BAYLOR GENETICS

Multiomic and Multimodal Technologies to Support Rare Disease Diagnostics

Introduction

Rare diseases are a significant cause of disability, chronic illness, and premature death in both children and adults. Although individually rare, in aggregate it is estimated that 350 million people worldwide are affected by a rare disease. **Approximately 80% of rare diseases are genetic in origin**.[1] Obtaining a molecular diagnosis through genetic testing can allow these individuals to receive more precise treatment and management. However, there are significant barriers to genetic testing including the complexity of detecting diverse types of disease-causing variants.[2]

Genome sequencing (GS) offers comprehensive sequencing of the genome, covering several variant types, and is useful for patients with a broad differential diagnosis and/or uninformative targeted testing. Over the last few years, **GS has been recommended as a first-tier test for patients with complex medical issues and suspected rare diseases** by professional societies such as the American College of Medical Genetics and Genomics, American Academy of Pediatrics, and National Society of Genetic Counselors.[3-5]

Across indications, up to 43% of all patients that have GS receive a molecular diagnosis that explains their phenotype.[3,6] **However, a negative or inconclusive result does not necessarily indicate the absence of a genetic etiology.** As a short-read sequencing-based test, GS may not capture certain types of pathogenic variants, such as complex structural variants and short tandem repeat expansions. Short-read sequencing also cannot provide gene expression or methylation status which are crucial for determining pathogenicity for certain variants. Additional technologies can address these distinct diagnostic gaps and provide more patients with the answers they and their clinicians are searching for.

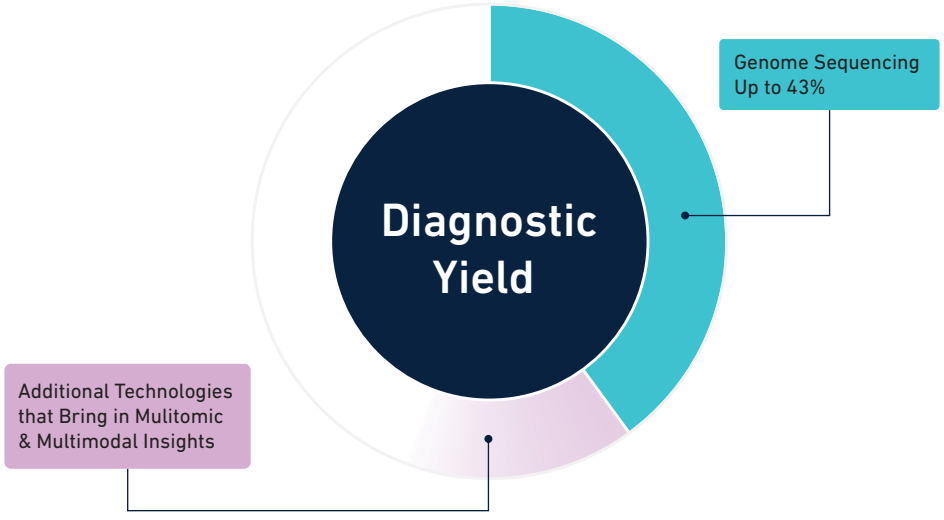

Figure 1: The diagnostic yield of genetic testing methods. Across indications, up to 43% of patients receive a positive result by genome sequencing. Additional technologies can help provide additional diagnoses and further reduce the diagnostic gap.

To address this diagnostic gap, Baylor Genetics is proud to offer the following enhancements to our Whole Genome Sequencing (WGS) test. Here, Baylor Genetics introduces how these advanced technologies complement WGS when qualified variants are identified to support their reporting and present clinical cases showing the diagnostic and clinical utility of these technologies.

	Enabled With
Complex Structural Variant (SV) Analysis[*†§] Clarify large or complex genomic rearrangements related to a wide spectrum of rare diseases	Optical Genome Mapping
Short Tandem Repeat (STR) Analysis[*†§] Detect STR expansions in **58 genes** to identify neurological, neuromuscular, and other genetic disorders	Long-Read Sequencing
Methylation Analysis of *FMR1*[*†§] Detect *FMR1* methylation status in larger premutation alleles for Fragile X syndrome assessment	Long-Read Sequencing
RNA Sequencing (RNA-Seq) Analysis[‡§] Supplemental targeted RNA analysis to support clearer result interpretation **(included with WGS)**	

* Long Read Sequencing (LRS) is used as a confirmation method for STR analysis. Optical Genome Mapping (OGM) may supplement WGS to augment detection and improve interpretation of potential complex structural variants identified through WGS.

† LRS and OGM are not yet approved by NY State CLEP.

‡ RNA-Seq is only performed on qualified variants that meet the prediction algorithm criteria that suggests additional functional evidence can be provided.

§ Additional specimens may be requested as needed.

Complex Structural Variant Analysis by Optical Genome Mapping

Structural variants (SV) include deletions, duplications, inversions, and translocations that are over 50 base pairs in size though are often significantly larger. SVs are clinically relevant and an often underrecognized contributors to genetic disease. **As many as 10-18% of patients with undiagnosed rare disease might have SVs that are not readily detected by conventional methods such as short-read sequencing.**[7-8] Larger SVs (>20Kb) are fifty times more likely to disrupt gene expression than point mutations, and 25-29% of protein truncating variants are SVs.[9]

Optical Genome Mapping (OGM) specializes in the high-resolution detection and characterization of complex SVs.[10] OGM uses fluorescent labels at specific DNA sequences to create predictable banding patterns throughout the genome that can be observed with high-powered microscopy. These labels bind to specific and unique intervals in the genome. Each image is digitized into molecular barcodes and aligned to a reference sequence for genome-wide interpretation of structural variation. OGM has also been called "molecular karyotype" and "next-generation cytogenetics." Unlike microarray, this method has the additional benefit of determining the location and orientation of duplicated material.[11]

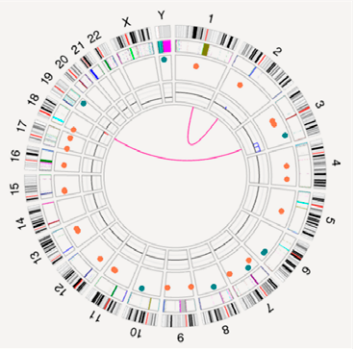

Figure 2: Example of a Circos plot. Circos plots are used to visualize SVs to support interpretation of results obtained by OGM.

Baylor Genetics validated OGM in blood for the identification of SVs including translocations, insertions, inversions, complex rearrangements, and mosaic SVs. Reagents, software, servers, and quality control metrics to support testing were provided by Bionano. In Baylor Genetics' validation, a total of 80 SVs were detected by standard of care (SOC) testing, including karyotype and chromosomal microarray. All variants were captured by OGM, leading to 100% concordance. The clinical sensitivity, clinical specificity, and reproducibility of OGM was 100%. Variants met specific internal quality and reporting criteria to be considered reportable.

Baylor Genetics now offers OGM as a reflex when complex SVs are identified by WGS. OGM can help discern the breakpoints, location, and orientation of SVs to allow for more precise clinical management.

OGM Case Example

OGM provided greater insights than WGS alone into a complex SV involving an inverted duplication in tandem with a copy number neutral inversion disrupting *STAG1* for an infant referred for encephalopathy, seizures, respiratory failure, lethargy, and hypotonia. Rapid trio WGS identified a de novo heterozygous 8.52 Mb inverted duplication at chromosome band 3q25. Duplications of 3q have been described in patients with congenital heart defects, intellectual disability, genitourinary malformations, and other congenital abnormalities. Reflex OGM revealed the duplication was in tandem with a copy neutral inversion at 3q22.3. This inversion interrupts *STAG1*, resulting in haploinsufficiency of the gene. This finding is associated with autosomal dominant intellectual developmental disorder 47, characterized by developmental delay, feeding difficulties, hypotonia, seizures, and intellectual disability. **This case demonstrates the utility of OGM for precisely evaluating SVs identified on WGS and identifying associated findings not captured by WGS.**

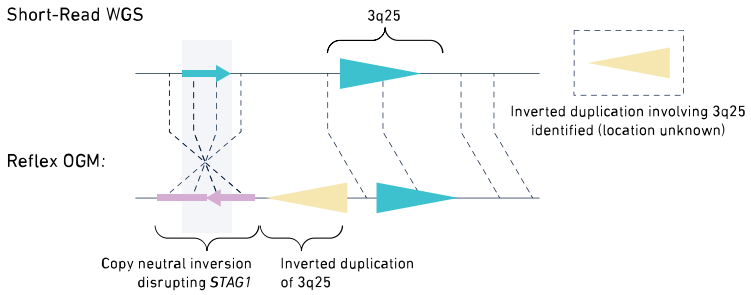

Figure 3: Depiction of findings identified in this patient by WGS and OGM. This image is a simplified representation of this genomic region.

Short Tandem Repeat and *FMR1* Methylation Analysis by Long-Read Sequencing

Short tandem repeat (STR) expansions are variants caused by abnormal increases in the number of repetitive DNA sequences at specific genomic loci; these expansions can disrupt gene function, leading to medically-complex and multisystem phenotypes.[12] Advances in molecular genetics have accelerated the discovery of STR disorders, with over 50 identified to date.[13] **These conditions are enriched in patients with neurological phenotypes, with as many as 1 in 62 having pathogenic expansions depending on clinical indication.**[14] STR expansions can be difficult to detect due to limitations of short-read sequencing to accurately analyze repetitive regions, requiring additional confirmation studies. In contrast, long-read sequencing (LRS) delivers high sensitivity for STR expansions.

LRS is a sequencing method with increased coverage for STR expansions without the need for targeted amplification such as polymerase chain reaction (PCR).[15] LRS eliminates amplification bias with more uniform coverage of the genome with each read spanning thousands of DNA bases. DNA is sheared and ligated to sequencing adapters. Real-time analysis of continuous DNA enables direct measurement of repeat length and assessment of structure. After sequencing, reads are aligned to the reference genome and quantified. Results are classified according to established thresholds for each STR based on published literature, disease-specific databases, and current clinical knowledge.

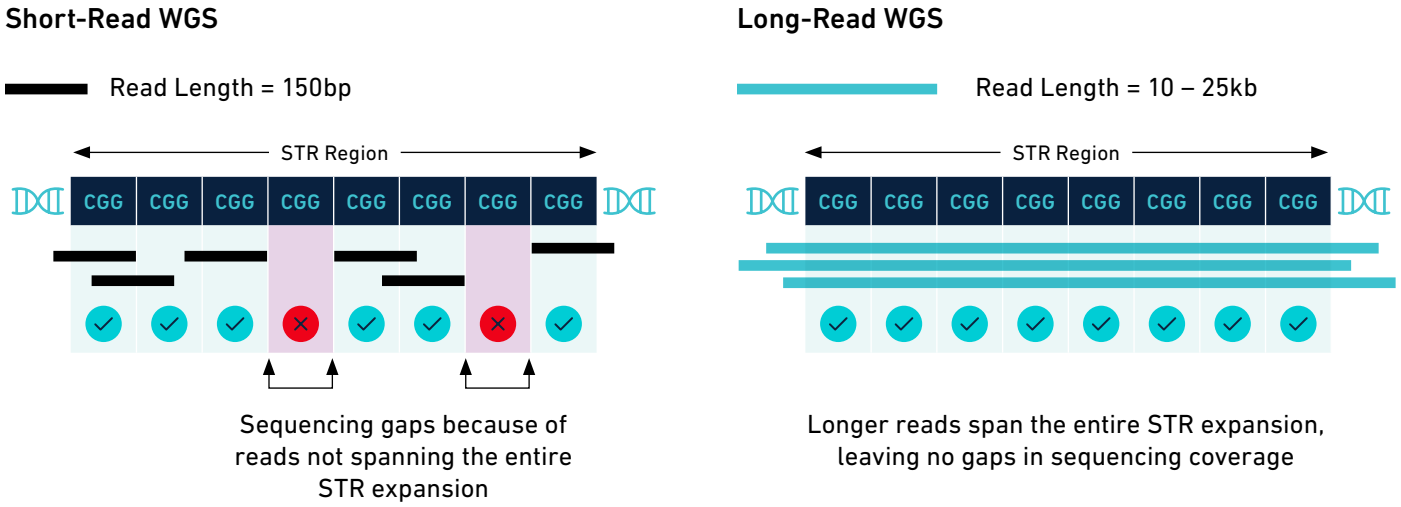

Figure 4: Comparison of long-read and short-read sequencing technology for STR detection. This image is a simplified representation of an STR and does not represent an actual gene associated with STRs.

LRS can also detect native DNA CpG methylation signals, allowing methylation analysis when clinically relevant. In rare cases, Fragile X (*FMR1*) CGG repeat size and methylation status uncouple.[16] Some large *FMR1* premutation alleles might have the same methylation status seen with full mutation alleles associated with Fragile X syndrome. **In these cases, knowing methylation status supports identifying the etiology in patients with Fragile X syndrome-like phenotypes. In addition, heterozygous females with full *FMR1* mutations might have skewed X-inactivation leading to Fragile X syndrome.**

Baylor Genetics validated LRS for the detection and confirmation of STR expansions in blood and other samples. Validation was performed for 31 unique samples, and all LRS results were 100% concordant with prior clinical results confirmed by PCR and/or Southern blot. LRS has been validated to detect STR expansions of varying size in our laboratory and provides accurate and reproducible results with 100% analytical sensitivity, 96.1% analytical specificity, and 100% reproducibility.

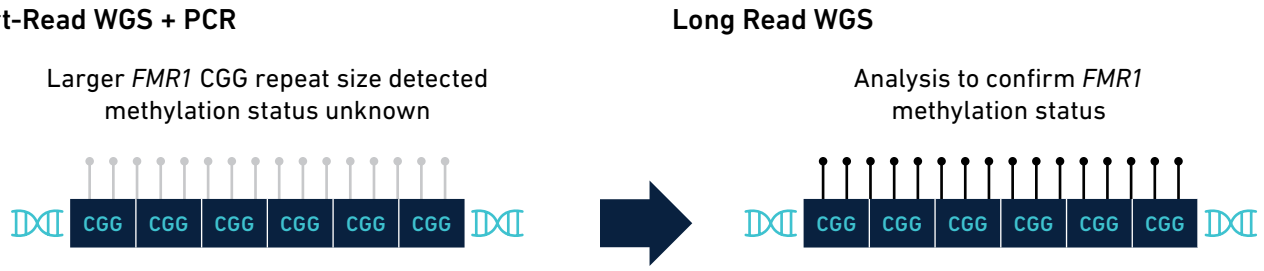

Figure 5: Comparison of long-read and short-read sequencing technology for assessment of *FMR1* methylation for patients with larger repeat sizes. This image is a simplified representation of *FMR1* and does not represent an actual repeat size or methylation status of this gene.

At Baylor Genetics, LRS in tandem with PCR and other technologies are indicated for patients identified to have possible expansions by short-read sequencing data from WGS. LRS may also be performed when precise repeat sizing and detection of large expansions is needed for clinical interpretation. This approach improves detection of STR expansions that may not be fully captured by short-read sequencing without additional orthogonal testing. For Fragile X syndrome assessment, LRS is also used with WGS for patients with approximately 150–200 CGG repeats and female patients with over 200 CGG repeats to clarify methylation and disease status.

LRS Case Example

LRS confirmed the presence and size of a large CAG repeat expansion in the *HTT* gene, associated with juvenile-onset Huntington disease (HD). A young patient was referred with a severe neurological phenotype including global developmental delay, microcephaly, developmental regression, and joint spasticity. The family history was significant for HD. Rapid proband WGS identified a heterozygous pathogenic CAG with over 200 repeats in the *HTT* gene. While the expansion was confirmed by PCR, the full extent of the expansion could only be approximated to 300–350 CAG repeats by subsequent Southern blot. **LRS precisely sized the expansion to 309 CAG repeats, guiding medical management and prognosis for the medical team as well as presenting an opportunity to minimize confirmatory assays for more rapid diagnosis.**

Figure 6: Depiction of findings identified by different test methods for a patient with a large *HTT* repeat expansion. This image is a simplified representation of this gene.

RNA Sequencing Analysis

Variants of uncertain significance (VUS) pose a barrier to rare disease diagnosis and clinical management, with an estimated 20% of patients receiving VUS from GS.[17] **Splicing variants comprise a significant proportion of disease-causing variations and are often classified as VUS due to challenges with capturing their effects on pathogenicity.**[18,19] VUS can be reclassified over time when publicly available gene and variant information is updated, placing a burden on ordering providers and laboratories to review periodically.[20] By bringing functional evidence to clinical care, RNA sequencing (RNA-Seq) offers greater insight into variant classification.

RNA-Seq can provide functional evidence on a variant's expression at the transcript level. For splicing variants, RNA-Seq can clarify the impact on splicing, possibly resulting in reclassification and confirmed diagnosis. In one study, it was estimated that 1.7% of all patients in a cohort of over 600,000 patients across nine clinical specialties could have their variants reclassified by RNA-Seq, potentially leading to a confirmed molecular diagnosis.[21]

Based on Baylor Genetics' internal data, approximately 4% of GS samples have a variant eligible for RNA-Seq. Several cases have more than one RNA-Seq eligible variant. Up to 50% of eligible variants are reclassified, with the vast majority of reclassifications resulting in an upgrade from VUS towards pathogenic; almost all reclassifications confirmed diagnoses for these patients. **Baylor Genetics' results suggest a 1.6% absolute increase in diagnostic yield for GS with RNA-Seq.**

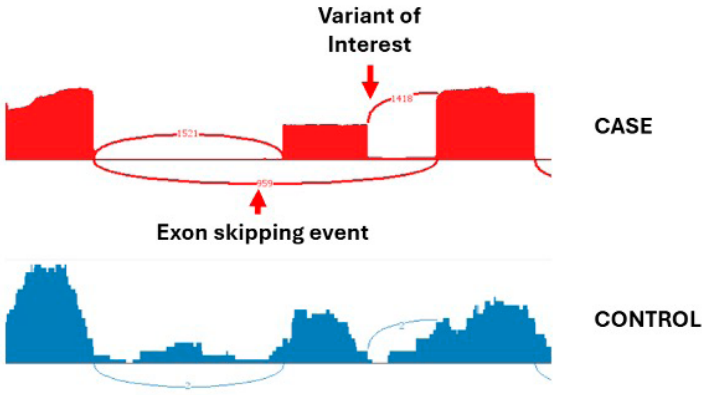

Figure 7: Sashimi plots, color-coded by sample, are used to visualize RNA-Seq. In both Case (red) and Control (blue) plots, exons are identified as plateaus and are connected by arches. In the Case plot, the arch under the X-axis indicates that the middle exon is skipped due to the variant of interest.

Baylor Genetics offers RNA-Seq as a reflex to GS when an eligible variant is identified. An eligible variant is located in a phenotypically concordant gene, classified as VUS or likely pathogenic, and meets splicing prediction algorithm criteria. RNA-Seq is a powerful tool that can add functional evidence to optimize variant classification for patients with inconclusive WGS results.

RNA-Seq Case Example

RNA-Seq clarified the impact of a novel variant in Jeffries-Lakhani neurodevelopmental syndrome (JELANS), a recently-described autosomal recessive condition associated with *CRELD1*. The JELANS phenotypic spectrum is still being defined but includes early-onset seizures, hypotonia, global developmental delay, and cardiac abnormalities. A 2-year-old male was admitted to the pediatric ICU for status epilepticus and a history of developmental delay, hypotonia, and prolonged QTc interval. Previous negative genetic testing included chromosomal microarray and multigene panels. Rapid trio WGS identified compound heterozygous *CRELD1* variants: pathogenic c.575G>A which had been previously reported in patients with JELANS and a novel VUS, c.637+3A>T. RNA-Seq for this VUS showed it causes abnormal splicing that leads to a premature stop codon. This provided functional evidence to upgrade this variant to likely pathogenic. **Reclassification of this variant by RNA-Seq provided clarity for their medical management including the avoidance of QTc prolonging agents.**

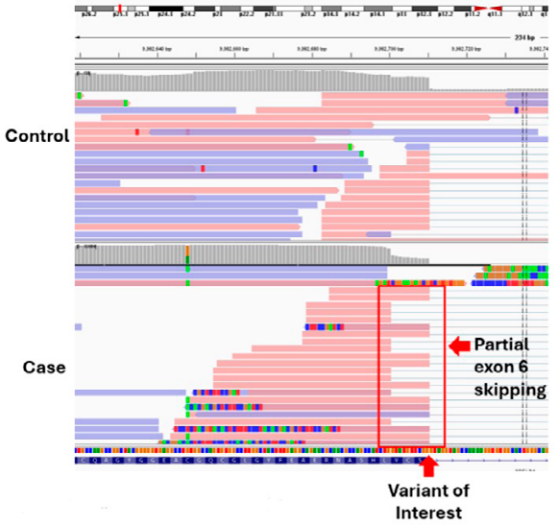

Figure 8: Visualization of partial exon skipping for *CRELD1* c.637+3A>T compared to the control.

Conclusion

Baylor Genetics is proud to offer WGS with OGM, LRS, and RNA-Seq as part of its commitment to translating scientific innovations into accessible clinical solutions for patients.

1. Combined with WGS, these advanced technologies support short-read sequencing, accelerating a quick resolution to the diagnostic odyssey for many patients that might not receive a diagnosis from WGS alone.
2. The breakpoints and orientation of complex structural variants can be more precisely captured with OGM.
3. STR repeat size and methylation status for larger *FMR1* CGG repeats can be supported by LRS for accurate, rapid assessment.
4. RNA-Seq clarifies the impact of splicing variants, leading to enhanced understanding of their pathogenicity.

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