



## Donor 5842

### Genetic Testing Summary

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

Last Updated: 11/2/20

Donor Reported Ancestry: German, Scottish, English, Belgian

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>Carrier: Congenital Adrenal Hyperplasia due to 21 Hydroxylase Deficiency (CYP21A2) Non-Classic variant</p> <p>Carrier: Limb-Girdle Muscular Dystrophy Type 2A (CAPN3)</p> <p>Negative for other genes sequenced.</p>	Partner testing is recommended before using this donor.
<b>Special Testing</b>		
DOCK8 gene	Negative by gene sequencing in the DOCK8 gene	
SPG7 gene	Negative by gene sequencing in the SPG7 gene	

\*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	Sample	Referring Doctor
<b>Patient Name:</b> 5842 Donor <b>Date of Birth:</b> [REDACTED] <b>Reference #:</b> [REDACTED] <b>Indication:</b> Carrier Testing <b>Test Type:</b> Expanded Carrier Screen (283)	<b>Specimen Type:</b> Blood <b>Lab #:</b> [REDACTED] <b>Date Collected:</b> 2/12/2019 <b>Date Received:</b> 2/13/2019 <b>Final Report:</b> 3/2/2019	[REDACTED] <b>Fairfax Cryobank, Inc.</b> [REDACTED] [REDACTED] [REDACTED]

## RESULT SUMMARY

### THIS PATIENT WAS TESTED FOR 283 DISEASES.

Please see Table 1 for list of diseases tested.

#### **POSITIVE for congenital adrenal hyperplasia (due to 21-hydroxylase deficiency)**

A heterozygous (one copy) pathogenic variant, c.1357C>T, p.P453S, was detected in the *CYP21A2* gene

#### **POSITIVE for limb-girdle muscular dystrophy, type 2A**

A heterozygous (one copy) likely pathogenic variant, c.2257G>A, p.D753N, was detected in the *CAPN3* gene

#### **NEGATIVE for the remaining diseases**

### 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. In addition, 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 for limb-girdle muscular dystrophy, type 2A

A heterozygous (one copy) likely pathogenic missense variant, c.2257G>A, p.D753N, was detected in the *CAPN3* gene (NM\_000070.2). When this variant is present in trans with a pathogenic variant, it is considered to be causative for limb-girdle muscular dystrophy, type 2A. Therefore, this individual is expected to be at least a carrier for limb-girdle muscular dystrophy, type 2A. Heterozygous carriers are not expected to exhibit symptoms of this disease.

Patient: 5842 Donor

DOB: [REDACTED]

Lab #: [REDACTED]

### What is limb-girdle muscular dystrophy, type 2A?

Limb-girdle muscular dystrophy, type 2A is an autosomal recessive, pan-ethnic disorder that is caused by pathogenic variants in the gene *CAPN3*. This form of muscular dystrophy presents with weakness of the pelvic girdle and legs, and eventually progresses to the upper limbs. Sometimes it presents with weakness of the upper limbs and progresses to the lower limbs. Onset is usually in childhood or early adolescence, although variability exists. Patients are usually wheelchair-bound about 20 years after diagnosis, and death usually occurs in middle age. Some patients also experience weakness of the facial muscles or contractures of the joints. Patients with at least one missense variant may experience a slightly slower rate of progression than those with two null variants, but the severity of the disease appears to be the same.

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 <http://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.

## TEST SPECIFIC RESULTS

### Alpha-thalassemia

#### **NEGATIVE for alpha-thalassemia**

*HBA1* copy number: 2

*HBA2* copy number: 2

No pathogenic copy number variants detected

*HBA1* and *HBA2* sequence analysis: No pathogenic or likely pathogenic variants identified

Reduced risk of being an alpha-thalassemia carrier (aa/aa)

**Genes analyzed:** *HBA1* (NM\_000558.4) and *HBA2* (NM\_000517.4)

**Inheritance:** Autosomal Recessive

### **Recommendations**

Individuals of Asian, African, Hispanic and Mediterranean ancestry should also be screened for hemoglobinopathies by CBC and hemoglobin electrophoresis.

### **Interpretation**

No pathogenic or likely pathogenic copy number variants or sequence variants were detected in this patient, suggesting that four copies of the alpha-globin gene are present (aa/aa). Typically, individuals have four functional alpha-globin genes: 2 copies of *HBA1* and 2 copies of *HBA2*, whose expression is regulated by a cis-acting regulatory element HS-40. Alpha-thalassemia carriers have three (silent carrier) or two (carrier of the

**Patient:** 5842 Donor

**DOB:** [REDACTED]

**Lab #:** [REDACTED]

alpha-thalassemia trait) functional alpha-globin genes with or without a mild phenotype. Individuals with only one functional alpha-globin gene have HbH disease with microcytic, hypochromic hemolytic anemia and hepatosplenomegaly. Loss of all four alpha-globin genes results in Hb Barts syndrome with the accumulation of Hb Barts in red blood cells and hydrops fetalis, which is fatal in utero or shortly after birth.

This individual was negative for all *HBA* deletions, duplications and variants that were tested. These negative results reduce but do not eliminate the possibility that this individual is a carrier. See *Table of Residual Risks Based on Ethnicity*. With individuals of mixed ethnicity, it is recommended to use the highest residual risk estimate.

**Table of Residual Risks Based on Ethnicity**

Ethnicity	Carrier Frequency	Detection Rate	Residual Risk
Caucasian	1 in 500	95%	1 in 10,000
African American	1 in 30	95%	1 in 580
Asian	1 in 20	95%	1 in 380
Worldwide	1 in 25	95%	1 in 480

**Congenital Adrenal Hyperplasia (21-Hydroxylase Deficiency)**

**POSITIVE for congenital adrenal hyperplasia (due to 21-hydroxylase deficiency)**

*CYP21A2* copy number: 2

No pathogenic copy number variants detected

Sequence analysis: A heterozygous (one copy) pathogenic variant, c.1357C>T, p.P453S, was detected in the *CYP21A2* gene

**Genes analyzed:** *CYP21A2* (NM\_000500.6)

**Inheritance:** Autosomal Recessive

**Recommendations**

Testing of the patient's partner and genetic counseling are recommended.

**Interpretation for congenital adrenal hyperplasia (due to 21-hydroxylase deficiency)**

A heterozygous (one copy) pathogenic missense variant, c.1357C>T, p.P453S, was detected in the *CYP21A2* gene (NM\_000500.6). Please note that this variant is typically causative for the non-classic form of congenital adrenal hyperplasia (PMID: 29450859). Variants associated with the non-classic form usually cause non-classic congenital adrenal hyperplasia when found in trans with a pathogenic allele, regardless of whether the second variant is associated with classic or non-classic disease (PMID: 29450859). Therefore, this individual is expected to be at least a carrier for non-classic congenital adrenal hyperplasia. Heterozygous carriers are not expected to exhibit symptoms of this disease.

Patient: 5842 Donor

DOB: [REDACTED]

Lab #: [REDACTED]

### What is congenital adrenal hyperplasia (due to 21-hydroxylase deficiency)?

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders resulting from deficiency in the enzymes involved in cortisol biosynthesis. The majority (95%) of CAH cases are due to 21-hydroxylase deficiency (21-OHD CAH), which is caused by homozygous or compound heterozygous pathogenic variants in the gene *CYP21A2*. Approximately 20% of mutant alleles have deletions of 30 kb that have been generated by unequal meiotic crossing-over between the two genes. Another 75% of mutant alleles are due to gene conversion events, where an inactivating mutation from the *CYP21A1P* pseudogene is introduced into one copy of the *CYP21A2* gene, thus making the gene non-functional. Three different forms of 21-OHD CAH have been reported: a classic salt wasting form, a classic simple virilizing form, and a non-classic form.

- The classic salt wasting form results from a nonfunctional enzyme and is the most severe. The phenotype includes prenatal onset of virilization and inadequate adrenal aldosterone secretion that can result in fatal salt-wasting crises.
- The classic simple virilizing form results from low levels of functional enzyme and involves prenatal virilization but no salt-wasting.
- The non-classic form, which results from a mild enzyme deficiency, occurs postnatally and involves phenotypes associated with hyperandrogenism, such as hirsutism, delayed menarche, and infertility.

Treatment for the classic forms of the disorder include glucocorticoid and mineralocorticoid replacement therapy, as well as the possibility of feminizing genitoplasty, while patients with the non-classic form usually do not require treatment. The life expectancy for this disorder can be normal with treatment, however the occurrence of salt-wasting crises can be fatal.

### Fragile X syndrome

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. Sequencing of the *FMR1* gene by next generation sequencing did not identify any clinically significant variants.

### Spinal Muscular Atrophy

#### **NEGATIVE for spinal muscular atrophy**

*SMN1* Copy Number: 2

*SMN2* Copy Number: 2

c.\*3+80T>G: Negative

#### **Negative copy number result**

**Decreased risk of being an *SMN1* silent (2+0) carrier (see *SMA Table*)**

Patient: 5842 Donor

DOB: [REDACTED]

Lab #: [REDACTED]

**Genes analyzed:** *SMN1* (NM\_000344.3) and *SMN2* (NM\_017411.3)

**Inheritance:** Autosomal Recessive

**Recommendations**

Consideration of residual risk by ethnicity after a negative carrier screen is recommended, especially in the case of a positive family history for spinal muscular atrophy.

**Interpretation**

This patient is negative for loss of *SMN1* copy number. Complete loss of *SMN1* is causative in spinal muscular atrophy (SMA). Two copies of *SMN1* were detected in this individual, which significantly reduces the risk of being an SMA carrier. Parallel testing to assess the presence of an *SMN1* duplication allele was also performed to detect a single nucleotide polymorphism (SNP), c.\*3+80T>G, in intron 7 of the *SMN1* gene. This individual was found to be negative for this change and is therefore, at a decreased risk of being a silent (2+0) carrier, see *SMA Table* for residual risk estimates based on ethnicity.

**SMA Table: Carrier detection and residual risk estimates before and after testing for c.\*3+80T>G**

Ethnicity	Carrier Frequency	Detection rate	Residual risk after negative result*	Detection rate with <i>SMN1</i> c.*3+80T>G	Residual risk c.*3+80T>G negative	Residual risk c.*3+80T>G positive
African American	1 in 85	71%	1 in 160	91%	1 in 455	1 in 49
Ashkenazi Jewish	1 in 76	90%	1 in 672	93%	1 in 978	1 in 10
East Asian	1 in 53	94%	1 in 864	95%	1 in 901	1 in 12
Caucasian	1 in 48	95%	1 in 803	95%	1 in 894	1 in 23
Latino	1 in 63	91%	1 in 609	94%	1 in 930	1 in 47
South Asian	1 in 103	87%	1 in 637	87%	1 in 637	1 in 608
Sephardic Jewish	1 in 34	96%	1 in 696	97%	1 in 884	1 in 12

\*Residual risk with two copies *SMN1* detected using dosage sensitive methods. The presence of three or more copies of *SMN1* reduces the risk of being an *SMN1* carrier between 5 - 10 fold, depending on ethnicity.

**FOR INDIVIDUALS WITH MIXED ETHNICITY, USE HIGHEST RESIDUAL RISK ESTIMATE**

^ Parental follow-up will be requested for confirmation

This case has been reviewed and electronically signed by Lisa Edelmann, Ph.D., FACMG, Laboratory Director

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

Patient: 5842 Donor

DOB: [REDACTED]

Lab #: [REDACTED]

## 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* 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<sup>®</sup> 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<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 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 silent 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

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).



Patient: 5842 Donor

DOB: [REDACTED]

Lab #: [REDACTED]

**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



Patient: 5842 Donor

DOB: [REDACTED]

Lab #: [REDACTED]

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.

#### 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 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:** 5842 Donor

**DOB:** [REDACTED]

**Lab #:** [REDACTED]

**Table 1. List of genes and diseases tested.**

Please see <http://go.sema4.com/residualrisk> for specific detection rates and residual risk by ethnicity.

Gene	Disease
<b>ACADM</b>	Medium Chain Acyl-CoA Dehydrogenase Deficiency
<b>ABCB11</b>	Progressive Familial Intrahepatic Cholestasis, Type 2
<b>ABCC8</b>	Familial Hyperinsulinism (ABCC8-Related)
<b>ABCD1</b>	Adrenoleukodystrophy, X-Linked
<b>ACAD9</b>	Mitochondrial Complex I Deficiency (ACAD9-Related)
<b>ACADVL</b>	Very Long Chain Acyl-CoA Dehydrogenase Deficiency
<b>ACAT1</b>	Beta-Ketothiolase Deficiency
<b>ACOX1</b>	Acyl-CoA Oxidase I Deficiency
<b>ACSF3</b>	Combined Malonic and Methylmalonic Aciduria
<b>ADA</b>	Adenosine Deaminase Deficiency
<b>ADAMTS2</b>	Ehlers-Danlos Syndrome, Type VIIC
<b>AGA</b>	Aspartylglycosaminuria
<b>AGL</b>	Glycogen Storage Disease, Type III
<b>AGPS</b>	Rhizomelic Chondrodysplasia Punctata, Type 3
<b>AGXT</b>	Primary Hyperoxaluria, Type 1
<b>AIRE</b>	Polyglandular Autoimmune Syndrome, Type 1
<b>ALDH3A2</b>	Sjogren-Larsson Syndrome
<b>ALDOB</b>	Hereditary Fructose Intolerance
<b>ALG6</b>	Congenital Disorder of Glycosylation, Type Ic
<b>ALMS1</b>	Alstrom Syndrome
<b>ALPL</b>	Hypophosphatasia
<b>AMT</b>	Glycine Encephalopathy (AMT-Related)
<b>AQP2</b>	Nephrogenic Diabetes Insipidus, Type II
<b>ARSA</b>	Metachromatic Leukodystrophy
<b>ARSB</b>	Mucopolysaccharidosis type VI
<b>ASL</b>	Argininosuccinic Aciduria
<b>ASNS</b>	Asparagine Synthetase Deficiency
<b>ASPA</b>	Canavan Disease
<b>ASS1</b>	Citrullinemia, Type 1
<b>ATM</b>	Ataxia-Telangiectasia
<b>ATP6V1B1</b>	Renal Tubular Acidosis and Deafness
<b>ATP7A</b>	Menkes Disease
<b>ATP7B</b>	Wilson Disease
<b>ATRX</b>	Alpha-Thalassemia Mental Retardation Syndrome
<b>BBS1</b>	Bardet-Biedl Syndrome (BBS1-Related)
<b>BBS10</b>	Bardet-Biedl Syndrome (BBS10-Related)
<b>BBS12</b>	Bardet-Biedl Syndrome (BBS12-Related)
<b>BBS2</b>	Bardet-Biedl Syndrome (BBS2-Related)
<b>BCKDHA</b>	Maple Syrup Urine Disease, Type 1a
<b>BCKDHB</b>	Maple Syrup Urine Disease, Type 1b
<b>BCS1L</b>	GRACILE Syndrome and Other BCS1L-Related Disorders
<b>BLM</b>	Bloom Syndrome
<b>BSND</b>	Bartter Syndrome, Type 4A
<b>BTD</b>	Biotinidase Deficiency
<b>CAPN3</b>	Limb-Girdle Muscular Dystrophy, Type 2A
<b>CBS</b>	Homocystinuria (CBS-Related)
<b>CDH23</b>	Usher Syndrome, Type ID
<b>CEP290</b>	Leber Congenital Amaurosis 10 and Other CEP290-Related Ciliopathies
<b>CERKL</b>	Retinitis Pigmentosa 26

Gene	Disease
<b>CFTR</b>	Cystic Fibrosis
<b>CHM</b>	Choroideremia
<b>CHRNE</b>	Congenital Myasthenic Syndrome (CHRNE-Related)
<b>CIITA</b>	Bare Lymphocyte Syndrome, Type II
<b>CLN3</b>	Neuronal Ceroid-Lipofuscinosis (CLN3-Related)
<b>CLN5</b>	Neuronal Ceroid-Lipofuscinosis (CLN5-Related)
<b>CLN6</b>	Neuronal Ceroid-Lipofuscinosis (CLN6-Related)
<b>CLN8</b>	Neuronal Ceroid-Lipofuscinosis (CLN8-Related)
<b>CLRN1</b>	Usher Syndrome, Type III
<b>CNGB3</b>	Achromatopsia
<b>COL27A1</b>	Steel Syndrome
<b>COL4A3</b>	Alport Syndrome (COL4A3-Related)
<b>COL4A4</b>	Alport Syndrome (COL4A4-Related)
<b>COL4A5</b>	Alport Syndrome (COL4A5-Related)
<b>COL7A1</b>	Dystrophic Epidermolysis Bullosa
<b>CPS1</b>	Carbamoylphosphate Synthetase I Deficiency
<b>CPT1A</b>	Carnitine Palmitoyltransferase IA Deficiency
<b>CPT2</b>	Carnitine Palmitoyltransferase II Deficiency
<b>CRB1</b>	Leber Congenital Amaurosis 8 / Retinitis Pigmentosa 12 / Pigmented Paravenous Chorioretinal Atrophy
<b>CTNS</b>	Cystinosis
<b>CTSK</b>	Pycnodysostosis
<b>CYBA</b>	Chronic Granulomatous Disease (CYBA-related)
<b>CYBB</b>	Chronic Granulomatous Disease (CYBB-related)
<b>CYP11B2</b>	Corticosterone Methyloxidase Deficiency
<b>CYP17A1</b>	Congenital Adrenal Hyperplasia due to 17-Alpha-Hydroxylase Deficiency
<b>CYP21A2</b>	Classic Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency
<b>CYP19A1</b>	Aromatase Deficiency
<b>CYP27A1</b>	Cerebrotendinous Xanthomatosis
<b>DCLRE1C</b>	Omenn Syndrome / Severe Combined Immunodeficiency, Athabaskan-Type
<b>DHCR7</b>	Smith-Lemli-Opitz Syndrome
<b>DHDDS</b>	Retinitis Pigmentosa 59
<b>DLG</b>	Lipoamide Dehydrogenase Deficiency
<b>DMD</b>	Duchenne Muscular Dystrophy / Becker Muscular Dystrophy
<b>DNAH5</b>	Primary Ciliary Dyskinesia (DNAH5-Related)
<b>DNAI1</b>	Primary Ciliary Dyskinesia (DNAI1-Related)
<b>DNAI2</b>	Primary Ciliary Dyskinesia (DNAI2-related)
<b>DYSF</b>	Limb-Girdle Muscular Dystrophy, Type 2B
<b>EDA</b>	Hypohidrotic Ectodermal Dysplasia 1
<b>EIF2B5</b>	Leukoencephalopathy with Vanishing White Matter
<b>EMD</b>	Emery-Dreifuss Myopathy 1
<b>ESCO2</b>	Roberts Syndrome
<b>ETFA</b>	Glutaric Acidemia, Type IIa
<b>ETFDH</b>	Glutaric Acidemia, Type IIc
<b>ETHE1</b>	Ethylmalonic Encephalopathy
<b>EVC</b>	Ellis-van Creveld Syndrome (EVC-Related)
<b>EYS</b>	Retinitis Pigmentosa 25
<b>F11</b>	Factor XI Deficiency
<b>F9</b>	Factor IX Deficiency
<b>FAH</b>	Tyrosinemia, Type I

**Patient:** 5842 Donor

**DOB:** [REDACTED]

**Lab #:** [REDACTED]

Gene	Disease
<b>FAM161A</b>	Retinitis Pigmentosa 28
<b>FANCA</b>	Fanconi Anemia, Group A
<b>FANCC</b>	Fanconi Anemia, Group C
<b>FANCG</b>	Fanconi Anemia, Group G
<b>FH</b>	Fumarase Deficiency
<b>FKRP</b>	Limb-Girdle Muscular Dystrophy, Type 2I
<b>FKTN</b>	Walker-Warburg Syndrome and Other FKTN-Related Dystrophies
<b>FMR1</b>	Fragile X Syndrome
<b>G6PC</b>	Glycogen Storage Disease, Type Ia
<b>GAA</b>	Glycogen Storage Disease, Type II
<b>GALC</b>	Krabbe Disease
<b>GALK1</b>	Galactokinase Deficiency
<b>GALT</b>	Galactosemia
<b>GAMT</b>	Cerebral Creatine Deficiency Syndrome 2
<b>GBA</b>	Gaucher Disease
<b>GBE1</b>	Glycogen Storage Disease, Type IV / Adult Polyglucosan Body Disease
<b>GCDH</b>	Glutaric Acidemia, Type I
<b>GFM1</b>	Combined Oxidative Phosphorylation Deficiency 1
<b>GJB1</b>	Charcot-Marie-Tooth Disease, X-Linked
<b>GJB2†</b>	Non-Syndromic Hearing Loss (GJB2-Related)
<b>GLA</b>	Fabry Disease
<b>GLB1</b>	Mucopolysaccharidosis Type IVb / GM1 Gangliosidosis
<b>GLDC</b>	Glycine Encephalopathy (GLDC-Related)
<b>GLE1</b>	Lethal Congenital Contracture Syndrome 1 / Lethal Arthrogryposis with Anterior Horn Cell Disease
<b>GNE</b>	Inclusion Body Myopathy 2
<b>GNPTAB</b>	Mucopolipidosis II / IIIA
<b>GNPTG</b>	Mucopolipidosis III Gamma
<b>GNS</b>	Mucopolysaccharidosis Type IIID
<b>GP1BA</b>	Bernard-Soulier Syndrome, Type A1
<b>GP9</b>	Bernard-Soulier Syndrome, Type C
<b>GPR56</b>	Bilateral Frontoparietal Polymicrogyria
<b>GRHPR</b>	Primary Hyperoxaluria, Type 2
<b>HADHA</b>	Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency
<b>HAX1</b>	Congenital Neutropenia (HAX1-Related)
<b>HBA1/HBA2</b>	Alpha-Thalassemia
<b>HBB</b>	Beta-Globin-Related Hemoglobinopathies
<b>HEXA</b>	Tay-Sachs Disease
<b>HEXB</b>	Sandhoff Disease
<b>HFE2</b>	Hemochromatosis, Type 2A
<b>HGSNAT</b>	Mucopolysaccharidosis Type IIIC
<b>HLCS</b>	Holocarboxylase Synthetase Deficiency
<b>HMGCL</b>	HMG-CoA Lyase Deficiency
<b>HOGA1</b>	Primary Hyperoxaluria, Type 3
<b>HPS1</b>	Hermansky-Pudlak Syndrome, Type 1
<b>HPS3</b>	Hermansky-Pudlak Syndrome, Type 3
<b>HSD17B4</b>	D-Bifunctional Protein Deficiency
<b>HSD3B2</b>	3-Beta-Hydroxysteroid Dehydrogenase Type II Deficiency
<b>HYAL1</b>	Mucopolysaccharidosis type IX
<b>HYLS1</b>	Hydroletharus Syndrome
<b>IDS</b>	Mucopolysaccharidosis Type II

Gene	Disease
<b>IDUA</b>	Mucopolysaccharidosis Type I
<b>IKBKAP</b>	Familial Dysautonomia
<b>IL2RG</b>	X-Linked Severe Combined Immunodeficiency
<b>IVD</b>	Isovaleric Acidemia
<b>KCNJ11</b>	Familial Hyperinsulinism (KCNJ11-Related)
<b>LAMA3</b>	Junctional Epidermolysis Bullosa (LAMA3-Related)
<b>LAMB3</b>	Junctional Epidermolysis Bullosa (LAMB3-Related)
<b>LAMC2</b>	Junctional Epidermolysis Bullosa (LAMC2-Related)
<b>LCA5</b>	Leber Congenital Amaurosis 5
<b>LDLR</b>	Familial Hypercholesterolemia
<b>LDLRAP1</b>	Familial Autosomal Recessive Hypercholesterolemia
<b>LHX3</b>	Combined Pituitary Hormone Deficiency 3
<b>LIFR</b>	Stuve-Wiedemann Syndrome
<b>LIPA</b>	Wolman Disease / Cholesteryl Ester Storage Disease
<b>LOXHD1</b>	Deafness, Autosomal Recessive 77
<b>LPL</b>	Lipoprotein Lipase Deficiency
<b>LRPPRC</b>	Leigh Syndrome, French-Canadian Type
<b>MAN2B1</b>	Alpha-Mannosidosis
<b>MCCC1</b>	3-Methylcrotonyl-CoA Carboxylase Deficiency (MCCC1-Related)
<b>MCCC2</b>	3-Methylcrotonyl-CoA Carboxylase Deficiency (MCCC2-Related)
<b>MCOLN1</b>	Mucopolipidosis IV
<b>MED17</b>	Infantile Cerebral and Cerebellar Atrophy
<b>MEFV</b>	Familial Mediterranean Fever
<b>MESP2</b>	Spondylothoracic Dysostosis
<b>MFSD8</b>	Neuronal Ceroid-Lipofuscinosis (MFSD8-Related)
<b>MKS1</b>	Meckel syndrome 1 / Bardet-Biedl Syndrome 13
<b>MLC1</b>	Megalencephalic Leukoencephalopathy with Subcortical Cysts
<b>MMAA</b>	Methylmalonic Acidemia (MMAA-Related)
<b>MMAB</b>	Methylmalonic Acidemia (MMAB-Related)
<b>MMACHC</b>	Methylmalonic Aciduria and Homocystinuria, Cobalamin C Type
<b>MMADHC</b>	Methylmalonic Aciduria and Homocystinuria, Cobalamin D Type
<b>MPI</b>	Congenital Disorder of Glycosylation, Type Ib
<b>MPL</b>	Congenital Amegakaryocytic Thrombocytopenia
<b>MPV17</b>	Mitochondrial DNA Depletion Syndrome 6 / Navajo Neurohepatopathy
<b>MTHFR</b>	Homocystinuria due to MTHFR Deficiency
<b>MTM1</b>	Myotubular Myopathy 1
<b>MTRR</b>	Homocystinuria, cbIE Type
<b>MTTP</b>	Abetalipoproteinemia
<b>MUT</b>	Methylmalonic Acidemia (MUT-Related)
<b>MYO7A</b>	Usher Syndrome, Type IB
<b>NAGLU</b>	Mucopolysaccharidosis Type IIIB
<b>NAGS</b>	N-Acetylglutamate Synthase Deficiency
<b>NBN</b>	Nijmegen Breakage Syndrome
<b>NDRG1</b>	Charcot-Marie-Tooth Disease, Type 4D
<b>NDUFAF5</b>	Mitochondrial Complex I Deficiency (NDUFAF5-Related)
<b>NDUFS6</b>	Mitochondrial Complex I Deficiency (NDUFS6-Related)
<b>NEB</b>	Nemaline Myopathy 2
<b>NPC1</b>	Niemann-Pick Disease, Type C (NPC1-Related)
<b>NPC2</b>	Niemann-Pick Disease, Type C (NPC2-Related)
<b>NPHS1</b>	Nephrotic Syndrome (NPHS1-Related) / Congenital Finnish Nephrosis

**Patient:** 5842 Donor

**DOB:** [REDACTED]

**Lab #:** [REDACTED]

Gene	Disease
<b>NPHS2</b>	Nephrotic Syndrome (NPHS2-Related) / Steroid-Resistant Nephrotic Syndrome
<b>NR2E3</b>	Enhanced S-Cone Syndrome
<b>NTRK1</b>	Congenital Insensitivity to Pain with Anhidrosis
<b>OAT</b>	Ornithine Aminotransferase Deficiency
<b>OPA3</b>	3-Methylglutaconic Aciduria, Type III
<b>OTC</b>	Ornithine Transcarbonylase Deficiency
<b>PAH</b>	Phenylalanine Hydroxylase Deficiency
<b>PCCA</b>	Propionic Acidemia (PCCA-Related)
<b>PCCB</b>	Propionic Acidemia (PCCB-Related)
<b>PCDH15</b>	Usher Syndrome, Type IF
<b>PDHA1</b>	Pyruvate Dehydrogenase E1-Alpha Deficiency
<b>PDHB</b>	Pyruvate Dehydrogenase E1-Beta Deficiency
<b>PEX1</b>	Zellweger Syndrome Spectrum (PEX1-Related)
<b>PEX10</b>	Zellweger Syndrome Spectrum (PEX10-Related)
<b>PEX2</b>	Zellweger Syndrome Spectrum (PEX2-Related)
<b>PEX6</b>	Zellweger Syndrome Spectrum (PEX6-Related)
<b>PEX7</b>	Rhizomelic Chondrodysplasia Punctata, Type 1
<b>PFKM</b>	Glycogen Storage Disease, Type VII
<b>PHGDH</b>	3-Phosphoglycerate Dehydrogenase Deficiency
<b>PKHD1</b>	Polycystic Kidney Disease, Autosomal Recessive
<b>PMM2</b>	Congenital Disorder of Glycosylation, Type Ia
<b>POMGNT1</b>	Muscle-Eye-Brain Disease and Other POMGNT1-Related Congenital Muscular Dystrophy-Dystroglycanopathies
<b>PPT1</b>	Neuronal Ceroid-Lipofuscinosis (PPT1-Related)
<b>PROP1</b>	Combined Pituitary Hormone Deficiency 2
<b>PRPS1</b>	Charcot-Marie-Tooth Disease, Type 5 / Arts syndrome
<b>PSAP</b>	Combined SAP Deficiency
<b>PTS</b>	6-Pyruvoyl-Tetrahydropterin Synthase Deficiency
<b>PUS1</b>	Mitochondrial Myopathy and Sideroblastic Anemia 1
<b>PYGM</b>	Glycogen Storage Disease, Type V
<b>RAB23</b>	Carpenter Syndrome
<b>RAG2</b>	Omenn Syndrome (RAG2-Related)
<b>RAPSN</b>	Congenital Myasthenic Syndrome (RAPSN-Related)
<b>RARS2</b>	Pontocerebellar Hypoplasia, Type 6
<b>RDH12</b>	Leber Congenital Amaurosis 13
<b>RMRP</b>	Cartilage-Hair Hypoplasia
<b>RPE65</b>	Leber Congenital Amaurosis 2 / Retinitis pigmentosa 20
<b>RPGRIP1L</b>	Joubert Syndrome 7 / Meckel Syndrome 5 / COACH Syndrome
<b>RS1</b>	X-Linked Juvenile Retinoschisis
<b>RTEL1</b>	Dyskeratosis Congenita (RTEL1-Related)
<b>SACS</b>	Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay
<b>SAMHD1</b>	Aicardi-Goutières Syndrome (SAMHD1-Related)
<b>SEPSECS</b>	Progressive Cerebello-Cerebral Atrophy

Gene	Disease
<b>SGCA</b>	Limb-Girdle Muscular Dystrophy, Type 2D
<b>SGCB</b>	Limb-Girdle Muscular Dystrophy, Type 2E
<b>SGCG</b>	Limb-Girdle Muscular Dystrophy, Type 2C
<b>SGSH</b>	Mucopolysaccharidosis Type IIIA
<b>SLC12A3</b>	Gitelman Syndrome
<b>SLC12A6</b>	Andermann Syndrome
<b>SLC17A5</b>	Salla Disease
<b>SLC22A5</b>	Primary Carnitine Deficiency
<b>SLC25A13</b>	Citrin Deficiency
<b>SLC25A15</b>	Hyperornithinemia-Hyperammonemia-Homocitrullinuria Syndrome
<b>SLC26A2</b>	Sulfate Transporter-Related Osteochondrodysplasia
<b>SLC26A4</b>	Pendred Syndrome
<b>SLC35A3</b>	Arthrogyposis, Mental Retardation, and Seizures
<b>SLC37A4</b>	Glycogen Storage Disease, Type Ib
<b>SLC39A4</b>	Acrodermatitis Enteropathica
<b>SLC4A11</b>	Corneal Dystrophy and Perceptive Deafness
<b>SLC6A8</b>	Cerebral Creatine Deficiency Syndrome 1
<b>SLC7A7</b>	Lysinuric Protein Intolerance
<b>SMARCAL1</b>	Schimke Immunoosseous Dysplasia
<b>SMN1</b>	Spinal Muscular Atrophy
<b>SMPD1</b>	Niemann-Pick Disease (SMPD1-Related)
<b>STAR</b>	Lipoid Adrenal Hyperplasia
<b>SUMF1</b>	Multiple Sulfatase Deficiency
<b>TCIRG1</b>	Osteopetrosis 1
<b>TECPR2</b>	Hereditary Spastic Paraparesis 49
<b>TFR2</b>	Hemochromatosis, Type 3
<b>TGM1</b>	Lamellar Ichthyosis, Type 1
<b>TH</b>	Segawa Syndrome
<b>TMEM216</b>	Joubert Syndrome 2
<b>TPP1</b>	Neuronal Ceroid-Lipofuscinosis (TPP1-Related)
<b>TRMU</b>	Acute Infantile Liver Failure
<b>TSM</b>	Combined Oxidative Phosphorylation Deficiency 3
<b>TTPA</b>	Ataxia With Isolated Vitamin E Deficiency
<b>TYMP</b>	Myoneurogastrointestinal Encephalopathy
<b>USH1C</b>	Usher Syndrome, Type IC
<b>USH2A</b>	Usher Syndrome, Type IIA
<b>VPS13A</b>	Choreoacanthocytosis
<b>VPS13B</b>	Cohen Syndrome
<b>VPS45</b>	Congenital Neutropenia (VPS45-Related)
<b>VRK1</b>	Pontocerebellar Hypoplasia, Type 1A
<b>VSX2</b>	Microphthalmia / Anophthalmia
<b>WNT10A</b>	Odonto-Onycho-Dermal Dysplasia / Schopf-Schulz-Passarge Syndrome

† Please note that GJB2 testing includes testing for the two upstream deletions, del(GJB6-D13S1830) and del(GJB6-D13S1854) (PMID: 11807148 and 15994881)

PATIENT INFORMATION	SPECIMEN INFORMATION	PROVIDER INFORMATION
5842, Donor ID#: 5842 DOB: [REDACTED] Sex: Male	Type: Whole Blood Collected: September 22, 2020 Received: September 24, 2020 PG ID: [REDACTED]	Harvey Stern, MD, PhD Suzanne Seitz, MS Fairfax Cyrobank

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

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

**RESULTS AND INTERPRETATIONS:** In this patient, for the *DOCK8* gene, we found no sequence variants that are likely to be a primary cause of disease.

This patient is 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 page(s).

**NOTES:** 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(S) ANALYZED:** *DOCK8*

**SUMMARY STATISTICS:**

Pipeline	Version	Average NGS Coverage	Fraction Bases Covered with NGS
Infinity_Pipeline	1.5.1	105x	99.7%

Minimum NGS coverage is ≥20x for all exons and +/-10bp of flanking DNA, and ≥10x from 11-20bp of flanking DNA.

Electronically signed on October 15, 2020 by:  
Megan Piazza, PhD, FACMG  
Clinical Molecular Geneticist

Electronically signed and reported on October 16, 2020 by:  
Luke Drury, PhD, HCLD(ABB)  
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.

PATIENT INFORMATION	SPECIMEN INFORMATION	PROVIDER INFORMATION
5842, Donor ID#: 5842 DOB: [REDACTED] Sex: Male	Type: DNA [REDACTED] [REDACTED] Requested: February 03, 2021 PG ID: [REDACTED]	Harvey Stern, MD, PhD Suzanne Seitz, MS Fairfax Cyrobank

**MOLECULAR GENETICS REPORT:**  
*SPG7* Gene Sequencing with CNV Detection

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

**RESULTS AND INTERPRETATIONS:** In this patient, for the *SPG7* 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:** *SPG7*

**SUMMARY STATISTICS:**

Pipeline	Version	Average NGS Coverage	Fraction Bases Covered with NGS
Infinity_Pipeline	1.7.3	160x	91.5%

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

Electronically signed on February 16, 2021 by:  
Diane Allingham-Hawkins, PhD, FCCMG, FACMG  
Clinical Molecular Geneticist

Electronically signed and reported on February 17, 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.