

## Combined Mito Genome Plus Mito Focused Nuclear Gene Panel

### Full Sequence Analysis and Deletion Testing of the Mitochondrial Genome Plus Sequence Analysis and Exon-Level Deletion/Duplication Testing of 202 Nuclear Genes

**Panel Gene List:** *AARS2, ABCB7, ACAD9, ACO2, AFG3L2, AGK, AIFM1, ALAS2, APOPT1, ATP5A1, ATP5E, ATP7B, ATPAF2, AUH, BCS1L, BOLA3, C12orf65, C19orf12, CARS2, CLPB, COA5, COA6, COASY, COQ2, COQ4, COQ6, COQ7, COQ8A, COQ8B, COQ9, COX10, COX14, COX15, COX20, COX6A1, COX6B1, COX8A, CYC1, DARS2, DGUOK, DLAT, DLD, DNA2, DNAJC19, DNM1L, EARS2, ECHS1, ELAC2, ETFA, ETFB, ETFDH, ETHE1, FARS2, FASTKD2, FBXL4, FDX1L, FH, FLAD1, FOXRED1, GARS, GCDH, GFER, GFM1, GFM2, GLRX5, GTPBP3, GYG2, HARS2, HMGCL, HTRA2, IARS2, IBA57, ISCA2, ISCU, LAMP2, LARS, LARS2, LIAS, LIPT1, LRPPRC, LYRM4, LYRM7, MARS2, MFF, MFN2, MGME1, MICU1, MPC1, MPV17, MRPL12, MRPL3, MRPL44, MRPS16, MRPS22, MRPS7, MTFMT, MTO1, MTPAP, NARS2, NDUFA1, NDUFA10, NDUFA11, NDUFA12, NDUFA2, NDUFA4, NDUFA9, NDUFAF1, NDUFAF2, NDUFAF3, NDUFAF4, NDUFAF5, NDUFAF6, NDUFAF7, NDUFB11, NDUFB3, NDUFB9, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS6, NDUFS7, NDUFS8, NDUFV1, NDUFV2, NFS1, NFU1, NR2F1, NUBPL, OPA1, OPA3, OTC, PARS2, PC, PCCA, PCCB, PDHA1, PDHB, PDHX, PDP1, PDSS1, PDSS2, PET100, PNPT1, POLG, POLG2, PRKAG2, PUS1, QARS, RARS, RARS2, RMND1, RNASEH1, RRM2B, SARS2, SCO1, SCO2, SDHA, SDHAF1, SERAC1, SFXN4, SLC19A2, SLC19A3, SLC22A5, SLC25A26, SLC25A3, SLC25A38, SLC25A4, SLC25A46, SPAST, SPG7, SUCLA2, SUCLG1, SURF1, TACO1, TARS2, TAZ, TFAM, TIMM8A, TK2, TMEM126A, TMEM126B, TMEM70, TPK1, TRIT1, TRMT10C, TRMU, TRNT1, TSFM, TTC19, TUFM, TWNK, TYMP, UQCC2, UQCC3, UQCRB, UQCRC2, UQCRQ, VARS2, WDR45, WFS1, YARS2*

#### **Clinical Features:**

Mitochondrial disorders are clinically heterogeneous and result from dysfunction of the mitochondrial respiratory chain, which can be caused by pathogenic variants in mitochondrial DNA (mtDNA) or in nuclear genes. Mitochondrial disorders may affect a single organ, but many involve multiple organ systems particularly those that are highly dependent on aerobic metabolism (brain, skeletal muscle, heart, kidney and endocrine system). Patients may present at any age; however, individuals with nuclear DNA variants generally present in childhood and those with mtDNA variants generally present in late childhood or in adults. Some affected individuals exhibit clinical features that fall into a discrete clinical syndrome, such as Leber Hereditary Optic Neuropathy (LHON), Kearns-Sayre syndrome (KSS), chronic progressive external ophthalmoplegia (CPEO), mitochondrial encephalomyopathy with lactic acidosis and

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 and many affected persons do not fit into one particular category. Similar clinical features can be caused by mtDNA variants or nuclear gene variants. Common features of mitochondrial disease may include ptosis, external ophthalmoplegia, proximal myopathy, exercise intolerance, cardiomyopathy, sensorineural deafness, optic atrophy, pigmentary retinopathy, diabetes mellitus, encephalopathy, seizures, dementia, migraine, stroke-like episodes, ataxia, spasticity, chorea and dementia. It has been estimated that approximately 7% of patients diagnosed with autism may have an underlying disorder of mitochondrial function<sup>1</sup>. The prevalence of mitochondrial disorders has been estimated 1/5000 to 1/8500.<sup>2-5</sup>

### **Genetics:**

Approximately 1500 gene products are involved in maintaining proper mitochondrial respiratory chain function.<sup>2</sup> The mtDNA encodes for ribosomal RNAs (two genes), transfer RNAs (22 genes) and 13 proteins that are part of the respiratory chain. Other genes required for mitochondrial function are nuclear. Variants in mtDNA arise de novo or are maternally inherited. In most cases, mtDNA point variants are inherited, whereas gross deletions arise de novo<sup>6</sup>. Each mitochondrion has multiple copies of mtDNA and there are hundreds to thousands of mitochondria per cell, dependent on the cell type. Usually, mtDNA variant affect only a fraction of the mtDNA; the coexistence of normal and variant mtDNA is called heteroplasmy. When the percentage of variant mtDNA (variant load) reaches a certain threshold that varies by tissue type, age, and specific variant, the function of that tissue may become impaired.<sup>6</sup> As the variant load varies within and between tissues, the manifestation of mitochondrial disease may reflect tissue-specific variant load.<sup>4</sup> Many factors can affect the percent heteroplasmy these include physiologic processes that are affected by the mtDNA variant, the function of the tissue, and the rate of cell division in that tissue. Variants in mtDNA may only be identified in specific tissues, particularly those with a lower rate of cell division such as skeletal muscle, heart and brain.<sup>6</sup> To date, around 200 nuclear genes have reported disease-causing variants associated with a primary mitochondrial disorder. Disorders due to nuclear gene variants that affect mitochondrial function may be inherited in an autosomal dominant, autosomal recessive or X-linked manner.

### **Test Methods:**

Genomic DNA is extracted from the submitted specimen. For the nuclear genome, the DNA is enriched for the complete coding regions and splice junctions of the genes on this panel using a proprietary targeted capture system developed by GeneDx for next-generation sequencing with CNV calling (NGS-CNV). The enriched targets are simultaneously sequenced with paired-end reads on an Illumina platform. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build

GRCh37/UCSC hg19. After gene specific filtering, data are analyzed to identify sequence variants and most deletions and duplications involving coding exons; however, technical limitations and inherent sequence properties effectively reduce this resolution for some genes. Alternative sequencing or copy number detection methods were used to analyze regions with inadequate sequence or copy number data by NGS. The entire mitochondrial genome from the submitted sample is also amplified and sequenced using Next Generation sequencing. DNA sequence is assembled and analyzed in comparison with the revised Cambridge Reference Sequence (rCRS GeneBank number NC\_012920) and the reported variants listed in the MITOMAP database (<http://www.mitomap.org>). Next generation sequencing may not detect large-scale mtDNA deletions present at 5% heteroplasmy or lower or mtDNA point variants present at 1.5% heteroplasmy or lower. Alternative sequencing or other detection methods may be used to analyze or confirm mtDNA variants. Reportable variants include pathogenic variants, likely pathogenic variants and variants of uncertain significance. Likely benign and benign variants, if present, are not routinely reported but are available upon request.

For the nuclear genome, the technical sensitivity of sequencing is estimated to be >99% at detecting single nucleotide events. It will not reliably detect deletions greater than 20 base pairs, insertions or rearrangements greater than 10 base pairs, or low-level mosaicism. The copy number assessment methods used with this test cannot reliably detect copy number variants of less than 500 base pairs or mosaicism and cannot identify balanced chromosome aberrations. Assessment of exon-level copy number events is dependent on the inherent sequence properties of the targeted regions, including shared homology and exon size. Due to the presence of non-functional pseudogenes, regions of the *GYG2*, *NR2F1*, *PDSS1*, and *TSMF*, and genes are not fully sequenced by this method. For the *COQ7*, *COX8A*, *HTRA2*, *NDUFB11*, *RNASEH1*, *SCO2*, *SDHA*, *SLC25A26*, *SLC25A46*, *TFAM*, *TMEM126B*, and *TRMT10C* genes, sequencing but not deletion/duplication analysis, was performed. In addition, for the *COA5* gene deletion/duplication analysis may only be able to detect full gene events. For the mitochondrial genome, next generation sequencing can detect mtDNA point variants as low as 1.5% heteroplasmy and large-scale deletions (2 kb or larger) as low as 5% heteroplasmy. However, for large-scale mtDNA deletions observed at less than 15% heteroplasmy a quantitative value will not be provided. This test is expected to detect greater than 98% of known pathogenic variants and deletions of the mitochondrial genome.

### **Clinical Sensitivity:**

The combination of full sequence analysis and deletion testing of the mitochondrial genome plus analysis of these 202 nuclear genes is estimated to identify disease-causing variant(s) in approximately 60-80% patients with a primary mitochondrial disorder.<sup>2, 7, 8, 9</sup>

## Specimen Requirements and Shipping/Handling:

Special Considerations for Mitochondrial Disorders: While variants in nuclear genes are easily detectable in whole blood specimens, some mtDNA variants may only be detectable in other tissues. Tissue biopsies are preferable for mtDNA analysis, therefore, sending a blood sample together with a tissue biopsy from the same patient is recommended for this test.

- **PREFERRED: TISSUE BIOPSIES** (muscle, liver, heart, kidney or brain) **AND BLOOD SPECIMEN:** For tissue, please submit 50 mg, frozen within minutes after collection, stored at -80°C and shipped on dry ice with overnight delivery. Whole blood in EDTA; Adults: 8-10 ml; Children: 4-6 ml; Infants: 2-3 ml. Ship blood separately, overnight at ambient temperature, using a cool pack in hot weather. Blood specimens may be refrigerated for up to 7 days prior to shipping. **DO NOT FREEZE BLOOD**

## References:

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3. Tarnopolsky et al. (2005) *Med Sci Sports Exerc* 37 (12):2086-93 (PMID: 16331134)
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6. Longo et al. (2003) *Neurol Clin* 21 (4):817-31 (PMID: 14743651)
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9. Taylor et al. (2014) *JAMA* 312 (1):68-77 (PMID: 25058219)