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Editorial Open Access

Should the Next Generation Sequencing be used as a Diagnostic Test?

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Editorial

More than ten years ago, next generation sequencing (NGS) technology surfaced and immediately became a favorable tool for scientists and industries. Growing usage and endless opportunities forecasted by scientific visionaries have accelerated the improvement of NGS technology. This technological advance has allowed for increased speed of testing, provoked the development of high throughput instruments, and dramatically decreased cost per test. As of today, the popularity of next generation sequencing is at its peak and every prestigious academic center and larger diagnostic laboratory possesses NGS instrumentation or plans to obtain one. The possibility to acquire a hefty amount of information by running just one test bends the rigid quality requirements and allows NGS to become a diagnostic tool used for patient care. But not all experts embrace the idea of giving an easy pass to this technology; they call for caution and perceive next generation sequencing as a promising child, which must grow and be polished before being allowed to play in the primary league of the diagnostic world.

In many institutions, an initial strategy of combining Sanger sequencing and pyrosequencing has been completely replaced by NGS or a combination of the "long reads" from pyrosequencing with the low operating cost of next generation sequencing. Such an approach allows for independent performance verification of both used systems. Some research laboratories strongly believe in the great performance of NGS. They use this technology not only as a primary method for discovering new genetic variations, but also as a reference system for error identification in pyrosequencing or for the assessment of the quality of "short read length" produced by other NGS platform. There are also some experts for whom conventional Sanger sequencing of small fragments of genome is no longer the gold standard for accuracy. They also believe that Sanger sequencing carries a higher risk of making errors than NGS. Nevertheless, for the majority of molecular groups, Sanger sequencing is still the gold standard method against which NGS is evaluated for accuracy and detection of systematic errors. It is undisputable and well documented that next generation sequencing tends to make more errors at the end of reads and at the GC rich regions, which are difficult to sequence. In addition, single base repeats are more susceptible to artificial insertion or deletion by the NGS procedure, and mapping of "reads" against a reference genome is a common source of error.

For oncological research, next-generation sequencing is a potentially perfect tool for discovering targets for pharmacotherapy, predicting outcome and response to the therapy, and tumor classification. Despite significant improvement in all aspects of NGS testing, accurate definition of genomic biomarkers and finding effective drug targets in cancer genomes remain a challenge. The only unquestionable advantage of NGS over the Sanger methodology is its ability to sequence ultra-long fragments. But when mapping a mutation other than a base substitution, the trade-off between longer reads and accuracy of testing still exists. An additional challenge in whole genome sequencing or whole exome sequencing by NGS for cancer testing is the requirement of a large amount of good quality DNA.

Compared to the classical sequencing methods, NGS produces a massive amount of data with the help of bioinformatics. The genomic rearrangement is performed by dedicated software and is perceived as a crucial step in the assembling of a correct sequence. As of today, a large number of genome mapping software applications is available on the market, but their performance is impossible to assess because consensus criteria for their evaluation do not exist. One of the reasons for this difficulty is the need for constant re-evaluation and updating, which makes any version of the program short-lived and quickly outdated. Additionally, unique evaluation criteria, such as thresholds for variant identification, make the software able to serve one testing platform only.

In order to be used as a diagnostic method, specific for NGS quality control (QC) program must be developed and implemented as part of testing. The separate elements of QC program may be found on the market, but they are not universally integrated into the one QC system. One of them is the evaluation of accuracy of "calling base in the sequence" which is controlled by a "Phred-like" scoring system. This system was developed based on a "Phred" scoring method used for Sanger sequencing. The limitations of the "Phred-like" system are that it is built exclusively for one platform, the scoring is unique for one system and it can't be used for measuring accuracy in comparison with other platforms.

The difficulties with NGS testing mentioned above are only a select few complications from a longer list of problems. Questionable reproducibility, the main elements in diagnostic testing, does not even make the list. How would the diagnostic community respond if the test offered to patients had the same performance characteristics as NGS testing?

One favorable argument for using NGS as a diagnostic test, despite its problems, is the potential of discovering new gene variations that would be otherwise missed with current technology. After careful clinical evaluation, these gene variants, may become the base for the patient therapeutic approach. However, the question about validity of results remains. Does the current status of technology and quality control programs guarantee that the discovered variants are real and not produced by technological error?

After honestly assessing such problems as lack of reproducibility, absence of standardized validation protocol, constantly evaluating sequence assembling software and lack of consistent evaluation for accuracy, we should admit that NSG, especially full genome sequencing, is not ready for wide spread use in the diagnostic world.

There may be some usefulness for using this technology for testing a small number of genes with well establish clinical significance, but the inherited technological glitches listed above must be addressed before patient testing. An efficient and restrictive QC program must also be in place and must run consistently?

The majority of molecular laboratories do an excellent job and perform high quality testing. However, in the current absence of official regulation, there are institutions that offer full genome screening performed by using a single drop of blood or a few oral cells. Certainly, laboratories are heavily regulated to the degree that patients' testing is becoming more and more difficult, and we should be careful with adding more restrictions. Yet on the other hand "when chaos governs the weakest get hurt". In an attempt to improve this situation, the College of American Pathologists, through the Laboratory Accreditation Committee, has released an updated "molecular checklist" that contains additional requirements for laboratories which seek CAP accreditation and perform NGS testing. Additionally, a new FDA regulation for the laboratory developed tests will also reinforce a better quality of the NGS testing for diagnostic purposes. But before any systematic regulation is in place, all laboratories performing NGS testing should develop a sound validation process with an emphasis on accuracy and reproducibility; establish internal and external QC programs; develop reliable control of accuracy and reproducibility; set up clear criteria for DNA quality that are specific for NGS; bring consistent, integrated and reliable software that can be operated by laboratory personnel which does not require constant supervision by an experienced bio-informatics department; and, specifically for oncologic diseases, establish a consistent set of genes which are tested for particular neoplasm. This list of suggestions does not address all the elements required for testing to be dependable and effective, but it may serve as a starting point for discussing what is needed for NGS to become a good diagnostic method.