



Central European Institute of Technology
BRNO | CZECH REPUBLIC

Boris Tichý

RNA-seq

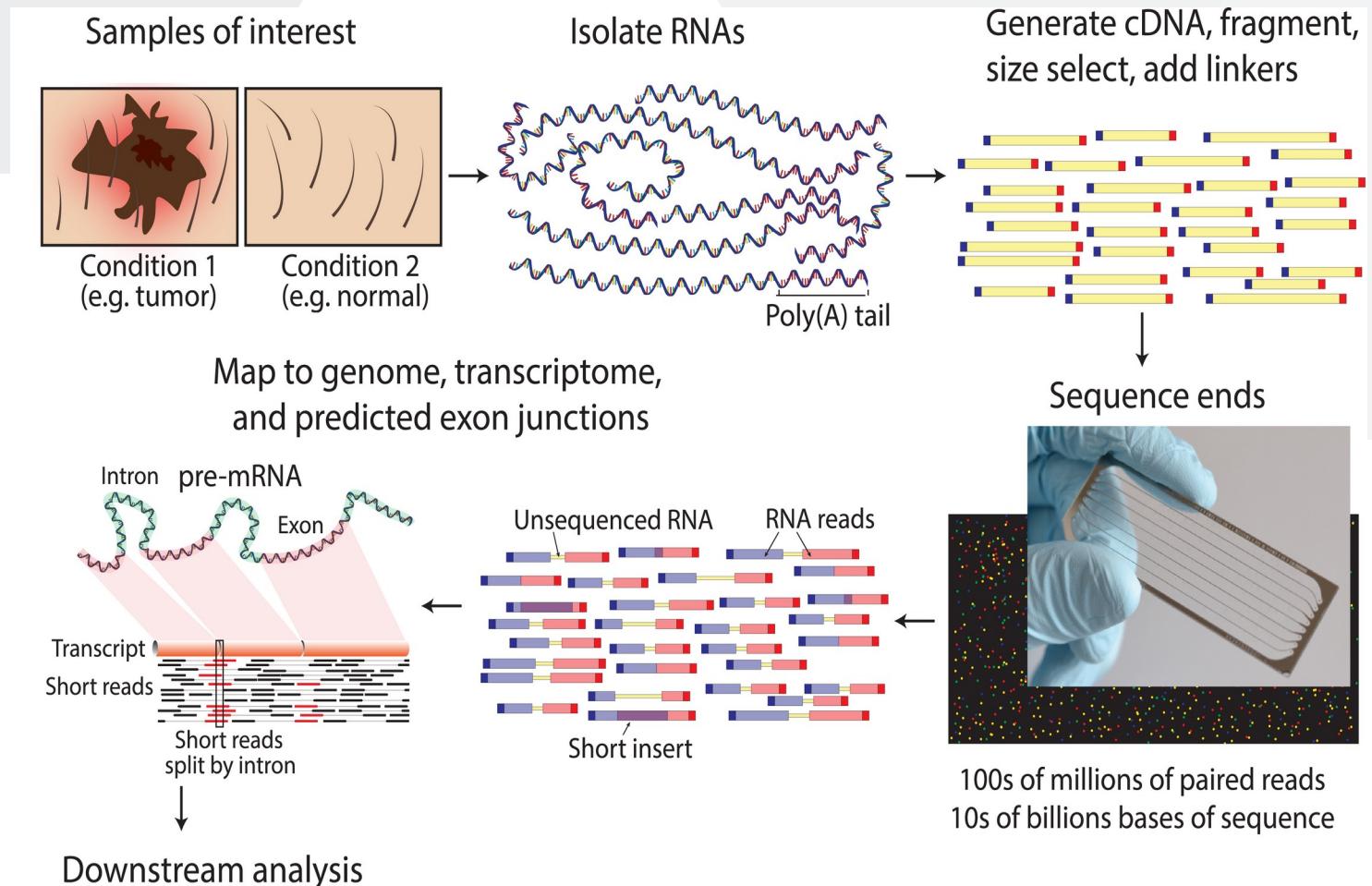
MUNI



RNA-seq

Genes/exons sequence and expression

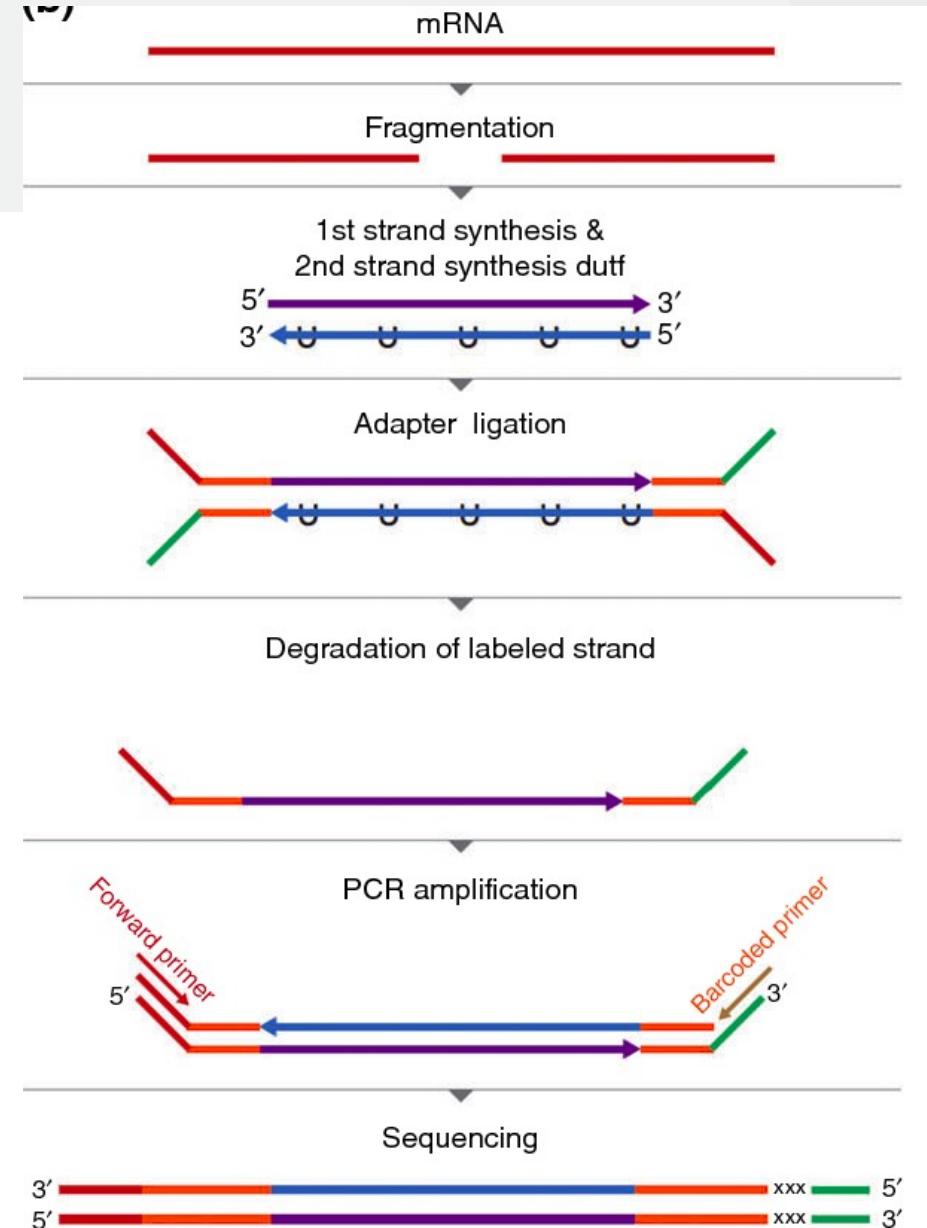
- “Classical” RNA-seq
 - Whole transcriptome
 - PolyA selection
 - rRNA depletion
- 3’ RNA-seq
- targeted RNA sequencing
- single-cell RNA sequencing
- smallRNA sequencing
- long-read RNA sequencing
- direct RNA sequencing



RNA-seq

„classical“ RNA-seq

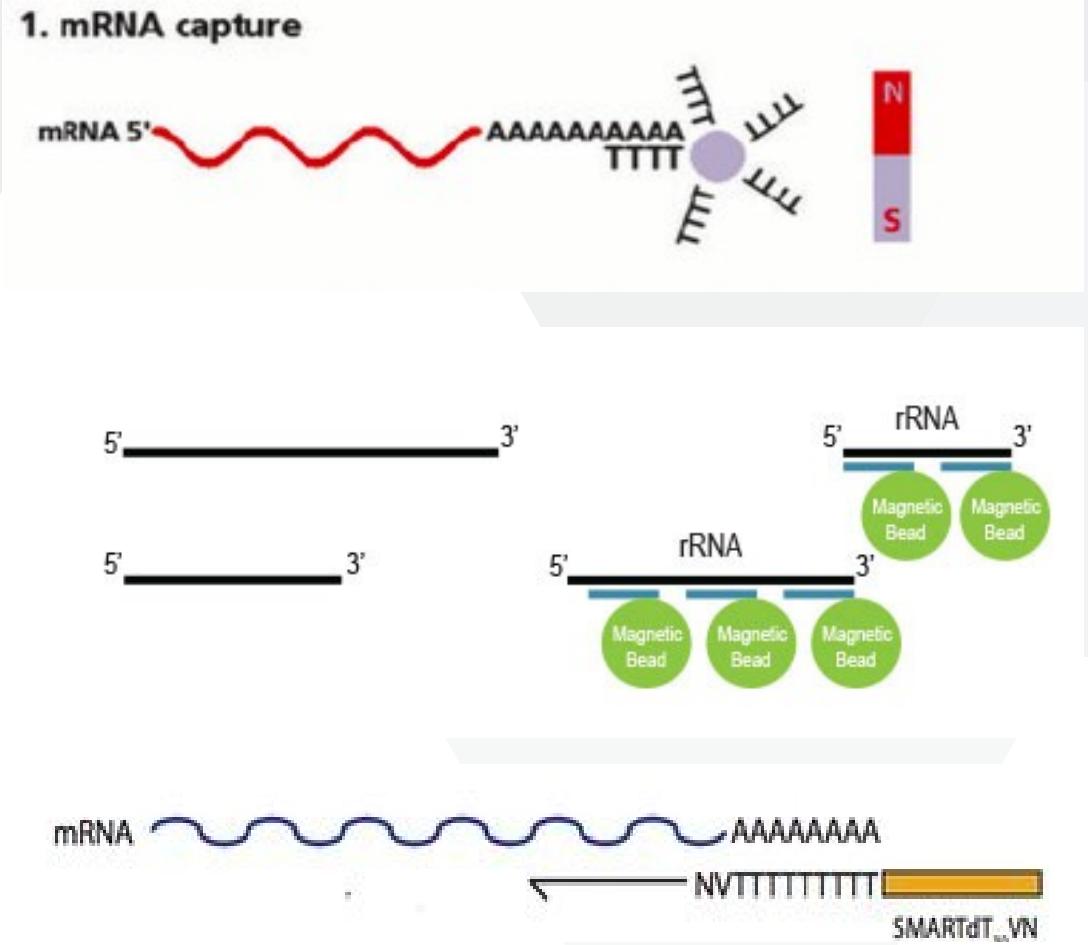
- RNA sequencing w/wo selection
- Fragmentation
 - temperature/chemic
 - primers
- Reverse transcription – random primers
- Double-stranded cDNA
 - directional/non-directional
- Adaptor ligation



RNA-seq

„classical“ RNA-seq

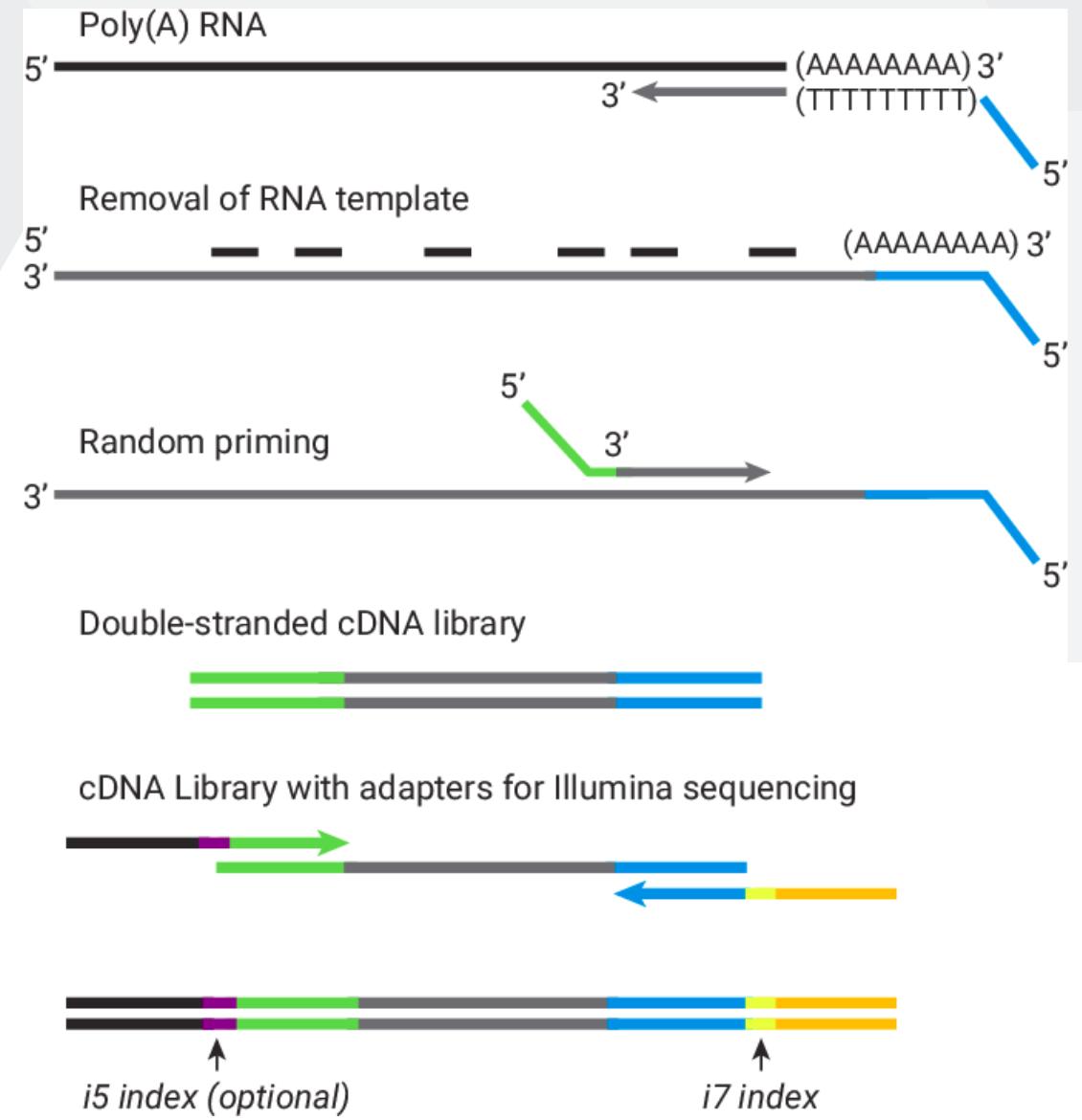
- RNA selection
 - polyA (mRNA)
 - beads
 - primer
 - rRNA depletion
- Differential expression
- Splicing analysis
- De-novo transcriptome
- Variant analysis



RNA-seq

3' RNA-seq

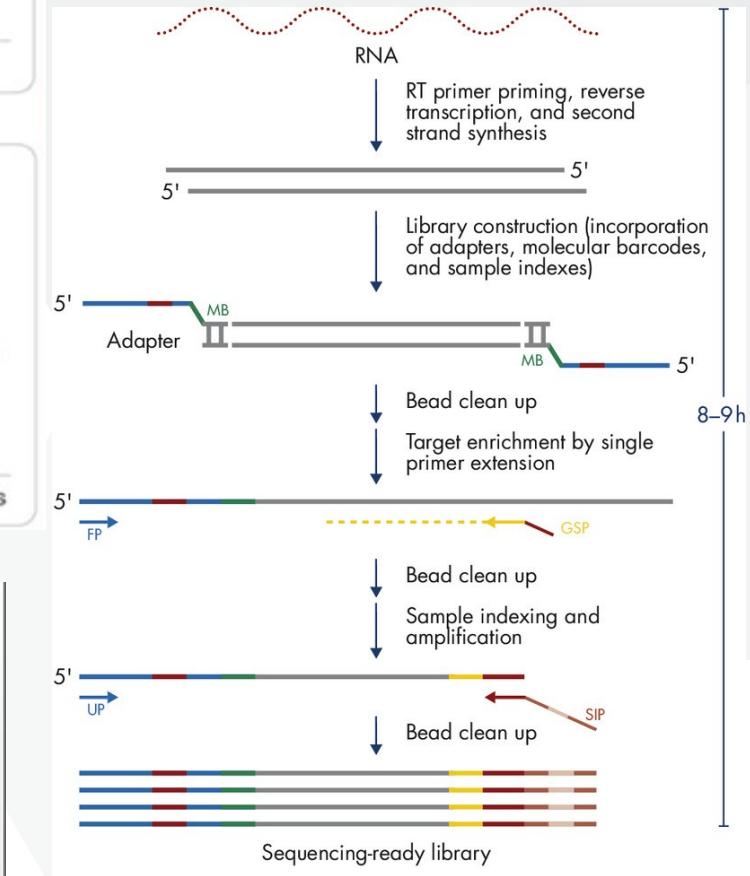
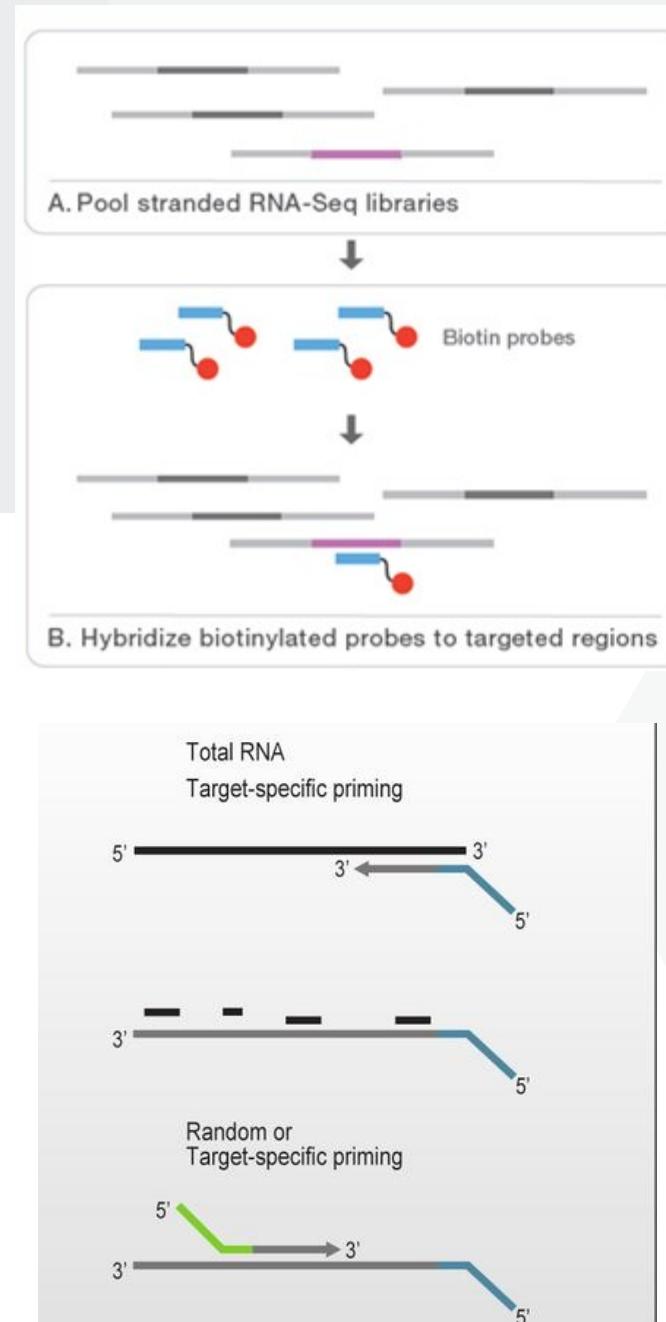
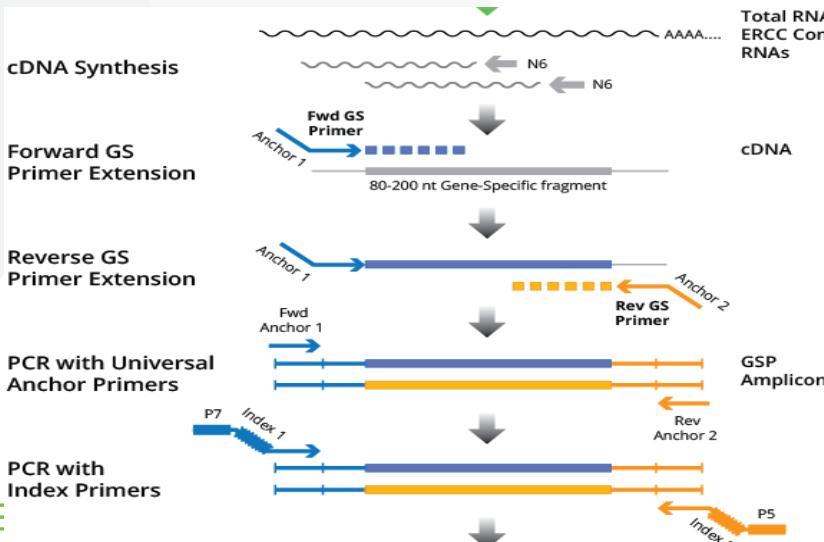
- 1 mRNA = 1 sequence
 - vs. 1 mRNA = n sequences
- Differential expression
-



RNA-seq

Targeted RNA-seq

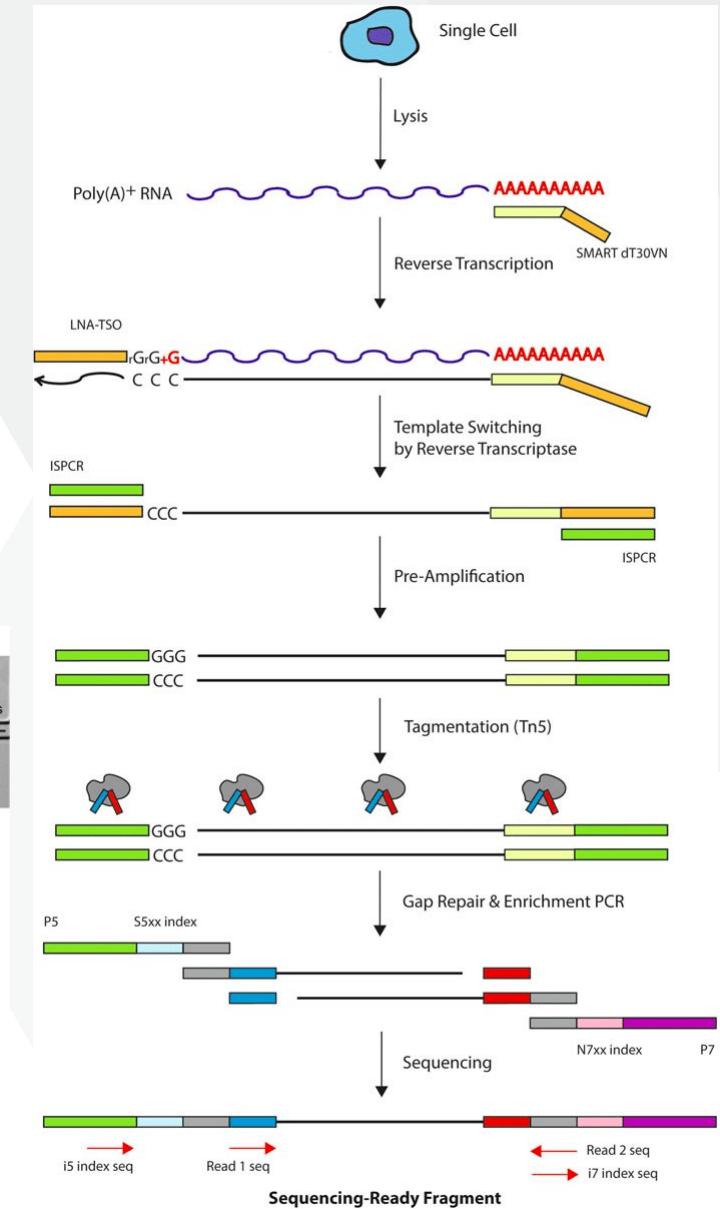
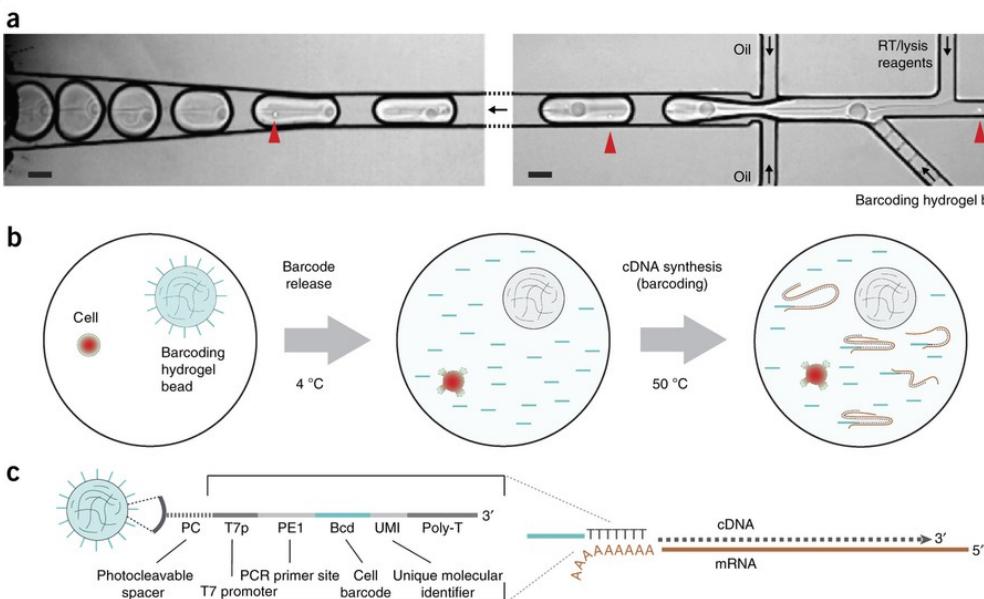
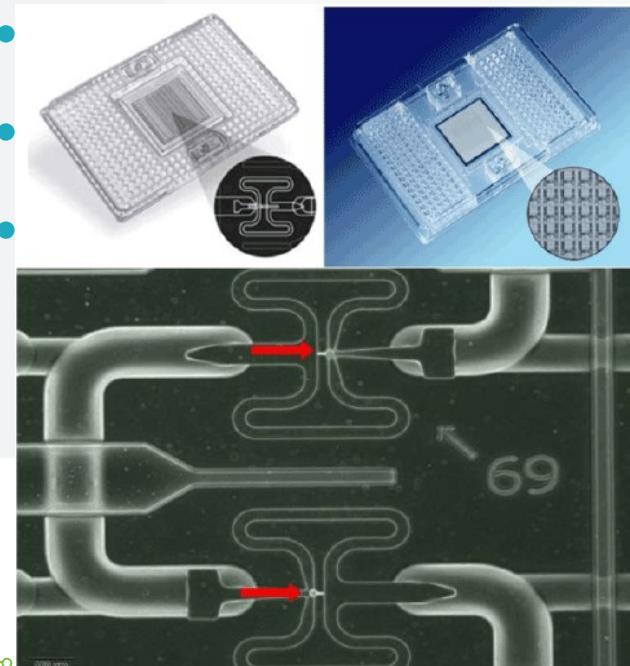
- Selection of genes/exons
 - During RT
 - PCR/SPE after RT
 - Capture after library prep



RNA-seq

Single-cell RNA-seq

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



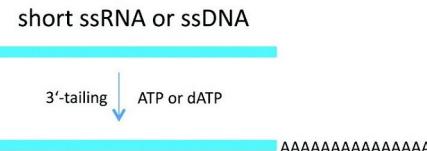
RNA-seq

SmallRNA sequencing

- RNA adaptors ligation
- CATS
- Size selection
- miRNA, piRNA, ...
-

Duration:

30 min



15 min



15 min

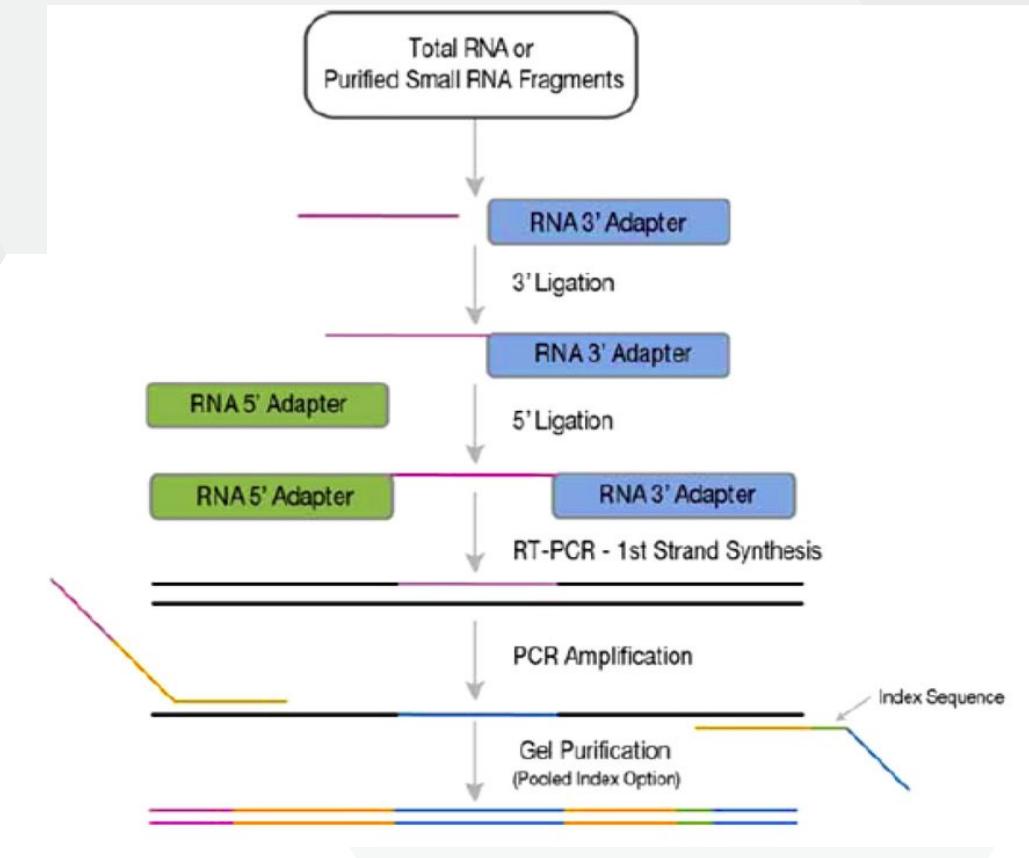
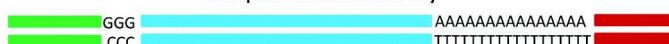
Template switch + Biotin- 5'-adaptor rGrGrG-OH



60 – 120 min

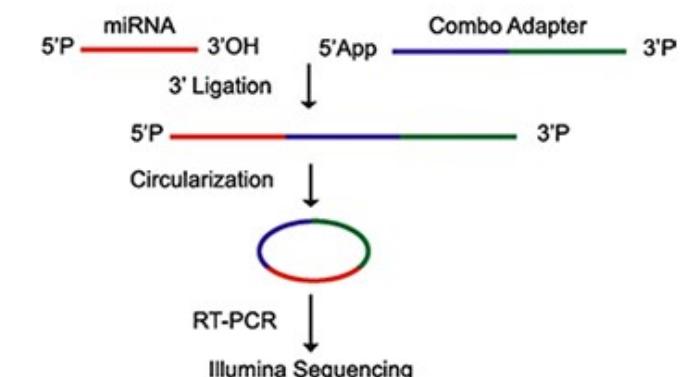
PCR + PCR forward
PCR reverse

Amplified DNA library



b

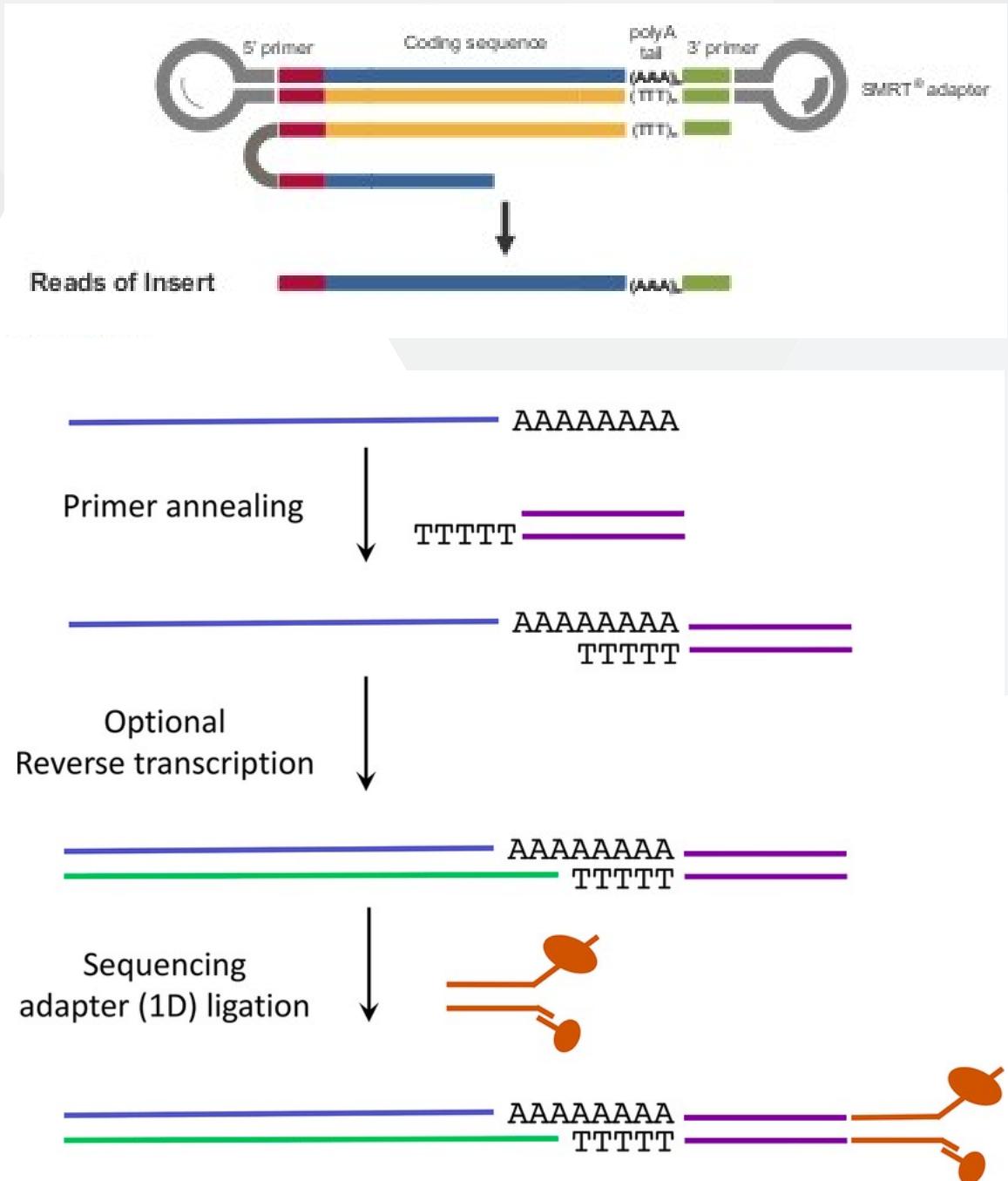
Single-adapter scheme



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

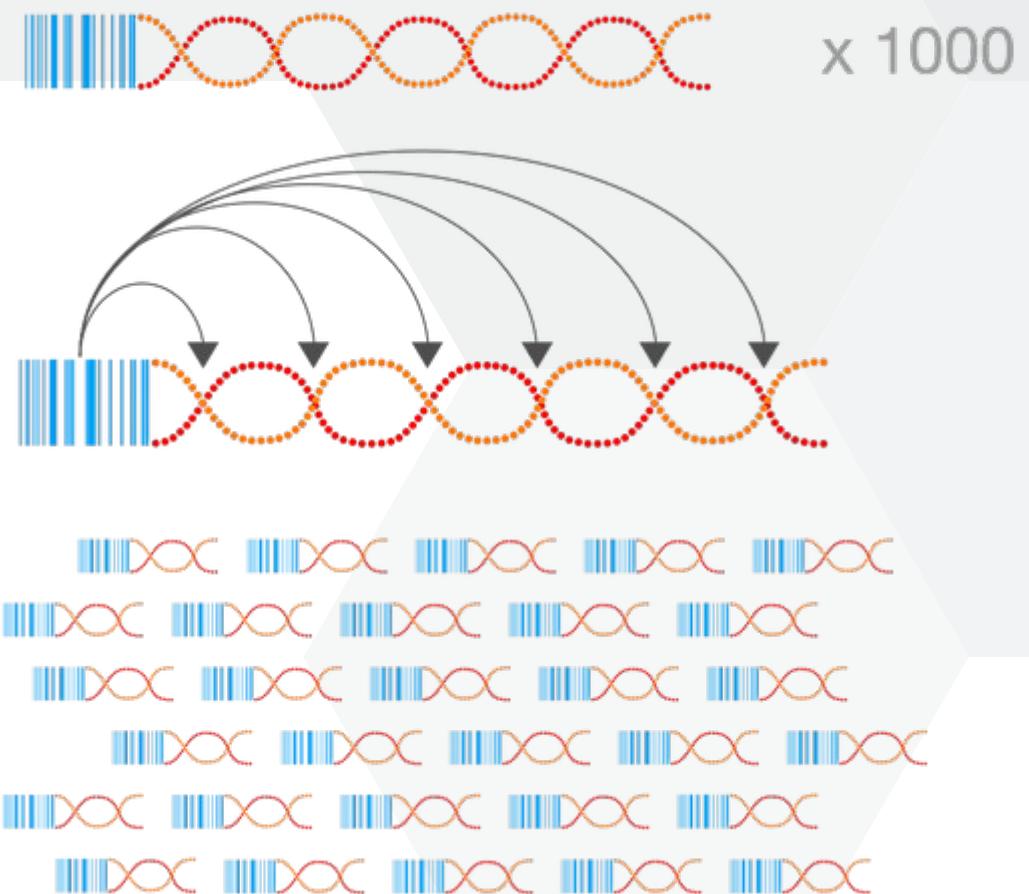
The logo for MUNI (Masaryk University) features the letters "MUNI" in a large, white, sans-serif font.



Metagenomics

Analýza komplexních populací

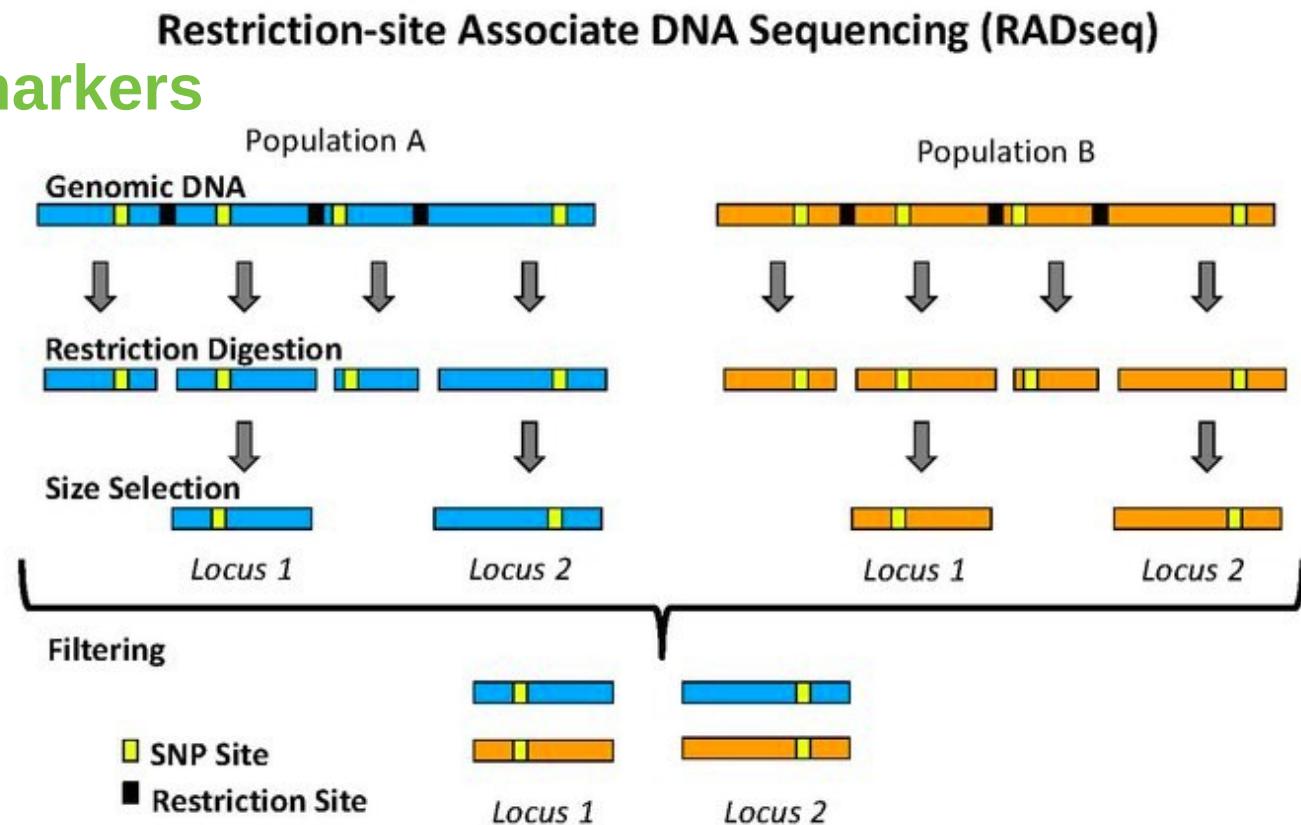
- Shotgun metagenome
- Markers
 - 16S rDNA
 - 18S, ITS, COX
 - Short vs. long amplicons



RAD, ddRAD

Restriction site associated DNA markers

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





A female scientist with long red hair is wearing a black head-mounted display (HMD) and a blue lab coat. She is examining a green plant with yellow flowers. The background shows a laboratory setting with other people and equipment. Overlaid on the image are several white icons: a DNA double helix, a brain, a cluster of circles, a gear, and an atom symbol.

Thank you
for your attention



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