RNA sequencing

Whole transcriptome sequencing, also known as RNA-Seq (RNA sequencing), is a powerful molecular biology technique used to comprehensively analyze the transcriptome of a biological sample. The transcriptome refers to the complete set of RNA molecules, including messenger RNAs (mRNAs), non-coding RNAs (such as microRNAs), and other types of RNA, produced in a cell or tissue at a specific moment in time. RNA-Seq allows researchers to profile and quantify all RNA molecules in a sample, providing valuable insights into gene expression, alternative splicing, and other aspects of RNA biology.

Liquid biopsy-derived RNA sequencing

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How it works

Sample Preparation: The process begins with the extraction of RNA from the biological sample of interest, such as cells, tissues, or even single cells. This RNA can be a mix of different RNA types, including mRNAs and non-coding RNAs.

RNA Library Preparation: The extracted RNA is then converted into a sequencing library. This involves steps like RNA fragmentation, reverse transcription into complementary DNA (cDNA), adapter ligation, and PCR amplification. The library preparation process can be tailored to focus on specific RNA types, such as polyadenylated mRNAs or total RNA (which includes non-coding RNAs).

Sequencing: The prepared library is then subjected to high-throughput sequencing using next-generation sequencing (NGS) platforms, such as Illumina or Ion Torrent. During sequencing, millions of short reads are generated from the library.

Data Analysis: The raw sequencing data is processed through a series of bioinformatics steps, including read alignment to a reference genome or transcriptome, quantification of gene expression levels, detection of alternative splicing events, and identification of differentially expressed genes or transcripts.

Key Applications and Advantages of Whole Transcriptome Sequencing:

Gene Expression Analysis: RNA-Seq provides precise measurements of gene expression levels, enabling the identification of differentially expressed genes under different conditions or in different tissues.

Alternative Splicing Detection: It allows researchers to study alternative splicing events, which can lead to the production of multiple protein isoforms from a single gene.

Discovery of Novel Transcripts: RNA-Seq can uncover previously unknown or unannotated transcripts, including non-coding RNAs and novel coding genes.

Functional Annotation: It aids in the functional annotation of genes by revealing the diversity of

transcripts and isoforms they produce.

Biomarker Discovery: RNA-Seq can be used to identify potential biomarkers associated with diseases or specific conditions.

Single-cell RNA sequencing

Whole transcriptome sequencing has become a fundamental tool in genomics and molecular biology, contributing to our understanding of gene regulation, disease mechanisms, and many other biological processes.

MI GPSai, a Genomic Prevalence Score, uses DNA sequencing and whole transcriptome sequencing data coupled with machine learning to aid in the diagnosis of cancer. The algorithm trained on genomic data from 34,352 cases and genomic and transcriptomic data from 23,137 cases and was validated on 19,555 cases. MI GPSai predicted the tumor type in the labeled data set with an accuracy of over 94% on 93% of cases while deliberating amongst 21 possible categories of cancer. When also considering the second highest prediction, the accuracy increases to 97%. Additionally, MI GPSai rendered a prediction for 71.7% of CUP cases. Pathologist evaluation of discrepancies between submitted diagnosis and MI GPSai predictions resulted in change of diagnosis in 41.3% of the time. MI GPSai provides clinically meaningful information in a large proportion of CUP cases and inclusion of MI GPSai in clinical routine could improve diagnostic fidelity. Moreover, all genomic markers essential for therapy selection are assessed in this assay, maximizing the clinical utility for patients within a single test [1)].

Single-cell transcriptome profiling is a powerful technique used in molecular biology and genomics to study gene expression patterns at the level of individual cells. Traditional bulk RNA sequencing provides an average gene expression profile for a population of cells, but it cannot capture the heterogeneity and diversity of gene expression within the population.

In contrast, single-cell transcriptome profiling allows researchers to analyze gene expression patterns in individual cells, providing insights into cellular heterogeneity, cell type identification, developmental processes, disease mechanisms, and more. This technique has revolutionized our understanding of cellular biology and has led to numerous discoveries in various fields.

The process of single-cell transcriptome profiling typically involves the following steps:

Cell isolation: Cells of interest are isolated and prepared for analysis. This can be done using different methods depending on the sample type, such as tissue dissociation, microfluidics, or fluorescence-activated cell sorting (FACS).

Cell lysis and RNA capture: Individual cells are lysed, and their RNA content is captured and converted into complementary DNA (cDNA). Various techniques are available for RNA capture, including oligo-dT priming, random priming, or unique molecular identifiers (UMIs).

Amplification and library preparation: The captured cDNA is amplified using techniques like polymerase chain reaction (PCR) or whole transcriptome amplification (WTA) to generate enough material for downstream analysis. Subsequently, libraries are prepared by adding sequencing adapters and unique barcodes to each cell's cDNA.

Sequencing: The prepared libraries are then subjected to high-throughput sequencing using next-generation sequencing platforms. The most commonly used sequencing methods for single-cell transcriptome profiling are RNA-Seq and Drop-seq.

Data analysis: After sequencing, the resulting data is processed and analyzed to identify individual gene expression profiles for each cell. This involves aligning the sequenced reads to a reference genome, quantifying gene expression levels, performing quality control, and applying specialized computational algorithms to cluster cells, identify different cell types, infer cell trajectories, and detect gene expression patterns.

Several technologies and platforms are available for single-cell transcriptome profiling, including but not limited to Fluidigm C1, 10x Genomics Chromium, and Drop-seq. Each platform has its own advantages and limitations, and the choice depends on the specific research goals and sample characteristics.

Single-cell transcriptome profiling has provided invaluable insights into cellular diversity, tissue development, disease progression, and therapeutic responses. It continues to advance our understanding of complex biological systems and holds great promise for personalized medicine and regenerative therapies.

Single-cell RNA sequencing (scRNA-Seq) technology is a powerful tool for studying intratumoral cellular heterogeneity and revealing the roles of various cell types in oncogenesis.

Traditional RNA sequencing methods typically analyze gene expression from a large population of cells, which can mask important biological variations that exist between individual cells. By analyzing the transcriptome of single cells, scRNA-seq provides a more precise view of gene expression heterogeneity within a tissue or population.

The scRNA-seq workflow involves isolating single cells, extracting RNA from each cell, and generating cDNA libraries for sequencing. The resulting data can be used to identify different cell types, characterize cell states, and discover novel cell populations or subtypes. scRNA-seq has many applications in biology, including the study of development, disease, and immune responses.

One of the challenges of scRNA-seq is the high degree of technical variability introduced by the low input of RNA from each cell, which can lead to technical noise and batch effects. As a result, careful experimental design, sample preparation, and computational analysis are crucial for obtaining high-quality data.

RNA sequencing technology has been recently used to investigate the mechanism of diseases owing to its ability to identify the gene changes on a transcriptome-wide level.

Targeted RNA-sequencing (RNA-Seq) is a highly accurate method for selecting and sequencing specific transcripts of interest. It offers both quantitative and qualitative information.

RNA-Seq (RNA sequencing), also called whole transcriptome shotgun sequencing (WTSS), uses next-generation sequencing (NGS) to reveal the presence and quantity of RNA in a biological sample at a given moment in time.

RNA-Seq is used to analyze the continually changing cellular transcriptome. Specifically, RNA-Seq facilitates the ability to look at alternative gene spliced transcripts, post-transcriptional modifications, gene fusion, mutations/SNPs and changes in gene expression.

In addition to mRNA transcripts, RNA-Seq can look at different populations of RNA including total RNA, small RNA, such as MicroRNA, tRNA, and ribosomal profiling.

RNA-Seq can also be used to determine exon/intron boundaries and verify or amend previously annotated 5' and 3' gene boundaries.

Prior to RNA-Seq, gene expression studies were done with hybridization-based microarrays. Issues with microarrays include cross-hybridization artifacts, poor quantification of lowly and highly expressed genes, and needing to know the sequence a priori.

Because of these technical issues, transcriptomics transitioned to sequencing-based methods. These progressed from Sanger sequencing of Expressed Sequence Tag libraries to chemical tag-based methods (e.g., serial analysis of gene expression), and finally to the current technology, NGS of cDNA (notably RNA-Seq).

A study aimed to gain insights into the potential mechanism involved in ruptured cerebral arteriovenous malformation.

Sixty-five cerebral arteriovenous malformation nidus samples were collected, among which 28 were ruptured and 37 were un-ruptured. Then, next-generation RNA sequencing was performed on all of them to obtain differential gene expression (DEGs) between the two groups. In addition, bioinformatics analysis was performed to evaluate the involved biological processes and pathways by Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analysis. Finally, they performed a univariate Cox regression analysis to obtain the early rupture-prone DEGs.

A total of 951 genes were differentially expressed between the ruptured and un-ruptured BAVM groups, of which 740 genes were upregulated and 211 genes were downregulated in ruptured BAVMs. Then, bioinformatics analysis showed the biological processes and pathways related to the inflammatory processes and extracellular matrix organization were significantly enriched. Meanwhile, some downregulated genes are involved in cell adhesion and genes participating in response to muscle activity and the terms of nervous system development. Finally, one hundred twenty-five genes, many were involved in inflammation, were correlated with the early rupture of BAVMs.

Conclusions: The upregulated genes in the ruptured BAVM group were involved in inflammatory processes and extracellular matrix organization. Some of the downregulated genes participated in cell adhesion and myofibril assembly, indicating the role of enhanced inflammation and reduced inflammation vessel strength in BAVMs rupture [2].

1)

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2)
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