



## Donor 5793

### Genetic Testing Summary

Fairfax Cryobank recommends reviewing this genetic testing summary with your healthcare provider to determine suitability.

Last Updated: 09/16/21

Donor Reported Ancestry: Irish

Jewish Ancestry: No

Genetic Test*	Result	Comments/Donor's Residual Risk**
Chromosome analysis (karyotype)	Normal male karyotype	No evidence of clinically significant chromosome abnormalities
Hemoglobin evaluation	Normal hemoglobin fractionation and MCV/MCH results	Reduced risk to be a carrier for sickle cell anemia, beta thalassemia, alpha thalassemia trait (aa/-- and a-/a-) and other hemoglobinopathies
Cystic Fibrosis (CF) carrier screening	Negative by gene sequencing in the CFTR gene	1/440
Spinal Muscular Atrophy (SMA) carrier screening	Negative for deletions of exon 7 in the SMN1 gene	1/894
Expanded Genetic Disease Carrier Screening Panel attached- 283 diseases by gene sequencing	<p><b>Carrier: Biotinidase Deficiency (BTD)</b></p> <p><b>Carrier: Methylmalonic Acidemia (MUT-Related)</b></p> <p>Negative for other genes sequenced.</p>	Partner testing is recommended before using this donor.
Special testing		
Gene: CRTAP	Negative by gene sequencing.	

\*No single test can screen for all genetic disorders. A negative screening result significantly reduces, but cannot eliminate, the risk for these conditions in a pregnancy.

\*\*Donor residual risk is the chance the donor is still a carrier after testing negative.



**Patient Information**

Name: 5793 Donor  
Date of Birth: [REDACTED]  
Sema4 ID: [REDACTED]  
Client ID: [REDACTED]  
Indication: Carrier Testing

**Specimen Information**

Specimen Type: Blood  
Date Collected: 08/14/2019  
Date Received: 08/15/2019  
Final Report: 08/29/2019

**Referring Provider**

[REDACTED]  
Fairfax Cryobank, Inc.  
[REDACTED]  
[REDACTED]

**Expanded Carrier Screen (283) Minus TSE**  
Number of genes tested: 283

**SUMMARY OF RESULTS AND RECOMMENDATIONS**

⊕ Positive	⊖ Negative
<p style="text-align: center;"><b>Carrier of Biotinidase Deficiency (AR)</b> Associated gene(s): <i>BTB</i> Variant(s) Detected: c.1330G&gt;C, p.D444H, Pathogenic, Heterozygous (one copy)</p> <p style="text-align: center;"><b>Carrier of Methylmalonic Acidemia (MUT-Related) (AR)</b> Associated gene(s): <i>MUT</i> Variant(s) Detected: c.1889G&gt;A, p.G630E, Pathogenic, Heterozygous (one copy)</p>	<p style="text-align: center;"><b>Negative for all other genes tested</b> To view a full list of genes and diseases tested please see Table 1 in this report</p>

AR=Autosomal recessive; XL=X-linked

**Recommendations**

- Testing the partner for the above positive disorder(s) and genetic counseling are recommended.
- Please note that for female carriers of X-linked diseases, follow-up testing of a male partner is not indicated.
- CGG repeat analysis of *FMR1* for fragile X syndrome is not performed on males as repeat expansion of premutation alleles is not expected in the male germline.
- Individuals of Asian, African, Hispanic and Mediterranean ancestry should also be screened for hemoglobinopathies by CBC and hemoglobin electrophoresis.
- Consideration of residual risk by ethnicity after a negative carrier screen is recommended for the other diseases on the panel, especially in the case of a positive family history for a specific disorder.



## Interpretation of positive results

### Biotinidase Deficiency (AR)

#### Results and Interpretation

A heterozygous (one copy) pathogenic missense variant, c.1330G>C, p.D444H, was detected in the *BTD* gene (NM\_000060.3). Please note that this is a mild variant and is not expected to result in a disease phenotype when homozygous, unless present as part of a complex allele. If found in trans with a severe pathogenic variant, the individual is expected to develop partial biotinidase deficiency. When this variant is present in trans with a pathogenic variant, it is considered to be causative for biotinidase deficiency. Therefore, this individual is expected to be at least a carrier for biotinidase deficiency. Heterozygous carriers are not expected to exhibit symptoms of this disease.

#### What is Biotinidase Deficiency?

Biotinidase deficiency is an autosomal recessive disorder caused by pathogenic variants in the gene *BTD*. This pan-ethnic disorder affects individuals within the first few months of life. Severe forms of the disorder cause children to experience neurological abnormalities such as seizures, hypotonia, developmental delay, and vision problems as well as hearing problems, respiratory problems, and cutaneous abnormalities. While effective treatment is available, symptoms such as vision problems, hearing loss, and developmental delay are irreversible. Several specific variants have been associated with full or partial biotinidase deficiency, and therefore the severity of the disease may be predicted based on the genotype.

### Methylmalonic Acidemia (*MUT*-Related) (AR)

#### Results and Interpretation

A heterozygous (one copy) pathogenic missense variant, c.1889G>A, p.G630E, was detected in the *MUT* gene (NM\_000255.3). When this variant is present in trans with a pathogenic variant, it is considered to be causative for methylmalonic acidemia (*MUT*-related). Therefore, this individual is expected to be at least a carrier for methylmalonic acidemia (*MUT*-related). Heterozygous carriers are not expected to exhibit symptoms of this disease.

#### What is Methylmalonic Acidemia (*MUT*-Related)?

Methylmalonic acidemia (*MUT*-related) is a pan-ethnic, autosomal recessive disease caused by pathogenic variants in the *MUT* gene. The most common presentation is during the newborn period, where a previously normal infant begins vomiting, and develops lethargy and hypotonia due to an excess of ammonia in the blood. Without immediate treatment, the resulting brain disease can be fatal. Patients can also present later in infancy or childhood after a period of normal development. Although the onset may be later, this form of the disease may also be fatal if not identified and treated promptly. Even with treatment, patients may develop intellectual disability, impaired function of the kidneys, vision loss, growth failure and pancreatitis. Life expectancy is variable depending on the number and length of metabolic crises and the severity of the resulting damage, but there is significant mortality associated with all ages. Several specific variants may be associated with the development of either the early or later-onset form, but some variants may not have a known genotype-phenotype correlation.

## Test description

This patient was tested for a panel of diseases using a combination of sequencing, targeted genotyping and copy number analysis. Please note that negative results reduce but do not eliminate the possibility that this individual is a carrier for one or more of the disorders tested. Please see Table 1 for a list of genes and diseases tested, and [go.sema4.com/residualrisk](https://go.sema4.com/residualrisk) for specific detection rates and residual risk by ethnicity. With individuals of mixed ethnicity, it is recommended to use the highest residual risk estimate. Only variants determined to be pathogenic or likely pathogenic are reported in this carrier screening test.



Wang

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## Genes and diseases tested

For specific detection rates and residual risk by ethnicity, please visit [go.sema4.com/residualrisk](https://go.sema4.com/residualrisk)

Table 1: List of genes and diseases tested with detailed results

Disease	Gene	Inheritance Pattern	Status	Detailed Summary
<b>⊕ Positive</b>				
Biotinidase Deficiency	<i>BTD</i>	AR	Carrier	c.1330G>C, p.D444H, Pathogenic, Heterozygous (one copy)
Methylmalonic Acidemia ( <i>MUT</i> -Related)	<i>MUT</i>	AR	Carrier	c.1889G>A, p.G630E, Pathogenic, Heterozygous (one copy)
<b>⊖ Negative</b>				
3-Beta-Hydroxysteroid Dehydrogenase Type II Deficiency	<i>HSD3B2</i>	AR	Reduced Risk	
3-Methylcrotonyl-CoA Carboxylase Deficiency ( <i>MCCC1</i> -Related)	<i>MCCC1</i>	AR	Reduced Risk	
3-Methylcrotonyl-CoA Carboxylase Deficiency ( <i>MCCC2</i> -Related)	<i>MCCC2</i>	AR	Reduced Risk	
3-Methylglutaconic Aciduria, Type III	<i>OPA3</i>	AR	Reduced Risk	
3-Phosphoglycerate Dehydrogenase Deficiency	<i>PHGDH</i>	AR	Reduced Risk	
6-Pyruvoyl-Tetrahydropterin Synthase Deficiency	<i>PTS</i>	AR	Reduced Risk	
Abetalipoproteinemia	<i>MTTP</i>	AR	Reduced Risk	
Achromatopsia	<i>CNGB3</i>	AR	Reduced Risk	
Acrodermatitis Enteropathica	<i>SLC39A4</i>	AR	Reduced Risk	
Acute Infantile Liver Failure	<i>TRMU</i>	AR	Reduced Risk	
Acyl-CoA Oxidase I Deficiency	<i>ACOX1</i>	AR	Reduced Risk	
Adenosine Deaminase Deficiency	<i>ADA</i>	AR	Reduced Risk	
Adrenoleukodystrophy, X-Linked	<i>ABCD1</i>	XL	Reduced Risk	
Aicardi-Goutieres Syndrome ( <i>SAMHD1</i> -Related)	<i>SAMHD1</i>	AR	Reduced Risk	
Alpha-Mannosidosis	<i>MAN2B1</i>	AR	Reduced Risk	
Alpha-Thalassemia	<i>HBA1/HBA2</i>	AR	Reduced Risk	<i>HBA1</i> Copy Number: 2 <i>HBA2</i> Copy Number: 2 No pathogenic copy number variants detected <i>HBA1/HBA2</i> Sequencing: Negative



Alpha-Thalassemia Mental Retardation Syndrome	<i>ATRX</i>	XL	Reduced Risk
Alport Syndrome ( <i>COL4A3</i> -Related)	<i>COL4A3</i>	AR	Reduced Risk
Alport Syndrome ( <i>COL4A4</i> -Related)	<i>COL4A4</i>	AR	Reduced Risk
Alport Syndrome ( <i>COL4A5</i> -Related)	<i>COL4A5</i>	XL	Reduced Risk
Alstrom Syndrome	<i>ALMS1</i>	AR	Reduced Risk
Andermann Syndrome	<i>SLC12A6</i>	AR	Reduced Risk
Argininosuccinic Aciduria	<i>ASL</i>	AR	Reduced Risk
Aromatase Deficiency	<i>CYP19A1</i>	AR	Reduced Risk
Arthrogryposis, Mental Retardation, and Seizures	<i>SLC35A3</i>	AR	Reduced Risk
Asparagine Synthetase Deficiency	<i>ASNS</i>	AR	Reduced Risk
Aspartylglycosaminuria	<i>AGA</i>	AR	Reduced Risk
Ataxia With Isolated Vitamin E Deficiency	<i>TTPA</i>	AR	Reduced Risk
Ataxia-Telangiectasia	<i>ATM</i>	AR	Reduced Risk
Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay	<i>SACS</i>	AR	Reduced Risk
Bardet-Biedl Syndrome ( <i>BBS10</i> -Related)	<i>BBS10</i>	AR	Reduced Risk
Bardet-Biedl Syndrome ( <i>BBS12</i> -Related)	<i>BBS12</i>	AR	Reduced Risk
Bardet-Biedl Syndrome ( <i>BBS1</i> -Related)	<i>BBS1</i>	AR	Reduced Risk
Bardet-Biedl Syndrome ( <i>BBS2</i> -Related)	<i>BBS2</i>	AR	Reduced Risk
Bare Lymphocyte Syndrome, Type II	<i>CIITA</i>	AR	Reduced Risk
Bartter Syndrome, Type 4A	<i>BSND</i>	AR	Reduced Risk
Bernard-Soutier Syndrome, Type A1	<i>GP1BA</i>	AR	Reduced Risk
Bernard-Soutier Syndrome, Type C	<i>GP9</i>	AR	Reduced Risk
Beta-Globin-Related Hemoglobinopathies	<i>HBB</i>	AR	Reduced Risk
Beta-Ketothiolase Deficiency	<i>ACAT1</i>	AR	Reduced Risk
Bilateral Frontoparietal Polymicrogyria	<i>GPR56</i>	AR	Reduced Risk
Bloom Syndrome	<i>BLM</i>	AR	Reduced Risk
Canavan Disease	<i>ASPA</i>	AR	Reduced Risk
Carbamoylphosphate Synthetase I Deficiency	<i>CPS1</i>	AR	Reduced Risk
Carnitine Palmitoyltransferase IA Deficiency	<i>CPT1A</i>	AR	Reduced Risk
Carnitine Palmitoyltransferase II Deficiency	<i>CPT2</i>	AR	Reduced Risk
Carpenter Syndrome	<i>RAB23</i>	AR	Reduced Risk
Cartilage-Hair Hypoplasia	<i>RMRP</i>	AR	Reduced Risk
Cerebral Creatine Deficiency Syndrome 1	<i>SLC6A8</i>	XL	Reduced Risk
Cerebral Creatine Deficiency Syndrome 2	<i>GAMT</i>	AR	Reduced Risk
Cerebrotendinous Xanthomatosis	<i>CYP27A1</i>	AR	Reduced Risk



Charcot-Marie-Tooth Disease, Type 4D	<i>NDRG1</i>	AR	Reduced Risk
Charcot-Marie-Tooth Disease, Type 5 / Arts Syndrome	<i>PRPS1</i>	XL	Reduced Risk
Charcot-Marie-Tooth Disease, X-Linked	<i>GJB1</i>	XL	Reduced Risk
Choreoacanthocytosis	<i>VPS13A</i>	AR	Reduced Risk
Choroideremia	<i>CHM</i>	XL	Reduced Risk
Chronic Granulomatous Disease (CYBA-Related)	<i>CYBA</i>	AR	Reduced Risk
Chronic Granulomatous Disease (CYBB-Related)	<i>CYBB</i>	XL	Reduced Risk
Citrin Deficiency	<i>SLC25A13</i>	AR	Reduced Risk
Citrullinemia, Type 1	<i>ASS1</i>	AR	Reduced Risk
Cohen Syndrome	<i>VPS13B</i>	AR	Reduced Risk
Combined Malonic and Methylmalonic Aciduria	<i>ACSF3</i>	AR	Reduced Risk
Combined Oxidative Phosphorylation Deficiency 1	<i>GFM1</i>	AR	Reduced Risk
Combined Oxidative Phosphorylation Deficiency 3	<i>TSFM</i>	AR	Reduced Risk
Combined Pituitary Hormone Deficiency 2	<i>PROP1</i>	AR	Reduced Risk
Combined Pituitary Hormone Deficiency 3	<i>LHX3</i>	AR	Reduced Risk
Combined SAP Deficiency	<i>PSAP</i>	AR	Reduced Risk
Congenital Adrenal Hyperplasia due to 17-Alpha-Hydroxylase Deficiency	<i>CYP17A1</i>	AR	Reduced Risk
Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency	<i>CYP21A2</i>	AR	Reduced Risk <i>CYP21A2</i> copy number: 2 <i>CYP21A2</i> sequencing: Negative
Congenital Amegakaryocytic Thrombocytopenia	<i>MPL</i>	AR	Reduced Risk
Congenital Disorder of Glycosylation, Type Ia	<i>PMM2</i>	AR	Reduced Risk
Congenital Disorder of Glycosylation, Type Ib	<i>MPI</i>	AR	Reduced Risk
Congenital Disorder of Glycosylation, Type Ic	<i>ALG6</i>	AR	Reduced Risk
Congenital Insensitivity to Pain with Anhidrosis	<i>NTRK1</i>	AR	Reduced Risk
Congenital Myasthenic Syndrome (CHRNE-Related)	<i>CHRNE</i>	AR	Reduced Risk
Congenital Myasthenic Syndrome (RAPSN-Related)	<i>RAPSN</i>	AR	Reduced Risk
Congenital Neutropenia (HAX1-Related)	<i>HAX1</i>	AR	Reduced Risk
Congenital Neutropenia (VPS45-Related)	<i>VPS45</i>	AR	Reduced Risk
Corneal Dystrophy and Perceptive Deafness	<i>SLC4A11</i>	AR	Reduced Risk
Corticosterone Methyloxidase Deficiency	<i>CYP11B2</i>	AR	Reduced Risk
Cystic Fibrosis	<i>CFTR</i>	AR	Reduced Risk
Cystinosis	<i>CTNS</i>	AR	Reduced Risk
D-Bifunctional Protein Deficiency	<i>HSD17B4</i>	AR	Reduced Risk
Deafness, Autosomal Recessive 77	<i>LOXHD1</i>	AR	Reduced Risk
Duchenne Muscular Dystrophy / Becker Muscular Dystrophy	<i>DMD</i>	XL	Reduced Risk



Dyskeratosis Congenita ( <i>RTEL1</i> -Related)	<i>RTEL1</i>	AR	Reduced Risk
Dystrophic Epidermolysis Bullosa	<i>COL7A1</i>	AR	Reduced Risk
Ehlers-Danlos Syndrome, Type VIIC	<i>ADAMTS2</i>	AR	Reduced Risk
Ellis-van Creveld Syndrome ( <i>EVC</i> -Related)	<i>EVC</i>	AR	Reduced Risk
Emery-Dreifuss Myopathy 1	<i>EMD</i>	XL	Reduced Risk
Enhanced S-Cone Syndrome	<i>NR2E3</i>	AR	Reduced Risk
Ethylmalonic Encephalopathy	<i>ETHE1</i>	AR	Reduced Risk
Fabry Disease	<i>GLA</i>	XL	Reduced Risk
Factor IX Deficiency	<i>F9</i>	XL	Reduced Risk
Factor XI Deficiency	<i>F11</i>	AR	Reduced Risk
Familial Autosomal Recessive Hypercholesterolemia	<i>LDLRAP1</i>	AR	Reduced Risk
Familial Dysautonomia	<i>IKBKAP</i>	AR	Reduced Risk
Familial Hypercholesterolemia	<i>LDLR</i>	AR	Reduced Risk
Familial Hyperinsulinism ( <i>ABCC8</i> -Related)	<i>ABCC8</i>	AR	Reduced Risk
Familial Hyperinsulinism ( <i>KCNJ11</i> -Related)	<i>KCNJ11</i>	AR	Reduced Risk
Familial Mediterranean Fever	<i>MEFV</i>	AR	Reduced Risk
Fanconi Anemia, Group A	<i>FANCA</i>	AR	Reduced Risk
Fanconi Anemia, Group C	<i>FANCC</i>	AR	Reduced Risk
Fanconi Anemia, Group G	<i>FANCG</i>	AR	Reduced Risk
Fragile X Syndrome	<i>FMR1</i>	XL	Reduced Risk <i>FMR1</i> CGG repeat sizes: Not Performed <i>FMR1</i> Sequencing: Negative Fragile X CGG triplet repeat expansion testing was not performed at this time, as the patient has either been previously tested or is a male.
Fumarase Deficiency	<i>FH</i>	AR	Reduced Risk
GRACILE Syndrome and Other <i>BCS1L</i> -Related Disorders	<i>BCS1L</i>	AR	Reduced Risk
Galactokinase Deficiency	<i>GALK1</i>	AR	Reduced Risk
Galactosemia	<i>GALT</i>	AR	Reduced Risk
Gaucher Disease	<i>GBA</i>	AR	Reduced Risk
Gitelman Syndrome	<i>SLC12A3</i>	AR	Reduced Risk
Glutaric Acidemia, Type I	<i>GCDH</i>	AR	Reduced Risk
Glutaric Acidemia, Type IIa	<i>ETFA</i>	AR	Reduced Risk
Glutaric Acidemia, Type IIc	<i>ETFDH</i>	AR	Reduced Risk
Glycine Encephalopathy ( <i>AMT</i> -Related)	<i>AMT</i>	AR	Reduced Risk
Glycine Encephalopathy ( <i>GLDC</i> -Related)	<i>GLDC</i>	AR	Reduced Risk
Glycogen Storage Disease, Type II	<i>GAA</i>	AR	Reduced Risk
Glycogen Storage Disease, Type III	<i>AGL</i>	AR	Reduced Risk



Glycogen Storage Disease, Type IV / Adult Polyglucosan Body Disease	<i>GBE1</i>	AR	Reduced Risk
Glycogen Storage Disease, Type Ia	<i>G6PC</i>	AR	Reduced Risk
Glycogen Storage Disease, Type Ib	<i>SLC37A4</i>	AR	Reduced Risk
Glycogen Storage Disease, Type V	<i>PYGM</i>	AR	Reduced Risk
Glycogen Storage Disease, Type VII	<i>PFKM</i>	AR	Reduced Risk
HMG-CoA Lyase Deficiency	<i>HMGCL</i>	AR	Reduced Risk
Hemochromatosis, Type 2A	<i>HFE2</i>	AR	Reduced Risk
Hemochromatosis, Type 3	<i>TFR2</i>	AR	Reduced Risk
Hereditary Fructose Intolerance	<i>ALDOB</i>	AR	Reduced Risk
Hereditary Spastic Paraparesis 49	<i>TECPR2</i>	AR	Reduced Risk
Hermansky-Pudlak Syndrome, Type 1	<i>HPS1</i>	AR	Reduced Risk
Hermansky-Pudlak Syndrome, Type 3	<i>HPS3</i>	AR	Reduced Risk
Holocarboxylase Synthetase Deficiency	<i>HLCS</i>	AR	Reduced Risk
Homocystinuria (CBS-Related)	<i>CBS</i>	AR	Reduced Risk
Homocystinuria due to <i>MTHFR</i> Deficiency	<i>MTHFR</i>	AR	Reduced Risk
Homocystinuria, cbIE Type	<i>MTRR</i>	AR	Reduced Risk
Hydrolethals Syndrome	<i>HYLS1</i>	AR	Reduced Risk
Hyperornithinemia-Hyperammonemia-Homocitrullinuria Syndrome	<i>SLC25A15</i>	AR	Reduced Risk
Hypohidrotic Ectodermal Dysplasia 1	<i>EDA</i>	XL	Reduced Risk
Hypophosphatasia	<i>ALPL</i>	AR	Reduced Risk
Inclusion Body Myopathy 2	<i>GNE</i>	AR	Reduced Risk
Infantile Cerebral and Cerebellar Atrophy	<i>MED17</i>	AR	Reduced Risk
Isovaleric Acidemia	<i>IVD</i>	AR	Reduced Risk
Joubert Syndrome 2	<i>TMEM216</i>	AR	Reduced Risk
Joubert Syndrome 7 / Meckel Syndrome 5 / COACH Syndrome	<i>RPGRIP1L</i>	AR	Reduced Risk
Junctional Epidermolysis Bullosa ( <i>LAMA3</i> -Related)	<i>LAMA3</i>	AR	Reduced Risk
Junctional Epidermolysis Bullosa ( <i>LAMB3</i> -Related)	<i>LAMB3</i>	AR	Reduced Risk
Junctional Epidermolysis Bullosa ( <i>LAMC2</i> -Related)	<i>LAMC2</i>	AR	Reduced Risk
Krabbe Disease	<i>GALC</i>	AR	Reduced Risk
Lamellar Ichthyosis, Type 1	<i>TGM1</i>	AR	Reduced Risk
Leber Congenital Amaurosis 10 and Other CEP290-Related Ciliopathies	<i>CEP290</i>	AR	Reduced Risk
Leber Congenital Amaurosis 13	<i>RDH12</i>	AR	Reduced Risk
Leber Congenital Amaurosis 2 / Retinitis Pigmentosa 20	<i>RPE65</i>	AR	Reduced Risk





Leber Congenital Amaurosis 5	LCA5	AR	Reduced Risk
Leber Congenital Amaurosis 8 / Retinitis Pigmentosa 12 / Pigmented Paravenous Chorioretinal Atrophy	CRB1	AR	Reduced Risk
Leigh Syndrome, French-Canadian Type	LRPPRC	AR	Reduced Risk
Lethal Congenital Contracture Syndrome 1 / Lethal Arthrogryposis with Anterior Horn Cell Disease	GLE1	AR	Reduced Risk
Leukoencephalopathy with Vanishing White Matter	EIF2B5	AR	Reduced Risk
Limb-Girdle Muscular Dystrophy, Type 2A	CAPN3	AR	Reduced Risk
Limb-Girdle Muscular Dystrophy, Type 2B	DYSF	AR	Reduced Risk
Limb-Girdle Muscular Dystrophy, Type 2C	SGCG	AR	Reduced Risk
Limb-Girdle Muscular Dystrophy, Type 2D	SGCA	AR	Reduced Risk
Limb-Girdle Muscular Dystrophy, Type 2E	SGCB	AR	Reduced Risk
Limb-Girdle Muscular Dystrophy, Type 2I	FKRP	AR	Reduced Risk
Lipoamide Dehydrogenase Deficiency	DLD	AR	Reduced Risk
Lipoid Adrenal Hyperplasia	STAR	AR	Reduced Risk
Lipoprotein Lipase Deficiency	LPL	AR	Reduced Risk
Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency	HADHA	AR	Reduced Risk
Lysinuric Protein Intolerance	SLC7A7	AR	Reduced Risk
Maple Syrup Urine Disease, Type 1a	BCKDHA	AR	Reduced Risk
Maple Syrup Urine Disease, Type 1b	BCKDHB	AR	Reduced Risk
Meckel 1 / Bardet-Biedl Syndrome 13	MKS1	AR	Reduced Risk
Medium Chain Acyl-CoA Dehydrogenase Deficiency	ACADM	AR	Reduced Risk
Megalencephalic Leukoencephalopathy with Subcortical Cysts	MLC1	AR	Reduced Risk
Menkes Disease	ATP7A	XL	Reduced Risk
Metachromatic Leukodystrophy	ARSA	AR	Reduced Risk
Methylmalonic Acidemia (MMAA-Related)	MMAA	AR	Reduced Risk
Methylmalonic Acidemia (MMAB-Related)	MMAB	AR	Reduced Risk
Methylmalonic Aciduria and Homocystinuria, Cobalamin C Type	MMACHC	AR	Reduced Risk
Methylmalonic Aciduria and Homocystinuria, Cobalamin D Type	MMADHC	AR	Reduced Risk
Microphthalmia / Anophthalmia	VSX2	AR	Reduced Risk
Mitochondrial Complex I Deficiency (ACAD9-Related)	ACAD9	AR	Reduced Risk
Mitochondrial Complex I Deficiency (NDUFAF5-Related)	NDUFAF5	AR	Reduced Risk
Mitochondrial Complex I Deficiency (NDUFS6-Related)	NDUFS6	AR	Reduced Risk
Mitochondrial DNA Depletion Syndrome 6 / Navajo Neurohepatopathy	MPV17	AR	Reduced Risk



Mitochondrial Myopathy and Sideroblastic Anemia 1	<i>PUS1</i>	AR	Reduced Risk
Mucopolidosis II / IIIA	<i>GNPTAB</i>	AR	Reduced Risk
Mucopolidosis III Gamma	<i>GNPTG</i>	AR	Reduced Risk
Mucopolidosis IV	<i>MCOLN1</i>	AR	Reduced Risk
Mucopolysaccharidosis Type I	<i>IDUA</i>	AR	Reduced Risk
Mucopolysaccharidosis Type II	<i>IDS</i>	XL	Reduced Risk
Mucopolysaccharidosis Type IIIA	<i>SGSH</i>	AR	Reduced Risk
Mucopolysaccharidosis Type IIIB	<i>NAGLU</i>	AR	Reduced Risk
Mucopolysaccharidosis Type IIIC	<i>HGSNAT</i>	AR	Reduced Risk
Mucopolysaccharidosis Type IIID	<i>GNS</i>	AR	Reduced Risk
Mucopolysaccharidosis Type IVb / GM1 Gangliosidosis	<i>GLB1</i>	AR	Reduced Risk
Mucopolysaccharidosis type IX	<i>HYAL1</i>	AR	Reduced Risk
Mucopolysaccharidosis type VI	<i>ARSB</i>	AR	Reduced Risk
Multiple Sulfatase Deficiency	<i>SUMF1</i>	AR	Reduced Risk
Muscle-Eye-Brain Disease and Other <i>POMGNT1</i> -Related Congenital Muscular Dystrophy-Dystroglycanopathies	<i>POMGNT1</i>	AR	Reduced Risk
Myoneurogastrointestinal Encephalopathy	<i>TYMP</i>	AR	Reduced Risk
Myotubular Myopathy 1	<i>MTM1</i>	XL	Reduced Risk
N-Acetylglutamate Synthase Deficiency	<i>NAGS</i>	AR	Reduced Risk
Nemaline Myopathy 2	<i>NEB</i>	AR	Reduced Risk
Nephrogenic Diabetes Insipidus, Type II	<i>AQP2</i>	AR	Reduced Risk
Nephrotic Syndrome ( <i>NPHS1</i> -Related) / Congenital Finnish Nephrosis	<i>NPHS1</i>	AR	Reduced Risk
Nephrotic Syndrome ( <i>NPHS2</i> -Related) / Steroid-Resistant Nephrotic Syndrome	<i>NPHS2</i>	AR	Reduced Risk
Neuronal Ceroid-Lipofuscinosis ( <i>CLN3</i> -Related)	<i>CLN3</i>	AR	Reduced Risk
Neuronal Ceroid-Lipofuscinosis ( <i>CLN5</i> -Related)	<i>CLN5</i>	AR	Reduced Risk
Neuronal Ceroid-Lipofuscinosis ( <i>CLN6</i> -Related)	<i>CLN6</i>	AR	Reduced Risk
Neuronal Ceroid-Lipofuscinosis ( <i>CLN8</i> -Related)	<i>CLN8</i>	AR	Reduced Risk
Neuronal Ceroid-Lipofuscinosis ( <i>MFSD8</i> -Related)	<i>MFSD8</i>	AR	Reduced Risk
Neuronal Ceroid-Lipofuscinosis ( <i>PPT1</i> -Related)	<i>PPT1</i>	AR	Reduced Risk
Neuronal Ceroid-Lipofuscinosis ( <i>TPP1</i> -Related)	<i>TPP1</i>	AR	Reduced Risk
Niemann-Pick Disease ( <i>SMPD1</i> -Related)	<i>SMPD1</i>	AR	Reduced Risk
Niemann-Pick Disease, Type C ( <i>NPC1</i> -Related)	<i>NPC1</i>	AR	Reduced Risk
Niemann-Pick Disease, Type C ( <i>NPC2</i> -Related)	<i>NPC2</i>	AR	Reduced Risk
Nijmegen Breakage Syndrome	<i>NBN</i>	AR	Reduced Risk
Non-Syndromic Hearing Loss ( <i>GJB2</i> -Related)	<i>GJB2</i>	AR	Reduced Risk



Odonto-Onycho-Dermal Dysplasia / Schopf-Schulz-Passarge Syndrome	WNT10A	AR	Reduced Risk
Omenn Syndrome (RAG2-Related)	RAG2	AR	Reduced Risk
Omenn Syndrome / Severe Combined Immunodeficiency, Athabaskan-Type	DCLRE1C	AR	Reduced Risk
Ornithine Aminotransferase Deficiency	OAT	AR	Reduced Risk
Ornithine Transcarbamylase Deficiency	OTC	XL	Reduced Risk
Osteopetrosis 1	TCIRG1	AR	Reduced Risk
Pendred Syndrome	SLC26A4	AR	Reduced Risk
Phenylalanine Hydroxylase Deficiency	PAH	AR	Reduced Risk
Polycystic Kidney Disease, Autosomal Recessive	PKHD1	AR	Reduced Risk
Polyglandular Autoimmune Syndrome, Type 1	AIRE	AR	Reduced Risk
Pontocerebellar Hypoplasia, Type 1A	VRK1	AR	Reduced Risk
Pontocerebellar Hypoplasia, Type 6	RARS2	AR	Reduced Risk
Primary Carnitine Deficiency	SLC22A5	AR	Reduced Risk
Primary Ciliary Dyskinesia (DNAH5-Related)	DNAH5	AR	Reduced Risk
Primary Ciliary Dyskinesia (DNAH1-Related)	DNAH1	AR	Reduced Risk
Primary Ciliary Dyskinesia (DNAH2-Related)	DNAH2	AR	Reduced Risk
Primary Hyperoxaluria, Type 1	AGXT	AR	Reduced Risk
Primary Hyperoxaluria, Type 2	GRHPR	AR	Reduced Risk
Primary Hyperoxaluria, Type 3	HOGA1	AR	Reduced Risk
Progressive Cerebello-Cerebral Atrophy	SEPSECS	AR	Reduced Risk
Progressive Familial Intrahepatic Cholestasis, Type 2	ABCB11	AR	Reduced Risk
Propionic Acidemia (PCCA-Related)	PCCA	AR	Reduced Risk
Propionic Acidemia (PCCB-Related)	PCCB	AR	Reduced Risk
Pycnodysostosis	CTSK	AR	Reduced Risk
Pyruvate Dehydrogenase E1-Alpha Deficiency	PDHA1	XL	Reduced Risk
Pyruvate Dehydrogenase E1-Beta Deficiency	PDHB	AR	Reduced Risk
Renal Tubular Acidosis and Deafness	ATP6V1B1	AR	Reduced Risk
Retinitis Pigmentosa 25	EYS	AR	Reduced Risk
Retinitis Pigmentosa 26	CERKL	AR	Reduced Risk
Retinitis Pigmentosa 28	FAM161A	AR	Reduced Risk
Retinitis Pigmentosa 59	DHDDS	AR	Reduced Risk
Rhizomelic Chondrodysplasia Punctata, Type 1	PEX7	AR	Reduced Risk
Rhizomelic Chondrodysplasia Punctata, Type 3	AGPS	AR	Reduced Risk
Roberts Syndrome	ESCO2	AR	Reduced Risk
Salla Disease	SLC17A5	AR	Reduced Risk



Sandhoff Disease	HEXB	AR	Reduced Risk	
Schimke Immunoosseous Dysplasia	SMARCAL1	AR	Reduced Risk	
Segawa Syndrome	TH	AR	Reduced Risk	
Sjogren-Larsson Syndrome	ALDH3A2	AR	Reduced Risk	
Smith-Lemli-Opitz Syndrome	DHCR7	AR	Reduced Risk	
Spinal Muscular Atrophy	SMN1	AR	Reduced Risk	SMN1 copy number: 2 SMN2 copy number: 0 c."3+80T>G Negative
Spondylothoracic Dysostosis	MESP2	AR	Reduced Risk	
Steel Syndrome	COL27A1	AR	Reduced Risk	
Stuve-Wiedemann Syndrome	LIFR	AR	Reduced Risk	
Sulfate Transporter-Related Osteochondrodysplasia	SLC26A2	AR	Reduced Risk	
Tay-Sachs Disease	HEXA	AR	Reduced Risk	
Tyrosinemia, Type I	FAH	AR	Reduced Risk	
Usher Syndrome, Type IB	MYO7A	AR	Reduced Risk	
Usher Syndrome, Type IC	USH1C	AR	Reduced Risk	
Usher Syndrome, Type ID	CDH23	AR	Reduced Risk	
Usher Syndrome, Type IF	PCDH15	AR	Reduced Risk	
Usher Syndrome, Type IIA	USH2A	AR	Reduced Risk	
Usher Syndrome, Type III	CLRN1	AR	Reduced Risk	
Very Long Chain Acyl-CoA Dehydrogenase Deficiency	ACADVL	AR	Reduced Risk	
Walker-Warburg Syndrome and Other FKTN-Related Dystrophies	FKTN	AR	Reduced Risk	
Wilson Disease	ATP7B	AR	Reduced Risk	
Wolman Disease / Cholesteryl Ester Storage Disease	LIPA	AR	Reduced Risk	
X-Linked Juvenile Retinoschisis	RS1	XL	Reduced Risk	
X-Linked Severe Combined Immunodeficiency	IL2RG	XL	Reduced Risk	
Zellweger Syndrome Spectrum (PEX10-Related)	PEX10	AR	Reduced Risk	
Zellweger Syndrome Spectrum (PEX1-Related)	PEX1	AR	Reduced Risk	
Zellweger Syndrome Spectrum (PEX2-Related)	PEX2	AR	Reduced Risk	
Zellweger Syndrome Spectrum (PEX6-Related)	PEX6	AR	Reduced Risk	

AR=Autosomal recessive; XL=X-linked

## Test methods and comments

Genomic DNA isolated from this patient was analyzed by one or more of the following methodologies, as applicable:

### Fragile X CGG Repeat Analysis (Analytical Detection Rate >99%)

PCR amplification using Asuragen, Inc. AmpliDeX® *FMR1* PCR reagents followed by capillary electrophoresis for allele sizing was performed. Samples positive for *FMR1* CGG repeats in the premutation and full mutation size range were further analyzed by Southern blot analysis to

assess the size and methylation status of the *FMR1* CGG repeat.

**Genotyping (Analytical Detection Rate >99%)**

Multiplex PCR amplification and allele specific primer extension analyses using the MassARRAY® System were used to identify variants that are complex in nature or are present in low copy repeats. Rare sequence variants may interfere with assay performance.

**Multiplex Ligation-Dependent Probe Amplification (MLPA) (Analytical Detection Rate >99%)**

MLPA® probe sets and reagents from MRC-Holland were used for copy number analysis of specific targets versus known control samples. False positive or negative results may occur due to rare sequence variants in target regions detected by MLPA probes. Analytical sensitivity and specificity of the MLPA method are both 99%.

For alpha thalassemia, the copy numbers of the *HBA1* and *HBA2* genes were analyzed. Alpha-globin gene deletions, triplications, and the Constant Spring (CS) mutation are assessed. This test is expected to detect approximately 90% of all alpha-thalassemia mutations, varying by ethnicity. Carriers of alpha-thalassemia with three or more *HBA* copies on one chromosome, and one or no copies on the other chromosome, may not be detected. With the exception of triplications, other benign alpha-globin gene polymorphisms will not be reported. Analyses of *HBA1* and *HBA2* are performed in association with long-range PCR of the coding regions followed by short-read sequencing.

For Duchenne muscular dystrophy, the copy numbers of all *DMD* exons were analyzed. Potentially pathogenic single exon deletions and duplications are confirmed by a second method. Analysis of *DMD* is performed in association with sequencing of the coding regions.

For congenital adrenal hyperplasia, the copy number of the *CYP21A2* gene was analyzed. This analysis can detect large deletions due to unequal meiotic crossing-over between *CYP21A2* and the pseudogene *CYP21A1P*. These 30-kb deletions make up approximately 20% of *CYP21A2* pathogenic alleles. This test may also identify certain point mutations in *CYP21A2* caused by gene conversion events between *CYP21A2* and *CYP21A1P*. Some carriers may not be identified by dosage sensitive methods as this testing cannot detect individuals with two copies (duplication) of the *CYP21A2* gene on one chromosome and loss of *CYP21A2* (deletion) on the other chromosome. Analysis of *CYP21A2* is performed in association with long-range PCR of the coding regions followed by short-read sequencing.

For spinal muscular atrophy (SMA), the copy numbers of the *SMN1* and *SMN2* genes were analyzed. The individual dosage of exons 7 and 8 as well as the combined dosage of exons 1, 4, 6 and 8 of *SMN1* and *SMN2* were assessed. Copy number gains and losses can be detected with this assay. Depending on ethnicity, 6 - 29 % of carriers will not be identified by dosage sensitive methods as this testing cannot detect individuals with two copies (duplication) of the *SMN1* gene on one chromosome and loss of *SMN1* (deletion) on the other chromosome (silent 2+0 carrier) or individuals that carry an intragenic mutation in *SMN1*. Please also note that 2% of individuals with SMA have an *SMN1* mutation that occurred *de novo*. Typically in these cases, only one parent is an SMA carrier.

The presence of the c.\*3+80T>G (chr5:70,247,901T>G) variant allele in an individual with Ashkenazi Jewish or Asian ancestry is typically indicative of a duplication of *SMN1*. When present in an Ashkenazi Jewish or Asian individual with two copies of *SMN1*, c.\*3+80T>G is likely indicative of a silent (2+0) carrier. In individuals with two copies of *SMN1* with African American, Hispanic or Caucasian ancestry, the presence or absence of c.\*3+80T>G significantly increases or decreases, respectively, the likelihood of being a silent 2+0 carrier.

Pathogenic or likely pathogenic sequence variants in exon 7 may be detected during testing for the c.\*3+80T>G variant allele; these will be reported if confirmed to be located in *SMN1* using locus-specific Sanger primers

Pathogenic or likely pathogenic sequence variants in exon 7 may be detected during testing for the c.\*3+80T>G variant allele; these will be reported if confirmed to be located in *SMN1* using locus-specific Sanger primers.

MLPA for Gaucher disease (*GBA*), cystic fibrosis (*CFTR*), and non-syndromic hearing loss (*GJB2/GJB6*) will only be performed if indicated for confirmation of detected CNVs. If *GBA* analysis was performed, the copy numbers of exons 1, 3, 4, and 6 - 10 of the *GBA* gene (of 11 exons total) were analyzed. If *CFTR* analysis was performed, the copy numbers of all 27 *CFTR* exons were analyzed. If *GJB2/GJB6* analysis was performed, the copy number of the two *GJB2* exons were analyzed, as well as the presence or absence of the two upstream deletions of the *GJB2* regulatory region, del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854).

**Next Generation Sequencing (NGS) (Analytical Detection Rate >95%)**

NGS was performed on a panel of genes for the purpose of identifying pathogenic or likely pathogenic variants.

Agilent SureSelect™QXT technology was used with a custom capture library to target the exonic regions and intron/exon splice junctions of the relevant genes, as well as a number of UTR, intronic or promoter regions that contain previously reported mutations. Samples were pooled and sequenced on the Illumina HiSeq 2500 platform in the Rapid Run mode or the Illumina NovaSeq platform in the Xp workflow, using 100 bp paired-end reads. The sequencing data was analyzed using a custom bioinformatics algorithm designed and validated in house. The coding exons and splice junctions of the known protein-coding RefSeq genes were assessed for the average depth of coverage (minimum of 20X) and data quality threshold values. Most exons not meeting a minimum of >20X read depth across the exon are further analyzed by Sanger sequencing. Please note that several genomic regions present difficulties in mapping or obtaining read depth >20X. The exons contained within these regions are noted within Table 1 (as "Exceptions") and will not be reflexed to Sanger sequencing if the mapping quality or coverage is poor. Any variants identified during testing in these regions are confirmed by a second method and reported if



determined to be pathogenic or likely pathogenic. However, as there is a possibility of false negative results within these regions, detection rates and residual risks for these genes have been calculated with the presumption that variants in these exons will not be detected, unless included in the MassARRAY® genotyping platform.

This test will detect variants within the exons and the intron-exon boundaries of the target regions. Variants outside these regions may not be detected, including, but not limited to, UTRs, promoters, and deep intronic areas, or regions that fall into the Exceptions mentioned above. This technology may not detect all small insertion/deletions and is not diagnostic for repeat expansions and structural genomic variation. In addition, a mutation(s) in a gene not included on the panel could be present in this patient.

Variant interpretation and classification was performed based on the American College of Medical Genetics Standards and Guidelines for the Interpretation of Sequence Variants (Richards et al, 2015). All potentially pathogenic variants may be confirmed by either a specific genotyping assay or Sanger sequencing, if indicated. Any benign variants, likely benign variants or variants of uncertain significance identified during this analysis will not be reported.

**Copy Number Variant Analysis (Analytical Detection Rate >95%)**

Large duplications and deletions were called from the relative read depths on an exon-by-exon basis using a custom exome hidden Markov model (XHMM) algorithm. Deletions or duplications determined to be pathogenic or likely pathogenic were confirmed by either a custom arrayCGH platform, quantitative PCR, or MLPA (depending on CNV size and gene content). While this algorithm is designed to pick up deletions and duplications of 2 or more exons in length, potentially pathogenic single-exon CNVs will be confirmed and reported, if detected.

**Exon Array (Confirmation method) (Accuracy >99%)**

The customized oligonucleotide microarray (Oxford Gene Technology) is a highly-targeted exon-focused array capable of detecting medically relevant microdeletions and microduplications at a much higher resolution than traditional aCGH methods. Each array matrix has approximately 180,000 60-mer oligonucleotide probes that cover the entire genome. This platform is designed based on human genome NCBI Build 37 (hg19) and the CGH probes are enriched to target the exonic regions of the genes in this panel.

**Quantitative PCR (Confirmation method) (Accuracy >99%)**

The relative quantification PCR is utilized on a Roche Universal Library Probe (UPL) system, which relates the PCR signal of the target region in one group to another. To test for genomic imbalances, both sample DNA and reference DNA is amplified with primer/probe sets that specific to the target region and a control region with known genomic copy number. Relative genomic copy numbers are calculated based on the standard  $\Delta\Delta C_t$  formula.

**Long-Range PCR (Analytical Detection Rate >99%)**

Long-range PCR was performed to generate locus-specific amplicons for *CYP21A2*, *HBA1* and *HBA2* and *GBA*. The PCR products were then prepared for short-read NGS sequencing and sequenced. Sequenced reads were mapped back to the original genomic locus and run through the bioinformatics pipeline. If indicated, copy number from MLPA was correlated with the sequencing output to analyze the results. For *CYP21A2*, a certain percentage of healthy individuals carry a duplication of the *CYP21A2* gene, which has no clinical consequences. In cases where two copies of a gene are located on the same chromosome in tandem, only the second copy will be amplified and assessed for potentially pathogenic variants, due to size limitations of the PCR reaction. However, because these alleles contain at least two copies of the *CYP21A2* gene in tandem, it is expected that this patient has at least one functional gene in the tandem allele and this patient is therefore less likely to be a carrier. When an individual carries both a duplication allele and a pathogenic variant, or multiple pathogenic variants, the current analysis may not be able to determine the phase (cis/trans configuration) of the *CYP21A2* alleles identified. Family studies may be required in certain scenarios where phasing is required to determine the carrier status.

**Residual Risk Calculations**

Carrier frequencies and detection rates for each ethnicity were calculated through the combination of internal curations of >28,000 variants and genomic frequency data from >138,000 individuals across seven ethnic groups in the gnomAD database. Additional variants in HGMD and novel deleterious variants were also incorporated into the calculation. Residual risk values are calculated using a Bayesian analysis combining the *a priori* risk of being a pathogenic mutation carrier (carrier frequency) and the detection rate. They are provided only as a guide for assessing approximate risk given a negative result, and values will vary based on the exact ethnic background of an individual. This report does not represent medical advice but should be interpreted by a genetic counselor, medical geneticist or physician skilled in genetic result interpretation and the relevant medical literature.

**Sanger Sequencing (Confirmation method) (Accuracy >99%)**

Sanger sequencing, as indicated, was performed using BigDye Terminator chemistry with the ABI 3730 DNA analyzer with target specific amplicons. It also may be used to supplement specific guaranteed target regions that fail NGS sequencing due to poor quality or low depth of coverage (<20 reads) or as a confirmatory method for NGS positive results. False negative results may occur if rare variants interfere with amplification or annealing.



Please note these tests were developed and their performance characteristics were determined by Mount Sinai Genomics, Inc. They have not been cleared or approved by the FDA. These analyses generally provide highly accurate information regarding the patient's carrier or affected status. Despite this high level of accuracy, it should be kept in mind that there are many potential sources of diagnostic error, including misidentification of samples, polymorphisms, or other rare genetic variants that interfere with analysis. Families should understand that rare diagnostic errors may occur for these reasons.

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##### Spinal Muscular Atrophy:

Luo M et al. An Ashkenazi Jewish SMN1 haplotype specific to duplication alleles improves pan-ethnic carrier screening for spinal muscular atrophy. *Genet Med.* 2014 16:149-56.

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Scott SA et al. Experience with carrier screening and prenatal diagnosis for sixteen Ashkenazi Jewish Genetic Diseases. *Hum. Mutat.* 2010 31:1-11.

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Flanigan KM et al. Mutational spectrum of DMD mutations in dystrophinopathy patients: application of modern diagnostic techniques to a large cohort. *Hum Mutat.* 2009 30:1657-66.

##### Variant Classification:

Richards S et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015 May;17(5):405-24

Additional disease-specific references available upon request.



PATIENT INFORMATION	SPECIMEN INFORMATION	PROVIDER INFORMATION
5793, Donor ID#: 5793 DOB: [REDACTED] Sex: Male	Type: Whole Blood Collected: July 06, 2021 Received: July 08, 2021 PG ID: [REDACTED]	Harvey Stern, MD, PhD Suzanne Seitz, MS, MPA Fairfax Cryobank

**MOLECULAR GENETICS REPORT:  
CRTAP Gene Sequencing with CNV Detection**

**SUMMARY OF RESULTS** **NEGATIVE**

**RESULTS AND INTERPRETATIONS:** In this patient, for the *CRTAP* gene, we found no sequence variants that are likely to be a primary cause of disease.

This patient is also apparently negative for copy number variants (CNVs) within the genomic regions of this test. These results should be interpreted in context of clinical findings, family history and other laboratory data. All genetic tests have limitations. See limitations and other information for this test on the following pages.

**NOTE:** Since this test is performed using exome capture probes, a reflex to any of our exome-based tests is available (PGxome, PGxome Custom Panels).

**GENE ANALYZED:** *CRTAP*

**SUMMARY STATISTICS:**

Pipeline	Version	Average NGS Coverage	Fraction Bases Covered with NGS
Infinity_Pipeline	1.8.7	84x	96.8%

Minimum NGS coverage is ≥20x for all exons and +/-10bp of flanking DNA.

Electronically signed on July 14, 2021 by:  
Allison Cox, PhD  
Human Molecular Geneticist

Electronically signed and reported on July 15, 2021 by:  
James L. Weber, PhD  
President and Human Molecular Geneticist



## SUPPLEMENTAL INFORMATION V.19.04 SEQUENCING WITH CNV DETECTION

### Limitations and Other Test Notes

Interpretation of the test results is limited by the information that is currently available. Better interpretation should be possible in the future as our knowledge about human genetics and the patient's condition improve.

When Next Gen or Sanger sequencing does not reveal any difference from the reference sequence, or when a sequence variant is homozygous, we cannot be certain that we were able to detect both patient alleles. Occasionally, a patient may carry an allele which does not capture or amplify due for example to a large deletion or insertion.

Copy number variants (CNVs) of four exons or more in size are detected with sensitivity approaching 100% through analysis of Next Gen sequence data. However, sensitivity for detection of CNVs smaller than four exons is lower (we estimate ~75%).

Coverage includes all coding exons of the gene(s) analyzed plus 10 bases of flanking noncoding DNA in all available transcripts along with other non-coding regions in which pathogenic variants have been identified at PreventionGenetics or reported elsewhere.

In most cases, we are unable to determine the phase of sequence variants. In particular, when we find two likely causative variants for recessive disorders, we cannot be certain that the variants are on different chromosomes.

Our ability to detect minor sequence variants due to somatic mosaicism is limited. Sequence variants that are present in less than 50% of the patient's nucleated cells may not be detected.

Unless present within coding regions, runs of mononucleotide repeats (eg (A)<sub>n</sub> or (T)<sub>n</sub>) with n >8 in the reference sequence) are generally not analyzed because of strand slippage during amplification.

Unless otherwise indicated, DNA sequence data is obtained from a specific cell type (often leukocytes from whole blood). Test reports contain no information about the DNA sequence in other cell types.

We cannot be certain that the reference sequences are correct. Genome build hg19, GRCh37 (Feb2009) is currently used as our reference in nearly all cases.

We have confidence in our ability to track a specimen once it has been received by PreventionGenetics. However, we take no responsibility for any specimen labeling errors that occur before the sample arrives at PreventionGenetics.

Genetic counseling to help to explain test results to the patients and to discuss reproductive options is recommended.

Reported results will typically not contain any additional information regarding pharmacogenetic analysis of genes, nor are these tests designed to help guide dosage requirements. Pharmacogenetic variant analysis is available, for a select list of genes, as an opt-in with PGxome® tests.

### Test Methods

We use Next Generation Sequencing (NGS) technologies to cover the coding regions of the targeted genes plus 10 bases of non-coding DNA flanking each exon. As required, genomic DNA is extracted from the specimen. The DNA corresponding to these regions is captured using Agilent Clinical Research Exome hybridization

probes. Captured DNA is sequenced using Illumina's Reversible Dye Terminator (RDT) platform NovaSeq 6000 using 150 by 150 bp paired end reads (Illumina, San Diego, CA, USA).

The following quality control metrics are generally achieved: >98% of target bases are covered at >20x, and mean coverage of target bases >120x. Data analysis is performed using the internally developed software Titanium-Exome. Specified genes for which the enhance option is selected are backfilled with Sanger sequencing to achieve 100% coverage.

For Sanger sequencing, Polymerase Chain Reaction (PCR) is used to amplify the necessary exons plus additional flanking non-coding sequence. After purification of the PCR products, cycle sequencing is carried out using the ABI Big Dye Terminator v.3.1 kit. PCR products are resolved by electrophoresis on an ABI 3730xl capillary sequencer. In most cases, cycle sequencing is performed separately in both the forward and reverse directions; in some cases, sequencing is performed twice in either the forward or reverse directions.

Copy number variants (CNVs) are also detected from NGS data. We utilize a CNV calling algorithm that compares mean read depth and distribution for each target in the test sample against multiple matched controls. Neighboring target read depth and distribution and zygosity of any variants within each target region are used to reinforce CNV calls. All reported CNVs are confirmed using another technology such as aCGH, MLPA, or PCR. On occasion, it will not be technically possible to confirm a smaller CNV called by NGS. In these instances, the CNV will not be included on the report.

All differences from the reference sequences (sequence variants) are assigned to one of five interpretation categories (Pathogenic, Likely Pathogenic, Variant of Uncertain Significance, Likely Benign and Benign) per ACMG Guidelines (Richards et al. 2015). Rare and undocumented synonymous variants are nearly always classified as likely benign if there is no indication that they alter protein sequence or disrupt splicing. Benign variants are not listed in the reports, but are available upon request.

Human Genome Variation Society (HGVS) recommendations are used to describe sequence variants (<http://www.hgvs.org>).

### FDA Notes

These results should be used in the context of available clinical findings, and should not be used as the sole basis for treatment. This test was developed and its performance characteristics determined by PreventionGenetics. US Food and Drug Administration (FDA) does not require this test to go through premarket FDA review. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high complexity clinical laboratory testing.