



Single cell sequencing

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24. 09. 2018

Genomics

Genomics Tools:

Microarrays

PCR

Sanger

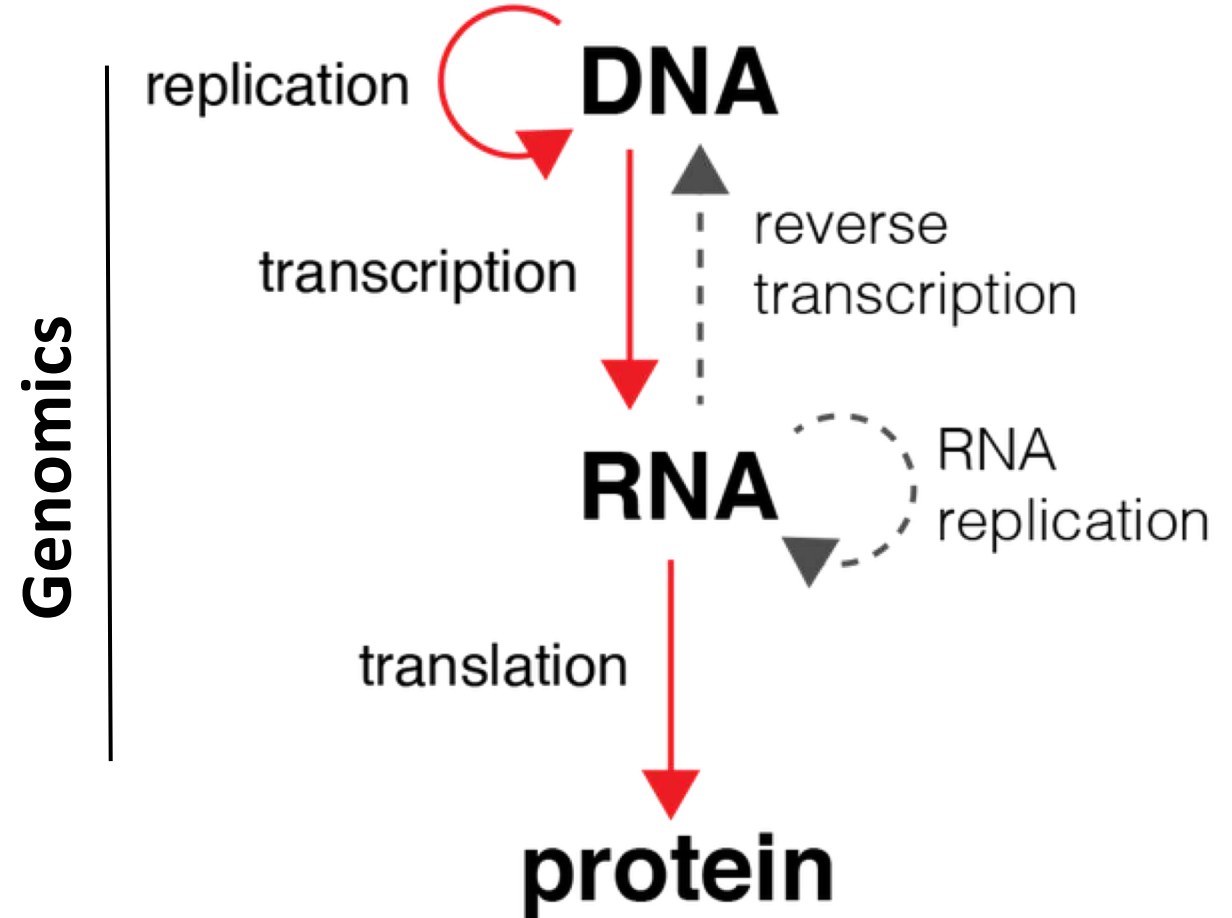
FISH

...

NGS

(Next Generation Sequencing)

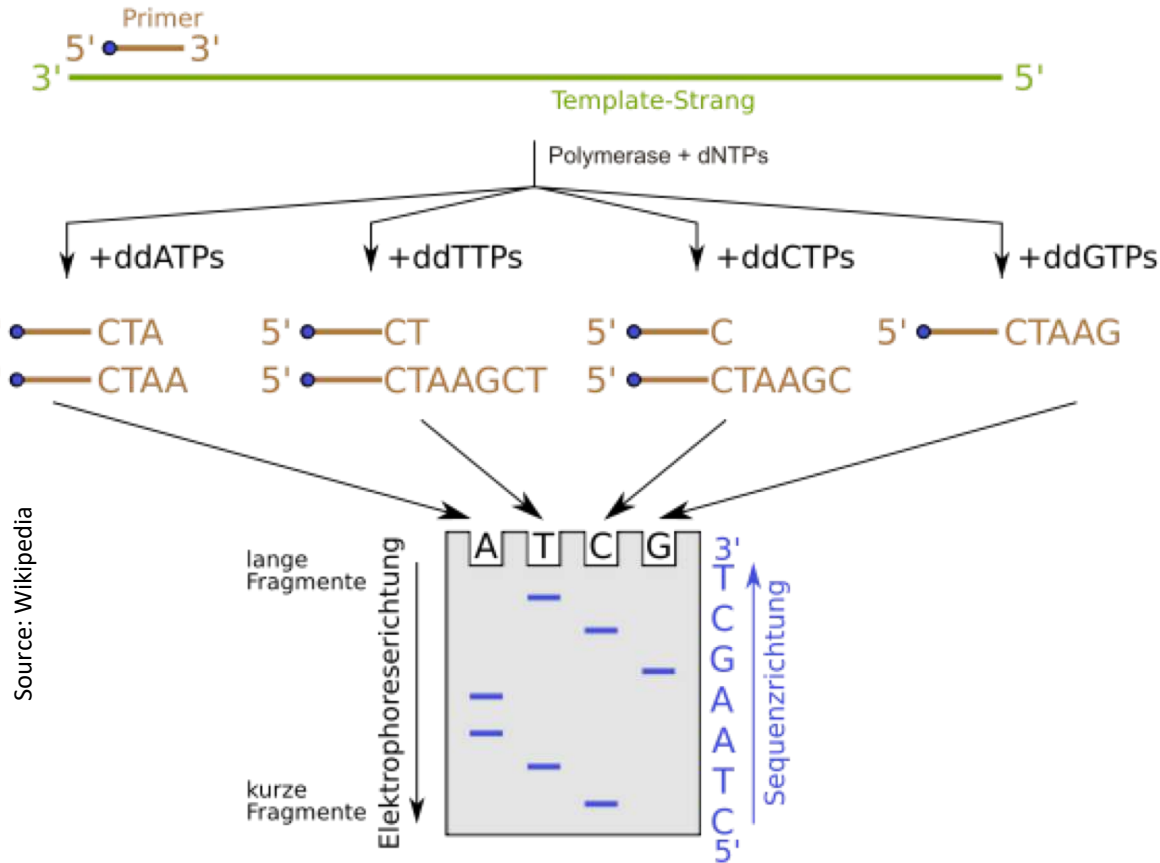
Single cell sequencing



Sanger



Frederick Sanger



Source: Wikipedia

Single cell sequencing



- ✧ First full human genome
- ✧ Large Sequencing centers from 6 countries,
- ✧ Hundreds of dedicated scientists
- ✧ Approx. 10 years
- ✧ > 3 billion USD



2005

2010

“A new generation of non-Sanger-based sequencing technologies has delivered on its promise of sequencing DNA at unprecedented speed, thereby enabling impressive scientific achievements and novel biological applications.”

nature methods: method of the Year 2007

**Massive Parallelization
of sequencing reactions!**

2014



2011



Pricing



Jul 24, 2018

Staphylococcus Genome Set Reveals Bug's Host-Switching History

When researchers sequenced *Staphylococcus aureus* isolates from domestic animals, they found evidence of host-switching events.

Aug 13, 2018

Deep Whole-Genome Sequencing Diagnoses Early Infantile Epileptic Encephalopathy

Researchers used deep whole-genome sequencing to identify a rare genetic mutation causing early infantile epileptic encephalopathy in a child.

Aug 17, 2018

International Consortium Sequences, Annotates Bread Wheat Reference Genome

The consortium, which has been led by researchers from the University of California, Davis, has completed the sequencing and annotation of the bread wheat reference genome.

Research | 17 September 2018

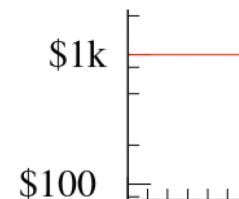
Genomic history of the Sardinian population

Analyses of 3,514 whole-genome-sequenced individuals from Sardinia indicate that within-island substructure and sex-biased processes have impacted the genetic history of Sardinia, providing new insight into the demography of ancestral Sardinians.

Charleston W. K. Chiang, Joseph H. Marcus [...] John Novembre

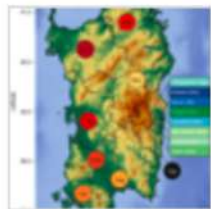
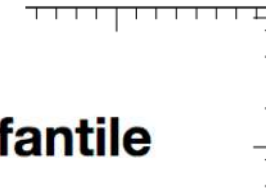
Nature Genetics, 1–9

Cost per

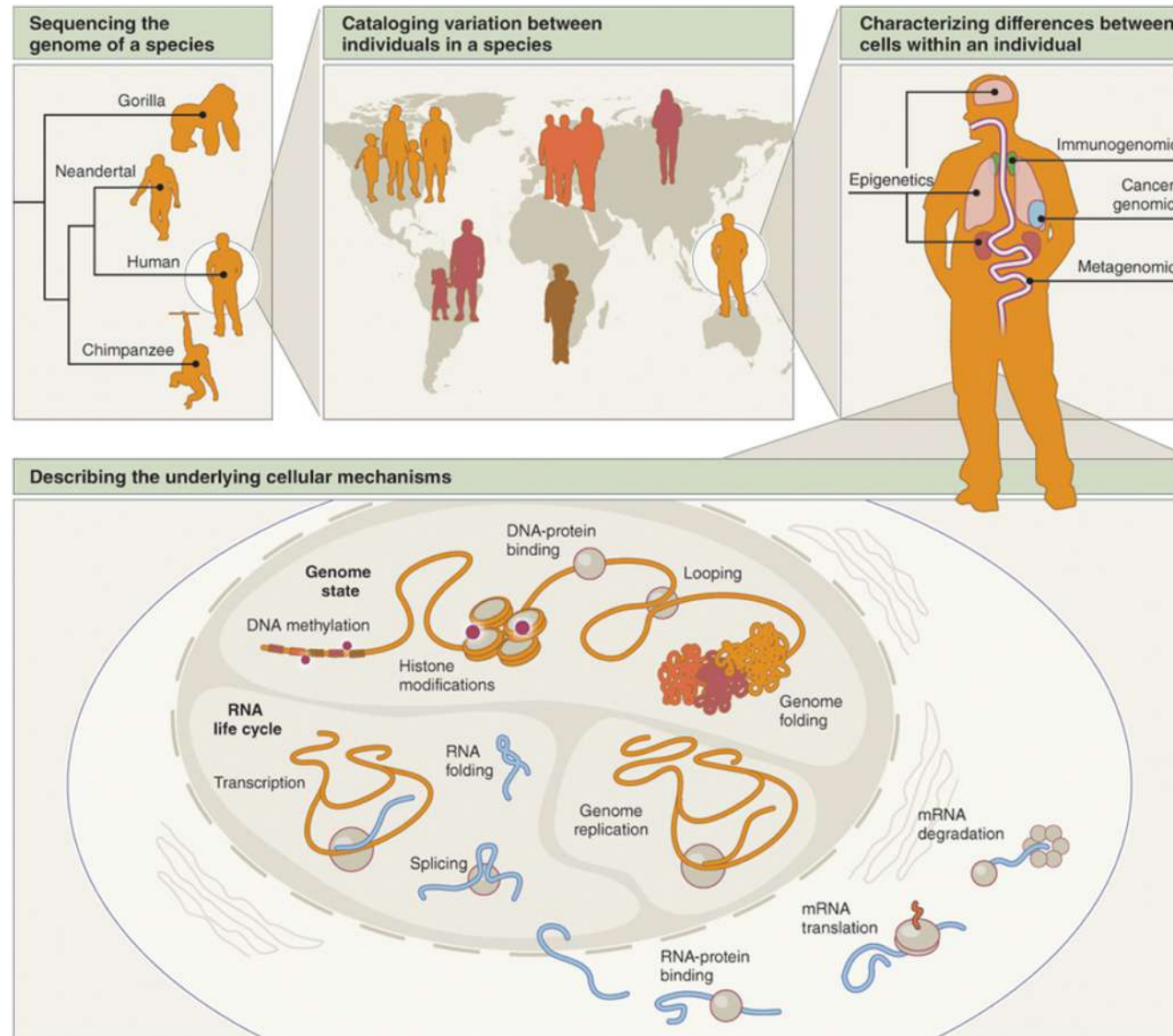


2001 2003 2005 2007 2009 2011 2013 2015 2017

[GRI]

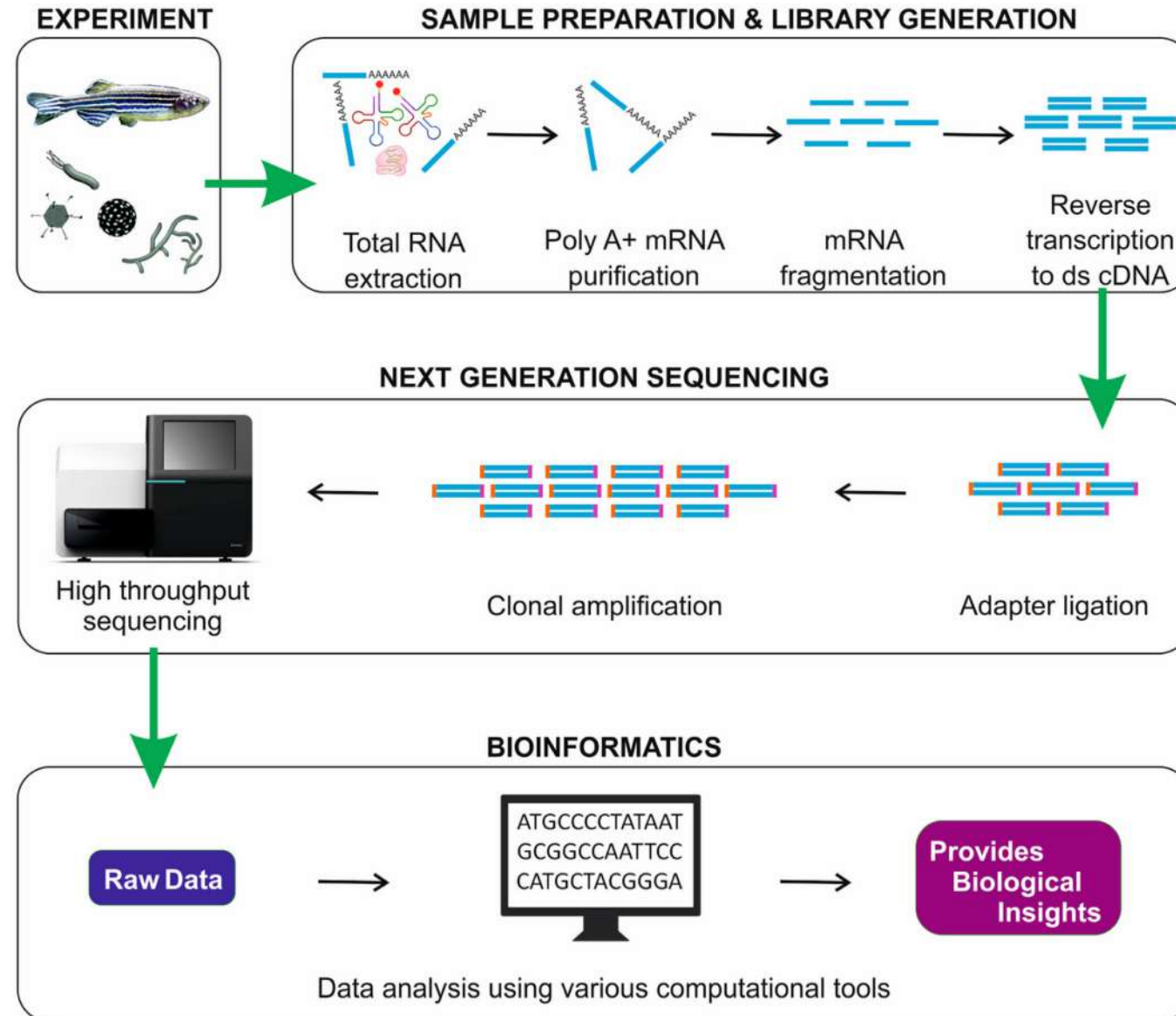


Protocols, Flexibility



Single cell sequencing

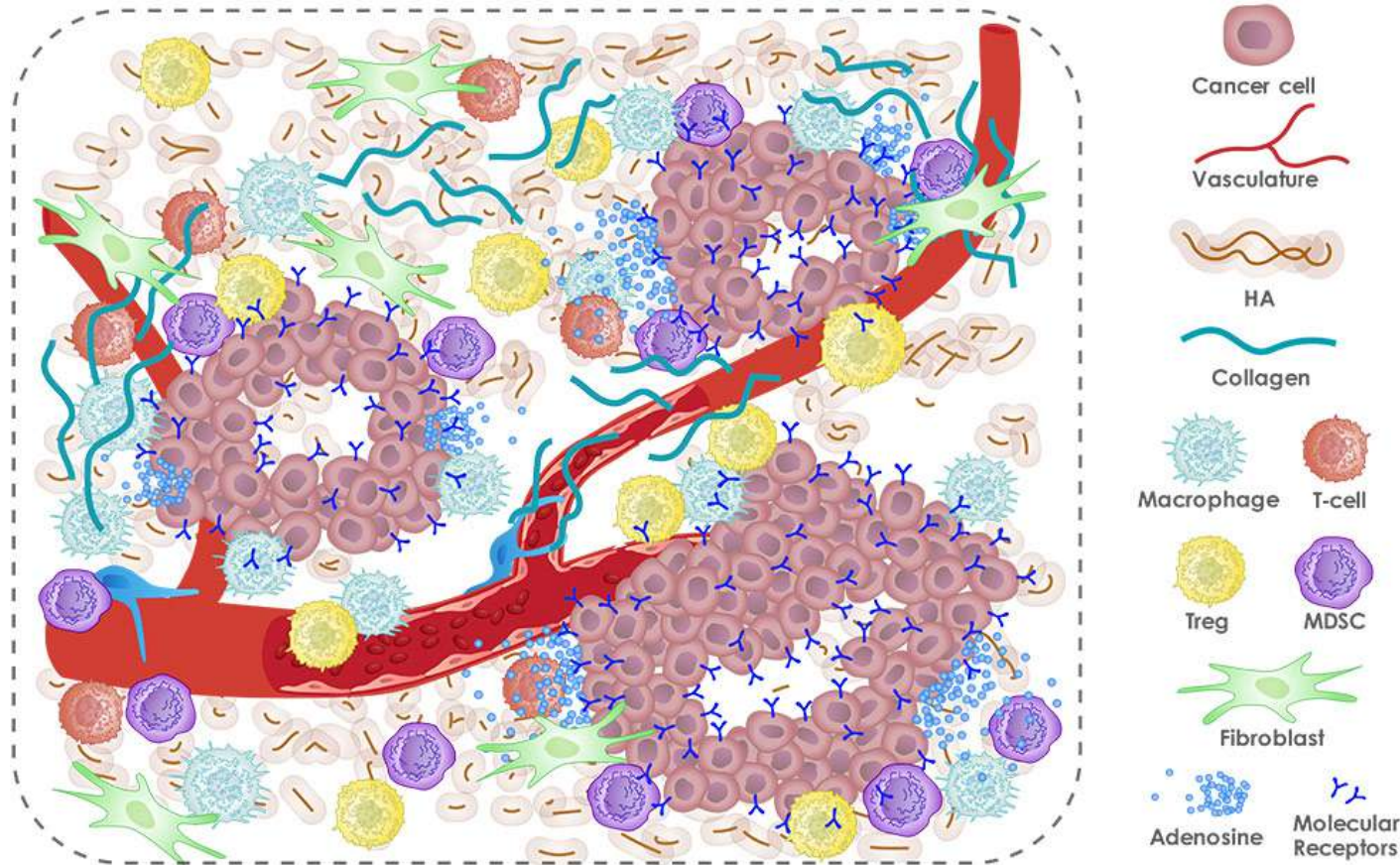
mRNA Sequencing



Single cell sequencing

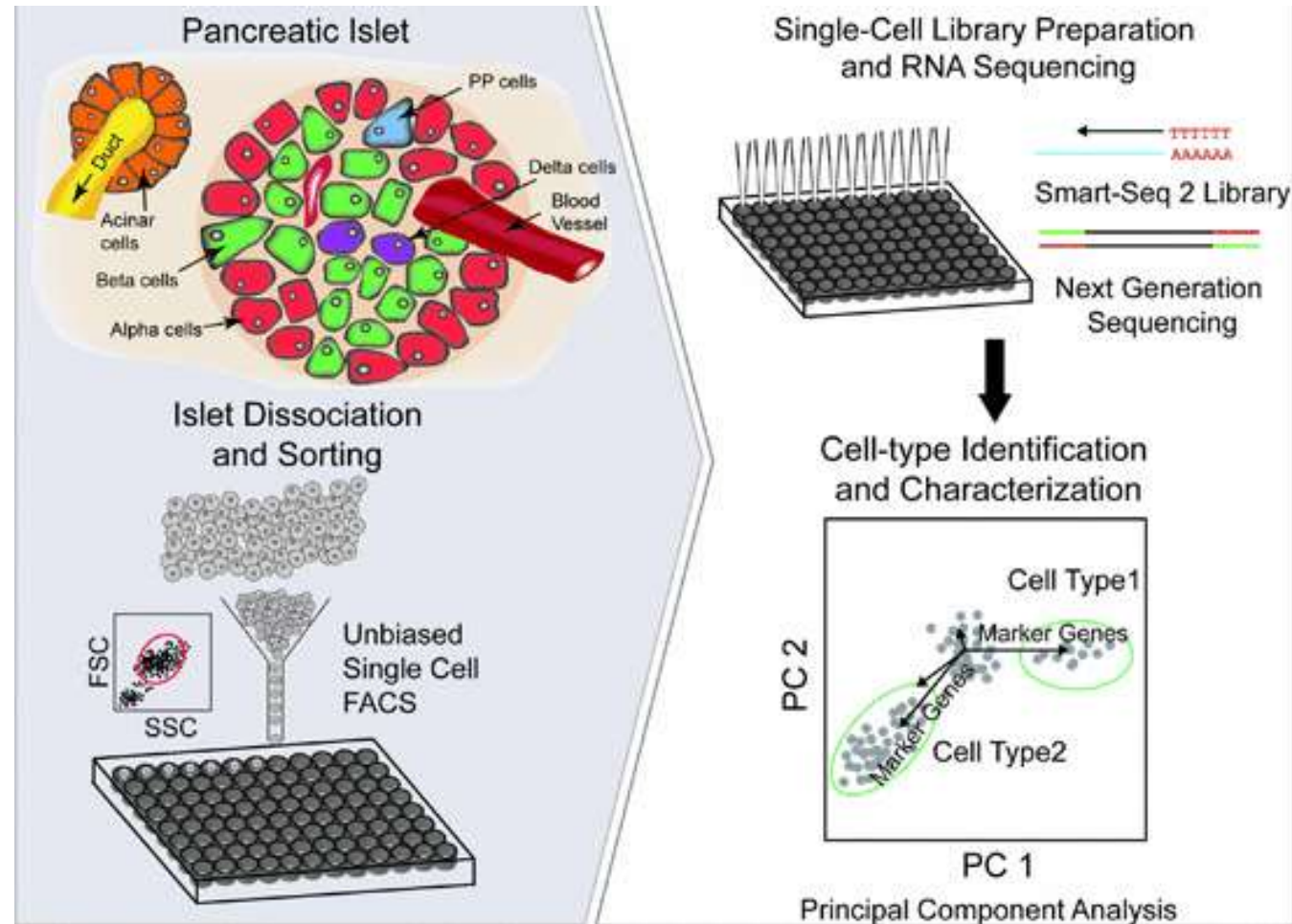
Heterogenous Material

The Tumor Microenvironment

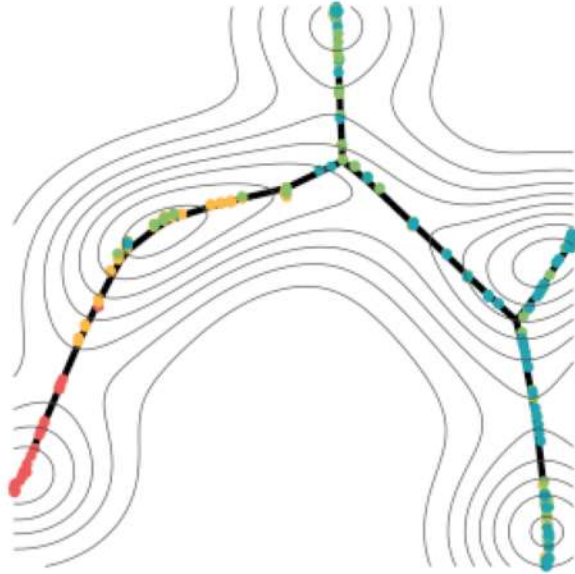


Source: <https://www.halozyme.com/science/>

Single RNA cell sequencing

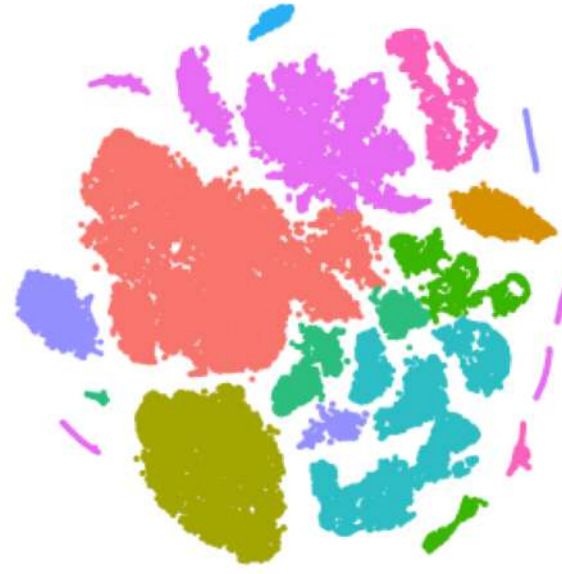


Data Analysis



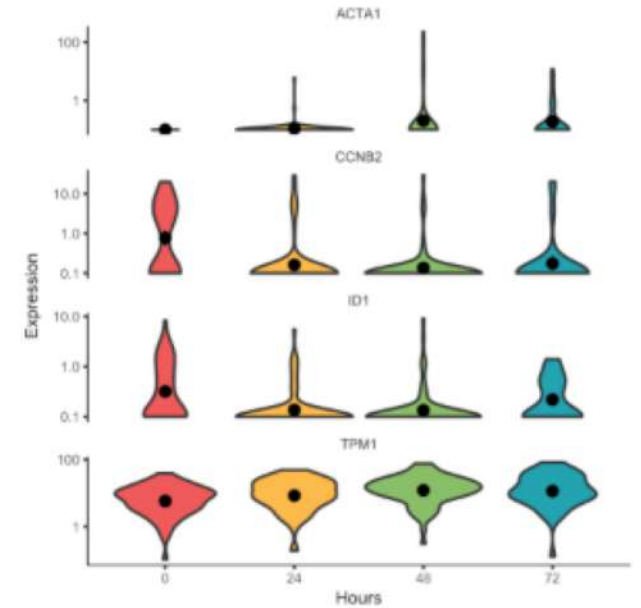
Pseudotime

Build single-cell trajectories with the software that introduced pseudotime. Find cell fate decisions and the genes regulated as they're made.



Clustering

Group and classify your cells based on gene expression. Identify new cell types and states and the genes that distinguish them.

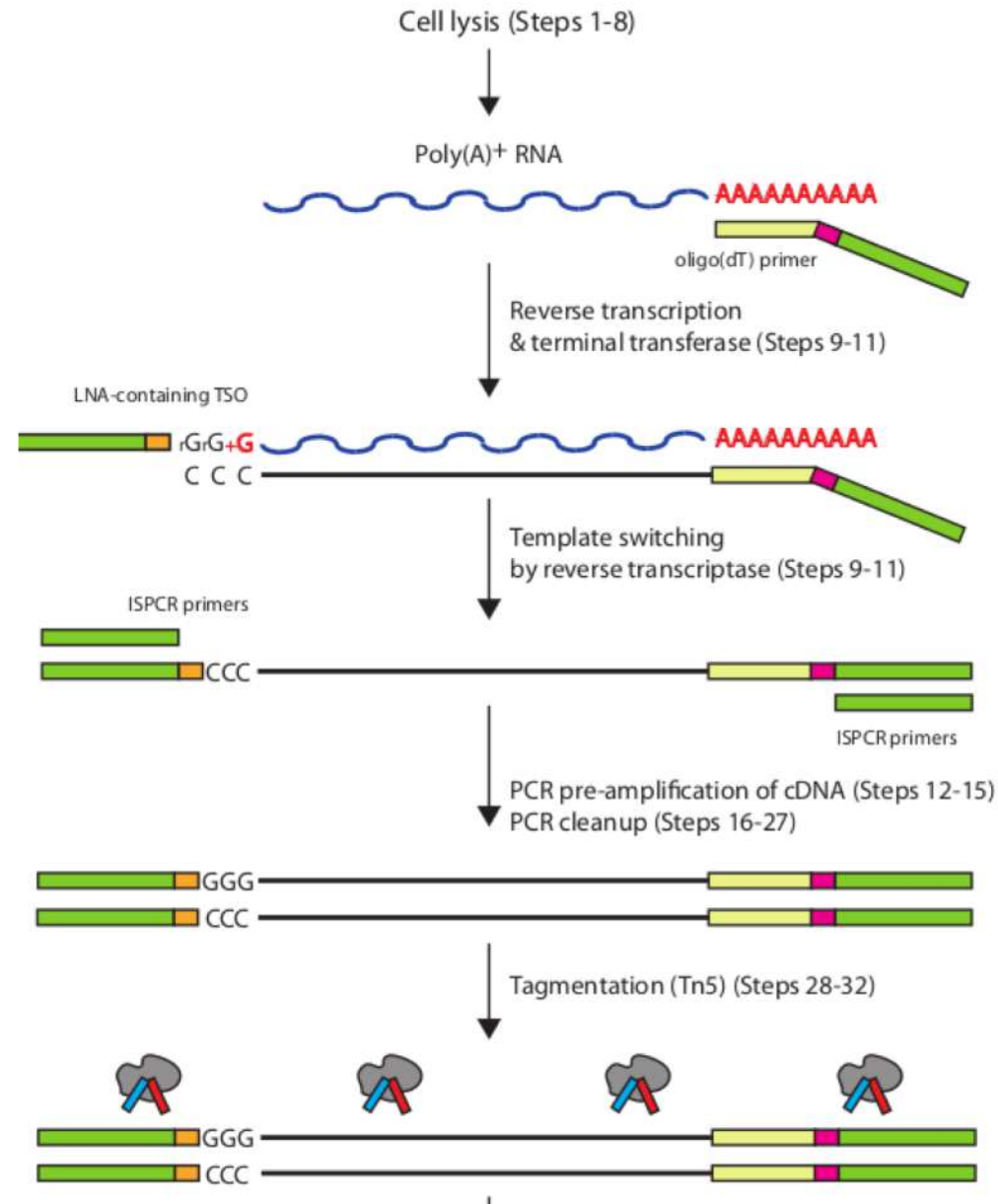


Differential expression

Find genes that vary between cell types and states, over trajectories, or in response to perturbations using statistically robust, flexible differential analysis.

Source: 10X Genomics

Smart-seq 2



Nature Protocols volume 9, pages 171–181 (2014)

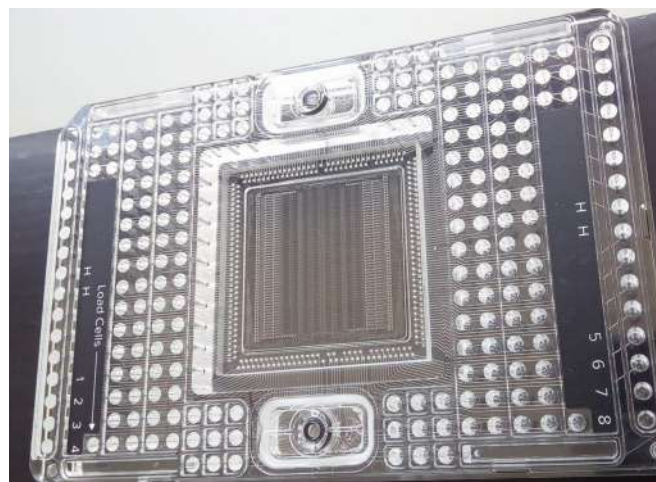
Pilot project introduction



- Project partners:
 - **VBCF – NGS Core Facility, CEITEC – Genomics Core Facility**
- Complementary equipment/expertise:
 - **Library preparation expertise, NGS sequencers, FACS, LCM, Fluidigm C1**
- Basic project idea:
 - **Set up a protocol for single cell RNA sequencing used at both sites**
- Project goal:
 - **Broadening the range of our services based on requirements of our users**
- End-users:
 - **Academic users (eg. oncology research)**

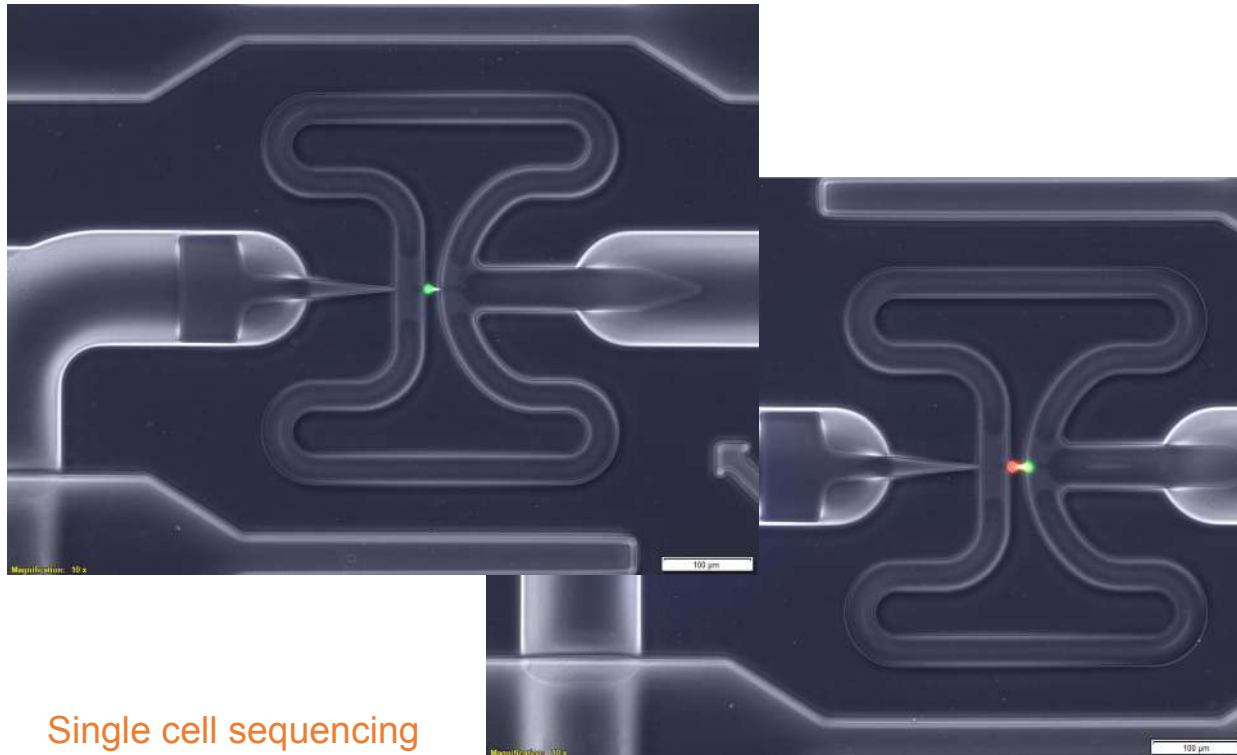
Project implementation

- Approach/methodology
 - **Library protocols based on SMART-Seq2 method**
 - Open solution according to *Picelli 2014*
 - Solution using Fluidigm C1 + commercial kit
- Implementation
 - **Implementation of Smart-Seq2 at VBCF** → transfer of the protocol to CEITEC
 - Staff exchange (Filip Pardy at VBCF)
 - **Implementation of Smart-Seq2 on Fluidigm C1 at CEITEC**

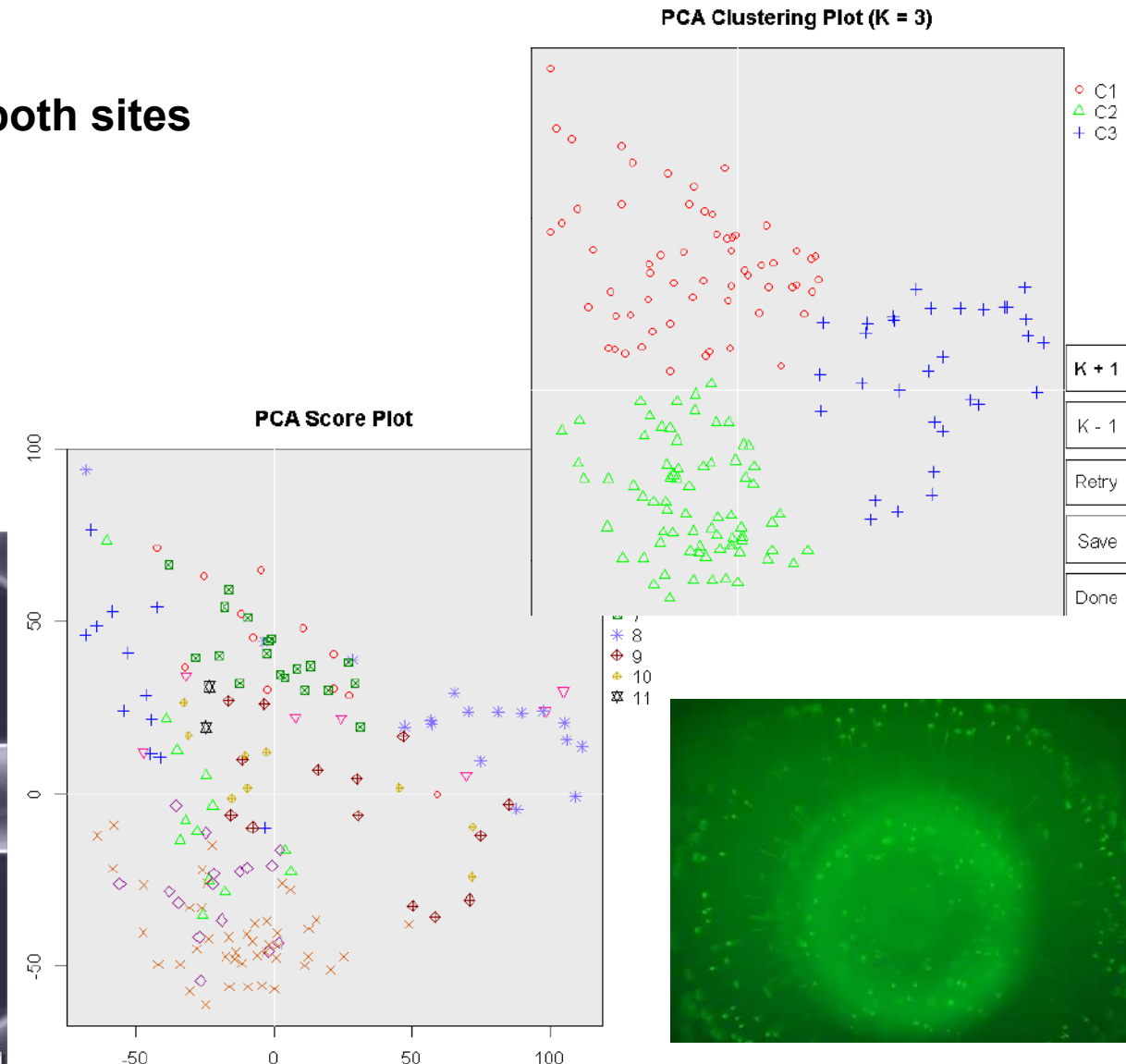


Project results

- Results:
 - Method for single-cell RNA sequencing at both sites**
- Benefits for the users:
 - No averaging of gene expression**
 - Screening for cell subpopulations**

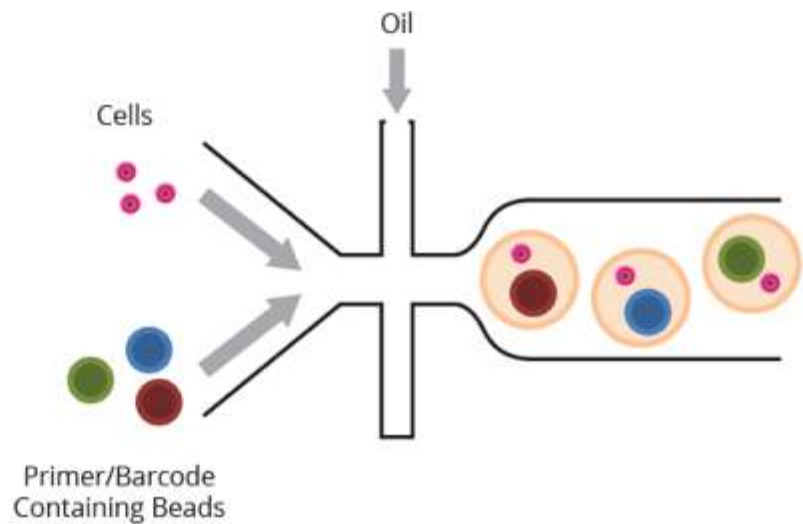


Single cell sequencing



Future

- New protocols?
 - 3' single-cell RNA-Seq
 - sci-RNA-Seq (single-cell combinatorial indexing RNA sequencing)
 - 10x Genomics single-cell RNA-Seq



Single cell sequencing

