

Test Menu Expansion Using WGS + In Silico Panels

Introduction

Next-generation sequencing (NGS) analysis of targeted sets of genes, also known as panels, has become one of the most widely used methods for molecular diagnostic testing of inherited disorders. Labs seeking to offer such tests have a choice of using commercially available NGS panels or of designing their own custom panels. This decision is most often based on the desired clinical outcome of the test - whether a diagnostic, prognostic or therapeutic application is intended - and the specific mutations or genes to be tested. Selection of the relevant mutation or gene content is based on the available scientific evidence for markers of clinical utility and validity.

Once the content of the panel has been determined, the appropriate sample and library preparation methods and sequencing platform are selected. The subsequent data analysis must be developed and the entire process tested and optimized, keeping in mind potential sources of error that must be guarded against. Once the test has been developed, it must ultimately be validated. An error-based validation approach is typically used for multigene panels because of the large amount of variation that is expected in samples put through such analyses. This is an intensive and lengthy process that must be undertaken, from start to finish, for each panel developed.

Whole genome sequencing (WGS), in combination with the use of in silico panels that are defined by genomic coordinates instead of amplification kits, is providing an alternative to traditional panel development.

Whole genome sequencing technology

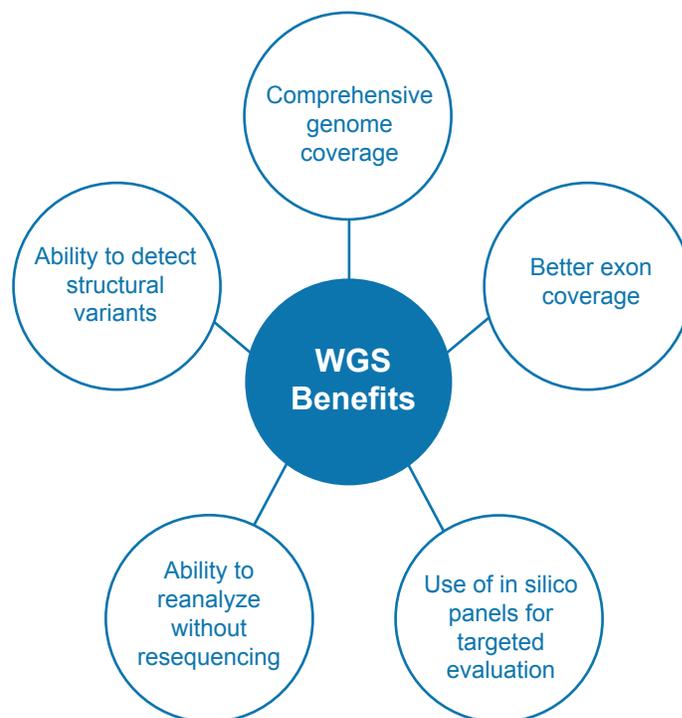
As a sequencing technology, WGS provides a number of significant advantages over other NGS methods, including comprehensive coverage of the genome, more consistent coverage of exonic regions, the ability to detect structural variants, and the ability to reanalyze a sample without resequencing.

Gene panels sequence only a fraction of a patient's DNA, typically covering <1 million bases, or about 0.02% of the genome. Exome sequencing covers much more, but still comes in at ~50 million bases, or about 1.5% of the genome. In contrast, whole genome sequencing provides a comprehensive view of a patient's genome. It covers the same bases covered by panel and exome sequencing, plus provides comprehensive coverage of intronic and intergenic regions enabling identification of potentially relevant transcription factor binding sites, enhancers and other regulatory variants.

PCR-free DNA preparation methods for WGS eliminate amplification-related issues that tend to arise during panel and exome sequencing. As a result, consistent read depth is generated across the entire genome, including amino acid coding regions.

Variant-containing regions are covered with high confidence and artifacts are unlikely to contaminate the sequencing results.

These features of WGS provide unique opportunities for detection of structural variants. Using a combination of breakpoint analysis, read depth analysis and de novo assembly, structural variants and trinucleotide tandem repeats can be identified down to single base pair resolution. All together, comprehensive coverage of the genome, more consistent exon coverage and the ability to detect structural variants means that WGS is less likely to miss variants relevant to a patient's diagnosis, prognosis or treatment. Annotation of identified variants is essential for interpretation, yet new information about variants and genes associated with disease becomes available every day. With other NGS methods the genes being considered in the analysis must be defined prior to sequencing to enable selection of the appropriate probes. With WGS, such decisions do not need to be made in advance. Its inherent comprehensive coverage of the genome means that no changes in sequencing reagents are required as knowledge grows and new genes become linked to disease. Just a simple change in the genomic coordinate filters at the data analysis stage is required.



In silico panels

In silico panel technology enables labs to benefit from WGS while retaining the ability to independently analyze discrete slices

of sequenced DNA ranging in size from a single gene to tens of thousands of genes. The genes to be covered by an in silico panel are identified in the same way as the genes to be covered by a traditional panel - using available scientific evidence to select relevant markers of clinical utility and validity. But instead of using the selected genes to guide sample preparation and sequencing, they are used to design genomic coordinate-based filters applied during the data analysis stage.

In silico panels are an effective tool for performing targeted, outcome-driven analyses of WGS data. For example, when considering cancer predisposition, it's possible to design an in silico panel that only considers alterations in the BRCA1 and BRCA2 genes as a first pass screen for the mutations most commonly associated with inherited breast cancer. Because it is based on WGS technology, the panel would detect SNVs, indels and structural variants, including CNVs, in a single analysis. Similarly, it's possible to design an in silico panel that considers a larger collection of genes associated with inherited breast cancer, including MLH1, MSH2, STK11 and others, for broader, second pass screening. Both sets of in silico panels could be applied to the same WGS sample.

Use of NGS methods for diagnosis of rare inherited diseases provides another example of how in silico panels can be used as an effective tool for targeted, outcome-driven analysis of WGS data. Taking the example of intellectual disability, a diagnostic path of increasing breadth could be created using a single DNA sample. An FMR1 in silico panel could be used to look for expansion mutations causing fragile X syndrome, the most common inherited cause of intellectual disability. In cases where no FMR1 mutations are identified, reflexive testing could utilize a larger in silico panel covering SNVs, small indels and structural variants in additional genes such as ATRX, IL1RAPL, RAB39B and others. Ultimately escalating to in silico exome analysis or in silico array CGH analysis if no causative mutation is found by the initial analyses. See the table below for a

summary of relevant CPT codes related to this intellectual disability example.

WGS cost and analysis considerations

The benefits of WGS technology are becoming clearer, but concerns over the cost of sequencing and the complexity of data analysis can create a barrier to entry for labs. Variantyx's Genomic Intelligence® platform was developed based on WGS technology. It provides sample to clinical report integration and automation for many types of genomic tests, but is specifically built to handle WGS data. Sequencing can be performed in-house at your site, but it can also be outsourced to our integrated, world-leading clinical sequence partners. Prearranged discounts keep the per-sample sequencing costs low, with no minimum volume and no investment in purchasing expensive sequencers. Genomic Intelligence® gold-standard tools and best practices, combined with proprietary algorithms, accurately and efficiently identify the most relevant variants. Preset and/or customized in silico panels are used for targeted analyses.

Summary

Whole genome sequencing provides the most comprehensive view of a patient's genome. In silico panel technology enables labs to benefit from WGS, while retaining the ability to offer targeted, outcome-driven analyses tailored to specific testing needs. Because WGS covers the complete genome, reflexive testing can be cost effectively performed on the original DNA sample, progressively using broader sets of in silico panels with no need for resequencing. Variantyx's Genomic Intelligence® platform for sample to clinical report generation addresses the barriers to entry associated with WGS, supporting on-premise as well as outsourced sequencing while providing gold-standard tools, best practices and proprietary algorithms for simple analysis of WGS data. To learn more about Genomic Intelligence®, contact us at info@variantyx.com or visit our website at www.variantyx.com.

Sample CPT codes for reimbursement of tests related to intellectual disability, as an example

Phenotype	CPT code	Description	Variants covered
Intellectual disability	81243	FMR1 (Fragile X mental retardation 1) (e.g., fragile X mental retardation) geneanalysis; evaluation to detect abnormal (e.g., expanded) alleles	SNVs, small indels, trinucleotide repeat expansions
Intellectual disability	81470	X-linked intellectual disability (XLID) (e.g., syndromic and non-syndromic XLID); genomic sequence analysis panel, must include sequencing of at least 60 genes, including ARX, ATRX, CDKL5, FGD1, FMR1, HUWE1, IL1RAPL, KDM5C, L1CAM, MECP2, MED12, MID1, OCRL, RPS6KA3, and SLC16A2	SNVs, small indels
Intellectual disability	81471	X-linked intellectual disability (XLID) (e.g., syndromic and non-syndromic XLID); duplication/deletion gene analysis, must include analysis of at least 60 genes, including ARX, ATRX, CDKL5, FGD1, FMR1, HUWE1, IL1RAPL, KDM5C, L1CAM, MECP2, MED12, MID1, OCRL, RPS6KA3, and SLC16A2	Structural variants, including CNVs
Intellectual disability	81415	Exome (e.g., unexplained constitutional or heritable disorder or syndrome); sequence analysis	SNVs, small indels
Intellectual disability	81229	Cytogenomic constitutional (genome-wide) microarray analysis; interrogation of genomic regions for copy number variants (e.g., Bacterial Artificial Chromosome [BAC] or oligo-based comparative genomic hybridization [CGH] microarray analysis)	Structural variants, including CNVs