Abstract

Multiple myeloma (MM) is a complex disease, characterized by genomic instability and high levels of clonal heterogeneity. As the disease evolves, the DNA found in plasma cells becomes increasingly disordered, and multiple clones of the malignant cells fight for dominance during progression.

Many new therapies for MM now provide improved outcomes, yet still, the complexity of the disease demands better tools for predicting and measuring treatment response.

Advances in Next-Generation Sequencing (NGS) technologies have enabled a deep understanding of the genomic basis of MM, and provide comprehensive insight into disease progression.

This white paper will examine how targeted NGS techniques will continue to enhance our understanding of MM cell biology, and in the future, will help to better predict therapy response, allowing for tailored treatment based on genomic profiles.
Introduction

NGS has been instrumental in shaping our understanding of the genomics of MM, and now has come center stage as one of the preferred methods for the detection of minimal residual disease (MRD) in MM.

This white paper is presented for a general audience. For those readers less conversant in the complexity of MM, we first provide an overview of the characteristics of MM.

The contributions made by NGS technology in understanding MM are also summarized, with additional discussion of what applications may pave the way for advantageous use of NGS in the future.

As different NGS methods have been used to analyze MM, and others are discussed herein, a glossary of terms related to NGS technologies is provided at the end of this whitepaper.

Disease overview

There are an estimated 229,000 existing cases of MM worldwide, and roughly 114,000 new cases are diagnosed each year [1-2].

Tracking the progression of MM is complicated due to the fact that the B cell compartment is a continually shifting population of cells of different lineage within the bone marrow and throughout the blood supply.

There are numerous host-related factors that affect the progression and outcome in MM, including age, frailty and comorbidities, among others [3].

It’s been observed that MM evolves from an asymptomatic pre-malignant stage known as monoclonal gammopathy of undetermined significance (MGUS) [4-5], and more than 50% of people diagnosed with MGUS will have had the condition for over 10 years prior to diagnosis [6-7].

An intermediate and more advanced asymptomatic stage known as smoldering multiple myeloma (SMM), progresses to the multiple myeloma stage over the course of five to ten years [8].

The final stage of disease progression is plasma cell leukemia (PCL), where the diseased plasma cell becomes proliferative, escaping the bone marrow with rapid expansion and ultimately leading to death [9].

Treatment of MM has advanced in recent years and includes numerous therapies, novel agents, multi-drug combinations, and autologous hematopoietic stem cell transplantation. However, the variations in disease progression make it difficult to predict which treatment will have the most impact [10].

Most MM cases now achieve some response to treatment. Yet relapse is inevitable in the majority of cases, which indicates a persistent disease that as yet remains uncontrollable by conventional treatments [11].

NGS approaches to MM

The genomics of multiple myeloma have been studied extensively with the aid of NGS technologies, including Whole Genome Sequencing (WGS), Whole Exome Sequencing (WES) and Targeted Sequencing (TS). Taken together, this

Figure 1. Clonal composition of multiple myeloma during disease progression and treatment. Derived from Morgan [9] and Röllig [35].
body of research has provided significant insight into the DNA alterations of MM cells, the characteristics of the disease and its progression.

Below is a brief summary of the broad knowledge that NGS has contributed to our understanding of MM, with an indication of how the management of MM will evolve as a result of these discoveries.

**Somatic mutations**

Several landmark studies have employed NGS to identify recurrent genetic mutations in MM that are believed to drive oncogenesis [9, 12, 13, 14, and Table 1]. These studies have opened avenues of research to clarify the role of recurrent mutations in progression, association with cytogenetic abnormalities and their usefulness for prognosis and risk stratification.

Common mutations have been found to occur through diverse pathways, and many have been shown to influence deregulation. NRAS, KRAS and BRAF are associated with disease progression, and have been observed within the MAPK pathway, but as yet show no association with progression-free survival (PFS) or overall survival (OS) [12]. Likewise, mutations found in the NF-kB pathway (TRAF3, CYLD and LTB) also appear to have no impact on survival [12].

Mutated genes found in the DNA-repair pathway (ATM, ATR and TP53) have been observed in roughly 15% of subjects, and these mutations are generally found to be associated with poor outcomes [12].

Among other recurrent mutations, DIS3 and FAM46C are thought to be tumor suppressor genes, while IRF4, LTB, PRDM1 and SP140, are involved in B cell lineage differentiation [12].

These mutations may potentially influence therapy response, and thus continued NGS studies will provide an informed base for the development of new MM treatments that can target specific pathways shown to influence progression and outcomes. This has fuelled complementary research to find drugs that may be combined with existing therapies such as monoclonal antibodies, immunomodulatory agents and proteasome inhibitors [15].

For example, it’s been shown that NRAS mutations are associated with a low response to bortezomib at the time of relapse [14], and IRF4 mutations have shown greater sensitivity to immunomodulatory agents. Several mutations have shown to be targeted by appropriate inhibitors, and one reported case indicated a patient with BRAF mutation responded favorably to the BRAF inhibitor vemurafenib [16].

A recent study also showed that a high level of somatic mutations may influence response to targeted immunotherapies, based on their correlation to neoantigen load [17].

Identifying these driver mutations in MM should eventually pave the way for precision medicine, where therapies can be tailored to each patient based on particular mutations, and for those that are “actionable”, therapies can inhibit the mutated or active genes.

**Clonal heterogeneity**

MM is marked by significant clonal heterogeneity, where multiple clones and subclones of malignant cells co-exist in the plasma cell population.

Our knowledge of clonal evolution has advanced significantly through the effective use of NGS technology. Sequential sequencing has shown how clones evolve over time, often first appearing at diagnosis and then evolving after treatment [13].

In some cases clonality is also characterized by gains and losses in copy number alterations (CNAs), indicating multiple clones found at baseline change during disease progression, and that clonal evolution may shift between different time points during progression [18].

When measured in response to therapy, clones have been shown to follow linear shifts, as seen in Figure 1, whereas new subclones evolve directly or find dominance in a later stage, or through branched evolution where clones evolve via multiple mutational pathways. In addition, stable heterogeneity can be found where the same clones and sub-

<table>
<thead>
<tr>
<th>Gene</th>
<th>Lohr et al (n = 203)</th>
<th>Bolli et al (n = 67)</th>
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clones are identified before and after therapy [18].

As clonal heterogeneity increases with disease progression and increases risk, it’s possible that subclones may harbor levels of resistance to drugs or treatment [19]. Thus more NGS studies are needed to identify the lesions (mutations, CNAs or rearrangements) that are truly clonal, and can be used to track disease persistence, from those that are acquired or lost during evolution and need to be tracked to inform treatment response.

In this context, the future use of NGS becomes a critical tool for the management of MM, where clinicians can effectively tune therapies to the exact clonal nature of the observed disease, and then provide data to detect, characterize and predict optimal treatment for each individual case, post-transplant, and possibly even after each round of therapy.

Diagnosis, prognosis and risk stratification

Historically, the diagnosis of MM has been largely indicated by 10% or more clonal plasma cells in bone marrow, and M protein found in serum [8].

Prognosis and risk stratification have been measured using a combination of Fluorescence in situ hybridization (FISH), G-banded karyotype and gene expression profiling. These methods have been invaluable, identifying the majority of cytogenetic abnormalities and structural rearrangements that drive MM. These are divided into two subtypes:

**Primary cytogenetic abnormalities** first occur when the normal plasma cell transitions to a mutated or clonal cell during the premalignant MGUS or SMM stages [9, 20], and include trisomies of chromosomes 3, 5, 7, 9, 11, 15, 19 and 21, and/or a combination of IgH translocations in t(4;14), t(6;14), t(11;14), t(14;16) and t(14;20).

**Secondary cytogenetic abnormalities** occur as the disease further progresses from SMM, and includes deletions (1p, 6q, 8p, 11q, 13, 14q, 16q, 17p), gains (1q, 12p, 17q) and MYC translocations [9, 20].

Methods for risk stratification have evolved over the last decade, effectively segmenting populations that respond to different treatments, and have helped to extend life expectancy for up to one decade or more [21-22].

The most current guideline is the Revised International Staging System (RISS). It offers a unified approach to prognosis, incorporating the prognostic indicators of disease biology indicated above, and outlined in Table 2.

Another risk stratification guideline (mSMART) was developed by the Mayo Clinic to provide therapeutic guidance for subjects in different risk categories [23, Table 2].

In recent years, translational researchers have utilized NGS to characterize the genomic changes in MM, adding new insight about indels, gene fusions, chromosomal abnormalities, single nucleotide variants (SNVs), and single nucleotide polymorphisms (SNPs), as well as larger shifts in DNA and RNA.

One landmark study by Bolli et al. used a targeted sequencing approach to illustrate how an NGS pipeline focused on the study of pathogenesis, diagnosis and prognosis of MM can be implemented in diagnostic laboratories that have limited NGS expertise [24]. This study showed that targeted NGS provides the necessary tools to analyze somatic mutations, IgH translocations and rearrangements, as well as CNAs in prognostically significant regions.

Importantly, several recent studies have echoed the results of Bolli, and also argue that NGS can characterize all of the cytogenetic abnormalities found in MM as effectively as FISH, and often for less cost.

A study by Fiala et al. used data from the CoMMpass study to show that when NGS is used for prognostication instead of FISH, subjects can be better stratified for risk according to the RISS model, especially in stages II and III [25]. Similarly, a study by Szalat et al. used WGS to perform a genome-wide analysis of all rearrangements in the IgH region, and delivered a comprehensive

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<td>Serum albumin  3.5 g/dL</td>
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<td>Serum β-2 microglobulin &lt;3.5 mg/L</td>
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<td>No high risk cytogenetics</td>
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<td>Normal serum lactate dehydrogenase level</td>
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<tr>
<th>Stage II</th>
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<tr>
<td>Not Stage I or III</td>
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<td>Serum β-2 microglobulin &gt;5.5 mg/L</td>
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<th>Stage III</th>
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<td>High risk cytogenetics [t(4;14), t(14;16), or del(17p)] or Elevated serum lactate dehydrogenase level</td>
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<th>Mayo Stratification of Myeloma and Risk-Adapted Therapy (mSmart)</th>
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<tr>
<td><strong>High Risk</strong> del(17p), t(14;16), t(14;20), High risk gene expression profiling signature</td>
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<td><strong>Intermediate Risk</strong> t(4;14), del(13), hypodiploidy, plasma cell labeling index (PCLI) ≥ 3%</td>
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<td><strong>Standard Risk</strong> All others including: t(11;14), t(6;14)</td>
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catalogue of rearrangements that proved more thorough than other technologies [26]. Finally, a study by Jiménez et al., produced results similar to Bolli, and furthermore showed that NGS can accurately identify V(D)J clonal rearrangements.

Minimal residual disease
The goal of MM treatment (in fact for all hematologic malignancies) is to eradicate the number of diseased plasma cells from within the body, leading to symptom-free remission. However, for all patients, while there can appear to be complete response (CR) following therapy that is indicative of remission, often there theoretically remains a very small number of diseased cells in the body, residing in the bone marrow or blood, which is believed to cause relapse. This is known as minimal residual disease (MRD).

MRD has become a hot topic in MM, as it provides a way to detect deep and sustained response to treatment, which is a powerful endpoint for clinical trials. Research has shown that following treatment, very low levels of MRD indicate improved clinical outcome and long-term survival, whereas high levels of MRD are associated with poor outcomes and disease relapse [11].

This has become important because a new drug or treatment may get accelerated regulatory approval when it’s attached to an endpoint that can predict clinical benefit, also known as a “surrogate marker”.

While there is considerable ongoing research to validate different technologies, as yet none have been qualified by the FDA or the European Medicines Agency to serve as a “drug development tool” for MRD [27].

As such, the International Myeloma Working Group noted in a recent update to their Response Criteria [11], that the measurement of MRD requires very sensitive technology that can detect disease in 1 out of 1 million MM cells, and recommended that NGS, with a sensitivity of at least 10^{-6} or greater [Figure 2], is one of the technologies that should be used to detect MRD [11].

A study by Martinez-Lopez using deep IgH sequencing [10] showed that MRD was prognostic for time to tumor progression. The results were compared with tests made by multi-color flow cytometry (MFC), and analysis showed that sequencing detected MRD-negative and predicted progression-free survival in 91% of subjects, compared with 83% for MFC.

The sensitivity of NGS was also demonstrated in an analysis of samples from the IFM/DFCI 2009 Trial [28], where in comparison with MFC, NGS delivered much more prognostic information, with a sensitivity of 1 in 1 million cells (10^{-6}). Moreover, Korde et al. found that in comparison with MFC, NGS detected 30% more MRD-positive cases [29].

Further research may prove that genome-wide resolution and greater sensitivity of NGS will find a strong correlation among MM genomics, treatment response and durable disease-free status, with the ultimate goal of keeping the disease in remission. A future trend will show the adoption of NGS as a critical tool for MRD assessment, where NGS data can guide treatment at multiple time-points during disease progression.

Figure 2. The evolution of technologies that measure minimal residual disease. Derived from Roche [36].
Study consortiums leverage NGS data

Recently, NGS has been integrated into several large international clinical trials, where various NGS methods are now used to advance the genomic characterization of MM with improved analysis and data sharing.

For example, the CoMMpass trial, which is organized by the Multiple Myeloma Research Foundation (MMRF), is a longitudinal, observational study of newly diagnosed symptomatic subjects. The study was initiated in 2011 and includes clinical sites in North America and Europe. Industry partners include Amgen, Inc., Bristol-Myers Squibb Co., Janssen Pharmaceuticals, Inc. and Takeda Pharmaceuticals Co. Ltd., as well as primary collaborators including the Translational Genomics Research Institute (TGen). In 2015, CoMMpass reached its target enrollment of 1,000 MM patients.

CoMMpass is focused on the study of disease progression and response to treatment based on genomic and molecular profiles. Clinical parameters are collected from subjects at diagnosis and every three months during an eight-year period. These parameters are used to establish a clinical baseline, and clinical samples are analyzed by WGS to identify somatic mutations, CNAs and structural changes, WES to identify single nucleotide variants and indels, and RNA-Seq to define transcript expression levels and fusion transcripts [30].

Another study supported by the MMRF is the Molecular Profiling Initiative (MPI), in partnership with the University of Michigan, which provides free clinical-grade genomic sequencing for study participants at sites across the USA. De-identified data from the MPI study will be added to the Multiple Myeloma Genomic Initiative. All data from these three MMRF initiatives are also shared with the Genomic Data Commons, a database of the National Cancer Institute (NCI), which in turn is publicly accessible to researchers.

The Myeloma Genome Project (MGP) is a large repository of molecular profiling data in MM, along with associated clinical outcome data. With data collected from the Myeloma XI trial in the UK, the Intergroupe Francophone du Myelome in France, the Dana-Farber Cancer Institute, and the Myeloma Institute at the University of Arkansas and the MMRF, this initiative intends to examine minor translocation and mutational groups that are often poorly described due to small sample numbers in limited data sets. Similar to the CoMMpass trial, MGP will analyze samples from a set of 2,161 subjects using WGS, WES, targeted sequencing, RNA-Seq and gene expression arrays.

These trials are representative of the coordinated global effort now made by researchers, using NGS technology to accelerate the translation of scientific discoveries that can improve MM management and outcomes. Key to these efforts is the way that the genomic data provided by NGS can be easily shared and analyzed among different researchers.

Future NGS applications

From the brief discussion above, we can conclude that NGS has significantly expanded our knowledge of MM, however, its pathogenesis still remains only partially understood.

For this reason, additional research using NGS is required to enable a deeper understanding of the myeloma genome, and provide better tools to help guide treatment decisions and improve outcomes.

As Morgan, Walker, Bolli, Munshi and others have independently observed, the technical challenge facing MM is in the development of better tools that can effectively measure recurrent gene mutations and expression signatures, IgH translocations, chromosome rearrangements, and CNAs.

At the same time, more comprehensive and standardized methods are required to measure clonal heterogeneity, characterize genomic pathways, identify post-translational regulation, and measure treatment response.

This section will briefly explore how NGS can contribute to the development of such tools.

Hybrid capture NGS

Among the many available NGS technologies, hybrid capture is quickly becoming a standard platform that can be used to reliably detect genetic mutations, CNAs, IgH translocations, and gene fusions. Hybrid capture has shown consistently greater uniformity in capture vs amplicon sequencing due to a greater on target rate [31, 32]. Furthermore, hybrid capture consistently picks up variants, such as indels or fusions, when compared to amplicon assays, which tend to be less reliable [32].

With these capabilities, hybrid capture seems uniquely fit to address the majority of requirements for the MM translational research community.

Like a multi-function Swiss Army knife, NGS is uniquely capable to deliver most of the tools required by stakeholders to more effectively characterize MM and assess treatment response.

The key benefit of sequence data is that one can test important segments of the genome and expressed genome, looking for any type of irregularity. This ability is powerfully different when compared to cellular or protein testing (i.e. FISH or MFC).

The flexibility of genomic sequencing allows for the coverage of a much larger and less predictable set of changes than other techniques.

Yet for NGS to be truly effective in clinical research, a standardized, inclusive NGS panel is required that can be easily deployed, with repeatable results across sequencing platforms and trials.

The primary advantage to using a standardized hybrid capture panel for
MM research is the flexibility it provides. A hybrid capture panel can be easily customized to add new markers at any time, and re-measure the same samples, thereby ensuring that no content is missed.

Other advantages of using a hybrid capture panel include:

- sequences within exons can be measured at specific genomic coordinates, using the same chemistry and analytical workflow, thereby minimizing batch processing and bias;
- when library preparation and sequencing methods are standardized, ground truth is known and assay troubleshooting becomes easier; and
- analytical workflows can become hardened and optimized to stretch sequencing budgets, yet provide the appropriate coverage for confident variant calls.

The flexibility of a standard hybrid capture panel can be further enhanced with custom panels. These are similar to add-on modules that can be used to focus on particular chromosomes and genes, tailored to targeted treatments, and even used for specific cohorts and samples.

This is commonly referred to as a “plus design strategy” [33]. For example, one interesting “plus” strategy could be to focus on genes that are linked to new drugs that are in clinical trials, where one can rapidly test drug susceptibility of a given clone, ensuring that novel and useful information will evolve rapidly, justifying further examination with each step. The core design could “plus” content when new critical targets or targeted therapies emerge.

In comparison with other tools used in the prognosis of MM, a standardized hybrid capture panel can be used to understand the biology of MM in the individual subject at initial diagnosis, as shown in Figure 3. Rather than waiting months or years for the disease to progress, use of NGS from initial diagnosis can reveal key data points that will likely inform clinicians if a subject can have reduced therapy or shorter courses of treatment.

Having such information early in the treatment cycle theoretically may reduce the number of high-risk patients that will relapse within the first 2 years by 5-10%.

Similar efforts already have been implemented in the MMRF CoMMpass trial, and a new study of MRD at the Memorial Sloan-Kettering Cancer Center also uses NGS from initial diagnosis to define subjects’ individual “genetic fingerprint” and then track MM subclones during therapy.

We are at a time in the study of multiple myeloma were NGS technology offers significant opportunities for matching subjects and therapies. What makes the use of hybrid capture so unique is the amount of information it can deliver with such little effort. The underlying implication is that with hybrid capture more detailed sequential patterns are found that will provide greater predictive power for both prognosis and therapy prediction.

Combined DNA-RNA sequencing

To get maximum predictive power from a standard NGS panel, hybrid capture also can be combined with RNA-Seq. Studies have shown that the mutation spectrum...
relevant to MM is often represented in RNA-Seq datasets [34]. By adding a targeted RNA-Seq approach, investigators can define minimal content with DNA-Seq panels and confirm active mutation spectra using RNA-Seq for the same panel.

With the broadly changing DNA clonality of MM, it’s critical to understand how cells adapt to modified DNA but still maintain viability. Monitoring the read count of RNA as well as the sequence dynamic of both DNA and RNA provides insight into the mechanism of DNA degradation and metabolic regulation of MM cells. This can be particularly useful for mapping clonal evolution. Moreover, differential gene expression measured by read counts in RNA-Seq provides a direct measurement of key metabolic activities that are critical to understand how cells adapt to modified DNA but still maintain viability.

Given the heterogeneity of cells found in MM samples, it’s possible that more information will be found in sequenced RNA than in DNA. If validated, such clone-specific information could help to better inform the selection of novel therapies.

Discussion
This paper suggests that the study of multiple myeloma is entering a “new knowledge cycle” where information and techniques afforded by NGS will significantly change the landscape of MM, from initial diagnosis through to treatment.

Adding to the substantial body of research to date and on-going studies, new NGS tools will enable a re-examination of the somatic plasma cell colonies at the single nucleotide level along with clonal population proportionality, providing considerable insight into the shifting cell colonies within bone marrow cell populations. This increased knowledge should allow for improved patient risk stratification, prognosis and therapy planning for clinical research studies.

Many studies have shown the predictive value of NGS for MM, and with the introduction of novel agents and new therapies, further validation of NGS is required with larger trials involving more subjects and proven outcomes to prove clinical efficacy.

The implementation of NGS workflows in clinical laboratories presents many challenges, but with those challenges also comes the opportunity to provide more actionable information that can ultimately improve the quality of care.

Finally, as many healthcare professionals were trained before molecular diagnostic techniques were introduced into clinical care, education and training is a critical path to translate research knowledge gained from NGS into the clinic.

Conclusion
One should conclude from this white paper that a significant paradigm shift is occurring in MM treatment, diagnosis, and in the way that principal investigators design clinical trials.

The power of NGS and hybrid capture techniques enables effective tools that support disease pathway-based predictive analysis in multiple myeloma. Once standardized, these tools will greatly aid efforts in new drug and therapy development in the future. Coupled with an improved understanding of the biological changes in MM, there is the potential to significantly alter treatment and outcomes.

Thus the key enabling feature of NGS is its ability to deliver individualized treatment decisions based on genomic profiling prior to therapy, and tracking clonal evolution during treatment.

Combined with more effective new therapies, the data afforded by NGS frames the paradigm shift in the way clinical trials for MM will be conducted in the future. Investigational use of NGS will be used to inform preclinical and clinical studies that provide better modeling, and testing genomic subsets of malignant and premalignant plasma cell disorders, and eventually set the stage for more informed use of NGS in the standard of care setting.

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Glossary

In this glossary we provide a brief overview of different NGS methodologies used in studies of MM.

Chromosomal rearrangement: Events mediated by double-strand breaks and subsequent repair in the genome. Different types of rearrangements include deletions (the removal of a DNA sequence) and insertions (the addition of a DNA sequence), among others.

Copy number alterations (CNAs): These refer to somatic changes to chromosome structure that result in gain or loss in copies of sections of DNA.

Copy number variations (CNVs): Refers to large-scale structural changes in DNA that vary from one individual to another. These changes include insertions, deletions, duplications and complex multi-site variants that range from kilobases to megabases in size.

Deep sequencing (DS): This sequencing method is used to sequence a specific genomic region multiple times, wherein a greater depth of coverage is achieved as the total number of reads is larger than the actual length of the sequence.

Exome: The portion of the genome that is most highly transcribed (expressed). Approximately 50 million base pairs in size.

Fluorescence in situ hybridization (FISH): This laboratory test is used for detecting and locating a specific DNA sequence on a chromosome. The technique relies on exposing chromosomes to a small DNA sequence called a probe that has a fluorescent molecule attached to it.

Fusion gene: A fusion gene is a hybrid gene formed from two previously separate genes. It can occur as a result of translocation, interstitial deletion, or chromosomal inversion.

Haplotype: A DNA ‘bar code’ composed of a combination of alleles at two different loci on the same DNA strand.

Hybrid capture or Hybridization capture: The most sensitive Targeted Sequencing method employs “target enrichment” where specific regions of the genome (up to several thousand genes) can be “captured” with a method known as “hybridization” using biotinylated probes, wherein the DNA is then isolated and pulled down with magnetic beads. This method can deliver up to 66Mb of a region with high specificity.

Karyotype: Refers to a laboratory technique that produces an image of an individual’s chromosomes. The karyotype is used to look for abnormal numbers or structures of chromosomes.

Next-generation sequencing (NGS): Next-generation sequencing (also referred to as high-throughput sequencing) refers to a set of methods and/or technologies that include genome sequencing, transcriptome sequencing (RNA-Seq), DNA-protein interactions (ChIP-sequencing), and epigenome characterization.

Read depth: The average number of representations of targeted nucleotides.

RNA sequencing (RNA-Seq): Sequencing RNA to detect differences in levels of expression and structure of particular RNAs. RNA-Seq uses NGS to sequence extracts of RNA from cells or tissues. It is an evolution of gene expression that provides for a snapshot of the whole transcriptome. The process of RNA-Seq normally begins with different methods for sample preparation to remove the ribosome, RNA is then converted into cDNA. Among the different types of RNA-Seq, Total RNA and mRNA sequencing provide a comprehensive biological snapshot of the entire cellular profile. In general, RNA-Seq allows for the detection of different isoforms, gene fusions and novel transcripts. Targeted RNA-Seq is another method that allows the measurement of transcripts that can identify coding SNPs and splice junctions, as well as allele-specific expression. This also allows for the creation of custom kits that can target specific biological events, cellular pathways, or diseases.

Whole genome sequencing (WGS): The most comprehensive method for interrogating the human genome is with Whole genome sequencing (WGS). By reading 3.2 billion bases, it is capable of detecting duplications, insertions, deletions, substitutions, gene and exon copy number changes, and chromosome inversions and translocations across the entire genome.

Whole exome sequencing (WES): Whole exome sequencing (WES) is one of the most widely-used sequencing methods. The exome represents less than 2% of the entire human genome, yet it contains the majority of all known disease-related mutations. In comparison with WGS, this method is extremely economical. WES can selectively capture and sequence the protein-coding regions of the human genome, effectively identifying the same number of variants as WGS.

Targeted sequencing (TS): Targeted sequencing represents a focused approach wherein specific genes or regions can be isolated and sequenced. TS allows for higher coverage of specific areas of the genome at 500-1000x, compared to WGS which achieves coverage of only 30-50x for each genome. Normally TS is used as a targeted panel from a manufacturer where the content of the panel is either fixed or custom designed. TS panels are also used in conjunction with kits for library preparation, representing probes for specific diseases such as cancer, or specific pathways.

Transcription: The synthesis of RNA complementary to DNA. The RNA transcript is often further processed and may encode protein, may have direct enzymatic or structural function, may be regulatory, or may be nonfunctional.

Translocation: Translocations are a chromosomal abnormality or rearrangement that occur when chromosomes break and the fragments rejoin to other chromosomes.
References


