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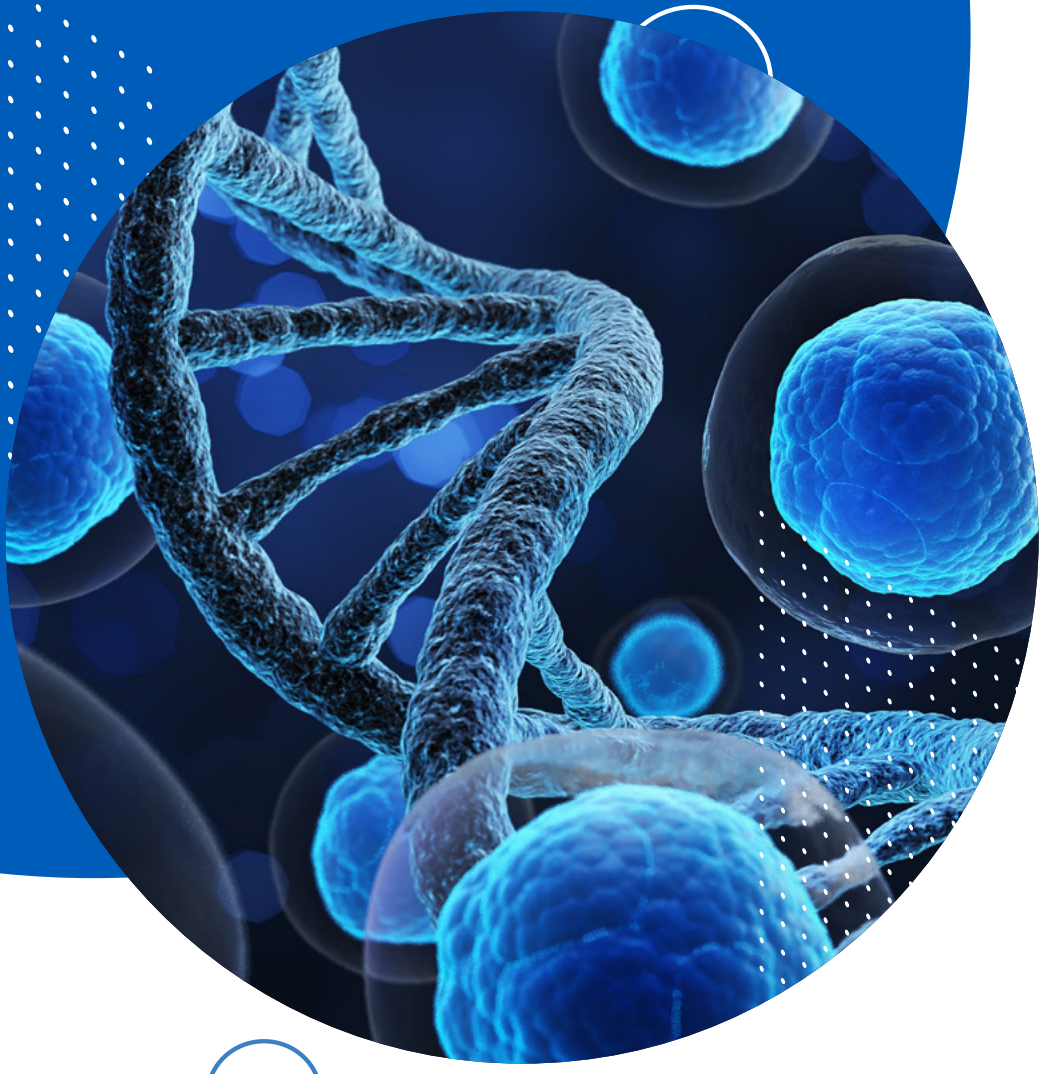

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ALL ABOUT -OMICS: SUBFIELDS AND APPLICATIONS

Biological research in the 21st century has been revolutionized by -omics technologies.[1] These techniques integrate technological innovations with advanced computational algorithms to probe and analyze a tissue's or cell's repertoire of DNA, RNA, proteins, and metabolites. Understanding a biological system's spatiotemporal changes at the molecular level using genomics, transcriptomics, proteomics, epigenomics, and metabolomics allows researchers to gain novel insights into human health and diseases.[2,3]

Genomics

Genomics involves reading the entire genome of an individual to identify gene variations and mutations. Profiling gene variants can provide deep insights into the workings of cells and tissues, disease mechanisms, and response to chemical and environmental changes. Genome-wide association studies (GWAS) are examples of successful genomics approaches that identify thousands of disease-associated and causative genetic variants in diverse human populations. Technologies such as genotype arrays, whole exome sequencing, and whole-genome sequencing using next-generation or Sanger sequencing allow researchers to read genome and exome sequences in depth to characterize mutations from single cell to whole population levels.

Transcriptomics

Unlike genomes, RNA expression and turnover are highly dynamic, constantly changing according to internal and external stimuli. Decoding mRNA species present within a tissue can reveal which biological pathways are active and how they influence physiological states. Similar to genomics, transcriptomics employs next-generation sequencing to generate sequencing reads from thousands of transcripts at once. Different transcriptomic analyses facilitate qualitative and/or quantitative determination of RNA at the tissue and single-cell level. Researchers isolate transcripts and generate bulk RNA sequence or single-cell RNA sequence datasets to catalog which transcripts are present in tissues, quantify the level of each transcript, and generate maps of cellular heterogeneity. Transcriptomics also reveals splice variants, novel transcripts in different cell types, and non-coding RNAs such as microRNA (miRNA), piwi-interacting RNA (piRNA), and small nuclear RNA (snRNA).

Proteomics

While transcriptomics uncovers numerous cellular pathways responsible for cell development and function, not all active RNA transcripts present in a cell have functional relevance. Proteins are the functional intermediaries that drive cellular signaling. Proteomics allows researchers to identify, characterize, and quantify multiple proteins at once and develop better understanding of protein-protein interactions and protein modifications. Researchers use liquid chromatography coupled with mass spectrometry for analysis of thousands of peptides to determine any protein's amino acid sequences, molecular weight, and post-translation modification sites, such as glycosylation, phosphorylation, and ubiquitination.

Epigenomics

Genomic DNA and associated histone proteins accumulate chemical modifications, such as DNA methylation and histone acetylation. These modifications alter chromatin and gene regulatory pathways that subsequently affect cell fate and behavior. Both genetic and environmental changes influence epigenetic modifications, contributing to a variety of human diseases. Epigenomics deals with characterizing genome-wide epigenetic modifications to understand disease mechanisms. Next-generation sequencing coupled with immunoprecipitation detects DNA modifications in broad genomic regions, whereas whole genome bisulfite sequencing allows researchers to identify methylated cytosines through genome-wide sequencing. Epigenetic signatures are tissue-specific, and probing differentiated epigenomic signatures between various organs helps reveal new mechanisms for complex diseases such as metabolic syndromes, cardiovascular diseases, and cancer.

Metabolomics

A cell contains a diverse host of low molecular weight (<1,500 Dalton) bioactive molecules, such as amino acids, fatty acids, carbohydrates, lipids, and other chemical metabolites. Metabolic regulation keeps their levels and relative ratios in check, and fluctuations from the normal range suggest the presence of disease. Metabolomics simultaneously identifies and quantifies small metabolites in plasma and relevant tissues to identify disease pathologies. Analytical approaches such as Fourier transform-infrared (FT-IR) spectroscopy, Raman spectroscopy, NMR spectroscopy, and chromatography coupled with mass spectrometry allow scientists to determine the structures of unknown metabolites and quantify them.

See References on Page 7

HARNESSING THE POWER OF ADAPTIVE FOCUSED ACOUSTICS® IN MULTIOMICS

Genomic and transcriptomic data generation involves nucleic acid extraction from cells of interest, library preparation, and next-generation sequencing (NGS). Because DNA and RNA endogenously exist as long molecules, researchers fragment them before library preparation to generate high-fidelity sequencing reads.[1]

Researchers in genomics laboratories use several DNA fragmentation approaches, such as chemical, enzymatic, and physical fragmentation. These approaches generate small DNA fragments that can be efficiently amplified by DNA polymerases. Chemical fragmentation uses cations such as magnesium and zinc to break down nucleic acids. However, this approach generates fragments of varying lengths, making sequencing less reproducible. Enzymatic fragmentation uses endonucleases that generate biased libraries due to enzymes' sequence preferences for fragmentation. Physical shearing is by far the most common fragmentation method, which can generate DNA fragments of uniform length without any bias.[1,2]

Breaking Down with Sound Waves

DNA strands bind to each other via covalent bonds. Physical fragmentation uses shear force to break covalent bonds and separate the two strands. The physical force further fragments every DNA and RNA single strand into smaller pieces. Sonication is a common physical fragmentation method, which breaks cells and nucleic acid molecules using sound waves. Typical sonicators produce high-frequency sound waves that generate a lot of heat. High heat creates oscillations in microscopic bubbles present in the sample, which become unstable and collapse, generating shear forces that cause fragmentation.[3,4]

Probe and bath sonicators are popular among molecular biologists for nucleic acid shearing. When using a probe sonicator, the sound wave-generating probe directly contacts the sample, providing more concentrated energy for fragmentation. However, probe sonicators run the risk of sample cross-contamination, and they do not work for small sample volumes. Bath sonicators first transmit the ultrasonic energy to a water bath and then to the sample tubes. While this energy transfer approach reduces sample contamination and is effective for very small samples, it is not efficient for high-throughput fragmentation applications due to insufficient energy transfer.[4] Both probe and bath sonicators generate a lot of heat that can destabilize the analytes.

Scientists at Covaris developed Adaptive Focused Acoustics® (AFA®) technology that uses focused-ultrasonicators to generate ultrasonic waves for the physical shearing of nucleic acids.[5,6] AFA focused-ultrasonicators generate short-wavelength bursts of acoustic energy that is precisely delivered to the biological sample. AFA delivers energy to the sample without any contact in a focused and efficient manner, controlling the total amount of heat, which in turn ensures efficient fragmentation of DNA and RNA. As a result, AFA helps in eliminating the drawbacks of probe and bath sonication.[6]

Empowering NGS with Focused Acoustic Shearing

AFA offers several advantages over other fragmentation methods, allowing researchers to process samples efficiently and reproducibly in a contact-free and temperature-controlled environment. AFA does not require a cleaning step after shearing, minimizing chances of cross-contamination and sample loss. Fragmentation with AFA is more consistent compared to other sonicators and enzymatic fragmentation. Large variability in DNA insert lengths used for library preparation results in inaccurate NGS results. Compared to enzymatic fragmentation, fragmentation with Covaris' focused-ultrasonicators dramatically reduces variability in sheared DNA fragment length, thereby ensuring higher accuracy in NGS results. In addition, Covaris' R230 focused-ultrasonicator is suited to address high throughput and scalability requirements and can be used with most automated liquid handlers.[7]

See References on Page 7

RELIABLE AND REPRODUCIBLE NUCLEIC ACID EXTRACTION FROM FFPE SAMPLES

Cancer researchers aim to generate -omics data from tissues obtained from a variety of sources. While cultured cells and freshly dissected or fresh frozen tissue samples are common DNA and RNA sources in research laboratories, most clinical samples are derived from tumors and are often preserved as formalin-fixed paraffin-embedded (FFPE) blocks for longer storage.[1]

Preserving Tissue Samples

FFPE is the oldest and the most common tissue fixation method, where tissue sections are immersed in formaldehyde, followed by paraffin embedding into a solid block for long-term preservation at room temperature. Although this method is time-consuming and involves toxic chemicals, it is convenient in surgical procedures where doctors can easily submerge tissues in formaldehyde.[2]

In recent years, researchers have become more inclined towards snap freezing for tissue storage, where samples are cryopreserved in liquid nitrogen. Compared to FFPE, snap freezing is easy, quick, and does not involve toxic chemicals. However, this method is relatively new to clinicians, and not all biobanks are equipped to store these samples. The need for liquid nitrogen containers, stable -80 °C freezers near surgery rooms, and expensive maintenance remains an obstacle to adopting fast-freezing in clinical settings.[2,3]

While frozen tissues remain the best DNA and RNA source in -omics applications, they have limited availability in clinical research. Cells grown in cultures are good nucleic acid sources; however, most human cell lines do not capture the structural and functional hallmarks of tissues and organs. FFPE tissues are popular for clinical studies because they are widely available and can be easily transported; however, they generate low nucleic acid yield and quality. By establishing new and improved extraction protocols from FFPE tissues, researchers can obtain high-quality DNA and RNA to investigate disease pathologies from clinical samples.[2-5]

Nucleic Acid Extraction from FFPE Tissue

Deparaffinization is the first step in nucleic acid extraction from FFPE samples. Researchers often employ chemical approaches to remove paraffin embedded inside the tissue, which affects the quality and quantity of extracted RNA and DNA. For example, harsh chemical paraffin removal treatment degrades RNA quality. Gentle chemical removal processes leave paraffin in the tissues, which makes it difficult for proteinase K to break down proteins. This makes nucleic acid extraction inefficient, reducing the total yield. Moreover, deparaffinization may include solvents such as xylene and hexadecane that are difficult to incorporate into automated extraction pipelines due to their toxicity and viscosity. Therefore, researchers first manually carry out deparaffinization before performing nucleic acid extraction kits in automated workflows. This increases the number of workflow steps, compromising throughput capabilities and also quality of data.[4]

AFA-Powered Nucleic Acid Extraction in Clinical Research

The truXTRAC FFPE extraction and purification kits incorporate deparaffinization and nucleic acid purification steps, allowing separate or sequential DNA and RNA extraction. For higher throughputs, kits include an array of 96 truTUBEs™ to carry out deparaffinization, sample lysis, and homogenization with Adaptive Focused Acoustics® (AFA®) technology and purification with either columns or magnetic beads. The AFA-based tissue shearing generates cavitation bubbles that actively deparaffinizes tissues without damaging nucleic acids and without using any harsh organic chemicals. The chemical-free process removes paraffin efficiently, enhancing total yield and improving reproducibility.

Researchers quantified the DNA and RNA obtained from brain, colon, breast tumor, and lung tumor FFPE tissues using the Covaris FFPE nucleic acid extraction kit and a competitor nucleic acid extraction kit with manual deparaffinization. The Covaris kit extracted equivalent or higher yield of nucleic acids compared to the competitor kit from all four tissue types. While the DNA quality determined by 260/280 nm and 260/230 nm ratios was the same for either set of samples, the DNA samples obtained with the Covaris kit showed higher consistency and fewer impurities in electropherogram analysis compared to DNA extracted using the competitor's product.[5]

See References on Page 7

IMPROVING REPRODUCIBILITY AND YIELD IN NGS ASSAYS

Next-generation sequencing (NGS) facilitates genomic and transcriptomic analyses in cancer research. Researchers aim to identify cancer-associated pathways using DNA sequencing data from thousands of tumor types to pinpoint causative genes and develop potential treatments.[1]

Next-Generation Sequencing 101

The NGS workflow begins with nucleic acid extraction, followed by fragmentation, library preparation, sequencing, and bioinformatics. Once researchers extract and quantify DNA from cells or tissues, they move on to library preparation, where they fragment sample nucleic acids into the appropriate lengths, amplify their numbers with PCR, and attach adapter oligos to these fragments to generate a sequencing-ready library.[2]

In cancer research, each NGS workflow step demands the parallel processing of numerous samples. Researchers cannot afford to lose precious samples as every tumor sample reveals critical information about cancer stage and progression. Therefore, obtaining a high-quality sequencing library in a high-throughput manner is imperative to successful NGS experiments. New technological innovations improve scalability and efficiency at each library preparation step.[1,2]

Innovation in Nucleic Acid Fragmentation

The fragmentation step involves breaking down long DNA strands into short sequences of specific length to obtain high-fidelity sequencing reads. Inconsistent fragmentation leads to incomplete sequencing of longer fragments and generates excessive copies of shorter fragments, resulting in low-quality data. Innovations in acoustic shearing of nucleic acids using ultrasonicators allow researchers to improve fragmentation reproducibility and uniformity.[3]

The input tissue sample's quality determines the success of the sequencing experiment. While fresh- or snap-frozen samples are ideal to collect good-quality nucleic acid for sequencing applications, their collection and maintenance is expensive and not always feasible in clinical research. Formalin-fixed paraffin-embedded (FFPE) tissue preservation of patient samples is a routine practice that powers large-scale -omics studies for cancer research. For some rare cancers, FFPE tumor samples are often the only source of DNA.[4,5]

Isolating nucleic acids from FFPE tumor samples is challenging due to the presence of paraffin, which lowers the yield and quality of the extracted DNA. Despite low-quality starting material, it is possible to generate high-quality sequencing libraries and sequencing results from FFPE samples. For a robust, reliable library preparation from FFPE tissues, generating the desired DNA fragment size using DNA fragmentation is a critical step prior to sequencing.[4,5]

Researchers tested whether Adaptive Focused Acoustics® (AFA®) technology, which uses high-frequency acoustic energy to shear DNA, can improve DNA extraction from FFPE tumor samples and enhance NGS library quality.[6] They isolated DNA from 32 different FFPE-preserved tumor tissue samples to probe cancer-associated genes using NovaSeq whole-genome Illumina® sequencing system. They incorporated Covaris AFA technology to generate a wide range of DNA fragment sizes using the ML230 Focused-ultrasonicator and obtained fragment lengths between 90 to 250 base pairs. Using a fragment analyzer high-sensitivity DNA kit that measures DNA size, the researchers determined that all 32 DNA samples sheared with the ML230 Focused-ultrasonicator were of the desired fragment length. The researchers used these fragments to generate genomic libraries and assessed them in a cancer screening assay, which resulted in the high-fidelity determination of 523 cancer-associated genes.[6]

See References on Page 7

IMPROVING REPRODUCIBILITY AND YIELD IN PROTEOMICS ASSAYS

Scientists extract proteins from tissues and cultured cells for biochemical and analytical characterization. Through proteomic analyses, quantifying protein levels in relation to each other helps researchers identify important biological pathways in health and disease.[1]

Challenges in Proteomics

Despite advances in end-detection technologies such as liquid chromatography (LC) coupled to mass spectrometry (MS), sample preparation is the critical step to ensure data quality in proteomic applications. Researchers typically use sonicators for cell breakdown and homogenization. Subsequent protein purification techniques, such as ultracentrifugation, chromatographic separation, immunoprecipitation, digestion and post-digestion purification, allow researchers to obtain pure peptides in greater quantities.[2]

Researchers commonly purify proteins from cultured cells, snap-frozen tissue, or formalin-fixed paraffin-embedded (FFPE) tissue samples. Each sample contains thousands of proteins in a wide range of concentrations and configurations. Therefore, protein extraction conditions and buffer preparations vary depending on the sample type. Depending on the nature of the proteins of interest and downstream assays, protein isolation and purification protocols require optimization. For example, SDS is necessary for the extraction buffer to maximize the yield of soluble proteins. However, SDS and high-concentration salts are not preferred in protein solutions if the samples were analyzed using mass spectrometry. Such discrepancies in protocols make proteomics data collection less efficient and reproducible.[1-3]

Reproducibility in Proteomics Powered By AFA

To streamline protein extraction workflows for proteomics, scientists can incorporate Covaris Adaptive Focused Acoustics® (AFA®) technology, which offers a one-plate sample preparation protocol utilizing controlled sound bursts to disrupt samples, with various commonly-used protein extraction protocols in which the Covaris ML230 Focused-ultrasonicator facilitates efficient cell lysis. When processing FFPE tissue sections, researchers dispense them in AFA-TUBEs® containing tissue lysis buffer. The initial AFA treatment emulsifies the paraffin and the second burst homogenizes the tissue, releasing proteins. Researchers then add capture proteins using magnetic beads and perform downstream purification in the same plate. With Covaris ultrasonicators and consumables, scientists can efficiently isolate and purify proteins from multiple samples at once, enhancing throughput and increasing protein yield.[4]

Automating Protein Extraction

To investigate the technical variability of different purification methods and the inherent biological differences among sample types used in a quantitative proteomics study, scientists employed SP3 clean-up and S-Trap protein purification methods to extract proteins from cultured cells, fresh tissues, and FFPE tissues.[4,5]

The researchers dispensed cultured HeLa cells, fresh rat liver tissue, and human liver FFPE tissue samples into AFA-TUBEs along with lysis buffer.[4] After AFA lysis, they performed downstream steps to obtain purified peptides and separated them using mass spectrometry.

The researchers isolated 30 µg of total protein from the HeLa cells and the mass spectrometry analysis identified 30,000 peptides and 4,000 protein groups. Similarly, proteins extracted from fresh rat liver and human liver FFPE tissues were in the 30-100 µg and 25-50 µg range, respectively. Proteomics analysis of the rat liver tissue found 17,500 peptides and 2,250 protein groups, whereas the human liver FFPE protein sample contained 15,000 different peptides and 2,500 protein groups. This analysis revealed robust proteome measurements across different sample types, showing AFA's ability to effectively extract good quality protein for proteomics.

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Fast and Efficient Protein Extraction — for All Sample Types

Covaris Focused-ultrasonicators

Covaris Focused-ultrasonicators, enabled by Adaptive Focused Acoustics® (AFA®) Technology, facilitate high-quality proteomics research with highly reproducible and reliable protein extraction. Achieve consistent results every time regardless of sample type (FFPE, LCM, fresh tissue, cells, bacteria, yeast, etc.).

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