STAT Whole Exome Sequencing Proband Only, plus CNGnome® Test

Test Code	D1010C
Test Summary	STAT diagnostic whole exome sequencing with CNGnome® analysis to detect large copy number changes (CNVs) of the proband only. Results are available in 7-10 days
Turn-Around-Time (TAT)*	7 - 10 days
Acceptable Sample Types	Dried Blood Spots (Preferred sample type) DNA, Isolated Saliva Whole Blood (EDTA)
Acceptable Billing Types	Self (patient) Payment Institutional Billing

Indications for Testing

- Genetically heterogeneous disease caused by likely pathogenic/pathogenic findings in multiple genes
- Condition suggestive of a genetic disorder with a long differential diagnosis list
- Unclear or atypical presentation of a genetic disorder
- Previous genetic testing did not yield a diagnosis

Test Description

This test involves sequencing of the whole exome with a mean coverage of 100x, enhanced coverage of known disease-causing genes, and curated deep-intronic variants. Our WES test will reliably detect the majority of copy number variations (CNVs) of 3 exons or greater. Smaller CNV events may also be detected and reported, but additional follow-up testing is recommended if a smaller CNV is suspected. CNGnome® analysis will detect CNVs greater than or equal to 25kb throughout the genome and reliably detects chromosome uniparental disomy using low pass genome sequencing (5x). In addition to the primary analysis, patients can opt-in to a comprehensive secondary analysis including the recommended list by the American College of Medical Genetics and Genomics (ACMG).

Test Methods and Limitations

Whole exome sequencing is performed on genomic DNA using the Agilent SureSelect Clinical Research Exome v3 targeted sequence capture method to enrich for the exome. Direct sequencing of the amplified captured regions was performed using 2X150bp reads on Illumina next generation sequencing (NGS) systems. A base is considered to have sufficient coverage at 20X and an exon is considered fully covered if all coding bases plus three nucleotides of flanking sequence on either side are covered at 20X or more. A list of low coverage regions, if any, is available upon request. Alignment to the human reference genome (hg19) is performed and annotated variants are identified in the targeted region. Variants reviewed have a minimum coverage of 8X and an alternate allele frequency of 20% or higher. Indels and single nucleotide variants (SNVs) may be confirmed by Sanger sequence analysis before reporting at director discretion. Mitochondrial DNA is sequenced and analyzed using the same pipeline. Mitochondrial variants are reported at a minimum of 5% heteroplasmy if the average coverage of the mitochondrial genome is 1000x. This assay cannot detect variants in regions of the exome that are not covered, such as deep intronic, promoter, and enhancer regions, and areas containing large numbers of tandem repeats. Genes and/or exons located in pseudogene regions are not covered in this assay. Copy number variation (CNV) analysis is designed to detect deletions and duplications of three exons or more; in some instances, due to the size of the exons or other factors, not all CNVs may be analyzed. This assay does not interrogate CNVs in mitochondrial DNA. CNV analysis will not detect tandem repeats, balanced alterations (reciprocal translocations, Robertsonian translocations, inversions, and balanced insertions), methylation abnormalities, triploidy, and genomic imbalances in segmentally duplicated regions. This assay is not designed to detect mosaicism; possible cases of mosaicism may be investigated at the discretion of the laboratory director. Primary data analysis is performed using Illumina bcl2fastq converter v2.19. Secondary analysis is performed using Illumina DRAGEN Bio-IT Platform v.3.4.12. Tertiary data analysis is performed using SnpEff v4.3t and PerkinElmer's internal ODIN v.1.01 software. CNV and absence of heterozygosity are assessed using BioDiscovery's NxClinical v5.1 software.

CNGnome consists of direct sequencing of genomic DNA was performed using 2X150bp reads on Illumina next generation sequencing (NGS)

systems at a mean coverage of 5X in the target region. Alignment to the human reference genome (hg19) was performed and copy number variant (CNV) calls made using the NxClinical software v5.0 (BioDiscovery, Inc., El Segundo, CA). CNVs meeting internal quality assessment guidelines are confirmed by real time quantitative PCR (qPCR) for records after results are reported. Some CNVs are confirmed by qPCR before reporting at a director's discretion. This assay cannot detect CNVs in regions of the genome that are not amenable to NGS and does not interrogate CNVs in mitochondrial DNA. This assay will not detect tandem repeats, balanced alterations (reciprocal translocations, Robertsonian translocations, inversions, and balanced insertions), point mutations, methylation abnormalities, genomic imbalances in segmentally duplicated regions and mosaicism; possible cases of mosaicism may be investigated at the discretion of the laboratory director. Small pathogenic CNVs within the exon, some small intragenic deletions or duplications, as well as complex rearrangements may not be detected. This assay has been validated to detect copy number variants >25 Kb and also has the ability to detect copy number changes such as homozygous deletions. For targeted CNV testing, smaller CNVs may be interrogated, analyzed, and reported per director discretion. This assay may not be able to discern between CNVs that are high copy number gains such as, duplication >=4X. CNVs involving genes with pseudogenes and pseudoexons may not be reliable detected or reported. Due to high similarity of certain regions on chromosome X and chromosome Y, CNVs in the following regions may not be detected for male patients (chrX: 60000-2699520; chrX:154930289-155260560; chrY:10000-2649520; chrY: :59033286-59363566).

Detailed Sample Requirements

Dried Blood Spots (Preferred sample type)

Collection Container(s): Dried blood spot card
Collection: Follow kit instructions. Briefly, allow blood to saturate card until indicated areas are filled and blood has soaked through card. Air dry card at ambient temperature for at least 3 hours.

- **NBS:** Please contact PKIG to request the StepOne® kit.
- **Gene Sequencing:** Please contact PKIG to request the DBS collection kit.

Shipping: Follow kit instructions. Double bag and ship overnight at ambient temperature.

DNA, Isolated

Collection: Required DNA Quantity by Test Type*:

- Next Generation Sequencing (NGS): Send >500ng total gDNA @ > 15ng/?L. Please ship samples in 10mM Tris. No EDTA.
- Sanger Sequencing: Send 5 to 25 micrograms (varies by size of gene). Please contact the laboratory for specificamounts.
- Non-Sequencing Tests: Send 20 micrograms
- Array-based Tests: Send 10 micrograms

*Required DNA Quality: High molecular weight DNA (>12kb). A260/A280 reading should be >= 1.8.
Shipping: Ship overnight at ambient temperature.

SPECIAL INSTRUCTIONS:

- **Research Laboratories:** DNA extracted in research laboratories is not acceptable. Only under exceptional circumstances (e.g. proband not available) will DNA extracted in a research laboratory be accepted for clinical testing. Additional testing (e.g. of other family members) may be required to confirm results.
- **Laboratories outside the United States:** Non-US laboratories are not subject to CLIA regulations and will be reviewed on a case-by-case basis. Please call to speak with a laboratory genetic counselor prior to submitting a DNA sample from any non-CLIA certified laboratory.
- **Special Notes**: If extracted DNA is submitted, information regarding the method used for extraction should be sent along with the sample.

Saliva

Collection Container(s): ORAcollect®•Dx Saliva Swab Collection Kit

Collection: Collect saliva in an ORAcollect®•Dx Saliva Swab Collection Kit according to the manufacturer's instructions. Please contact PerkinElmer Genomics to request the saliva swab collection kit for patients that cannot provide a blood sample as whole blood is the preferred sample.
Sample Condition: Store at ambient temperature. Do not refrigerate or freeze.
Shipping: Ship overnight at ambient temperature.

Whole Blood (EDTA)

Collection Container(s):

EDTA (purple top)

Collection:

Infants (< 2-years): 2 to 3 mL; Children (>2-years): 3 to 5 mL; Older children and adults: 5 to 10 mL Southern Blot Analysis requires 3 mL blood.

Sample Condition: Store at ambient temperature. Do not refrigerate or freeze.
Shipping: Ship overnight at ambient temperature ensuring receipt within 4-days of collection.

SPECIAL INSTRUCTIONS: Clotted or hemolyzed samples are not accepted.