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Next-generation sequencing: Pyrosequencing and its utilization in medical field

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Abstract

Next-generation sequencing (NGS) can be an idle innovation utilized for DNA and RNA sequencing and variant or mutation study. NGS can arrangement huge qualities or entire genome in a brief period of time. The arrangement variants or mutations recognized by NGS have been broadly utilized for infection. DNA sequencing is one of the foremost critical stages for consideration of organic frameworks nowadays. Arrangement assurance is most commonly performed utilizing deoxy chain end innovation. As of late, pyrosequencing has developed as an unused sequencing methodology. This strategy could be a broadly appropriate, elective innovation for the point by point characterization of nucleic acids. Pyrosequencing has the potential focal points of exactness, adaptability, parallel preparing, and can be effortlessly mechanized. Besides, the strategy apportions with the requirement for abled groundworks, labeled nucleotides, and gel-electrophoresis. This article considers key highlights with respect to distinctive viewpoints of pyrosequencing innovation, counting the common standards, chemical properties, sequencing modes, instrumented, and potential applications. By using these techniques, the DNA sequencing subsequently the precision medicinal field can be explore for the therapeutics approaches for different diseases.

Introduction

DNA sequencing is a biochemical method for determining the of nucleotide base, for example Guanine, Thymine, Adenine and Cytosine. Through DNA sequencing easily detect the genomic map and proper position of nitrogenous base. If any mutation occurs in DNA, easily detected through genome sequencing. Now a day DNA sequencing is cost effective and rapidly used technology. So it is used in diagnostics and therapies. Many medical house have begun to use DNA sequencing for treatment of some diseases. In case cancer patient medical physician must be recommended for DNA sequencing for identifying which type of cancer. In 2-3 years ago SARS (Severe Acute Respiratory syndrome) cov-2 infection effect in our life. But drug used against SARS cov and MERS (closely related species with SARS cov-2) are ineffective against SARS Cov2, cause of inactivation of drug revealed by DNA sequencing (Bau et al., 2009).

A whole new era in version/mutation detection and DNA and RNA sequencing has begun with NGS1-4. The advantages of distinct sequencing chemicals, various sequencing matrices, and bioinformatics generation are combined in this era. This combination enables massively parallel sequencing of different lengths of DNA or RNA sequences, or even the entire genome, in a very short amount of time. Following Sanger sequencing, it's a far more advanced sequencing technique. NGS requires a lot of advanced sequencing procedures. DNA NGS, for example, entails DNA fragmentation, bioinformatics analysis, massively parallel sequencing, library instruction, and variation/mutation annotation and interpretation. (Gargis et al., 2015).

DNA Fragment

DNA into multiple little sections, typically ranging from 100 to 300 bp. This can be done in a number of ways. Enzymatic digestion, mechanical techniques, and other techniques can all break apart DNA. For instance, DNA can be broken via sonication. Specific complimentary probes with distinct structures are used to extract brief fragments associated with the target DNA sequences. This process is commonly referred to as hybridization capture. Polymerase chain reaction-PCR amplification is another technique. This method makes use of several primer pairs to amplify specific DNA regions via PCR. Target DNA is divided into brief fragments by PCR products. One common name for this technique is amplicon analysis. After that, a library is created using the DNA segments. (Ferrando & Lopez-Otin, 2015).

DNA segments are modified during library preparation

In the course of preparing the library, each DNA sample will be given a sample-specific index, such as sample identifier, to help identify the patient whose DNA was sequenced. This process can also be used to provide sequencing adaptors for the DNA segments. This alteration permits massively parallel sequencing in the future by enabling the sequencing primers to bind to every segment of DNA.

Using an NGS sequencer

There is massively parallel sequencing. The library is uploaded onto a sequencing matrix by a particular sequencer. Depending on the sequencer, different sequencing matrices exist. For example, sequencing chips and flow cells are used by Illumina and Ion Torrent's NGS sequencers, respectively. It still seeks to allow massively parallel sequencing of every DNA segment at the same time. Software for bioinformatics is used to analyse the sequence data generated by massively parallel sequencing. Variant annotation, variant detection, and read alignment. To determine whether the targeted sequences have undergone any alterations or mutations, the sequencing data is compared to a reference sequence derived from the human genome. The final sequencing findings for the entire length of the targeted DNA are obtained by combining the data from each sequenced segment. The ultimate sequencing findings and interpretation techniques help to determine each mutation's possible clinical relevance (Shah et al., 2012; Quin, 2019).

NGS applications in Tumors

There is a need for NGS technology in medicine. One of the advantages of NGS is the capacity to analyse hundreds of thousands or even millions of targets at once. Because of these characteristics, NGS has a great deal of potential applications in clinical settings. For example, a single tumour in cancer patients may have several mutations during treatment. In these clinical settings, using traditional molecular diagnostics might require doing multiple assays for different mutations. A larger amount of tissue might be needed for these several studies. NGS technology allows questions to be asked about these targets in a single test. As a result, less tissue is needed, and results for dozens to hundreds of DNA targets can be obtained from a single test. The frequency of mutations in various diseases has been growing in recent years, according to scientific study. For instance, several mutations have been found in various hematological leukemias. Acute myeloid leukemia (AML) has been linked to several AML subtypes by mutations in the RUNX1 genes NPM1 and CEBPA12. Numerous mutations connected to various clinical consequences have been discovered in myelodysplastic syndrome. Many oncogenes and tumor supressor genes are susceptible for mutations, such as p53, KRAS, etc. It is believed that a single clone is responsible for the leukemic cells with the identical mutation found in a patient's sample. Multiple leukemic cell clones may be present in a patient's sample if there are numerous mutations. Multiple clones of leukemic cells may be involved in a leukemic illness, and as the disease progresses, the clones may differ. Clonal evolution is the term used to describe this phenomena. Solid tumours have also been linked to a similar phenomenon. The outdated theory of "one tumour, one mutation" has been changed by this discovery. Multiple mutations may be present in a solid tumour. These alterations could have originated from one or more clones. Tumour mutation heterogeneity is the term for this. Consequently, the management of cancer patients necessitates regular testing for different gene changes. Clonal evolution during

follow-up necessitates assessing multiple different gene variants. Furthermore, malignancies that have progressed from the original tumour and become metastatic. These results further suggest the necessity of doing diagnostic and follow-up molecular studies to examine various mutations (Gerlinger et al., 2012).

In the era of immunotherapy, tumour mutation burden has become an essential measure to be evaluated23. This necessitates reexamining many mutations in a tumour sample. Conventional molecular test methods are useless in these kinds of circumstances. Therefore, NGS technology is needed for these patient care tasks. Furthermore, in modern medicine, more information regarding mutation must be extracted from microscopic biopsy samples, even as biopsy specimens are becoming smaller and smaller. Molecular tests that are frequently used often do not meet these requirements.

Performance of NGS technology

Several crucial measures must be followed in order to apply NGS in a clinical setting properly. The application of NGS technology for clinical diagnosis has been the subject of guidelines and recommendations published by the College of American Pathology (CAP), the Association of Molecular Pathology (AMP), and other organisations. NGS can be used on a variety of levels. It may be used to sequence the entire genome. This level of analysis covers almost all nucleotides in the genome, including mitochondrial and chromosomal DNA. In research contexts, whole genome sequencing is used more frequently than in therapeutic settings. It is applied to constitutional genetic disorders in clinical settings more often than to somatic cancer mutations. It is quite beneficial for many different kinds of diagnoses.

In addition, NGS can be performed at the transcriptome level, which encompasses the complete assembly of mRNA, rRNA, tRNA, micro-RNA, and noncoding RNA in a particular cell type. This is called RNA sequencing, as opposed to DNA sequencing. Fusion gene detection is also routinely accomplished using specially engineered mRNA sequencing. Focused panel sequencing, which typically cross-examines dozens or even hundreds of centred genes, is the most commonly utilised next-generation sequencing (NGS) analysis for cancer patients. These focused NGS analyses are typically created for a specific disease or group of related disorders, such as a panel created for carcinoma or myeloid leukaemia. In contrast to whole genome sequencing, such a focused panel has limited objectives. Therefore, it allows huge extra depth in sequencing, which is essential to cover selective mutations with distinct allelic mutation frequencies (Yachida et al., 2010; Steuer & Ramalingam, 2018; Jennings et al., 2017)

NGS for clinical utility

Entailing a variety of tasks, including as verifying assay precision, accuracy, reportable range, reference range, analytical sensitivity, and specificity. There must be a plan in place for validation. Authentication plans typically specify which subpar and excellent control samples

will be used. It is also possible to combine the positive and negative samples at remarkable ratios to create excellent controls with unique allelic mutation frequencies. The fundamentally excellent controls should ideally include several types of mutations, such as sensitive deletions, insertions, and single nucleotide mutations in particular. Furthermore, the validation format essentially needs to specify the relatively large number of samples that genuinely select to be examined, which is somewhat delicate (Faehling et al., 2017; Sholl et al., 2015).

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