



## Donor 4767

### Genetic Testing Summary

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

Last Updated: 07/19/23

Donor Reported Ancestry: French, Dutch

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 genotyping of 99 mutations in the CFTR gene	1/300
Spinal Muscular Atrophy	Negative for deletion in the exon 7	1/610
Hb Beta Chain related Hemoglobinopathies	Negative for 28 mutations in the HBB gene	1/290
<b>Special Testing</b>		
Genes: BBS1, BBS10, ACADM	Negative by genotyping (see attached)	

\*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.



RESULT RECIPIENT  
FAIRFAX CRYOBANK -

MALE  
DONOR 4767

FEMALE  
N/A

DOB: [REDACTED]  
Ethnicity: Northern European  
Sample Type: EDTA Blood  
Date of Collection: 10/10/2014  
Date Received: 10/13/2014  
Date Tested: 10/16/2014  
Barcode: [REDACTED]  
Indication: Egg or Sperm Donor

Report Date: 10/16/2014

## Family Prep Screen

**NEGATIVE**

### ABOUT THIS TEST

The Counsyl Family Prep Screen (version 1.0) tests known mutations to help you learn about your chance to have a child with a genetic disease.

### PANEL DETAILS

Fairfax Cryobank Fundamental Panel (3 diseases tested)

### VERSION

DONOR 4767 (Family Prep Screen 1.0)

### RESULTS SUMMARY

#### **NEGATIVE**

No known or potential disease-causing mutations were detected.

### CLINICAL NOTES

- None

### NEXT STEPS

- If necessary, patients can discuss residual risks with their physician or a genetic counselor.
- To schedule a complimentary appointment to speak with a clinical expert about these results, please visit [counsyl.com/my/consults/](http://counsyl.com/my/consults/).



RESULT RECIPIENT  
FAIRFAX CRYOBANK  
Report Date: 10/16/2014

MALE  
DONOR 4767  
DOB: [REDACTED]  
Ethnicity: Northern European  
Barcode: [REDACTED]  
FEMALE  
N/A

## Methods and Limitations

### DONOR 4767 [Family Prep Screen 1.0]: targeted genotyping and copy number analysis.

**Targeted genotyping:** Targeted DNA mutation analysis is used to simultaneously determine the genotype of 127 variants associated with 2 diseases. The test is not validated for detection of homozygous mutations, and although rare, asymptomatic individuals affected by the disease may not be genotyped accurately.

**Copy number analysis:** Targeted copy number analysis is used to determine the copy number of exon 7 of the SMN1 gene relative to other genes. Other mutations may interfere with this analysis. Some individuals with two copies of SMN1 are carriers with two SMN1 genes on one chromosome and a SMN1 deletion on the other chromosome. In addition, a small percentage of SMA cases are caused by nondeletion mutations in the SMN1 gene. Thus, a test result of two SMN1 copies significantly reduces the risk of being a carrier; however, there is still a residual risk of being a carrier and subsequently a small risk of future affected offspring for individuals with two or more SMN1 gene copies. Some SMA cases arise as the result of de novo mutation events which will not be detected by carrier testing.

**Limitations:** In an unknown number of cases, nearby genetic variants may interfere with mutation detection. Other possible sources of diagnostic error include sample mix-up, trace contamination, bone marrow transplantation, blood transfusions and technical errors. If more than one variant is detected in a gene, additional studies may be necessary to determine if those variants lie on the same chromosome or different chromosomes. The Counsyl test does not fully address all inherited forms of intellectual disability, birth defects and genetic disease. A family history of any of these conditions may warrant additional evaluation. Furthermore, not all mutations will be identified in the genes analyzed and additional testing may be beneficial for some patients. For example, individuals of African, Southeast Asian, and Mediterranean ancestry are at increased risk for being carriers for hemoglobinopathies, which can be identified by CBC and hemoglobin electrophoresis or HPLC (*ACOG Practice Bulletin No. 78, Obstet. Gynecol. 2007;109:229-37*).

This test was developed and its performance characteristics determined by Counsyl, Inc. It has not been cleared or approved by the US Food and Drug Administration (FDA). The FDA does not require this test to go through premarket 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 testing. These results are adjunctive to the ordering physician's workup. CLIA Number: #05D1102604.

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#### LAB DIRECTORS

*Hyunseok Kang*

H. Peter Kang, MD, MS, FCAP



RES RECIPIENT  
FAIRFAX CRYOBANK -  
Report Date: 10/16/2014

MALE ( FEMALE  
DONOR 4767 N/A  
DOB:   
Ethnicity: Northern European  
Barcode:

## Diseases Tested

### Autosomal Recessive Disorders

#### TARGETED GENOTYPING

**Cystic Fibrosis** - Gene: CFTR. Variants (99): G85E, R117H, R334W, R347P, A455E, G542\*, G551D, R553\*, R560T, R1162\*, W1282\*, N1303K, c.1521\_1523delCTT, c.1519\_1521delATC, c.2052delA, c.3528delC, c.489+1G>T, c.579+1G>T, c.1585-1G>A, c.1766+1G>A, 2789+5G>A, c.2988+1G>A, 3849+10kbC>T, E60\*, R75\*, E92\*, Y122\*, G178R, R347H, Q493\*, V520F, S549N, P574H, M1101K, D1152H, c.2012delT, c.262\_263delTT, c.313delA, c.948delT, c.3744delA, c.3773dupT, c.1680-1G>A, 3272-26A>G, c.2051\_2052delAAinsG, S549R, R117C, L206W, G330\*, T338I, R352Q, S364P, G480C, C524\*, S549R, Q552\*, A559T, G622D, R709\*, K710\*, R764\*, Q890\*, R1066C, W1089\*, Y1092X, R1158\*, S1196\*, W1204\*, Q1238\*, S1251N, S1255\*, c.3067\_3072del6, c.442delA, c.531delT, c.803delA, c.805\_806delAT,

c.1545\_1546delTA, M607\_Q643del, c.1911delG, c.1923\_1931del9ins1, c.1976delA, c.3039delC, c.3536\_3539delCCAA, c.3659delC, c.1155\_1156dupTA, c.2052dupA, c.2175dupA, c.2738insG, 296+12T>C, c.273+1G>A, 405+3A>C, c.274-1G>A, 711+5G>A, c.580-1G>T, c.1766+1G>T, 1898+5G>T, Q996, c.325\_327delTATinsG, 3849+4A>G, c.1075\_1079del5ins5. IVS8-5T allele analysis is only reported in the presence of the R117H mutation. Detection rate: Northern European 91%.

**Hb Beta Chain-Related Hemoglobinopathy (Including Beta Thalassemia and Sickle Cell Disease)** - Gene: HBB. Variants (28): E7V, K18\*, Q40\*, c.126\_129delCTTT, c.27dupG, IVS-II-65A, IVS-II-745, c.315+1G>A, IVS-I-6, IVS-I-110, IVS-I-5, c.92+1G>A, -88C>T, -28A>G, -29A>G, c.25\_26delAA, c.217dupA, c.316-2A>C, c.316-2A>G, G25, -87C>G, E7K, W16\*, c.51delC, c.20delA, E27K, E122Q, E122K. Detection rate: Northern European 83%.

#### COPY NUMBER ANALYSIS

**Spinal Muscular Atrophy** - Gene: SMN1. Variant (1): SMN1 copy number. Detection rate: Northern European 95%.



RESUL RECIPIENT  
FAIRFAX CRYOBANK -  
Report Date: 10/16/2014

MALE  
DONOR 4767  
DOB:  
Ethnicity: Northern European  
Barcode:

FEMALE  
N/A

## Risk Calculations

Below are the risk calculations for all diseases tested. Since negative results do not completely rule out the possibility of being a carrier, the **residual risk** represents the patient's post-test likelihood of being a carrier and the **reproductive risk** represents the likelihood the patient's future children could inherit each disease. These risks are inherent to all carrier screening tests, may vary by ethnicity, are predicated on a negative family history and are present even after a negative test result. Inaccurate reporting of ethnicity may cause errors in risk calculation.

Disease	DONOR 4767 Residual Risk	Reproductive Risk
Cystic Fibrosis	1 in 300	1 in 33,000
Hb Beta Chain-Related Hemoglobinopathy (Including Beta Thalassemia and Sickle Cell Disease)	1 in 290	1 in 58,000
Spinal Muscular Atrophy	SMN1: 2 copies 1 in 610	1 in 84,000



GENETICS & IVF  
Institute

Cytogenetic Report

Client Fairfax Cryobank - [REDACTED]

Address [REDACTED]

Reporting Phone # [REDACTED]

Patient name/Donor Alias Donor # 4767

Patient DOB N/A

Donor # 4767 [REDACTED]

Specimen type Peripheral Blood

Collection Date 10/10/2014

Accession # [REDACTED]

Date Received 10/11/2014

RESULTS

CYTOGENETIC ANALYSIS

FISH

Cells counted 21

Type of banding GTG

Probe(s) N/A

Cells analyzed 5

Band resolution 550

Nuclei scored N/A

Cells karyotyped 2

Modal chromosome # 46

KARYOTYPE 46,XY

INTERPRETATION

Normal male karyotype

No clonal numerical or structural abnormalities were identified. This normal cytogenetic result does not exclude the possibility of the presence of subtle rearrangements beyond the technical limits of detection with this test.

Comments



Wayne S. Stanley, M.D., FACMG  
Clinical Cytogeneticist

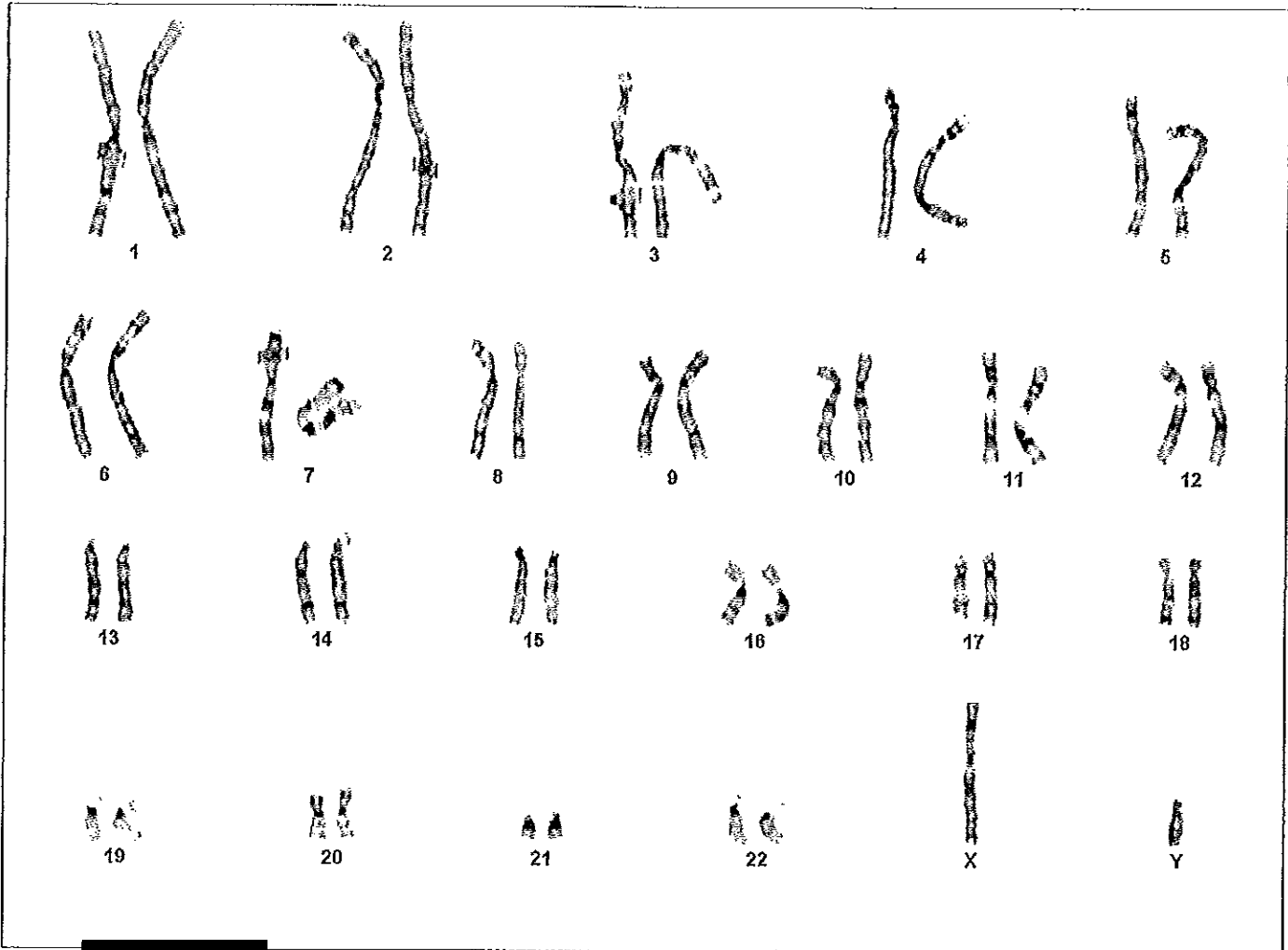
10/20/14  
Date

Genetics and IVF Preimplantation Genetics Laboratory

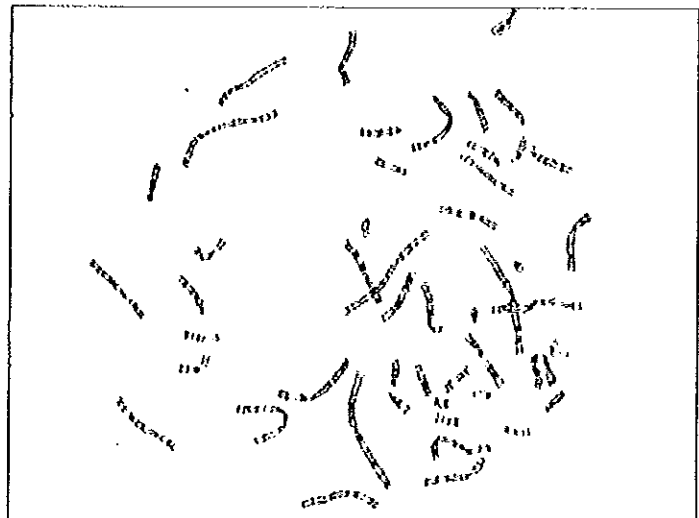
Patient name: DONOR # 4767

Case name: [REDACTED]

46,XY



Case: [REDACTED] Slide: B4 Cell: 14





Patient Information	Specimen Information	Client Information
<b>ID4767, DONOR</b>  DOB: [REDACTED] AGE: [REDACTED] Gender: M Fasting: U Phone: NG Patient ID: 4767-[REDACTED]	Specimen: [REDACTED] Requisition: [REDACTED]  Collected: 10/10/2014 / 07:30 CDT Received: 10/11/2014 / 06:39 CDT Faxed: 10/14/2014 / 17:01 CDT	Client #: 41550 AUS0000 STERN FAIRFAX CRYOBANK [REDACTED]

Test Name	In Range	Out Of Range	Reference Range	Lab
HEMOGLOBINOPATHY EVALUATION				
RED BLOOD CELL COUNT	5.49		4.20-5.80 Million/uL	IG
HEMOGLOBIN	16.4		13.2-17.1 g/dL	
HEMATOCRIT	49.6		38.5-50.0 %	
MCV	90.4		80.0-100.0 fL	
MCH	30.0		27.0-33.0 pg	
RDW	12.9		11.0-15.0 %	
HEMOGLOBIN A	97.5		>96.0 %	IG
HEMOGLOBIN F	<1.0		<2.0 %	
HEMOGLOBIN A2 (QUANT)	2.5		1.8-3.5 %	
INTERPRETATION				
Normal phenotype.				

## PERFORMING SITE:

IG QUEST DIAGNOSTICS-IRVING, 4770 REGENT BLVD., IRVING, TX 75063 Laboratory Director: ELISABETH S BROCKIE, DO, CLIA: 45D0697943



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Ordering Practice:

Practice Code: 1172  
Fairfax Cryobank - [REDACTED]  
[REDACTED]  
[REDACTED]  
Physician: [REDACTED]  
Report Generated: 2016-01-26

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Donor 4767

DOB: [REDACTED]  
Gender: Male  
Ethnicity: European  
Procedure ID: 41232  
Kit Barcode: [REDACTED]  
Method: Genotyping  
Specimen: Blood, #43043  
Specimen Collection: 2016-01-15  
Specimen Received: 2016-01-19  
Specimen Analyzed: 2016-01-26

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Partner Not Tested

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SUMMARY OF RESULTS

## NO MUTATIONS IDENTIFIED


Donor 4767 was not identified to carry any of the mutations tested.

All mutations analyzed were not detected, reducing but not eliminating your chance to be a carrier for the associated genetic diseases. A list of all the diseases and mutations you were screened for is included later in this report. The test does not screen for every possible genetic disease.

For disease information, please visit [www.recombine.com/diseases](http://www.recombine.com/diseases). To speak with a Genetic Counselor, call [855.OUR.GENES](tel:855.OUR.GENES).

♂ Male

Panel: Custom Panel , Diseases Tested: 2, Mutations Tested: 6, Genes Tested: 2, Null Calls: 0

Assay performed by   
Reprogenetics

CLIA ID: 31D1054821  
3 Regent Street, Livingston, NJ 07039  
Lab Technician Bo Chu

Recombine CLIA # 31D2100763  
Reviewed by Pere Colls, PhD, HCLD, Lab Director

*This test was developed and its performance determined by Recombine Inc. and it has not been cleared or approved by the U.S. Food and Drug Administration.*

## Methods and Limitations

**Genotyping:** Genotyping is performed using the Illumina Infinium Custom HD Genotyping assay to identify mutations in >200 genes. The assay is not validated for homozygous mutations, and it is possible that individuals affected with disease may not be accurately genotyped.

**Limitations:** In some cases, genetic variations other than that which is being assayed may interfere with mutation detection, resulting in false-negative or false-positive results. Additional sources of error include, but are not limited to: sample contamination, sample mix-up, bone marrow transplantation, blood transfusions, and technical errors.

The test does not test for all forms of genetic disease, birth defects, and intellectual disability. All results should be interpreted in the context of family history; additional evaluation may be indicated based on a history of these conditions. Additional testing may be necessary to determine mutation phase in individuals identified to carry more than one mutation in the same gene. All mutations included within the genes assayed may not be detected, and additional testing may be appropriate for some individuals.

## Diseases &amp; Mutations Assayed

● High Impact
 ● Treatment Benefits
 ● X-Linked
 ● Moderate Impact

H	T	X	M	Disease	#	Mutations
<span style="color: red;">●</span>	<span style="color: gray;">○</span>	<span style="color: gray;">○</span>	<span style="color: gray;">○</span>	Bardet-Biedl Syndrome: BBS1 Related	3	♂ Genotyping   c.851delA, c.G1645T (p.E549X), c.T1169G (p.M390R)
<span style="color: red;">●</span>	<span style="color: gray;">○</span>	<span style="color: gray;">○</span>	<span style="color: gray;">○</span>	Bardet-Biedl Syndrome: BBS10 Related	3	♂ Genotyping   c.271_273ins1bp (p.C91fsX95), c.G101C (p.R34P), c.T931G (p.S311A)

Patient	Sample	Referring Doctor
<b>Patient Name:</b> Donor 4767 <b>Date of Birth:</b> [REDACTED] <b>Reference #:</b> [REDACTED] <b>Indication:</b> Carrier Testing <b>Test Type:</b> Custom Carrier Screen (ECS)	<b>Specimen Type:</b> Blood <b>Lab #:</b> [REDACTED] <b>Date Collected:</b> 3/12/2019 <b>Date Received:</b> 3/13/2019 <b>Final Report:</b> 3/27/2019	<b>Fairfax Cryobank, Inc.</b> [REDACTED] [REDACTED] [REDACTED] [REDACTED]

## Results

**Negative: No clinically significant variant(s) detected**

**Gene(s) analyzed:** *ACADM*

### Recommendations:

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

### Interpretation:

Screening for the presence of pathogenic variants in the *ACADM* gene which is associated with medium chain acyl-CoA dehydrogenase deficiency was performed by next generation sequencing and possibly genotyping for select variants on DNA extracted from this patient's sample. No clinically significant variants were detected during this analysis.

Please note that negative results reduce but do not eliminate the possibility that this individual is a carrier for the disorder(s) tested. Please see table of residual risks 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.

### Comments:

This carrier screening test masks likely benign variants and variants of uncertain significance (VUS) if there are any. Only known pathogenic variants or likely pathogenic variants which are expected to result in deleterious effects on protein function are reported. If reporting of likely benign variants and VUS is desired in this patient, please contact the laboratory (tel. 212-241-2537) to request an amended report.

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.

**Patient:** Donor 4767

**DOB:** [REDACTED]

**Lab #:** [REDACTED]

## Table of Residual Risks by Ethnicity

Please note: This table displays residual risks after a negative result for each of the genes and corresponding disorders. **If a patient is reported to be a carrier of a disease, their residual risk is 1 and this table does not apply for that disease.**

Disease (Inheritance)	Gene	Ethnicity	Carrier Frequency	Detection Rate	Residual Risk	Analytical Detection Rate
Medium Chain Acyl-CoA Dehydrogenase Deficiency (AR) NM_000016.5	ACADM	African	1 in 172	77%	1 in 740	99%
		Ashkenazi Jewish	1 in 133	99%	1 in 13,200	
		East Asian	1 in 255	35%	1 in 390	
		Finnish	1 in 383	96%	1 in 8,700	
		Caucasian	1 in 56	95%	1 in 1,100	
		Latino	1 in 92	63%	1 in 250	
		South Asian	1 in 142	51%	1 in 290	
		Worldwide	1 in 82	85%	1 in 560	

AR: Autosomal Recessive

This case has been reviewed and electronically signed by Anastasia Larmore, PhD, Assistant Director

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

**Patient:** Donor 4767

**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. AmpliX<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:** Donor 4767

**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:** Donor 4767

**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.

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Additional disease-specific references available upon request.



**Patient:** Donor 4767

**DOB:** [REDACTED]

**Lab #:** [REDACTED]