

## Deletion Testing of mtDNA

### Clinical Features:

Mitochondrial DNA (mtDNA) deletion syndromes predominately consist of three overlapping phenotypes that usually occur in a single individual in a family.<sup>1</sup> The three phenotypes are Kearns-Sayre syndrome (KSS), progressive external ophthalmoplegia (PEO), and Pearson syndrome (Table 1).

Table 1. Characteristics of Mitochondrial DNA Deletion Syndromes

mtDNA Deletion Syndromes	Disease Characteristics	Characteristics of mtDNA Deletions <sup>1</sup>
KSS	A triad of (1) onset < 20 y/o, (2) pigmentary retinopathy, and (3) PEO, plus at least one of the following: cardiac conduction block, cerebrospinal fluid protein concentration greater than 100 mg/dL, or cerebellar ataxia	~90% have a large-scale 1.3-10 kb deletion usually present in all tissues, but most abundant in muscle, and often undetectable in blood cells. A deletion of 4977 bp is the most common. Over 150 deletions have been associated with KSS. Large-scale duplications have also been reported.
CPEO	Ptosis, ophthalmoplegia, and variably severe proximal limb weakness may be the early sign of KSS.	Deletion/duplication analysis is estimated to identify a deletion in approximately 50% of patients. Deletions are confined to skeletal muscle.
Pearson Syndrome	Sideroblastic anemia, exocrine pancreas dysfunction, usually fatal in infancy: children who survive the disease usually go on to develop KSS.	Deletions are usually more abundant in blood than other tissue types. Deletion load gradually decreases in blood and increases in muscle as the disease evolves to PEO and KSS over time.

### Genetics:

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>2</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 variants affect only a fraction of the mtDNA; the coexistence of normal and mutant mtDNA is called heteroplasmy. When the percentage of mutant 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>2</sup> As the variant load varies within and between tissues, the manifestation of mitochondrial disease may reflect tissue-specific variant load.<sup>3</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>2</sup> Large deletions of mtDNA associated with Pearson syndrome are detectable in blood, while large deletions associated with KSS and CPEO are detectable in skeletal muscle.

### Test Methods:

Using genomic DNA, the entire mitochondrial genome is amplified by long-range PCR and sequenced for the detection of large mtDNA deletions using a novel solid-state sequencing-by-synthesis process that allows sequencing a large number of amplicons in parallel.<sup>5</sup> DNA sequences are assembled and compared to the published mitochondrial genome reference sequences for analysis. The presence of any variants is confirmed by conventional dideoxy sequence analysis, custom-designed microarray or other methods.

### Test Sensitivity:

Approximately 90% of individuals with Pearson syndrome or KSS, and 50% of patients with CPEO have a large-scale (2-10 kb) mtDNA deletion.<sup>1</sup> MtDNA deletions larger than 2 kb account for >95% of the reported disease causing mtDNA deletions and are responsible for >99% cases of mtDNA deletion-associated mitochondrial disease ([www.mitomap.org](http://www.mitomap.org)). Deletion/duplication analysis, as performed by GeneDx, is expected to be highly sensitive and able to detect all large mtDNA deletions/duplications associated with mitochondrial disease including those associated with KSS, CPEO, and Pearson syndrome. Heteroplasmy as low as 2.5%-5% is expected to be detected. However, for large-scale mtDNA deletions observed at less than 15% heteroplasmy a quantitative value will not be provided.

### References:

1. DiMaruo, S. and Hirano, M. (Updated [May 3, 2011]). Mitochondrial DNA Deletion Syndromes In: GeneReviews at Genetests: Medical Genetics Information Resource (database online). Copyright, University of Washington, Seattle. 1997-2012. Available at <http://www.genetests.org>
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5. Bennett S.(2004) *Pharmacogenomics* 5:433-8.