Next Generation Sequencing

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NGS (Next generation sequencing) is a newly discovered technology that has been first invented in the early two thousand. The first implementation of NGS is called MPSS (Massively parallel signature sequencing). MPSS is a process which quantifies and identifies transcripts of mRNA. Its results are comparable with SAGE (serial analysis of gene expression), but the MPSS technique is able to store twenty times more information than SAGE.

With the development of the NGS technology, the Sanger method has become outdated and slow, but, due to constant research, the Sanger method has been the most versatile sequencing method for many of the present applications. The research around the Sanger method has brought to light new innovations in the domain such as:

* Invention of terminator dyes (fluorescently colored)
* Reduction of the necessary quantity of input DNA by using thermal-cycle sequencing
* Accurate and efficient way of incorporating the fluorescent terminating dyes into the developing strands of DNA by using a method called thermostable polymerases.
* Software applications and implementations that play a crucial role for analyzing and interpreting the sequences.

The Sanger method still shines when the application does not require a large throughput. For this particular reason, DNA sequencing companies and facilities sell and provide services and solutions based on Sanger sequencing.

A better name for NGS would be SGS (Second generation sequencing). This is due to the fact that science is always progressing and evolving, and, inevitably, a more efficient and accurate method would arise.

All of the Second gene sequencing methods can be grouped into two significant categories:

* sequencing by hybridization (SBH)
* sequencing by synthesis (SBS)

SBS technology is a more advanced and developed variation of Sanger sequencing, with the following changes: the dideoxy terminators are absent, recurrent cycles of synthesis, imaging, and methods that add nucleotides in the evolving chain. All of the before mentioned changes appear to be costly, but, by running the reactions in parallel and in reduced volumes (nanoliters, zeptoliters), the cost per base is manageable.

The cost is determined by a plethora of factors that are usually grouped under the estimate of „cost per base”. In most of the cases, the total cost is heavily dependent on the quantity requested and it is thoroughly negotiated between the client and the vendor. For example, sequencing facilities and centers usually benefit from discounts when ordering large quantities. Due to the nature of the process, the „cost per base” does not include the bioinformatics workflow and the labor.

Despite all of the progress and optimization made, the industry holds high two golden standards: reducing the „cost per base” and achieving the „thousand-dollar human genome”.

## Usage of NGS in clinical practices

Patient’s well-being can be easily improved by the right usage of the Next generation sequencing techniques, for example:

**By using NGS instead of the Sanger sequencing method, a larger range of mutations can be captured.** The human genome’s DNA variation range includes substitutions (small base changes), deletions of DNA, insertions, big genomic deletions of whole genes or exons, and repositioning such as translocations and inversions. The Sanger method is limited to the detecting of small insertions, substitutions and deletions. However, a full range of the genomic fluctuations and variations can be extracted from a singular experiment.

**Genomes can be interrogated with no bias**. Other methods, such as Capillary sequencing, need previous context such as pre-knowledge of the analyzed gene, but NGS doesn’t. It is totally unselective and it can interrogate entire genomes in order to discover new genes that provoke diseases and mutations.

**Mosaic mutations can be detected by the NGS’s increased accuracy and sensitivity**. Mosaic mutation occur as a result of the postfertilization phenomenon. These mutations can be found in varying quantities throughout the tissue and cells of individuals. Methods such as Capillary sequencing, due to their low sensitivity, might miss these kind of mutations as they are subtle enough to go undetected by traditional methods. However, Second generation sequencing (SGS) outputs a better read-out due to its increased sensitivity. This read-out contains valuable information about mutations that can be found in only a small number of cells, including the mosaic mutations. Furthermore, the sensitivity of SGS can be easily empowered, by simply increasing the depth of the sequencing.

## Usage of NGS in Oncology

The base idea of cancer causes and origins is that the disease is provoked by mutations that have been obtained somatically and, by consequence, cancer is categorized as a genome disease. Even though the Capillary sequencing cancer research has been going on for more than a decade, the analysis were limited to a reduced and small quantity of candidate samples and genes. With the apparition of Next generation sequencing, the genomes that have cancer can be thoroughly researched in their entireness.

## Limitation of NGS

The primary downside of NGS in the context of clinical applications is the infrastructure. NGS requires a complex infrastructure that is demands high computing power and storage, as well as trained personnel that are required to interpret the data and to analyze it in a comprehensive way.

Bibliography:

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