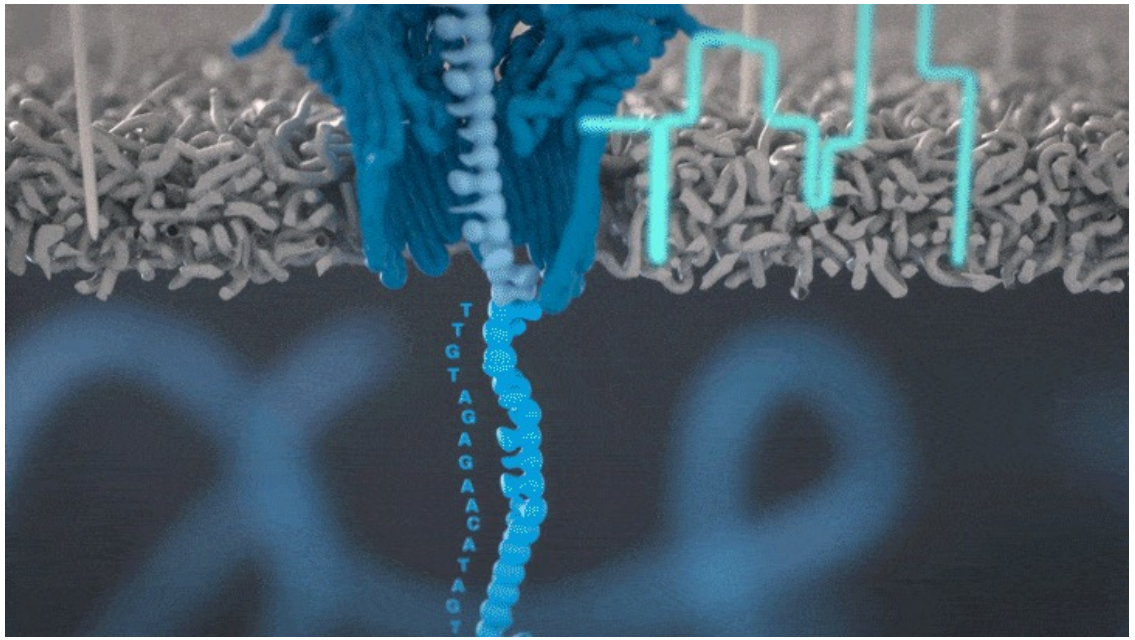
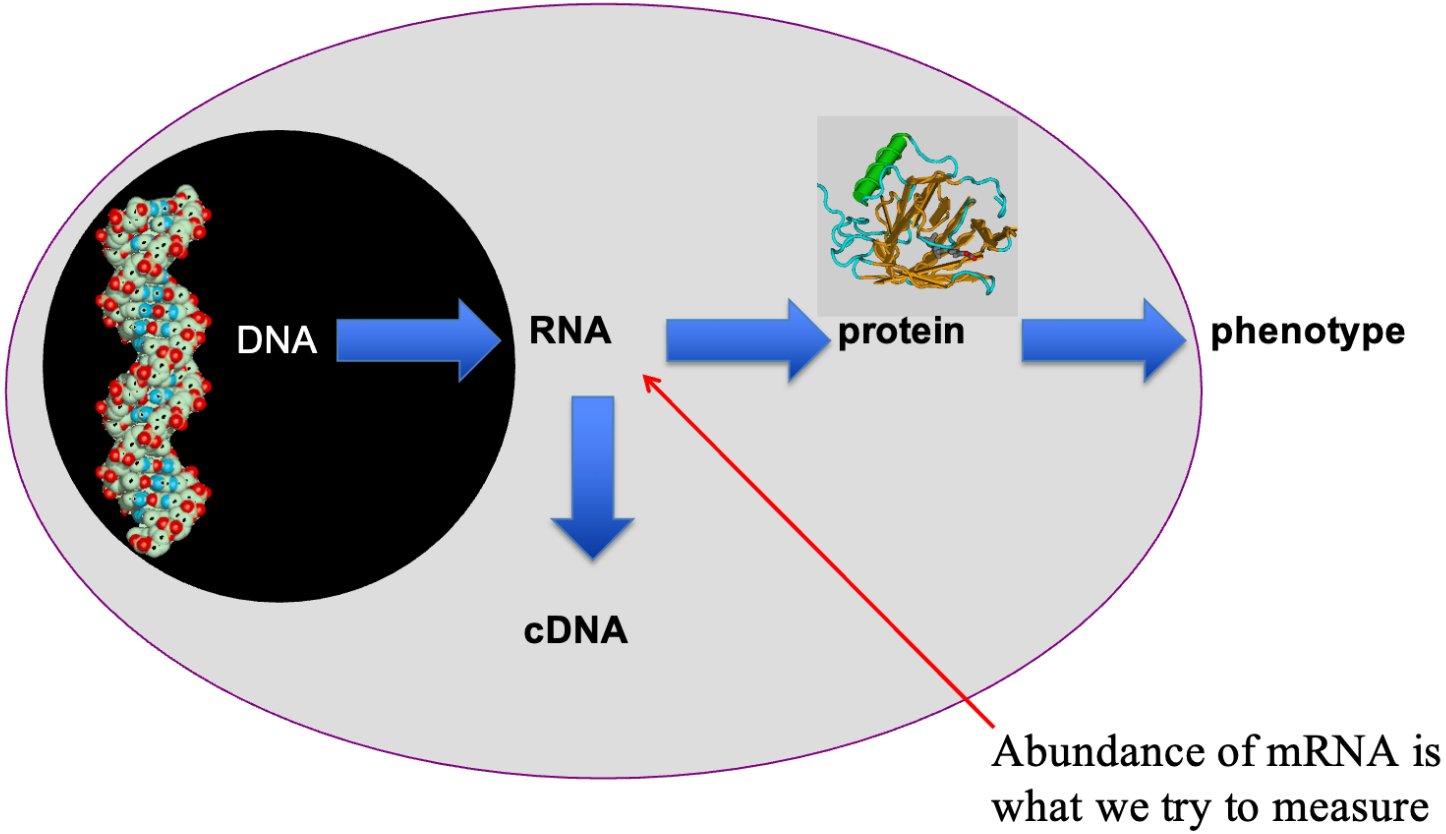


Direct RNA Sequencing: Technique and Applications

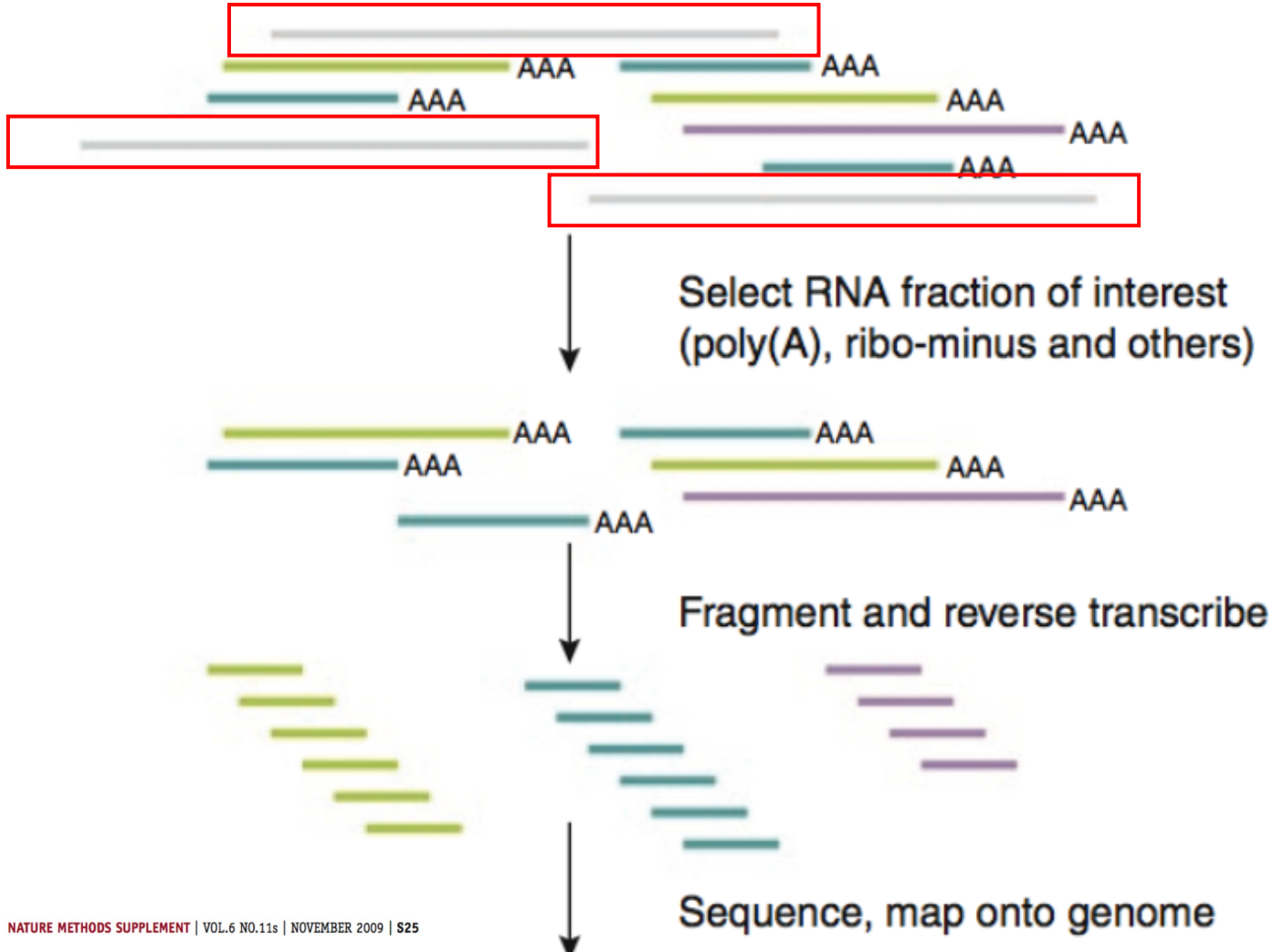
Joseph Won

Traditional RNA Sequencing



- In classic RNA-seq, complementary DNA (cDNA) is required
- Requires fragments with a poly-A tail attached
- Therefore, portions of the of the transcriptome are not sequenced

Transcriptomics using RNA-seq



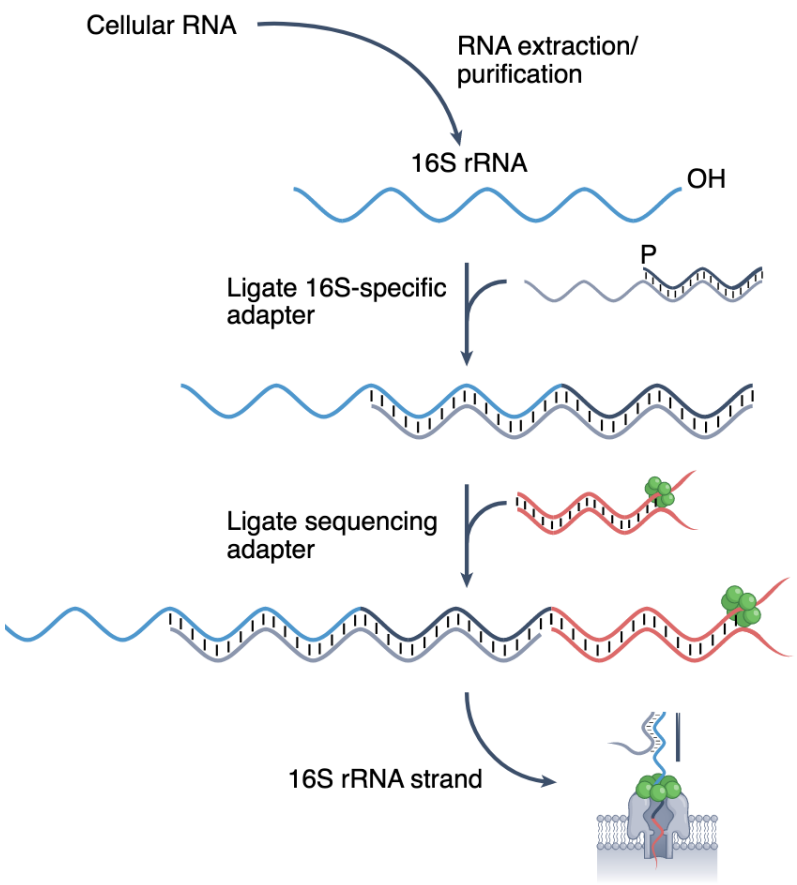
To differentiate from rRNA, all portions without a poly(a) are ignored

PCR amplification can introduce significant biases

Shirley Pepke[1], Barbara Wold[2] & Ali Mortazavi[2]

Direct RNA Sequencing

- Nanopore Sequencing: reads individual DNA or RNA strands by passing them through tiny protein nanopores, detecting changes in electrical current as different nucleotides pass through
- Benefits for RNA:
  - No conversion to cDNA
  - Full length RNA reads
  - Detection of RNA modifications (i.e. methylation)



Library preparation for MinION sequencing

Requirements and Costs:

- Equipment:
  - Sequencing device (MinION)
  - Library Prep Kit
- Software:
  - Minimap2 (alignment tool)
  - Nanopolish (variant calling tool)

The MinION Flow Cell can generate up to 50 Gb of data for sequencing DNA, cDNA or native RNA in real-time.

$1,999.00

The PromethION Flow Cell can generate up to 290 Gb for sequencing DNA, cDNA or native RNA in real-time.

$436,000.00

Nanopolish

nanopolish passing

Software package for signal-level analysis of Oxford Nanopore sequencing data. Nanopolish can calculate an improved consensus sequence for a draft genome assembly, detect base modifications, call SNPs and indels with respect to a reference genome and more (see Nanopolish modules, below).

Users' Guide

Minimap2 is a versatile sequence alignment program that aligns DNA or mRNA sequences against a large reference database. Typical use cases include: (1) mapping PacBio or Oxford Nanopore genomic reads to the human genome; (2) finding overlaps between long reads with error rate up to ~15%; (3) splice-aware alignment of PacBio Iso-Seq or Nanopore cDNA or Direct RNA reads against a reference genome; (4) aligning Illumina single- or paired-end reads; (5) assembly-to-assembly alignment; (6) full-genome alignment between two closely related species with divergence below ~15%.

Direct RNA Sequencing Kit

SQK-RNA004

A sequencing kit optimised for sequencing native RNA with improved output and accuracy.

This is an Early Access product.

Product lead time: 1 week

US$599.00

− 1 + Add to basket 2 Early Access

Applications

Article | Open access | Published: 19 December 2022

Nano3P-seq: transcriptome-wide analysis of gene expression and tail dynamics using end-capture nanopore cDNA sequencing

Oguzhan Begik, Gregor Diensthuber, Huanle Liu, Anna Delgado-Tejedor, Cassandra Kontur, Adnan Muhammad Niazi, Eivind Valen, Antonio J. Giraldez, Jean-Denis Beaudoin, John S. Mattick & Eva Maria Novoa ✉

Nature Methods **20**, 75–85 (2023) | Cite this article

19k Accesses | **18** Citations | **54** Altmetric | Metrics

Article | Open access | Published: 14 February 2019

Direct RNA sequencing on nanopore arrays redefines the transcriptional complexity of a viral pathogen

Daniel P. Depledge ✉, Kalanghad Puthankalam Srinivas, Tomohiko Sadaoka, Devin Bready, Yasuko Mori, Dimitris G. Placantonakis, Ian Mohr & Angus C. Wilson ✉

Nature Communications **10**, Article number: 754 (2019) | Cite this article

32k Accesses | **138** Citations | **60** Altmetric | Metrics

Identification of differential RNA modifications from nanopore direct RNA sequencing with xPore

Ploy N. Pratanwanich ✉, Fei Yao, Ying Chen, Casslynn W. Q. Koh, Yuk Kei Wan, Christopher Hendra, Polly Poon, Yeek Teck Goh, Phoebe M. L. Yap, Jing Yuan Chooi, Wee Joo Chng, Sarah B. Ng, Alexandre Thiery, W. S. Sho Goh ✉ & Jonathan Göke ✉

Nature Biotechnology **39**, 1394–1402 (2021) | Cite this article

23k Accesses | **128** Citations | **134** Altmetric | Metrics

- **Gene expression analysis**: Capturing full-length transcripts including exons, introns, and untranslated regions
- **Splicing and isoform diversity**: Detecting alternative splicing events
- **Epitranscriptomics**: Identification of RNA modification directly on native RNA molecules

Limitations

Error rates: Higher error rates compared to DNA sequencing

"Since 2019, two publications have reported median accuracy of ~91% for *Brassica napus* and ~88–90% for *E. coli*"

Lower throughput: Therefore, lower depth in sequencing and requires higher input RNA quantity

Less computational tools: By virtue of its novelty, there are less tools and datasets to utilize nanopore sequencing data

Question: Where do you see application of dRNA sequencing in evolutionary biology?

This is what we are interested in…right?

Species

Group

Organism

Cellular

Molecular

Kin Selection?

Gene expression?

Keep in mind MLS is controversial because there is no good way to link different "levels" of biological organization – even through we can obviously observe the patterns (differential gene expression, phenotypes, social behavior, etc…)

Multilevel selection – Wilson and Wilson 2008

Pattern vs. Process

Traditional RNA Seq:

- Gene expression quantification
- Differential gene expression

Direct RNA Seq:

- Isoform Diversity
- RNA Modifications
- Methylation
- Alternative Splicing

*as related to the transcriptome