Genetic Testing for Mitochondrial Disorders at GeneDx:  
Mitochondrial Complex IV Deficiency Nuclear Gene Panel  
Sequence Analysis and Exon-Level Deletion/Duplication* Testing of 18 Nuclear Genes

<table>
<thead>
<tr>
<th>COA5 (C2orf64)</th>
<th>COX6B1</th>
<th>OPA3</th>
<th>SPG7</th>
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<tbody>
<tr>
<td>COX10</td>
<td>ETHE1</td>
<td>POLG (POLG1)</td>
<td>SURF1</td>
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<tr>
<td>COX14 (C12orf62)</td>
<td>FASTKD2</td>
<td>RRM2B</td>
<td>TACO1</td>
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<tr>
<td>COX15</td>
<td>LRPPRC</td>
<td>SCO1</td>
<td></td>
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<tr>
<td>COX20 (FAM36A)*</td>
<td>OPA1</td>
<td>SCO2</td>
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*Exon-level deletion/duplication testing is not currently available for this gene

Complex IV (cytochrome c oxidase or COX) is the terminal component of the respiratory chain catalyzing the reduction of molecular oxygen to water. COX is composed of 3 catalytic subunits encoded by mtDNA genes and ten accessory subunits encoded by nuclear genes. The accessory subunits are involved in structural stabilization, assembly of the complex and in the modulation of its catalytic activity. Many other nuclear gene encoded proteins needed for the assembly of complex IV (referred to as assembly factors) are not themselves components of the final complex IV structure. Complex IV deficiency is the second most common defect of the oxidative phosphorylation (OXPHOS) system accounting for nearly 30% of all OXPHOS disorders (1). Patients with COX deficiency can present with a broad and heterogeneous range of clinical phenotypes. This panel includes the nuclear genes with reported mutations associated with complex IV deficiency.

Clinical features of mitochondrial disorders:
Mitochondrial disorders are clinically heterogeneous and result from dysfunction of the mitochondrial respiratory chain, which can be caused by mutations 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, nuclear DNA mutations generally present in childhood and mtDNA mutations 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 mutations or nuclear gene mutations. 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 (2). The prevalence of mitochondrial disorders has been estimated 1/5000 to 1/8500. (3-6)

Genetics of mitochondrial disorders:
To date, around 120 nuclear genes have reported disease-causing mutations associated with a primary mitochondrial disorder. Disorders due to nuclear gene mutations that affect mitochondrial function may be inherited in an autosomal dominant, autosomal recessive or X-linked manner.
Reasons for referral:
1. Molecular confirmation of a clinical diagnosis
2. Testing of patients suspected of having a mitochondrial disorder
3. Prenatal diagnosis for known familial mutation(s) in nuclear genes in at-risk pregnancies.
4. Genetic counseling

Post-PCR Next-Generation Sequencing and Deletion/Duplication Analysis of 18 Nuclear Genes Associated with Complex IV Deficiency

Method:
Using genomic DNA obtained from blood (2-5 mL in EDTA), the coding exons of the 18 genes including their splice junctions are PCR amplified and sequenced using a novel solid-state sequencing-by-synthesis process that allows sequencing a large number of amplicons in parallel. (7) DNA sequence is then assembled and compared to the published genomic reference sequences. The presence of any potentially disease-associated sequence variant(s) is confirmed by conventional dideoxy DNA sequence analysis. In addition, targeted array CGH analysis with exon-level resolution is performed concurrently to evaluate for a deletion or duplication of one or more exons in most of the genes included in the panel(s). The technical sensitivity of next-generation sequencing is estimated to be 98%. Next-generation sequencing will not detect large chromosomal aberrations and deletions, insertions, or rearrangements greater than or equal to 5 base pairs. The targeted array CGH is expected to detect most exonic deletions or duplications as small as 150-300 bp.

Advantages of Post PCR Next-Generation Sequencing Versus Capture Next-Generation Sequencing:
Pseudogenes and homologous sequences can lead to both false positive and false negative results. Approximately 25% of human genes and approximately 30% of the genes included in this panel have homologous, but non-functional pseudogenes in the genome (www.pseudogene.org). In contrast to capture next-generation sequencing (NGS), post-PCR NGS uses PCR-based sequence enrichment with unique primers that selectively amplify only the gene of interest, thus avoiding many pseudogene problems.

Test Sensitivity:
This panel includes more than 95% of the known nuclear gene mutations associated with isolated mitochondrial complex IV deficiency. Most isolated COX deficiencies are caused by mutations in nuclear genes; mutations in the mtDNA-encoded COX subunit genes are relatively rare. To our knowledge, there has not been a large study examining the frequency of mutations in the genes on this panel in patients with isolated COX deficiency. Therefore, the clinical sensitivity of this panel is not known.

Complex IV (Cytochrome C Oxidase) Deficiency
18 genes: COA5 (C2ORF64); COX10; COX14 (C12orf62); COX15; COX20 (FAM36A); COX6B1; ETHE1; FASTKD2; LRPPRC; OPA1; OPA3; POLG; RRM2B; SCO1; SCO2; SPG7; SURF1; TACO1

Specimen Requirements and Shipping/Handling:
- Blood: Whole blood in EDTA; Adults: 8-10 ml; Children: 4-6 ml; Infants: 2-3 ml. Ship blood overnight at ambient temperature, using a cool pack in hot weather. Blood specimens may be refrigerated for up to 7 days prior to shipping.
- Extracted DNA: A minimum amount of 20 micrograms of high quality DNA, with a concentration of at least 50 ng/ul (50 nanograms per microliter).
- Buccal Brushes: NOT accepted for this test.
- Cultured fibroblasts NOT accepted for this test
- Prenatal Diagnosis (for specific known familial mutation(s) or deletion(s) only): please refer to the specimen requirements table on our website at: http://www.genedx.com/test-catalog/prenatal/. Ship specimen overnight at ambient temperature, using a cool pack in hot weather.
Required Forms:
- Sample Submission (Requisition) Form – complete all relevant pages
- Payment Options Form or Institutional Billing Instructions

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<tr>
<th>Test#</th>
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<td>613</td>
<td>Complex IV Deficiency Nuclear Gene Panel (sequencing and del/dup analysis for 18 genes)</td>
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Possible ICD9 Codes:
- 277.87 Disorder of mitochondrial metabolism
- 276.2 Lactic acidosis
- 250 Diabetes
- 330.8 Leigh syndrome
- 389.10 Hearing loss, sensorineural
- 425.1 Hypertrophic cardiomyopathy

References: