Single cell sequencing

Boris Tichý (CEITEC-MU), Andreas Sommer (VBCF)

24. 09. 2018

Genomics

Genomics Tools:
Microarrays
PCR
Sanger
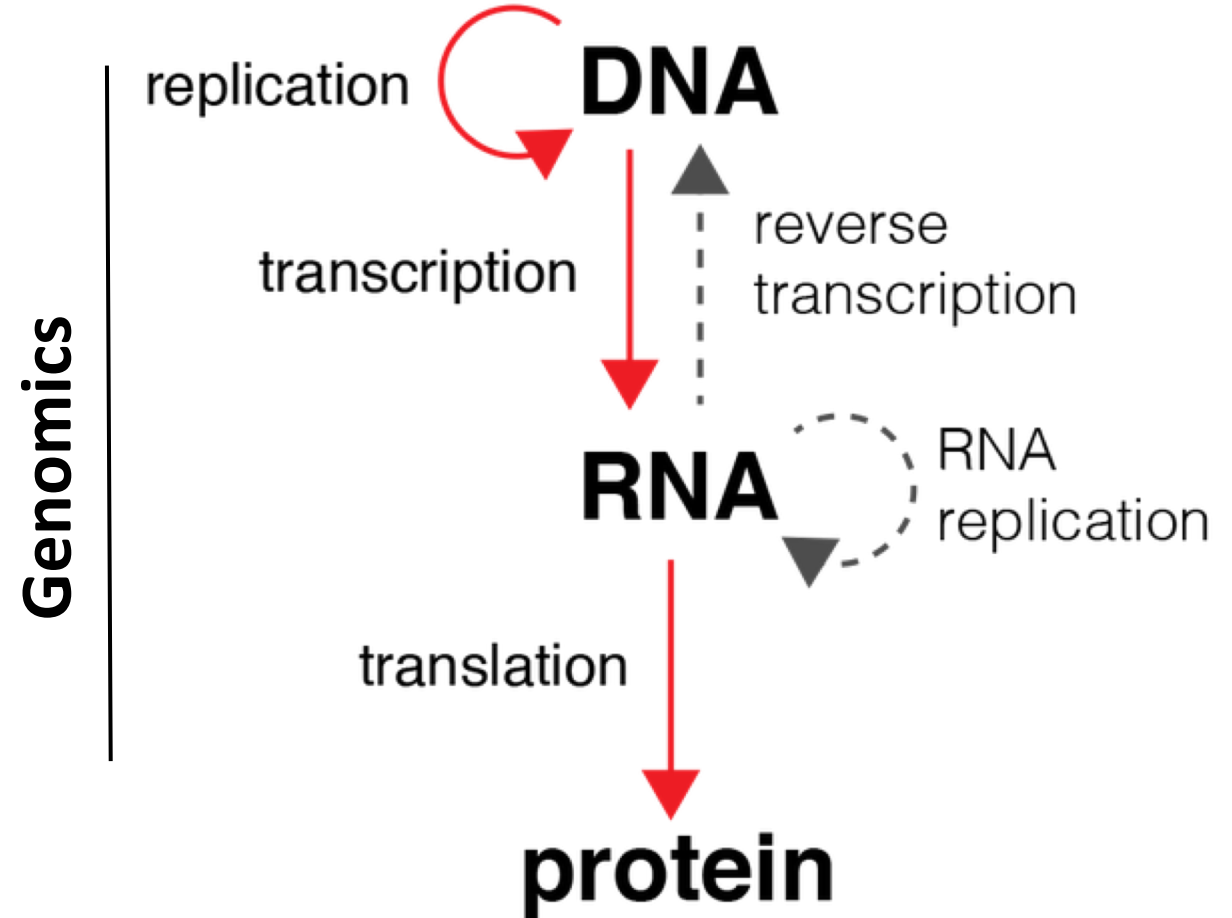FISH
…
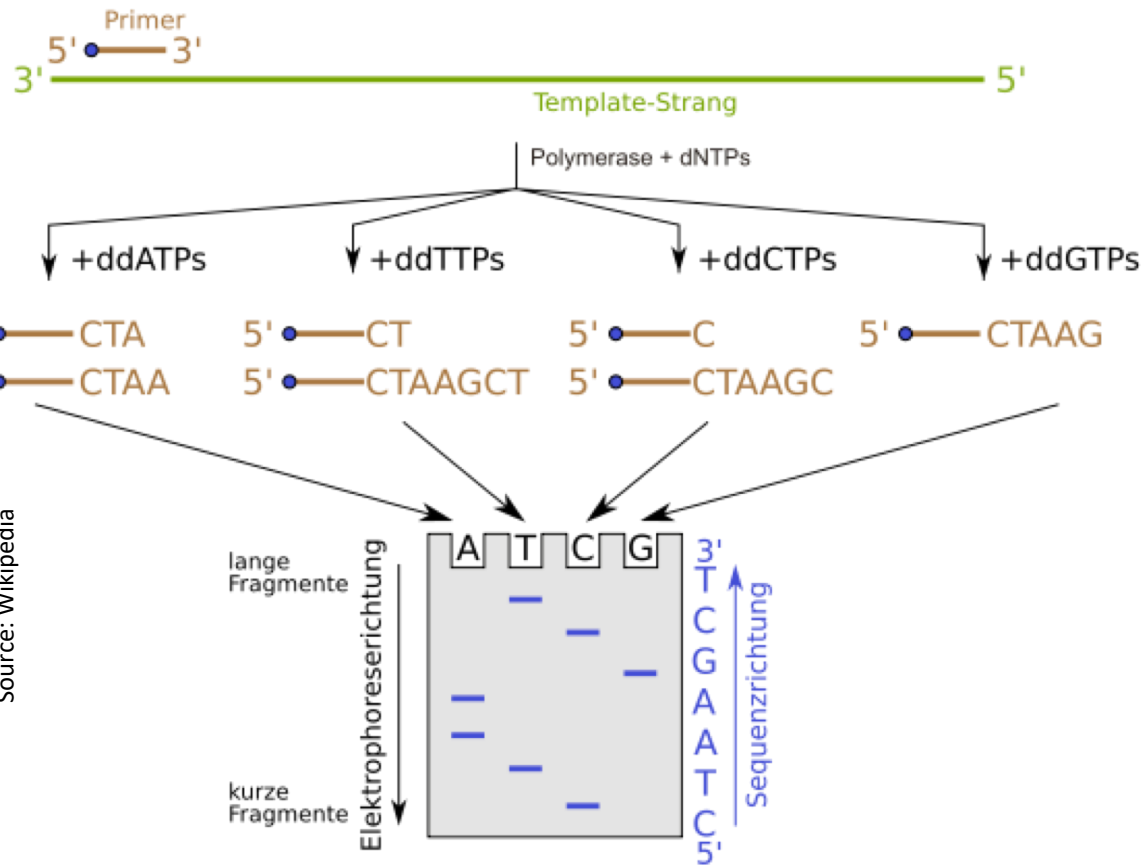**NGS
(Next Generation Sequencing)**

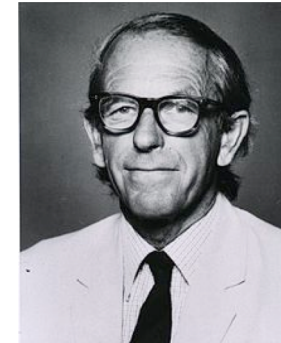Single cell sequencing

Sanger

Frederick Sanger

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

Single cell sequencing

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

EUROPEAN UNION

Interreg
Austria-Czech Republic
European Regional Development Fund

Jul 24, 2018
Staphylococcus Genome Set Reveals Bug's Host-Switching History

When rese
aureus isol
domestic a

Aug 13, 2018
Deep Whole-Genome Sequencing Diagnoses Early Infantile Epileptic Encephalopathy

Researchers
testing coul

Aug 17, 2018
International Consortium Sequences, Annotates Bread Wheat Reference Genome

The consortiu
which has be
researchers.

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.
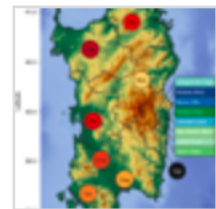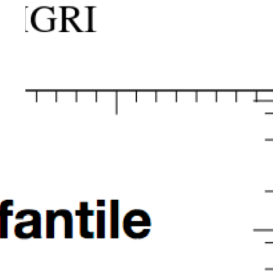
Charleston W. K. Chiang, Joseph H. Marcus [...] John Novembre
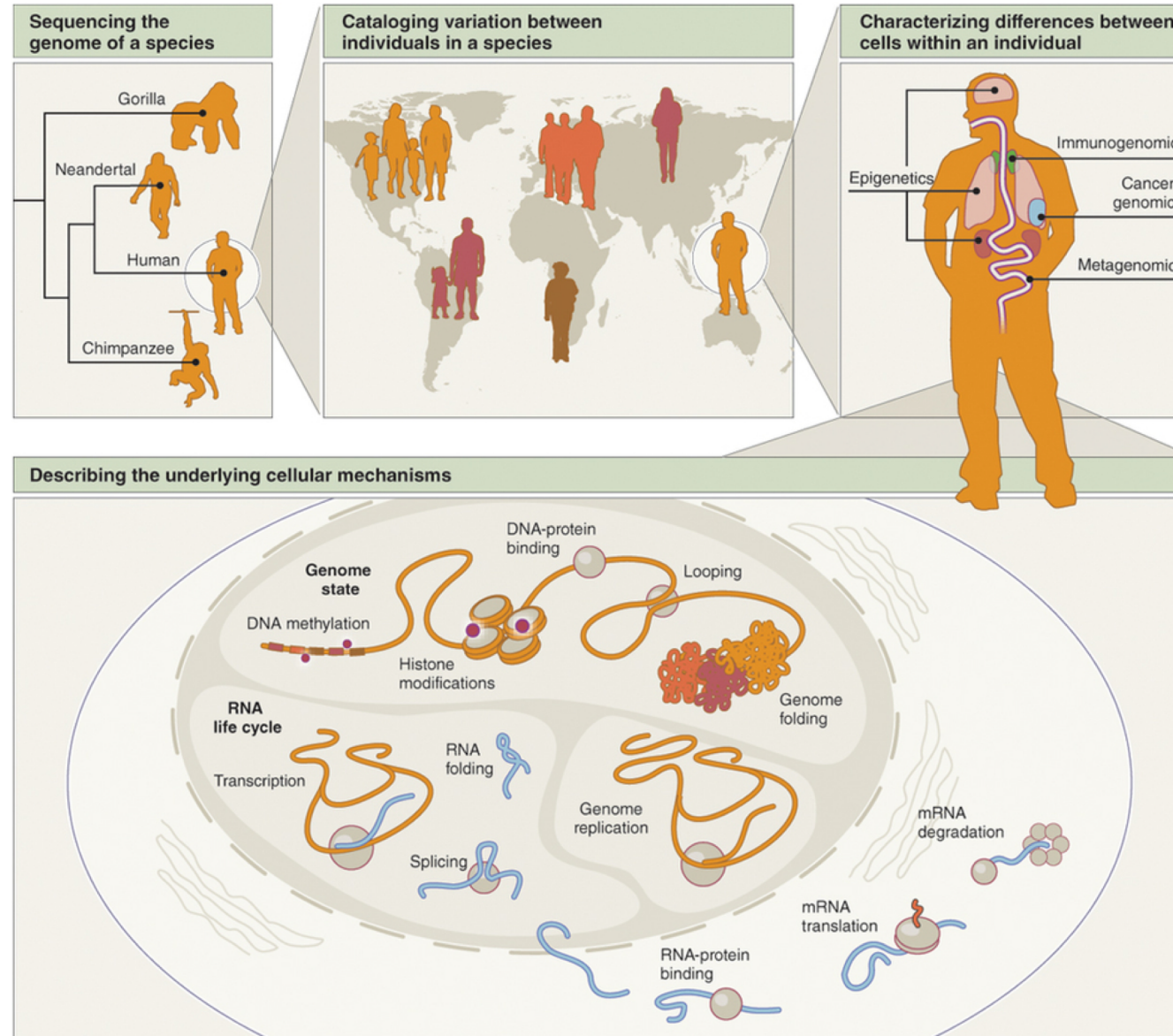
Nature Genetics, 1–9

Cost pe

$1k

$100
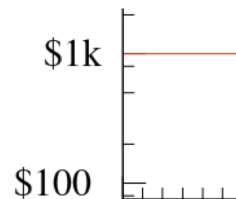
2001   2003   2005   2007   2009   2011   2013   2015   2017
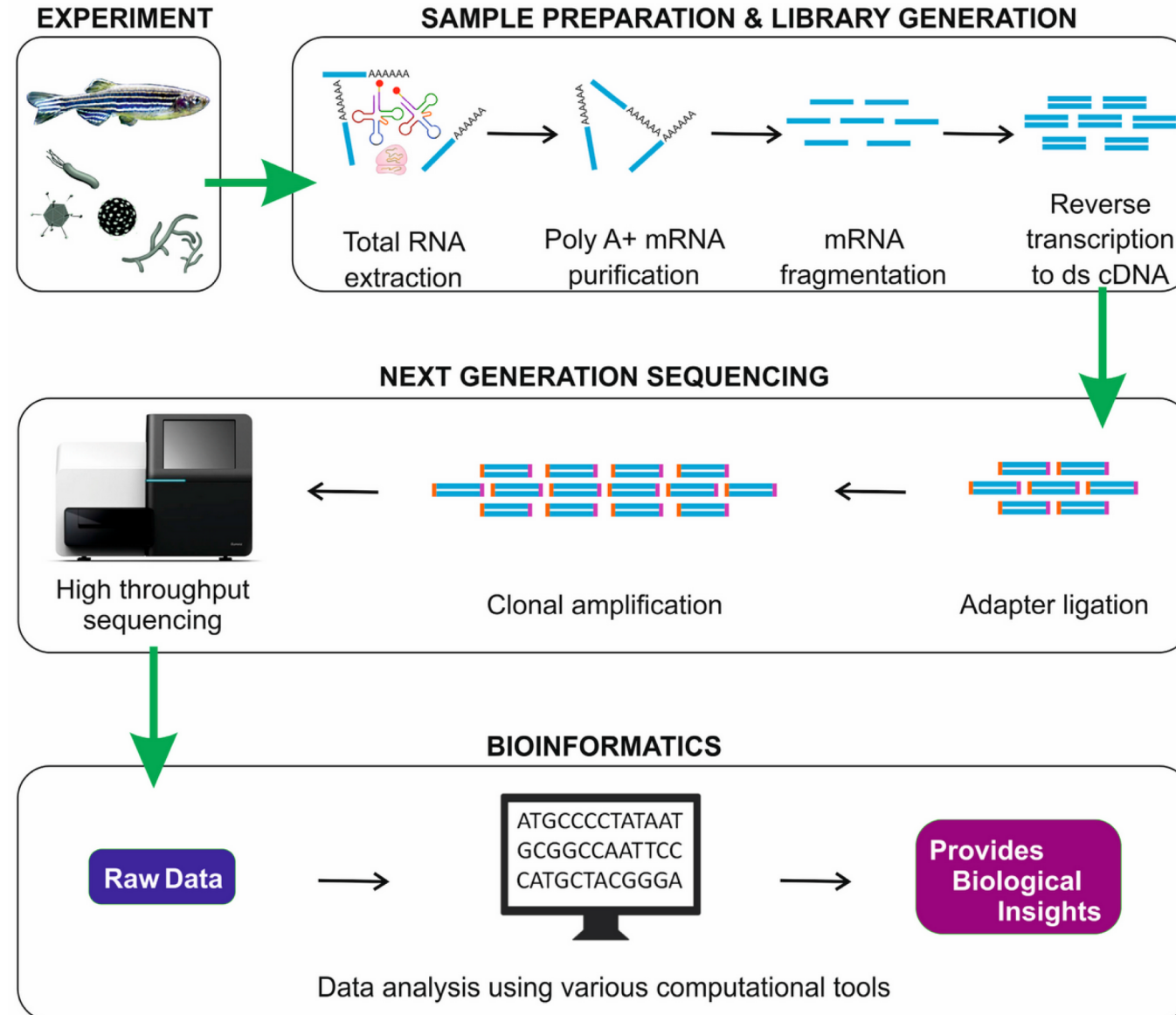
GRI

Single cell sequencing

Protocols, Flexibility

Single cell sequencing

Nature Biotechnology 30, 1084–1094 (2012)

mRNA Sequencing

Single cell sequencing

Heterogenous Material

The Tumor Microenvironment

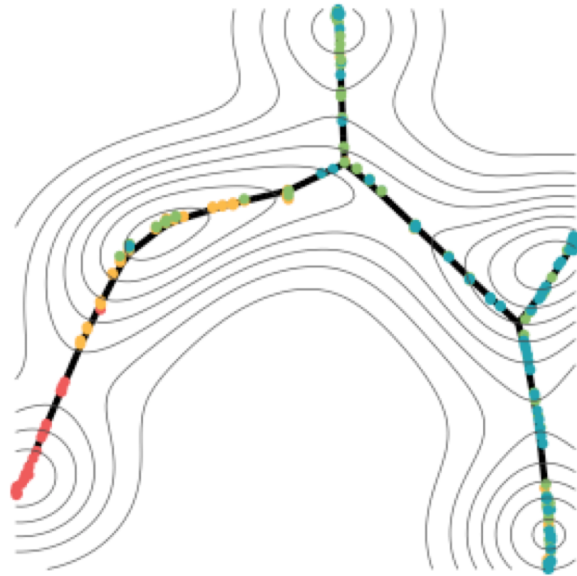Source: https://www.halozyme.com/science/
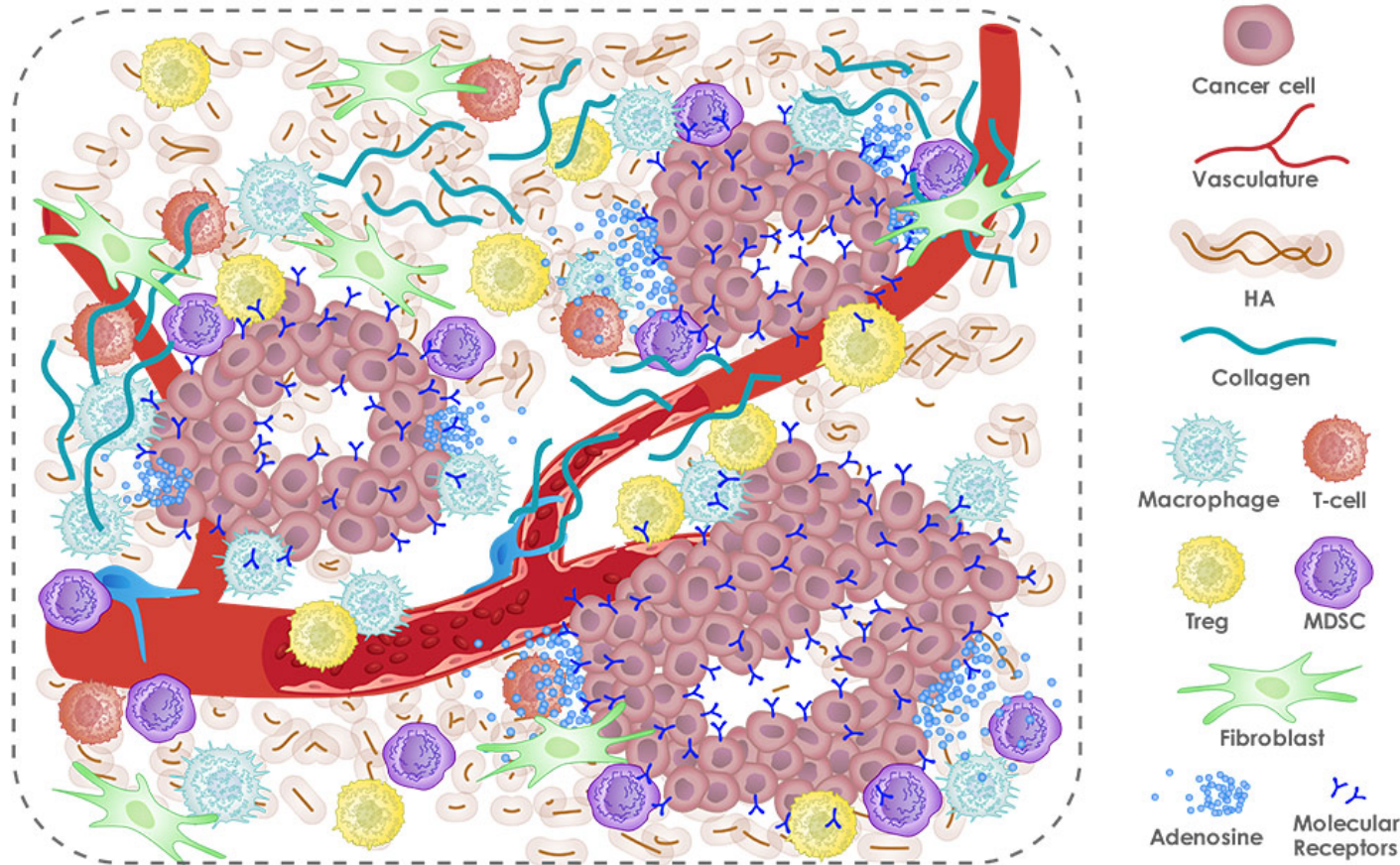
Single cell sequencing

Single RNA cell sequencing

Single cell sequencing
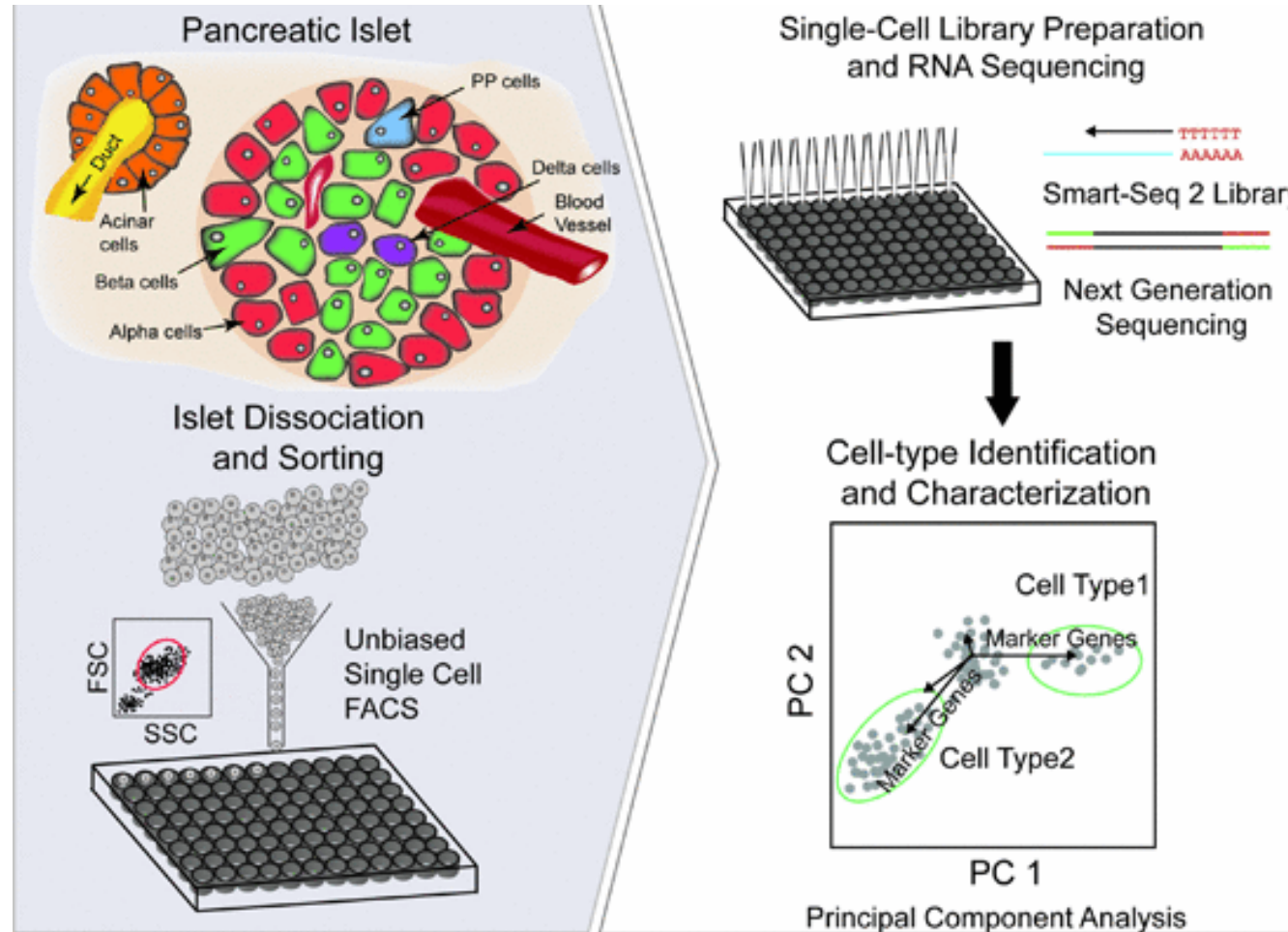
Data Analysis

Pseudotime
Build single-cell trajectories with the software that ntroduced 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.
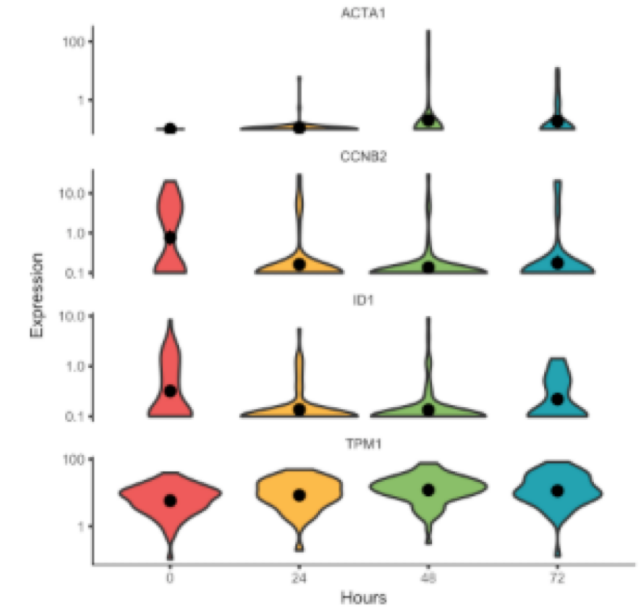
Differential expression
Find genes that vary between cell types and states, over trajectories, or in response to perturbations using statistically robust, flexible differential analysis.

Source: 10X Genomics

Single cell sequencing

Smart-seq 2

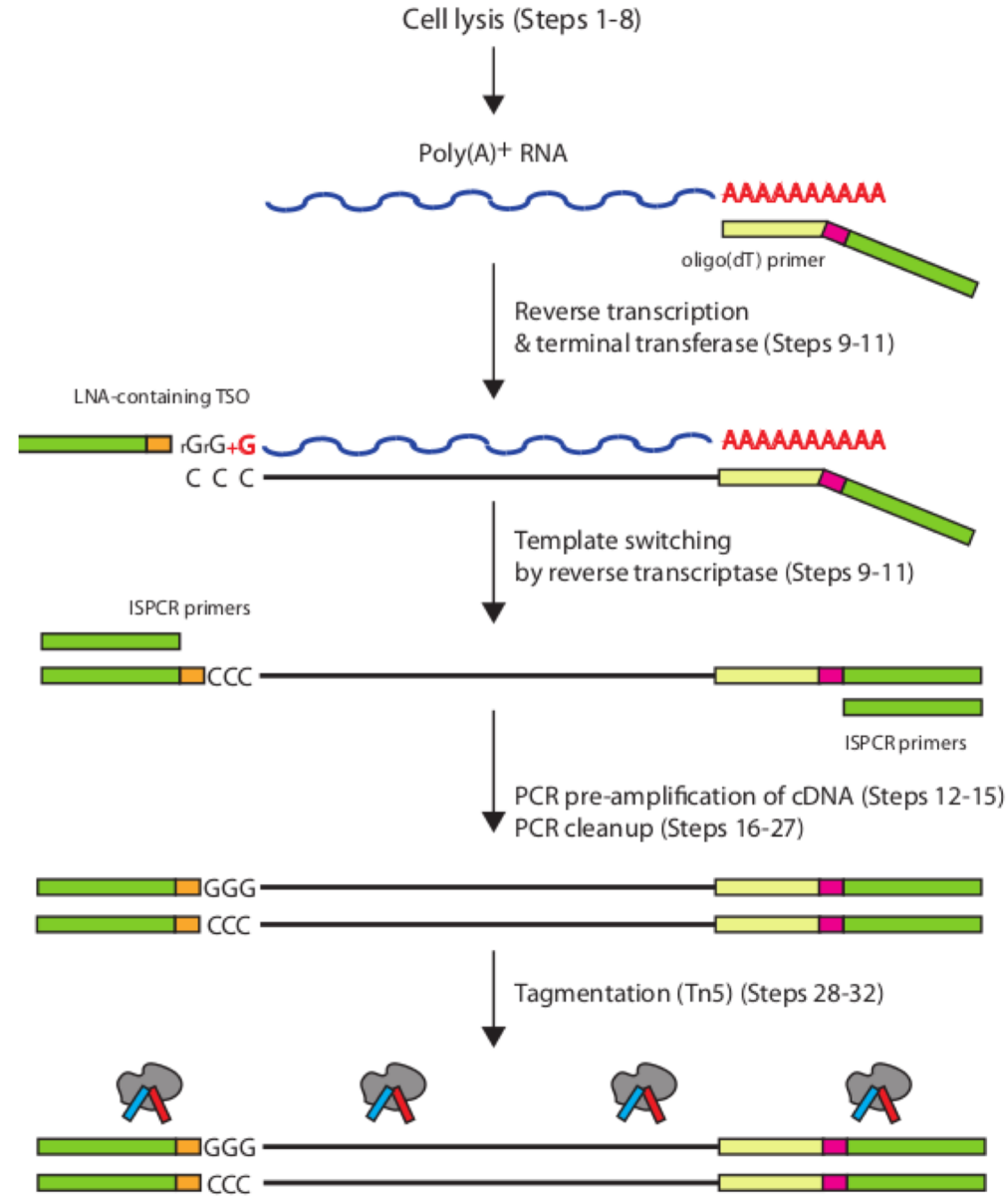Cell lysis (Steps 1-8)

Poly(A)$^+$ RNA

AAAAAAAAAA

oligo(dT) primer

Reverse transcription
& terminal transferase (Steps 9-11)

LNA-containing TSO

rGrG+G   AAAAAAAAAA
C C C

Template switching
by reverse transcriptase (Steps 9-11)

ISPCR primers

CCC

ISPCR primers

PCR pre-amplification of cDNA (Steps 12-15)
PCR cleanup (Steps 16-27)

GGG
CCC

Tagmentation (Tn5) (Steps 28-32)

GGG
CCC

Single cell sequencing

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)**

Single cell sequencing

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**
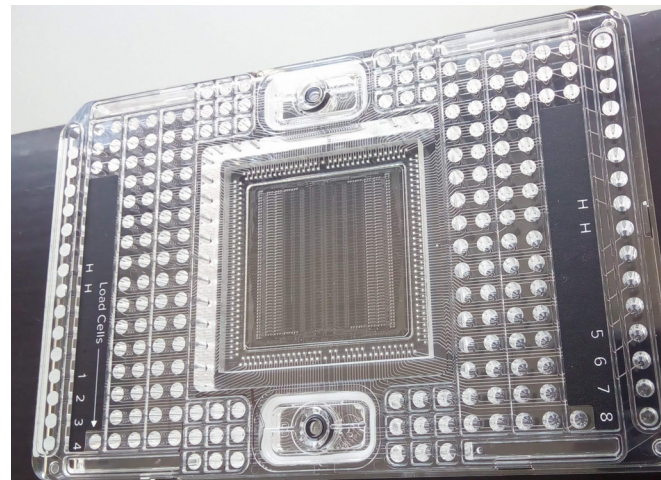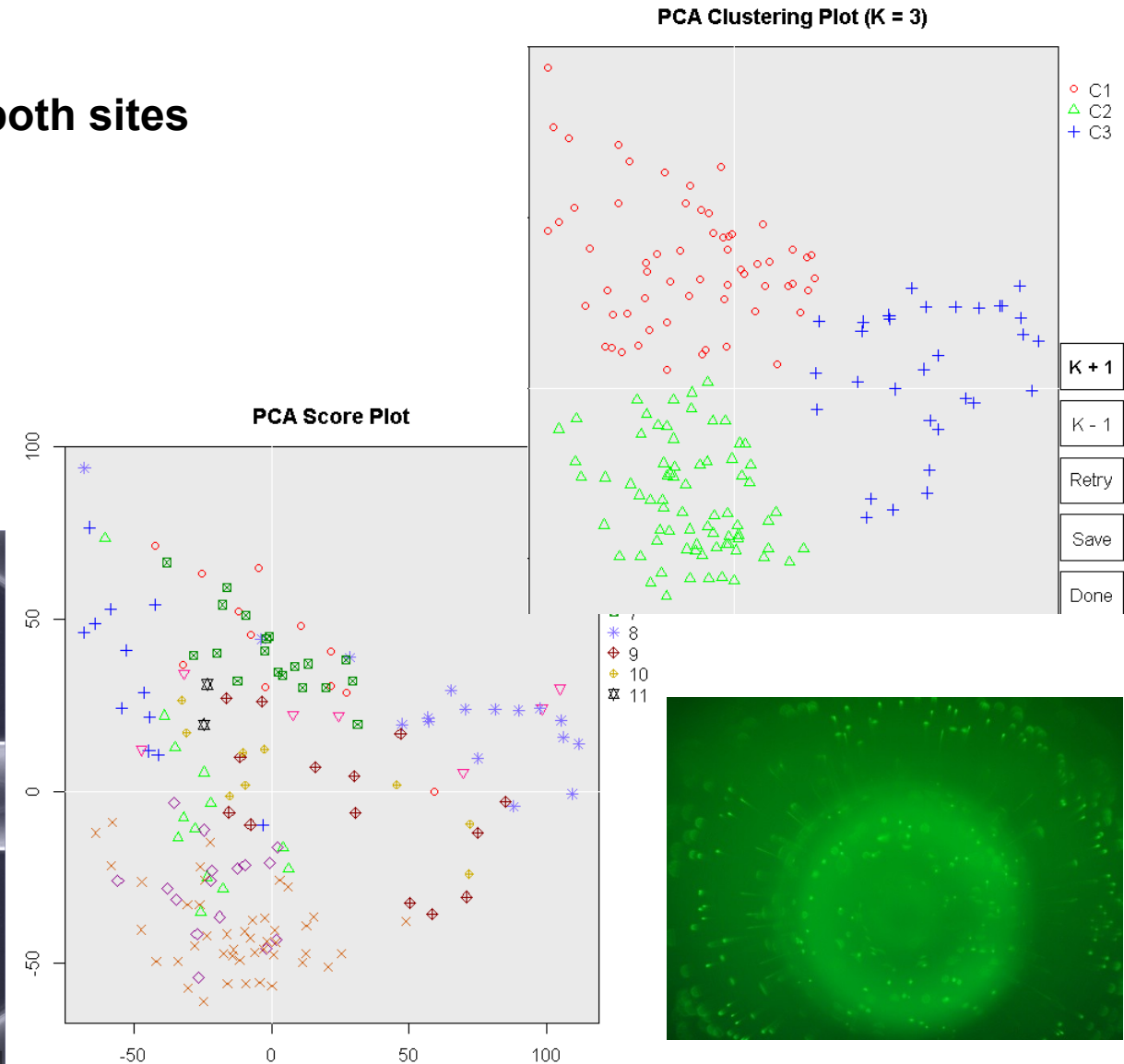

Picelli et al., Nature Protocols 2014

Single cell sequencing

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

PCA Clustering Plot (K = 3)

PCA Score Plot
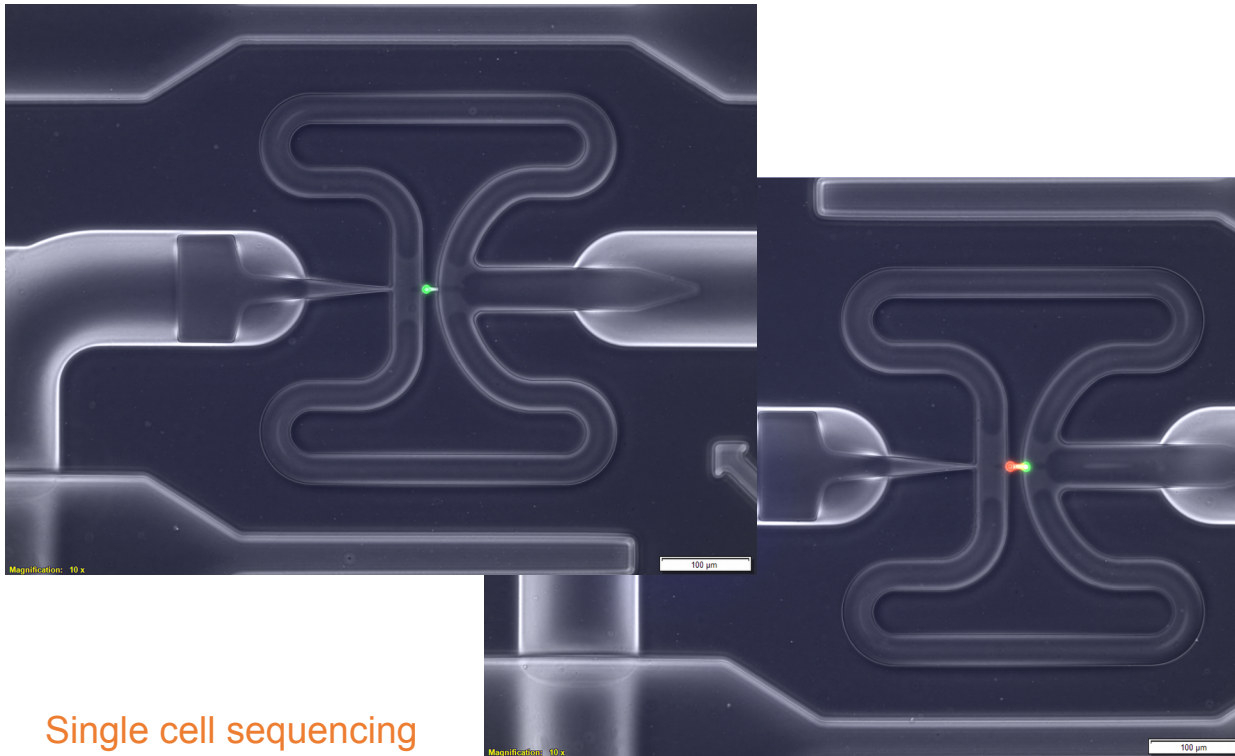
Future

- 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