



Central European Institute of Technology  
BRNO | CZECH REPUBLIC

Boris Tichý

RNA-seq

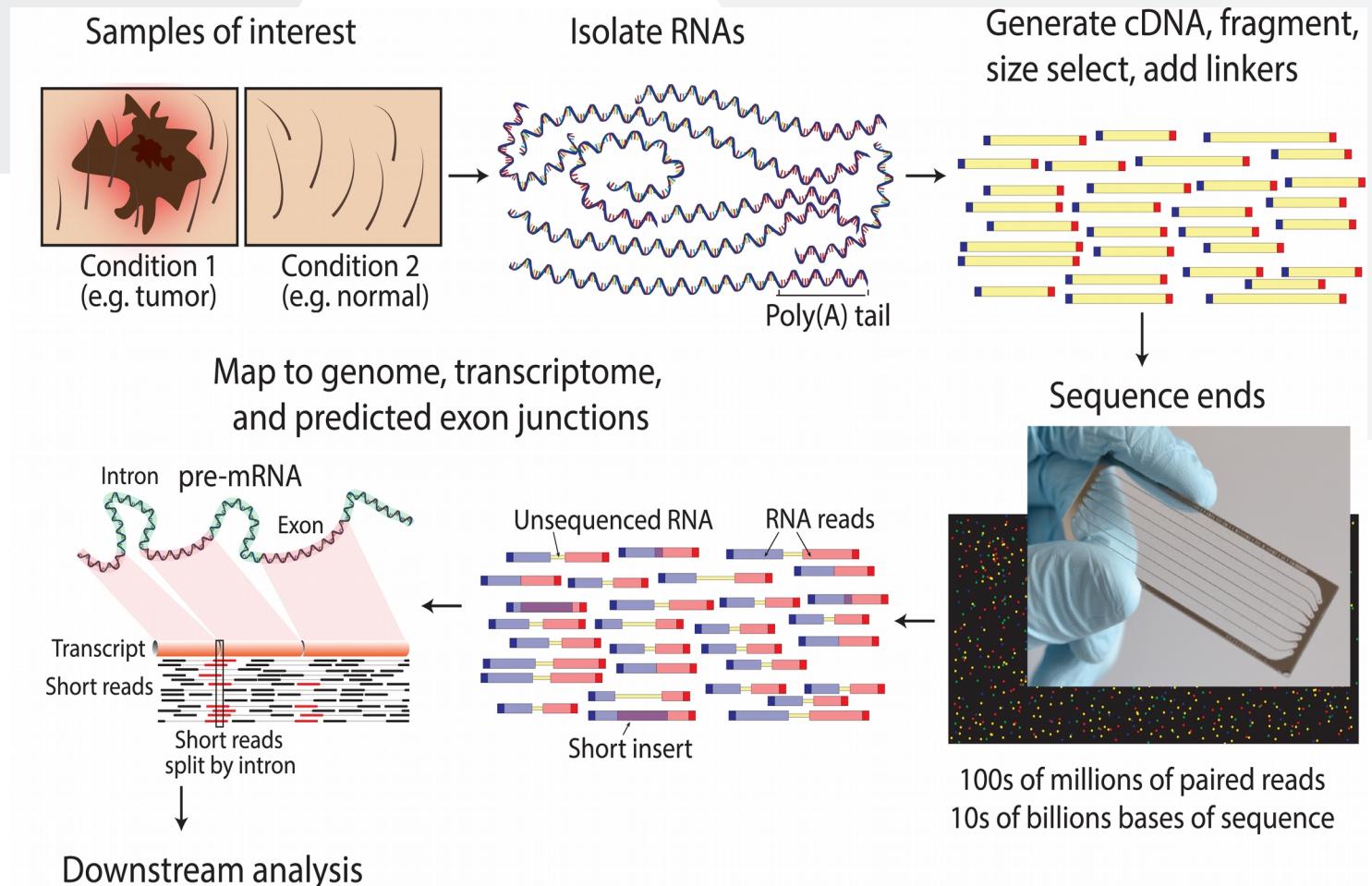
MUNI



# RNA-seq

## Stanovení sekvence a exprese genů/exonů

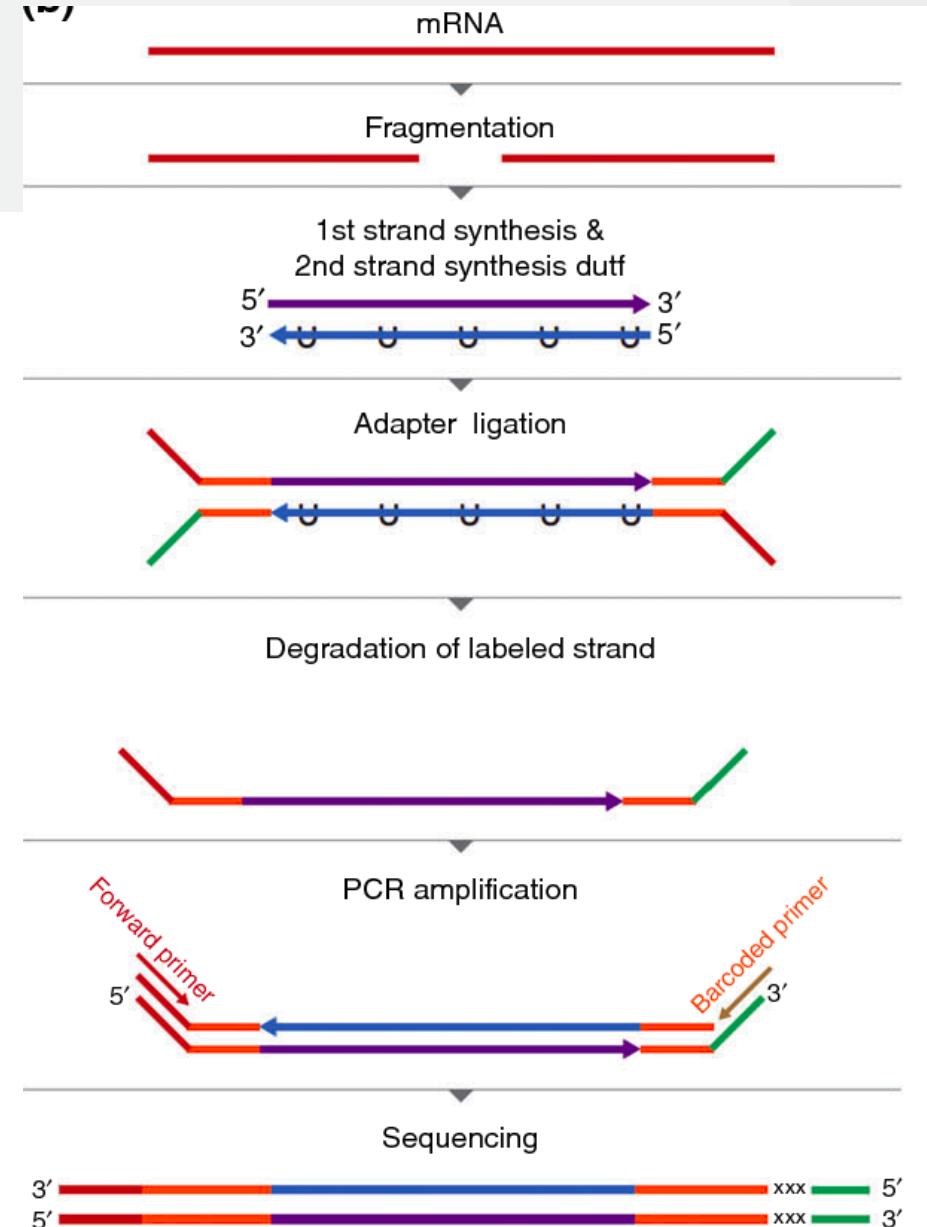
- “Clasical” RNA-seq
  - Whole transcriptome
  - PolyA selection
  - rRNA depletion
- 3' RNA-seq
- targeted RNA sequencing
- single-cell RNA sequencing
- smallRNA sequencing
- long-read RNA sequencing
- direct RNA sequencing



# RNA-seq

## „classical“ RNA-seq

- Sekvenace RNA s/bez selekce
- Fragmentace
  - teplota/chemicky
  - primery
- Reverzní transkripce – random primery
- Double-stranded cDNA
  - directional/non-directional
- Ligace adaptorů

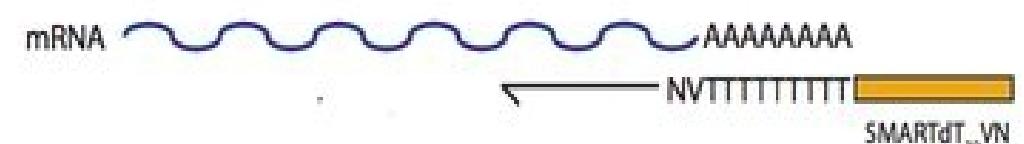
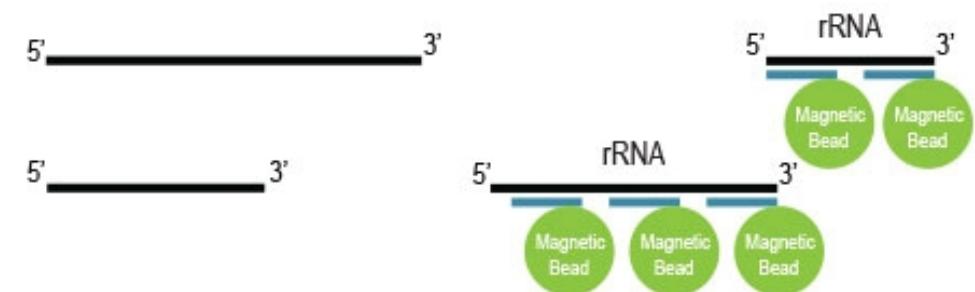


# RNA-seq

## „classical“ RNA-seq

- Selekcje
  - polyA (mRNA)
    - beads
    - primer
  - rRNA deplece
- Differential expression
- Splicing analysis
- De-novo transcriptome
- Variant analysis

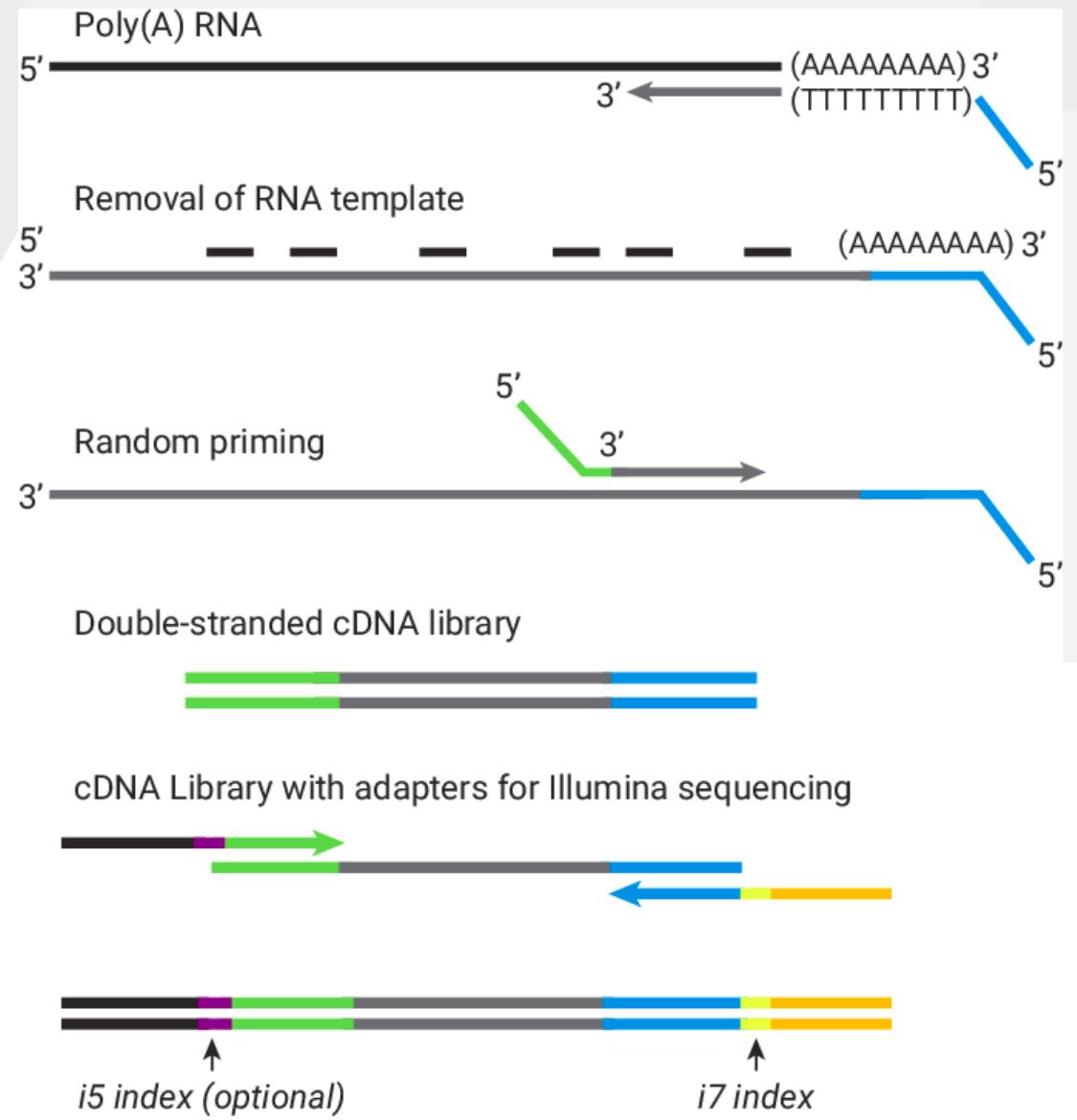
### 1. mRNA capture



# RNA-seq

## 3' RNA-seq

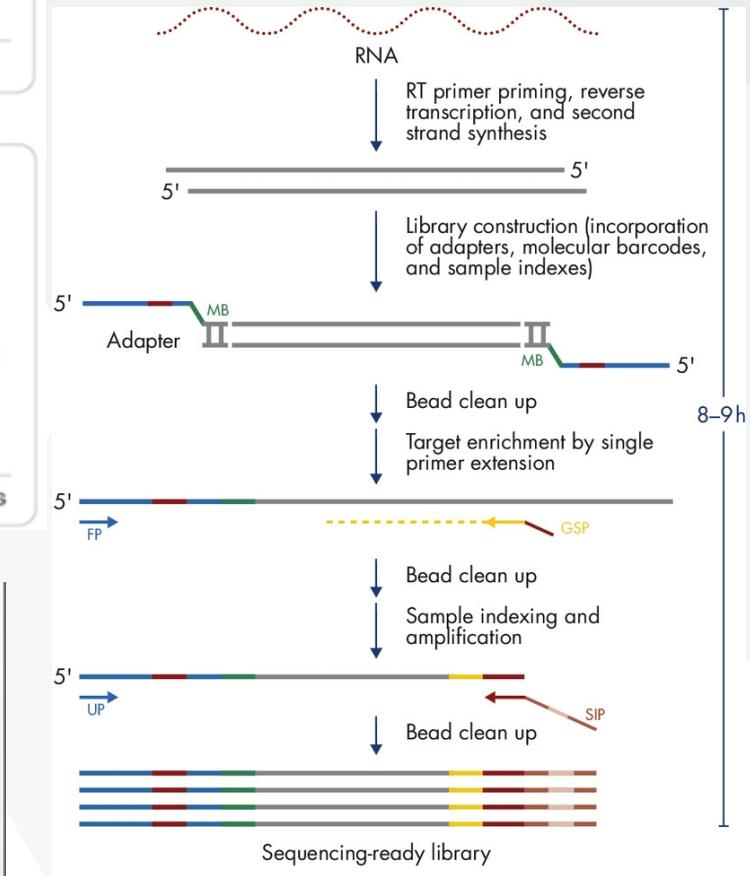
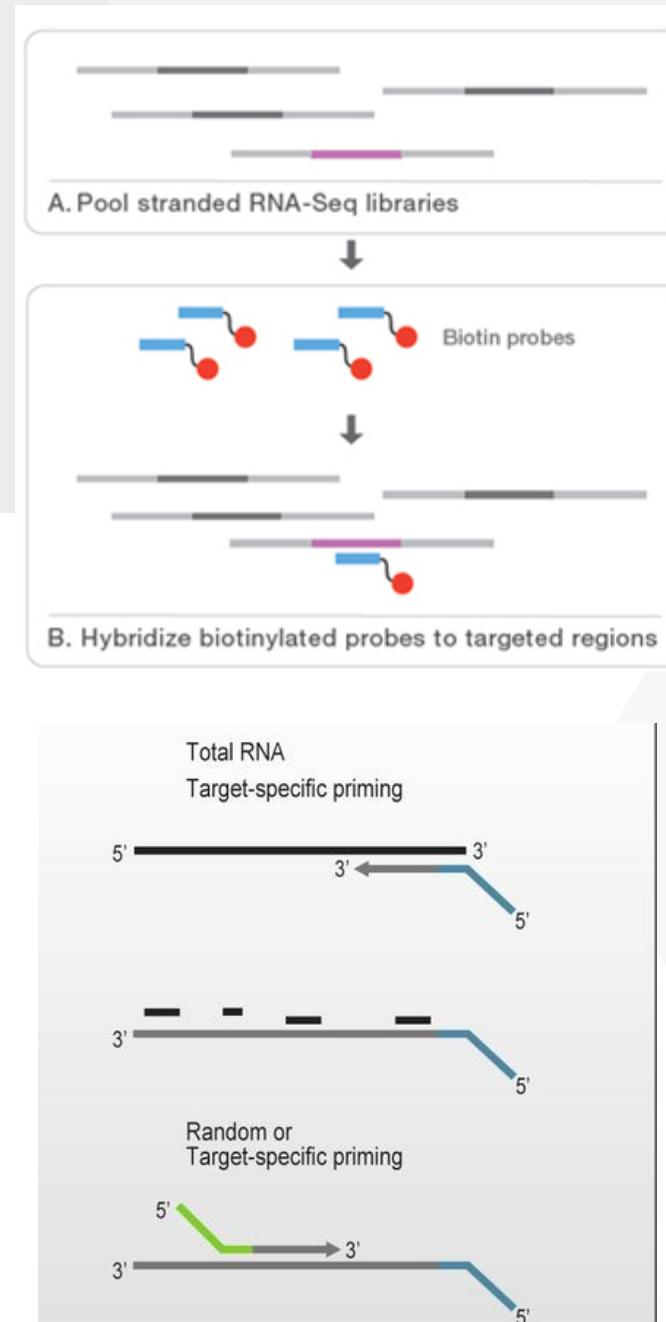
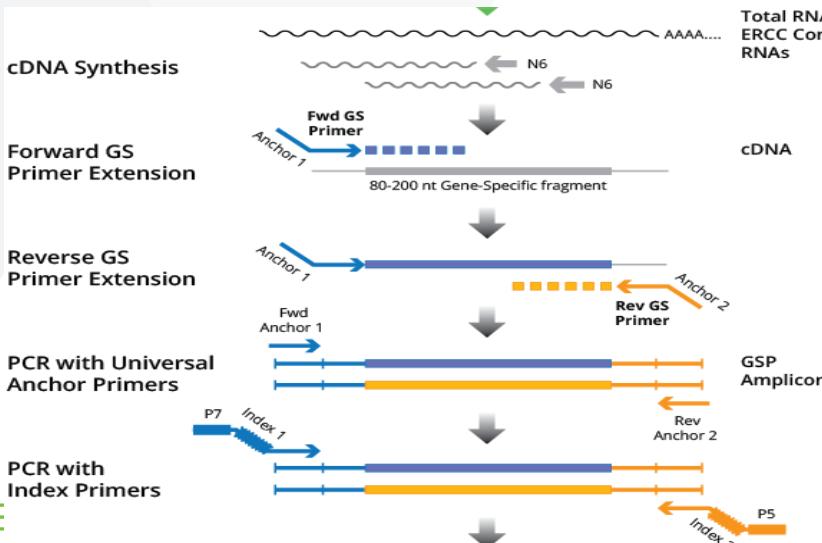
- 1 mRNA = 1 sekvence
  - vs. 1 mRNA = n sekvencí
- Differential expression
- 



# RNA-seq

## Targeted RNA-seq

- Selekce vybraných genů
- Během Rev. transkripce
- PCR/SPE po cDNA syntéze
- Capture po přípravě knihoven



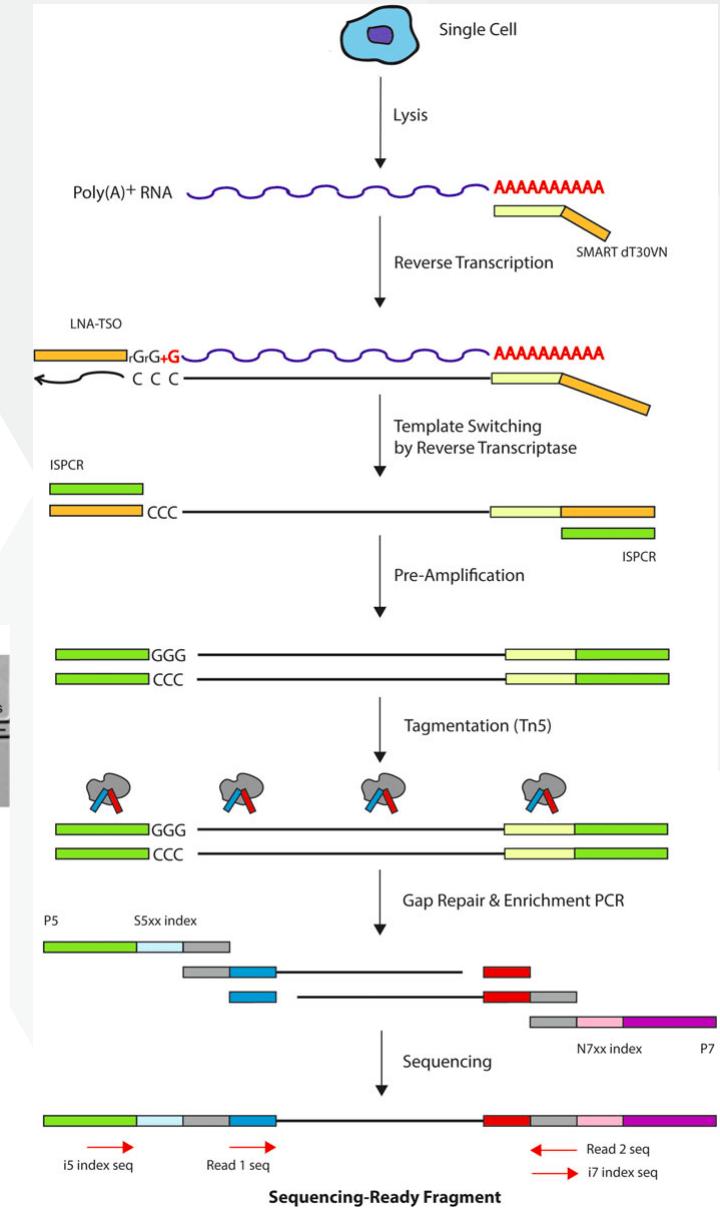
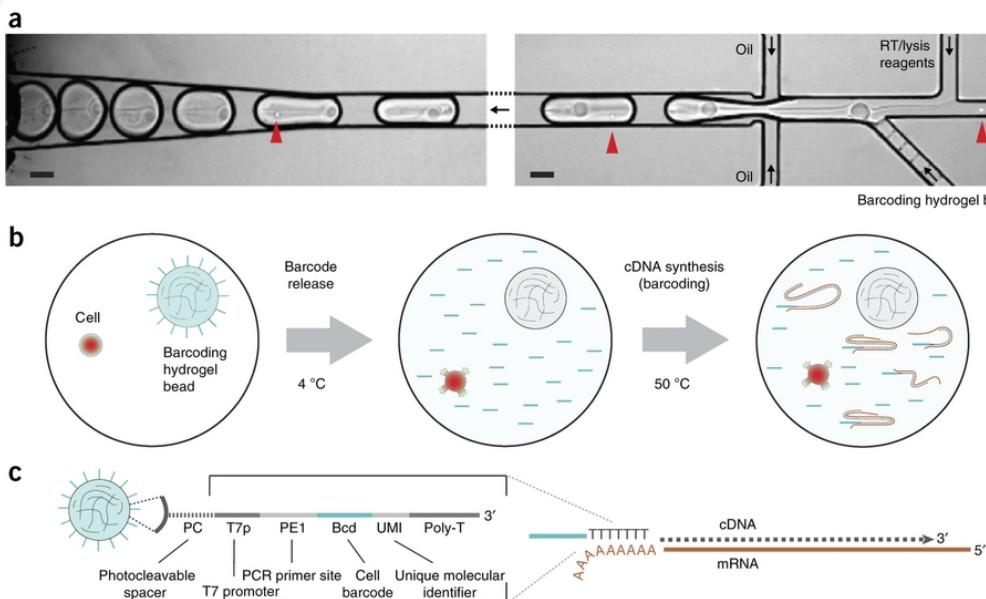
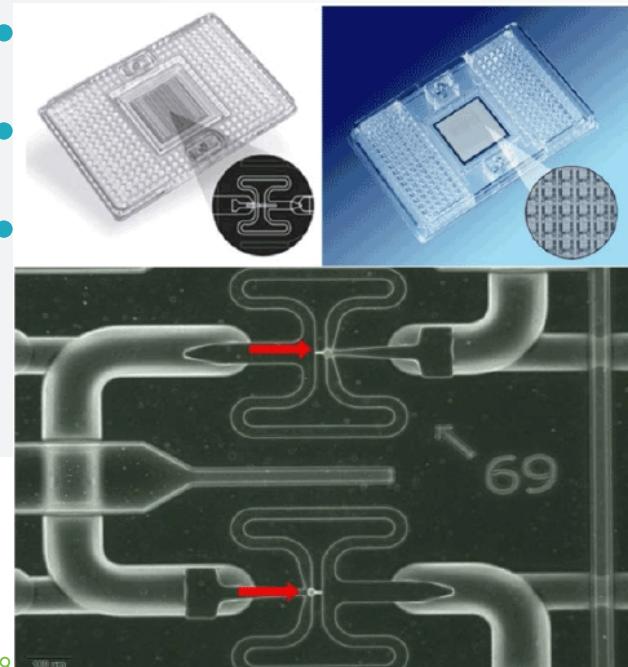
MB: Molecular barcode  
GSP: Gene specific primer  
FP: Forward primer

UP: Universal primer  
SIP: Sample index primer

# RNA-seq

## Single-cell RNA-seq

- 3' sequencing / whole transcript
- Droplets, microfluidics, nanowells, plates
- UMI, cell barcodes



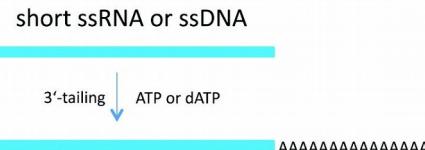
# RNA-seq

## SmallRNA sequencing

- Ligace RNA adaptoru
- CATS
- Size selection
- miRNA, piRNA, ...
- 

Duration:

30 min



15 min



15 min

Template switch + Biotin- 5'-adapter rGrGrG-OH



60 – 120 min

PCR + PCR forward  
PCR reverse

Amplified DNA library



Total RNA or  
Purified Small RNA Fragments

RNA 3' Adapter

3' Ligation

RNA 3' Adapter

5' Ligation

RNA 5' Adapter

RNA 3' Adapter

RT-PCR - 1st Strand Synthesis

PCR Amplification

Gel Purification  
(Pooled Index Option)

Index Sequence

b

Single-adapter scheme



Circularization



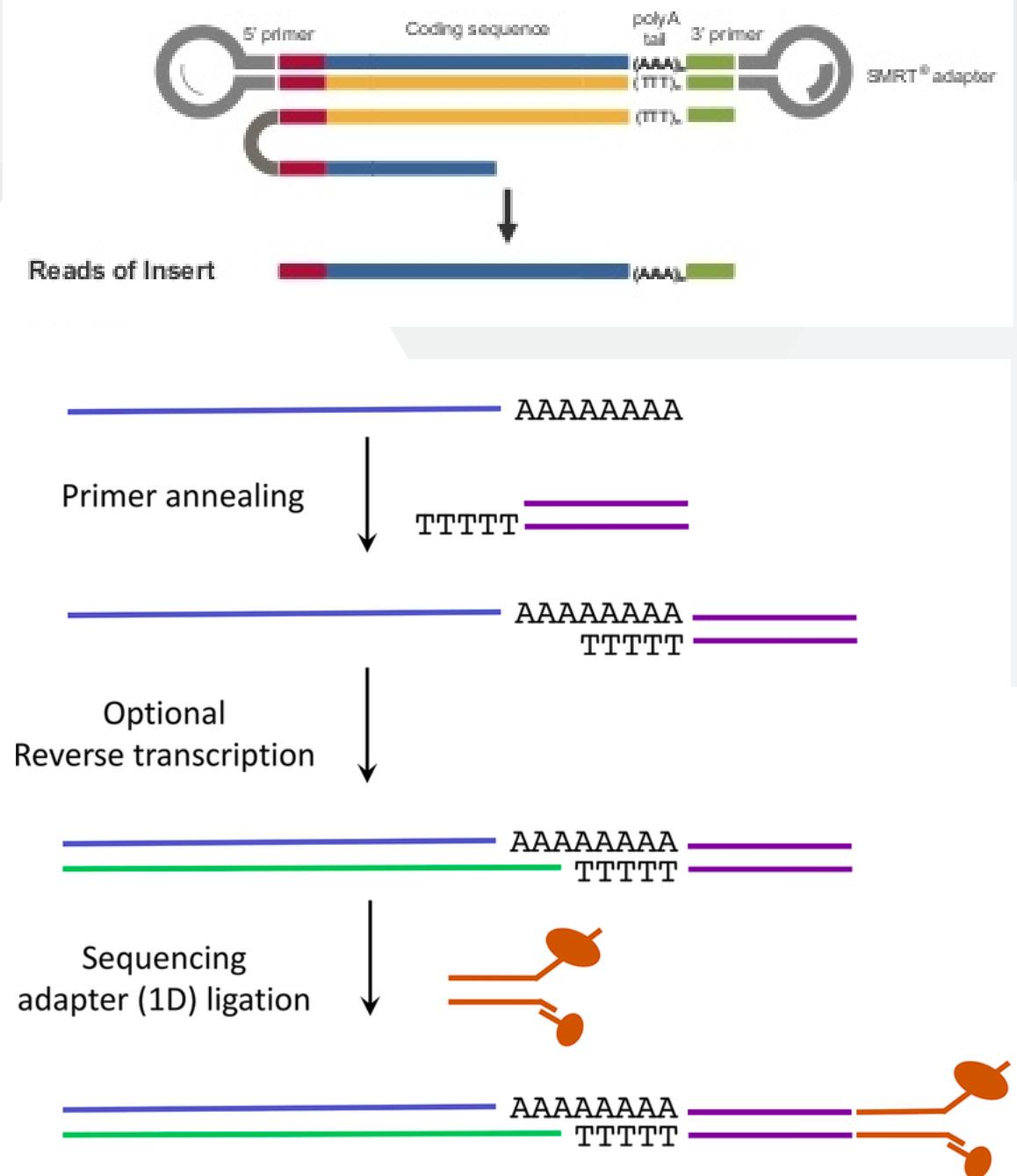
RT-PCR

Illumina Sequencing

# RNA-seq

## Long-read + direct RNA sequencing

- PacBio
  - Iso-seq
  - Isoform analysis
- Oxford Nanopore
  - Direct RNA sequencing
  - Isoform analysis
  - Base modifications





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16S, RAD

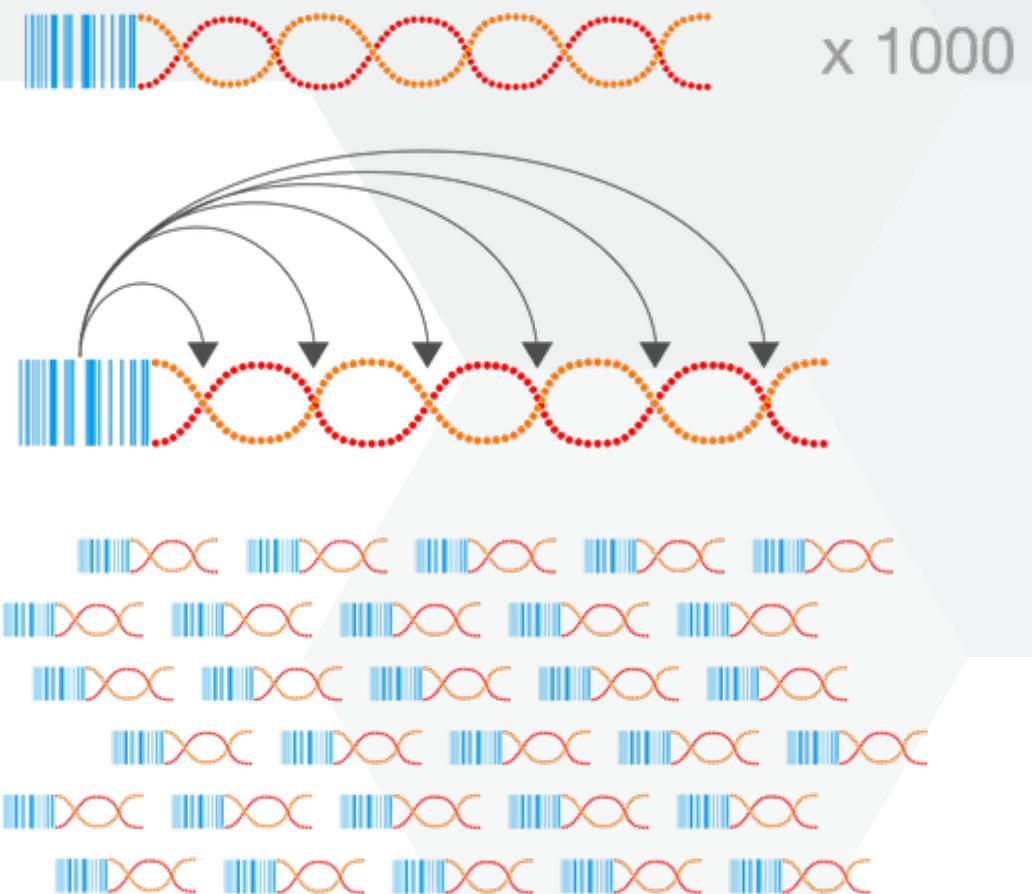
The logo for MUNI (Masaryk University) features the letters "MUNI" in a large, white, sans-serif font.



# Metagenomics

## Analýza komplexních populací

- Shotgun metagenome
- Markers
  - 16S rDNA
  - 18S, ITS, COX
  - Short vs. long amplicons

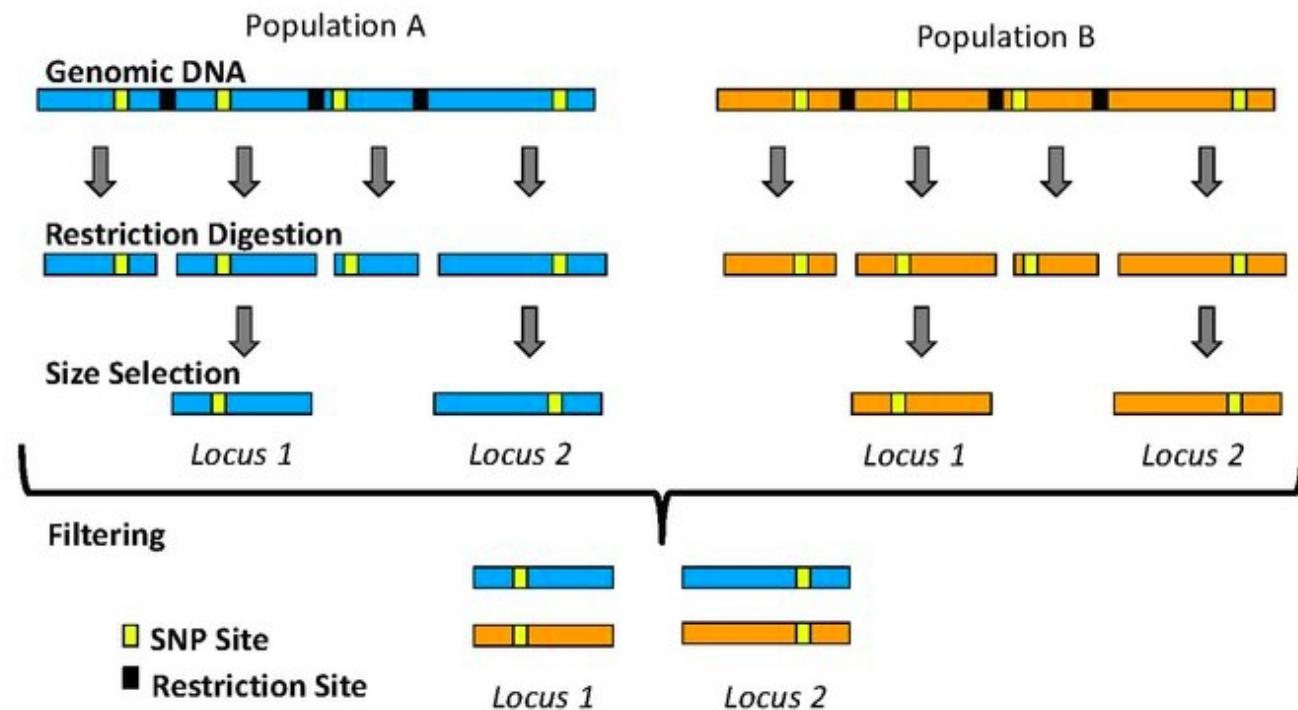


# RAD, ddRAD

## Restriction site associated DNA markers

- Analýza variabilité populací
- ddRAD = double digest
- Alternativa – capture enrichment
- 

### Restriction-site Associate DNA Sequencing (RADseq)





A background photograph shows a female scientist with long red hair wearing a grey head-mounted display (HMD). She is focused on examining a small yellow flower held in her hands. The setting appears to be a modern laboratory or greenhouse. Overlaid on the image are several white icons: a DNA double helix symbol near the top left, a cluster of circles near the center, a brain icon on the left side, a gear icon at the bottom center, and an atom symbol on the right side.

# Thank you for your attention



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