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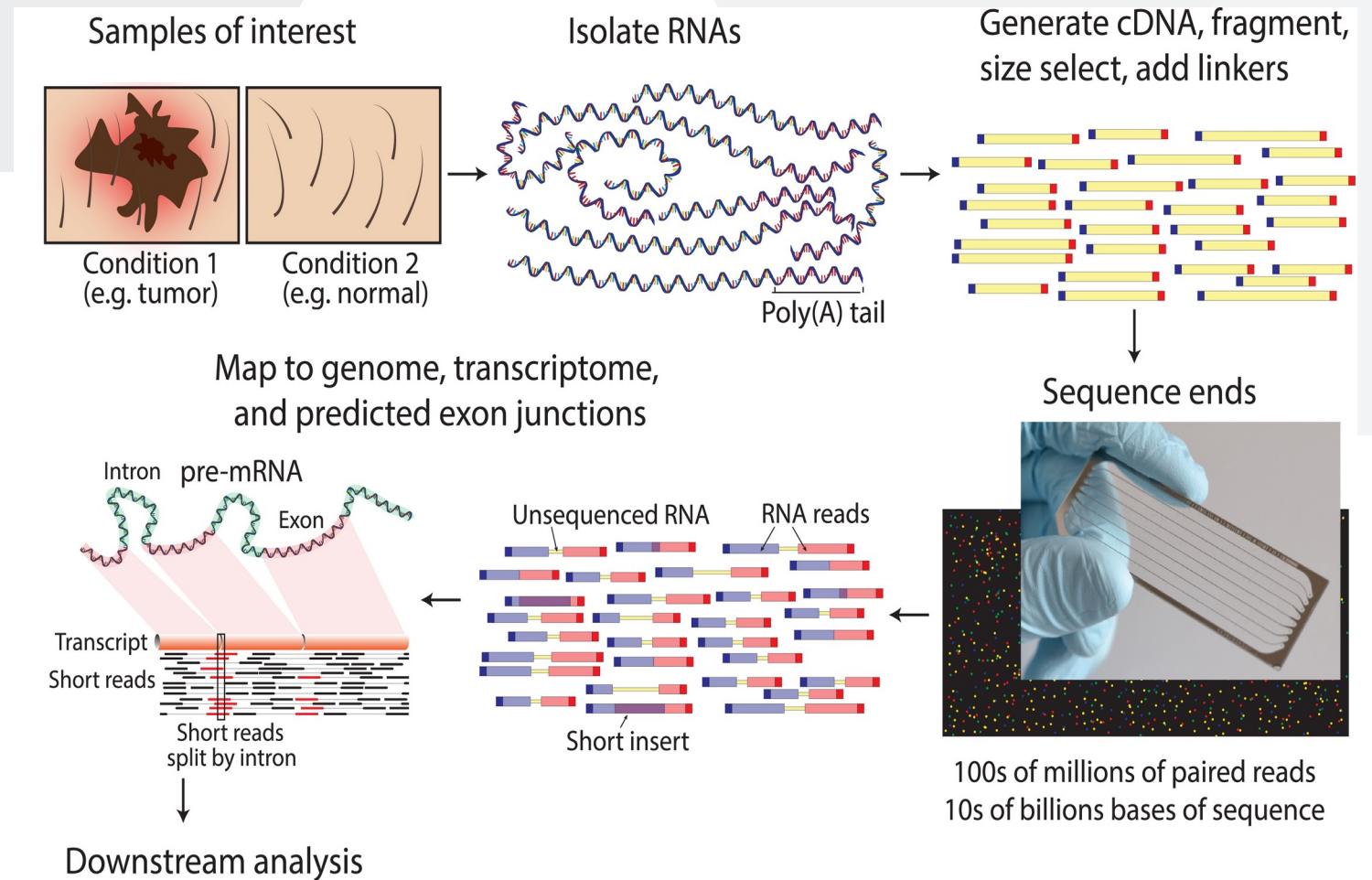
Boris Tichý

RNA-seq

# RNA-seq

## Genes/exons sequence and expression

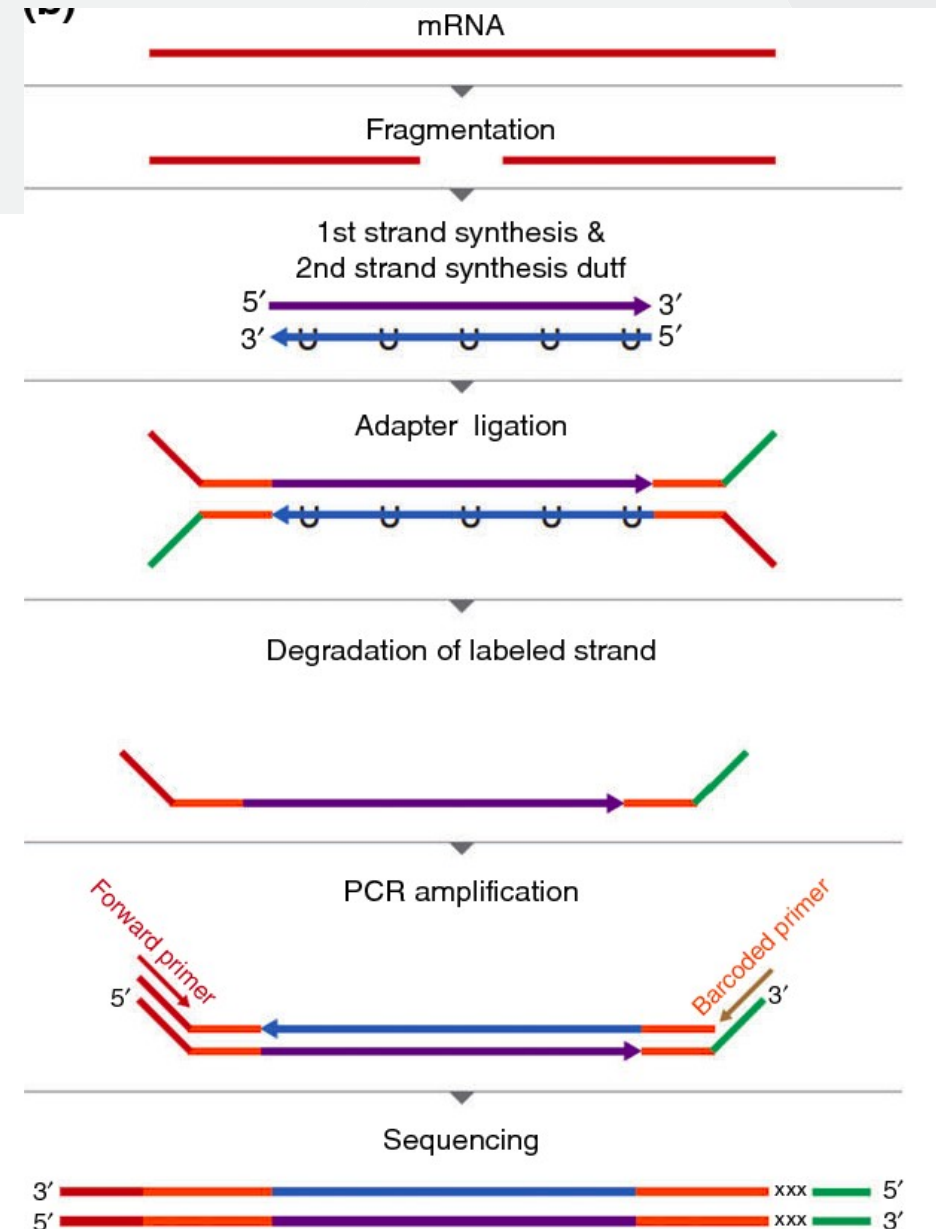
- “Classical” RNA-seq
  - Whole transcriptome
  - PolyA selection
  - rRNA depletion
- 3' RNA-seq
- targeted RNA sequencing
- single-cell RNA sequencing
- smallRNA sequencing
- long-read RNA sequencing
- direct RNA sequencing



# 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

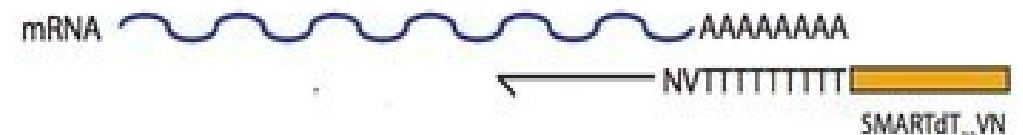
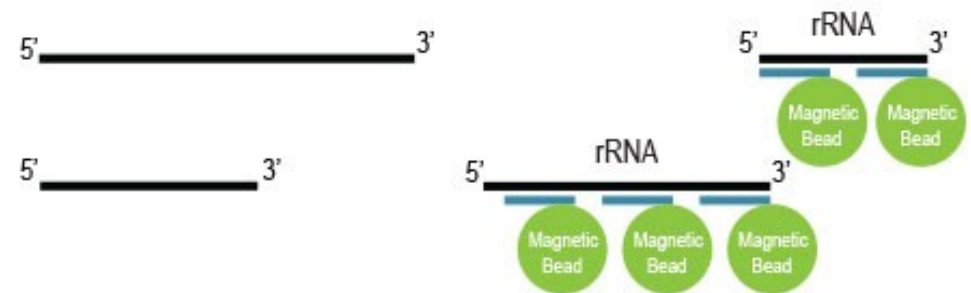


# RNA-seq

## „classical“ RNA-seq

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

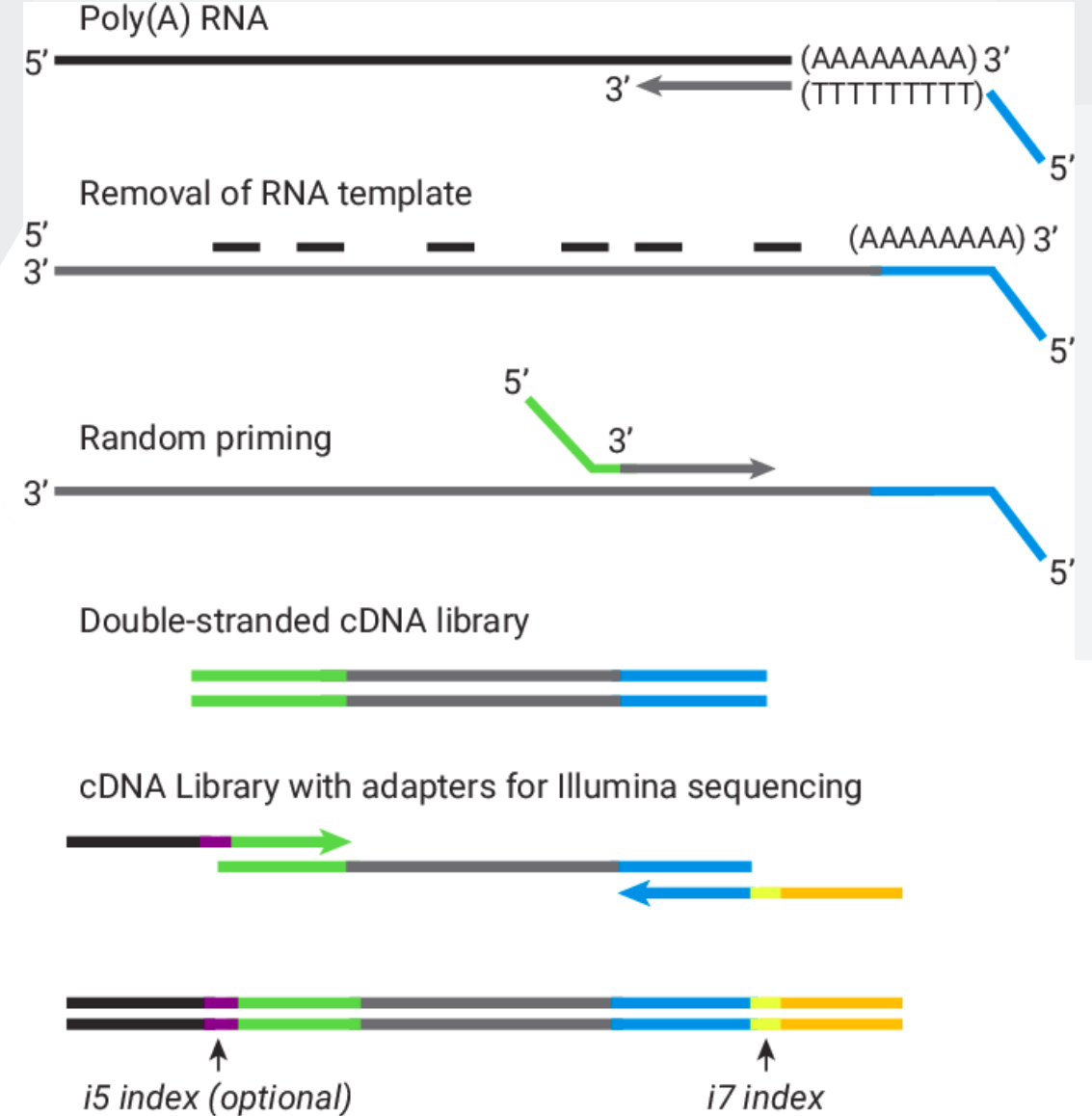
### 1. mRNA capture



# RNA-seq

## 3' RNA-seq

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

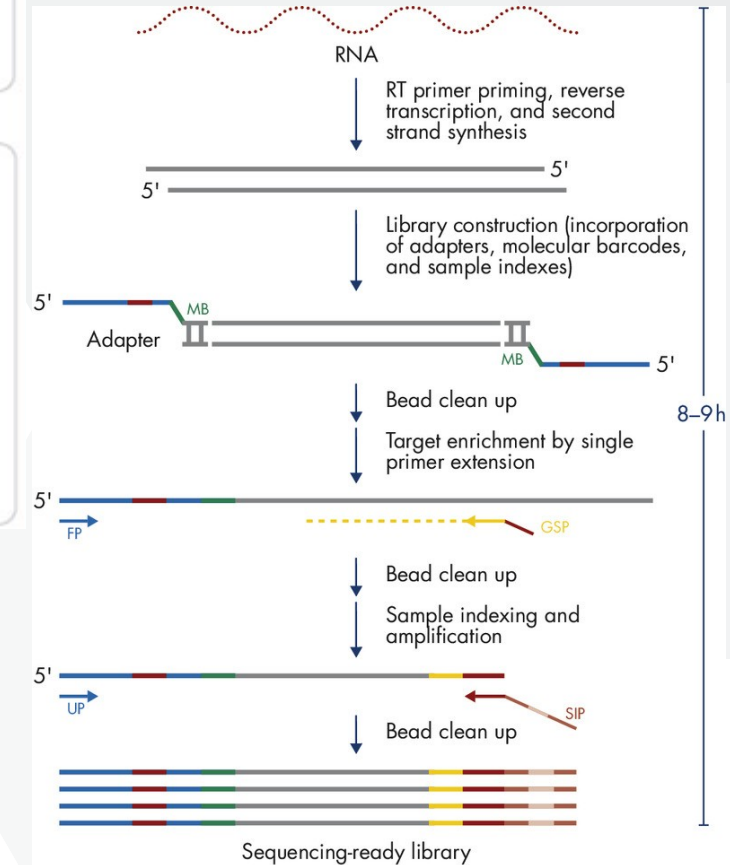
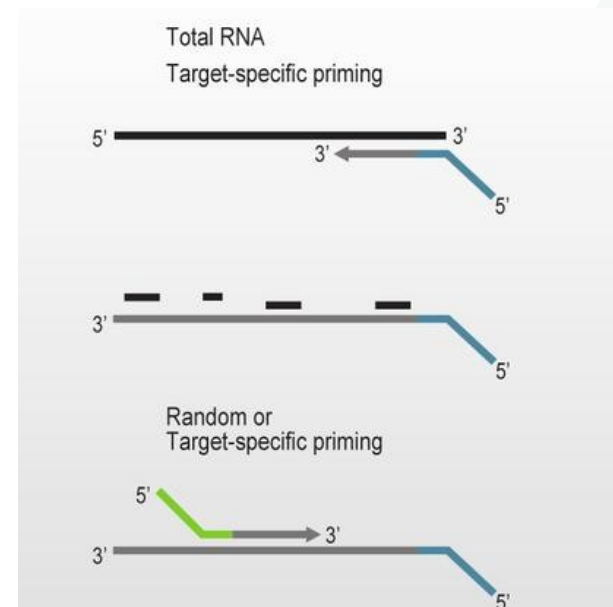
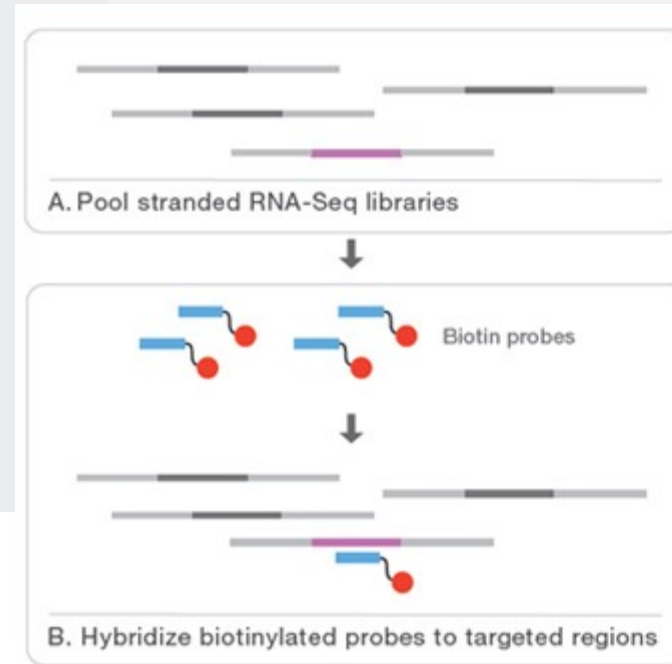
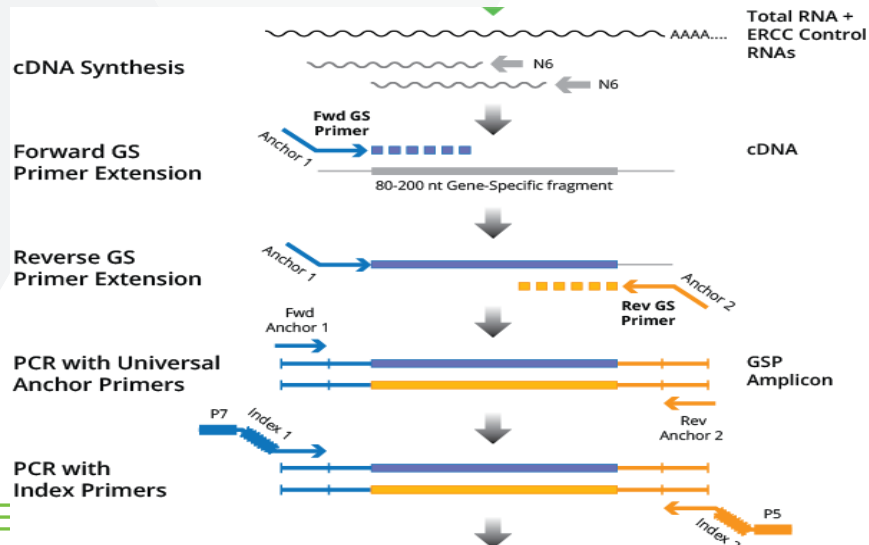




# RNA-seq

## Targeted RNA-seq

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

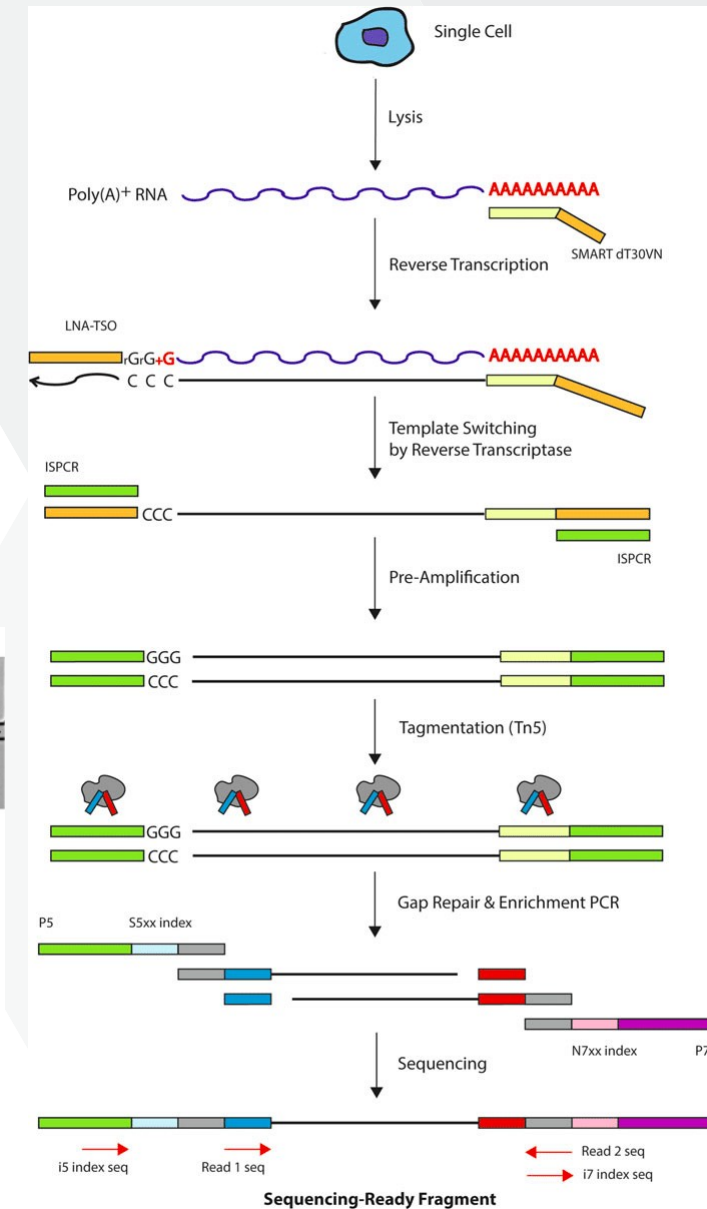
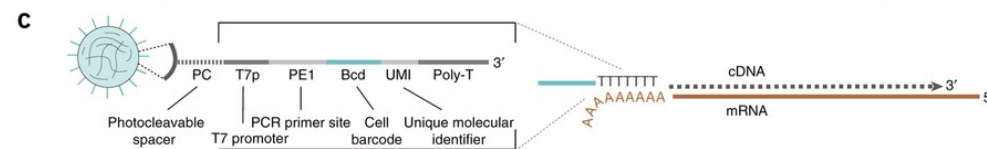
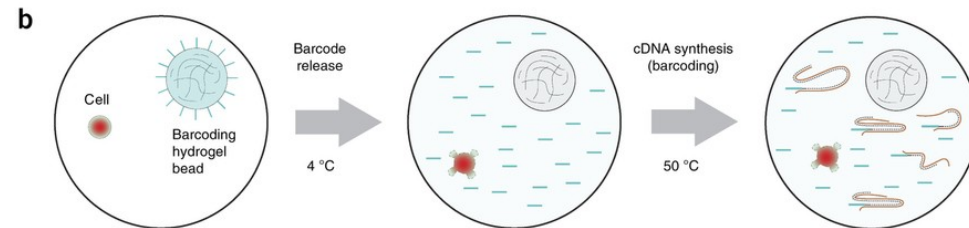
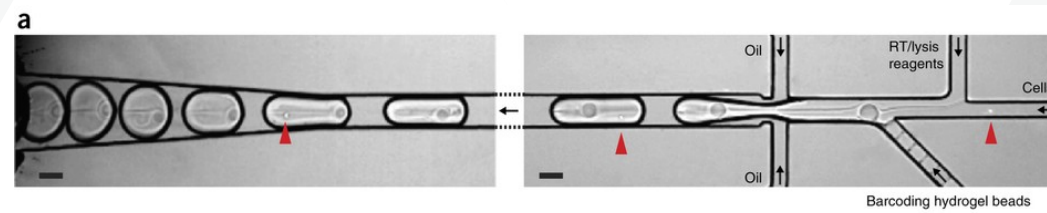
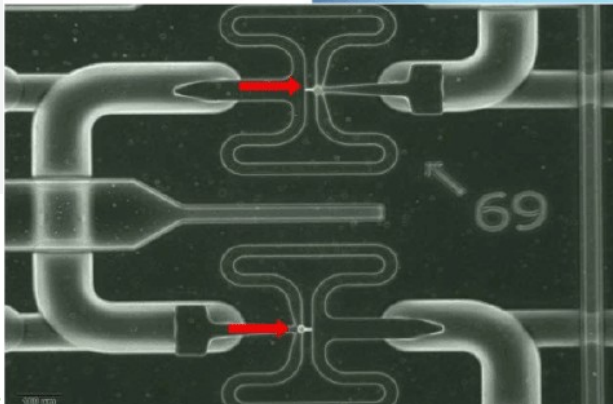
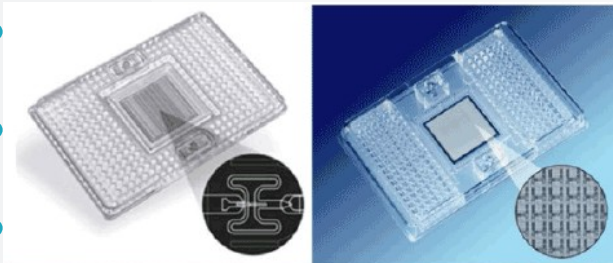


MB: Molecular barcode  
 GSP: Gene specific primer  
 FP: Forward primer  
 UP: Universal primer  
 SIP: Sample index primer

# RNA-seq

## Single-cell RNA-seq

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



# RNA-seq

## SmallRNA sequencing

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

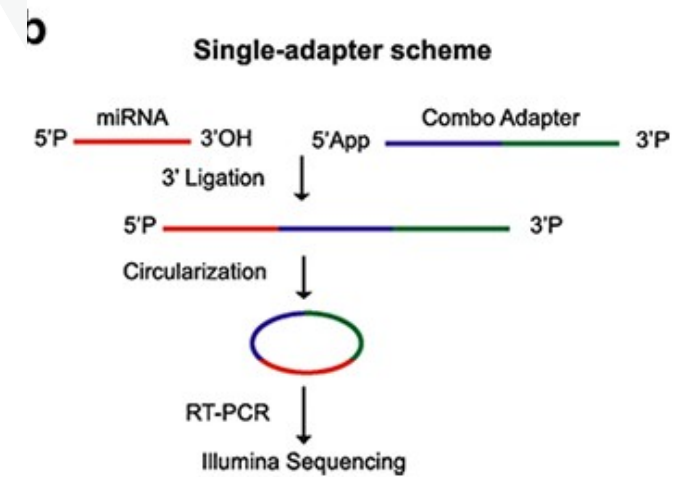
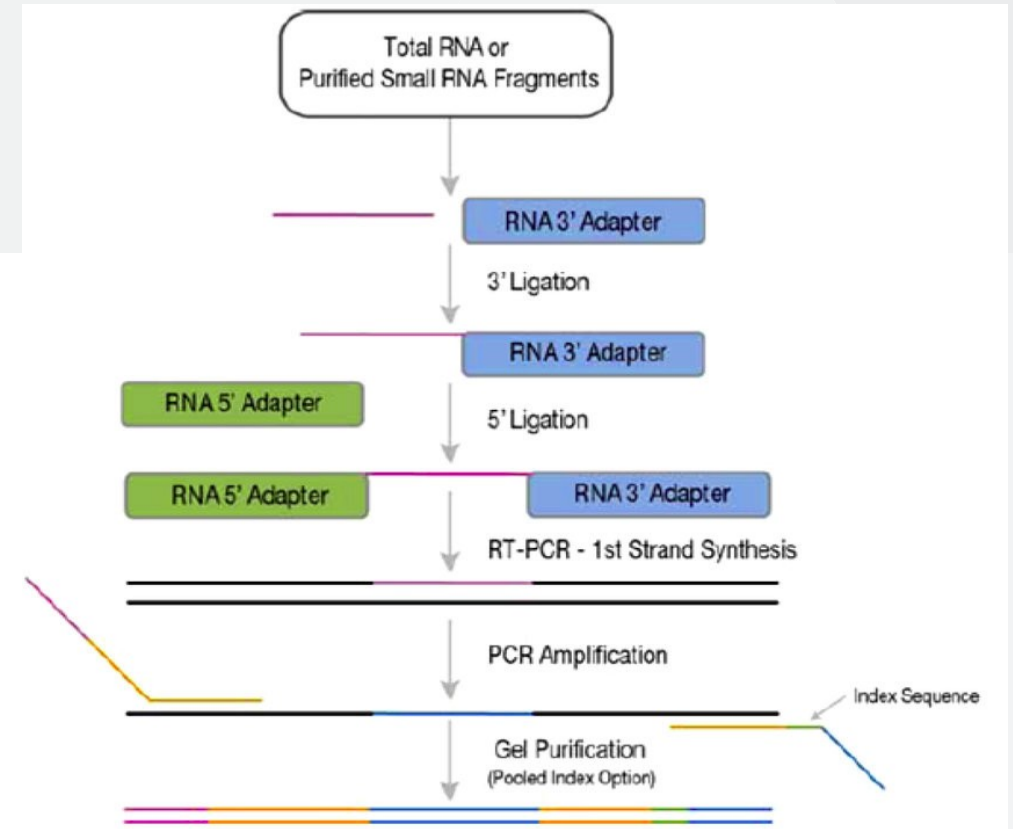
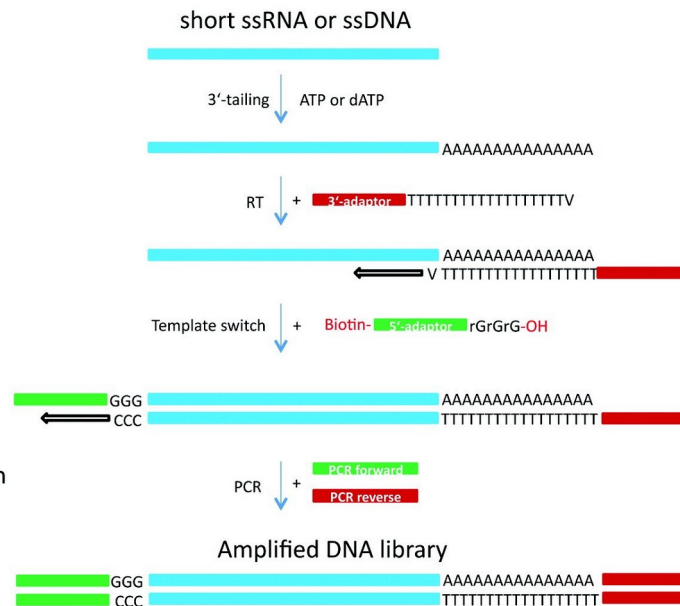
Duration:

30 min

15 min

15 min

60 – 120 min

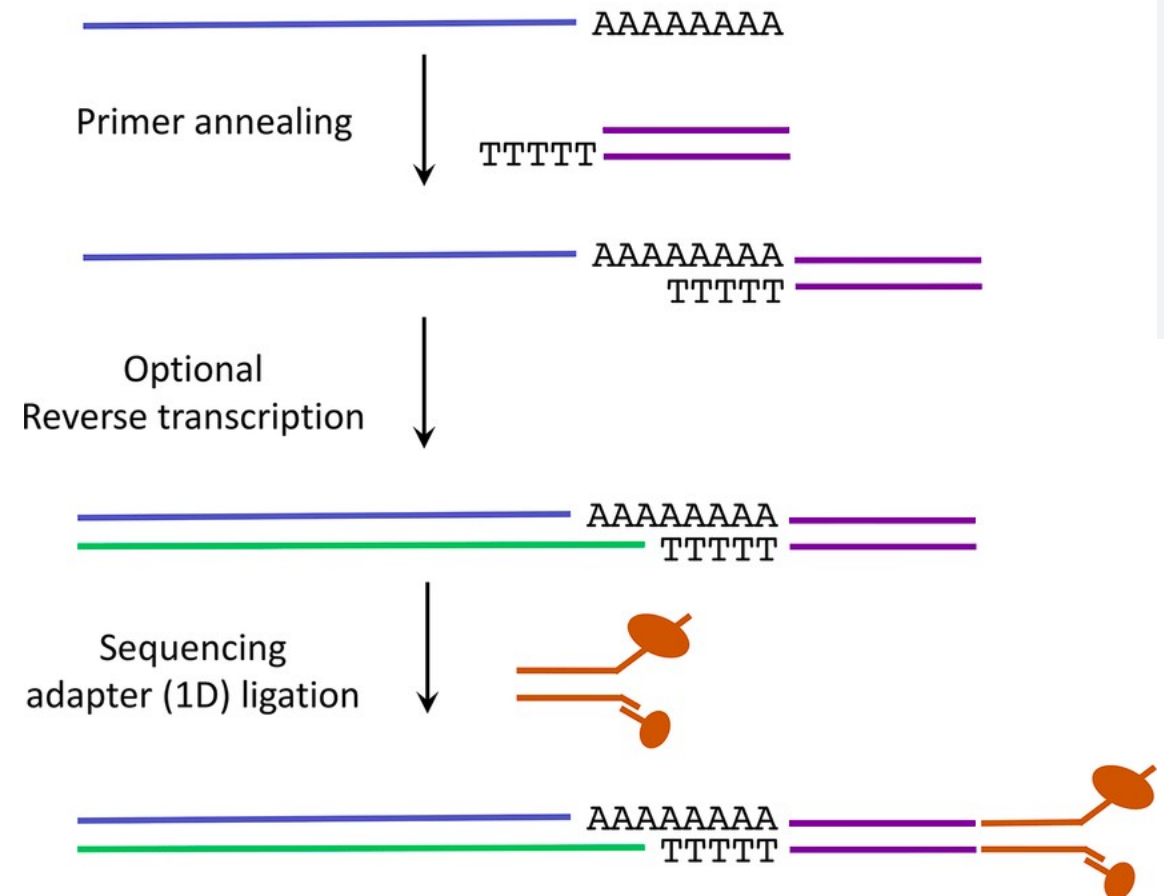
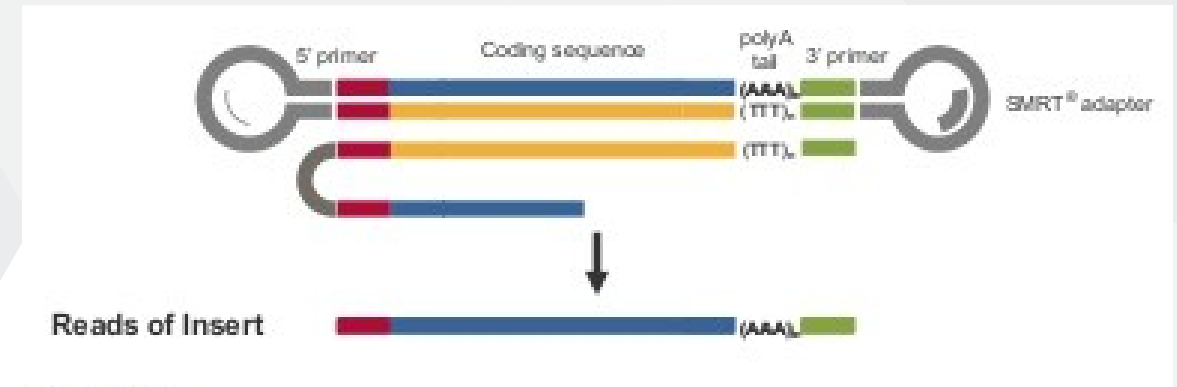




# RNA-seq

## Long-read + direct RNA sequencing

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





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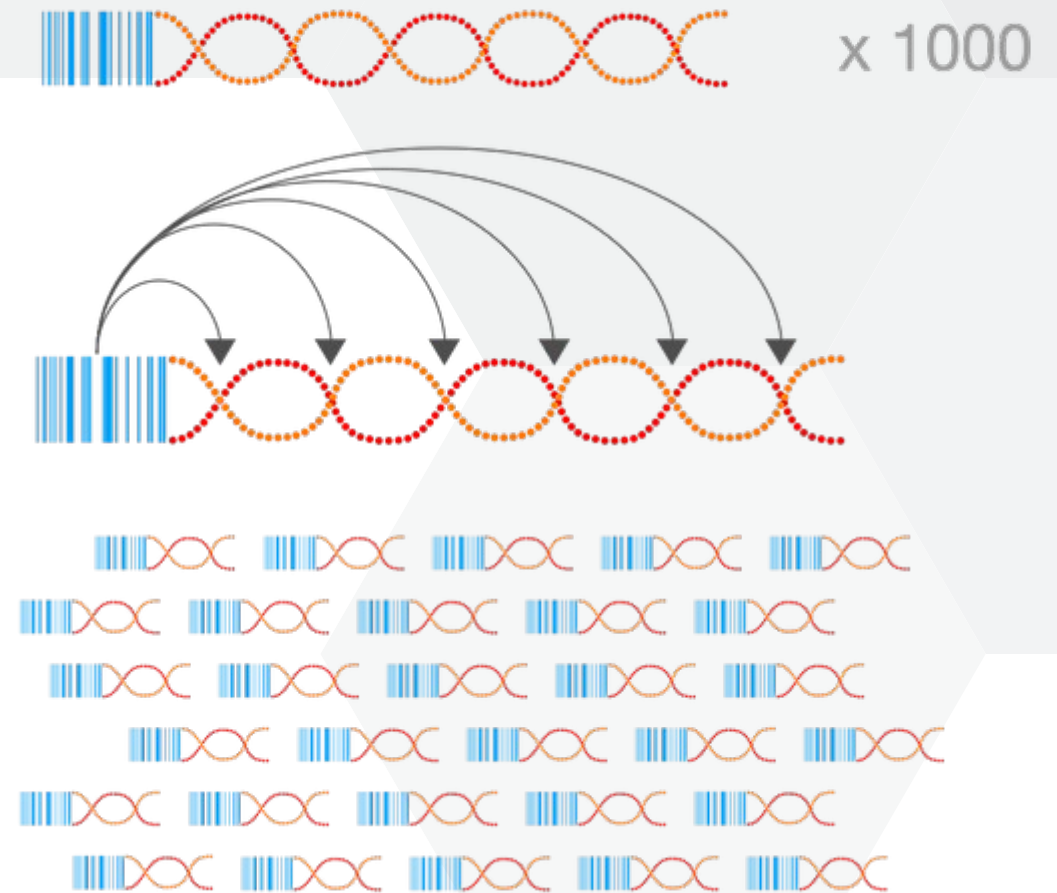
16S, RAD



# Metagenomics

## Analýza komplexních populací

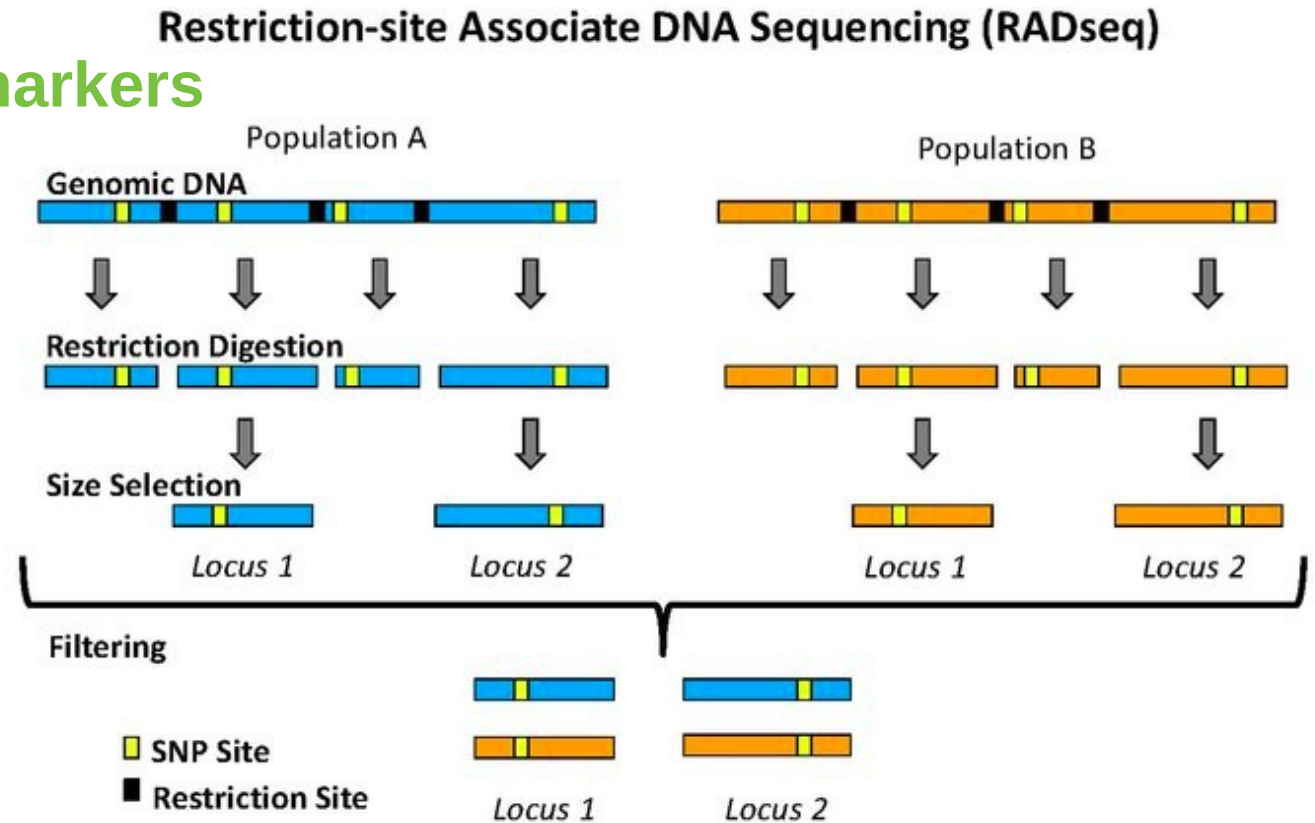
- Shotgun metagenome
- Markers
  - 16S rDNA
  - 18S, ITS, COX
  - Short vs. long amplicons



# RAD, ddRAD

## Restriction site associated DNA markers

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







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Thank you  
for your attention