Rady Children's Institute for Genomic Medicine | Clinical Genome Center

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Whole Genome Sequencing Test Definition

This document outlines test specifications for Whole Genome Sequencing (WGS) at the Rady Children's Institute for Genomic Medicine Clinical Genome Center (RCIGM CGC). This test is a laboratory developed test (LDT) that adheres to the guidelines established by CLIA '-88. The WGS test must be ordered by an authorized provider.

Test Type

Laboratory Developed Test (LDT)

Turnaround Time Definitions

Turnaround Time (TAT) is defined as the duration from when a test is accessioned to the time results are signed out and reported.

Operational definitions for WGS TAT for the FINAL report are as follows:

- Ultra-Rapid: ≤ 7 calendar days (preliminary positive reports ≤ 3 calendar days)
- Rapid: ≤ 5 business days
- Standard: ≤ 45 calendar days

Background and Methodology

Genomic DNA is sequenced via next-generation sequencing (NGS) technology. PCR-free library preparation is performed prior to whole genome sequencing (WGS). An average genomic coverage of at least 35x, and/or at least 90% of OMIM genes will achieve 100% of coding base coverage of >10x for each proband. This ensures robust and uniform genome coverage. The current version of this test assesses single nucleotide variants (SNVs), small deletions and insertions, larger deletions and duplications, the mitochondrial genome, SMN1 and SMN2 copy number analysis, repeat expansions in PHOX2B and DMPK. Additionally, this test assesses UPD15 on trio WGS (proband and biological parents). Alignment and variant calling are performed using the Illumina DRAGEN pipeline using the official reference build GRCh38 (hg38). Copy number variation (CNV) calling is performed using a combination of CNV callers. Interpretation of CNVs is focused on variants that overlap or have a boundary that lies within 1 kb of an exon in all coding genes. Repeat expansion calling for PHOX2B and DMPK only are performed using Expansion Hunter algorithm. Analysis for Uniparental Disomy (UPD) associated with imprinting disorders is performed using a RCIGM proprietary algorithm on trio WGS only.

Orthogonal Confirmation Policy

Reported sequencing variants are confirmed by Sanger sequencing at RCIGM or at an external CAP/CLIA laboratory, but may not be confirmed using orthogonal technologies if the following criteria are met:

1) the coverage at the variant's position is ≥ 20x;
2) the allelic balance for heterozygous calls is between 0.3-0.7;
3) the allelic balance is 0 (wild type allele as reference) for homozygous and hemizygous calls;
4) no systematic sequencing errors or local alignment problems are observed;

5) the call is not located in a difficult sequence context (highly homologous and repetitive regions);
6) the call is not a complex insertion/deletion call resulting from nearby variants that may be difficult to align.
If specific protocols require orthogonal confirmations of all reported variants, confirmations will be performed.

Reported copy number variants are confirmed using orthogonal technologies including Multiplex ligation-dependent probe amplification (MLPA), but may not be confirmed using orthogonal technologies if the following criteria are met:
1) the deletion or duplication event contains robust coverage and/or NGS read support;
2) no systematic sequencing errors or local alignment problems are observed;
3) the call is not located in a difficult sequence context (highly homologous, repetitive regions, or segmental duplication regions).
If specific protocols require orthogonal confirmations of all reported variants, confirmations will be performed.

Reporting Categories
Variants related to patient's phenotype – findings with strong evidence of variant pathogenicity and strong evidence that the reported gene-disease association overlaps with the patient's phenotype.

Variants possibly related to patient's phenotype - findings that are suggestive of a diagnosis but lacks either conclusive variant pathogenicity evidence or lack conclusive gene-disease association evidence.

Variants in genes of uncertain significance – findings in genes that lack strong or supporting evidence for association with human disease.

Variants in the mitochondrial genome – findings located within the mitochondrial genome.

Incidental findings - findings in genes that do not overlap with the patient's reported phenotype but may be medically actionable for the patient or tested family members.

Test Specifications
The sensitivity and specificity for SNVs (single nucleotide variants) and small insertions and deletions up to 50 base pairs is greater than 99%. The analytical validity of SNVs was assessed using reference samples provided through the Genome in a Bottle (Zook et al. 2019, PMID: 30936564).

The sensitivity for larger deletions and duplications from WGS is estimated to be greater than 85%, although reliable reference data for these types of events are not well established. Deletions and duplications from 1 kb to whole chromosomal abnormalities are detected with this test.

The sensitivity for detecting GCN trinucleotide repeat expansions within the PHOX2B carboxy terminal polyalanine repeat region by Expansion Hunter on short-read NGS is >90% for expansions greater than 20 repeats. The sensitivity for detecting 3' UTR DMPK trinucleotide repeat expansion (CTG repeats) is >90% for expansions greater than 50 repeats. However, due to short-read NGS technology's limitations, repeat expansion size cannot be precisely determined and reported (see Limitations section). The sensitivity for detecting in trio genome sequencing (proband and biological parents) only clinically relevant UPD associated with imprinting disorders at chromosome 15 is >90% (see Limitations section). This validation does not include distinction between pure isodisomy, pure heterodisomy or mixed hetero/iso-disomy calls; complete (affecting the whole chromosome) or segmental (affecting a part of a chromosome) UPD calls. In addition, the test is not validated to

distinguish between Identity By Descent (IBD) and UPD and to detect mosaic UPD calls. This test is validated for copy number analysis of exons 7 and 8 of the SMN1 and SMN2 genes. Over 95% of pathogenic variation for SMA involves biallelic loss of exon 7 of the SMN1 gene (Prior et al. 2010, PMID: 20057317). Other variation within the SMN1 gene is not detected with this assay. Results will only be reported in the proband if 0 copies of SMN1 are detected. If parental samples are available, only parental SMN1 copy number will be reported. Whole genome sequencing is unable to determine the phase of SMN1 variants in the absence of parental testing. Therefore, in the absence of phasing, this assay does not exclude the possibility than an individual harbors two copies of SMN1 on the same allele and no copies on the other allele (silent carriers), two pathogenic sequence variants on the same SMN1 allele, two pathogenic sequence variants on opposite SMN1 alleles, and one pathogenic sequence variant and the loss of exon 8 on the opposite SMN1 allele.

Non-PCR amplified whole-genome sequencing (WGS) provides a stable, at least ~1,000-fold average, coverage across the entire mitochondrial genome (mtDNA). This test can detect SNVs, small insertions and deletions, as well as large deletions in the mtDNA. Variants that are classified as pathogenic or likely pathogenic that overlap with the patient's phenotype, with levels of heteroplasmy of >5% will be reported. However, suspicious variants of uncertain clinical significance will only be reported if heteroplasmy levels are >20%. If the sample has a SNV with heteroplasmy of >20%, Sanger sequencing will be performed for sequence confirmation. The revised Cambridge Reference sequence is used as a reference (rCRS NC_012920). Interpretations are made with the assumption that any information provided on family relationship is accurate.

Both phenotype-informed and phenotype-agnostic analyses are performed. Likely pathogenic and pathogenic variants that may explain the patient's phenotype will be reported as related/possibly related to the patient phenotype. Selected variants of uncertain significance may be reported as well. Should an incidental finding be revealed during genomic analysis for proband, and proband and parents have opted-in to receive incidental findings, it will be included on the proband's report. Parents do not have the option to opt-in for incidental findings if proband has opted-out. Reported variants are curated and classified in accordance with the American College of Medical Genetics and Genomics Guidelines (PMID: 25741868, 21681106, 24071793, 31690835).

Incidental findings may be reported if the patient and/or patient's family do not opt-out of receiving these results. RCIGM's internal incidental finding policy includes the following: 1) variant must be classified as pathogenic per ACMG guidelines and in alignment with the known inheritance pattern of the genetic condition; 2) the variant is located in a gene with a well-established gene-disease relationship; 3) the gene and associated condition is shown to be medically actionable as established by RCIGM policy and in consultation with the RCIGM clinical team. Pathogenic variants included within the ACMG secondary findings genes that are identified will be reported as well as other findings that meet the above criteria.

Assay Limitations

Full coverage of the genome is not currently possible due to technically challenging repetitive elements and duplicated regions within the genome. Thus, not all regions of the genome are sequenced and/or uniquely aligned to the reference genome. Mosaic variant detection is limited using whole genome sequencing. Repeat expansion detection is limited to the DMPK and PHOX2B genes. The exact number of repeats cannot be determined by the current methodology and therefore orthogonal confirmation for precise sizing may be required. UPD calling is limited to trio WGS on chr15 associated with imprinting disorders. Non-diagnostic

findings do not rule out the diagnosis of a genetic disorder since some genetic abnormalities may be undetectable with the current version of this test. False negative results may occur in the setting of bone marrow transplantation, recent blood transfusion, or suboptimal DNA quality. The chance of false positive or false negative result due to laboratory errors incurred during any phase of testing cannot be completely excluded. Interpretations are made with the assumption that any clinical information provided, including family relationships, are accurate. This test is set up to evaluate the potential contribution of rare disease-causing variants in known disease genes. It is not designed to evaluate for common variants in genes that might contribute to disease risk or for disorders that have a multigenic inheritance. Based on current knowledge, potential disease-causing variants may not always be recognized at the time of testing.

Regulatory Disclosures

This test was developed, and its performance characteristics determined by the Rady Children's Hospital and the Rady Children's Institute for Genomic Medicine. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. RCIGM has established and verified the test's accuracy and precision as outlined in the requirements of CLIA '88. The test is used for clinical purposes. It should not be regarded as investigational or for research. Analytical portions of the testing process may have been performed at one of these locations: 1 – 30. The Rady Children's Institute for Genomic Medicine is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing.