



## Donor 6230

### Genetic Testing Summary

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

Last Updated: 09/23/22

Donor Reported Ancestry: Irish, Swedish, Norwegian, Mexican

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	<b>Carrier: Wilson Disease (ATP7B)</b>	Partner testing recommended before using this donor.
<b>Special Testing</b>		
Gene: IGHMBP2	Negative by gene sequencing	See attached results for residual risks

\*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: Donor 6230  
Date of Birth: [REDACTED]  
Sema4 ID: [REDACTED]  
Client ID: [REDACTED]  
Indication: Carrier Testing

**Specimen Information**

Specimen Type: Blood  
Date Collected: 09/30/2019  
Date Received: 10/01/2019  
Final Report: 10/15/2019

**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><b>Carrier of Wilson Disease (AR)</b> Associated gene(s): <i>ATP7B</i> Variant(s) Detected: c.122A&gt;G, p.N41S, Likely Pathogenic, Heterozygous (one copy)</p>	<p><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

### Wilson Disease (AR)

#### Results and Interpretation

A heterozygous (one copy) likely pathogenic missense variant, c.122A>G, p.N41S, was detected in the *ATP7B* gene (NM\_000053.3). When this variant is present in trans with a pathogenic variant, it is considered to be causative for Wilson disease. Therefore, this individual is expected to be at least a carrier for Wilson disease. Heterozygous carriers are not expected to exhibit symptoms of this disease.

#### What is Wilson Disease?

Wilson disease is an autosomal recessive disease caused by pathogenic variants in the gene *ATP7B*. While it is a pan-ethnic disease, it is found more frequently in individuals of Sephardic and Ashkenazi Jewish descent, as well as individuals from the Canary Islands and from Sardinia. As the protein encoded by *ATP7B* plays a role in copper transport, pathogenic variants in this gene result in the toxic accumulation of copper in different tissues in the body, particularly the liver, nervous system and eyes. Liver disease includes cirrhosis caused by chronic hepatitis, leading to liver failure. Copper depositions in the nervous system can cause neurologic symptoms including changes in behavior, parkinsonism, ataxia and dystonia, and psychiatric symptoms including anxiety, depression and psychosis. While the presence of two null variants is often associated with a more severe disease phenotype, the severity of the disease can vary within families, thereby making it difficult to predict disease severity based on genotype. Without treatment, life expectancy is estimated to be 40 years, but with prompt and efficient treatment, patients may have a normal lifespan.

## 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.

Anastasia Larmore, Ph.D., Assistant Laboratory Director

Laboratory Medical Consultant: George A. Diaz, M.D., Ph.D.

## 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
⊕ Positive				
Wilson Disease	<i>ATP7B</i>	AR	Carrier	c.122A>G, p.N41S, Likely Pathogenic, Heterozygous (one copy)



⊖ Negative

3-Beta-Hydroxysteroid Dehydrogenase Type II Deficiency	<i>HSD3B2</i>	AR	Reduced Risk
3-Methylcrotonyl-CoA Carboxylase Deficiency (MCCC1-Related)	<i>MCCC1</i>	AR	Reduced Risk
3-Methylcrotonyl-CoA Carboxylase Deficiency (MCCC2-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 (SAMHD1-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 (COL4A3-Related)	<i>COL4A3</i>	AR	Reduced Risk
Alport Syndrome (COL4A4-Related)	<i>COL4A4</i>	AR	Reduced Risk
Alport Syndrome (COL4A5-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 (BBS10-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
Barter Syndrome, Type 4A	<i>BSND</i>	AR	Reduced Risk
Bernard-Soulier Syndrome, Type A1	<i>GP1BA</i>	AR	Reduced Risk
Bernard-Soulier 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
Biotinidase Deficiency	<i>BTD</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 ( <i>CYBA</i> -Related)	<i>CYBA</i>	AR	Reduced Risk
Chronic Granulomatous Disease ( <i>CYBB</i> -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	PSAP	AR	Reduced Risk
Congenital Adrenal Hyperplasia due to 17-Alpha-Hydroxylase Deficiency	CYP17A1	AR	Reduced Risk
Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency	CYP21A2	AR	Reduced Risk CYP21A2 copy number: 2 CYP21A2 sequencing: Negative
Congenital Amegakaryocytic Thrombocytopenia	MPL	AR	Reduced Risk
Congenital Disorder of Glycosylation, Type Ia	PMM2	AR	Reduced Risk
Congenital Disorder of Glycosylation, Type Ib	MPI	AR	Reduced Risk
Congenital Disorder of Glycosylation, Type Ic	ALG6	AR	Reduced Risk
Congenital Insensitivity to Pain with Anhidrosis	NTRK1	AR	Reduced Risk
Congenital Myasthenic Syndrome (CHRNE-Related)	CHRNE	AR	Reduced Risk
Congenital Myasthenic Syndrome (RAPSN-Related)	RAPSN	AR	Reduced Risk
Congenital Neutropenia (HAX1-Related)	HAX1	AR	Reduced Risk
Congenital Neutropenia (VPS45-Related)	VPS45	AR	Reduced Risk
Corneal Dystrophy and Perceptive Deafness	SLC4A11	AR	Reduced Risk
Corticosterone Methyloxidase Deficiency	CYP11B2	AR	Reduced Risk
Cystic Fibrosis	CFTR	AR	Reduced Risk
Cystinosis	CTNS	AR	Reduced Risk
D-Bifunctional Protein Deficiency	HSD17B4	AR	Reduced Risk
Deafness, Autosomal Recessive 77	LOXHD1	AR	Reduced Risk
Duchenne Muscular Dystrophy / Becker Muscular Dystrophy	DMD	XL	Reduced Risk
Dyskeratosis Congenita (RTEL1-Related)	RTEL1	AR	Reduced Risk
Dystrophic Epidermolysis Bullosa	COL7A1	AR	Reduced Risk
Ehlers-Danlos Syndrome, Type VIIC	ADAMTS2	AR	Reduced Risk
Ellis-van Creveld Syndrome (EVC-Related)	EVC	AR	Reduced Risk
Emery-Dreifuss Myopathy 1	EMD	XL	Reduced Risk
Enhanced S-Cone Syndrome	NR2E3	AR	Reduced Risk
Ethylmalonic Encephalopathy	ETHE1	AR	Reduced Risk
Fabry Disease	GLA	XL	Reduced Risk
Factor IX Deficiency	F9	XL	Reduced Risk
Factor XI Deficiency	F11	AR	Reduced Risk
Familial Autosomal Recessive Hypercholesterolemia	LDLRAP1	AR	Reduced Risk
Familial Dysautonomia	IKBKAP	AR	Reduced Risk
Familial Hypercholesterolemia	LDLR	AR	Reduced Risk
Familial Hyperinsulinism (ABCC8-Related)	ABCC8	AR	Reduced Risk
Familial Hyperinsulinism (KCNJ11-Related)	KCNJ11	AR	Reduced Risk



Familial Mediterranean Fever	MEFV	AR	Reduced Risk	
Fanconi Anemia, Group A	FANCA	AR	Reduced Risk	
Fanconi Anemia, Group C	FANCC	AR	Reduced Risk	
Fanconi Anemia, Group G	FANCG	AR	Reduced Risk	
Fragile X Syndrome	FMR1	XL	Reduced Risk	FMR1 CGG repeat sizes: Not Performed FMR1 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	FH	AR	Reduced Risk	
GRACILE Syndrome and Other BCS1L-Related Disorders	BCS1L	AR	Reduced Risk	
Galactokinase Deficiency	GALK1	AR	Reduced Risk	
Galactosemia	GALT	AR	Reduced Risk	
Gaucher Disease	GBA	AR	Reduced Risk	
Gitelman Syndrome	SLC12A3	AR	Reduced Risk	
Glutaric Acidemia, Type I	GCDH	AR	Reduced Risk	
Glutaric Acidemia, Type IIa	ETFA	AR	Reduced Risk	
Glutaric Acidemia, Type IIc	ETFDH	AR	Reduced Risk	
Glycine Encephalopathy (AMT-Related)	AMT	AR	Reduced Risk	
Glycine Encephalopathy (GLDC-Related)	GLDC	AR	Reduced Risk	
Glycogen Storage Disease, Type II	GAA	AR	Reduced Risk	
Glycogen Storage Disease, Type III	AGL	AR	Reduced Risk	
Glycogen Storage Disease, Type IV / Adult Polyglucosan Body Disease	GBE1	AR	Reduced Risk	
Glycogen Storage Disease, Type Ia	G6PC	AR	Reduced Risk	
Glycogen Storage Disease, Type Ib	SLC37A4	AR	Reduced Risk	
Glycogen Storage Disease, Type V	PYGM	AR	Reduced Risk	
Glycogen Storage Disease, Type VII	PFKM	AR	Reduced Risk	
HMG-CoA Lyase Deficiency	HMGCL	AR	Reduced Risk	
Hemochromatosis, Type 2A	HFE2	AR	Reduced Risk	
Hemochromatosis, Type 3	TFR2	AR	Reduced Risk	
Hereditary Fructose Intolerance	ALDOB	AR	Reduced Risk	
Hereditary Spastic Paraparesis 49	TECPR2	AR	Reduced Risk	
Hermansky-Pudlak Syndrome, Type 1	HPS1	AR	Reduced Risk	
Hermansky-Pudlak Syndrome, Type 3	HPS3	AR	Reduced Risk	
Holocarboxylase Synthetase Deficiency	HLCS	AR	Reduced Risk	
Homocystinuria (CBS-Related)	CBS	AR	Reduced Risk	
Homocystinuria due to MTHFR Deficiency	MTHFR	AR	Reduced Risk	



Homocystinuria, cbIE Type	<i>MTRR</i>	AR	Reduced Risk
Hydrolethalus Syndrome	<i>HYLS1</i>	AR	Reduced Risk
Hyperomithinemia-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	<i>LCA5</i>	AR	Reduced Risk
Leber Congenital Amaurosis 8 / Retinitis Pigmentosa 12 / Pigmented Paravenous Chorioretinal Atrophy	<i>CRB1</i>	AR	Reduced Risk
Leigh Syndrome, French-Canadian Type	<i>LRPPRC</i>	AR	Reduced Risk
Lethal Congenital Contracture Syndrome 1 / Lethal Arthrogryposis with Anterior Horn Cell Disease	<i>GLE1</i>	AR	Reduced Risk
Leukoencephalopathy with Vanishing White Matter	<i>EIF2B5</i>	AR	Reduced Risk
Limb-Girdle Muscular Dystrophy, Type 2A	<i>CAPN3</i>	AR	Reduced Risk
Limb-Girdle Muscular Dystrophy, Type 2B	<i>DYSF</i>	AR	Reduced Risk
Limb-Girdle Muscular Dystrophy, Type 2C	<i>SGCG</i>	AR	Reduced Risk
Limb-Girdle Muscular Dystrophy, Type 2D	<i>SGCA</i>	AR	Reduced Risk
Limb-Girdle Muscular Dystrophy, Type 2E	<i>SGCB</i>	AR	Reduced Risk
Limb-Girdle Muscular Dystrophy, Type 2I	<i>FKRP</i>	AR	Reduced Risk
Lipoamide Dehydrogenase Deficiency	<i>DLD</i>	AR	Reduced Risk
Lipoid Adrenal Hyperplasia	<i>STAR</i>	AR	Reduced Risk
Lipoprotein Lipase Deficiency	<i>LPL</i>	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 Acidemia (MUT-Related)	MUT	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	PUS1	AR	Reduced Risk
Mucopolipidosis II / IIIA	GNPTAB	AR	Reduced Risk
Mucopolipidosis III Gamma	GNPTG	AR	Reduced Risk
Mucopolipidosis IV	MCOLN1	AR	Reduced Risk
Mucopolysaccharidosis Type I	IDUA	AR	Reduced Risk
Mucopolysaccharidosis Type II	IDS	XL	Reduced Risk
Mucopolysaccharidosis Type IIIA	SGSH	AR	Reduced Risk
Mucopolysaccharidosis Type IIIB	NAGLU	AR	Reduced Risk
Mucopolysaccharidosis Type IIIC	HGSNAT	AR	Reduced Risk
Mucopolysaccharidosis Type IIID	GNS	AR	Reduced Risk
Mucopolysaccharidosis Type IVb / GM1 Gangliosidosis	GLB1	AR	Reduced Risk
Mucopolysaccharidosis type IX	HYAL1	AR	Reduced Risk
Mucopolysaccharidosis type VI	ARSB	AR	Reduced Risk
Multiple Sulfatase Deficiency	SUMF1	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	<i>WNT10A</i>	AR	Reduced Risk
Omenn Syndrome ( <i>RAG2</i> -Related)	<i>RAG2</i>	AR	Reduced Risk
Omenn Syndrome / Severe Combined Immunodeficiency, Athabaskan-Type	<i>DCLRE1C</i>	AR	Reduced Risk
Ornithine Aminotransferase Deficiency	<i>OAT</i>	AR	Reduced Risk
Ornithine Transcarbonylase Deficiency	<i>OTC</i>	XL	Reduced Risk
Osteopetrosis 1	<i>TCIRG1</i>	AR	Reduced Risk
Pendred Syndrome	<i>SLC26A4</i>	AR	Reduced Risk
Phenylalanine Hydroxylase Deficiency	<i>PAH</i>	AR	Reduced Risk
Polycystic Kidney Disease, Autosomal Recessive	<i>PKHD1</i>	AR	Reduced Risk
Polyglandular Autoimmune Syndrome, Type 1	<i>AIRE</i>	AR	Reduced Risk
Pontocerebellar Hypoplasia, Type 1A	<i>VRK1</i>	AR	Reduced Risk
Pontocerebellar Hypoplasia, Type 6	<i>RARS2</i>	AR	Reduced Risk
Primary Carnitine Deficiency	<i>SLC22A5</i>	AR	Reduced Risk



Primary Ciliary Dyskinesia (DNAH5-Related)	DNAH5	AR	Reduced Risk	
Primary Ciliary Dyskinesia (DNAI1-Related)	DNAI1	AR	Reduced Risk	
Primary Ciliary Dyskinesia (DNAI2-Related)	DNAI2	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: 2 c.*3+80T>G: Negative
Spondylthoracic 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	<i>USH1C</i>	AR	Reduced Risk
Usher Syndrome, Type ID	<i>CDH23</i>	AR	Reduced Risk
Usher Syndrome, Type IF	<i>PCDH15</i>	AR	Reduced Risk
Usher Syndrome, Type IIA	<i>USH2A</i>	AR	Reduced Risk
Usher Syndrome, Type III	<i>CLRN1</i>	AR	Reduced Risk
Very Long Chain Acyl-CoA Dehydrogenase Deficiency	<i>ACADVL</i>	AR	Reduced Risk
Walker-Warburg Syndrome and Other <i>FKTN</i> -Related Dystrophies	<i>FKTN</i>	AR	Reduced Risk
Wolman Disease / Cholesteryl Ester Storage Disease	<i>LIPA</i>	AR	Reduced Risk
X-Linked Juvenile Retinoschisis	<i>RS1</i>	XL	Reduced Risk
X-Linked Severe Combined Immunodeficiency	<i>IL2RG</i>	XL	Reduced Risk
Zellweger Syndrome Spectrum ( <i>PEX10</i> -Related)	<i>PEX10</i>	AR	Reduced Risk
Zellweger Syndrome Spectrum ( <i>PEX1</i> -Related)	<i>PEX1</i>	AR	Reduced Risk
Zellweger Syndrome Spectrum ( <i>PEX2</i> -Related)	<i>PEX2</i>	AR	Reduced Risk
Zellweger Syndrome Spectrum ( <i>PEX6</i> -Related)	<i>PEX6</i>	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.

## SELECTED REFERENCES

### Carrier Screening

Grody W et al. ACMG position statement on prenatal/preconception expanded carrier screening. *Genet Med*. 2013 15:482-3.

### Fragile X syndrome:

Chen L et al. An information-rich CGG repeat primed PCR that detects the full range of Fragile X expanded alleles and minimizes the need for Southern blot analysis. *J Mol Diag* 2010 12:589-600.

### 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.

### Ashkenazi Jewish Disorders:

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

### Duchenne Muscular Dystrophy:

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**

Name: Donor 6230  
 Date of Birth: [REDACTED]  
 Sema4 ID: [REDACTED]  
 Client ID: [REDACTED]  
 Indication: Carrier Screening

**Specimen Information**

Specimen Type: Purified DNA  
 Date Collected: 08/24/2022  
 Date Received: 08/26/2022  
 Final Report: 09/06/2022

**Referring Provider**

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

Custom Carrier Screen (1 gene)  
 with Personalized Residual Risk

**SUMMARY OF RESULTS AND RECOMMENDATIONS**

⊖ Negative

Negative for all genes tested: *IGHMBP2*  
 To view a full list of genes and diseases tested  
 please see Table 1 in this report

AR=Autosomal recessive; XL=X-linked

**Recommendations**

- 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. Please note that residual risks for X-linked diseases (including full repeat expansions for Fragile X syndrome) may not be accurate for males and the actual residual risk is likely to be lower.

**Test description**

This patient was tested for the genes listed above using one or more of the following methodologies: target capture and short-read sequencing, long-range PCR followed by short-read sequencing, targeted genotyping, and/or 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 with the patient's personalized residual risk. If personalized residual risk is not provided, please see the complete residual risk table at [go.sema4.com/residualrisk](http://go.sema4.com/residualrisk). Only known pathogenic or likely pathogenic variants are reported. This carrier screening test does not report likely benign variants and variants of uncertain significance (VUS). If reporting of likely benign variants and VUS are desired in this patient, please contact the laboratory at 800-298-6470, option 2 to request an amended report.



Alice Tanner, Ph.D., M.S., CGC, FACMG, Laboratory Director  
 Laboratory Medical Consultant: George A. Diaz, M.D., Ph.D



## Genes and diseases tested

The personalized residual risks listed below are specific to this individual. The complete residual risk table is available at [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>Negative</b>				
<b>Spinal Muscular Atrophy with Respiratory Distress 1 / Charcot-Marie-Tooth Disease, Type 2S</b>	<i>IGHMBP2</i>	AR	Reduced Risk	<b>Personalized Residual Risk: 1 in 1,200</b>

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<sup>®</sup> *FMR1* PCR reagents followed by capillary electrophoresis for allele sizing was performed. Samples positive for *FMR1* premutations and full mutations greater than 90 CGG repeats in length were further analyzed by Southern blot analysis or methylation PCR to assess the size and methylation status of the *FMR1* CGG repeat. Additional testing to determine the status of AGG interruptions within the *FMR1* CGG repeat will be automatically performed for premutation alleles ranging from 55 to 90 repeats. These results, which may modify risk for expansion, will follow in a separate report.

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

Multiplex PCR amplification and allele specific primer extension analyses using the MassARRAY<sup>®</sup> System were used to identify certain recurrent 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<sup>®</sup> 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 typically due to unequal meiotic crossing-over between *CYP21A2* and the pseudogene *CYP21A1P*. Classic 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 diagnosed with SMA have a causative *SMN1* variant that occurred de novo, and therefore cannot be picked up by carrier screening in the parents. Analysis of *SMN1* is performed in association with short-read sequencing of exons 2a-7, followed by confirmation using long-range PCR (described below).

In individuals with two copies of *SMN1* with Ashkenazi Jewish, East Asian, African American, Native American 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 silent carrier.

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™XT Low Input 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. Libraries were pooled and sequenced on the Illumina NovaSeq 6000 platform, using paired-end 100 bp 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. These regions, which are described below, 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.

**Exceptions:** *ABCD1* (NM\_000033.3) exons 8 and 9; *ACADSB* (NM\_001609.3) chr10:124,810,695-124,810,707 (partial exon 9); *ADA* (NM\_000022.2) exon 1; *ADAMTS2* (NM\_014244.4) exon 1; *AGPS* (NM\_003659.3) chr2:178,257,512-178,257,649 (partial exon 1); *ALDH7A1* (NM\_001182.4) chr5:125,911,150-125,911,163 (partial exon 7) and chr5:125,896,807-125,896,821 (partial exon 10); *ALMS1* (NM\_015120.4) chr2:73,612,990-73,613,041 (partial exon 1); *APOPT1* (NM\_032374.4) chr14:104,040,437-104,040,455 (partial exon 3); *CDAN1* (NM\_138477.2) exon 2; *CEP152* (NM\_014985.3) chr15:49,061,146-49,061,165 (partial exon 14) and exon 22; *CEP290* (NM\_025114.3) exon 5, exon 7, chr12:88,519,017-88,519,039 (partial exon 13), chr12:88,514,049-88,514,058 (partial exon 15), chr12:88,502,837-88,502,841 (partial exon 23), chr12:88,481,551-88,481,589 (partial exon 32), chr12:88,471,605-88,471,700 (partial exon 40); *CFTR* (NM\_000492.3) exon 10; *COL4A4* (NM\_000092.4) chr2:227,942,604-227,942,619 (partial exon 25); *COX10* (NM\_001303.3) exon 6; *CYP11B1* (NM\_000497.3) exons 3-7; *CYP11B2* (NM\_000498.3) exons 3-7; *DNAI2* (NM\_023036.4) chr17:72,308,136-72,308,147 (partial exon 12); *DOK7* (NM\_173660.4) chr4:3,465,131-3,465,161 (partial exon 1) and exon 2; *DUOX2* (NM\_014080.4) exons 6-8; *EIF2AK3* (NM\_004836.5) exon 8; *EVC* (NM\_153717.2) exon 1; *F5* (NM\_000130.4) chr1:169,551,662-169,551,679 (partial exon 2); *FH* (NM\_000143.3) exon 1; *GAMT* (NM\_000156.5) exon 1; *GLDC* (NM\_000170.2) exon 1; *GNPTAB* (NM\_024312.4) chr17:4,837,000-4,837,400 (partial exon 2); *GNPTG* (NM\_032520.4) exon 1; *GHR* (NM\_000163.4) exon 3; *GYS2* (NM\_021957.3) chr12:21,699,370-21,699,409 (partial exon 12); *HGSNAT* (NM\_152419.2) exon 1; *IDS* (NM\_000202.6) exon 3; *ITGB4* (NM\_000213.4) chr17:73,749,976-73,750,060 (partial exon 33); *JAK3* (NM\_000215.3) chr19:17,950,462-17,950,483 (partial exon 10); *LIFR* (NM\_002310.5) exon 19; *LMBRD1* (NM\_018368.3) chr6:70,459,226-70,459,257 (partial exon 5), chr6:70,447,828-70,447,836 (partial exon 7) and exon 12; *LYST* (NM\_000081.3) chr1:235,944,158-235,944,176 (partial exon 16) and chr1:235,875,350-235,875,362 (partial exon 43); *MLYCD* (NM\_012213.2) chr16:83,933,242-83,933,282 (partial exon 1); *MTR* (NM\_000254.2) chr1:237,024,418-237,024,439 (partial exon 20) and chr1:237,038,019-237,038,029 (partial exon 24); *NBEAL2* (NM\_015175.2) chr3:47,021,385-47,021,407 (partial exon 1); *NEB* (NM\_001271208.1) exons 82-105; *NPC1* (NM\_000271.4) chr18:21,123,519-21,123,538 (partial exon 14); *NPHP1* (NM\_000272.3) chr2:110,937,251-110,937,263 (partial exon 3); *OCRL* (NM\_000276.3) chrX:128,674,450-128,674,460 (partial exon 1); *PHKB* (NM\_000293.2) exon 1 and chr16:47,732,498-47,732,504 (partial exon 30); *PIGN* (NM\_176787.4) chr18:59,815,547-59,815,576 (partial exon 8); *PIP5K1C* (NM\_012398.2) exon 1 and chr19:3637602-3637616 (partial exon 17); *POU1F1* (NM\_000306.3) exon 5; *PTPRC* (NM\_002838.4) exons 11 and 23; *PUS1* (NM\_025215.5) chr12:132,414,446-132,414,532 (partial exon 2); *RPGRIP1L* (NM\_015272.2) exon 23; *SGSH* (NM\_000199.3) chr17:78,194,022-78,194,072 (partial exon 1); *SLC6A8* (NM\_005629.3) exons 3 and 4; *ST3GAL5* (NM\_003896.3) exon 1; *SURF1* (NM\_003172.3) chr9:136,223,269-136,223,307 (partial exon 1); *TRPM6* (NM\_017662.4) chr9:77,362,800-77,362,811 (partial exon 31); *TSEN54* (NM\_207346.2) exon 1; *TYR* (NM\_000372.4) exon 5; *VWF* (NM\_000552.3) exons 24-26, chr12:6,125,675-6,125,684 (partial exon 30), chr12:6,121,244-6,121,265 (partial exon 33), and exon 34.

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.

#### Next Generation Sequencing for *SMN1*

Exonic regions and intron/exon splice junctions of *SMN1* and *SMN2* were captured, sequenced, and analyzed as described above. Any variants located within exons 2a-7 and classified as pathogenic or likely pathogenic were confirmed to be in either *SMN1* or *SMN2* using gene-specific long-range PCR analysis followed by Sanger sequencing. Variants located in exon 1 cannot be accurately assigned to either *SMN1* or *SMN2* using our current methodology, and so these variants are not 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. Deletions and duplications near the lower limit of detection may not be detected due to run variability.

#### 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. Please note that in rare cases, allele drop-out may occur, which has the potential to lead to false negative results. For *CYP21A2*, a certain percentage of healthy individuals carry a duplication of the *CYP21A2* gene, which has no clinical consequences. In cases where multiple copies of *CYP21A2* are located on the same chromosome in tandem, only the last 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. A *CYP21A1P/CYP21A2* hybrid gene detected only by MLPA but not by long-range PCR will not be reported when the long-range PCR indicates the presence of two full *CYP21A2* gene copies (one on each chromosome), as the additional hybrid gene is nonfunctional. Classic 30-kb deletions are identified by MLPA and are also identified by the presence of multiple common pathogenic *CYP21A2* variants by long-range PCR. Since multiple pseudogene-derived variants are detected in all cases with the classic 30kb deletion, we cannot rule out the possibility that some variant(s) detected could be present in trans with the chimeric *CYP21A1P/CYP21A2* gene created by the 30kb deletion. 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 >30,000 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.

#### Personalized Residual Risk Calculations

Agilent SureSelect™XT Low-Input technology was utilized in order to create whole-genome libraries for each patient sample. Libraries were then pooled and sequenced on the Illumina NovaSeq platform. Each sequencing lane was multiplexed to achieve 0.4-2x genome coverage, using paired-end 100 bp reads. The sequencing data underwent ancestral analysis using a customized, licensed bioinformatics algorithm that was validated in house. Identified sub-ethnic groupings were binned into one of 7 continental-level groups (African, East Asian, South Asian, Non-Finnish European, Finnish, Native American, and Ashkenazi Jewish) or, for those ethnicities that matched poorly to the continental-level groups, an 8<sup>th</sup> "unassigned" group, which were then used to select residual risk values for each gene. For individuals belonging to multiple high-level ethnic groupings, a weighting strategy was used to select the most appropriate residual risk. For genes that had insufficient data to calculate ethnic-specific residual risk values, or for sub-ethnic groupings that fell into the "unassigned" group, a "worldwide" residual risk was used. This "worldwide" residual risk was calculated using data from all available continental-level groups.

Several genes have multiple residual risks associated to reflect the likelihood of the tested individual being a carrier for different diseases that are attributed to non-overlapping pathogenic variants in that gene. When calculating the couples' combined reproductive risk, the highest residual risk for each patient was selected.

#### 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.

#### Tay-Sachs Disease (TSD) Enzyme Analysis (Analytical Detection Rate ≥98%)

Hexosaminidase activity and Hex A% activity were measured by a standard heat-inactivation, fluorometric method using artificial 4-MU-β-N-acetyl glucosaminide (4-MUG) substrate. This assay is highly sensitive and accurate in detecting Tay-Sachs carriers and individuals affected with TSD. Normal ranges of Hex A% activity are 55.0-72.0 for white blood cells and 58.0-72.0 for plasma. It is estimated that less than 0.5% of Tay-Sachs carriers have non-carrier levels of percent Hex A activity, and therefore may not be identified by this assay. In addition, this assay may detect individuals that are carriers of or are affected with Sandhoff disease. False positive results may occur if benign variants, such as pseudodeficiency alleles, interfere with the enzymatic assay. False negative results may occur if both *HEXA* and *HEXB* pathogenic or pseudodeficiency variants are present in the same individual.

Please note that it is not possible to perform Tay-Sachs disease enzyme analysis on saliva samples, buccal swabs, tissue samples, semen samples, or on samples received as extracted DNA.

This test was developed, and its performance characteristics determined by Sema4 Opco, Inc. It has not been cleared or approved by the 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 (CLIA) as qualified to perform high complexity clinical laboratory testing. 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.

## SELECTED REFERENCES

### Carrier Screening

Grody W et al. ACMG position statement on prenatal/preconception expanded carrier screening. *Genet Med*. 2013 15:482-3.

### Fragile X syndrome:

Chen L et al. An information-rich CGG repeat primed PCR that detects the full range of Fragile X expanded alleles and minimizes the need for Southern blot analysis. *J Mol Diag* 2010 12:589-600.

### 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.

### Ashkenazi Jewish Disorders:



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

Akler G et al. Towards a unified approach for comprehensive reproductive carrier screening in the Ashkenazi, Sephardi, and Mizrahi Jewish populations. *Mol Genet Genomic Med.* 2020 Feb 8(2):e1053.

**Duchenne Muscular Dystrophy:**

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.