



scRNA-Seq methods and NGI services

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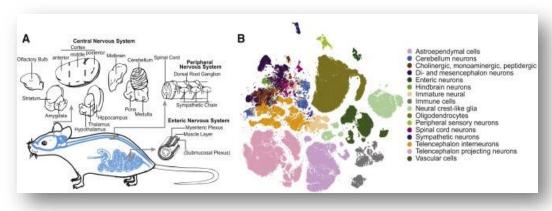
Outline

- The workflow
- Various methods
- General principles
- Examples of common methods
- Summary
- NGI services

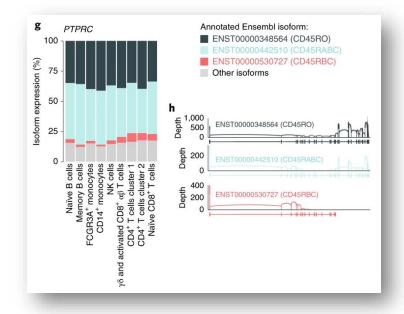
Applications



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



Zeisel et al, Cell 2018



Hagemann-Jensen et al, Nat Biotech 2020

Single cell RNA-seq workflow



I. Tissue Procurement



Source:

- Primary human

- Model organism
- Cell culture

Key considerations:

- Biological variation
- Sampling/handling variation
- Duration of sourcing

Study design:

- Biological replicates
- Technical replicates
- Cell number calculation
- Workflow optimization

II. Tissue Dissociation



Mechanical mincing

- Enzymatic digestion
- Automated blending
- Microfluidics devices

Key considerations:

- Experimental consistency
- Shortest duration
- Highest cell/nucleus quality

Quality control:

- FACS analysis
- qPCR for marker genes
- Imaging of cell integrity
- Representation of all cell types RNA quality (RIN)

III. Cell Enrichment (optional)



- Differential centrifugation, sedimentation, filtration
- Antibody labeling for positive/negative selection
- Flow cytometry or bead-based enrichment
- Dead cell removal

Key considerations:

- Additional handling
- Longer duration
- Loss of RNA quality
- Transcriptome changes

IV. Single Cell RNAseq Platform



Method:

- Droplet-based
- Tube-based after FACS
- Microwell-based
- Microfluidics-enabled

Key considerations:

- Cell throughput and handling time
- Gene coverage and cell type detection
- Whole transcript versus 3'end counting
- Imaging capability for doublet detection

V. Library Sequencing



Method:

- Illumina NGS
- Compatible with cDNA library

Sequencing depth considerations:

- 3'end counting: low depth ~50K RPC
- Whole transcript: high depth ~1M RPC
- Alternative splicing: ~20-30M RPC
- Iterative optimization for biological system

VI. Computational Analysis



Key considerations:

- Separation of batch and condition
- Technical vs. biological variation

Sample Batch correction approaches:

- Cell Hashing
- Demuxlet
- Canonical correlation analysis (CCA)
- MAST

Verify your results with orthogonal method!

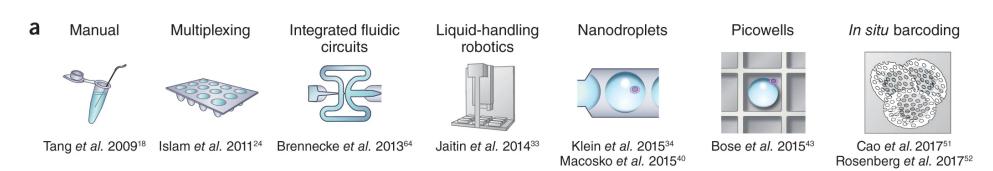
⁴ SciLifeLab	Omics type	Read out		Complexity	Sample requirements			Spatial resolution
		NGS	Imaging	(number of targets)	Fresh- frozen	FFPE	TMAs	
Spatial transcriptimics (10X Visium)	RNA	✓	✓	Unbiased transcriptomome-wide	✓	(✓)	×	Anatomical features of 55 μm
In situ sequencing	RNA	×	V	200-300	V	✓	✓	Subcellular
Spatial proteomics (Codex)	Protein	×	✓	40	✓	✓	✓	Subcellular
Advanced FISH technologies (smFISH)	DNA/RNA	×	✓	6	V	✓	V	Subcellular
Spatial Mass Spectometry	Small molecules	×	✓	Mutliplexed, targeted or untargeted	✓	×	×	Anatomical features of 15 μm

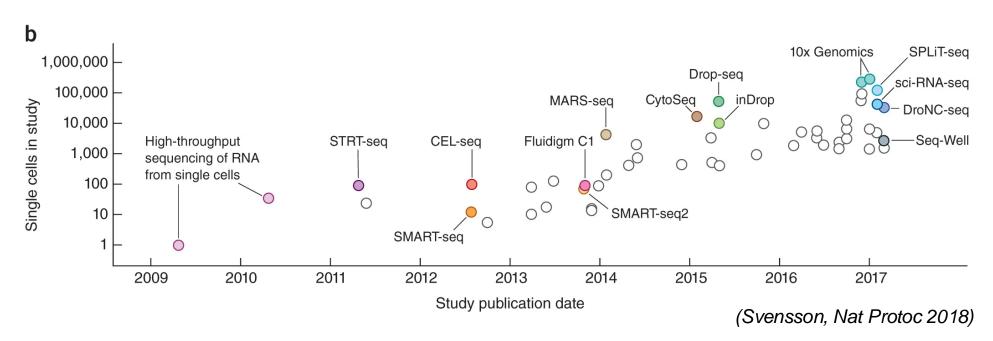


Nguyen et al., "Experimental Considerations for Single-Cell RNA Sequencing Approaches." Frontiers in Cell and Developmental Biology 2018

Short history of scRNA-seq methods

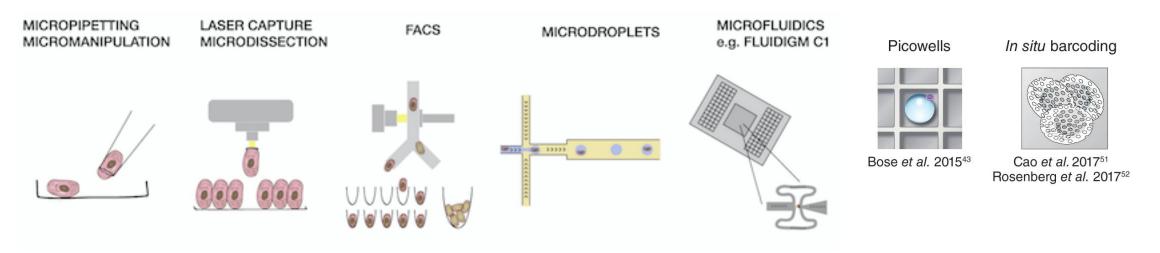






Single-cell isolation or capture





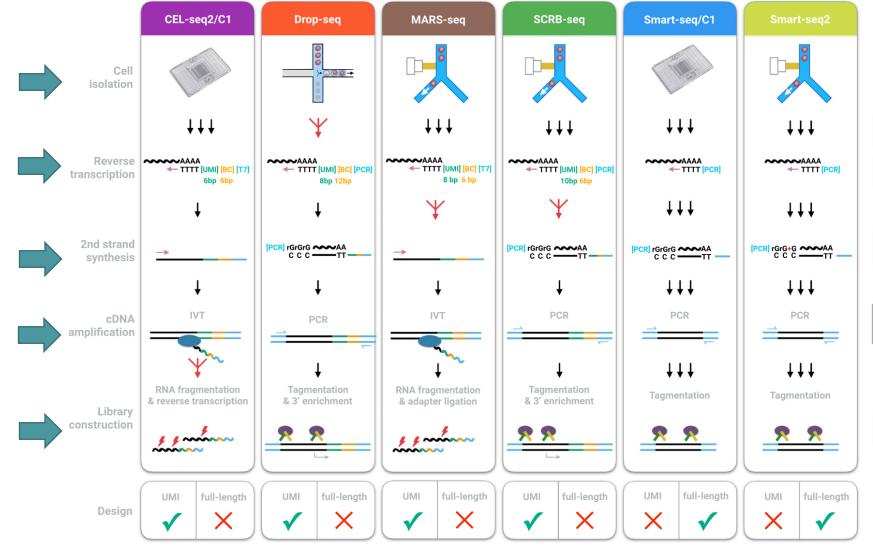
Adapted from: Kolodziejczyk A et al, Molecular Cell, 2015

(Adapted from: Svensson, Nat Protoc 2018)

Add a barcode to the cell in the compartment

scRNA-sequencing protocol principles





BC (cell unique sequence)

UMI (random sequence)

TSO (annealing handle)

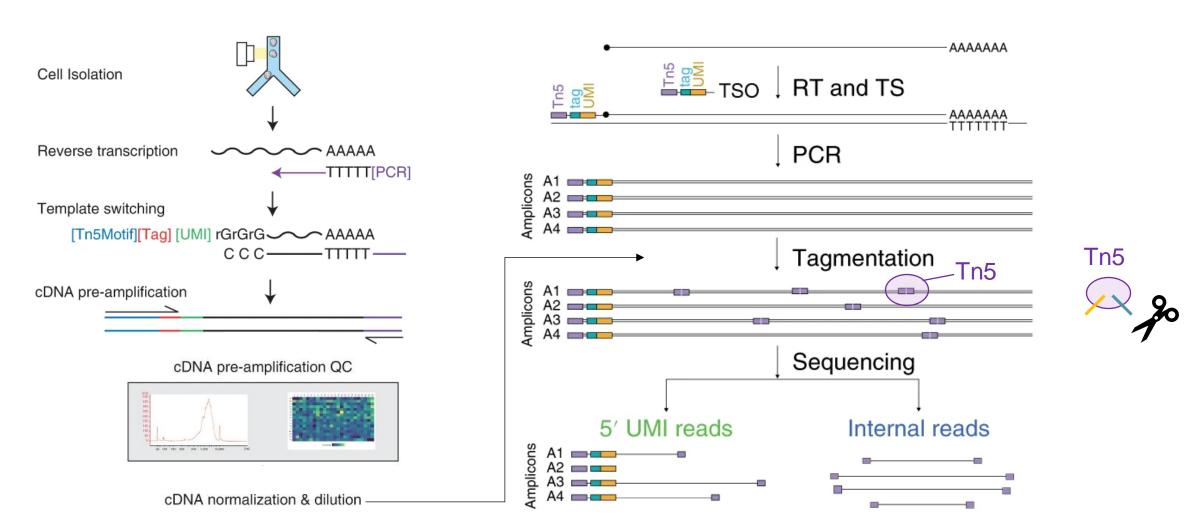
PCR (or in vitro transcript)

Fragmentation / Tagment-

Zieghain et al. Mol Cell 2017

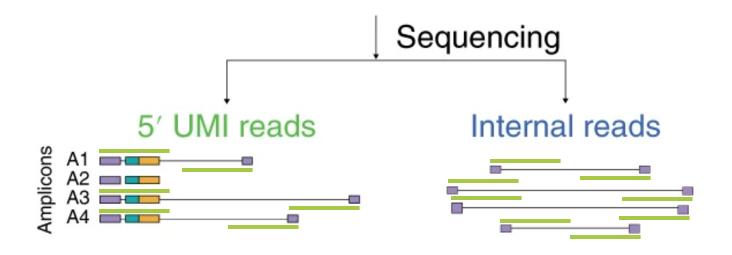
Example scRNA-seq: SMART-seq3

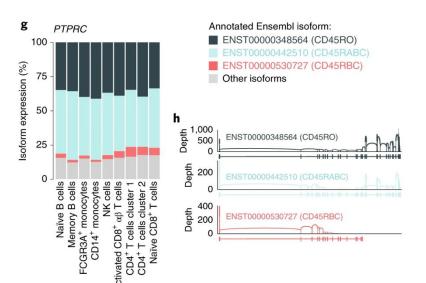




SMART-seq3 Sequencing

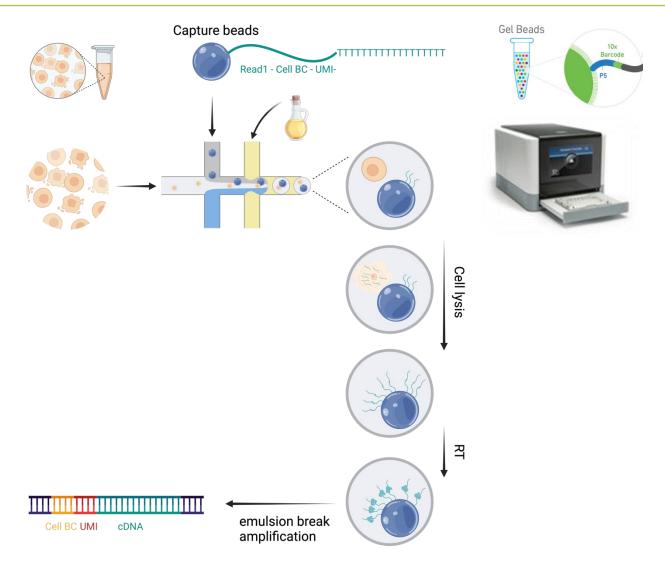






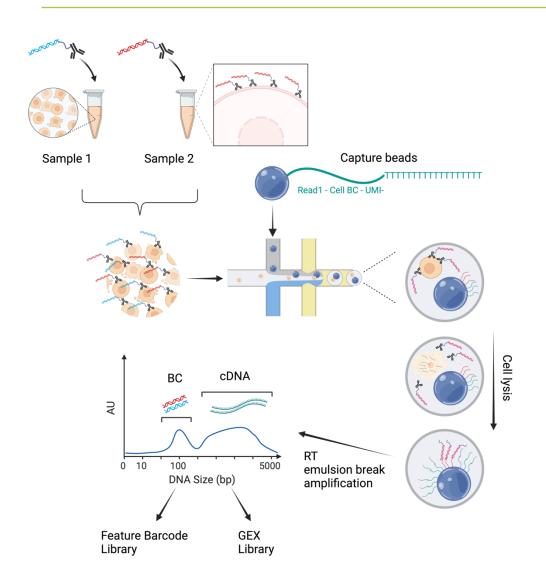
10x Genomics Chromium 3'



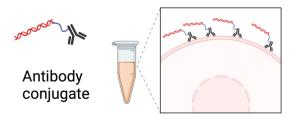


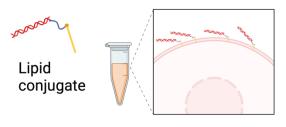
Cell multiplexing in 10x Chromium

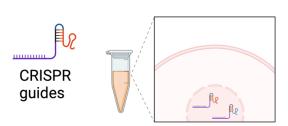


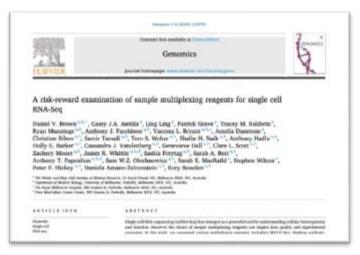












(Brown et al. 2024)

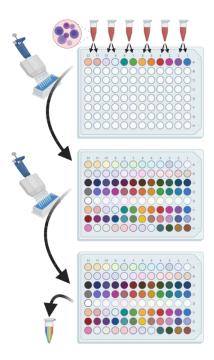
SPLiT-seq workflow

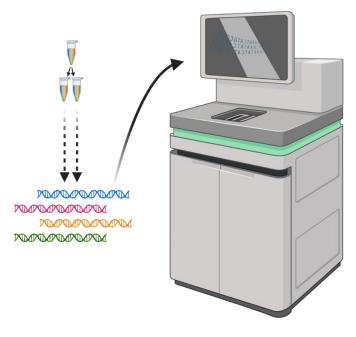


In situ barcoding



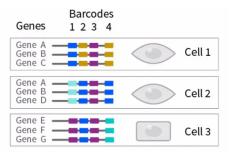
Cao *et al.* 2017⁵¹ Rosenberg *et al.* 2017⁵²





- 12 (BC 1)
- 96 (BC 2)
- 96 (BC 3)
- 2 (BC 4)
- = 221 184

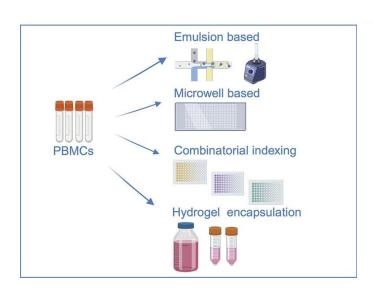
Possible combinations

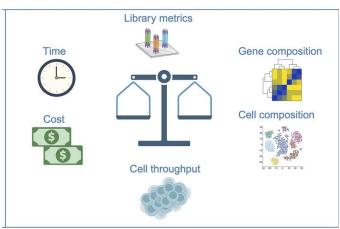


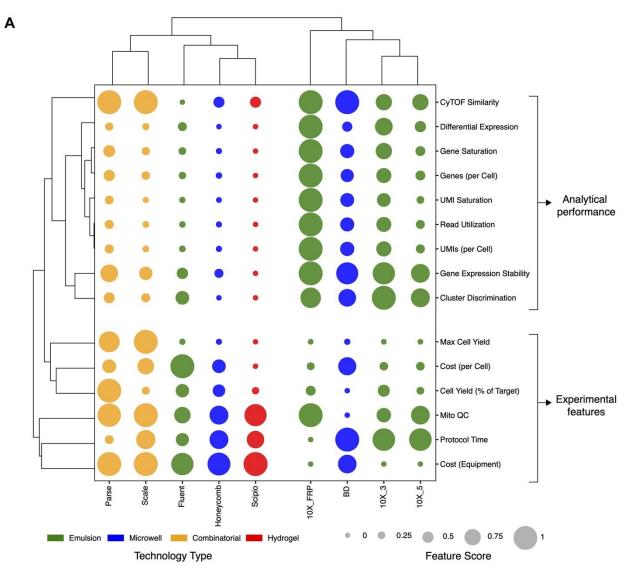
Up to 10 000 cells (with low doublet rate) 100 000 cell with 48 initial barcode 1 000 000 cells with 96 initial barcodes

Comparison of current methods



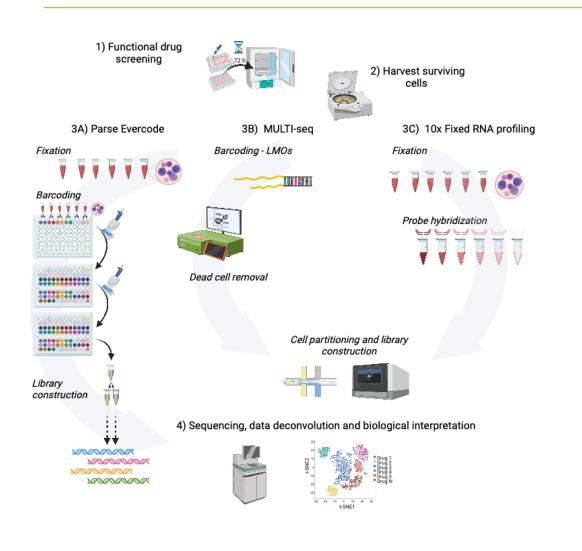


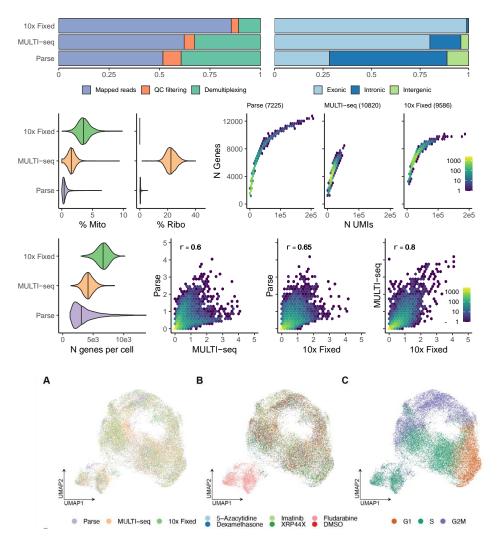




Comparison of multiplexing methods

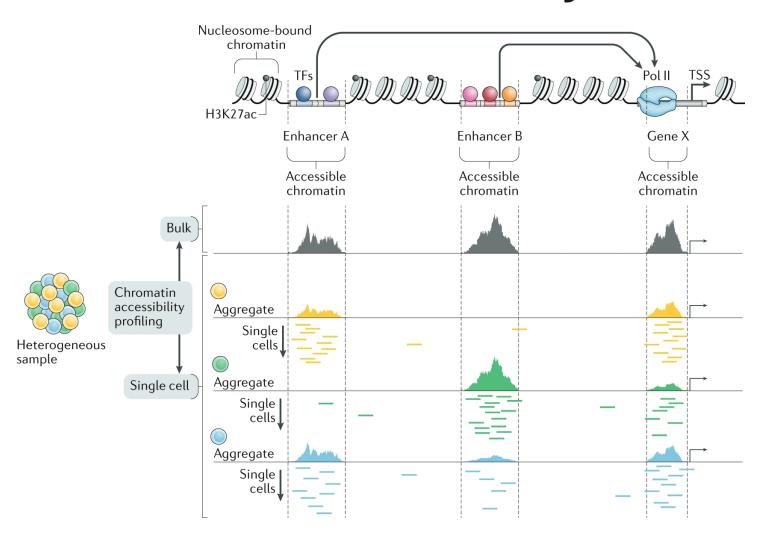


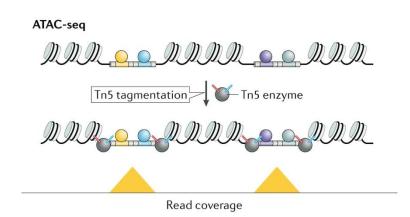




Beyond transcriptomics - Chromatin accessibility







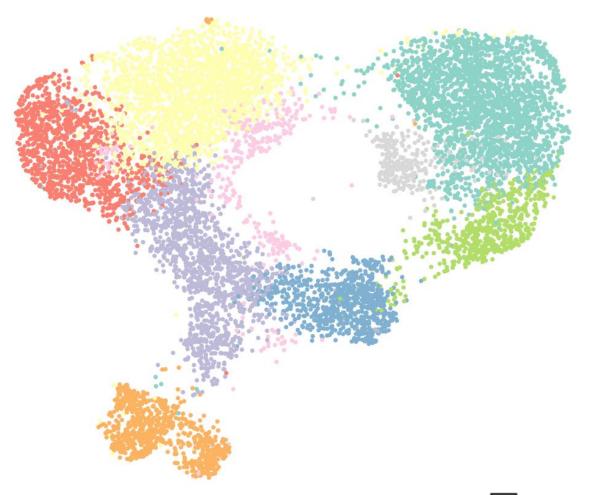
Summary single cell sequencing



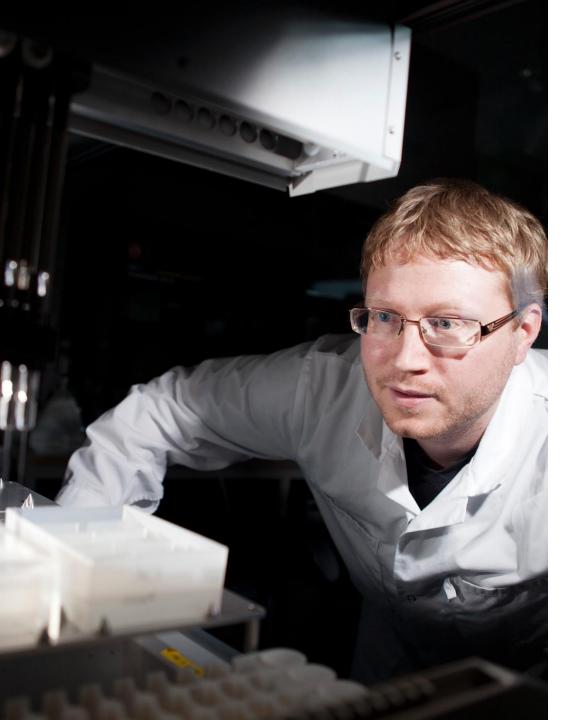
- ✓ Isolate cells in compartments
- Add UMI & barcode for later pooling
- Amplify
- Fragment (e.g Tn5 tagmentation)
- Sequence pooled libraries
- Analyze your data!

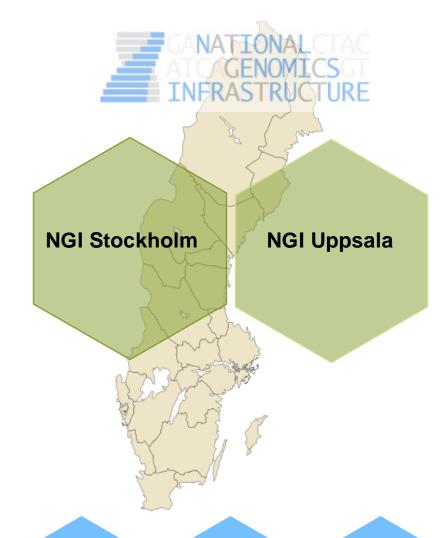
Questions?





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NGI-Stockholm

NGI-Uppsala Uppsala Genome Center NGI-Uppsala SNP&SEQ Technology platform

services Multi-omics

Infrastructure, services and expertise in genomic technologies and applications



Genome Sequencing De novo, re-seq, targeted... **Epigenomics** Methylation, chromatin state, HiC...

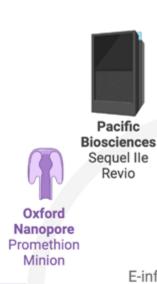
Transcriptomics Short-read, long-read

Proteomics Olink Explore

Arrays SNPs, methylation

Source material

Tissues Cells Microbes Plasma Nucleic acids Archaeological material **Environmental samples** Read-made libraries



Proteomics Olink Explore

Ancient DNA







Element AVITI24



E-infrastructure & pipelines for FAIR data processing and management

Smart-seq



BSL-3



Methods development

Disease genetics

Cancer research

Molecular biology

Drug development

Infectious diseases

Population genetics

Ecology & biodiversity

Evolutionary genetics

Biotechnology

Archaeology

Agriculture

Forestry

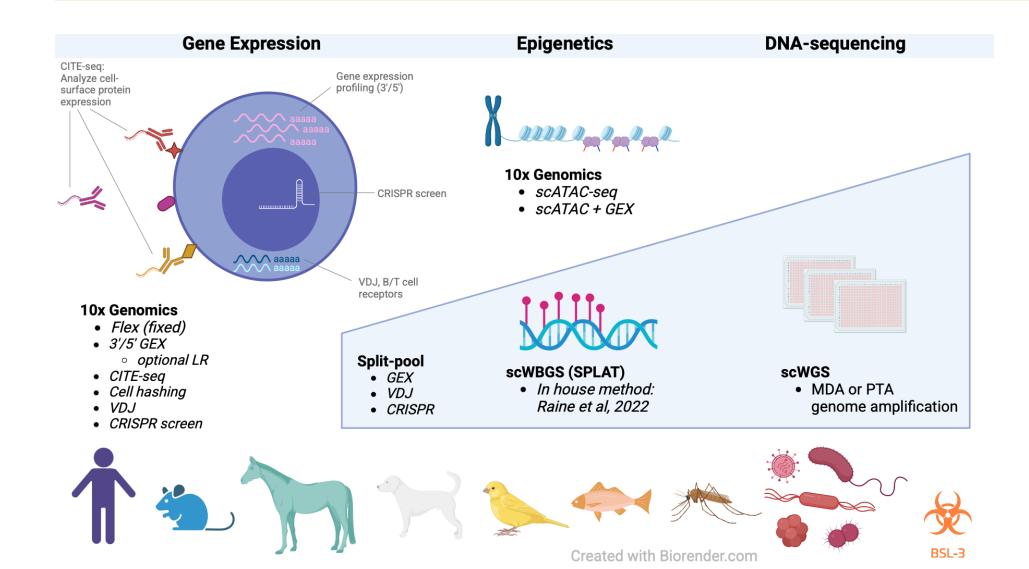


Transcriptomics 10x Genomics Single-cell Visium 10x Genomics Chromium

capabilities

NGI Single Cell services

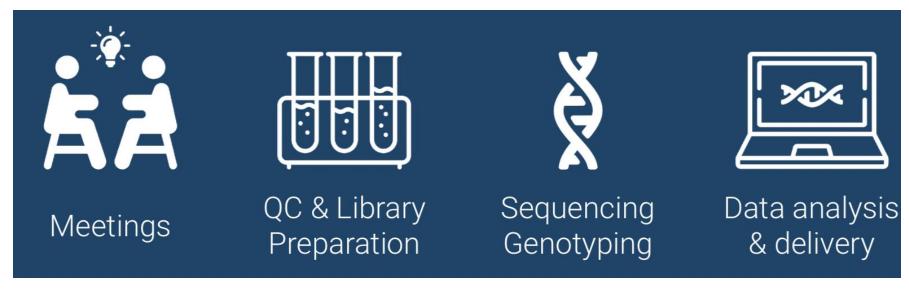




Project workflow at NGI







NGI OpenLab

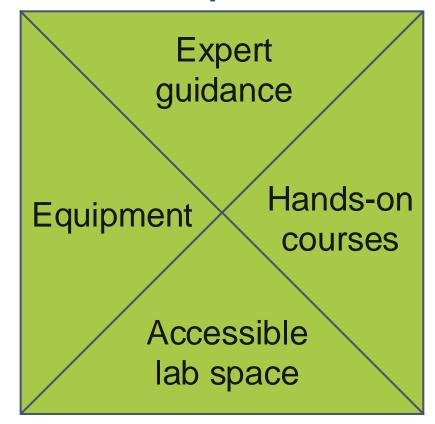


User needs in niche assays, e.g.

- Complex biospecimens
- Customization
- New assays



NGI OpenLab



Platform service

- Assays with robust demand
- Automization
- Large-scale equipment
- Strategic and selective R&D

Users

Platform





For more details and project requests

Contact NGI at support@ngisweden.se

or place your order or meeting request in our order portal at

https://ngisweden.scilifelab.se/