SickKids | Genome Diagnostics

A Guide to Next-Generation Sequencing

As we move towards testing of larger targeted gene panels, whole exomes, and whole genomes, next generation sequencing (NGS) technologies will be increasingly used for their high-throughput abilities and cost-efficiency. NGS methods represent a variety of technologies that are able to sequence a large number of segments of DNA concurrently. This is in contrast to Sanger sequencing, which typically analyzes one sequence at a time. Both of these technologies have advantages and limitations: Sanger sequencing is currently the gold standard for detecting sequence variations and is more effective at sequencing repetitive DNA regions, small insertions and deletions; while NGS is less expensive than Sanger sequencing (when comparing cost per base pair) and allows for the analysis of tens to thousands of genes in one test.

Next Generation Sequencing Tests

A number of tests can be performed using NGS, each of which has its advantages and limitations. It is important to thoroughly evaluate which is the appropriate test to meet your patient's needs.

- **Targeted Gene Panels (TGPs):** sequencing of one to hundreds of pre-selected genes based on a particular disease indication.
- **Whole Exome Sequencing (WES):** sequencing of the "exome," which includes all of the exons (DNA sequences within genes that predominantly codes for proteins). The exome encompasses approximately 2% of the entire genome.
- **Whole Genome Sequencing (WGS):** sequencing of the genome, the entire genetic code in an individual, including coding and non-coding regions.

TGPs may be useful for patients that have a relatively defined disease aetiology, for which there are known causative genes. WES or WGS may be useful for patients with: 1) Many possible diagnoses; 2) A complex phenotype that suggests a genetic aetiology but does not correspond to a known condition or; 3) A condition with a likely genetic aetiology, but other targeted tests have failed to provide a diagnosis.

WES and WGS can reveal new disease-causing variants whose clinical significance in causing particular phenotypes was not previously known. Unlike TGPs, WES and WGS may reveal secondary findings, which are defined as medically relevant variants not related to the primary disease indication. WES is less costly and easier to analyze than WGS; however, WGS may cover some parts of the exome better than WES due to its more comprehensive coverage and the ability to detect structural variations.

Clinical Considerations and Recommendations

- Ensure that appropriate consent is obtained to facilitate informed decision-making, including choices for disclosure of secondary findings.
- Discuss the possibility of finding variants of uncertain significance with your patients *before* ordering testing. Please refer to "A Guide to Interpreting Sequence Variations" information sheet on our website for further information on variants of unknown significance.
- For WES and WGS tests, discuss the possibility of secondary findings with your patient *before* submitting for testing and ensure that they understand their choices for learning about secondary findings including what can be reported and why identifying them could be beneficial or harmful.
- Information such as clinical findings, family history, ethnic background, and other laboratory data must be effectively communicated to the lab to guide the most thorough investigation of variant interpretation.

Technological Limitations

The reliability of each variant found by NGS and whether it is heterozygous, homozygous or hemizygous is determined by the read-depth (also called fold-coverage), which is the number, on average, that each base has been sequenced. There are a number of ways in which data can be lost throughout the process of NGS testing:

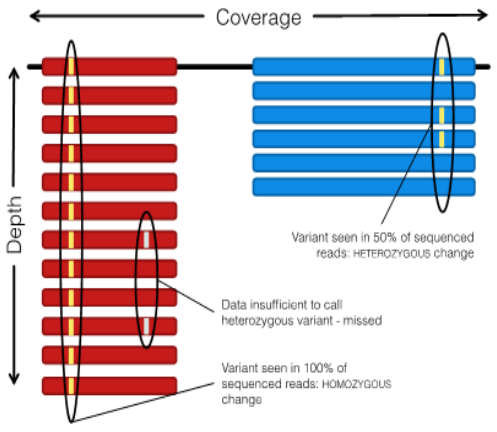
During Capture or Amplification

- Regions with high GC-content are more difficult to sequence
- Allele dropout: when one allele fails to be captured or amplify such that it is not represented in the sequencing data

During Analysis

Errors in alignment between the reference and sample sequence can be caused by:

- Inaccuracies in the reference genome
- Highly repetitive regions
- Highly polymorphic regions (sequences with extensive variability between individuals)
- Homologous regions (sequences with high similarity in the genome)
- Larger insertions or deletions



Topper S and Wagonner DJ (2013). Syllabus from the 2013 ACMG Annual Clinical

For more information, contact the Genome Diagnostics Laboratory at SickKids at 416-813-7200 x1 or www.sickkids.ca/genome-diagnostics
To locate a genetics center near you, visit the Canadian Association of Genetic Counsellors website at www.cagc-accg.ca
or the National Society of Genetic Counsellors website at www.nsgc.org.