CEITEC

Central European Institute of Technology
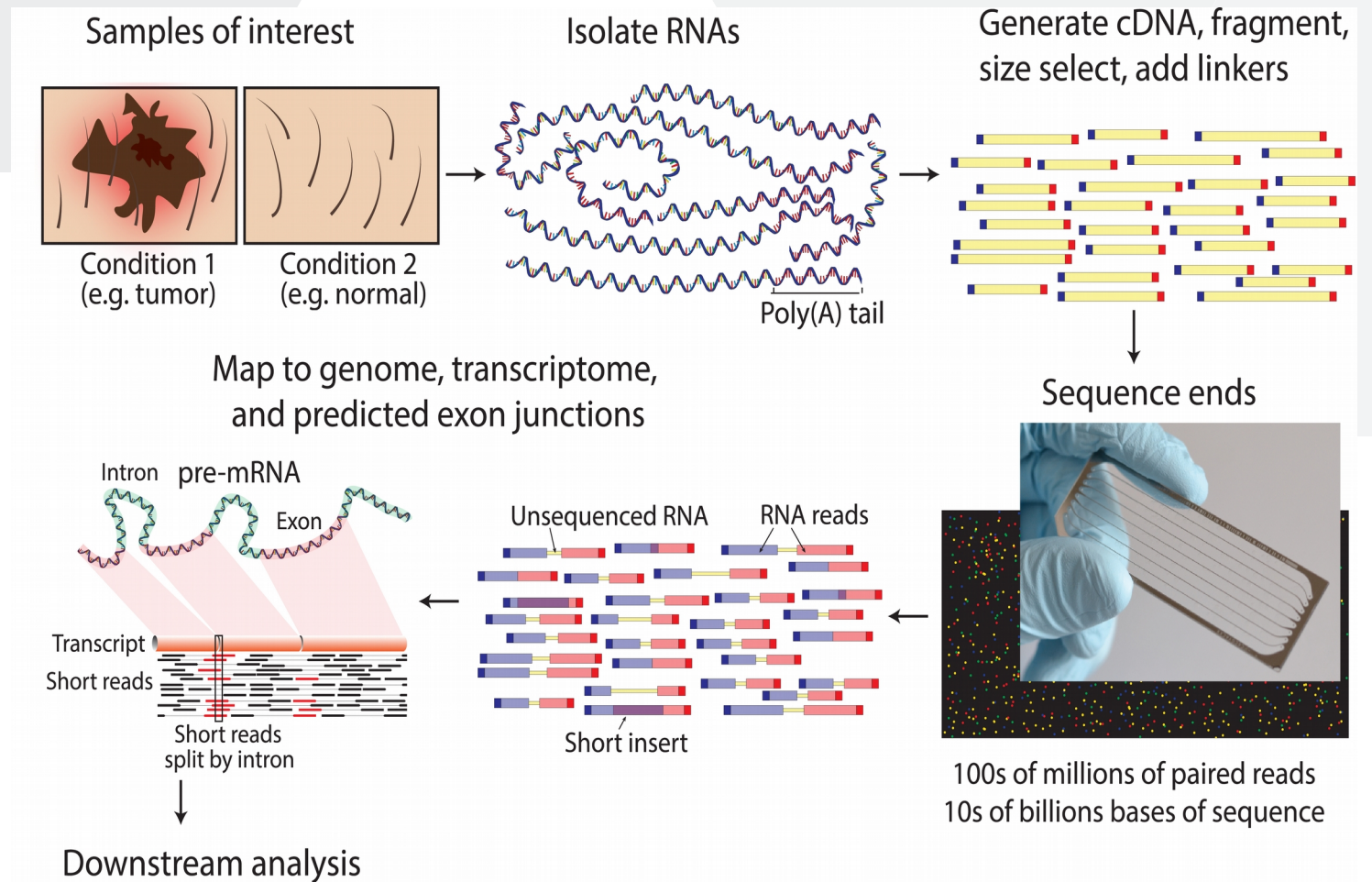### BRNO | CZECH REPUBLIC

MUNI

Boris Tichý

RNA-seq

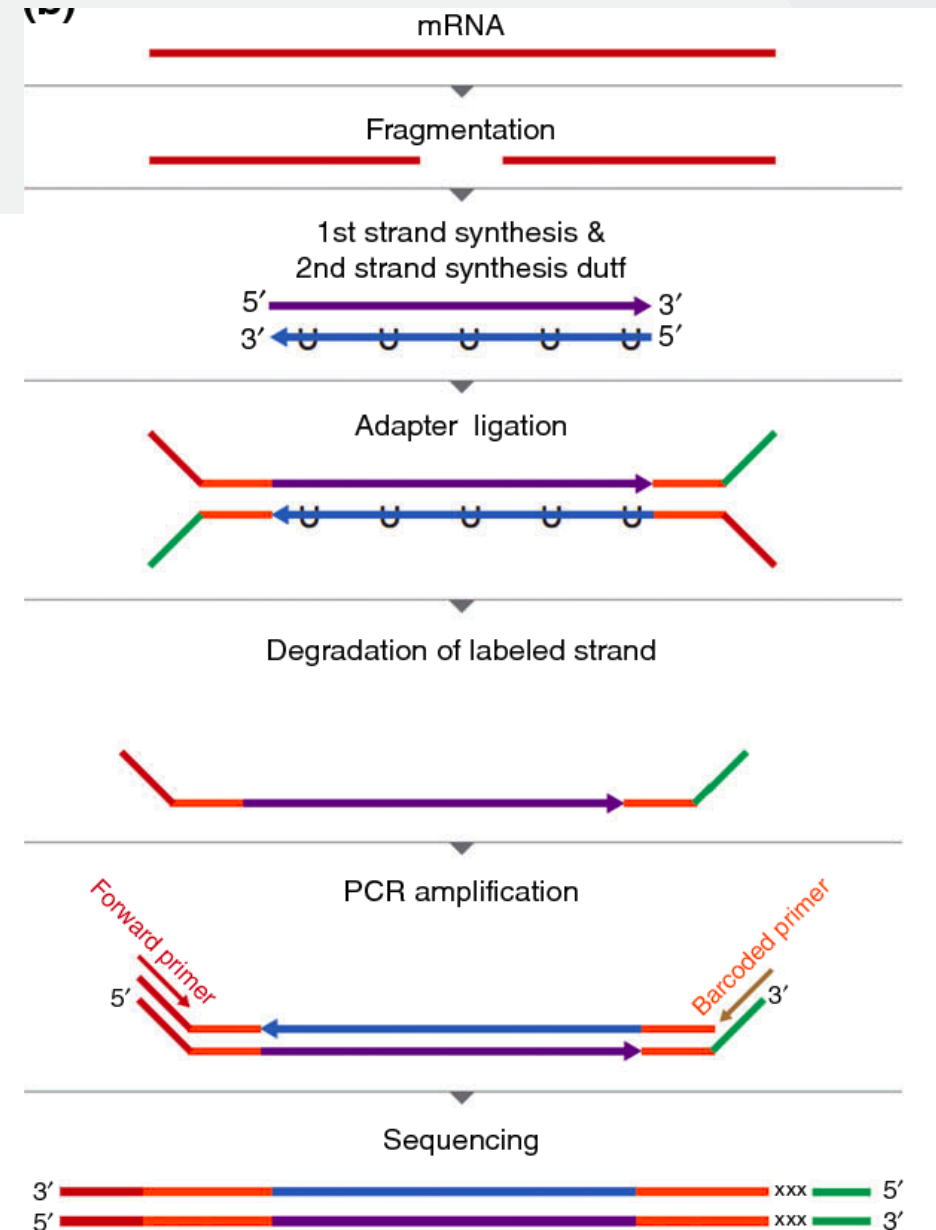# RNA-seq

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
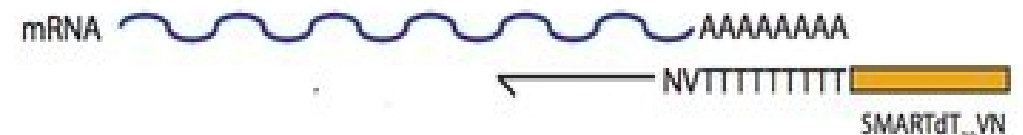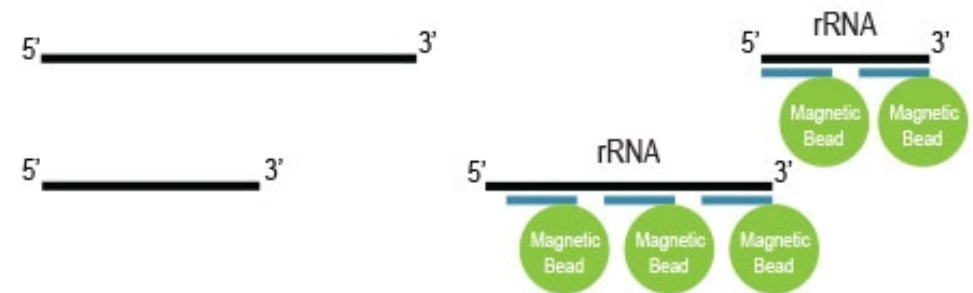 - directional/non-directional
- Ligace adaptorů

RNA-seq

„classical" RNA-seq

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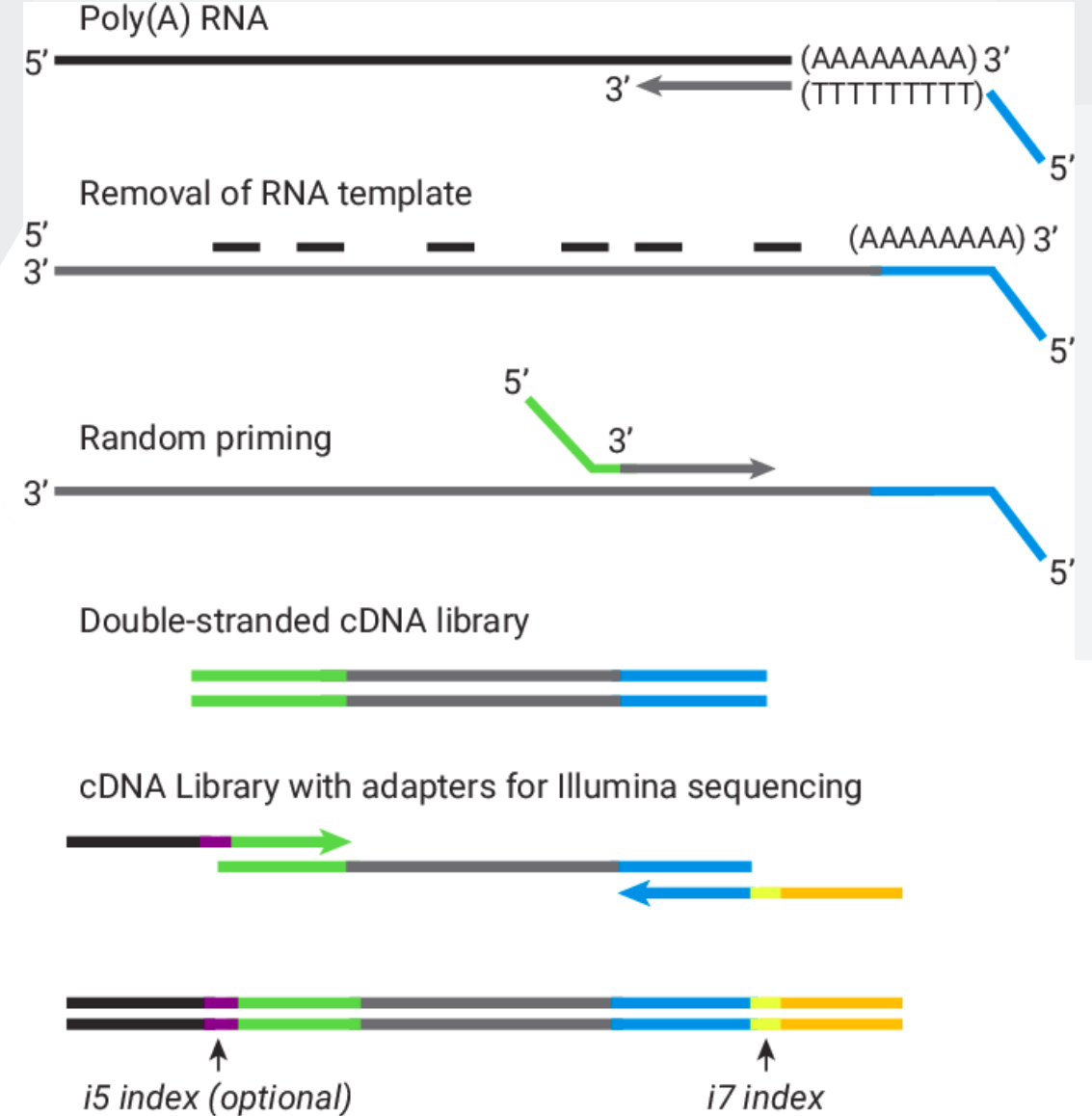- De-novo transcriptome
- Variant analysis

RNA-seq

3' RNA-seq

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

Poly(A) RNA

Removal of RNA template

Random priming

Double-stranded cDNA library

cDNA Library with adapters for Illumina sequencing

i5 index (optional)   i7 index

RNA-seq

Targeted RNA-seq

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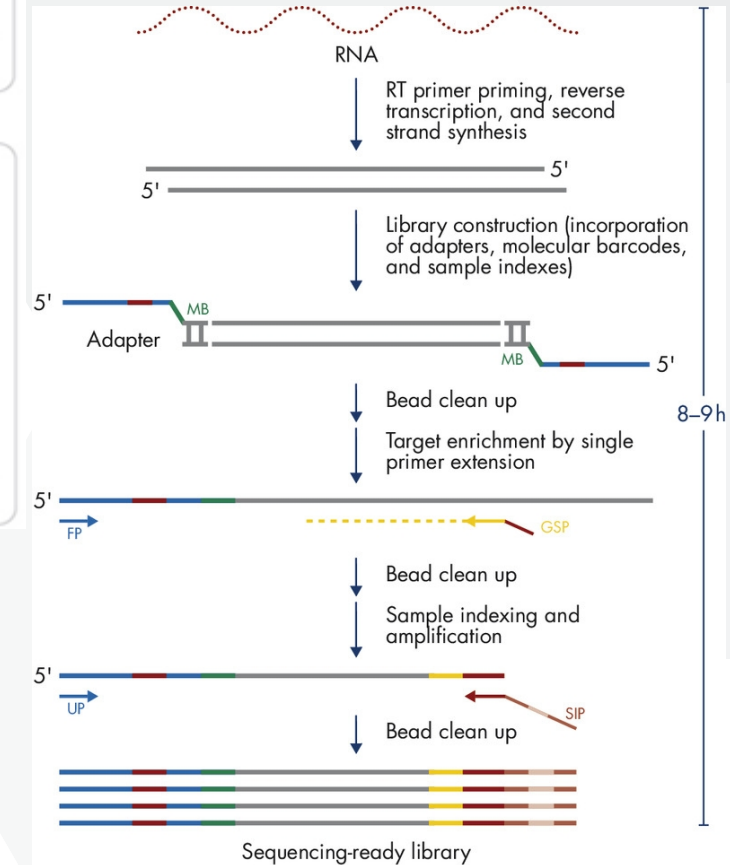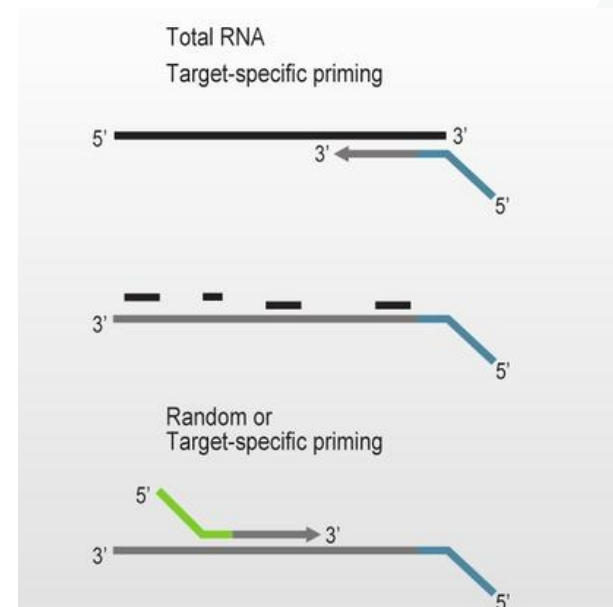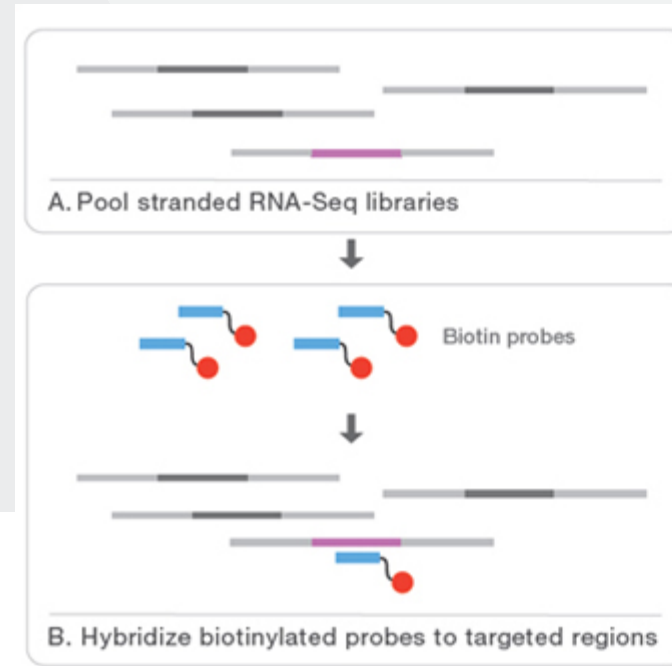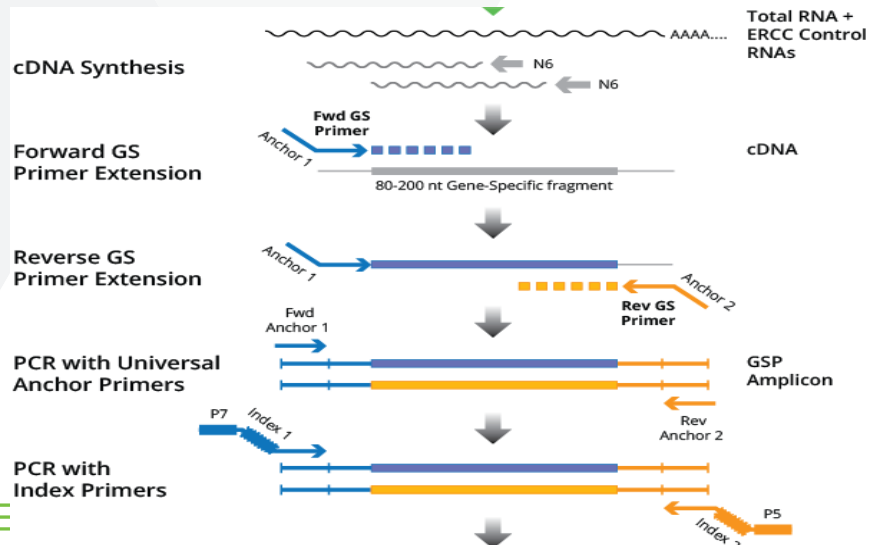
RNA-seq
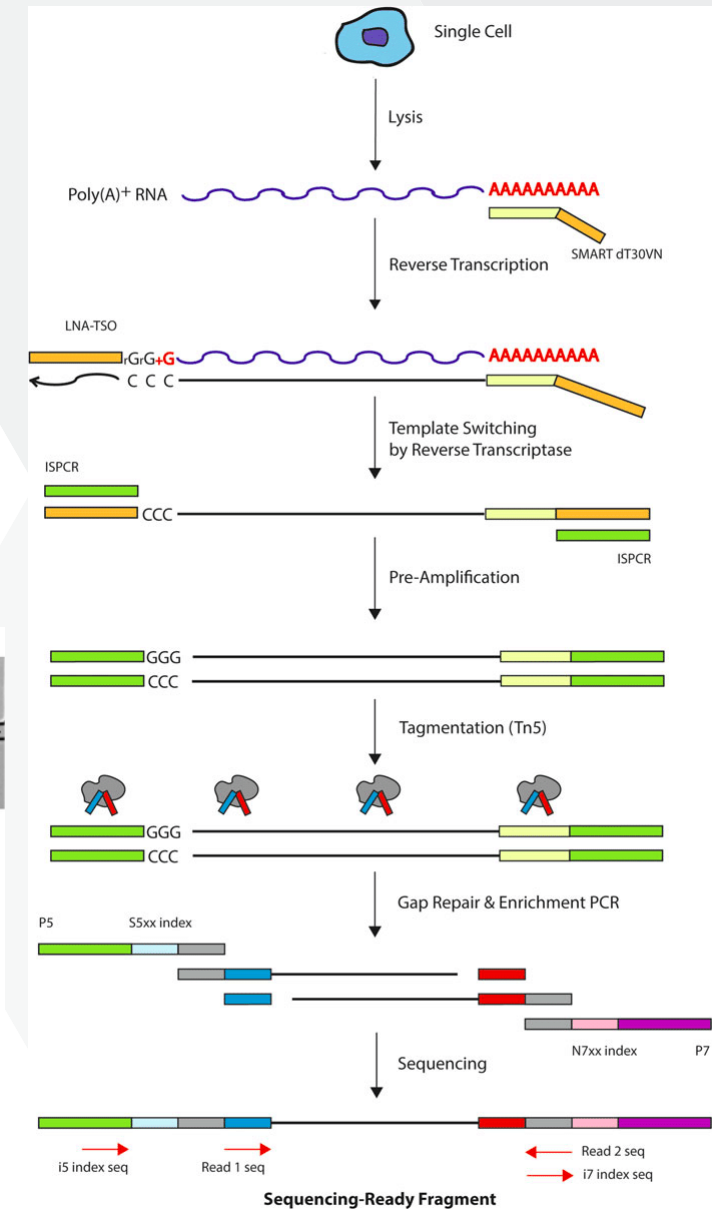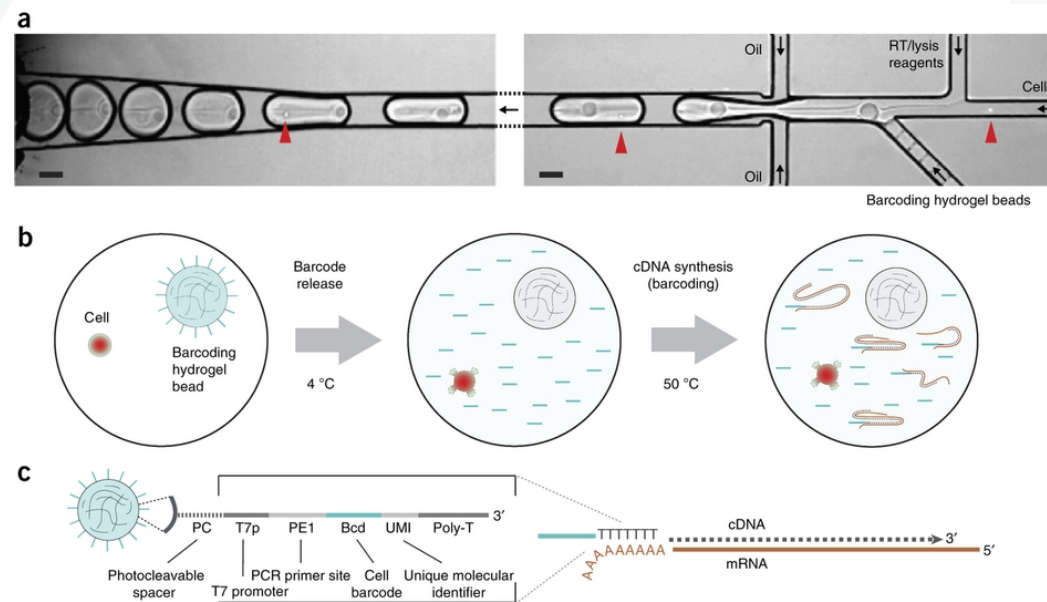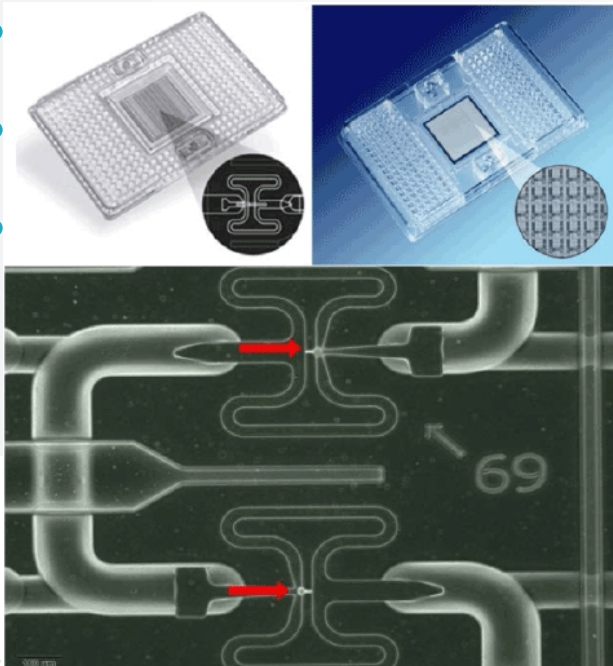
Single-cell RNA-seq

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

RNA-seq

SmallRNA sequencing

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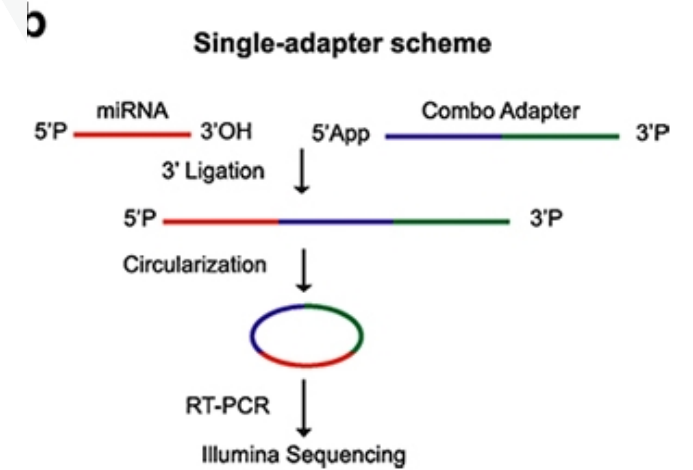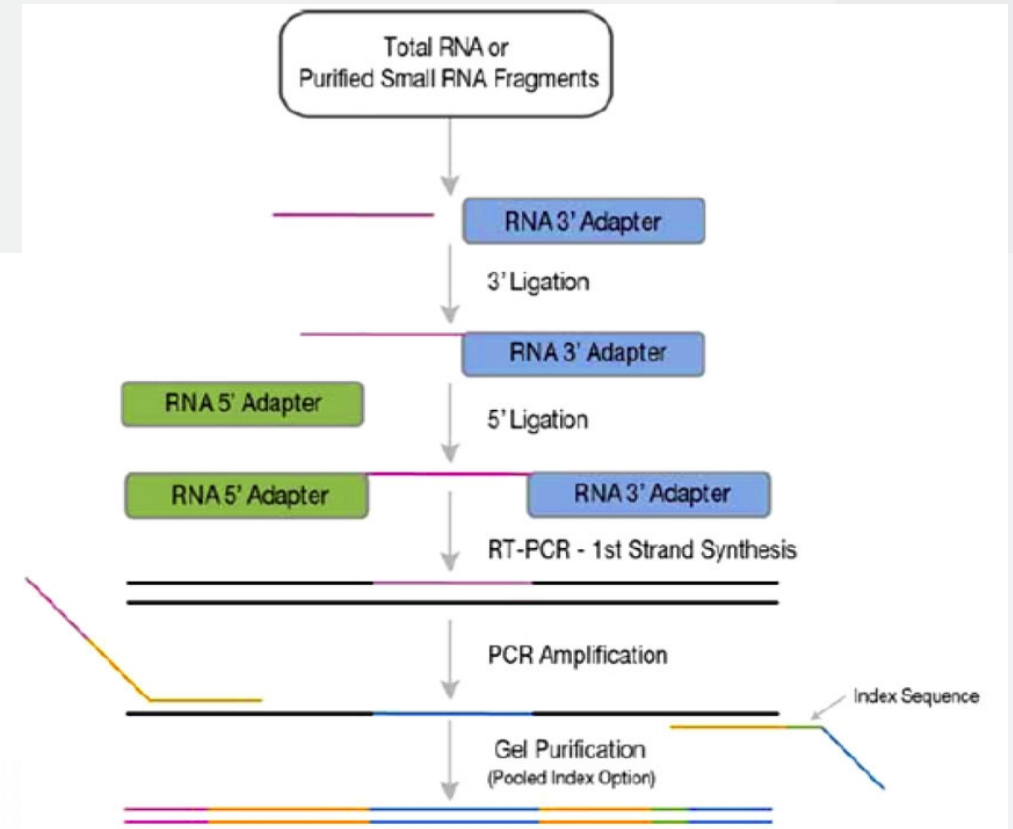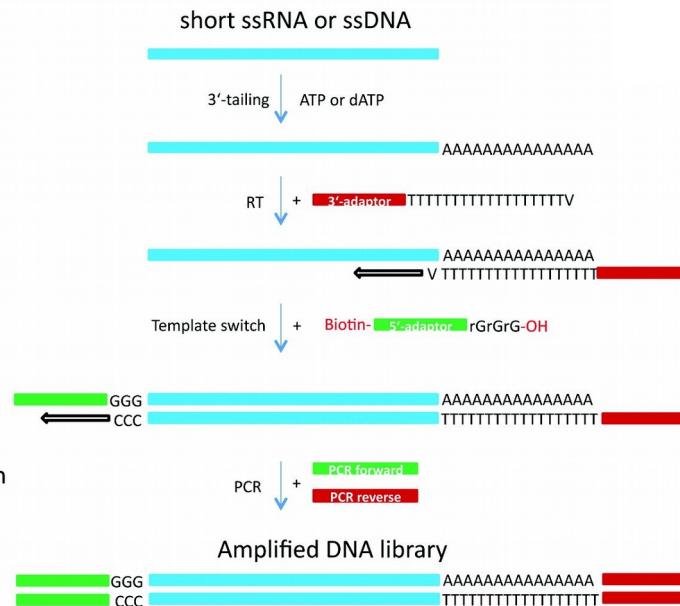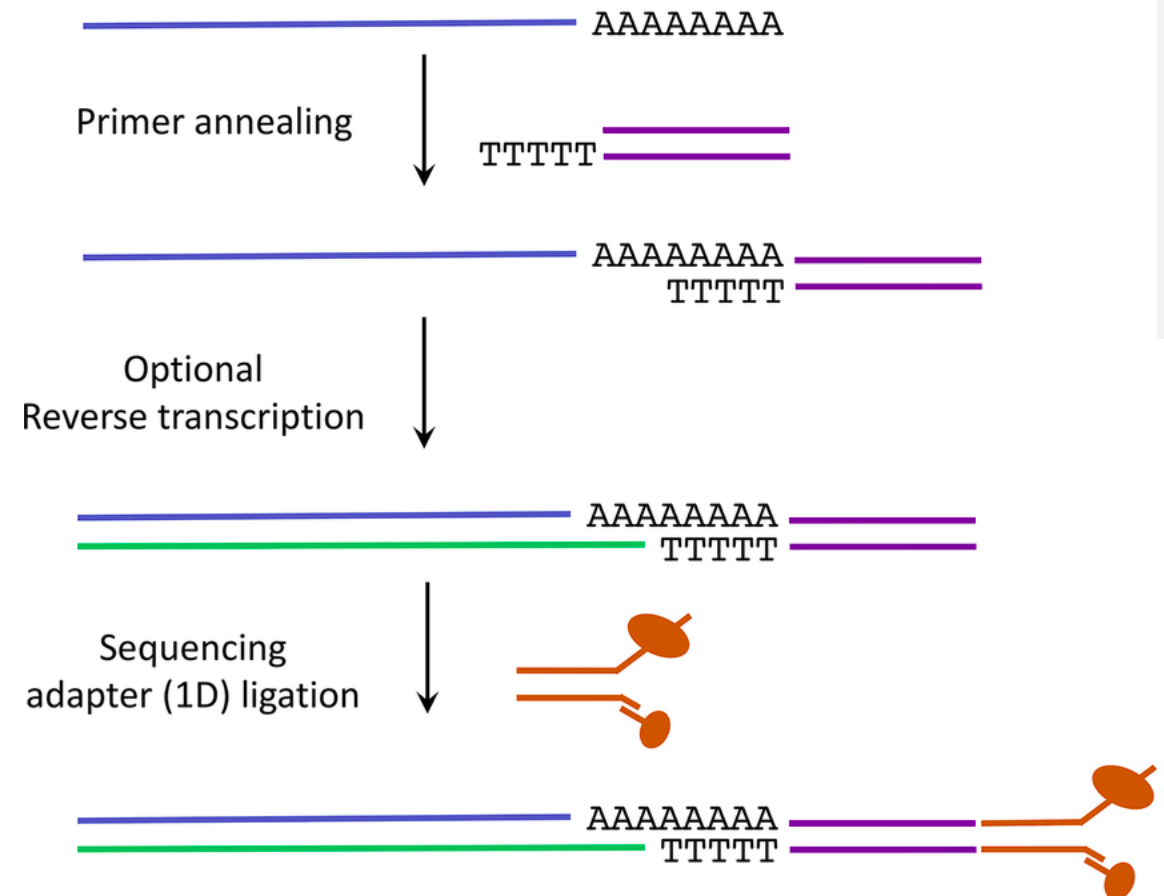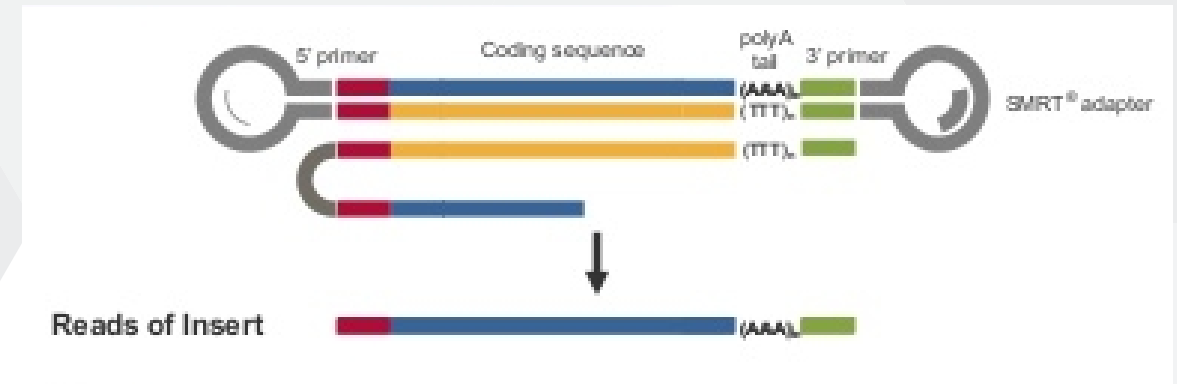- Size selection
- miRNA, piRNA, ...
-

RNA-seq

Long-read + direct RNA sequencing

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

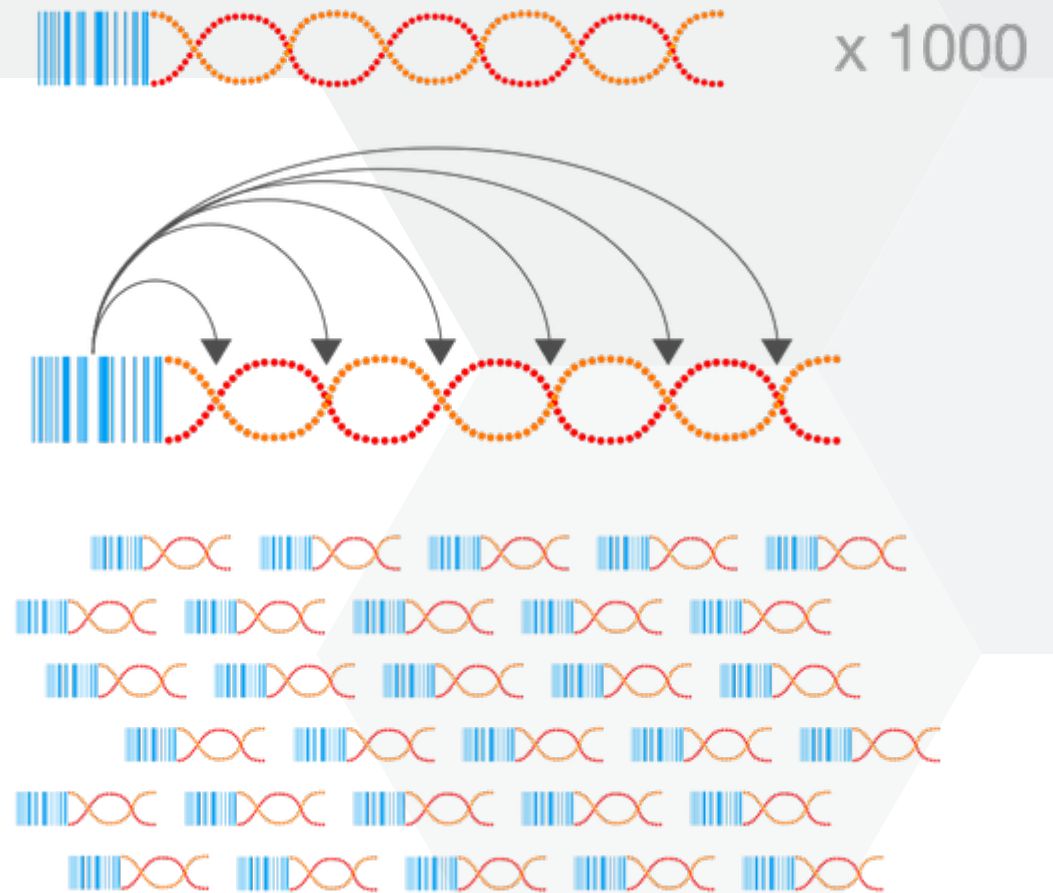16S, RAD

Metagenomics

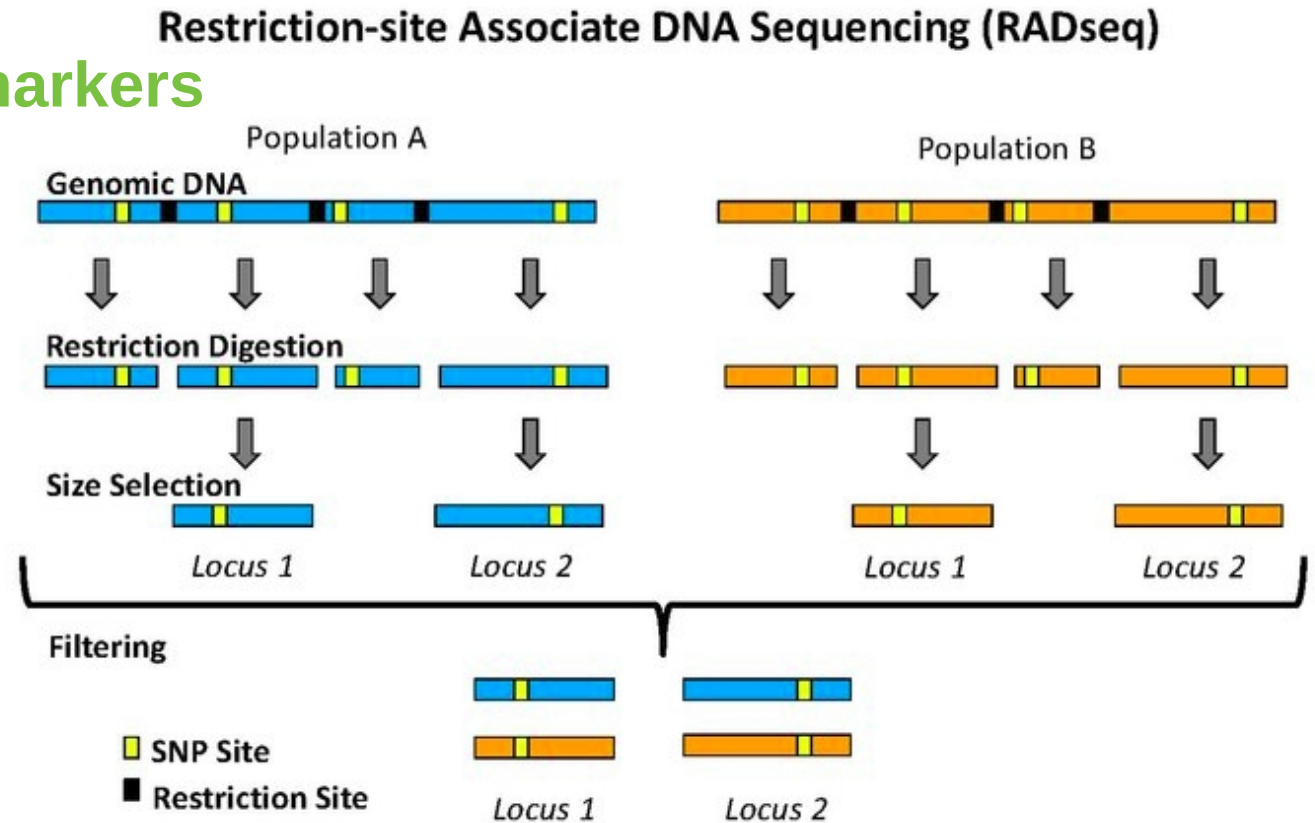**Analýza komplexních populací**

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

x 1000

RAD, ddRAD

Restriction site associated DNA markers

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

Thank you
for your attention

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