GENOMIKI

Empowering Medical Decisions

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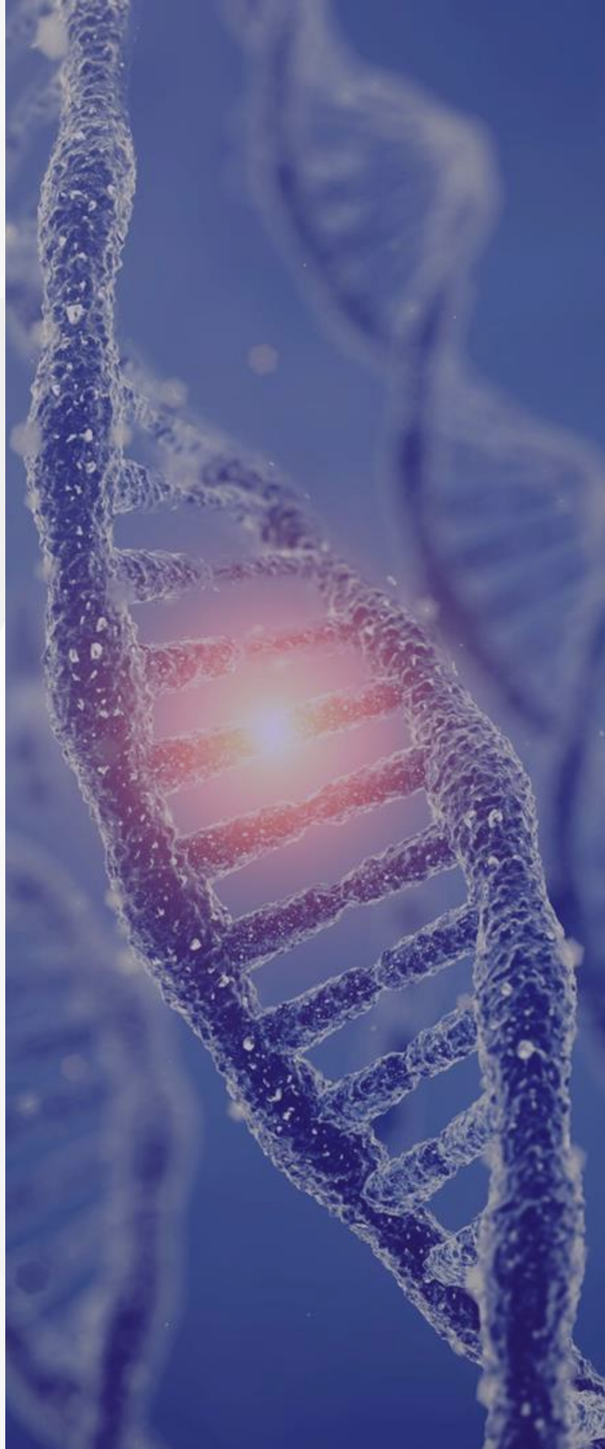
Beyond the Basics: Innovative Applications of RNA Sequencing in Research and Diagnostics



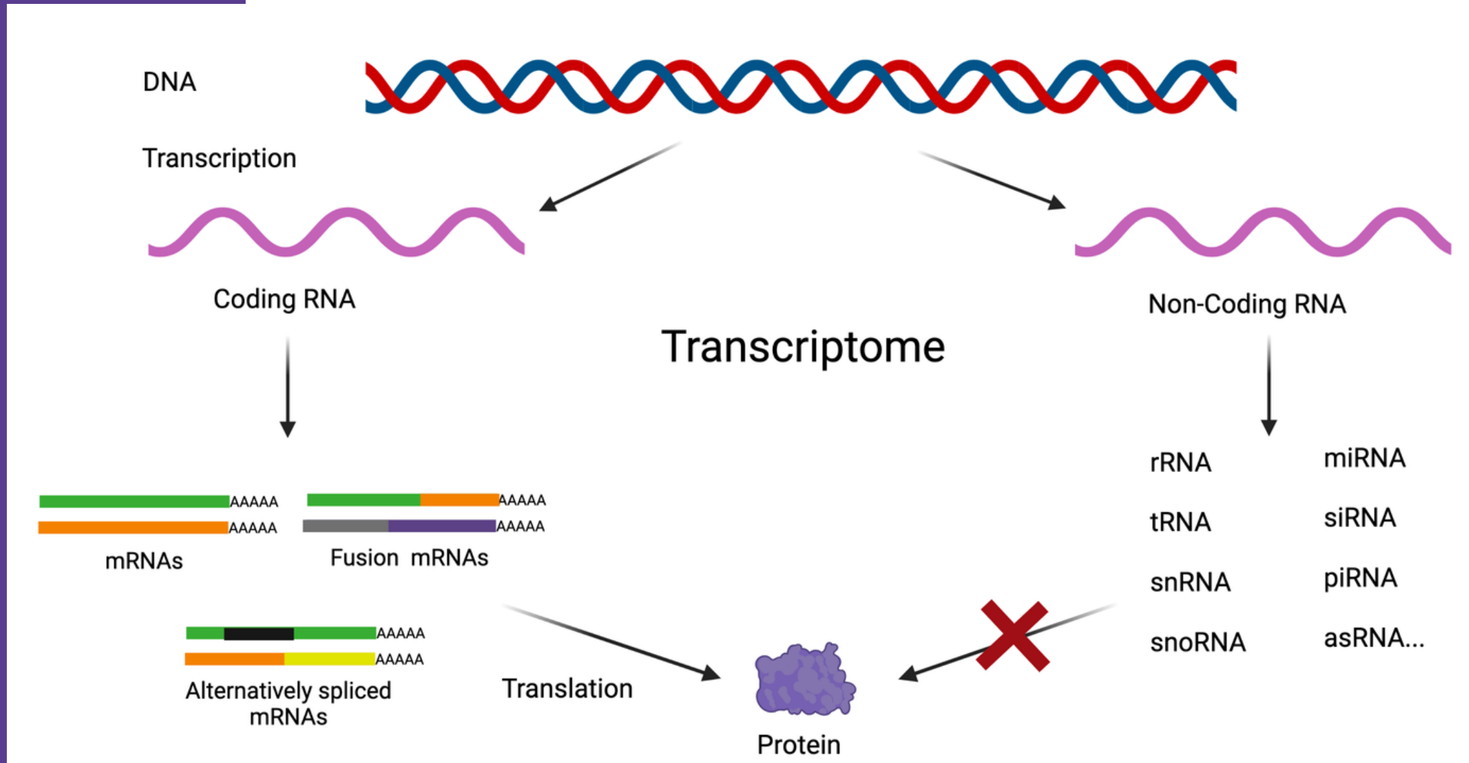
Founder & Director

Dr. Deeksha Bhartiya

Founder & Director Genomiki Solutions Private Limited
PhD (Bioinformatics)
Postdoctoral fellow (Sweden)
10+ years of experience as Bioinformatics scientist post Phd

WHAT IS TRANSCRIPTOME?

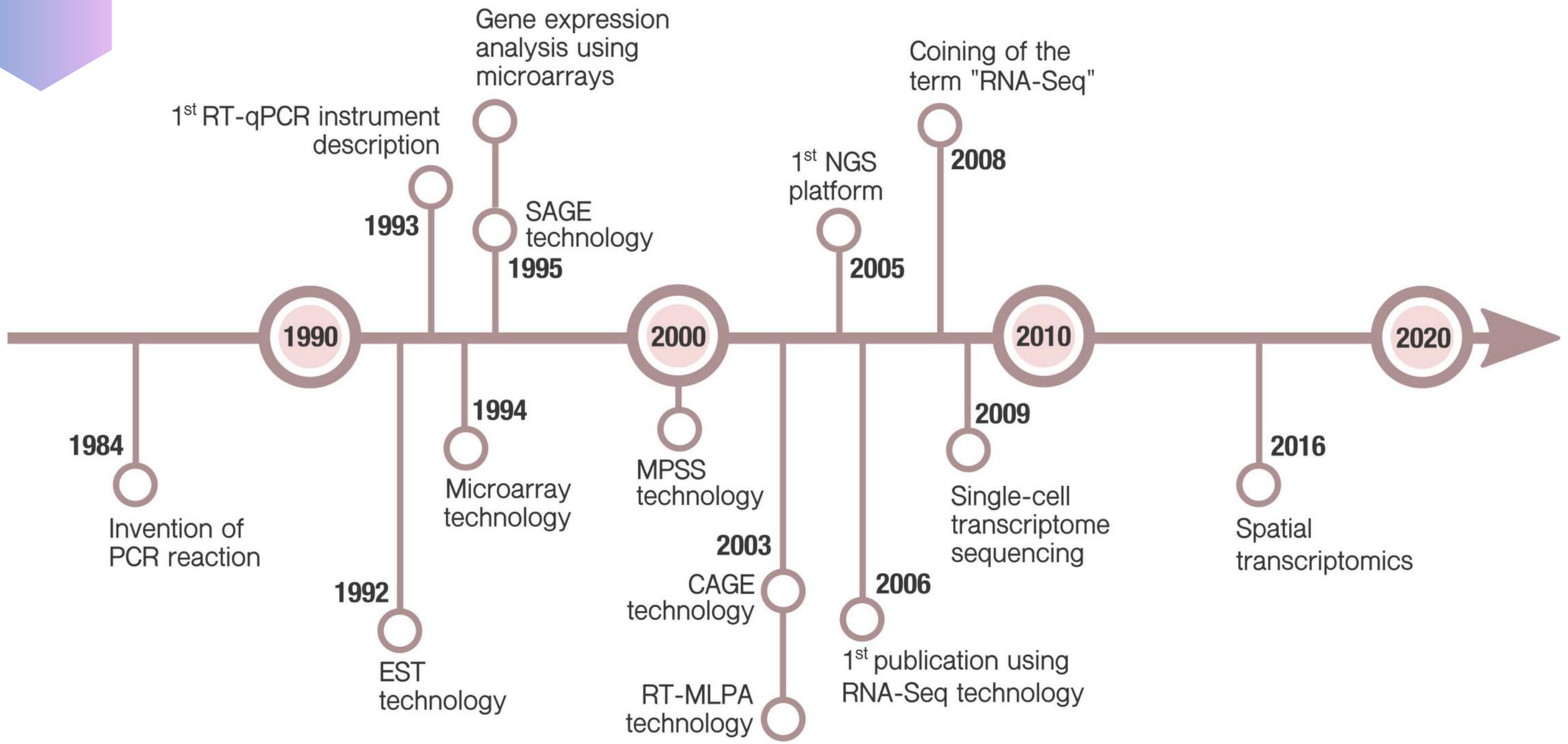
- The transcriptome is the set of all RNA produced in a cell (or population of cells)
- The transcriptome of a cell varies over time and with environmental conditions
- The mRNA transcripts reflect which genes are actively expressed
- The median size of the human transcriptome is 16,342 genes, or 65.44% of all human genes



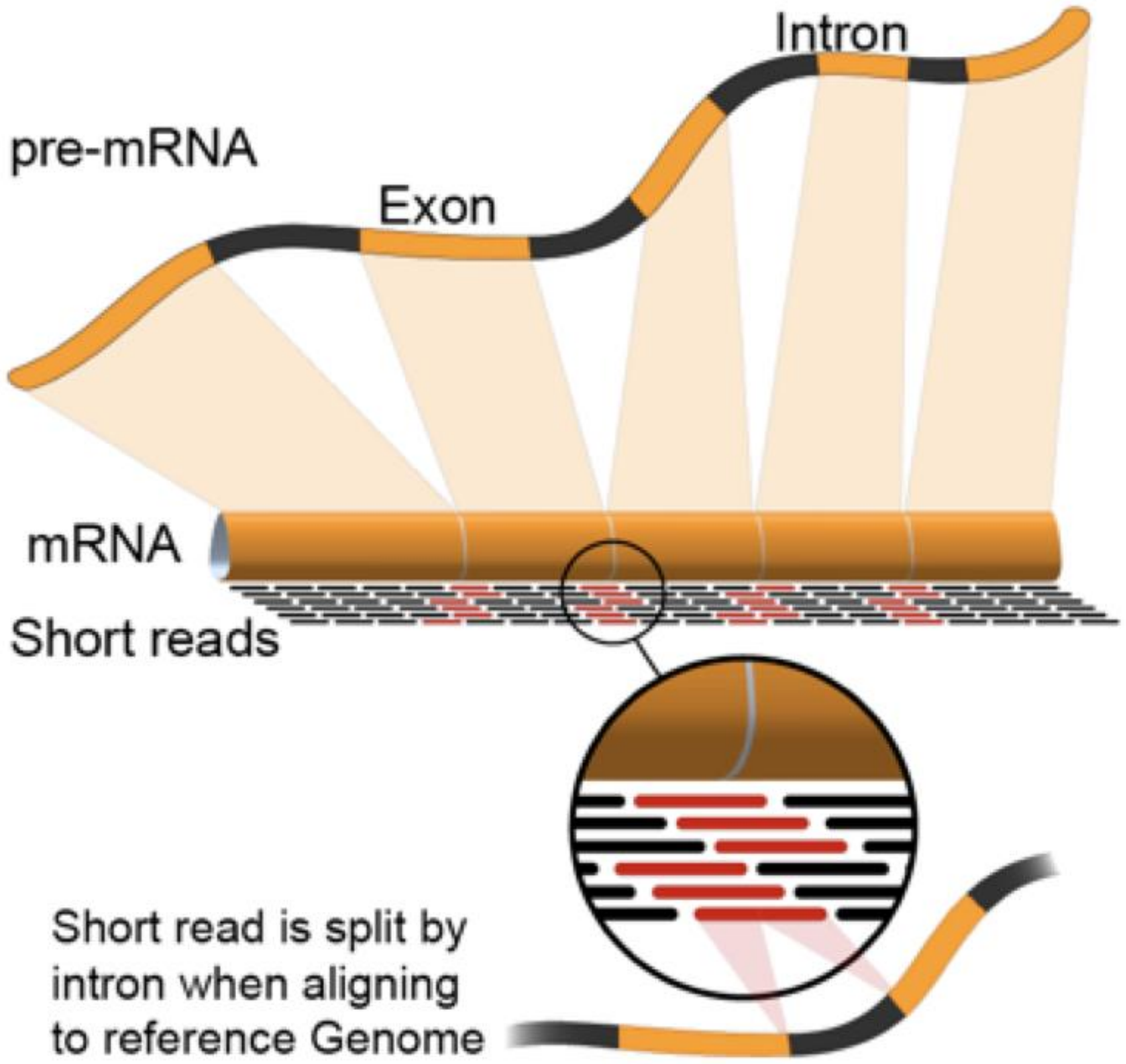
What is RNA Sequencing?

- Uses the capabilities of NGS to identify all the RNA molecules in cell or tissue
- Help study the transcriptome, entire RNA content, of organism. This method is cost effective and accurate for RNA studies than other available methods.

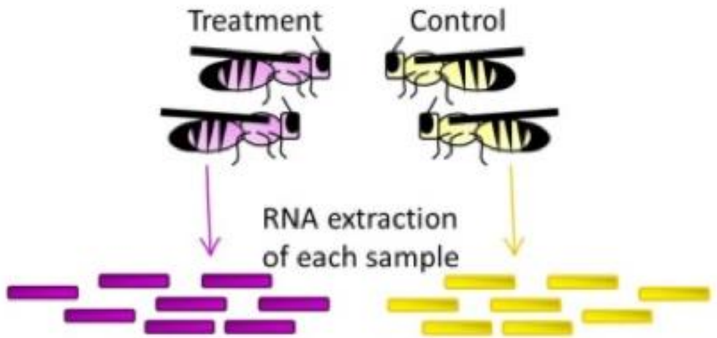
History


Gene expression analysis using microarrays
1st RT-qPCR instrument description
Coining of the term "RNA-Seq"
1st NGS platform
2008
1993
SAGE technology
1995
2005
1990
2000
2010
2020
1984
Invention of PCR reaction
1994
Microarray technology
MPSS technology
2009
Single-cell transcriptome sequencing
2016
Spatial transcriptomics
1992
EST technology
2003
CAGE technology
2006
1st publication using RNA-Seq technology
RT-MLPA technology

Principles of RNA seq Alignment



Exploring Transcriptomes



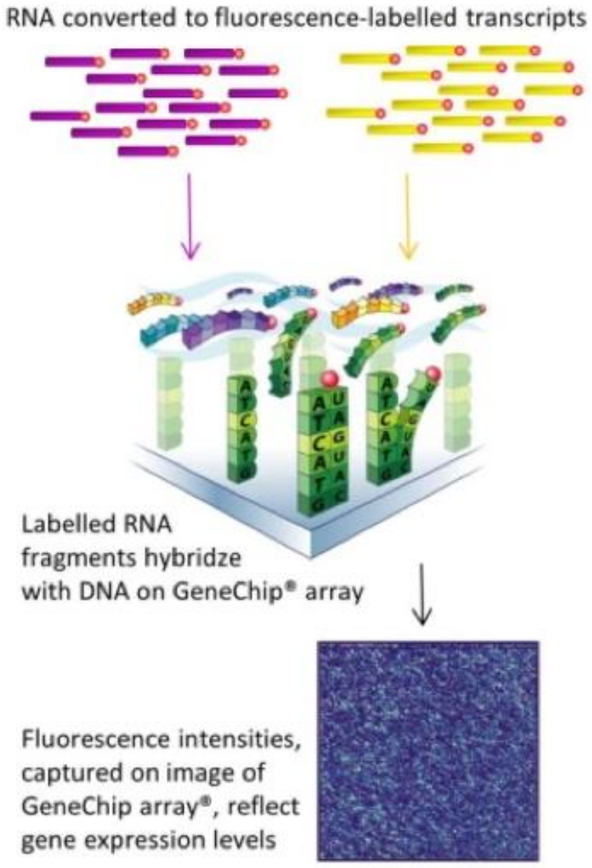
Treated Samples:

Samples exposed to a specific treatment (e.g., growth factors, drugs, environmental stimuli).

Control Samples:

Samples without treatment or exposed to a placebo, serving as a baseline for comparison.

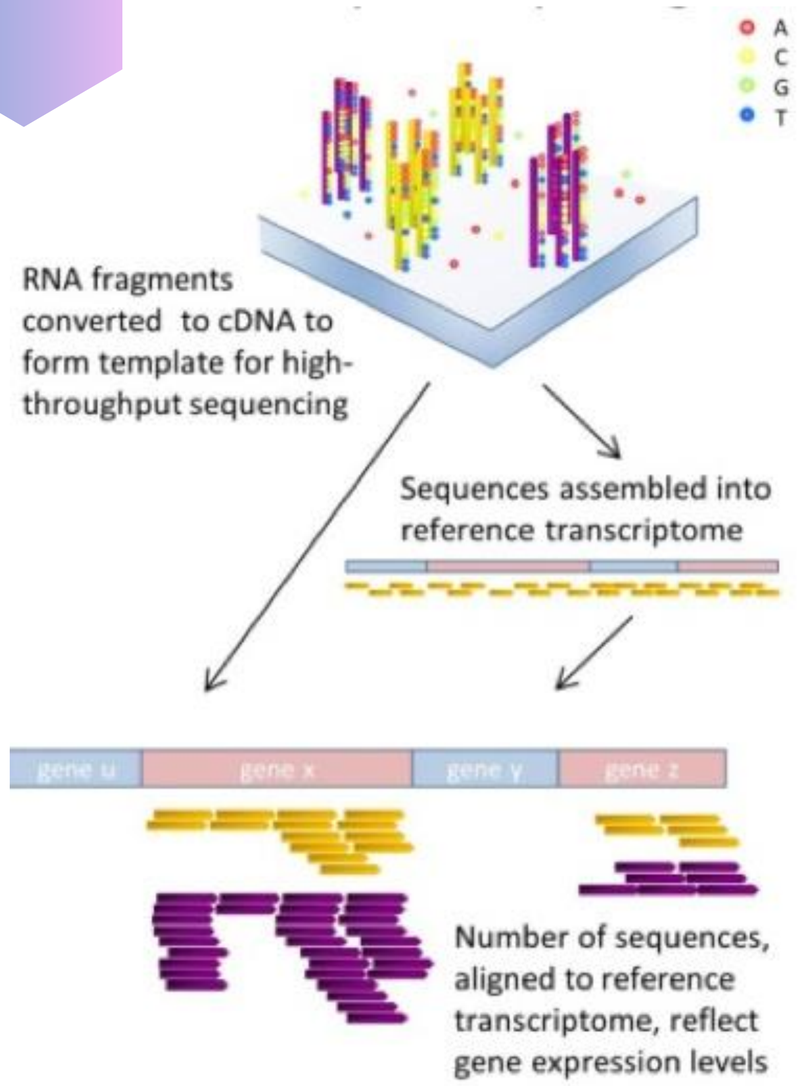
Microarray



- A microarray is a technology used to measure the expression levels of thousands of genes simultaneously.
- It involves **hybridizing labeled** cDNA or RNA samples to a set of pre-designed probes (short DNA sequences) that represent known genes.
- Microarrays provide data on **relative gene expression** by detecting hybridization signals between the probes and RNA samples.

Source: https://mindthegraph.com/infographic-templates/rna-extraction/

Transcriptome Sequencing



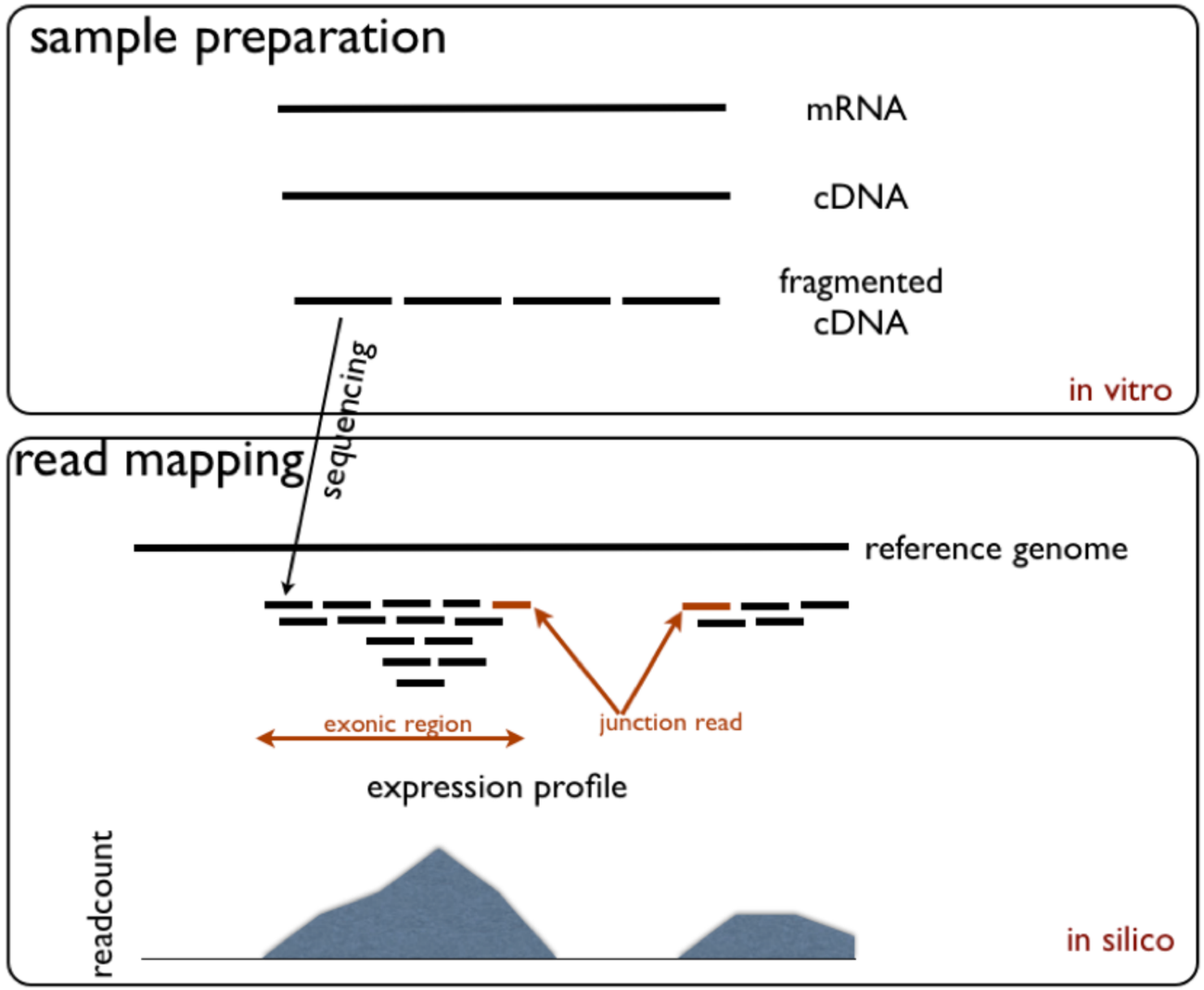
- RNA-Seq is a next-generation sequencing (NGS) technology that provides a comprehensive and unbiased view of the entire transcriptome by sequencing the cDNA generated from RNA.

- RNA-Seq offers the ability to quantify gene expression and explore more complex aspects of the transcriptome, such as alternative splicing, gene fusions, and novel transcript discovery.

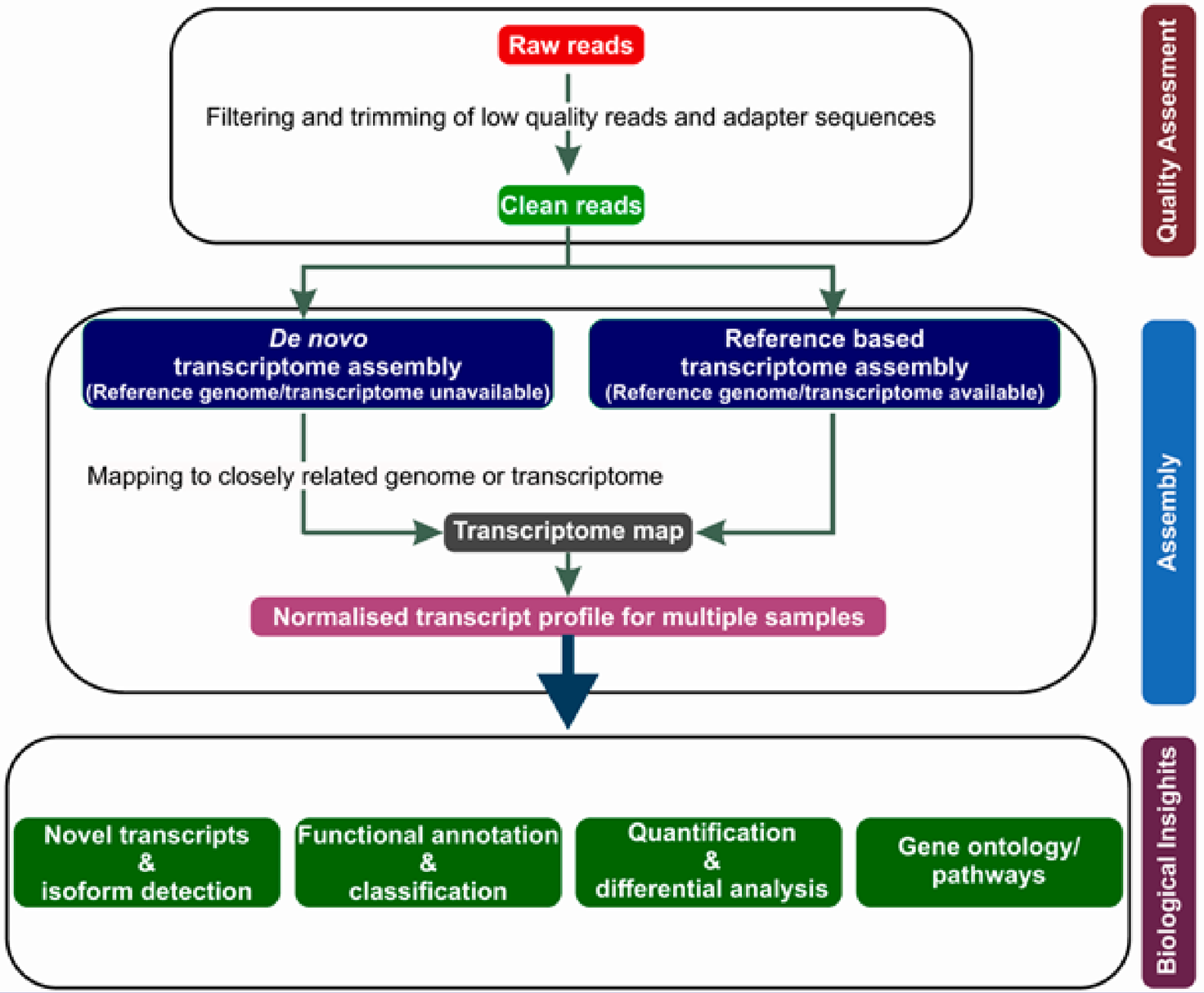
Gene ID	Transcript abundance in Treatment sample	Transcript abundance in Control sample
ABc00001	3778	3894
ABc00002	768	189
ABc00003	1087	1476

Transcript Abundance in Control vs. Treated Samples

RNA Sequencing Process



Bioinformatics Analysis Steps



RNA seq Analysis Steps

RNA-seq data

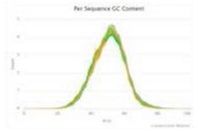
Reads mapping

Expression quantification

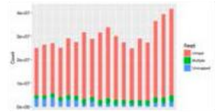
Differential expression

Functional enrichment

Reads QC

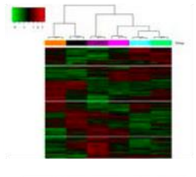


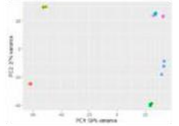
Mapping QC

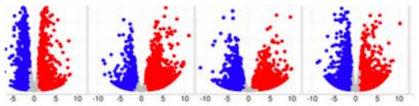


	Input reads	Uniquely mapped.reads	mapped.to multiple.loci	mapped.to too.many.loci	Ratio
Sample 1	23129942	20037403	1437979	102781	0.856165207
Sample 2	26437651	21418385	1535786	111894	0.868237916
Sample 3	27058294	21294319	1587209	107564	0.845638236
Sample 4	23214088	20953523	1579915	66478	0.895684435
Sample 5	29131506	24037114	1855183	80866	0.888807363
Sample 6	27589547	22785569	1721950	75914	0.888290011
.....	.....	.....	.....	.....	.....

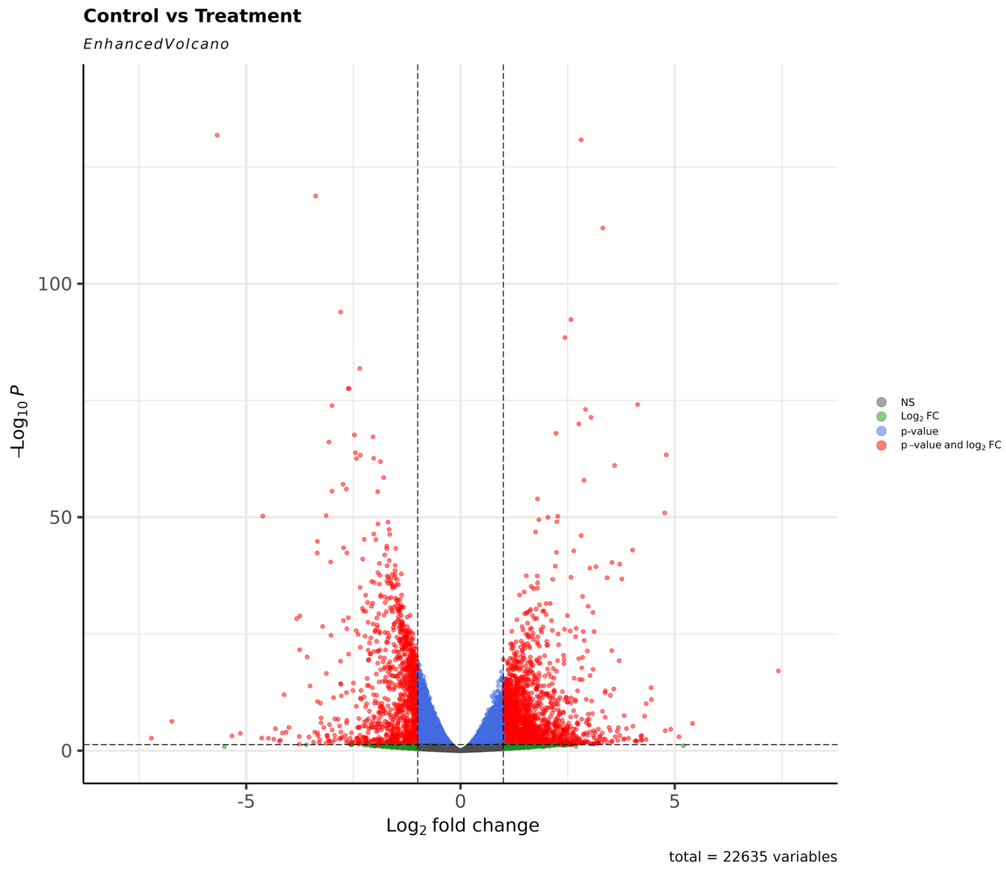
Experiment QC

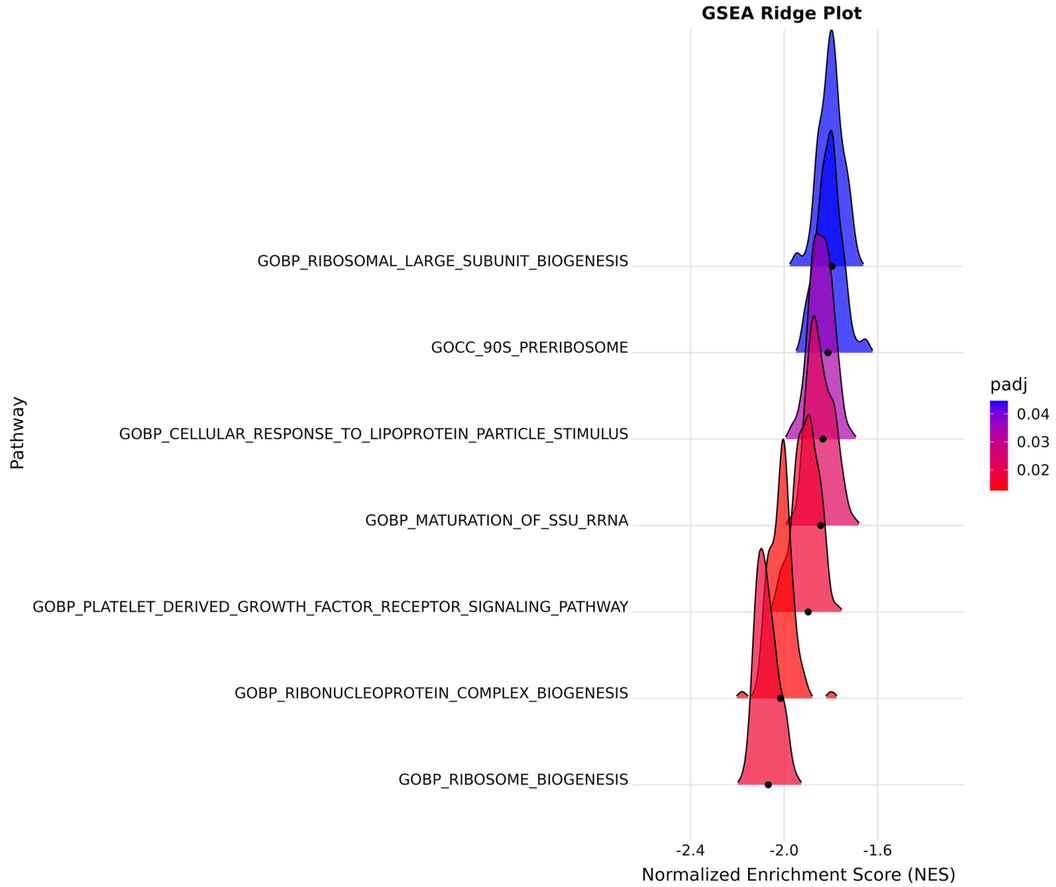






GSEA (Gene Set Enrichment Analysis)

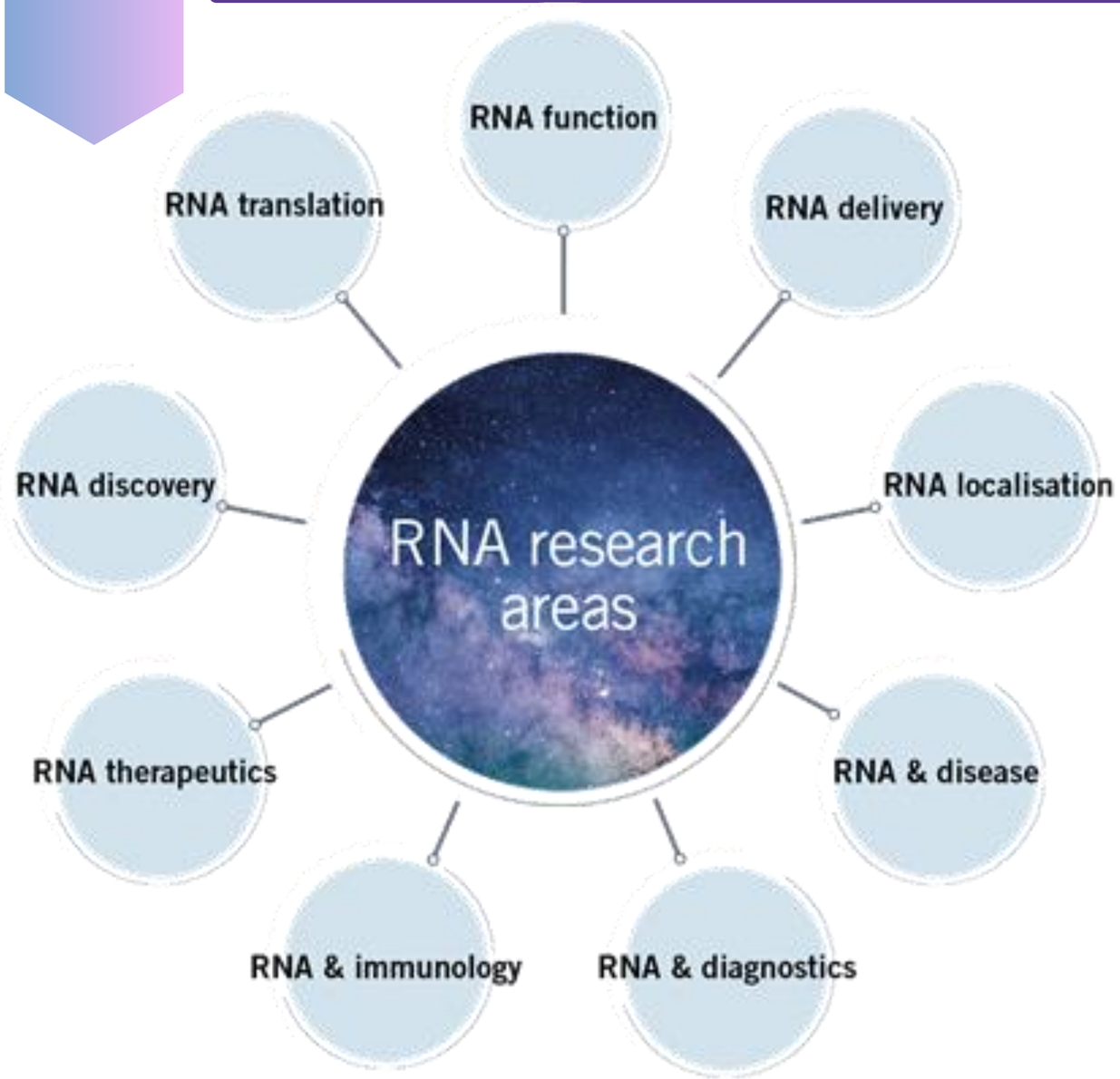




GSEA in RNA-Seq Analysis involves identifying enriched biological pathways or gene sets in RNA sequencing (RNA-Seq) data.

It helps link differential gene expression to broader biological processes, providing insights into functional changes between conditions.

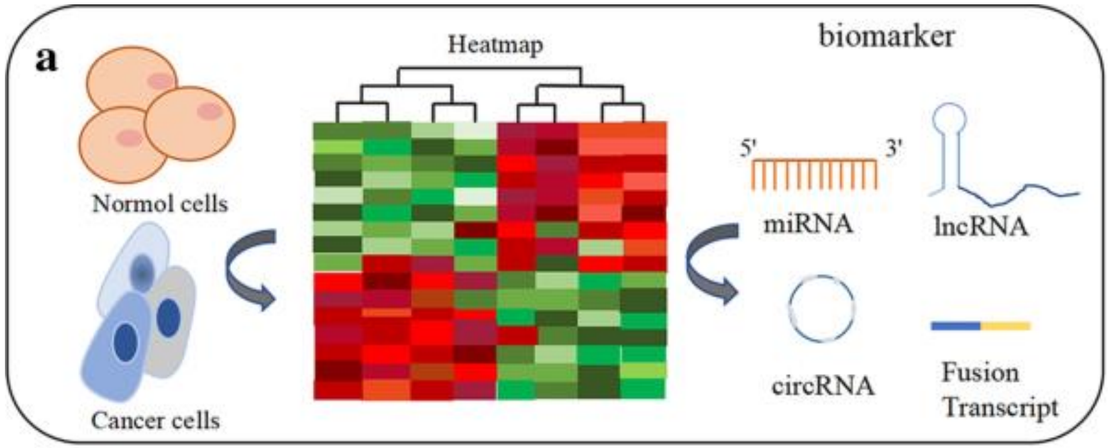
Applications of RNA Sequencing



- Gene Expression Profiling
- Discovery of Novel Transcripts
- Disease Diagnostics & Biomarkers
- Single-Cell RNA-Sequencing (scRNA-seq)
- Non-Coding RNA Analysis
- Pathway and Network Analysis
- Cancer Research

Source: https://eurofinsgenomics.eu/en/eurofins-genomics/material-and-methods/whole-transcriptome-

Differential expression analysis by RNA sequencing



- Detects changes in gene expression levels across conditions (e.g., disease vs. healthy).
- Helps discover novel fusion transcripts linked to specific diseases or cancer subtypes.
- Identifies differentially expressed long non-coding RNAs (lncRNAs) involved in gene regulation.
- Detects dysregulated microRNAs (miRNAs) with potential roles in disease pathogenesis.
- Reveals expression patterns of circular RNAs (circRNAs) with potential regulatory and biomarker functions.

Source: https://jhoonline.biomedcentral.com/articles/10.1186/s13045-020-01005-x

Can we use RNA-Seq in disease diagnosis?

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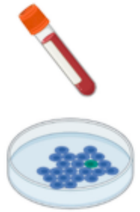
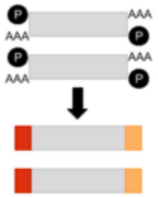
Individual with a rare disease

Sample preparation



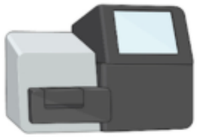
Days - weeks

RNA-seq library preparation



2-3 Days / batch

Sequencing and alignment



1 Day / batch

DROP workflow

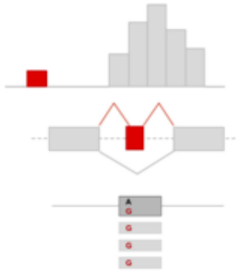
2 Days / cohort

1. Quality control

- DNA-RNA sample matching
- Sequencing depth
- # Expressed genes

2. Aberrant events detection

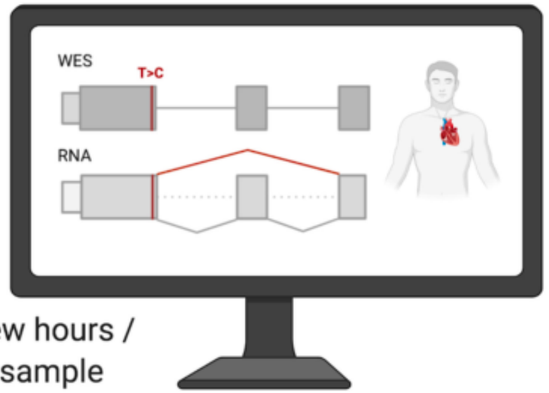
- Aberrant expression
- Aberrant splicing
- Mono-allelic expression



Results interpretation

1. Gene prioritization

- ✓ Aberrant event(s)
- ✓ Rare variant(s)
- ✓ Matching phenotype

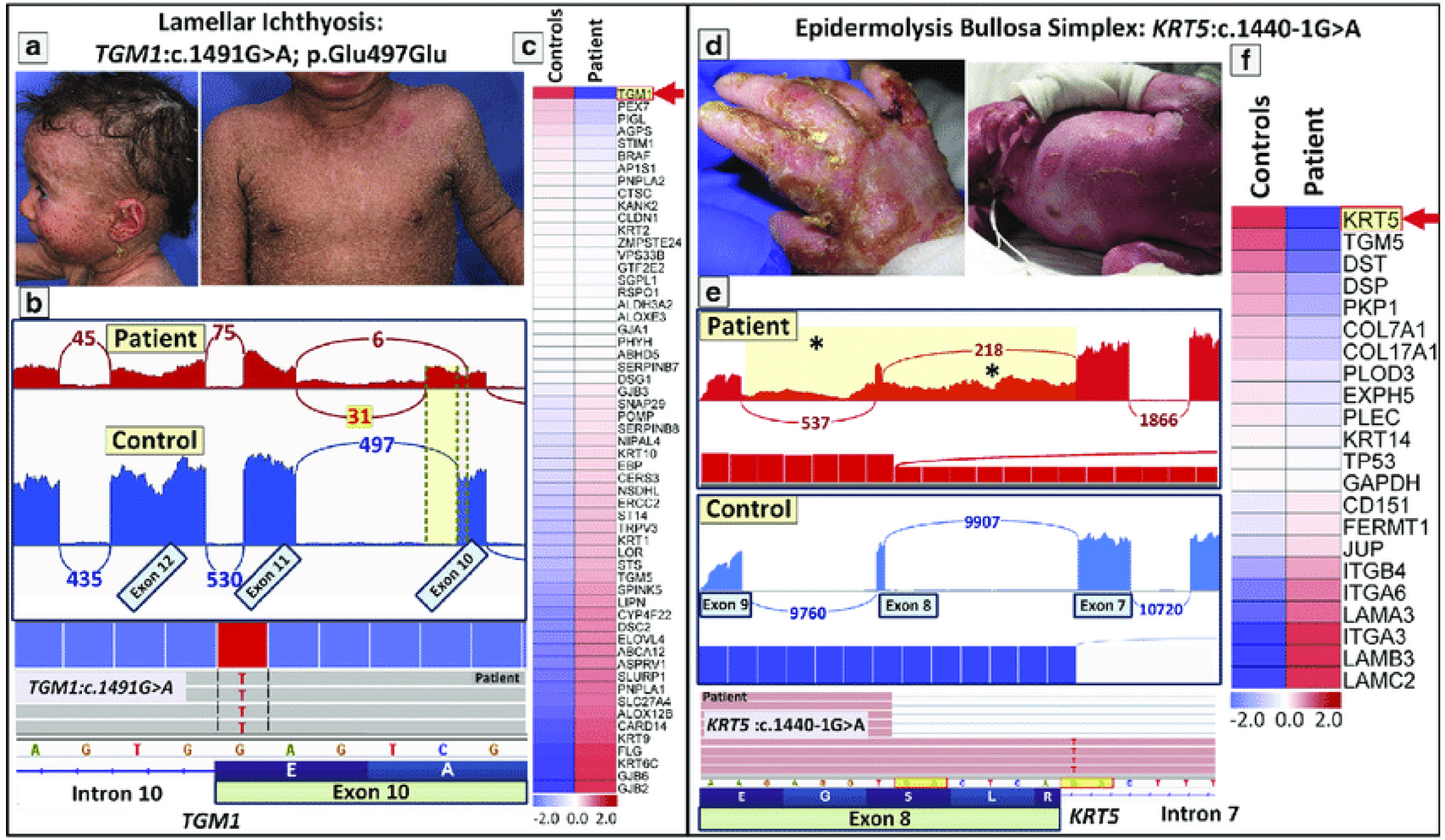


Few hours / sample

2. Report

- ✓ Diagnosis
- ? Candidate
- ✕ No candidate

RNA-Seq for Disorder Diagnosis

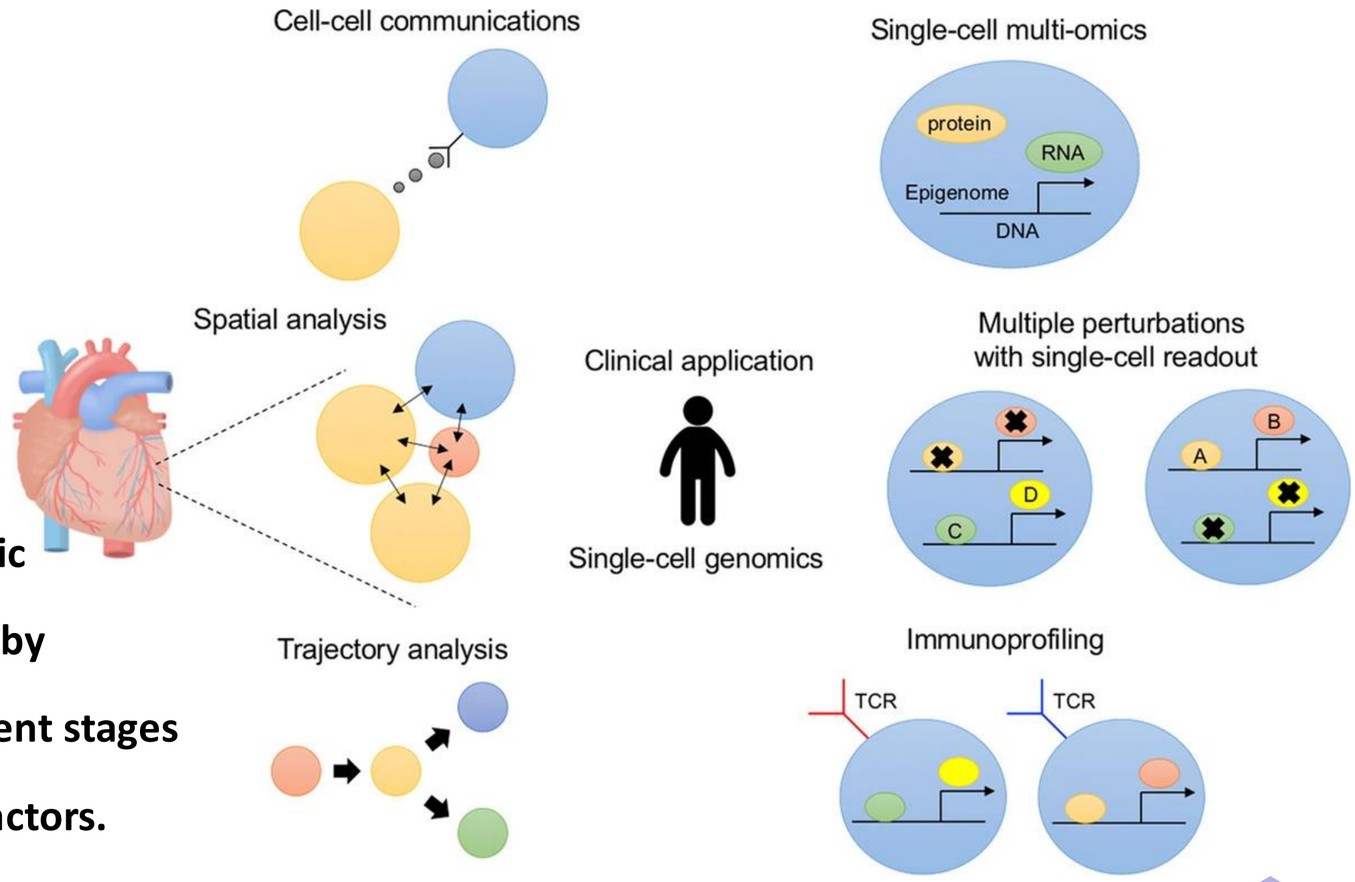


Utility of RNA-Seq in mutation detection in challenging cases of heritable skin diseases

Source:https://www.researchgate.net/publication/341524501_Research_Techniques_Made_Simple_Whole-Transcriptome_Sequencing_by_RNA-Seq_for_Diagnosis_of_Monogenic_Disorders

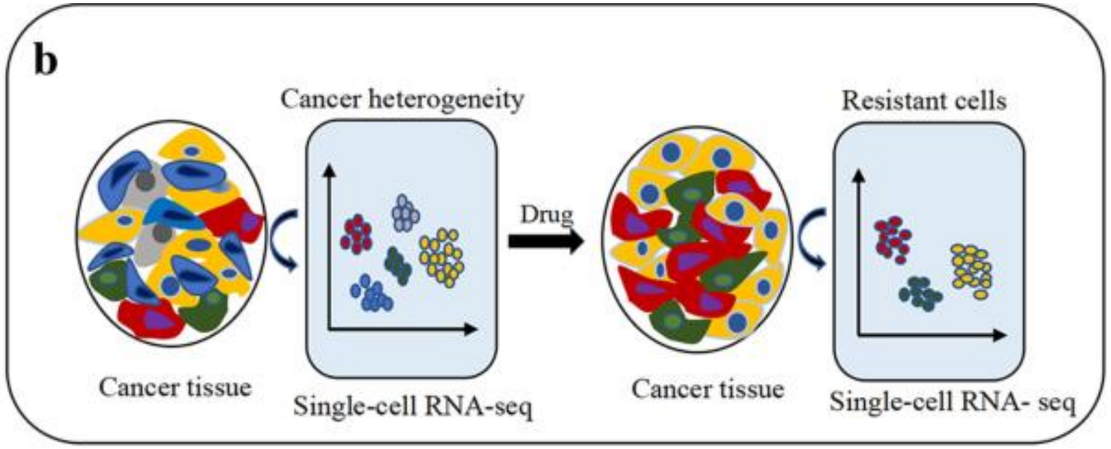
RNA-Seq for Disorder Diagnosis

- **Gene Expression Profiling**
- **Identification of Disease-Associated Genes**
- **Detection of Rare Variants and Mutations**
- **Understanding Pathophysiology**
- **Personalized Medicine**
- **RNA-Seq is especially useful for diagnosing pediatric disorders, including inherited metabolic disorders, by analyzing how gene expression varies across different stages of development or in response to environmental factors.**



Source:https://www.nature.com/articles/s10038-020-00844-

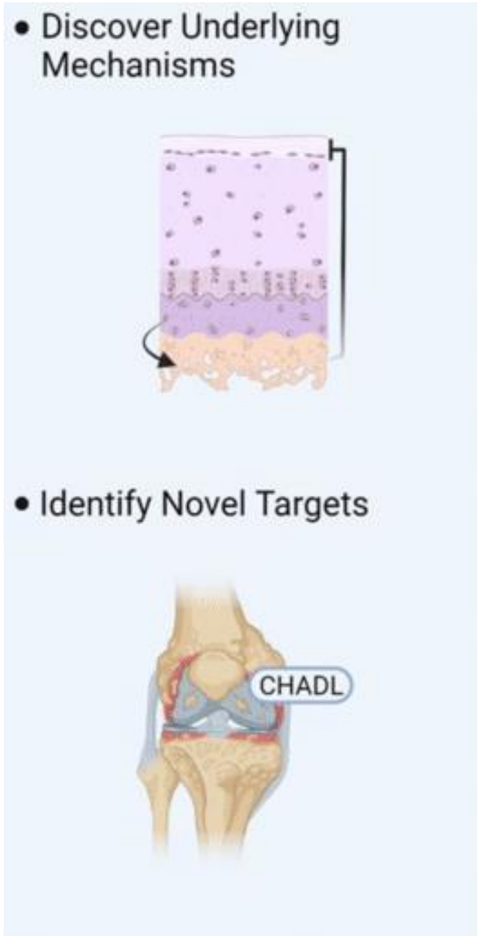
The heterogeneity and drug resistance of cancer cells identified by RNA-seq.



- RNA-seq uncovers intratumoral heterogeneity, revealing diverse cancer cell subpopulations.
- Detects resistance-associated gene expression changes after treatment.
- Identifies upregulation of drug efflux pumps, anti-apoptotic pathways, and metabolic reprogramming.
- Highlights alterations in signaling pathways (e.g., PI3K/AKT, MAPK) driving resistance.
- Reveals roles of lncRNAs, miRNAs, and circRNAs in modulating drug sensitivity.
- Identifies non-coding RNAs that influence epithelial-to-mesenchymal transition (EMT) and immune evasion.

Source: https://jhoonline.biomedcentral.com/articles/10.1186/s13045-020-01005-x

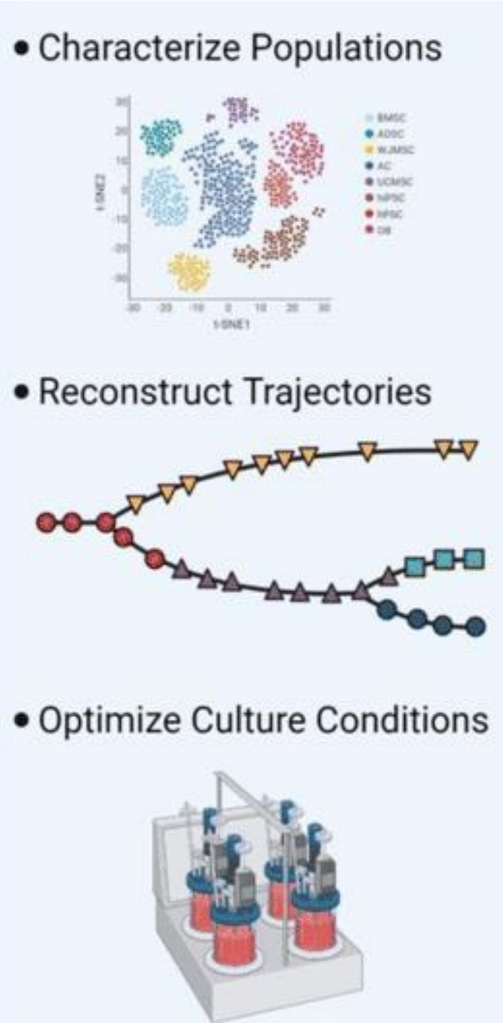
Applications of RNA-sequencing in Pathophysiology



- Gene Expression Profiling
- Detects RNA-based biomarkers for early diagnosis and prognosis
- Discovers disease-specific splicing events
- Explores the function of microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) in regulating disease processes
- Immune Response and Inflammation
- Novel Peptides and Neoantigens

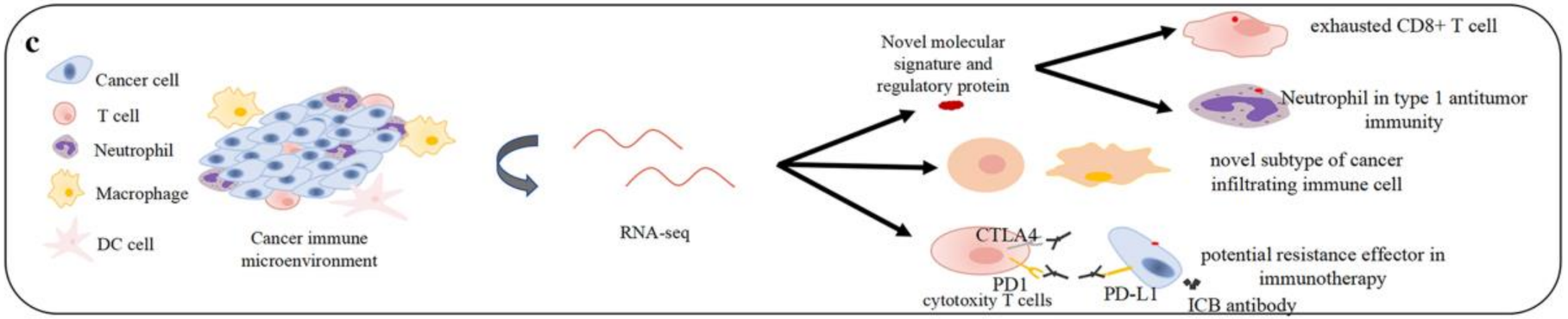
Source:https://www.researchgate.net/figure/Applications-of-RNA-sequencing-in-regenerative-medicine-RNA-seq-can-help-decipher_fig1_358590757

Applications of RNA-sequencing in Stem Cells



- Tracks gene expression changes during stem cell differentiation into various lineages
- Identifying Key Regulatory Pathways
- Single-Cell RNA-Seq for Heterogeneity
- Explores how stem cells interact with their microenvironment (niche) to regulate fate decisions
- Uses RNA-Seq to study stem cell models of developmental diseases, such as congenital disorders or genetic mutations
- Cell Fate Decisions and Lineage Tracing

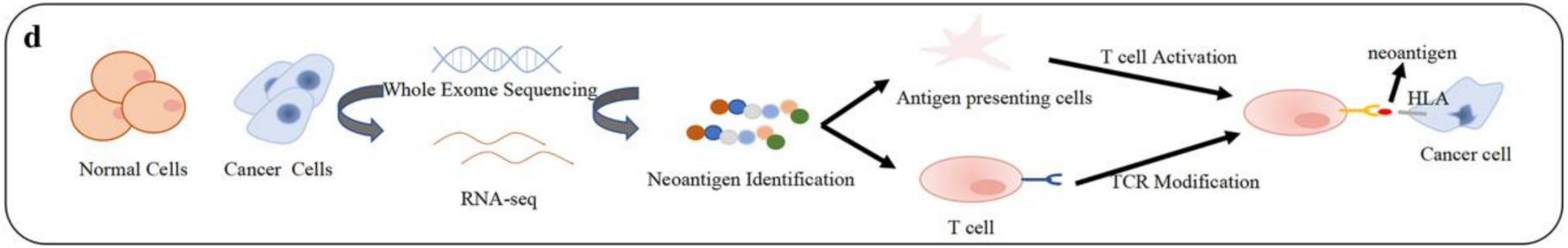
Identifying Regulatory Proteins and Unknown Subtypes in Tumor-Immune Interactions via RNA-Seq



- Identifies unique gene expression profiles in tumor-infiltrating immune cells.
- Detects key proteins that modulate immune responses.
- Reveals immune checkpoints and effectors linked to therapy resistance.
- Maps diverse immune cells at single-cell resolution.
- Supports tailored immunotherapy strategies.

Source: https://jhoonline.biomedcentral.com/articles/10.1186/s13045-020-01005-x

Neoantigen profiling by RNA-seq and TCR modification targeted neoantigens

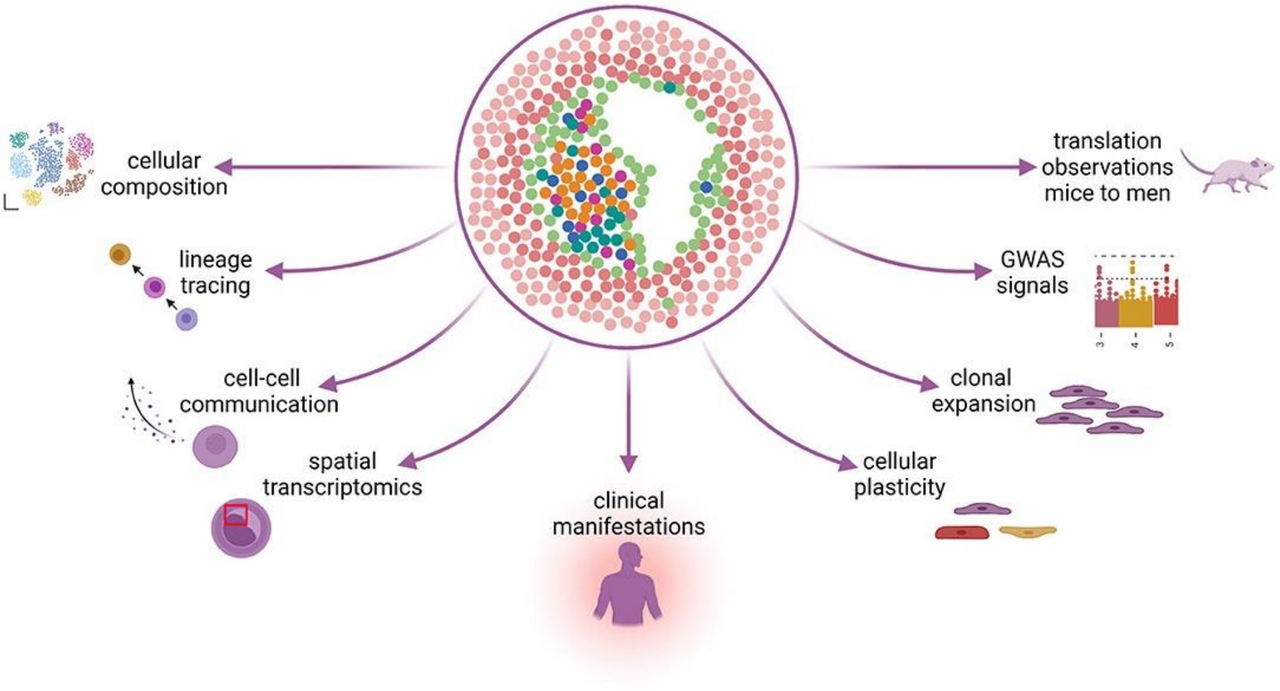


- **Neoantigen Discovery: RNA-seq identifies tumor-specific neoantigens by analyzing RNA from tumor vs. normal tissues**
- **TCR Engineering: Tumor-specific T cells can be modified to express TCRs targeting neoantigens, enhancing immune recognition**
- **Personalized Immunotherapy:This approach allows for tailored immunotherapies based on an individual's tumor mutations**

Source: https://jhoonline.biomedcentral.com/articles/10.1186/s13045-020-01005-x

Application of single-cell RNA Sequencing

Single-cell RNA sequencing (scRNA-Seq) is a powerful technology that enables the analysis of gene expression at the individual cell level.



- **Define The Heterogeneity Within The Cell Population**
- **Rare Cell Type Identification**
- **Look Of Cell In Different Developemtal Stages**
- **Discover New Drug Targets And Biomarkers**
- **Investigate The Effects Of Existing Treatments**

Source: https://www.frontiersin.org/journals/cardiovascular-medicine/articles/10.3389/fcvm.2022.826103/full

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Empowering
Medical Decisions

Genome-informatics Solutions and Services

OUR PRODUCTS

ANUVANSHIKI

Designed to identify germline mutations in rare inherited pediatric disorders.

ONQUER

Transforming genetic data into actionable insights and AI-driven therapy recommendations with our advanced oncopanel solution.

CUSTOMISED SOLUTIONS

Solutions tailored to your needs, ensuring precision and efficiency in genomic analysis.

NeoCheck

An app for symptom checking in newborns, designed to match phenotypes with potential genetic conditions.

NeoScreen

A comprehensive newborn screening panel for early diagnosis of metabolic and genetic disorders.

GENOMIKI™

OUR SERVICES

- Genome-Informatics Services
- Transcriptomics (RNA-Seq)
- Whole Genome Sequencing (WGS)
- Whole Exome Sequencing (WES)
- Epigenomics (ChIP-Seq)
- Variant Annotation and Interpretation
- Functional Genomics and Pathway Analysis

Source: https://www.csbj.org/article/S2001-0370%2821%2900072-6/fulltext

GENOMIKI

Core Team

Dr. Deeksha Bhartiya, Founder
PhD (Bioinformatics)
Postdoctoral fellow (Sweden)
10+ years of experience as Bioinformatics scientist post Phd

Dr. Sunil Tadepalli, Scientific Advisor, MBBS
MD
18+ years experience as a Healthcare professional and physician

Dr. Tani Agarwal,
VP (Advisory and strategy)
PhD, 10+ years of experience post PhD

Dr. Priyanshu Mathur
Associate Professor
Pediatric Medicine
SMS Medical College, Jaipur

Mr. Abhijit Swain, Bioinformatician
M.Sc. Bioinformatics
Expert in Genome analysis

Mr. Prashant Kumar Dhangar,
Bioinformatician
M.Sc Bioinformatics

Ms. Anchal Negi
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THANK YOU

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