Mitochondrial Genome Sequencing by LR-PCR followed by NGS

Test Code	D5230
Test Summary	Long-range PCR to amplify mitochondrial DNA followed by NGS
Turn-Around-Time (TAT)*	3 weeks
Acceptable Sample Types	Whole Blood (EDTA) (Preferred sample type) DNA, Isolated Saliva
Acceptable Billing Types	Self (patient) Payment Institutional Billing

Indications for Testing

Mitochondrial disorders can be difficult to diagnose due to their heterogeneous genocopies and phenocopies. It should be considered in the differential diagnosis of any progressive multisystem disorder or suspected mitochondria dysfunction in which no clear evidence of a nuclear origin of the condition. A full evaluation for a mitochondrial disorder is often warranted in individuals with a complex neurologic picture or a single neurologic manifestation and other system involvement. The pattern of symptoms may be suggestive of a specific mitochondrial condition in some cases. Genetic testing can be a useful tool to confirm disease-causing mutations in mtDNA.

Test Description

The entire mitochondrial genome is amplified by long-range PCR and sequenced using 2X150bp reads on Illumina next-generation sequencing (NGS) systems. The mitochondrial DNA sequence was evaluated for variants using the revised Cambridge reference sequence (rCRS GenBank number NC_01290). Variants are evaluated by their reported frequency in databases such as MitoMap, Genome Aggregation Database (gnomAD), Human Gene Mutation Database (HGMD), ClinVar, and other disease-specific or population-specific databases when applicable. Variants that have a population frequency greater than expected given the prevalence of the disease in the general population are considered to be benign variants. Pathogenic, likely pathogenic, and variants of uncertain significance (VUS) are reported. Benign and likely benign variants are not reported. Silent variants are not reported unless known or suspected to be pathogenic. This assay can detect mtDNA variants as low as 1.5% heteroplasmy. This analysis is not designed to detect variants in the nuclear DNA and it is not designed to detect copy number variants (i.e., deletions and duplications) within the mitochondrial genome.

Condition Description

Mitochondrial disorders refer to a group of clinically and genetically heterogeneous conditions resulting from pathogenic variants in mitochondrial DNA (mtDNA) or in nuclear genes. A mitochondrial genome contains 37 genes that encode ribosomal RNAs (2 genes), transfer RNAs (22 genes), and proteins that are part of the respiratory chain (13 genes). Some affected individuals exhibit clinical features that fall into a discrete clinical syndrome, such as Leber's Hereditary Optic Neuropathy (LHON), Kearns-Sayre syndrome (KSS), chronic progressive external ophthalmoplegia (CPEO), mitochondrial encephalomyopathy with lactic acidosis, stroke-like episodes (MELAS), myoclonic epilepsy with ragged-red fibers (MERRF), neurogenic weakness with ataxia, and retinitis pigmentosa (NARP) or Leigh syndrome (LS). However, often the clinical features are highly variable and non-specific. Common clinical features of mitochondrial disorders include ptosis, external ophthalmoplegia, proximal myopathy, exercise intolerance, cardiomyopathy, sensorineural deafness, optic atrophy, pigmentary retinopathy, and diabetes mellitus. The symptom and severity of mitochondrial diseases depend on the number of defective mitochondria and their distribution in tissues. Mutations in mtDNA are transmitted by maternal inheritance while a mutation in nucleus gene-causing mitochondria disorders can follow an autosomal or x-linked pattern.

Test Methods and Limitations

Mitochondria genome is amplified by long-range PCR followed by library preparation and sequencing

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.

DNA, Isolated

Collection:

Required DNA Quantity by Test Type*:

- **Next Generation Sequencing (NGS):** Send >1000 ng total gDNA @ >15 ng/?L. Please ship samples in 10mM Tris. Do not use EDTA.
- **Sanger Sequencing:** Send >500 ng total gDNA @ >15 ng/?L (varies by the size of the gene and the variants requested).
- **Non-Sanger Sequencing Tests:** Send >500 ng total gDNA @ >15 ng/?L.

Sample Condition: * Required DNA Quality: High molecular weight DNA (>12kb). A260/A280 reading should be ? 1.8. A260/230 a ratio range of 1.8 to 2.2. Contact the laboratory for specific amounts if total ng cannot be met.
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):

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.