

# Next generation sequencing: from research area to clinical practice

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## ARTICLE INFO

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## ABSTRACT

Translating the power of high-throughput sequencing technologies from research area into clinical medicine is one of the major goal for several researchers and health-care providers. One of the important advantages of these technologies is that they can be successfully used in a numerous range of clinical applications. The efficiency of sequencing, that can now be achieved, is leading impressive progress in the diagnostics of common and rare genetic disorders, inherited forms of cancer, prenatal testing or infectious diseases, to cite some examples. Despite several challenges and limitations still remain to overcome, the high-throughput sequencing technologies are leading to real and unprecedented benefits for the medical care of patients.

## GENERAL OVERVIEW

Over the past decade great advances have been done in sequencing technologies. After Sanger Sequencing, the current gold standard approach, also known as dideoxy method [1], high-throughput sequencing has been developed and widespread in biomedical laboratories. The first one allows to analyse one DNA segment at time in laborious and time-consuming way while the second approach has the great advantage of performing a simultaneous analysis of several genomic regions, with a dramatic reduction also of the cost of sequencing per base [2].

Today the high-throughput next generation sequencing (NGS) instruments mainly used in biomedical laboratories are the Ion Torrent sequencers (Life Technologies) and the Illumina platforms (Illumina) [3,4].

All NGS technologies are based on the same general process, comprising template preparation, sequencing and data analysis. The unique combination of specific technical details distinguishes one technology from another and determines the type of data produced from each platform [5].

After the extraction of DNA, the first step of the sequencing process is the library preparation, which consists on the ligation of DNA fragments to platform specific oligonucleotide adapters [2]. After that each fragment is immobilized and clonally amplified. In Life Technologies approach, clonal amplification is performed by emulsion PCR, in which DNA fragments are amplified on the beads surface in oil-aqueous mixture [6–8]. Illumina approach otherwise is based on a unique “isothermal bridge amplification” reaction that occurs on the surface of the flow cell [9].

For sequencing Life Technologies exploited the native dNTP chemistry during base incorporation by DNA polymerase, that relies hydrogen ions, causing the pH modification that is detected by a modified silicon chip [10]. Illumina sequencing is

instead performed on a flow-cell and it is based on the existing Solexa *sequencing by synthesis* chemistry, based on the fluorescent detection released when the complementary fluorescently tagged nucleotides are incorporated [11].

In recent years, the advent of these benchtop NGS platforms on the marketplace has had an impressive impact in *-omics*, thanks to the huge amount of data obtained with a significant reduction of time and costs [12]. Indeed NGS has been applied in varied contexts, restricted not only to genomics but also to transcriptomics and epigenomics, such as in non-coding RNA expression profiling, finding transcription factor binding sites, RNA seq, ChIP-Seq or MeDIP, to cite few examples [13].

In research genetic studies NGS has been successfully exploited to identify new causative genes or variants associated to inherited diseases, especially in genetically heterogenous disorders, whose genetic basis was partially unknown and in which gene-by-gene Sanger sequencing approach would not have been economical or efficient [2]. For this purpose, NGS has been applied to whole-genome, exome or targeted sequencing, leading to the improvement of the current knowledge of genetic basis of several pathologies, such as retinitis pigmentosa, cardiomyopathies or inherited cancer [14–17].

More recently the widespread use of these rapid high-throughput technologies, the improvement of their performance and the overcoming of initial technical limitations are encouraging their transition from basic research into clinics with important benefits for routine patient management.

## USE OF NGS IN THE CLINICAL PRACTICE

Now NGS is an established test method in many clinical laboratories, in particular for the detection of germline and somatic genetic mutations.

The analysis of causative mutations in inherited diseases is performed using different approaches, exploiting targeted panel, whole exome, whole genome or mitochondrial DNA sequencing [3,18,19]. More in details, targeted panel analysis is usually applied to genetic test for different genetically heterogeneous disorders, such as renal, neurologic, connective tissue disorders, cardiomyopathies, immune deficiencies, blindness, deafness, and several forms of inherited cancer [15,18,20–23].

Even if the analyzed gene panel may vary between laboratories, target sequencing is the first approach of genetic test for inherited disorders, while whole exome sequencing is exploited for negative cases, in which targeted testing has not been informative. Moreover, whole exome approach is useful in rare diseases for trio testing, sequencing the child and both parents [24–26].

In oncology, targeted testing is widely used, exploiting two different approaches. In the first one the targeted panel may be focused only on principle genes associated to a particular type of malignancy, for example *BRCA1* and *BRCA2* gene for breast and ovarian cancer, while in the other one NGS approach allows to analyze a broader panel including genes associated with other cancers. Given the clinical overlapping between different forms of cancer, for example between ovarian cancer and Lynch syndrome, this latter approach may be useful to enhance the diagnostic yield [27–29]. In oncology, whole exome and whole genome sequencing are not currently used for clinical purpose, in order to avoid the potential risk of unactionable incidental findings [19,29].

More recently, several new NGS applications moved to the research area to clinical use, citing for example the analysis of cell-free DNA in the prenatal genetic-testing [30], circulating tumor DNA testing [31,32], human leukocyte antigen

(HLA) typing [33], microbial analysis [19], RNA sequencing and expression [19], and methylation [19], even if there are yet some challenges to overcome.

For example in HLA typing, it is difficult to differentiate low-frequency alleles from high-frequency artifacts and newer data analysis approach or the development of instruments for single-molecule sequencing, called third-generation sequencers, are solving these limitations [33,34].

Today testing of circulating tumor DNA (ctDNA), often referred to as “liquid biopsy”, is now available in clinics [35,36]. One possible approach is NGS, that presents a lot of potential applications, including diagnosis of cancer, monitoring for progression or relapse, and targeted therapy for a patient with a known cancer diagnosis [32,37,38]. Indeed, several studies have shown that ctDNA sequencing allows at first to detect somatic mutation in patients with known cancer diagnosis and then to monitor it in correlation with the relapse and progression of disease [39]. Without doubt the detection of ctDNA using NGS presents the great advantage to be a reasonable alternative to the repeated invasive biopsies for patients with metastatic cancers. However, it still presents some limitations due to a low sensitivity to detect early-stage cancer (false negatives), limiting until now the practical use of ctDNA for early cancer diagnosis or screening [38].

Other clinical applications of NGS include pharmacogenetics and microbial sequencing but these topics are beyond the scope of this article.

Although NGS is now widely used in clinics, several challenges still remain to overcome.

The main issues are, for example, the sequencing of genomic portions that are difficult to analyze, due their intrinsic features (pseudogenes, homologous regions, repetitive regions, GC-rich regions) and the limited ability to detect structural gene variation and copy number variation

(CNV) [40–43]. Sometimes the storage and the interpretation of huge amounts of sequence data, mainly of several novel or rare mutations, by trained health care professionals may be still an open challenge [44,45]. Moreover, a successful NGS testing need a collaborative effort between geneticists and physicians to combine and integrate clinical data and genetic analyses to guide medical care of patient.

## CONCLUSIONS

NGS technologies have revolutionized biological research and have deeply transform the fields of diagnostic pathology and clinical medicine. In the future, the use of NGS in clinical laboratories will surely increase as technology and bioinformatics, in order to address the current limitations, improve the quality of results, and increase the number of possible clinical applications.

However, the challenge for clinical laboratories will be to perform the most appropriate approach of NGS testing taking into account the clinical relevance, cost-effectiveness and clinical care of patient.

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