Built To Adapt

Comprehensive next-generation sequencing promotes efficiencies in rare disease analysis

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Move

traditional methods

The pace of genomic discovery in rare disease is breathtaking. Two hundred and fifty new gene-disease associations are identified annually. Over nine thousand new variant-disease associations are reported per year.[1]

Deeper understanding of the genome is being uncovered with each new day.

Chromosomal microarrays (CMA) are a traditional testing method used by investigators in individuals with unexplained developmental disabilities.[2]

It enables profiling of chromosomal abnormalities, such as duplications and microdeletions, down to 5-10kb in size.[3] While highly effective, CMA accesses only a portion of the genome and does not enable interrogation of sequence variants.

WGS and WES offer higher diagnostic utility than CMA

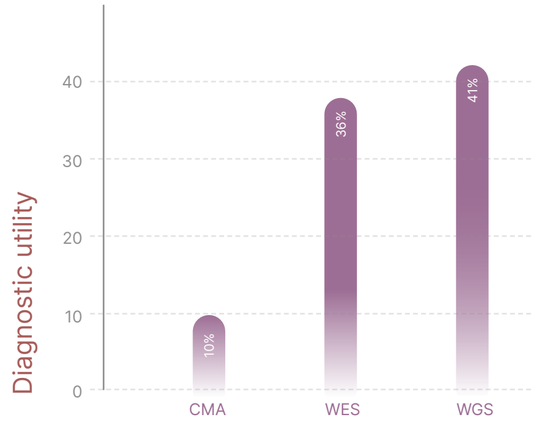


Figure 1:
Thirty-seven studies comprising 20,068 children published between January 2011 and August 2017 were reviewed for the diagnostic utility of CMA, WES, and WGS. 95% CI: 4.7-14.9,P < 0.0001.[4]

Evidence-based guidelines issued by the American College of Genetics and Genomics (ACMG) have recognized the value of whole-genome sequencing (WGS) or whole-exome sequencing (WES) in first or second tier use. Improved management, higher diagnostic yield, and improved costs were cited as support to using early in a genomic evaluation.[5]

for the future

The burden of multigene panels

The velocity of change brings challenges for the modern molecular genomics laboratory to stay current. One lab found 23% of positive WES findings were in genes described within the last two years, while 7% of positive variants were in novel gene discoveries.[6]

Labs face a continuous cycle of new panel design and validation with every new gene or variant association with rare disease, requiring significant expenditure of time and resources, all while being unable to engage in gene discovery themselves.

In contrast, with WGS and WES labs can create a comprehensive assay, amenable to the latest genomic discoveries. New findings can be incorporated into existing workflows and "future-proof" the test menu.

Re-analysis of existing data sets can identify novel associations without the need to re-sequence samples or re-validate an assay. "Virtual panels" can be created out of a genome or exome output, providing ordering health care providers a bespoke panel of their choosing (Figure 2).

Create "virtual panels" with a genome or exome foundation

Use of whole-genome or whole-exome sequencing as an assay foundation enables dynamic creation and modification of "virtual panels" as more is understood about the genome.

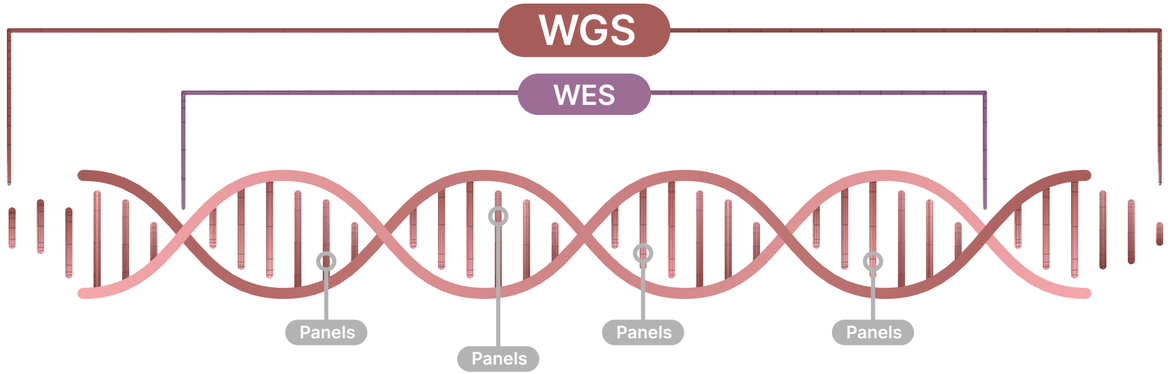


Figure 2: Genome as a foundation

your analysis with WES

Scale variant interpretation and benefit from Next-Generation Sequencing (NGS)

For labs that want to increase capabilities and gain proficiency in comprehensive NGS analysis, WES is a targeted sequencing approach that enables them to focus resources on genes likely to affect the phenotype.

WES targets protein-coding regions, which comprise less than 2% of the genome but contain ~90-95% of known disease-related variants.[6] It produces a manageable data set for focused analysis that can help build competencies.

With WES

~30%

find a molecular etiology[7]

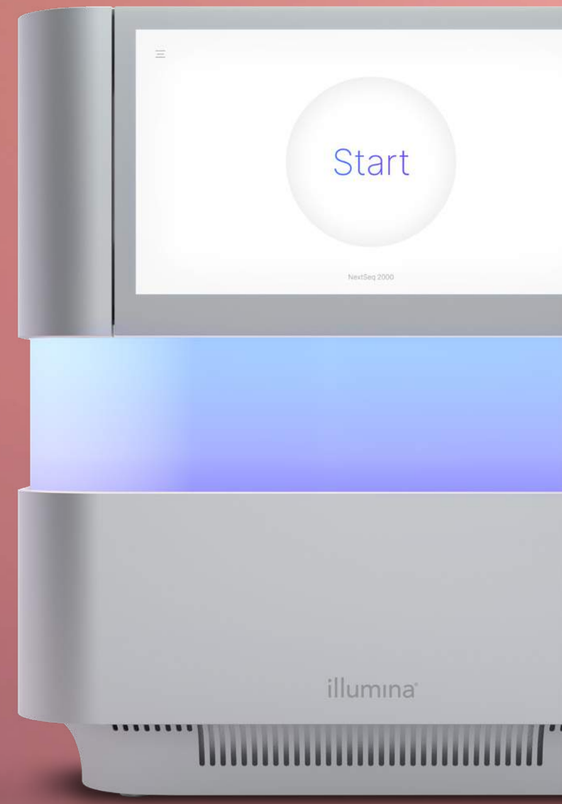
WES can:

CTTGTCTA
TACTTGTC
GCAGCTAC
TGTCTAGC

Provide the laboratory professional a broad view of coding variants.

Enhance laboratory proficiencies associated with data management and interpretation at scale.

Offer greater opportunity for re-analysis or discovery potential than CMA or gene panels.



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No single test is more

WGS offers unparalleled analysis

For labs that want to streamline operational efficiency, WGS provides the most comprehensive view of the human genome.

WGS enables simultaneous analysis of thousands of genes with known or suspected associations with rare disease, as well as discovery of novel causative variants. Enabling uniform coverage of coding regions, WGS provides advantages evaluating exons compared to WES.
As a single assay, there is no other test that can detect as many diverse variant types (Table 1).

Comprehensive variant calling can result in greater opportunities to interrogate the genome, as 13% of WGS diagnoses in a large national pilot study were not expected to be discovered with exome sequencing.[7] WGS also provides a foundational assay for other emerging applications including pharmacogenomics, human leukocyte antigen (HLA) typing, and polygenic risk scores.

WGS provides the most comprehensive analysis of geneomic variants among all clinical genomic testing methods[9]

	Sanger*	Targeted NGS*	PCR*	CMA*	WES*	WGS*
Single-Nucleotide Variants (SNVs)	✓	✓	✓		✓	✓
Insertions & Deletions (Indels)	✓	✓	✓	✓	✓	✓
Copy Number Variants (CNVs)		✓	✓	✓	✓	✓
Repeat Expansions			✓			✓
Structural Variants (SVs)				✓	✓	✓
Mitochandrial	✓	✓			✓	✓
Paralogs	✓		✓			✓

Table 1: Comparaison of WGS to Standard Testing

✓ Limited capabilities ✓ Capable

It is clear whole-genome sequencing is contributing significantly to end diagnostic odysseys in rare disease. With guidelines advocating use as a first–tier test[5], inclusion in national health care systems[7], and increasing evidence of economic value when used as a first–tier test[8], genome sequencing appears to be on the path toward standard of care.

*Variant detection may vary depending on laboratory and test offering
CMA = chromosomal microarray, CNV = copy number variant, FISH = fluorescence in-situ hybridization, indel = small insertion deletion, NGS = next-generation sequencing, PCR = polymerase chain reaction, SNV=single nucleotide variant, WES = whole-exome sequencing, WGS = whole-genome sequencing

workflows for NGS methods

Illumina offers investigators integrated, streamlined workflows for WES and WGS research that follow the same three steps labs may be familiar with and already use for targeted sequencing (Figure 4). Regardless of the method, prepared libraries are loaded onto an Illumina platform for sequencing.

WES research can be performed on a range of Illumina systems from the benchtop MiSeq™ System to the NovaSeq™ 6000 System. The output capabilities and scalability of the NextSeq™ 1000, NextSeq 2000, and NovaSeq 6000 Systems make them ideal for WGS investigations.

Illumina sequencing systems are powered by the same sequencing by synthesis (SBS) chemistry, so data generated across systems can be compared and integrated, enabling labs to transition to new methods with confidence.

The Illumina NGS workflow

Regardless of the specific method used, all Illumina NGS workflows consist of three basic steps: library prep, sequencing, and data analysis.

Figure 4:
The Illumina NGS workflow

with automated interpretation and XAI

The cornerstone of rare disease analysis is interpretation. With variability in the method, the genes interrogated, and the output generated by an application, a software solution to provide an investigator a complete view of the data is crucial.

Illumina's Emedgene tertiary analysis platform has been designed to translate the vast amounts of data produced by WGS, WES and virtual panels into meaningful insights, enabling rapid analysis.

Illumina's Emedgene intuitive genomic analysis platform enables 2-5x improvement in efficiency:

- Streamline interpretation and automate evidence curation with explainable artificial intelligence (XAI) and machine-learning
- Integrate with the cloud-based DRAGEN™ Bio-IT Platform to enable comprehensive, streamlined secondary and tertiary analysis workflows and ultrarapid variant calling

Illumina offers users an ecosystem of end-to-end high-throughput products, designed for diverse researcher needs. Whether it is including automation to increase efficiency, ensuring quality of a run, or providing a seamless experience with scalable software for sample-to-report generation, laboratories can have confidence knowing they have the very latest to equip them in their search for answers.

Learn more

→ Whole-genome sequencing

→ Whole-exome sequencing

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