RNA-seq Introduction

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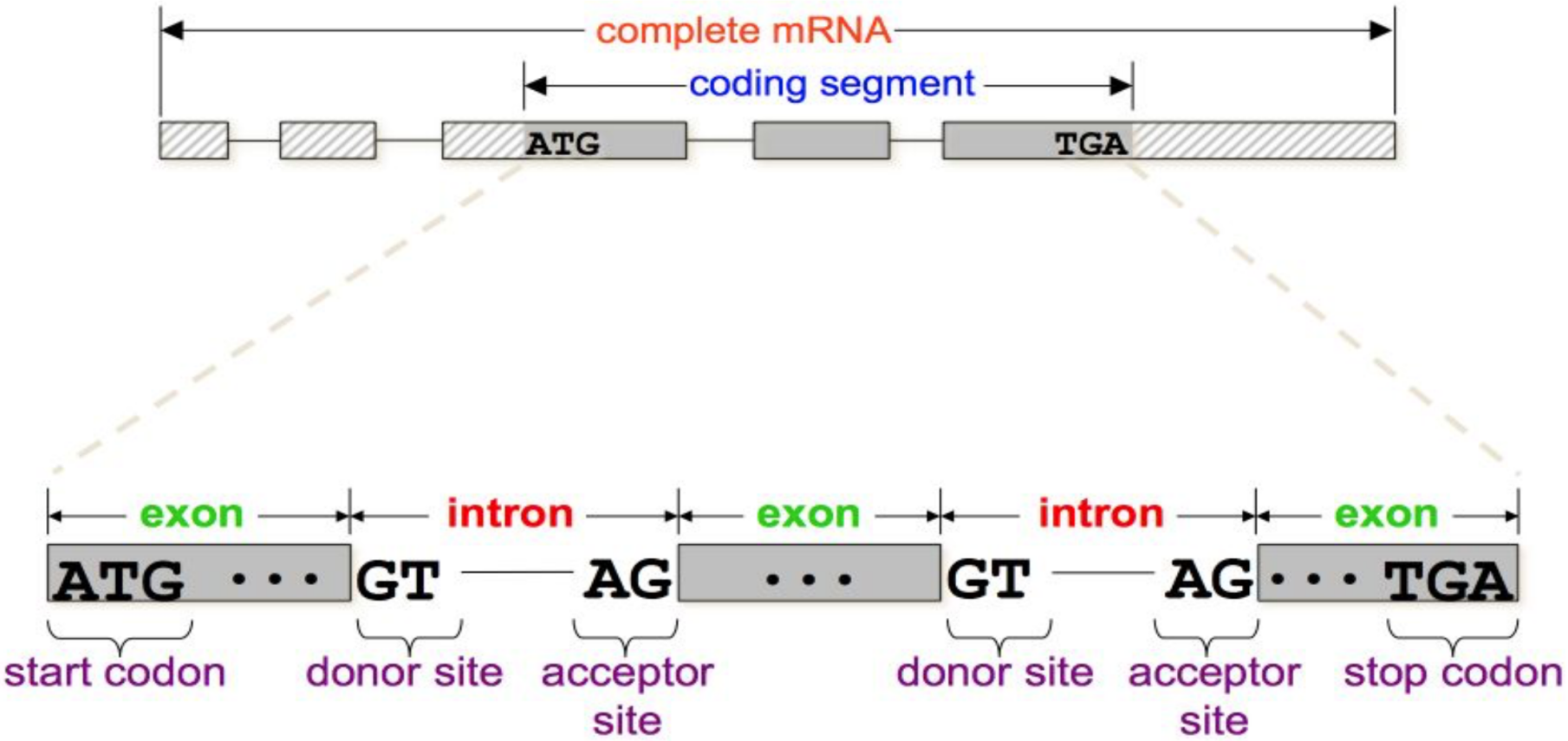
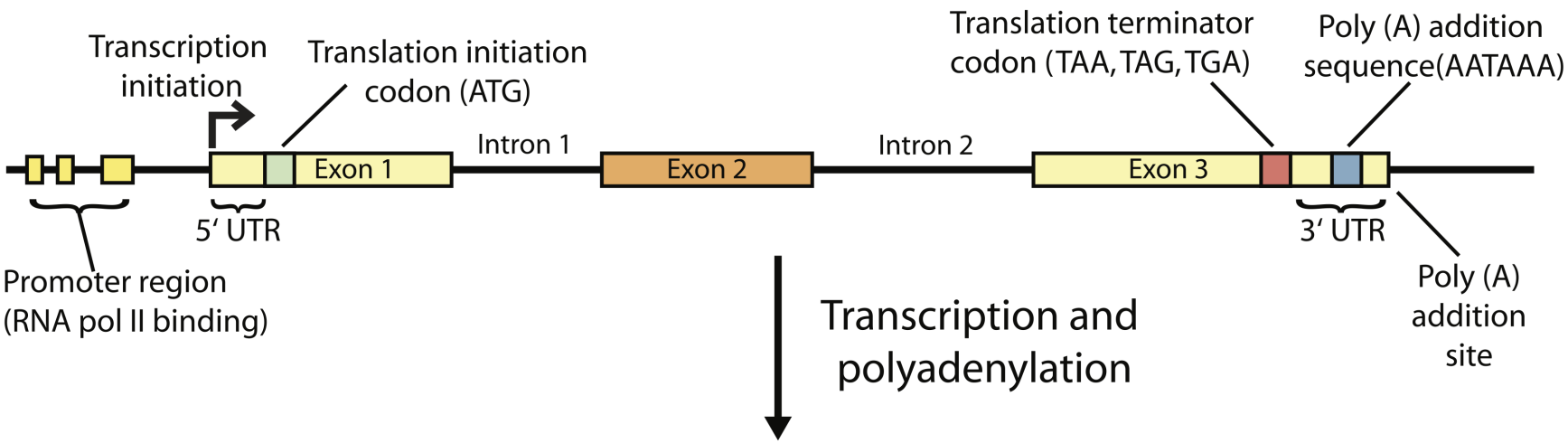
2021-03-03

Eukaryotic gene structure



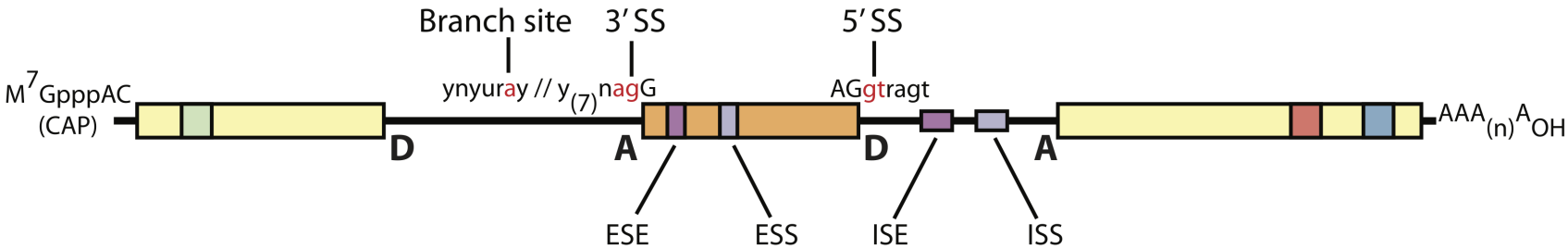
Gene expression

Double-stranded genomic DNA template



Transcription and polyadenylation

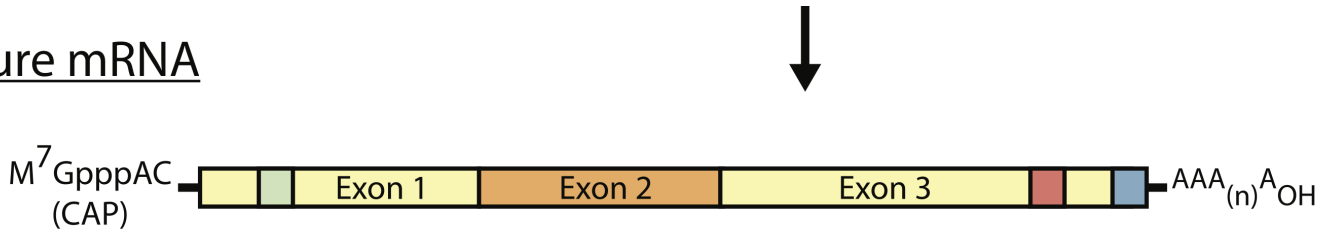
Single-stranded pre-mRNA (nuclear RNA)



RNA processing

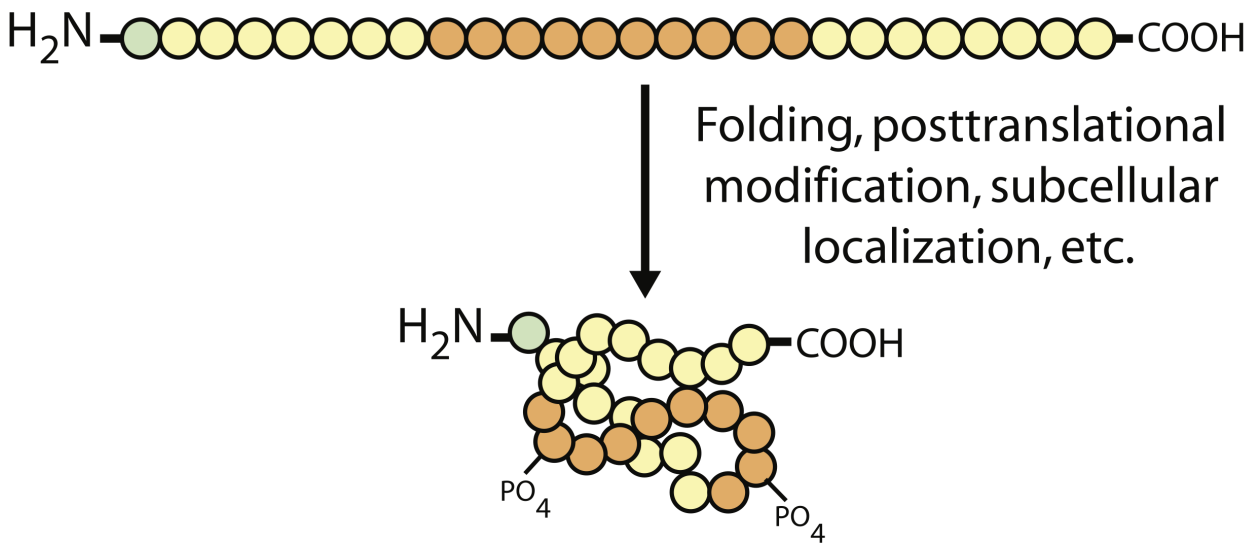
Gene expression

Mature mRNA



Export to cytoplasm and translation

Protein (amino acid sequence)



What is RNA sequencing?

- Massive parallel sequencing to **characterize and quantify transcriptomes** (all actively transcribed genes)
- Detection of **differential gene expression**
- **Transcriptome reconstruction**, identification of **new transcripts**
- Detection of **alternative splicing events**
- Detection of **structural variants**, e.g., fusion transcripts
- **Allele-specific** gene expression measurements
- **Mutation analysis** – presence of genomic mutations and their effect on gene expression

RNA-seq analysis techniques

Sequencing technologies

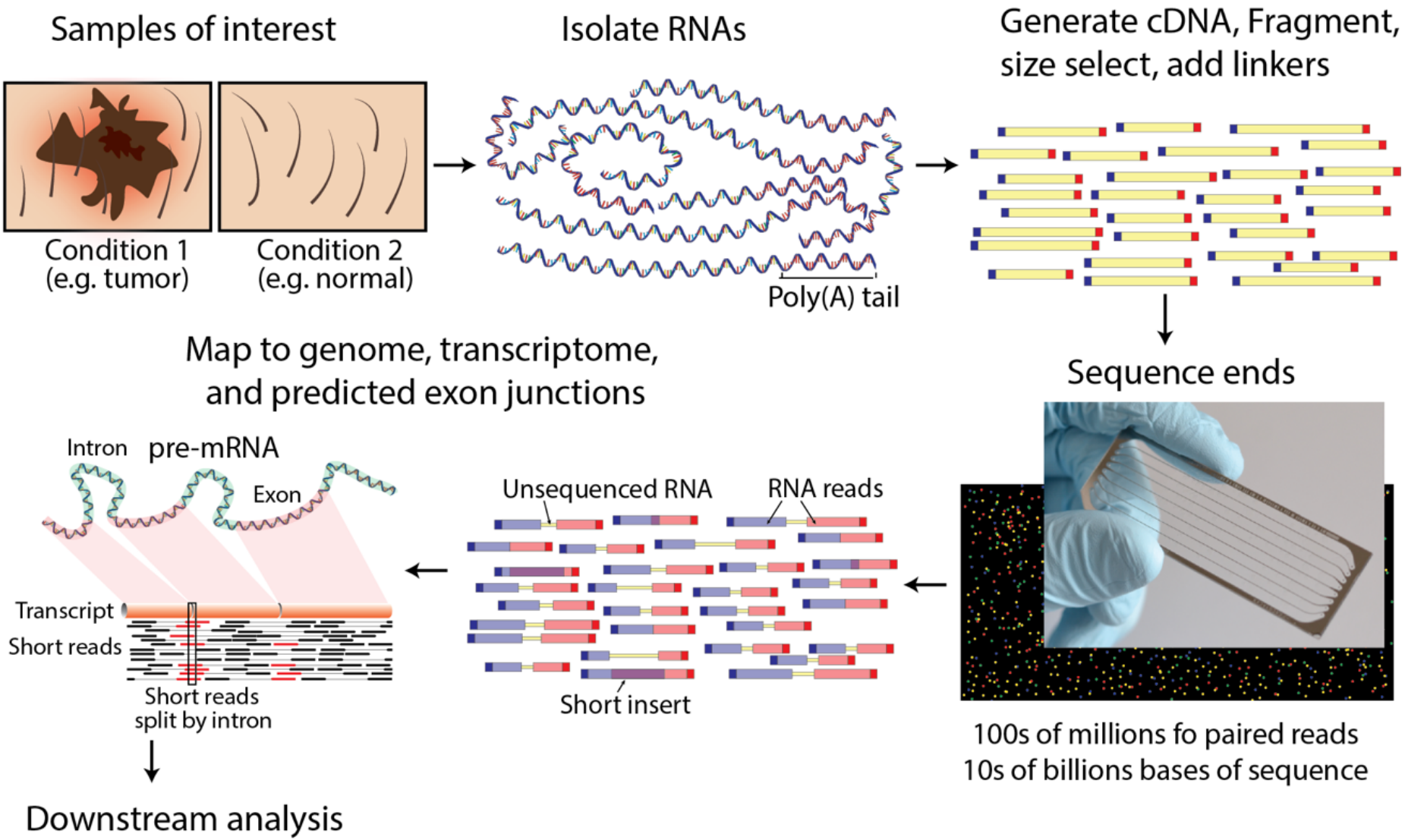
Commercially available

- **Illumina/Solexa** - short reads, sequencing-by-synthesis
- **Life Technologies Ion Torrent/Proton** - short reads, Ion Semiconductor sequencing
- **Pacific Biosciences** - long reads, Single Molecule Real Time sequencing

Experimental

- **Nanopore sequencing** - continuous sequencing (very long reads), fluctuations of the ionic current from nucleotides passing through the nanopore

Overview of RNA sequencing technology



Source: http://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1004393

RNA-Seq Limitations

Quantitation influenced by many confounding factors

- "Sequenceability" - varying across genomic regions, local GC content and structure-related
- Varying length of gene transcripts and exons
- Bias in read ends due to reverse transcription, subtle but consistent
- Varying extent of PCR amplification artifacts
- Effect of RNA degradation in the real world
- Computational bias in aligning reads to genome due to aligners

RNA-Seq Limitations

SNP discovery in RNA-seq is more challenging than in DNA

- Varying levels of coverage depth
- False discovery around splicing junctions due to incorrect mapping

De novo assembly of transcripts without genome sequence:
computationally intensive but possible, technical improvements will help

- Longer read length
- Lower error rate
- More uniform nucleotide coverage of transcripts - more equalized transcript abundance