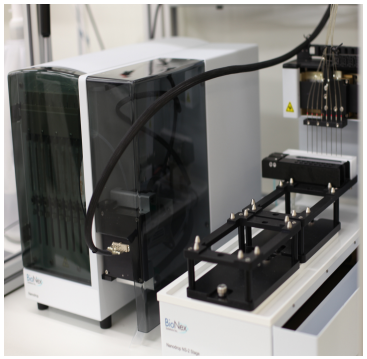
- Project discussion
  - Feasibility
  - Tissue, cells
  - Project size
  - Time line
- Choice of method
  - Data output
  - Number of cells to be analyzed
  - Location, cell delivery
- Bioinformatics
  - Early contact
  - National Bioinformatics Infrastructure Sweden (NBIS)
- Data delivery
- User fees

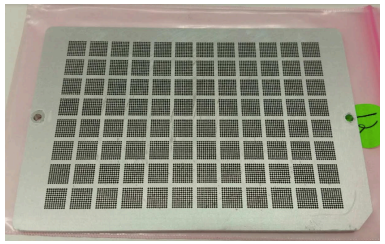


ESCG facility services

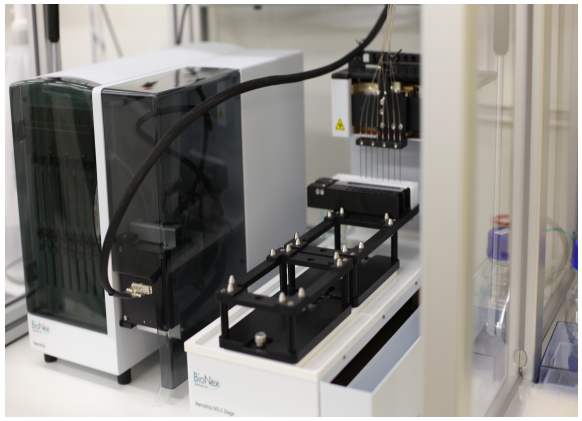
SciLifeLab

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



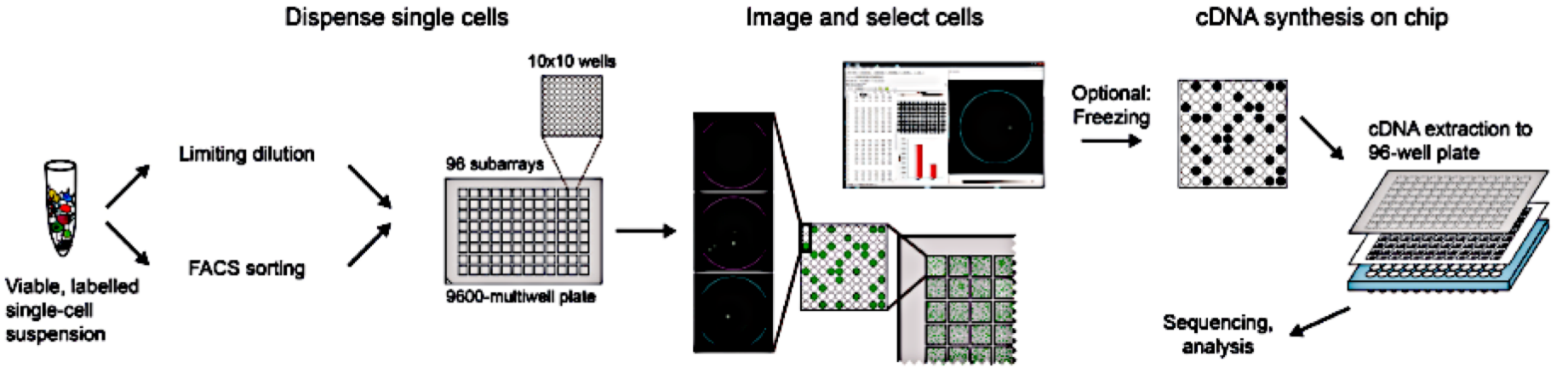


Smart-seq2 at ESCG

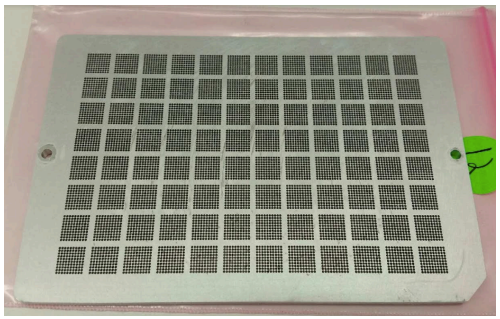


- 384 well plates
- Isolation: FACS
- Input: cells/nuclei
- Full-length
- Sequencing: 50bp single-read
- ERCC spike-ins
  - Two different dilutions
- Flexible delivery (shipment)

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

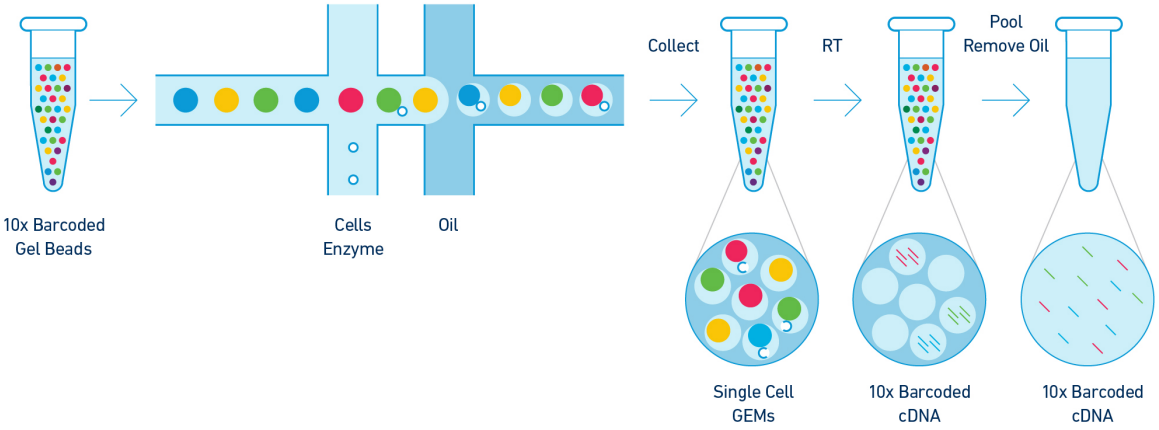


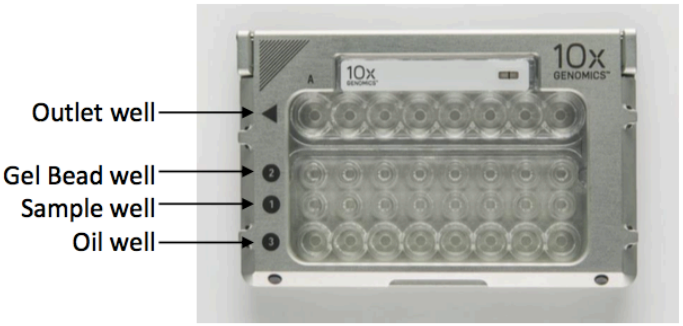
Adapted from: Hochgerner H, et al, SciRep, 2017

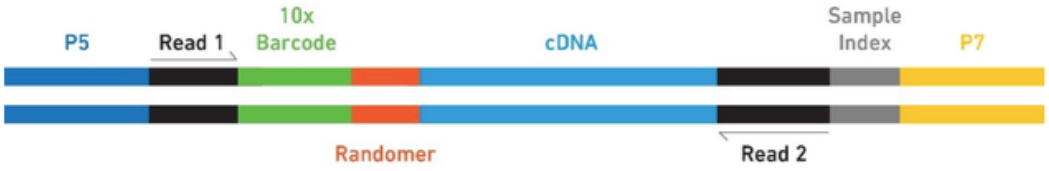


- Isolation: FACS/dispensing
- Input: Cells/nuclei
- Scale: 9600 wells (~2500 cells)
- Sequencing: 5'-tag (50 bp single read)
- Up to 8 samples in a chip
- No size limitation
- UMI:s

10X Genomics Chromium -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
- Size: up to 30µm (channels 50µm)
- UMI, cell barcode, sample barcode
- CellRanger

Data delivery

SciLifeLab

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

Comparing our services

SciLifeLab

	Full-length	Quantitative	
	Smart-seq2	**STRT-seq-2i**	**10xGenomics**
Format	384-well plate	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	• Flexible delivery • Isoforms, SNPs, mutations • Nuclei • ERCC spike-ins	• Limiting dilution/ FACS • Cell selection • Unbiased • 8 samples parallel • Nuclei	• High throughput • 8 samples parallel • Nuclei • Sample pooling

User fees

Smart-seq2 384 well plate	STRT-seq-2i 9600 wells chip (~2,500 cells)	10XGenomics 1 sample (~3,000 cells)
• Validation • Smart-seq2 library • Sequencing (50 bp, single-read	• Validation • STRT library (dual index) • Sequencing (50 bp single-read)	• Validation • Illumina library • Sequencing (paired-end, dual index)
~45,000 SEK	~60,000 SEK	~50,000 SEK

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

Single cell submission guidelines

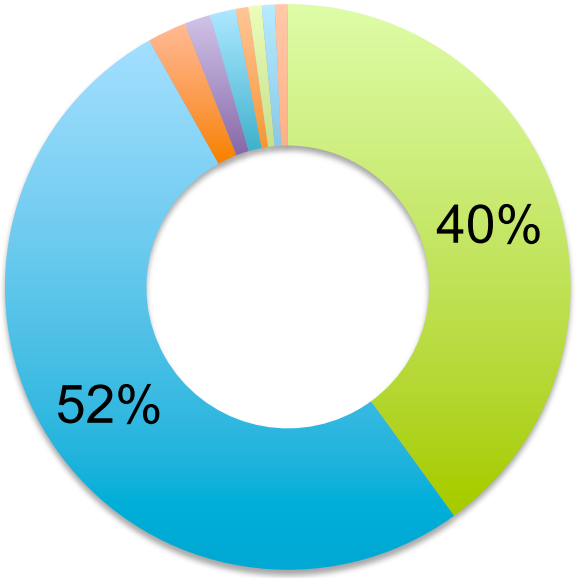
SciLifeLab

- 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 in numbers

SciLifeLab

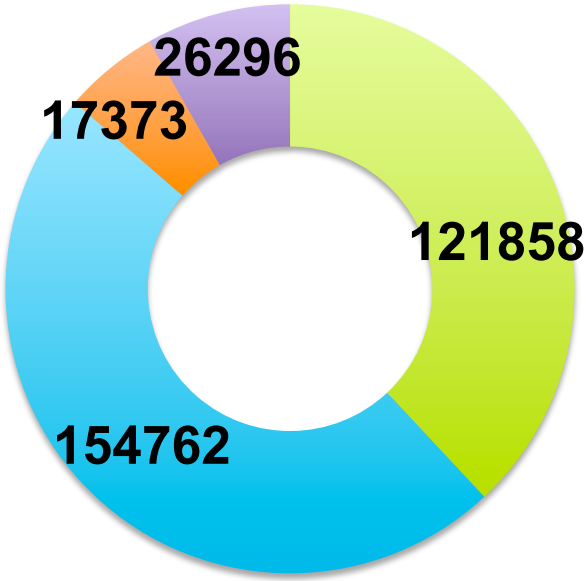
137 projects on 9 species



- Human projects
- Mouse projects
- Newt projects
- Zebrafish
- Non-human primate
- Mosquito
- Plasmodium sp
- Pig
- Protist

To see all cell types come to our poster!

Over 320 000 cells sequenced



- Smart-seq2
- 10X
- STRT-C1
- STRT-Seq2i

Cell types analyzed at ESCG

SciLifeLab

Brain	Immune system	Cancer / tumor	Other
Oligodendrocyte	B cells	CLL tumor cells	Embryonic stem cells
Ependymal cells	T cells	CAFs from colon tumors	Hematopoietic stem cell (HSC, mouse)
Motor Neurons	Tumor macrophages	Leukemia cells	iPS cell lines
All cell types: Nuclei-Frozen	B-cells from RA patients	Cancer cell lines co-cultured with Immune cells	Pluripotent stem cells
Spinal cord	CD4 T-cells	myeloid cells from solid tumors	Human Neuronal Stem cells
Neurons (sensory ganglia)	inactive T-cells	Patient Tongue tumor cells	trophectoderm
Neurons/glia	All immune cells		Neural Crest Cells
Primany neurons		**Pancreas**	Mesenchymal progenitors
Spinal cord injuries	**Skin**	pancreatic islets or islets of Langerhans	ILCs
Smaller and Large DRG neurons	Keratinocytes		Primary bone marrow (BM, human)
Interneurons	Endothelial cells	**Bladder**	Fibroblasts from POMPE patients
Embryonic neural crest cells	Skin: All cell types	Bladder normal epithelium	vascular smooth muscle cells
Pericytes		Bladder cancer cell line	Artery cells
Sensory Neurons	**Heart**		Thymus cells
Glioblastoma (GBM) cells	Cardiomyocytes	**Endometrium**	Thymic epithelial cells
Microglia	Mouse Embryonic Progenitor Heart Cells	Stromal Progenitor - Epithelium	Kidney cells
Retina/Spinal Cord	All cell types		Kidney pericytes
Enteric cells (neuron, glia)		**Cell lines**	Liver cells
OPCs	**Breast**	HCT116 - instestinal epithelial cell line	Spermatids & spermatogonia
Schwann cells	Fibroblasts from mammary tumor	Human HeLa cells	vascular smooth muscle cells
NES cells	Breast cancer cells	HEK293	Intestinal ILC
Astrocytes	Mammary gland epithelial cells	C2C12 cells	Blastema
Human Dopaminergic Neurons			Mosquito hemocytes
			Plasmodium (MALARIA) eukaryotic cells
			Protists

What lays ahead?

SciLifeLab

- Emerging techniques
  - Single cell ATAC-seq (under test/evaluation)
  - Transcriptome + Epigenome (future)
  - Transcriptome + Proteome (future)
  - CRISPR-Cas9 + Transcriptome (future)
  - ‘split-pooling’ scRNA-seq (future?)
  - non-coding RNA-seq (future?)
- Validation
  - Small molecule FISH
- Human Cell Atlas
  - Sten Linnarsson lab among the involved

SciLifeLab

Karolinska Institutet | KTH | Stockholms universitet | Uppsala Universitet

Eukaryotic Single Cell Genomics facility

escg@scilifelab.se
http://escg.se