Blueprint Genetics

Mitochondrial Genome Test

Test code: MI0101

Is a 37 mtDNA gene panel with extremely high sequencing coverage. Is ideal for patients who have a suspicion of mitochondrial disease and have been tested negative using a targeted nuclear gene panel or whole exome test that did not cover mitochondrial DNA at the time of testing.

Is not available for prenatal samples from ongoing pregnancies.

About mitochondrial disorders

This panel includes the 16.5-kb mitochondrial genome (mtDNA) containing 37 genes, all of which are essential for normal mitochondrial function. Thirteen of these genes encode for polypeptides that form structural subunits of the respiratory chain (RC complexes I, III, IV, and V), which is functionally essential and evolutionarily constrained, and the RNA necessary for mtDNA translation, namely 2 rRNAs (MT-RNR1 and MT-RNR2, encoding 12S and 16S rRNA) and 22 transfer RNAs (tRNA, e.g. tRNALys), which are interspaced between the protein-encoding genes. The vast majority of over 1000 mitochondrial proteins are encoded by nuclear genes. There are several unique properties associated with the mitochondrial genome that are important in understanding the primary mitochondrial DNA disease: 1) there are multiple copies of mtDNA in each cell; 2) mtDNA is maternally inherited, 3) mutated mtDNA coexists with wild type mtDNA (heteroplasmy), 4) minimum critical proportion of mutated mtDNAs is necessary before tissue dysfunction comes apparent (threshold effect), 5) mutation load (heteroplasmy level) may vary among different tissues.Mitochondrial diseases are a clinically heterogeneous group of disorders that arise as a result of dysfunction of the mitochondrial respiratory chain. While some mitochondrial disorders only affect a single organ, many involve multiple organ systems. Patients' symptoms can range from mild to severe and can occur at any age. Common clinical features of mitochondrial disease include fatigue, weakness, metabolic strokes, seizures, cardiomyopathy, arrhythmias, developmental or cognitive disabilities, diabetes mellitus, impairment of hearing, vision, growth, liver, gastrointestinal, or kidney function, and more. Typical early onset mitochondrial diseases include Leigh syndrome, depletions syndromes, Kearns-Sayre (KSS) and Pearson syndrome, whereas chronic progressive external ophthalmoplegia (CPEO), Leber's hereditary optic neuropathy (LHON), neuropathy, ataxia, and retinitis pigmentosa (NARP), mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) are seen in later childhood or adult life. To date, close to 400 mutations have been reported, that are known to cause a spectrum of mitochondrial diseases.Most mtDNA alterations are neutral polymorphisms, which define different population haplogroups and have been used for example in tracking human migrations. Mitochondrial genetics differ considerably from Mendelian genetics. Uniparental inheritance, cellular polyploidy and a deviation from the standard genetic code are just some of the characteristics of mitochondrial genetics. Although mitochondrial variants are typically maternally inherited, they can be sporadic (de novo). There are currently no standard guidelines for reporting and classifying mtDNA variants. The nomenclature differs slightly from the standard HGVS nomenclature for nuclear genes. Mitochondrial variants are reported using gene name and m. numbering (e.g. m.8993T>C), and p. numbering for protein coding changes. The current accepted reference sequence is the Revised Cambridge Reference Sequence (rCRS) of the Human Mitochondrial DNA: GenBank sequence NC_012920 gi:251831106.4. The multi-copy nature of the mitochondrial genome leads to complicated genetics. MtDNA mutations can be either heteroplasmic (where both mutated and wild type mtDNA co-exist within the cell) or homoplasmic (only mutated species are present). Heteroplasmy percentages may vary in different tissue types from the sample tested; therefore, interpreting low heteroplasmic levels also must be done in the context of the tissue tested, and may be meaningful only in the affected tissue such as muscle. Lack of correlation between the heteroplasmy level and disease severity further complicate the interpretation of mtDNA variants. The genotype–phenotype correlation is not always clear, and most patients do not fit within any defined syndrome and even within a family the expressivity of the disease can be extremely variable.Diagnostics areas affected most: Cardiology (cardiomyopathy), ENT (hearing loss), Metabolic, Neurology, Ophthalmology

Gene set description

Genes in the Mitochondrial Genome Test and their clinical significance

Gene	Associated phenotypes	Inheritance	ClinVar	HGMD

MT-ATP6	Neuropathy, ataxia, and retinitis pigmentosa, Leber hereditary optic neuropathy, Ataxia and polyneuropathy, adult-onset, Cardiomyopathy, infantile hypertrophic, Leigh syndrome, Striatonigral degeneration, infantile, mitochondrial	Mitochondrial	19
MT-ATP8	Cardiomyopathy, apical hypertrophic, and neuropathy, Cardiomyopathy, infantile hypertrophic	Mitochondrial	4
MT-CO1	Myoglobinuria, recurrent, Leber hereditary optic neuropathy, Sideroblastic anemia, Cytochrome C oxidase deficiency	Mitochondrial	17
MT-CO2	Cytochrome c oxidase deficiency	Mitochondrial	8
MT-CO3	Cytochrome c oxidase deficiency, Leber hereditary optic neuropathy	Mitochondrial	9
MT-CYB	Leber hereditary optic neuropathy	Mitochondrial	69
MT-ND1	Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes, Leber hereditary optic neuropathy, Leber optic atrophy and dystonia	Mitochondrial	21
MT-ND2	Leber hereditary optic neuropathy, Mitochondrial complex I deficiency	Mitochondrial	6
MT-ND3	Leber optic atrophy and dystonia, Mitochondrial complex I deficiency	Mitochondrial	7
MT-ND4	Leber hereditary optic neuropathy, Leber optic atrophy and dystonia, Mitochondrial complex I deficiency	Mitochondrial	11
MT-ND4L	Leber hereditary optic neuropathy	Mitochondrial	2
MT-ND5	Myoclonic epilepsy with ragged red fibers, Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes, Leber hereditary optic neuropathy, Mitochondrial complex I deficiency	Mitochondrial	19
MT-ND6	Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes, Oncocytoma, Leber hereditary optic neuropathy, Leber optic atrophy and dystonia, Mitochondrial complex I deficiency	Mitochondrial	16
MT-RNR1	Deafness, mitochondrial	Mitochondrial	3
MT-RNR2	Chloramphenicol toxicity/resistance	Mitochondrial	2
MT-TA	Leber hereditary optic neuropathy, Mitochondrial multisystemic disorder, Progressive external ophthalmoplegia, Dilated cardiomyopathy (DCM)	Mitochondrial	4
MT-TC	Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes	Mitochondrial	3
MT-TD	Mitochondrial multisystemic disorder	Mitochondrial	1
MT-TE	Diabetes-deafness syndrome, Mitochondrial myopathy, infantile, transient, Mitochondrial myopathy with diabetes	Mitochondrial	5
MT-TF	Myoclonic epilepsy with ragged red fibers, Nephropathy, tubulointerstitial, Encephalopathy, mitochondrial, Epilepsy, mitochondrial, Myopathy, mitochondrial, Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes	Mitochondrial	7
MT-TG	Hypertrophic cardiomyopathy, Encephalopathy, Myopathy	Mitochondrial	3

MT-TH	Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes	Mitochondrial	4
MT-TI	Progressive external ophthalmoplegia	Mitochondrial	7
MT-TK	Myoclonic epilepsy with ragged red fibers	Mitochondrial	5
MT-TL1	Cytochrome c oxidase deficiency, Myoclonic epilepsy with ragged red fibers, Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes, Diabetes-deafness syndrome, Cyclic vomiting syndrome, SIDS, susceptibility to	Mitochondrial	14
MT-TL2	Progressive external ophthalmoplegia, Mitochondrial multisystemic disorder	Mitochondrial	5
MT-TM	Mitochondrial Myopathy, Leigh syndrome, Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes	Mitochondrial	1
MT-TN	Progressive external ophthalmoplegia	Mitochondrial	3
MT-TP	Mitochondrial multisystemic disorder	Mitochondrial	2
MT-TQ	Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes, Encephalopathy	Mitochondrial	2
MT-TR	Dilated cardiomyopathy (DCM)	Mitochondrial	2
MT-TS1	Myoclonic epilepsy with ragged red fibers, Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes	Mitochondrial	10
MT-TS2	Mitochondrial multisystemic disorder	Mitochondrial	2
MT-TT		Mitochondrial	5
MT-TV	Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes	Mitochondrial	3
MT-TW	Leigh syndrome, Mitochondrial Myopathy	Mitochondrial	8
MT-TY		Mitochondrial	4

*Some regions of the gene are duplicated in the genome. Read more.

The gene has suboptimal coverage (means <90% of the gene's target nucleotides are covered at >20x with mapping quality score (MQ>20) reads), and/or the gene has exons listed under Test limitations section that are not included in the panel as they are not sufficiently covered with high quality sequence reads.

The sensitivity to detect variants may be limited in genes marked with an asterisk (*) or number sign (#)

Gene refers to the HGNC approved gene symbol; Inheritance refers to inheritance patterns such as autosomal dominant (AD), autosomal recessive (AR), mitochondrial (mi), X-linked (XL), X-linked dominant (XLD) and X-linked recessive (XLR); ClinVar refers to the number of variants in the gene classified as pathogenic or likely pathogenic in this database (ClinVar); HGMD refers to the number of variants with possible disease association in the gene listed in Human Gene Mutation Database (HGMD). The list of associated, gene specific phenotypes are generated from CGD or Mitomap databases.

Test Strengths

The strengths of this test include:

- CAP accredited laboratory
- CLIA-certified personnel performing clinical testing in a CLIA-certified laboratory
- Powerful sequencing technologies, advanced target enrichment methods and precision bioinformatics pipelines ensure superior analytical performance
- Careful construction of clinically effective and scientifically justified gene panels
- Some of the panels include the whole mitochondrial genome (please see the Panel Content section)
- Our Nucleus online portal providing transparent and easy access to quality and performance data at the patient level
- Our publicly available analytic validation demonstrating complete details of test performance
- ~2,000 non-coding disease causing variants in our clinical grade NGS assay for panels (please see 'Non-coding disease causing variants covered by this panel' in the Panel Content section)
- Our rigorous variant classification scheme
- Our systematic clinical interpretation workflow using proprietary software enabling accurate and traceable processing of NGS data
- Our comprehensive clinical statements

Test Limitations

This test does not detect the following:

- Complex inversions
- Gene conversions
- Balanced translocations
- Some of the panels include the whole mitochondrial genome but not all (please see the Panel Content section)
- Repeat expansion disorders unless specifically mentioned
- Non-coding variants deeper than ±20 base pairs from exon-intron boundary unless otherwise indicated (please see above Panel Content / non-coding variants covered by the panel).

This test may not reliably detect the following:

- Low level mosaicism in nuclear genes (variant with a minor allele fraction of 14.6% is detected with 90% probability)
- Stretches of mononucleotide repeats
- Low level heteroplasmy in mtDNA (>90% are detected at 5% level)
- Indels larger than 50bp
- Single exon deletions or duplications
- Variants within pseudogene regions/duplicated segments
- Some disease causing variants present in mtDNA are not detectable from blood, thus post-mitotic tissue such as skeletal muscle may be required for establishing molecular diagnosis.

The sensitivity of this test may be reduced if DNA is extracted by a laboratory other than Blueprint Genetics.

For additional information, please refer to the Test performance section and see our Analytic Validation.

Test performance

test performance

The genes on the panel have been carefully selected based on scientific literature, mutation databases and our experience.

Our panels are sectioned from our high-quality, clinical grade NGS assay. Please see our sequencing and detection performance table for details regarding our ability to detect different types of alterations (Table).

	Sensitivity % (TP/(TP+FN)	Specificity %
Single nucleotide variants	99.89% (99,153/99,266)	>99.9999%
Insertions, deletions and indels by sequence analysis		

1-10 bps	96.9% (7,563/7,806)	>99.9999%
11-50 bps	99.13% (2,524/2,546)	>99.9999%
Copy number variants (exon level dels/dups)		
1 exon level deletion (heterozygous)	100% (20/20)	NA
1 exon level deletion (homozygous)	100% (5/5)	NA
1 exon level deletion (het or homo)	100% (25/25)	NA
2-7 exon level deletion (het or homo)	100% (44/44)	NA
1-9 exon level duplication (het or homo)	75% (6/8)	NA
Simulated CNV detection		
5 exons level deletion/duplication	98.7%	100.00%
Microdeletion/-duplication sdrs (large CNVs, n=37))		
Size range (0.1-47 Mb)	100% (37/37)	

The performance presented above reached by Blueprint Genetics high-quality, clinical grade NGS sequencing assay with the following coverage metrics

Mean sequencing depth	143X
Nucleotides with >20x sequencing coverage (%)	99.86%

Performance of Blueprint Genetics Mitochondrial Sequencing Assay.

	Sensitivity %	**Specificity %**
ANALYTIC VALIDATION (NA samples; n=4)		
Single nucleotide variants		
Heteroplasmic (45-100%)	100.0% (50/50)	100.0%
Heteroplasmic (35-45%)	100.0% (87/87)	100.0%
Heteroplasmic (25-35%)	100.0% (73/73)	100.0%
Heteroplasmic (15-25%)	100.0% (77/77)	100.0%
Heteroplasmic (10-15%)	100.0% (74/74)	100.0%
Heteroplasmic (5-10%)	100.0% (3/3)	100.0%
Heteroplasmic (<5%)	50.0% (2/4)	100.0%
CLINICAL VALIDATION (n=76 samples)		
All types		

Single nucleotide variants n=2026 SNVs		
Heteroplasmic (45-100%)	100.0% (1940/1940)	100.0%
Heteroplasmic (35-45%)	100.0% (4/4)	100.0%
Heteroplasmic (25-35%)	100.0% (3/3)	100.0%
Heteroplasmic (15-25%)	100.0% (3/3)	100.0%
Heteroplasmic (10-15%)	100.0% (9/9)	100.0%
Heteroplasmic (5-10%)	92.3% (12/13)	99.98%
Heteroplasmic (<5%)	88.9% (48/54)	99.93%
Insertions and deletions by sequence analysis n=40 indels		
Heteroplasmic (45-100%) 1-10bp	100.0% (32/32)	100.0%
Heteroplasmic (5-45%) 1-10bp	100.0% (3/3)	100.0%
Heteroplasmic (<5%) 1-10bp	100.0% (5/5)	99,997%
SIMULATION DATA /(mitomap mutations)		
Insertions, and deletions 1-24 bps by sequence analysis; n=17		
Homoplasmic (100%) 1-24bp	100.0% (17/17)	99.98%
Heteroplasmic (50%)	100.0% (17/17)	99.99%
Heteroplasmic (25%)	100.0% (17/17)	100.0%
Heteroplasmic (20%)	100.0% (17/17)	100.0%
Heteroplasmic (15%)	100.0% (17/17)	100.0%
Heteroplasmic (10%)	94.1% (16/17)	100.0%
Heteroplasmic (5%)	94.1% (16/17)	100.0%
Copy number variants (separate artifical mutations; n=1500)		
Homoplasmic (100%) 500 bp, 1kb, 5 kb	100.0%	100.0%
Heteroplasmic (50%) 500 bp, 1kb, 5 kb	100.0%	100.0%
Heteroplasmic (30%) 500 bp, 1kb, 5 kb	100.0%	100.0%
Heteroplasmic (20%) 500 bp, 1kb, 5 kb	99.7%	100.0%
Heteroplasmic (10%) 500 bp, 1kb, 5 kb	99.0%	100.0%
The performance presented above reached by following coverage metrics at assay level (n=66)		
	Mean of medians	Median of medians
Mean sequencing depth MQ0 (clinical)	18224X	17366X

Nucleotides with >1000x MQ0 sequencing coverage (%) (clinical)	100%
rho zero cell line (=no mtDNA), mean sequencing depth	12X

Bioinformatics

The target region for each gene includes coding exons and ±20 base pairs from the exon-intron boundary. In addition, the panel includes non-coding variants if listed above (Non-coding variants covered by the panel). Some regions of the gene(s) may be removed from the panel if specifically mentioned in the 'Test limitations" section above. The sequencing data generated in our laboratory is analyzed with our proprietary data analysis and annotation pipeline, integrating state-of-the art algorithms and industry-standard software solutions. Incorporation of rigorous quality control steps throughout the workflow of the pipeline ensures the consistency, validity and accuracy of results. Our pipeline is streamlined to maximize sensitivity without sacrificing specificity. We have incorporated a number of reference population databases and mutation databases such as, but not limited, to 1000 Genomes Project, gnomAD, ClinVar and HGMD into our clinical interpretation software to make the process effective and efficient. For missense variants, *in silico* variant prediction tools such as SIFT, PolyPhen, MutationTaster are used to assist with variant classification. Through our online ordering and statement reporting system, Nucleus, the customer has an access to details of the analysis, including patient specific sequencing metrics, a gene level coverage plot and a list of regions with inadequate coverage if present. This reflects our mission to build fully transparent diagnostics where customers have easy access to crucial details of the analysis process.

Clinical interpretation

We provide customers with the most comprehensive clinical report available on the market. Clinical interpretation requires a fundamental understanding of clinical genetics and genetic principles. At Blueprint Genetics, our PhD molecular geneticists, medical geneticists and clinical consultants prepare the clinical statement together by evaluating the identified variants in the context of the phenotypic information provided in the requisition form. Our goal is to provide clinically meaningful statements that are understandable for all medical professionals regardless of whether they have formal training in genetics.

Variant classification is the corner stone of clinical interpretation and resulting patient management decisions. Our classifications follow the Blueprint Genetics Variant Classification Schemes based on the ACMG guideline 2015. Minor modifications were made to increase reproducibility of the variant classification and improve the clinical validity of the report. Our experience with tens of thousands of clinical cases analyzed at our laboratory allowed us to further develop the industry standard.

The final step in the analysis of sequence variants is confirmation of variants classified as pathogenic or likely pathogenic using bi-directional Sanger sequencing. Variant(s) fulfilling the following criteria are not Sanger confirmed: the variant quality score is above the internal threshold for a true positive call, and visual check-up of the variant at IGV is in-line with the variant call. Reported variants of uncertain significance are confirmed with bi-directional Sanger sequencing only if the quality score is below our internally defined quality score for true positive call. Reported copy number variations with a size <10 exons are confirmed by orthogonal methods such as qPCR if the specific CNV has been seen less than three times at Blueprint Genetics.

Our clinical statement includes tables for sequencing and copy number variants that include basic variant information (genomic coordinates, HGVS nomenclature, zygosity, allele frequencies, in silico predictions, OMIM phenotypes and classification of the variant). In addition, the statement includes detailed descriptions of the variant, gene and phenotype(s) including the role of the specific gene in human disease, the mutation profile, information about the gene's variation in population cohorts and detailed information about related phenotypes. We also provide links to the references used, congress abstracts and mutation variant databases used to help our customers further evaluate the reported findings if desired. The conclusion summarizes all of the existing information and provides our rationale for the classification of the variant.

Identification of pathogenic or likely pathogenic variants in dominant disorders or their combinations in different alleles in recessive disorders are considered molecular confirmation of the clinical diagnosis. In these cases, family member testing can be used for risk stratification within the family. In the case of variants of uncertain significance (VUS), we do not recommend family member risk stratification based on the VUS result. Furthermore, in the case of VUS, we do not recommend the use of genetic information in patient management or genetic counseling.

Our interpretation team analyzes millions of variants from thousands of individuals with rare diseases. Thus, our database, and

our understanding of variants and related phenotypes, is growing by leaps and bounds. Our laboratory is therefore well positioned to re-classify previously reported variants as new information becomes available. If a variant previously reported by Blueprint Genetics is re-classified, our laboratory will issue a follow-up statement to the original ordering health care provider at no additional cost.

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ICD codes

Commonly used ICD-10 codes when ordering the Mitochondrial Genome Test

ICD-10	Disease
F84.2	Rett syndrome
H49.40	Progressive external ophthalmoplegia
G11.9	Hereditary ataxia
C94.2	Acute Megakaryoblastic Leukemia
K59.8	Chronic Intestinal Pseudoobstruction
T36.5	Adverse effect of aminoglycosides
G93.41	Metabolic Encephalopathy
H49.81	Kearns Sayre Syndrome
E88.42	MERFF Syndrome
H47.013	Nonarteritic Anterior Ischemic Optic Neuropathy
G60.2	Neuropathy in association with hereditary ataxia
G30	Alzheimer's Disease
G25.5	Chorea
G40	Epilepsy and recurrent seizures
I42	Cardiomyopathy
N26.9	Focal Segmental Glomerulosclerosis
G31.82	Leigh's Disease
H47.2	Leber's hereditary optic neuropathy
G71.3	Mitochondrial Myopathy
I42.1	Hypertrophic Cardiomyopathy
E11.9	Non-Insulin Dependent Diabetes Mellitus
Z86.74	Personal history of sudden cardiac arrest
H90.3	Sensorineural Hearing Loss