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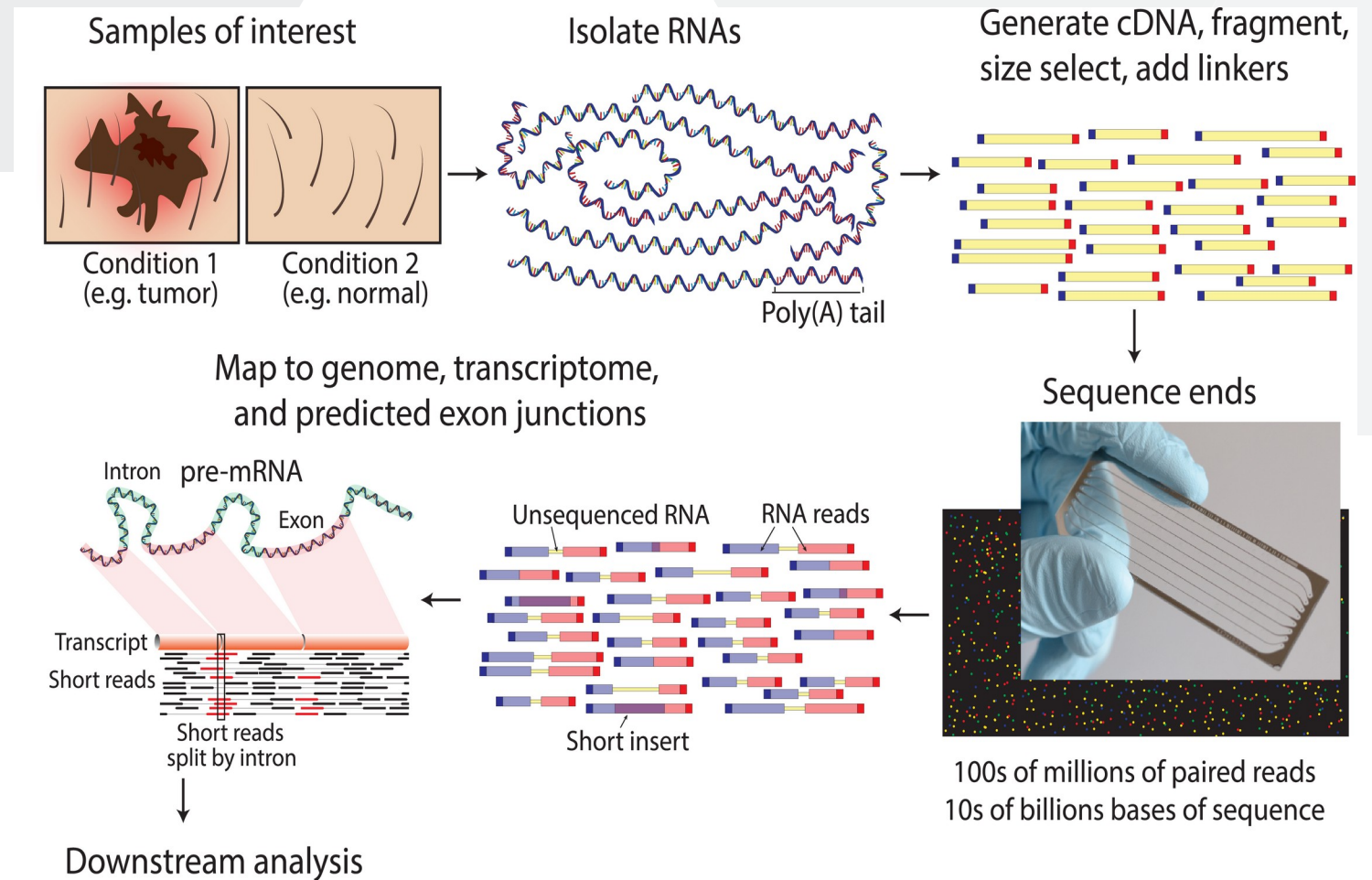
Boris Tichý

RNA-seq

RNA-seq

Genes/exons sequence and expression

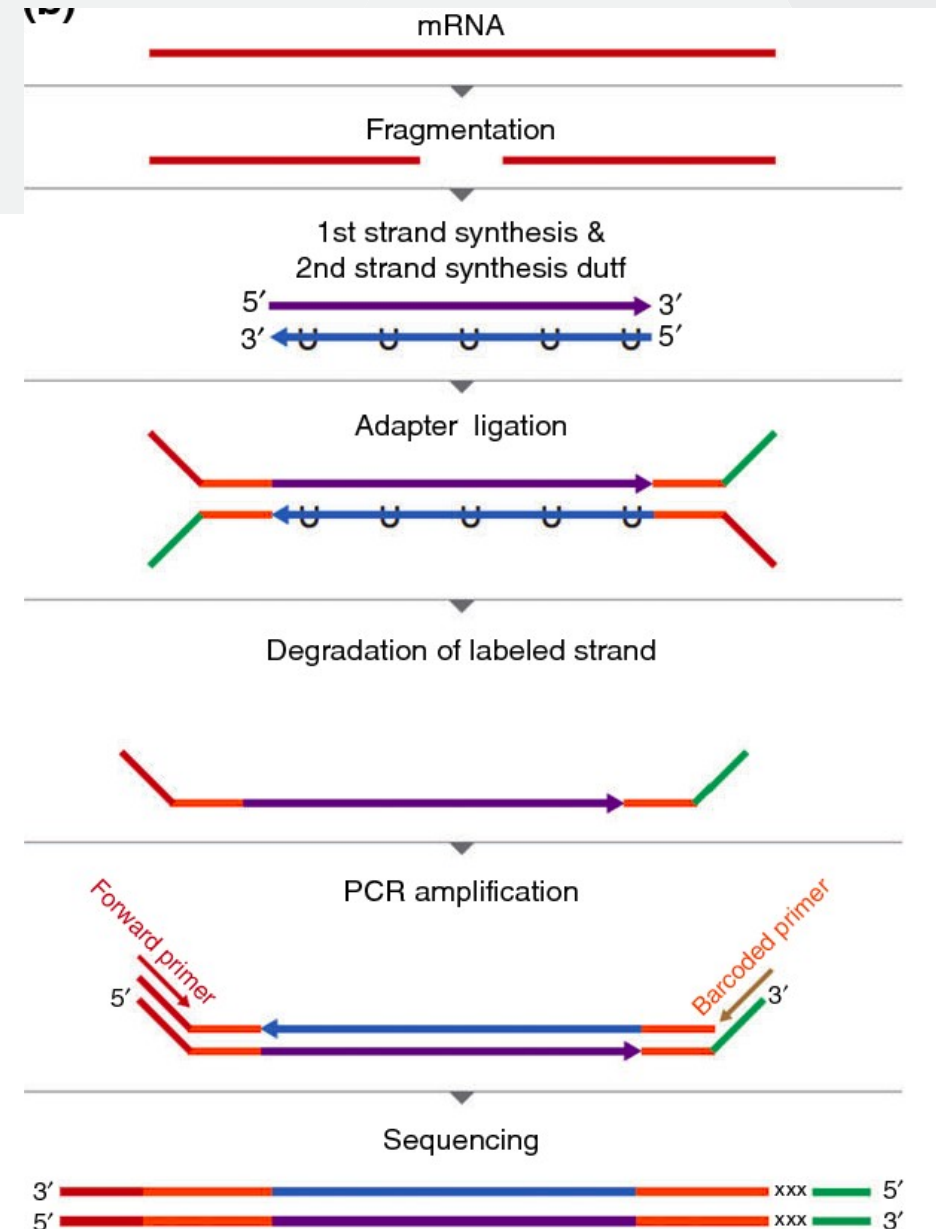
- “Classical” RNA-seq
 - Whole transcriptome
 - PolyA selection
 - rRNA depletion
- 3' RNA-seq
- targeted RNA sequencing
- single-cell RNA sequencing
- smallRNA sequencing
- long-read RNA sequencing
- direct RNA sequencing



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

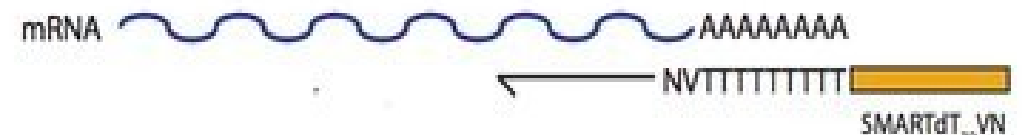
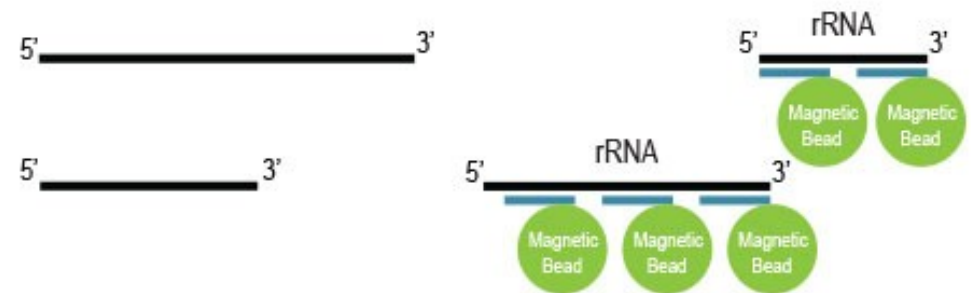


RNA-seq

„classical“ RNA-seq

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

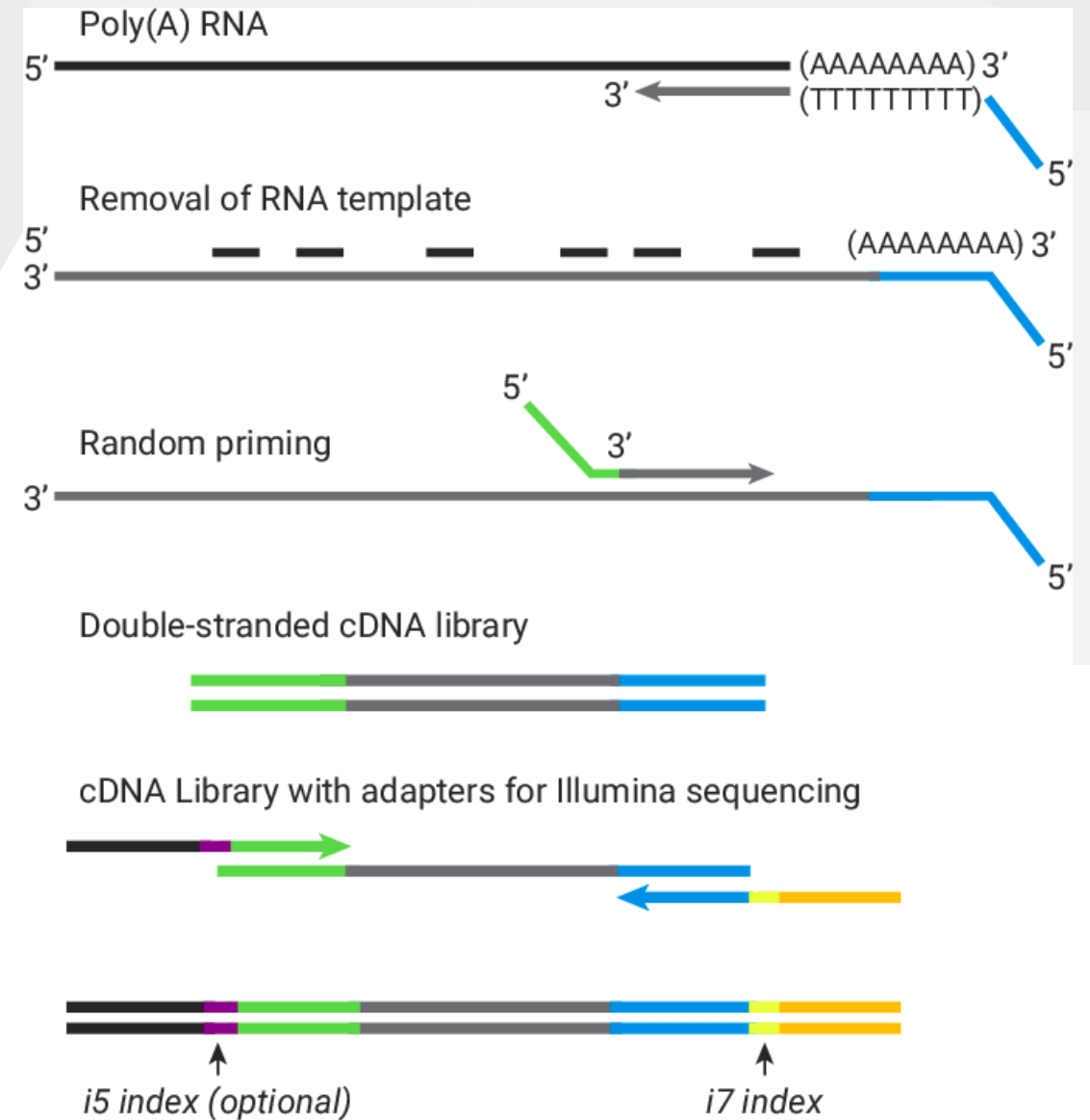
1. mRNA capture



RNA-seq

3' RNA-seq

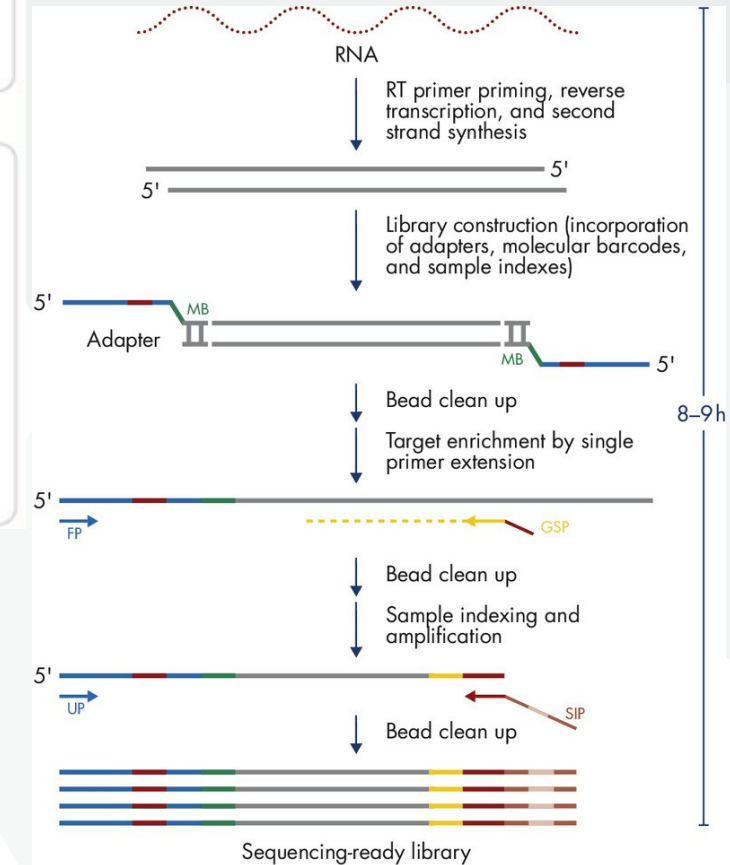
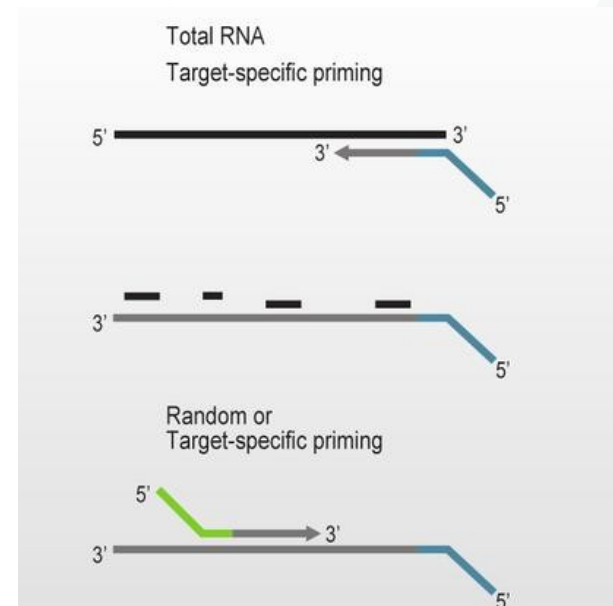
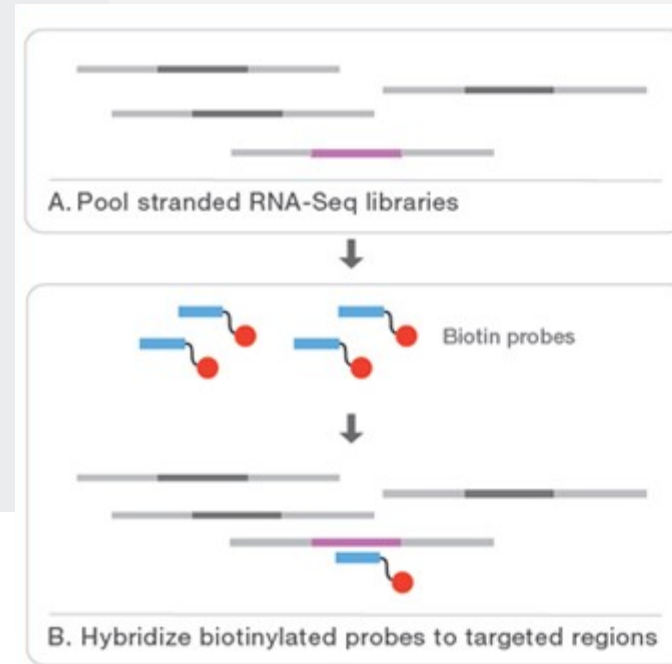
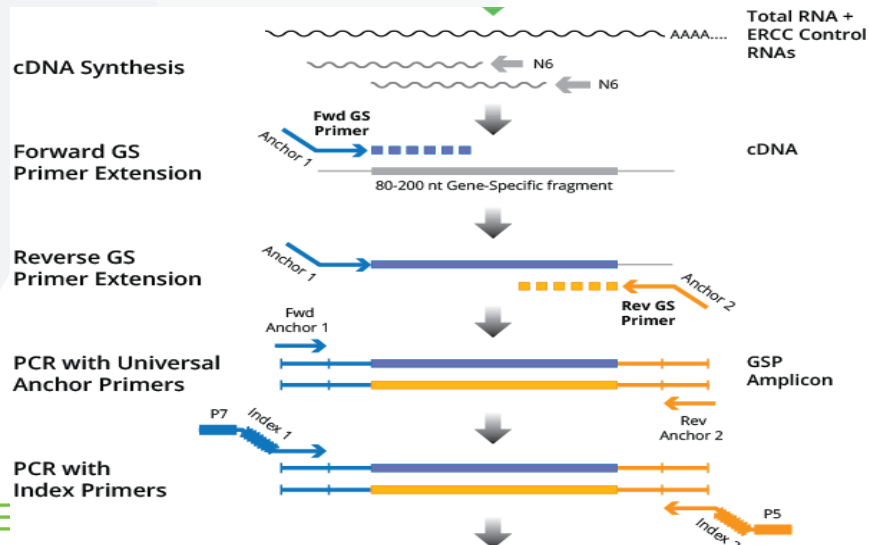
- 1 mRNA = 1 sequence
 - vs. 1 mRNA = n sequences
- Differential expression
-



RNA-seq

Targeted RNA-seq

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

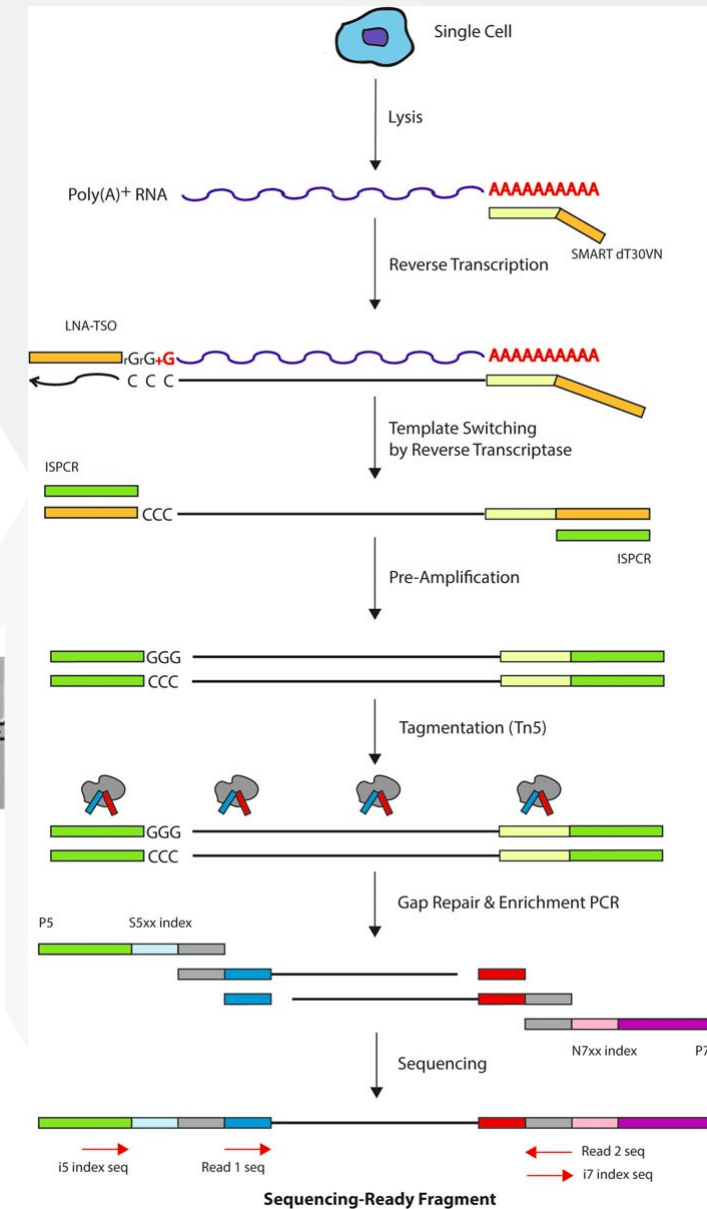
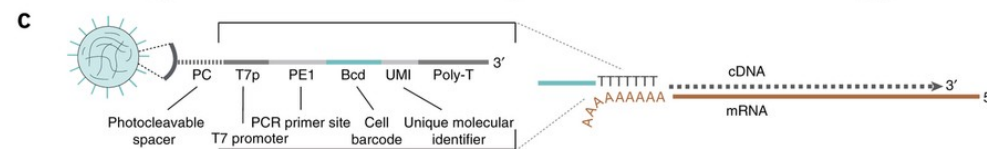
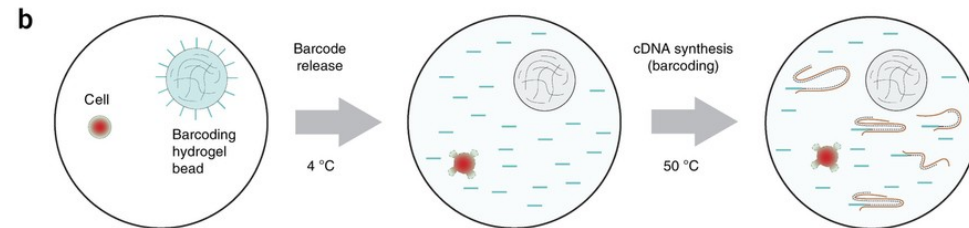
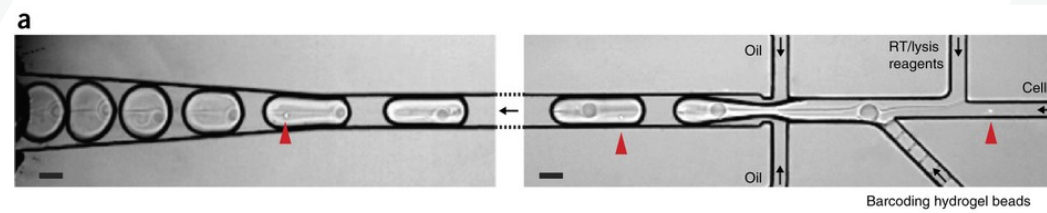
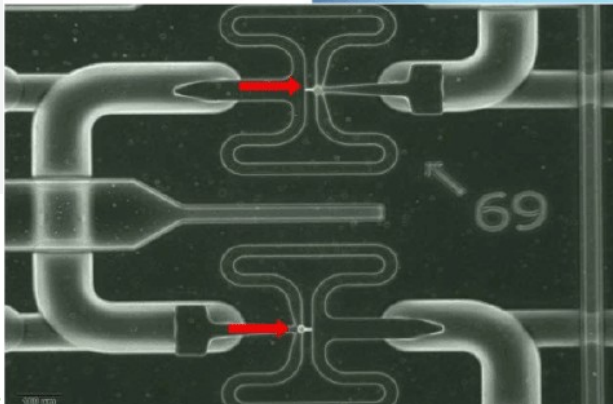
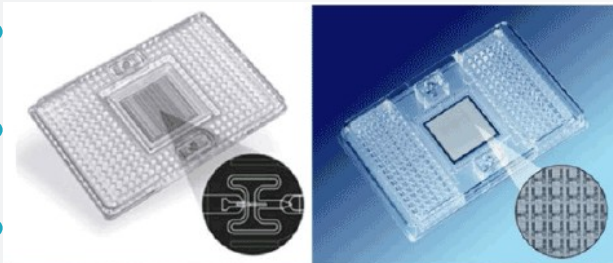


MB: Molecular barcode
 UP: Universal primer
 GSP: Gene specific primer
 SIP: Sample index primer
 FP: Forward primer

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, ...
-

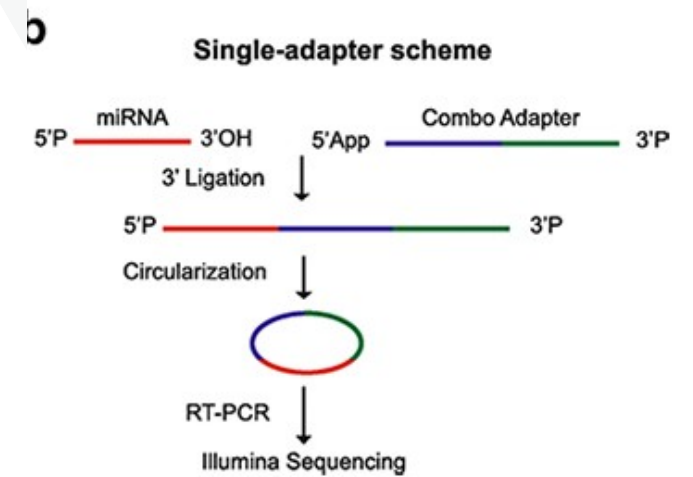
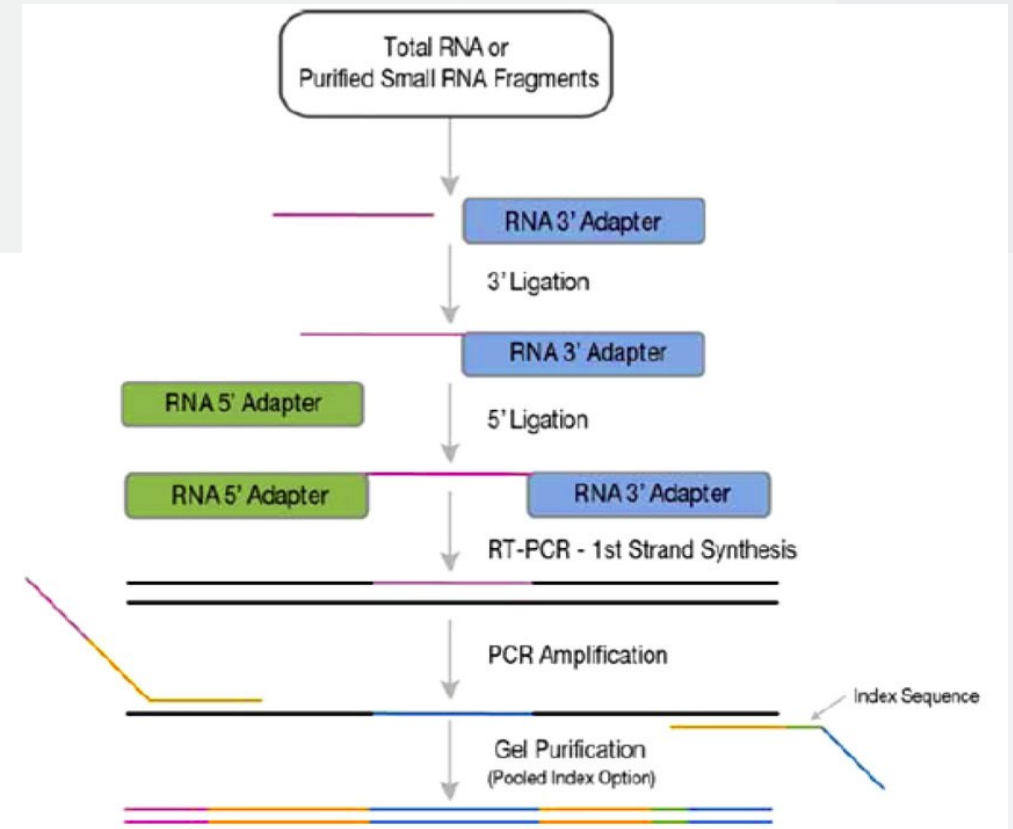
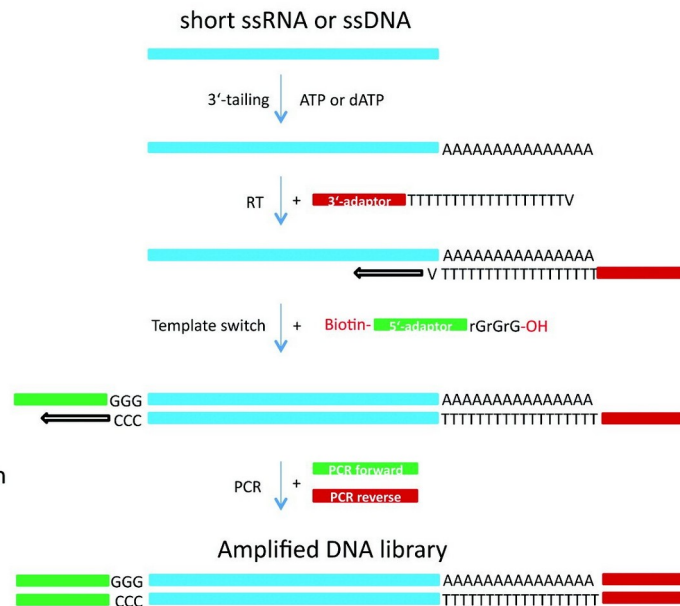
Duration:

30 min

15 min

15 min

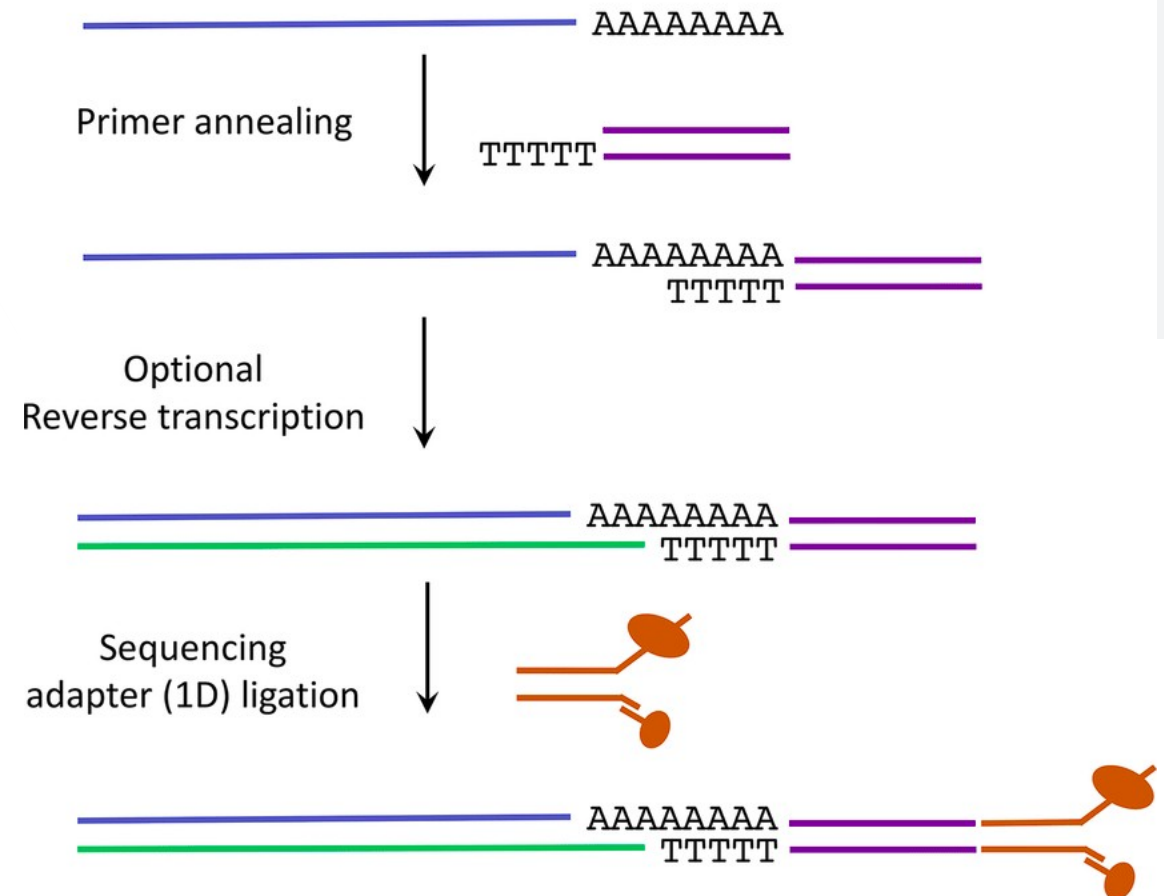
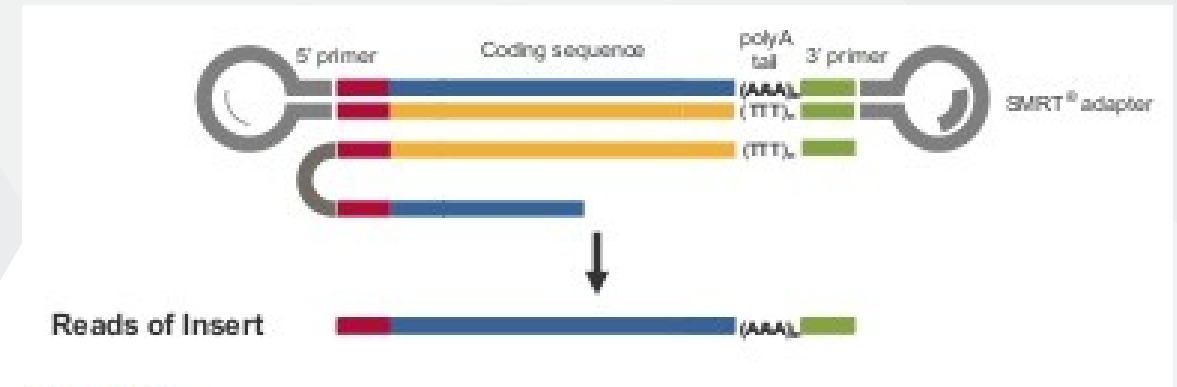
60 – 120 min



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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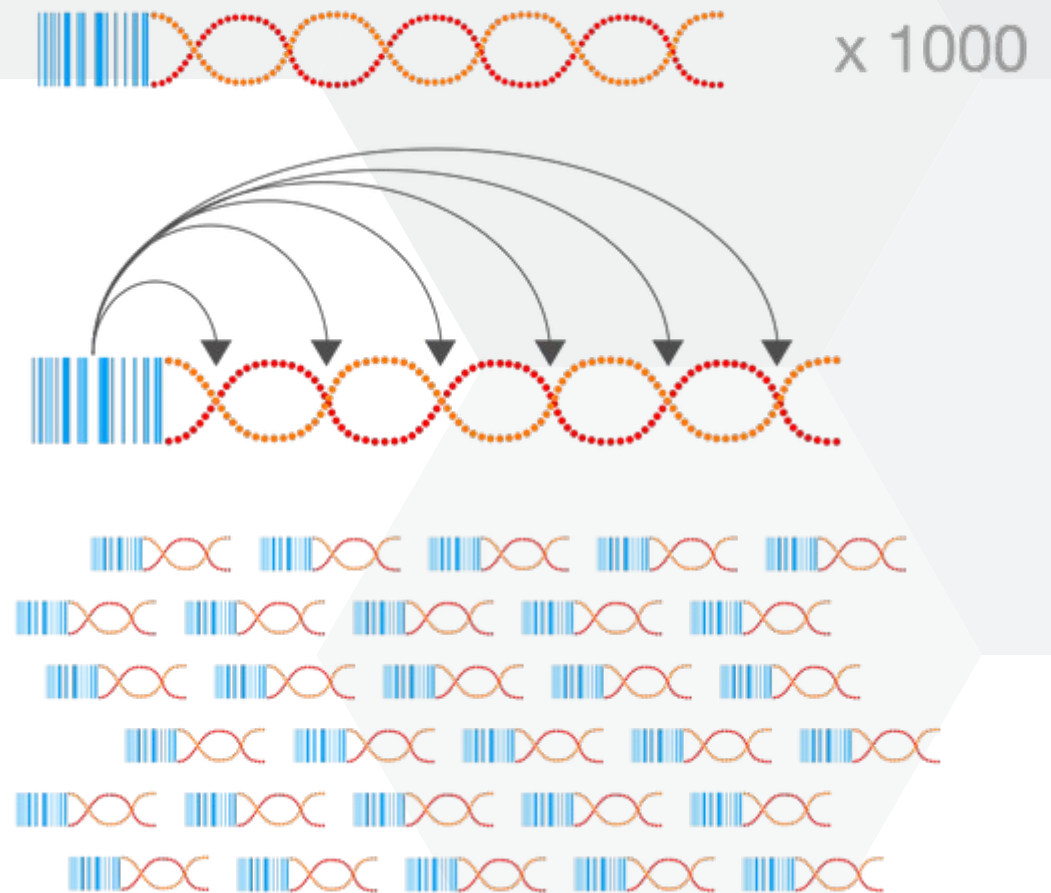
16S, RAD



Metagenomics

Analýza komplexních populací

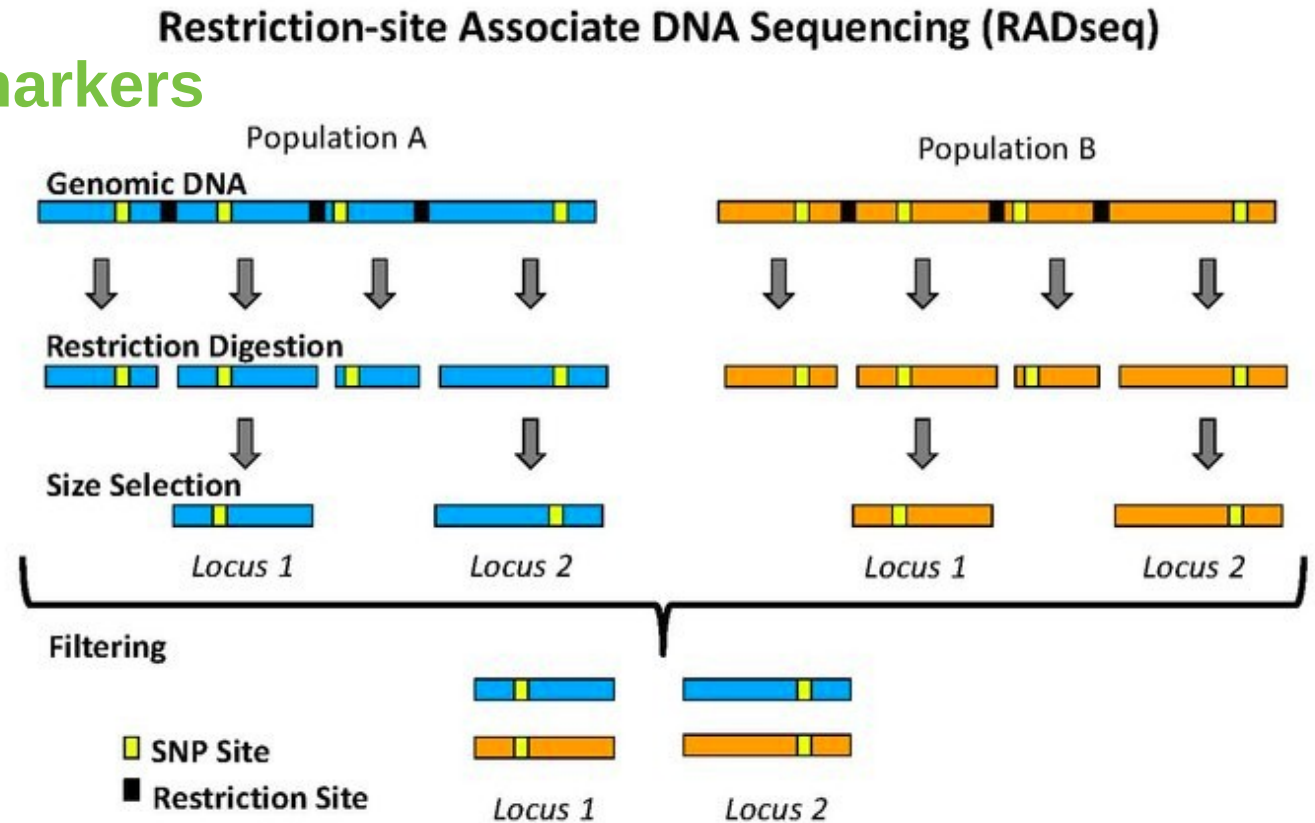
- Shotgun metagenome
- Markers
 - 16S rDNA
 - 18S, ITS, COX
 - Short vs. long amplicons



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