Abstract
Specific genomic colorectal cancer alterations are increasingly linked to prognosis and/or response to specific anticancer agents. The identification of KRAS mutations as markers of resistance to epidermal growth factor receptor (EGFR) inhibitors has paved the way to the interrogation of numerous other markers of resistance to anti-EGFR therapy, such as NRAS, BRAF, and PIK3CA mutations. Other genomic and protein expression alterations have recently been identified as potential targets of treatment or as markers of chemotherapy or targeted-therapy resistance, including ERCC1 expression, c-Met expression, PTEN expression, HER2 amplification, HER3 expression, and rare KRAS mutations. As the number of distinct validated intratumor genomic assays increases, numerous molecular assays will need to be compiled into one multigene panel assay. Several companies and academic centers are now offering multigene assays to patients with metastatic colorectal cancer and other solid tumors. This article discusses the technology behind multigene assays, its limitations, its current advantages, and its potential in the clinical care of metastatic colorectal cancer. (JNCCN 2013;11[Suppl 4]:S9–S17)

With an estimated excess of 50,000 deaths per year, colorectal cancer continues to be the second leading cause of cancer death in the United States. Despite FDA approval of 10 different agents for the treatment of metastatic colorectal cancer, improvement in overall survival has been modest and the median survival is estimated at 20 to 30 months. One of the main obstacles toward significant improvements in overall survival is related to innate or acquired tumor resistance to chemotherapy and targeted agents. Furthermore, clinical studies and molecular genotyping have now clearly indicated the heterogeneity of colorectal cancer and the inadequacy of the concept of “one size fits all” in the systemic treatment of metastatic colorectal cancer.

For example, one of the major advances in the past 5 to 7 years has been the interrogation of the epidermal growth factor receptor (EGFR) pathway vis-à-vis cetuximab-based therapy in colorectal cancer. Retrospective analyses of tissue obtained from prospective studies of cetuximab or panitumumab in colorectal cancer indicate a lack of clinical benefit from anti-EGFR therapy in colorectal cancer tumors with KRAS mutations. However, some debate continues regarding the degree of resistance to anti-EGFR therapy among the various KRAS mutations, specifically with KRAS codon 13 mutations. Furthermore, colorectal tumors with BRAF V600E mutation have been associated with a more aggressive clinical behavior and increased resistance to chemotherapy and refractoriness to anti-EGFR therapy. Other mutations, such as PIK3CA and PTEN mutations, have been linked to resistance to anti-EGFR therapy in some studies, but their predictive role is yet to be conclusively validated. Other molecular characteristics have been implicated in the prognosis and response of colorectal cancer to various therapeutics agents. C-met overexpression has been associated with resistance to cetuximab treatment in primary xenograft models. Insulin-like growth factor 1 (IGF-1) overexpression has been linked to a benefit from IGF receptor (IGFR) targeting. MTHFR methylation and silencing have been associated with an increased likelihood of re-
response to temozolomide. Finally, ERCC-1 overexpression has been associated with resistance to platinum therapy. These are only a few examples of the changing landscape of target-directed therapy and patient selection in the management of metastatic colorectal cancer.

This increasingly complex treatment landscape highlights the need for a refined, molecularly characterized approach to treating metastatic colorectal cancer. Molecular diagnostics has now transcended beyond the application of mutation assays of a single genetic mutation, such as KRAS, to a more comprehensive assessment of genomic alterations. This review describes next-generation sequencing (NGS)–based multigene assays in clinical practice, their distinct advantages, and their limitations.

Application of Multigene Assays in Clinical Practice

The identification of multiple clinically relevant driver mutations in molecular and cellular mechanisms involved in tumor initiation, maintenance, and progression has improved the understanding of cancer pathogenesis and led to the discovery of novel drug targets and development of new treatment paradigms for patients with cancer. The standard of care for patients with metastatic colorectal cancer has shifted from selecting conventional chemotherapy based on the patient’s clinicopathologic features, to using biomarker-driven targeted treatment algorithms based on the molecular profile of a patient’s tumor, such as assessing the status of KRAS and BRAF. These genotype-based targeted therapies represent the first step toward personalizing the treatment of different types of cancers. Multiple commercial molecular laboratories and numerous academic centers have begun to develop multiplex mutational profiling assays in clinical molecular diagnostic laboratories to sequence and genotype tumors and use the mutational profiles for clinical decision-making (Table 1). Recent technological advances in high-throughput target gene panel sequencing and genomic profiling (exome capture or whole genome) using NGS technology offer the opportunity to broadly interrogate a patient’s cancer genome from tumor biopsies for determining personalized therapy (Figure 1). Therefore, molecularly defined subsets of patients with metastatic colorectal cancer will be given the opportunity to clinically explore, at least through appropriate clinical trials, a growing list of novel molecularly targeted therapeutics that are currently available.

NGS Platforms

Cancer molecular diagnosis and treatment have co-evolved through the decades from single gene–based assays toward genomic profiling of individual patients. Currently, clinicopathologic features of tumors remain the primary standard to select available drugs for an individual patient. Traditional single-gene molecular tests, using Sanger DNA sequencing, pyrosequencing, or allele-specific or melting curve real-time polymerase chain reaction (RT-PCR), offer varying degrees of sensitivity but also have major disadvantages: they 1) are time-consuming (Sanger); 2) have high costs and are labor-intensive; 3) shorten length read limits (pyrosequencing); 4) lack the ability to detect deletions, translocations, and copy number changes; and 5) have limited scalability and multiplex capability. Single gene–based tests for KRAS and BRAF mutations in colorectal cancer have been incorporated into oncology practice for cetuximab and panitumumab therapy since 2008. High-throughput technologies using NGS that enable massively parallel sequencing of nucleic acid (DNA and RNA) at a significantly lower cost per base, faster speed, higher sensitivity, and reduced error rate (Table 2) continue to be deployed in clinical laboratories. The 3 levels of analysis that can be performed by NGS, with increasing complexity, are targeted-gene panels, exome sequencing, and whole-genome sequencing. Disease-targeted gene panels focus on a limited set of genes known to be disease-associated and allow greater depth of coverage for increased analytical sensitivity and specificity, and better interpretation of findings in a clinical context. Exome sequencing covers all the coding regions of the genome with approximately 85% of recognized disease-causing mutations. Exome sequencing is often used to detect variants in known disease-associated genes and discovering new gene–disease associations. Whole-genome sequencing covers both coding and noncoding regions that may affect gene expression of disease-associated genes of the genome.

The 3 major components in NGS involve sample preparation, sequencing, and data analysis. Because clinical samples available for molecular studies...
are from formalin-fixed paraffin-embedded (FFPE) tissue, in which DNA quality and amount can be limiting, minimal amount of input sample can be used for a massively parallel sequencing approach to detect tumor genomic alterations in FFPE tumor samples.\textsuperscript{30–32} Genomic DNA extracted from a patient sample is enriched for a subset of genomic targets (targeted-gene panels or exome sequencing). These targets, flanked by platform-specific adapters, are the required input for the currently available NGS platforms. Multiple NGS platforms have been developed by Roche, Illumina, and Life Technologies with the capacity to massively sequence millions of DNA fragments in parallel, which differ in sequencing chemistry and impact the differences in total sequence capacity, sequence read length, sequence run time, and quality and accuracy of the data (Table 2).\textsuperscript{27,33,34} Depending on the platform, a series of processing steps are required to convert the DNA sample into appropriate sequencing format. A brief overview and the relative strengths and disadvantages of the different NGS platforms are summarized in Table 2.

**Targeted Multigene DNA Sequencing**

Targeted multigene panels use several targeted enrichment strategies: PCR-amplicon–based and hybridization capture approaches.\textsuperscript{35} PCR-based approaches, if adequately normalized before pooling and sequencing, are highly specific and generate more uniform coverage than capture hybridization approaches. Ion AmpliSeq technology (Life Tech-

### Table 1 Multigene Assays Using Different Methodologies\textsuperscript{a}

<table>
<thead>
<tr>
<th>Institute</th>
<th>Type of Test</th>
<th>Multigene Assay</th>
<th>Number of Genes</th>
<th>Web Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quest Diagnostics</td>
<td>PCR: Sanger sequencing</td>
<td>Colorectal Cancer Mutation Panel</td>
<td>4 (KRAS, PIK3CA, BRAF, NRAS)</td>
<td><a href="http://www.questdiagnostics.com">www.questdiagnostics.com</a></td>
</tr>
<tr>
<td>CARIS Life Sciences</td>
<td>NGS panel: Illumina MiSeq (PCR-based)</td>
<td>Molecular Intelligence for Colorectal Cancer</td>
<td>44</td>
<td><a href="http://www.carismolecularintelligence.com">www.carismolecularintelligence.com</a></td>
</tr>
<tr>
<td>City of Hope</td>
<td>NGS panel: AmpliSeq Ion Torrent PGM</td>
<td>Onco-44-Gene Panel</td>
<td>44</td>
<td><a href="http://www.cityofhope.org/mdl">www.cityofhope.org/mdl</a></td>
</tr>
<tr>
<td>Foundation Medicine, Inc.</td>
<td>NGS panel: Illumina HiSeq (hybrid capture)</td>
<td>FoundationOne pan-cancer test</td>
<td>236</td>
<td><a href="http://www.foundationone.com">www.foundationone.com</a></td>
</tr>
<tr>
<td>Knight Diagnostic Laboratories</td>
<td>NGS panel: AmpliSeq Ion Torrent PGM</td>
<td>GeneTrials 38 Cancer Gene Panel</td>
<td>38</td>
<td><a href="http://www.knightdxlabs.com">www.knightdxlabs.com</a></td>
</tr>
<tr>
<td>Baylor College of Medicine</td>
<td>NGS panel: AmpliSeq Ion Torrent PGM</td>
<td>Cancer Gene Mutation Panel (version 2)</td>
<td>50</td>
<td><a href="http://www.bcm.edu/geneticlabs">www.bcm.edu/geneticlabs</a></td>
</tr>
<tr>
<td>Washington University</td>
<td>NGS panel: HiSeq Illumina</td>
<td>UW-OncoPlex cancer gene panel</td>
<td>194</td>
<td>menu.labmed.washington.edu</td>
</tr>
<tr>
<td>AI BioTech</td>
<td></td>
<td>KRAS-BRAF complete</td>
<td>23 most common KRAS and BRAF mutations</td>
<td><a href="http://www.aiibiotech.com">www.aiibiotech.com</a></td>
</tr>
<tr>
<td>Amby Genetics</td>
<td></td>
<td>Exome sequencing</td>
<td></td>
<td><a href="http://www.ambygen.com">www.ambygen.com</a></td>
</tr>
<tr>
<td>Memorial Sloan-Kettering Cancer Center</td>
<td>NGS panel: MiSeq Illumina</td>
<td>TruSeq Amplicon - Cancer Panel</td>
<td>48</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: NGS, next-generation sequencing; PCR, polymerase chain reaction.

\textsuperscript{a}Table does not represent a comprehensive list of current institutes providing multigene assays.
Technologies, Carlsbad, CA) is based on ultrahigh-multiplex PCR in a single PCR reaction, and provides simple and fast library construction for the targeted sequencing of a target set of genes with very little input DNA (10 ng). The TruSeq Amplicon (Illumina, San Diego, CA) allows amplification of multiple targets with minimum genomic DNA (150 ng) through hybridizing 2 independent 5′ and 3′ flanking oligonucleotides to a genomic DNA template, enabling polymerase extension and ligation, and incorporation of universal barcoded indices and Illumina sequencing adapters. However, multiplex PCR raises the challenge of uniform reads across targeted amplicons and may require rebalancing of oligonucleotide pools to achieve adequate and uniform coverage. In the target hybridization capture approach, adaptor-modified genomic DNA libraries are hybridized to target-specific probes either immobilized on a microarray surface or in solution. The sample quality, presence of variants within the capture region, and DNA fragment size (ie, shorter fragments being captured with higher specificity than longer ones) have a large influence over the outcome of target enrichment. Compared with PCR, hybridization capture may lack specificity because of cross-hybridization and GC content of target sequence. Genes high in GC content are difficult to capture or amplify and are poorly represented in NGS data; however, optimized methods attempt to overcome this obstacle. These target-enrichment methods aim to increase the scale of PCR, and minimize reagent use, technical labor, and amount of DNA template required. Reliability of targeted enrichment, albeit hybridization capture–based or multiplex PCR–based, is very important in obtaining adequate representation, coverage, and sequence depth for all targeted regions.

Because biopsy samples and amount of genomic DNA from FFPE tissue are limiting, the concept of a single test and a single drug is becoming unsustainable, with a growing list of molecular targets and need for assessment of multiple genomic alterations. Several companies and academic institutions (Table 1) are offering pan-cancer NGS panels for solid tumors, comprising many prognostic and actionable genes across multiple tumor types, to simultaneously identify actionable driver mutations in an individual patient’s tumor. These pan-cancer NGS panels are not tumor-specific because of the overlap of many driver gene mutations in the various tumor types. The NGS gene panels differ in the number of targeted genes (ie, full-length and mutation hotspots), platform used, and target enrichment methods. Although bioinformatics for NGS analysis remains a significant challenge, most centers have analysis pipelines for targeted NGS panels, in which data analysis becomes more manageable compared with whole-genome or exome sequencing. The Founda-
Table 2  Overview of Next-Generation Sequencing Platforms Commonly Used for Multigene Assays

<table>
<thead>
<tr>
<th>Platform</th>
<th>Technique</th>
<th>Amplification</th>
<th>Sequencing Run Time</th>
<th>Read Lengths (Bases)</th>
<th>Error Rate (in 100 Bp)</th>
<th>Major Uses</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Illumina – MiSeq</td>
<td>Flow cell–based, reversible dye termination, and 4-color optical imaging(^{a})</td>
<td>Bridge PCR(^{a})</td>
<td>4–27 h</td>
<td>36–300 bp (single-end, paired-end(^{a}))</td>
<td>0.1(^{44})</td>
<td>Targeted gene panels</td>
<td>Widest use; automated workflow (MiSeq); low error rates, high-quality sequence; user-friendly sample preparation</td>
<td>Need balanced nucleotide in first 4 bases</td>
</tr>
<tr>
<td>Illumina – HiSeq</td>
<td>Flow cell–based, reversible dye termination, and 4-color optical imaging(^{a})</td>
<td>Bridge PCR(^{a})</td>
<td>2.5–11.0 d</td>
<td>36–300 bp (single-end, paired-end(^{a}))</td>
<td>≥0.1</td>
<td>Exome and whole-genome sequencing, targeted gene panels</td>
<td>Highest throughput; lowest cost per base</td>
<td>High cost of instrument and maintenance; high computation needs</td>
</tr>
<tr>
<td>Roche 454 – GS FLX+/GS Junior</td>
<td>Sequence-by-synthesis pyrosequencing(^{b}); CCD light imaging(^{b})</td>
<td>Emulsion PCR(^{c})</td>
<td>GS Junior: 10 h</td>
<td>FLX+: 700 bp(^{d})</td>
<td>FLX+: 1.5(^{54})</td>
<td>Targeted exon sequencing; confirmatory sequencing; SNP sequencing</td>
<td>Fast, high accuracy; long reads useful for mapping and confirming</td>
<td>Homopolymer errors; expensive reagents; high cost per Mb</td>
</tr>
<tr>
<td>Life Technologies – Ion Torrent PGM</td>
<td>Semiconductor-based hydrogen ion detection(^{e}); standard dNTP sequencing chemistry</td>
<td>Emulsion PCR(^{c})</td>
<td>Fast, 3 h</td>
<td>200–400 bp (depends on chip used)</td>
<td>0.38(^{48})</td>
<td>Targeted-gene panels, SNP detection</td>
<td>Label-free chemistry; lowest cost per instrument, fastest sequencing time, highly scalable, long read potential</td>
<td>Homopolymer errors; no paired-end read; high indel error rate; laborious template preparation but semiautomatable</td>
</tr>
</tbody>
</table>

Abbreviations: bp, base pairs; dNTP, deoxynucleotide; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism

Adapted from Metzker ML. Sequencing technologies - the next generation. Nat Rev Genet 2010;11:31–46

\(^{a}\)With the Illumina platform, DNA clusters are created directly on a glass surface, known as a flow cell, through bridge amplification (PCR that occurs between primers bound to a surface) and sequenced.\(^{45}\) Illumina uses reversible dye terminator chemistry. During sequencing, all 4 nucleotides, each labeled with a different dye, are incorporated and imaged simultaneously (4-color optical imaging). After imaging, a cleavage step removes the fluorescent dyes and regenerates the 3’-OH group, in which iterative cycles of single-base incorporation, imaging, and cleavage of the terminator chemistry can proceed.\(^{27}\) Single-end reads are read from one direction and are similar to Sanger sequences. Paired-end reads allow both ends of a template to be sequenced.

\(^{b}\)A total of 454 platforms use pyrosequencing, whereby chemiluminescent signal, detected by a high-resolution charge-coupled device (CDD) camera, indicates base incorporation and the intensity of signal correlates to the number of bases incorporated.\(^{51}\)

\(^{c}\)For Roche (454) and Life Technologies (Ion Torrent), emulsion PCR is used to generate clonal DNA fragments on beads in emulsion oil droplet containing a single bead and single DNA molecule.\(^{22}\) The amplified templates, enriched on capture beads, are subsequently deposited into separate wells and sequenced in massively parallel manner.\(^{52,53}\)

\(^{d}\)Mode read length: the individual fragment read length is variable.

\(^{e}\)Information based on company sources.

\(^{f}\)Homopolymer repeats are contiguous runs of the same base pair (eg, 5’-AAAAA-3’) that exist in the human genome. Homopolymer-associated sequence errors are unique to NGS that reads signal intensity (eg, pyrosequencing and light imaging) as a measure of homopolymer length, and can contribute to false insertions and deletions (indels).\(^{55,56}\) The Illumina MiSeq had the lowest indels, whereas the GS Junior and PGM had higher indel error rates because of substantial homopolymers.\(^{55,56}\)

\(^{g}\)Ion Torrent uses detects signal through the release of hydrogen ions resulting from the activity of DNA polymerase during nucleotide incorporation.\(^{57}\)
Challenges remain for clinical oncologists regarding how to select, interpret, and apply these new genetic alterations in metastatic colorectal cancer. The only genetic mutation currently endorsed by NCCN and ASCO to guide anti-EGFR therapy in metastatic colorectal cancer is KRAS. Many other genetic alterations have been associated with chemotherapy or targeted therapy resistance, such as PIK3CA mutations, HER2 amplification, PTEN mutations, BRAF mutations, and NRAS and HRAS mutations, as the authors previously reviewed. Furthermore, increasing evidence suggests that KRAS mutations involving exons 3 and 4, which are not captured in most currently used KRAS assays (focused on six mutations in codon 12 and one mutation in codon 13 of exon 2), may also be associated with anti-EGFR resistance. Although multigene assays can identify patients with these genetic alterations and direct them to specific clinical trials, no definitive clinical evidence at this point suggests that this strategy is beneficial to the individual patients. Early clinical trials do not support a robust clinical activity to PIK3CA or BRAF targeting in metastatic colorectal cancer, and clinical data continue to be lacking in the setting of HER2 amplification.

The understanding of molecular biomarkers that drive tumorigenesis and maintenance of malignancy has led to the rational utility of clinically targeted therapy, and patient relapse risk assessment and cancer prognosis. Although single targets for personalized therapy are currently exploited (KRAS and BRAF), future treatment strategies may

Discussion

NGS-based multigene panels are currently being integrated into clinical practice as a suitable platform to provide quantitative, sensitive, and accurate sequencing data on a constantly increasing number of molecular mutations. NGS provided sensitivity superior to Sanger sequencing and pyrosequencing in detecting EGFR and KRAS mutations in lung cancer specimens and clinical response to EGFR inhibitor. Challenges remain for clinical oncologists regarding how to select, interpret, and apply these new genetic and genomic assays for patient treatment to improve clinical outcome. The ultimate goal of NGS-based multigene cancer sequencing panels is to provide oncologists with timely information on potentially actionable mutations to help guide patient management. The term actionable remains elusive, because it is dependent on the definition by different providers of NGS-based cancer panels. Several academic centers defined actionable to include FDA-approved drugs for the patient’s cancer type, and off-label use of FDA-approved drugs. Foundation Medicine, Inc., provider of the 236-gene FoundationOne pan-cancer test, more broadly defined actionable as FDA-approved targeted therapy in the solid tumor under study or in another tumor type, and any clinical trial under investigation for a therapy targeting the alteration. Currently available multigene NGS-based cancer panels include genes for which the FDA has approved single-gene companion diagnostics, and also allow interrogation of a patient’s other cancer-related mutations in a single assay instead of a series of tests, which is much more affordable, and saves time and tissues. Recently, standards and professional practice guidelines were established for NGS for clinical applications to assist clinical laboratories with the validation of NGS methods and platforms, monitoring of NGS testing, and interpretation and reporting of variants found using these technologies. NCCN has not recommended the standard use of multigene assays in metastatic colorectal cancer. The only genetic mutation currently endorsed by NCCN and ASCO to guide anti-EGFR therapy in metastatic colorectal cancer is KRAS. Many other genetic alterations have been associated with chemotherapy or targeted therapy resistance, such as PIK3CA mutations, HER2 amplification, PTEN mutations, BRAF mutations, and NRAS and HRAS mutations, as the authors previously reviewed. Furthermore, increasing evidence suggests that KRAS mutations involving exons 3 and 4, which are not captured in most currently used KRAS assays (focused on six mutations in codon 12 and one mutation in codon 13 of exon 2), may also be associated with anti-EGFR resistance. Although multigene assays can identify patients with these genetic alterations and direct them to specific clinical trials, no definitive clinical evidence at this point suggests that this strategy is beneficial to the individual patients. Early clinical trials do not support a robust clinical activity to PIK3CA or BRAF targeting in metastatic colorectal cancer, and clinical data continue to be lacking in the setting of HER2 amplification.

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Multigene Assays

depend on therapies directed toward multiple targets to avoid relapses and may allow monitoring of disease response to therapy (ie, emerging resistance and acquired mutations). Therefore, the utility of pan-cancer NGS multigene panels may be advantageous in building a comprehensive knowledge base of genes and mutations that may direct patients to future targeted therapies.

A genomic revolution is occurring that will transform medicine and how patients are treated. Multigene assays undoubtedly have the potential to provide a more efficient platform for personalized medicine in colorectal cancer. However, the reality is that the clinical data provided from these assays do not, to date, confirm an advantage over assays focusing on 2 genes in colorectal cancer (KRAS and BRAF). This will likely change as the utility of additional genomic alterations in the clinical management of patients with colorectal cancer is confirmed. Therefore, multigene assays, such as those developed by Foundation Medicine and CARIS, can be regarded as potential strategies to direct patients and providers to more patient-selective clinical trials, rather than a superior standard clinical practice at this time. These multigene assays are more consistent with experimental screening assays, rather than reflective of standard-of-care practice.

Some considerations exist for clinical NGS: sequencing performance, amplicon coverage and sensitivity, and variant detection. Challenges for variant detection in cancer result from inherent characteristics of tumor samples: DNA quality from FFPE tissues, aneuploidy, tumor heterogeneity, and contamination with normal tissue. Mutations can be observed in the range of 1% to 20%, which is below the currently accepted cutoff value of conventional Sanger-based sequencing and pyrosequencing. Sensitivities of well below 1% can be seen if tens of thousands of reads are generated. One can envision that a patient with a specific molecular mutation or a disease-relevant hot spot region can be targeted and sequenced with sufficient depth, so that monitoring minimal residual disease will be possible (eg, RUNX1 runt-related transcription factor 1 and CEBPA CCAAT/enhancer binding protein α).48,49

The real drive for NGS-based multigene panels will be the ability to rapidly and comprehensively assess tumors at the molecular level, in a cost-effective manner, with the short turnaround time that is required by individualized treatment regimens. Small molecule inhibitors and antibodies against druggable gene targets will continue to evolve, and these multigene panels need to be just as flexible. Pan-cancer multigene panels, with high representation of cancer-related genes, increase the complexity of analysis and interpretation: variants of unknown significance and function are overstated for clinical care given that not all genes have clear clinical value. Generic “cancer” multigene gene panels may evolve toward more disease-specific gene panels, wherein data are more manageable and relevant to cancer type in regard to actionable, targeted therapies, therapeutic implications (resistance), prognosis, and recurrent risks. This will allow multiplexing of multiple samples per run, thereby reducing cost and affordability of sequencing. NGS technology is evolving and progressing fast, allowing the possibility that this point will be reached in the foreseeable future.

References


