Whole Genome Sequencing, TRIO

Test Code	D2300
Test Summary	Diagnostic whole genome sequencing and mitochondrial genome sequencing of the proband and 2 family members
Turn-Around-Time (TAT)*	6 - 8 weeks
Acceptable Sample Types	Whole Blood (EDTA) (Preferred sample type) Dried Blood Spots Saliva
Acceptable Billing Types	Self (patient) Payment Institutional Billing Commercial Insurance

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 the whole genome with a mean coverage of 40X with a phenotype-driven variant analysis to minimize VUS. All variants identified will be analyzed according to American College of Medical Genetics and Genomics (ACMG) guidelines. This test includes the reliable detection of deletions, duplications, and other gene- and chromosomal-level events. Mitochondrial DNA analysis is included. In addition to the primary analysis, patients can opt-in to a comprehensive secondary analysis including the recommended list by ACMG. Family samples are tested concurrently with the proband sample to further elucidate potential pathogenic changes.

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. 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 the director's 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.3.4.12. Secondary and tertiary data analysis is performed using PerkinElmer's internal ODIN v.1.01 software for SNVs and Biodiscovery's NxClinical v.6.1 or Illumina DRAGEN Bio-IT Platform v.3.4.12 for CNV and the 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: Minimum 5mL. The blood tube should be inverted several times immediately after blood collection to prevent coagulation.

Sample Condition: Store at ambient temperature. Do not refrigerate or freeze.
Shipping: Ship overnight at ambient temperature ensuring receipt within 5-days of collection.

SPECIAL INSTRUCTIONS: Clotted or hemolyzed samples are not accepted.

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.
- **For pre-punched DBS:** The required minimum 6 punches with 3.2 mm or 4 punches 4.75 mm.
  Sample Condition: Follow the instructions provided with the collection set. Store the dried blood at ambient temperature for up to two days. If the specimen cannot be sent as soon as it is dry, the filter paper should be placed in a sealable plastic bag and stored in a refrigerator (? 8°C) or preferably in a freezer.
  Shipping: Follow kit instructions. Double bag and ship overnight at ambient temperature.

Saliva

Collection Container(s):

Oragene™ Saliva Collection Kit or ORAcollect-Dx kit

Collection:

Collect saliva on an Oragene™ Saliva Collection Kit ORAcollect-Dx kit according to the manufacturer's instructions.

Sample Condition: Store at ambient temperature. Do not refrigerate or freeze.
Shipping: Ship overnight at ambient temperature.

SPECIAL INSTRUCTIONS: Please contact PerkinElmer to request the saliva collection kit for patients that cannot provide a blood sample as whole blood is the preferred sample.