Al-Farabi Kazakh National University
Higher School of Medicine
Department of Fundamental Medicine

Transcriptomics. Methods of transcriptome investigation.

Lecturer and creator: PhD Pinsky Ilya Vladimirovich

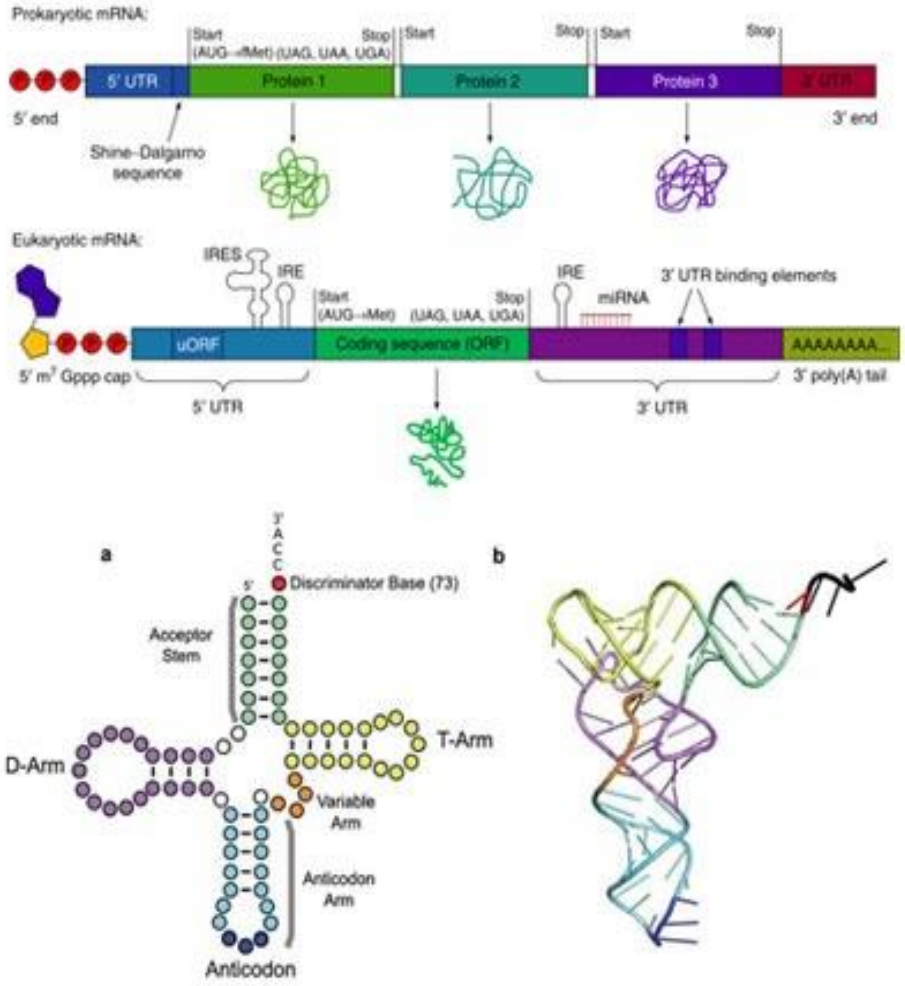
LEARNING OUTCOMES
As a result of the lesson you will be able to:

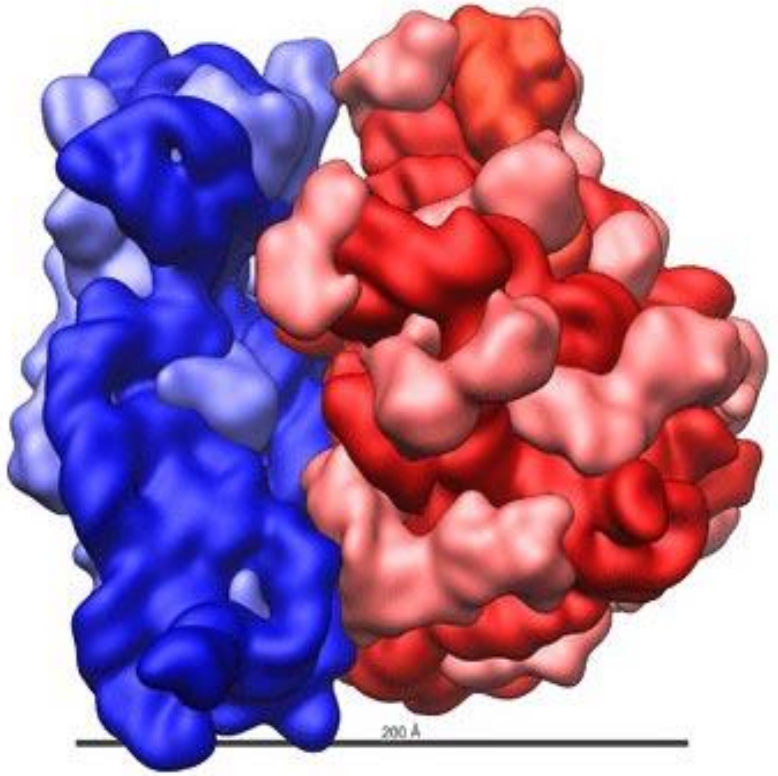
- 1. Give the definition to the following terms: “transcript”, “transcriptome”, “transcriptomics”, “gene expression profile”.
- 2. Describe and analyze the different types of RNA by their structure and functions.
- 3. Explain the methods of different RNA extraction, amplification and sequencing.
- 4. Explain how the methods of gene expression profiling, RNA microarray and RNA-seq can be used for diagnostics of different diseases?

Definition

Transcriptomics technologies are the techniques used to study an organism's **transcriptome**, the sum of all of its RNA **transcripts**. The information content of an organism is recorded in the DNA of its genome and expressed through **transcription**. Here, mRNA serves as a transient intermediary molecule in the information network, whilst non-coding RNAs perform additional diverse functions. A transcriptome captures a snapshot in time of the total transcripts present in a cell. Transcriptomics technologies provide a broad account of which cellular processes are active and which are dormant. A major challenge in molecular biology lies in understanding how the same genome can give rise to different cell types and how gene expression is regulated [1].

Types of RNA

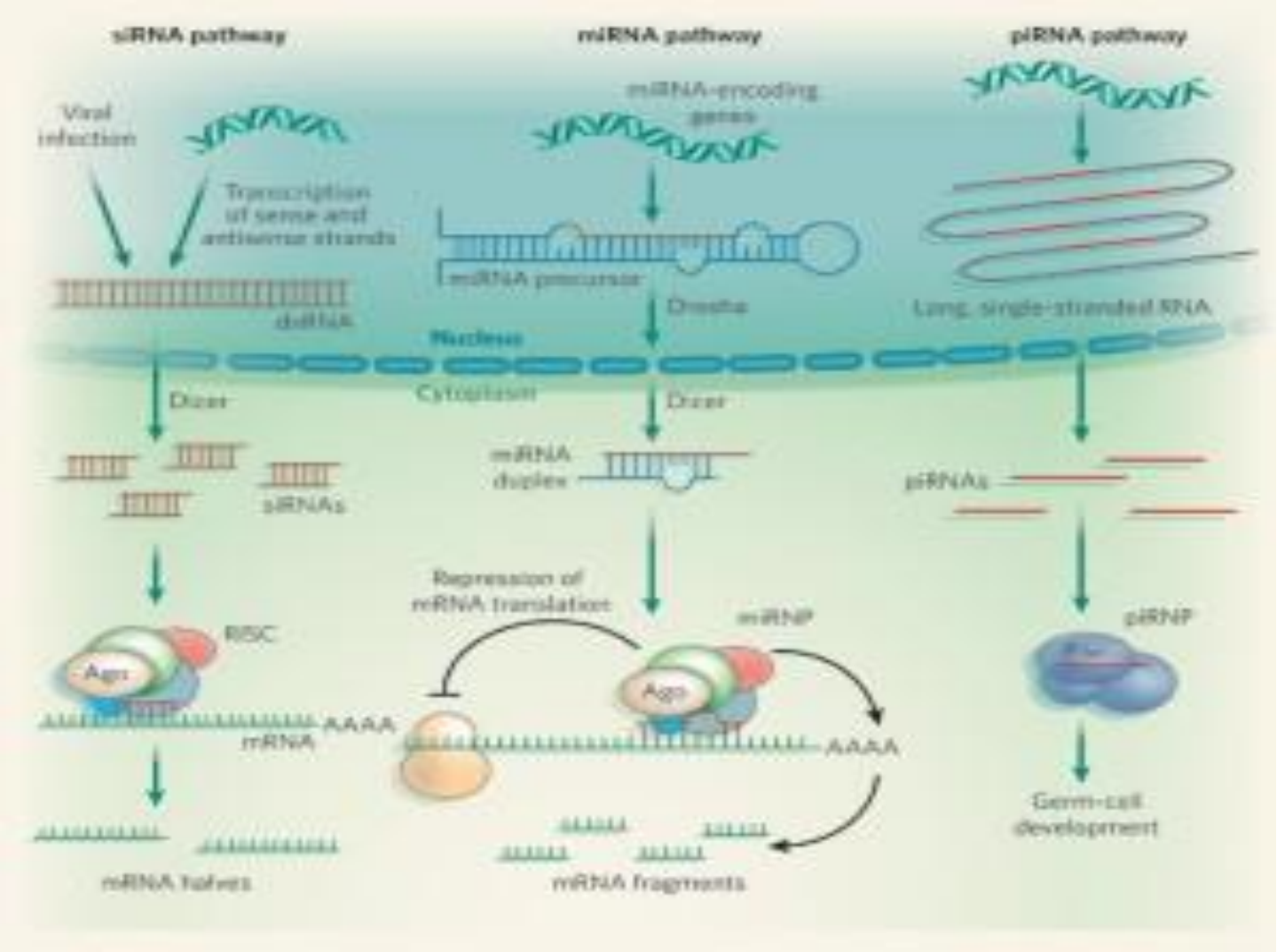




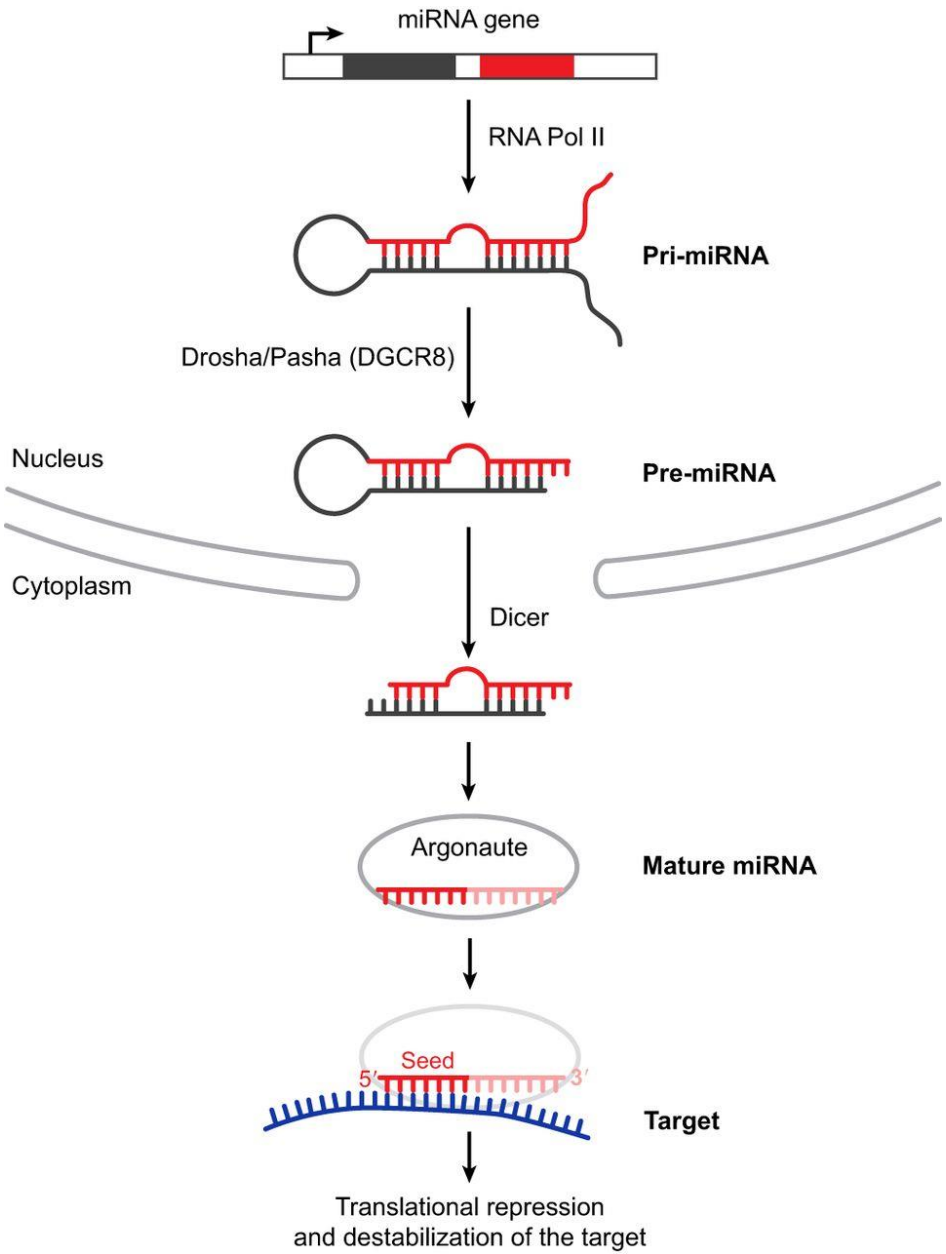
https://microbenotes.com/types-of-rna/

TABLE 6–1 Principal Types of RNAs Produced in Cells	
Type of RNA	Function
mRNAs	Messenger RNAs, code for proteins
rRNAs	Ribosomal RNAs, form the basic structure of the ribosome and catalyze protein synthesis
tRNAs	Transfer RNAs, central to protein synthesis as adaptors between mRNA and amino acids
snRNAs	Small nuclear RNAs, function in a variety of nuclear processes, including the splicing of pre-mRNA
snoRNAs	Small nucleolar RNAs, help to process and chemically modify rRNAs
miRNAs	MicroRNAs, regulate gene expression by blocking translation of specific mRNAs and cause their degradation
siRNAs	Small interfering RNAs, turn off gene expression by directing the degradation of selective mRNAs and the establishment of compact chromatin structures
piRNAs	Piwi-interacting RNAs, bind to piwi proteins and protect the germ line from transposable elements
lncRNAs	Long noncoding RNAs, many of which serve as scaffolds; they regulate diverse cell processes, including X-chromosome inactivation

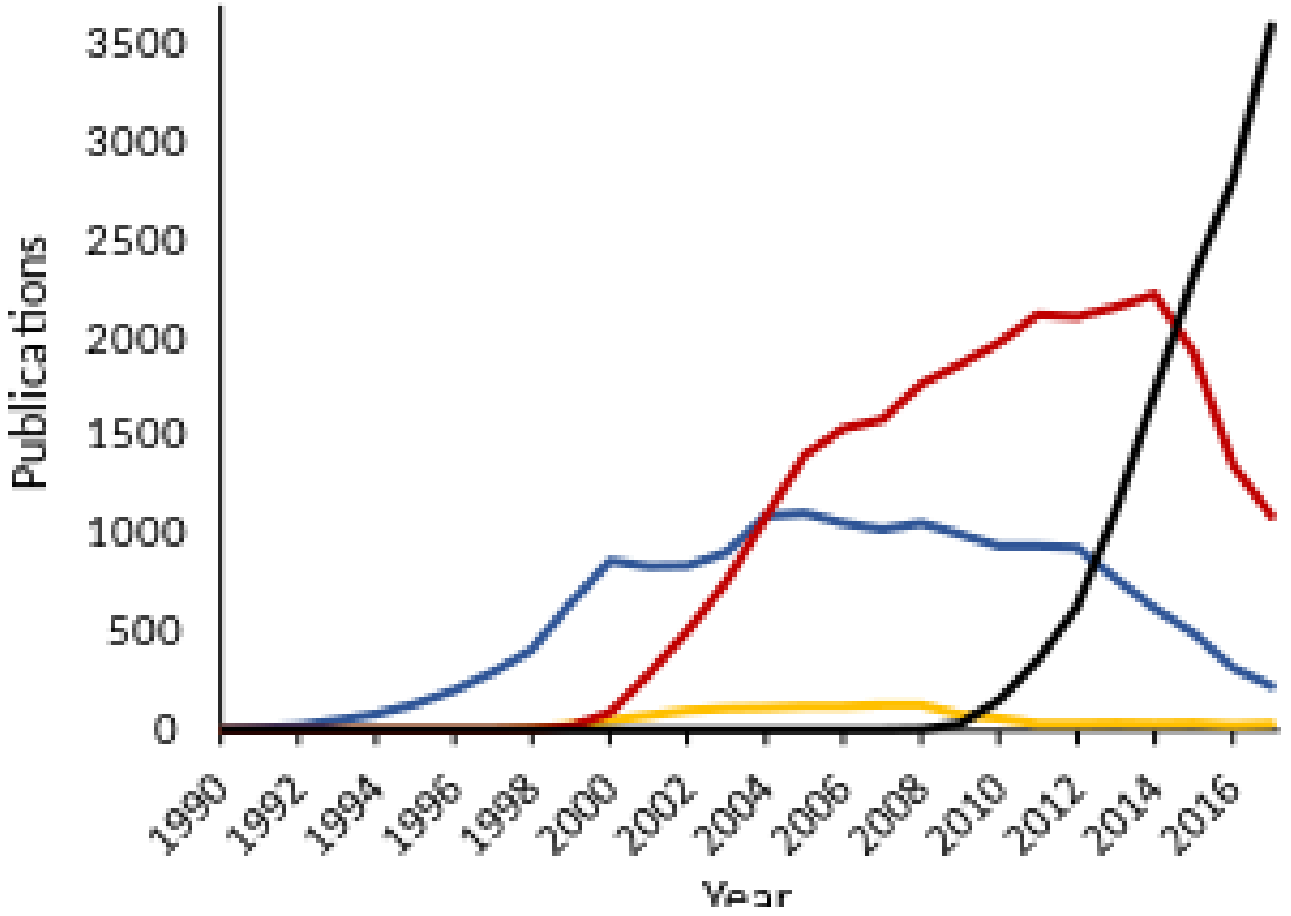
Alberts B. et al. Molecular biology of the cell. 6th ed. 2015. Garland Science. – p. 305.



https://www.nature.com/articles/451414a

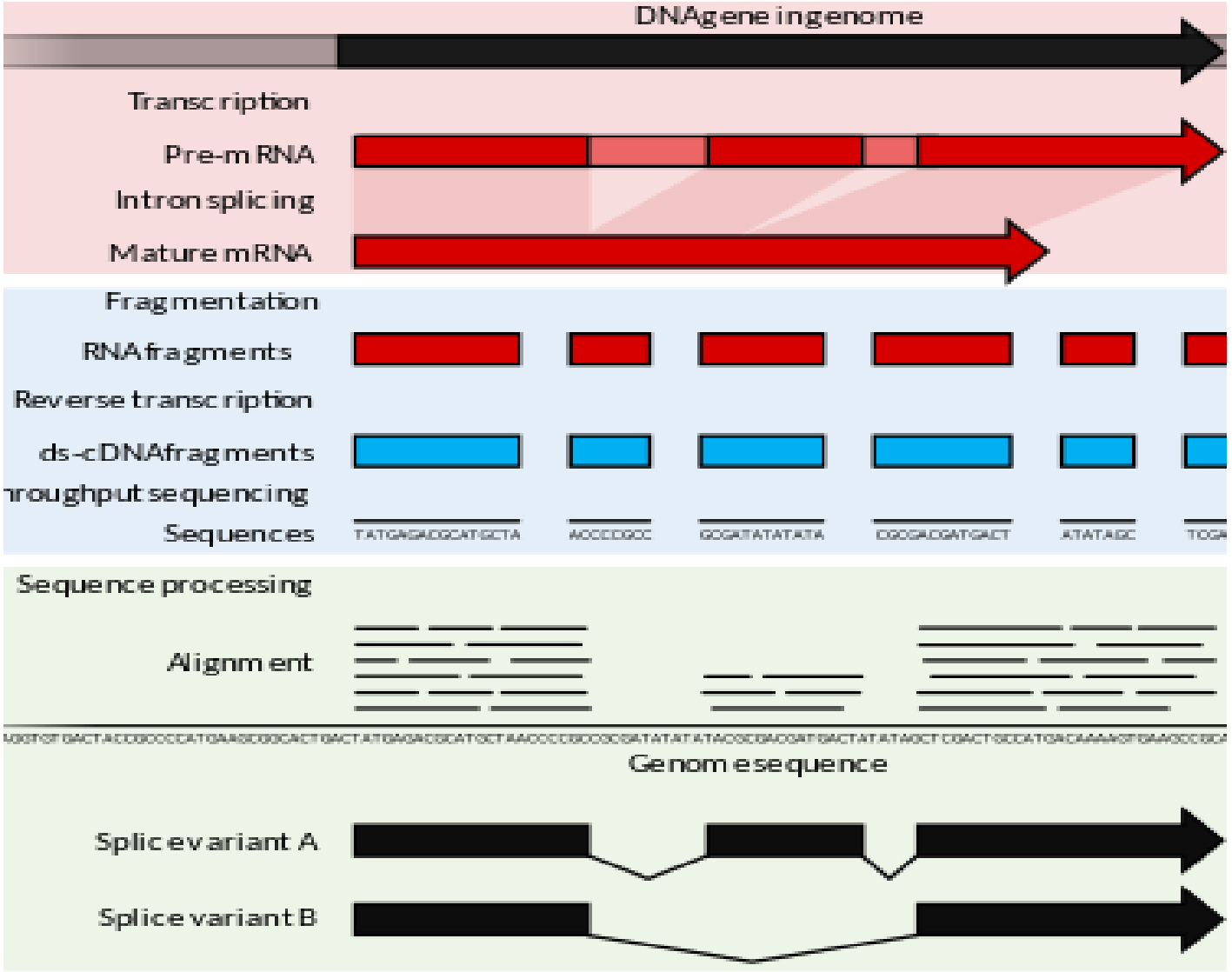


https://dev.biologists.org/content/144/14/2548



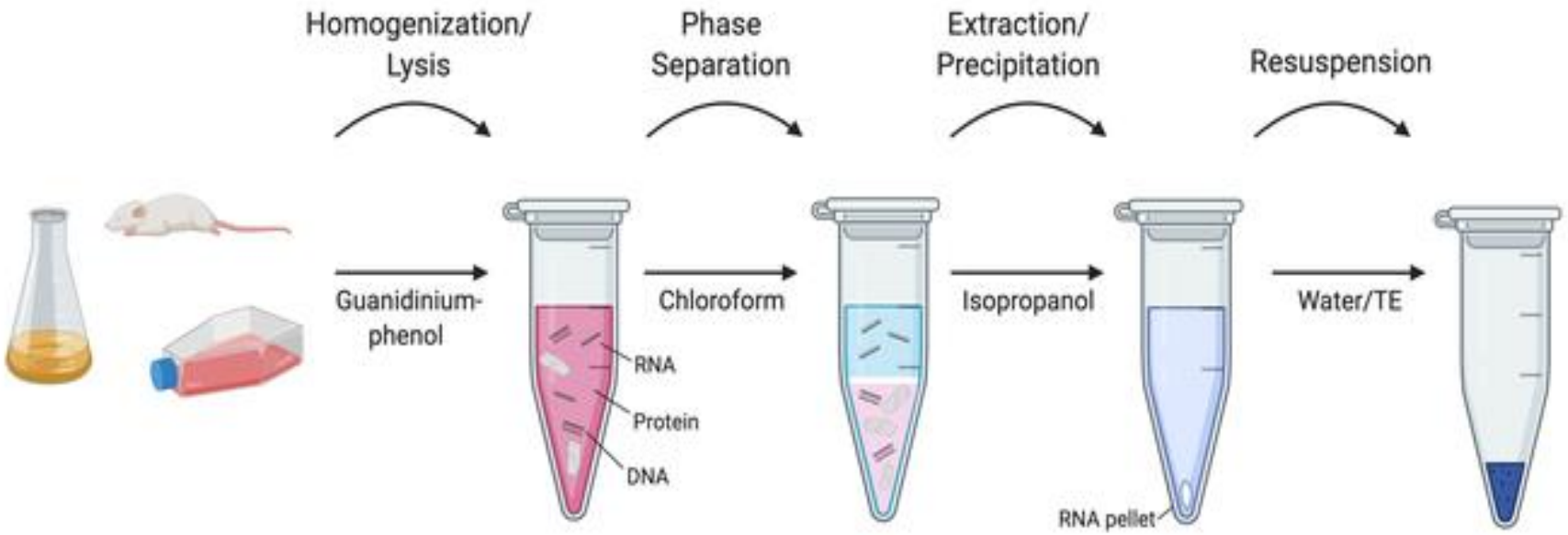
Transcriptomics method use over time. Published papers referring to RNA-Seq (black), RNA microarray (red), expressed sequence tag (blue) and serial/cap analysis of gene expression (yellow) since 1990.[1]

RNA-seq

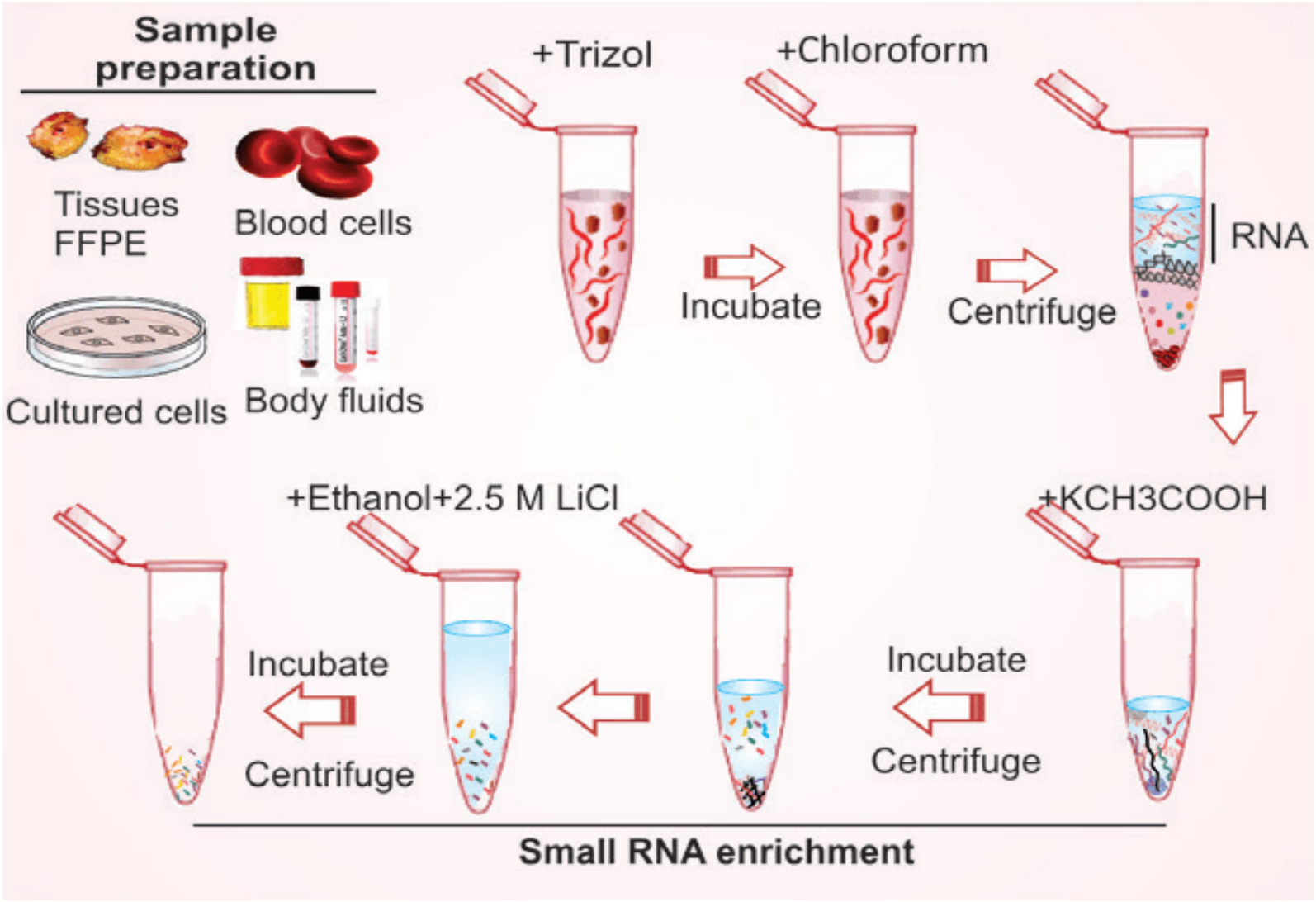


Shafee T, Lowe R (2017). "Eukaryotic and prokaryotic gene structure". WikiJournal of Medicine. 4 (1). doi:10.15347/wjm/2017.002.

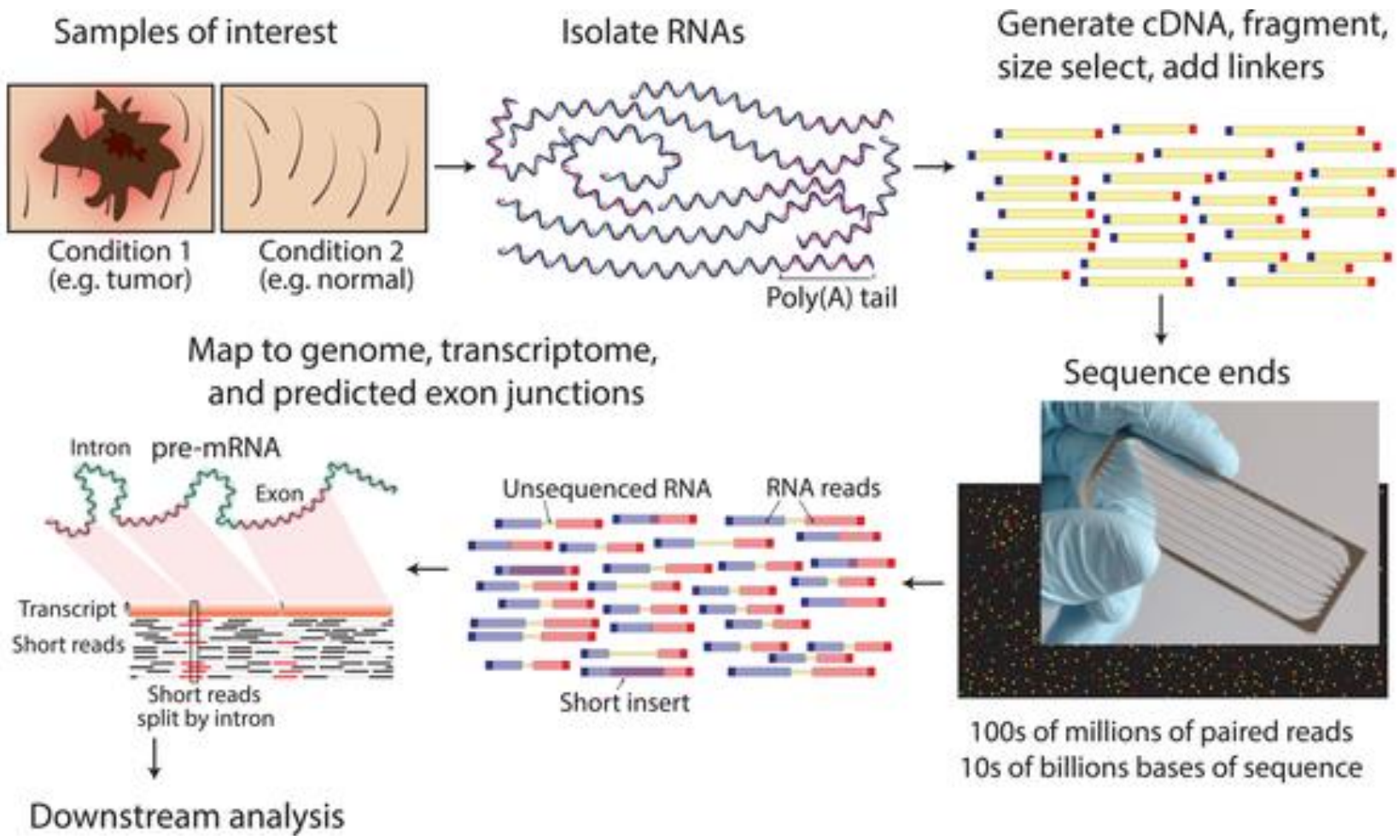
RNA extraction



Addgene: Kit Free RNA Extraction
addgene.org



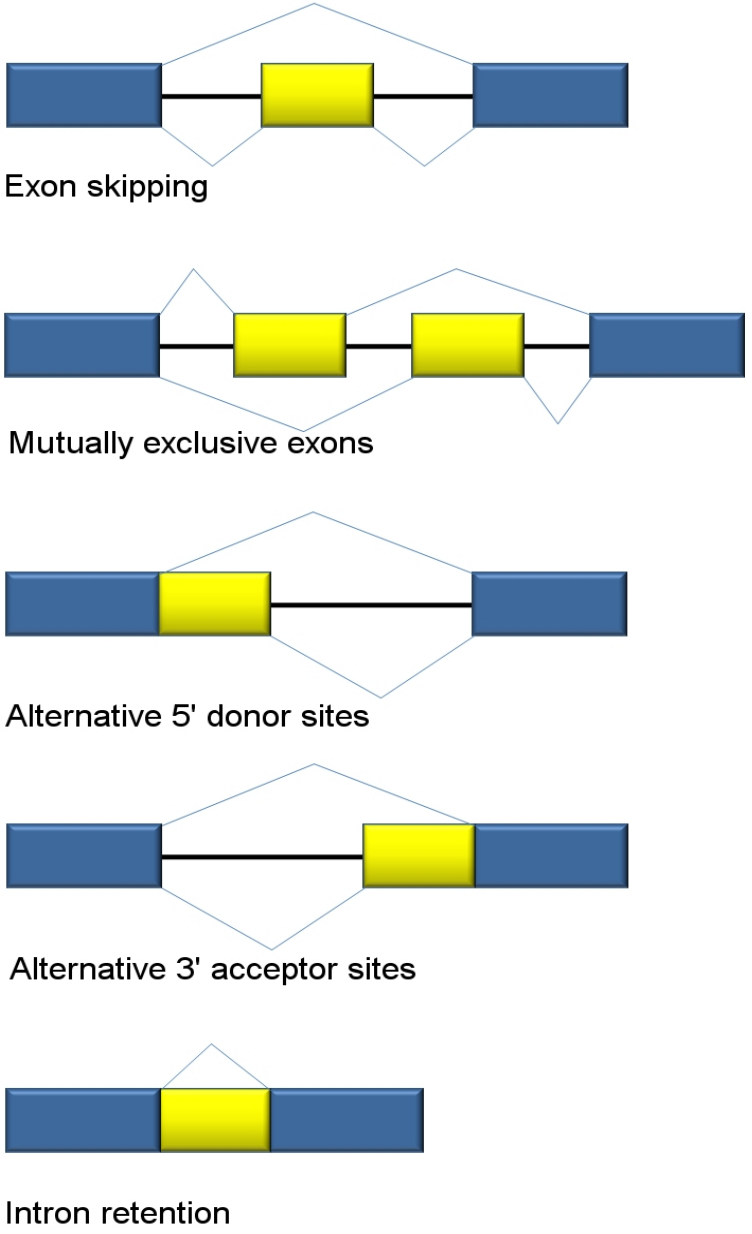
Isolation of RNA from Blood - Principle ...
howbiotech.com

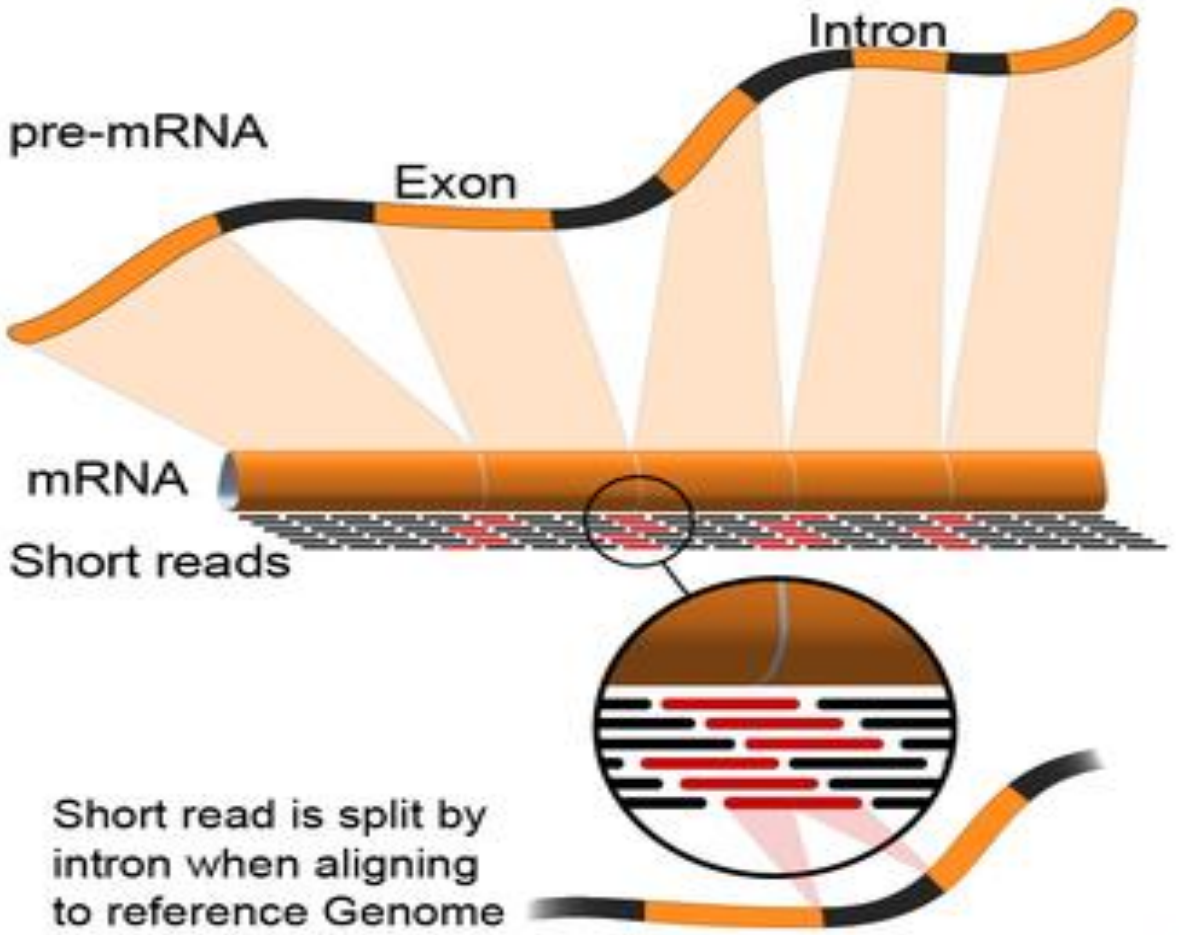


Griffith M, Walker JR, Spies NC, Ainscough BJ, Griffith OL (August 2015). "Informatics for RNA Sequencing: A Web Resource for Analysis on the Cloud". PLOS Computational Biology. 11 (8): e1004393. Bibcode:2015PLSCB..11E4393G. doi:10.1371/journal.pcbi.1004393. PMC 4527835. PMID 26248053.

Alternative RNA splicing event types.
Exons are represented as blue and yellow blocks, introns as lines in between.

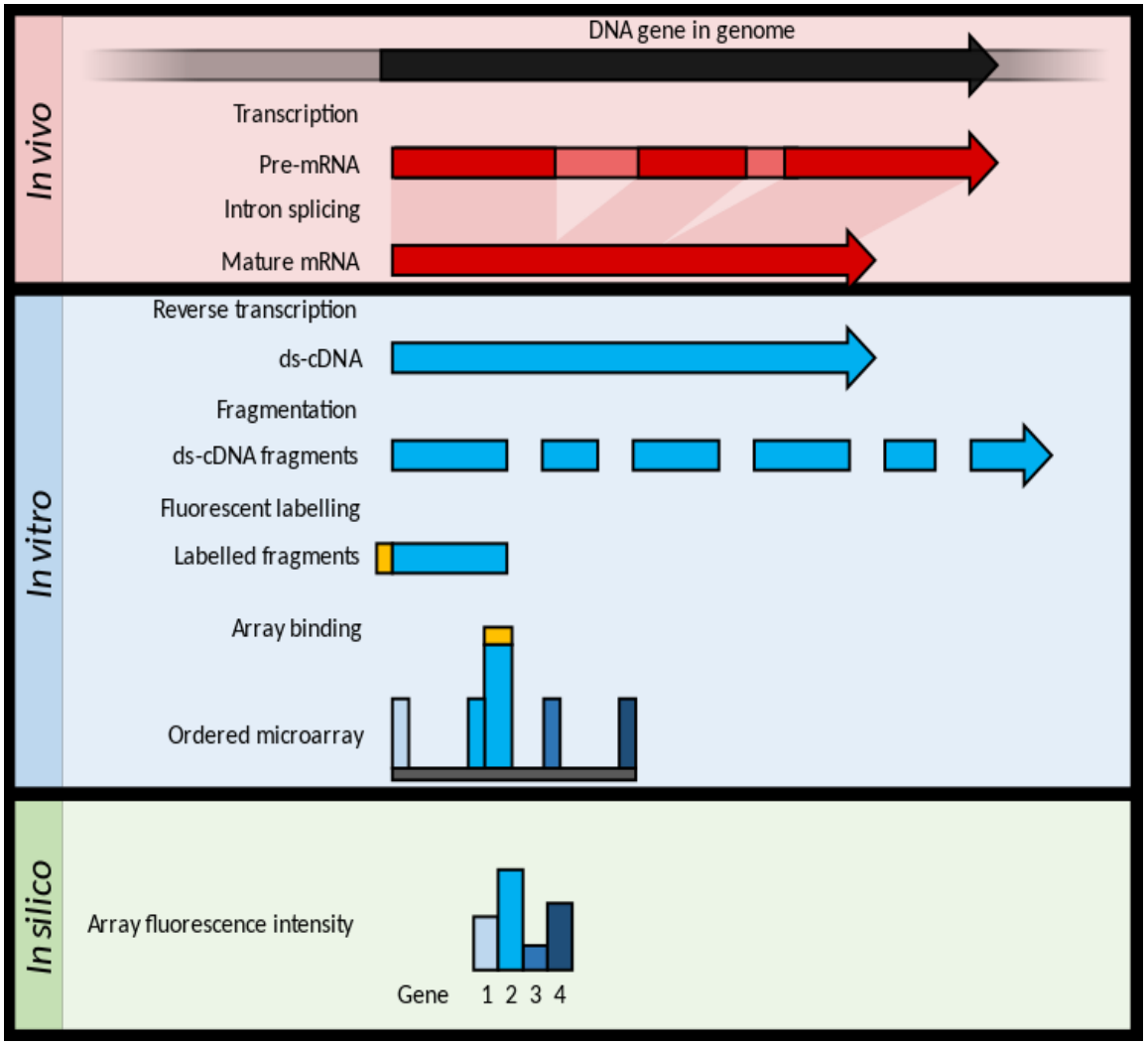
https://en.wikipedia.org/wiki/RNA-Seq#/media/File:Alt_splicing_bestiary2.jpg



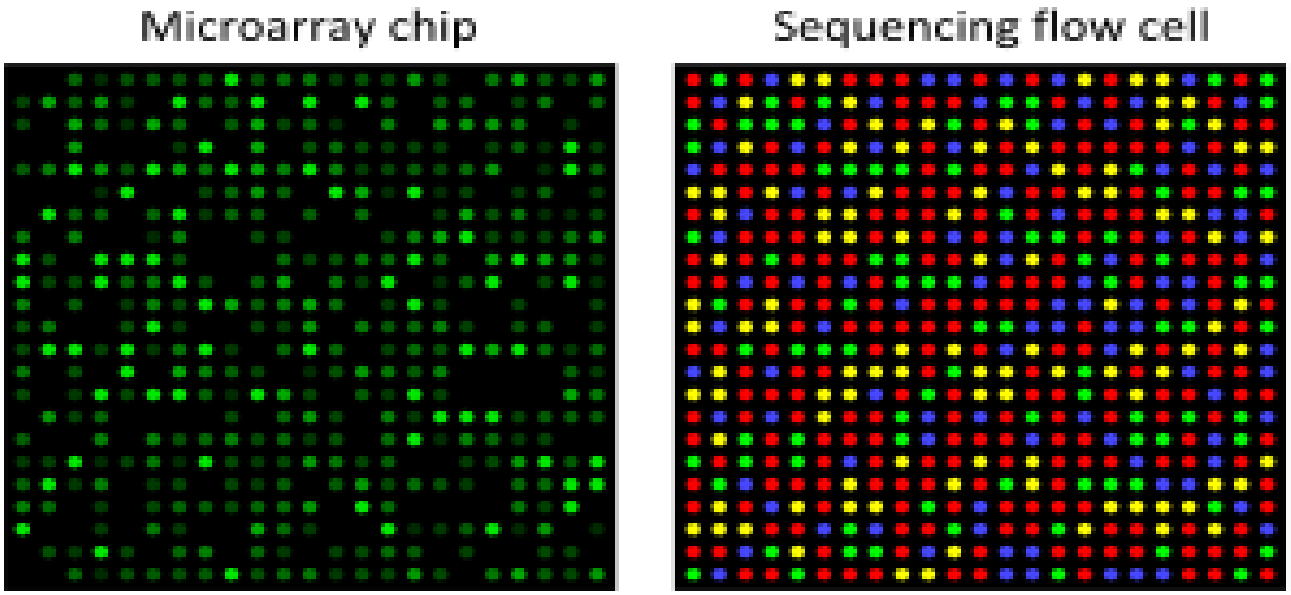


https://en.wikipedia.org/wiki/RNA-Seq#cite_note-ReferenceA-8

DNA Microarrays

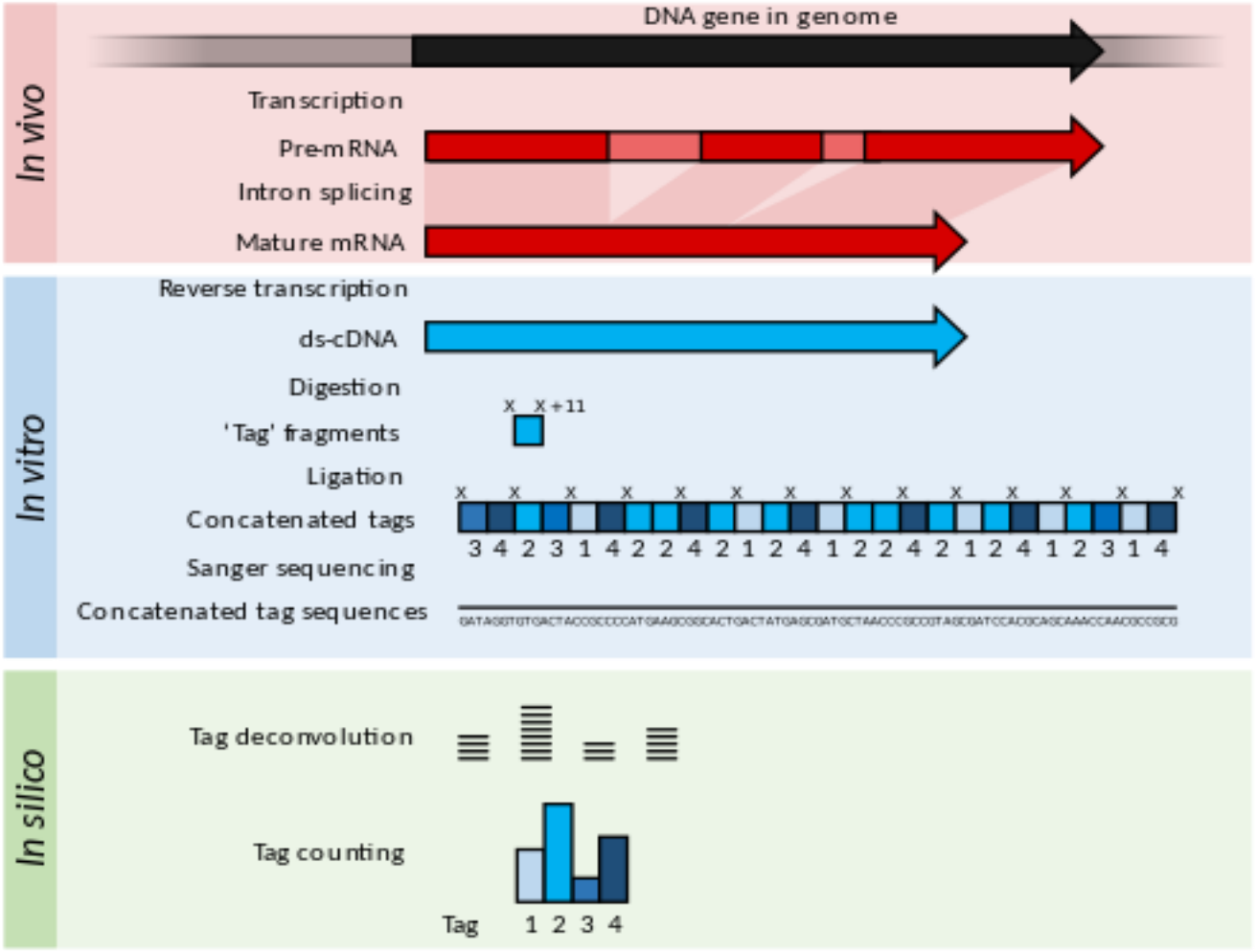


https://en.wikipedia.org/wiki/Transcriptomics_technologies#/media/File:Summary_of_RNA_Microarray.svg



Microarray and sequencing flow cell. Microarrays and RNA-seq rely on image analysis in different ways. In a microarray chip, each spot on a chip is a defined oligonucleotide probe, and fluorescence intensity directly detects the abundance of a specific sequence (Affymetrix). In a high-throughput sequencing flow cell, spots are sequenced one nucleotide at a time, with the colour at each round indicating the next nucleotide in the sequence (Illumina Hiseq). Other variations of these techniques use more or fewer colour channels. [2], [3]

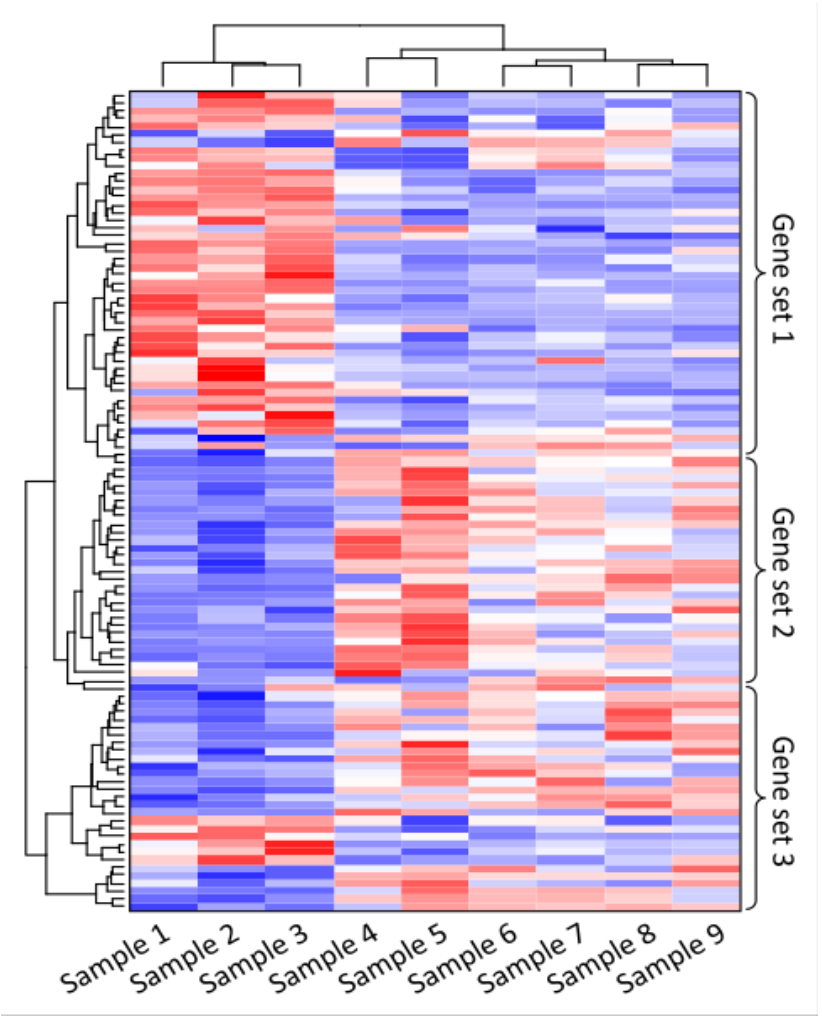
Serial and cap analysis of gene expression (SAGE/CAGE)



Lowe R, Shirley N, Bleackley M, Dolan S, Shafee T (May 2017). "Transcriptomics technologies". PLOS Computational Biology. 13 (5): e1005457. Bibcode:2017PLSCB..13E5457L. doi:10.1371/journal.pcbi.1005457. PMC 5436640. PMID 28545146.

Gene expression profiling

Heatmap identification of gene co-expression patterns across different samples. Each column contains the measurements for gene expression change for a single sample. Relative gene expression is indicated by colour: high-expression (red), median-expression (white) and low-expression (blue). Genes and samples with similar expression profiles can be automatically grouped (left and top trees). Samples may be different individuals, tissues, environments or health conditions. In this example, expression of gene set 1 is high and expression of gene set 2 is low in samples 1, 2, and 3. [3], [4]



https://en.wikipedia.org/wiki/Transcriptomics_technologies#/media/File:Transcriptomes_heatmap_example.svg

References

1. "Transcriptomics technologies". PLOS Computational Biology. 13 (5): e1005457. 18 May 2017. doi:10.1371/JOURNAL.PCBI.1005457. ISSN 1553-734X. PMC 5436640. PMID 28545146. S2CID 3714586. Wikidata Q33703532.
2. Lowe R, Shirley N, Bleackley M, Dolan S, Shafee T (May 2017). "Transcriptomics technologies". PLOS Computational Biology. 13 (5): e1005457. Bibcode:2017PLSCB..13E5457L. doi:10.1371/journal.pcbi.1005457. PMC 5436640. PMID 28545146.
3. Petrov A, Shams S (2004-11-01). "Microarray Image Processing and Quality Control". Journal of VLSI Signal Processing Systems for Signal, Image and Video Technology. 38 (3): 211–226.
4. Lowe R, Shirley N, Bleackley M, Dolan S, Shafee T (May 2017). "Transcriptomics technologies". PLOS Computational Biology. 13 (5): e1005457. Bibcode:2017PLSCB..13E5457L. doi:10.1371/journal.pcbi.1005457. PMC 5436640. PMID 28545146.