






## STAT Whole Genome Sequencing, Proband ONLY

	<b>Test Code</b>	D2010
	<b>Test Summary</b>	STAT Diagnostic whole genome sequencing and mitochondrial genome sequencing of the proband only
	<b>Turn-Around-Time (TAT)*</b>	10 - 12 days
	<b>Acceptable Sample Types</b>	Whole Blood (EDTA) (Preferred sample type) DNA, Isolated Dried Blood Spots Saliva
	<b>Acceptable Billing Types</b>	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, including exome sequencing

### Test Description

This test involves sequencing of the whole genome with mean coverage of 40X and complete coverage of over 5,400 disease-associated genes. All variants identified will be analyzed according to American College of Medical Genetics and Genomics (ACMG) guidelines. In addition to SNVs, our WGS test will reliably detect CNVs of 3 exons or greater as well as large-scale CNVs such as microdeletions and other gene/chromosomal-level events. CNVs of 1-2 exons may be detected and reported with the recommendation for follow-up testing. Mitochondrial DNA analysis is included. In addition to primary analysis, patients can opt-in to a comprehensive secondary analysis including the recommended list by ACMG.

### Test Methods and Limitations

Whole genome sequencing is performed on genomic DNA using 2X150bp reads on Illumina next generation sequencing (NGS) systems at a mean coverage of 40X in the target region. The target region includes coding exons and 10bp of flanking intronic sequence of the known protein coding RefSeq genes. This sequencing provides >97% coverage of the 22,000 genes in the genome at >40x. 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 is available upon request. Alignment to the human reference genome (hg19) is performed and annotated variants are identified in the targeted region. Variants are called at a minimum coverage of 8X and an alternate allele frequency of 20% or higher. Single nucleotide variants (SNVs) meeting internal quality assessment guidelines are confirmed by Sanger sequence analysis for records after results are reported. Indels and SNVs may be confirmed by Sanger sequence analysis before reporting at director discretion. This assay cannot detect variants in areas containing large numbers of tandem repeats. Mitochondrial DNA is sequenced and analyzed using the same pipeline. 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. Only CNVs related to phenotype are reported. 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 DRAGEN Bio-IT Platform v.2.03. Secondary and tertiary data analysis is performed using PerkinElmer's internal ODIN v.1.01 software for SNVs and Biodiscovery's NxClinical v.4.3 or Illumina DRAGEN Bio-IT Platform v.2.03 for CNV and absence of heterozygosity (AOH).

### Detailed Sample Requirements

#### Whole Blood (EDTA) (Preferred sample type)

*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.

## 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 specific amounts.
- 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  $\geq 1.8$ .

*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.

## Dried Blood Spots

*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.

## 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.