



## Donor 5447

### Genetic Testing Summary

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

Last Updated: 06/10/19

Donor Reported Ancestry: German, Polish, Italian

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	1/1250
Spinal Muscular Atrophy (SMA) carrier screening	Negative for deletions of exon 7 in the SMN1 gene	1/632
Standard testing attached- 22 diseases by gene sequencing	Negative for genes sequenced	
<b>Special Testing</b>		
21 Hydroxylase Deficiency Congenital Adrenal Hypoplasia	Negative by gene sequencing in the CYP21A2 gene	1/201
Niemann Pick Disease Type B	Negative by gene sequencing in the SMPD1 gene	1/33,333
Smith Lemli Opitz Syndrome	Negative by gene sequencing in the DHCR7 gene	1/750

Gaucher Disease	Negative by gene sequencing in the GBA gene	1/1300
Methylmalonic Aciduria	Negative by gene sequencing in the MMAA gene	1/1200
Familial Mediterranean Fever	Negative by gene sequencing in the MEFV gene	1/1200

\*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.

## Ordering Practice:

Practice Code: [REDACTED]

Fairfax Cryobank - [REDACTED]

[REDACTED]

Physician: [REDACTED]

Report Generated: 2017-08-15

Report Updated: 2017-08-15

5447 [REDACTED]

DOB: [REDACTED]

Gender: Male

Ethnicity: European

Procedure ID: 100775

Kit Barcode: [REDACTED]

Specimen: Blood, #102156

Specimen Collection: 2017-07-31

Specimen Received: 2017-08-01

Specimen Analyzed: 2017-08-15

Partner Not Tested

## TEST INFORMATION

Test: CarrierMap<sup>SEQ</sup> (Genotyping & Sequencing)

Panel: Fairfax Cryobank Panel V2- Sequencing

Diseases Tested: 22

Genes Tested: 22


Genes Sequenced: 18

## SUMMARY OF RESULTS: NO MUTATIONS IDENTIFIED

5447 [REDACTED] was not identified to carry any pathogenic mutations in the gene(s) tested.

No pathogenic mutations were identified in the genes tested, reducing but not eliminating the chance to be a carrier for the associated genetic diseases. CarrierMap assesses carrier status for genetic disease via molecular methods including targeted mutation analysis and/ or next-generation sequencing; other methodologies such as CBC and hemoglobin electrophoresis for hemoglobinopathies and enzyme analysis for Tay-Sachs disease may further refine risks for these conditions. Results should be interpreted in the context of clinical findings, family history, and/or other testing. A list of all the diseases and mutations screened for is included at the end of the report. This test does not screen for every possible genetic disease.

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

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

## ADDITIONAL RESULTS: NO INCREASED REPRODUCTIVE RISK

The following results are not associated with an increased reproductive risk.

Disease (Gene)	5447 [REDACTED]	Partner Not Tested
Spinal Muscular Atrophy: SMN1 Linked (SMN1) *	SMN1 Copy Number: 2 or more copies Method: Genotyping & dPCR	

### \* SMA Risk Information for Individuals with No Family History of SMA

	Detection Rate	Pre-Test Carrier Risk	Post-Test Carrier Risk (2 SMN1 copies)	Post-Test Carrier Risk (3 SMN1 copies)
European	95%	1/35	1/632	1/3,500
Ashkenazi Jewish	90%	1/41	1/350	1/4,000
Asian	93%	1/53	1/628	1/5,000
African American	71%	1/66	1/121	1/3,000
Hispanic	91%	1/117	1/1,061	1/11,000

For other unspecified ethnicities, post-test carrier risk is assumed to be <1%. For individuals with multiple ethnicities, it is recommended to use the most conservative risk estimate.

## Methods and Limitations

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

**Sequencing:** Sequencing is performed using a custom next-generation sequencing (NGS) platform. Only the described exons for each gene listed are sequenced. Variants outside of these regions may not be identified. Some splicing mutations may not be identified. Triplet repeat expansions, intronic mutations, and large insertions and deletions may not be detected. All identified variants are curated, and determination of the likelihood of their pathogenicity is made based on examining allele frequency, segregation studies, predicted effect, functional studies, case/control studies, and other analyses. All variants identified via sequencing that are reported to cause disease in the primary scientific literature will be reported. Variants considered to be benign and variants of unknown significance (VUS) are NOT reported. In the sequencing process, interval drop-out may occur, leading to intervals of insufficient coverage. Intervals of insufficient coverage will be reported if they occur.

**Spinal Muscular Atrophy:** Carrier status for SMA is assessed via copy number analysis by dPCR and via genotyping. Some individuals with a normal number of SMN1 copies (2 copies) may carry both copies of the gene on the same allele/chromosome; this analysis is not able to detect these individuals. Thus, a normal SMN1 result significantly reduces but does not eliminate the risk of being a carrier. Additionally, SMA may be caused by non-deletion mutations in the SMN1 gene; CarrierMap tests for some, but not all, of these mutations. Some SMA cases arise as the result of de novo mutation events which will not be detected by carrier testing.

**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.

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 (FDA). The FDA has determined that such clearance or approval is not necessary.

## Diseases & Mutations Assayed

**Alpha Thalassemia (HBA1, HBA2):** Mutations (9): ♂ Genotyping | SEA deletion, c.207C>A (p.N69K), c.223G>C (p.D75H), c.2T>C (p.M1T), c.207C>G (p.N69K), c.340\_351delCTCCCGCCGAG (p.L114\_E117del), c.377T>C (p.L126P), c.427T>C (p.X143Qext32), c.\*+94A>G

**Beta Thalassemia (HBB):** Mutations (81): ♂ Genotyping | c.124\_127delTTCT (p.F42Lfs), c.17\_18delCT, c.20delA (p.E7Gfs), c.217insA (p.S73Kfs), c.223+702\_444+342del620insAAGTAGA, c.230delC, c.25\_26delAA, c.315+1G>A, c.315+2T>C, c.316-197C>T, c.316-146T>G, c.315+745C>G, c.316-1G>A, c.316-1G>C, c.316-2A>G, c.316-3C>A, c.316-3C>G, c.4delG (p.V2Cfs), c.51delC (p.K18Rfs), c.93-21G>A, c.92+1G>A, c.92+5G>A, c.92+5G>C, c.92+5G>T, c.92+6T>C, c.93-1G>A, c.93-1G>T, c.-50A>C, c.-78a>g, c.-79A>G, c.-81A>G, c.52A>T (p.K18X), c.-137c>g, c.-138c>t, c.-151C>T, c.118C>T (p.Q40X), c.169G>C (p.G57R), c.295G>A (p.V99M), c.415G>C (p.A139P), c.47G>A (p.W16X), c.48G>A (p.W16X), c.-80t>a, c.2T>C (p.M1T), c.75T>A (p.G25G), c.444+111A>G, c.-29g>a, c.68\_74delAAGTTGG, c.92G>C (p.R31T), c.92+1G>T, c.93-15T>G, c.93-1G>C, c.112delT, c.113G>A (p.W38X), c.114G>A (p.W38X), c.126delC, c.444+113A>G, c.250delG, c.225delC, c.383\_385delAGG (p.Q128\_A129delQAlnsP), c.321\_322insG (p.N109fs), c.316-1G>T, c.316-2A>C, c.287\_288insA (p.L97fs), c.271G>T (p.E91X), c.203\_204delITG (p.V68Afs), c.154delC (p.P52fs), c.135delC (p.F46fs), c.92+2T>A, c.92+2T>C, c.90C>T (p.G30G), c.84\_85insC (p.L29fs), c.59A>G (p.N20S), c.580delT (p.W16Gfs), c.45\_46insG (p.L16fs), c.36delT (p.T13fs), c.2T>G (p.M1R), c.1A>G (p.M1V), c.-137c>t, c.-136C>G, c.-142c>t, c.-140c>t Sequencing | NM\_000518:1-3

**Bloom Syndrome (BLM):** Mutations (25): ♂ Genotyping | c.2207\_2212delATCTGAinsTAGATTC (p.Y736Lfs), c.2407insT, c.557\_559delCAA (p.S186X), c.1284C>A (p.W428X), c.1701G>C (p.Y657R), c.1933C>T (p.Q645X), c.2528C>T (p.T843I), c.2695C>T (p.R899X), c.3107G>T (p.C1036F), c.2923delC (p.Q975K), c.3558+1G>T, c.3875-2A>G, c.2074+2T>A, c.2343\_2344dupGA (p.781EfsX), c.318\_319insT (p.L107fs), c.380delC (p.127Tfs), c.3564delC (p.1188Dfs), c.4008delG (p.1336Rfs), c.947C>G (p.S316X), c.2193+1\_2193+9del9, c.1642C>T (p.Q548X), c.3143delA (p.1048NfsX), c.356\_357delITA (p.C120Hfs), c.4076+1delG, c.3281C>A (p.S1094X) Sequencing | NM\_000057:2-22

**Canavan Disease (ASPA):** Mutations (8): ♂ Genotyping | c.433-2A>G, c.854A>C (p.E285A), c.693C>A (p.Y231X), c.914C>A (p.A305E), c.71A>G (p.E24G), c.654C>A (p.C218X), c.2T>C (p.M1T), c.79G>A (p.G27R) Sequencing | NM\_000049:1-6

**Cystic Fibrosis (CFTR):** Mutations (150): ♂ Genotyping | c.1029delC, 1153\_1154insAT, c.1477delCA, c.1519\_1521delATC (p.507delI), c.1521\_1523delCTT (p.508delF), c.1545\_1546delTA (p.Y515Xfs), c.1585-1G>A, c.164+12T>C, c.1680-886A>G, c.1680-1G>A, c.1766+1G>A, c.1766+1G>T, c.1766+5G>T, c.1818del84, c.1911delG, c.1923delCTCAAAACtinsA, c.1973delGAAATCAATCTCTinsAGAAA, c.2052delA (p.K684fs), c.2052insA (p.Q685fs), c.2051\_2052delAAinsG (p.K684SfsX38), c.2174insA, c.261delTT, c.2657+5G>A, c.273+1G>A, c.273+3A>C, c.274-1G>A, c.2988+1G>A, c.3039delC, c.3140-26A>G, c.325delATinsG, c.3527delC, c.3535delACCA, c.3691delT, c.3717+12191C>T, c.3744delA, c.3773\_3774insT (p.L1258fs), c.442delA, c.489+1G>T, c.531delT, c.579+1G>T, c.579+5G>A (IVS4+5G>A), c.803delA (p.N268fs), c.805\_806delAT (p.I269fs), c.933\_935delCTT (p.311delF), c.946delT, c.1645A>C (p.S549R), c.2128A>T (p.K710X), c.1000C>T (p.R334W), c.1013C>T (p.T338I), c.1364C>A (p.A455E), c.1477C>T (p.Q493X), c.1572C>A (p.C524X), c.1654C>T (p.Q552X), c.1657C>T (p.R553X), c.1721C>A (p.P574H), c.2125C>T (p.R709X), c.223C>T (p.R75X), c.2668C>T (p.Q890X), c.3196C>T (p.R1066C), c.3276C>G (p.Y1092X), c.3472C>T (p.R1158X), c.3484C>T (p.R1162X), c.349C>T (p.R117C), c.3587C>G (p.S1196X), c.3712C>T (p.Q1238X), c.3764C>A (p.S1255X), c.3909C>G (p.N1303K), c.1040G>A (p.R347H), c.1040G>C (p.R347P), c.1438G>T (p.G480C), c.1558G>T (p.V520F), c.1624G>T (p.G542X), c.1646G>A (p.S549N), c.1646G>T (p.S549I), c.1652G>A (p.G551D), c.1675G>A (p.A559T), c.1679G>C (p.R560T), c.178G>T (p.E60X), c.1865G>A (p.G622D), c.254G>A (p.G85E), c.271G>A (p.G91R), c.274G>T (p.E92X), c.3209G>A (p.R1070Q), c.3266G>A (p.W1089X), c.3454G>C (p.D1152H), c.350G>A (p.R117H), c.3611G>A (p.W1204X), c.3752G>A (p.S1251N), c.3846G>A (p.W1282X), c.3848G>T (p.R1283M), c.532G>A (p.G178R), c.988G>T (p.G330X), c.1090T>C (p.S364P), c.3302T>A (p.M1101K), c.617T>G (p.L206W), c.114C>T (p.P51), c.19G>T (p.E7X), c.171G>A (p.W57X), c.313delA (p.I105fs), c.328G>C (p.D110H), c.580-1G>T, c.1055G>A (p.R352Q), c.1075C>A (p.Q359K), c.1079C>A (p.T360K), c.1647T>G (p.S549R), c.1976delA (p.N659fs), c.2290C>T (p.R764X), c.2737\_2738insG (p.Y913X), c.3067\_3072delATAGTG (p.I1023\_V1024delIT), c.3536\_3539delCCAA (p.T1179fs), c.3659delC (p.T1220fs), c.54-5940\_273+10250del21080bp (p.S18fs), c.4364C>G (p.S1455X), c.4003C>T (p.L1335F), c.2538G>A (p.W846X), c.200C>T (p.P67L), c.4426C>T (p.Q1476X), c.1116+1G>A, c.1986\_1989delAACT (p.T663R), c.2089\_2090insA (p.R697Kfs), c.2215delG (p.V739Y), c.263T>G (p.L196X), c.3022delG (p.V1008S), c.3908dupA (p.N1303Kfs), c.658C>T (p.Q220X), c.868C>T (p.Q290X), c.1526delG (p.G509fs), c.2908+1085-3367+260del7201, c.11C>A (p.S4X), c.3878\_3881delATT (p.V1293fs), c.3700A>G (p.I1234V), c.416A>T (p.H139I), c.366T>A (p.Y122X), c.3767\_3768insC (p.A1256fs), c.613C>T (p.P205S), c.293A>G (p.Q98R), c.3731G>A (p.G1244E), c.535C>A (p.Q179K), c.3368-2A>G, c.455T>G (p.M152R), c.1610\_1611delAC (p.D537fs), c.3254A>G (p.H1085R), c.496A>G (p.K166E), c.1408\_1417delGTGATTATGG (p.V470fs), c.1585-8G>A, c.2909G>A (p.G970D), c.653T>A (p.L218X), c.1175T>G (p.V392G), c.3139\_3139+1delGG, c.3717+4A>G (IVS22+4A>G) Sequencing | NM\_000492:1-27

**Familial Dysautonomia (IKBKAP):** Mutations (4): ♂ Genotyping | c.2204+6T>C, c.2741C>T (p.P914L), c.2087G>C (p.R696P), c.2128C>T (p.Q710X) Sequencing | NM\_003640:2-37

**Familial Hyperinsulinism: Type 1: ABCC8 Related (ABCC8):** Mutations (11): ♂ Genotyping | c.3989-9G>A, c.4159\_4161delITC (p.1387delF), c.4258C>T (p.R1420C), c.4477C>T (p.R1493W), c.2147G>T (p.G716V), c.4055G>C (p.R1352P), c.560T>A (p.V187D), c.4516G>A (p.E1506K), c.2506C>T (p.Q836X), c.579+2T>A, c.1333-1013A>G (IVS8-1013A>G) Sequencing | NM\_000352:1-39

**Fanconi Anemia: Type C (FANCC):** Mutations (8): ♂ Genotyping | c.456+4A>T, c.67delG, c.37C>T (p.Q13X), c.553C>T (p.R185X), c.1661T>C (p.L554P), c.1642C>T (p.R548X), c.66G>A (p.W22X), c.65G>A (p.W22X) Sequencing | NM\_000136:2-15

**Gaucher Disease (GBA):** Mutations (6): ♂ Genotyping | c.84\_85insG, c.1226A>G (p.N409S), c.1343A>T (p.D448V), c.1504C>T (p.R502C), c.1297G>T (p.V433L), c.1604G>A (p.R535H)

**Glycogen Storage Disease: Type IA (G6PC):** Mutations (13): ♂ Genotyping | c.376\_377insTA, c.79delC, c.979\_981delITC (p.327delF), c.1039C>T (p.Q347X), c.247C>T (p.R83C), c.724C>T (p.Q242X), c.248G>A (p.R83H), c.562G>C (p.G188R), c.648G>T, c.809G>T (p.G270V), c.113A>T (p.D38V), c.975delG (p.L326fs), c.724delC Sequencing | NM\_000151:1-5

**Joubert Syndrome (TMEM216):** Mutations (2): ♂ Genotyping | c.218G>T (p.R73L), c.218G>A (p.R73H) Sequencing | NM\_001173991:1-5

**Maple Syrup Urine Disease: Type 1B (BCKDHB):** Mutations (6): ♂ Genotyping | c.1114G>T (p.E372X), c.548G>C (p.R183P), c.832G>A (p.G278S), c.970C>T (p.R324X), c.487G>T (p.P163X), c.853C>T (p.R285X) Sequencing | NM\_183050:1-10

**Maple Syrup Urine Disease: Type 3 (DLD):** Mutations (8): ♂ Genotyping | c.104\_105insA, c.685G>T (p.G229C), c.214A>G (p.K72E), c.1081A>G (p.M361V), c.1123G>A (p.E375K), c.1178T>C (p.I393T), c.1463C>T (p.P488L), c.1483A>G (p.R495G) Sequencing | NM\_000108:1-14

**Mucopolidiosis: Type IV (MCOLN1):** Mutations (5): ♂ Genotyping | c.-1015\_788del6433, c.406-2A>G, c.1084G>T (p.D362Y), c.304C>T (p.R102X), c.244delC (p.L82fsX) Sequencing | NM\_020533:1-14

**Nemaline Myopathy: NEB Related (NEB):** Mutations (2): ♂ Genotyping | c.7434\_7536del2502bp, c.8890-2A>G (IVS63-2A>G) Sequencing | NM\_001164508:63-66,86,95-96,103,105,143,168-172, NM\_004543:3-149

**Niemann-Pick Disease: Type A (SMPD1):** Mutations (6): ♂ Genotyping | c.996delC, c.1493G>T (p.R498L), c.911T>C (p.L304P), c.1267C>T (p.H423Y), c.1734G>C (p.K578N), c.1493G>A (p.R498H) Sequencing | NM\_000543:1-6

**Sickle-Cell Anemia (HBB):** Mutations (1): ♂ Genotyping | c.20A>T (p.E7V) Sequencing | NM\_000518:1-3

**Spinal Muscular Atrophy: SMN1 Linked (SMN1):** Mutations (19): ♂ Genotyping | DEL EXON 7, c.22\_23insA, c.43C>T (p.Q15X), c.91\_92insT, c.305G>A (p.W102X), c.400G>A (p.E134K), c.439\_443delGAAGT, c.558delA, c.585\_586insT, c.683T>A (p.L228X), c.734C>T (p.P245L), c.768\_778dupTGCTGATGCTT, c.815A>G (p.Y272C), c.821C>T (p.T274I), c.823G>A (p.G275S), c.834+2T>G, c.835-18\_835-12delCCTTTAT, c.835G>T, c.836G>T dPCR | DEL EXON 7

**Tay-Sachs Disease (HEXA):** Mutations (78): ♂ Genotyping | c.1073+1G>A, c.1277\_1278insATC, c.1421+1G>C, c.805+1G>A, c.532C>T (p.R178C), c.533G>A (p.R178H), c.805G>A (p.G269S), c.1510C>T (p.R504C), c.1496G>A (p.R499H), c.509G>A (p.R170Q), c.1003A>T (p.I335F), c.910\_912delITC (p.305delF), c.749G>A (p.G250D), c.632T>C (p.F211S), c.629C>T (p.S210F), c.613delC, c.611A>G (p.H204R), c.598G>A (p.V200M), c.590A>C (p.K197T), c.571-1G>T, c.540C>G (p.Y180X), c.538T>C (p.Y180H), c.553G>T (p.R178L), c.508C>T (p.R170W), c.409C>T (p.R137X), c.380T>C (p.L127R), c.346+1G>C, c.116T>G (p.L39R), c.788G>A (p.W26X), c.1A>G (p.M1V), c.1495C>T (p.R499C), c.459+5G>A (IVS4+5G>A), c.1422-2A>G, c.535C>T (p.H179Y), c.1141delG (p.V381fs), c.796T>G (p.W266G), c.155C>A (p.S52X), c.426delT (p.F142fs), c.413-2A>G, c.570+3A>G, c.536A>G (p.H179R), c.1146+1G>A, c.736G>A (p.A246T), c.1302C>G (p.F434L), c.778C>T (p.P260S), c.1008G>T (p.Q336H), c.1385A>T (p.E462V), c.964G>A (p.D322N), c.340G>A (p.E114K), c.1432G>A (p.G478R), c.1178G>C (p.R393P), c.805+1G>C, c.1426A>T (p.R476X), c.623A>T (p.D208V), c.1537C>T (p.Q513X), c.1511G>T (p.R504L), c.1307\_1308delTA (p.I436fs), c.571-8A>G, c.624\_627delTCTT (p.D208fs), c.1211\_1212delITG (p.L404fs), c.621T>G (p.D207E), c.1511G>A (p.R504H), c.1177C>T (p.R393X), c.2T>C (p.M1T), c.1292G>A (p.W431X), c.947\_948insA (p.Y316fs), c.607T>G (p.W203G), c.1061\_1063delITCT (p.F354\_Y355delinsX), c.615delG (p.L205fs), c.805+2T>C, c.1123delG (p.E375fs), c.1121A>G (p.Q374R), c.1043\_1046delTCAA (p.F348fs), c.1510delC (p.R504fs), c.1451T>C (p.L484P), c.964G>T (p.D322Y), c.1351C>G (p.L451V), c.571-2A>G (IVS5-2A>G) Sequencing | NM\_000520:1-14

**Usher Syndrome: Type 1F (PCDH15):** Mutations (7): ♂ Genotyping | c.733C>T (p.R245X), c.2067C>A (p.Y684X), c.7C>T (p.R3X), c.1942C>T (p.R648X), c.1101delT (p.A367fsX), c.2800C>T (p.R934X), c.4272delA (p.L1425fs) Sequencing | NM\_001142763:2-35

**Usher Syndrome: Type 3 (CLRN1):** Mutations (5): ♂ Genotyping | c.144T>G (p.N48K), c.131T>A (p.M120K), c.567T>G (p.Y189X), c.634C>T (p.Q212X), c.221T>C (p.L74P) Sequencing | NM\_001195794:1-4

**Walker-Warburg Syndrome (FKTN):** Mutations (5): ♂ Genotyping | c.1167insA (p.F390fs), c.139C>T (p.R47X), c.748T>G (p.C250G), c.648-1243G>T (IVS5-1243G>T), c.515A>G (p.H172R) Sequencing | NM\_006731:2-10



## Residual Risk Information

Detection rates are calculated from the primary literature and may not be available for all ethnic populations. The values listed below are for genotyping. Sequencing provides higher detection rates and lower residual risks for each disease. More precise values for sequencing may become available in the future.

Disease	Carrier Rate	Detection Rate	Residual Risk
Alpha Thalassemia	♂ General: 1/48	50.67%	1/97
Beta Thalassemia	♂ African American: 1/75	84.21%	1/475
	♂ Indian: 1/24	74.12%	1/93
	♂ Sardinians: 1/23	97.14%	1/804
	♂ Spaniard: 1/51	93.10%	1/739
Bloom Syndrome	♂ Ashkenazi Jewish: 1/134	96.67%	1/4,020
	♂ European: Unknown	66.22%	Unknown
	♂ Japanese: Unknown	50.00%	Unknown
Canavan Disease	♂ Ashkenazi Jewish: 1/55	98.86%	1/4,840
	♂ European: Unknown	53.23%	Unknown
Cystic Fibrosis	♂ African American: 1/62	69.99%	1/207
	♂ Ashkenazi Jewish: 1/23	96.81%	1/721
	♂ Asian: 1/94	65.81%	1/275
	♂ European: 1/25	94.96%	1/496
	♂ Hispanic American: 1/48	77.32%	1/212
	♂ Native American: 1/53	84.34%	1/338
Familial Dysautonomia	♂ Ashkenazi Jewish: 1/31	>99%	<1/3,100
Familial Hyperinsulinism: Type 1: ABCC8 Related	♂ Ashkenazi Jewish: 1/52	98.75%	1/4,160
	♂ Finnish: 1/101	45.16%	1/184
Fanconi Anemia: Type C	♂ Ashkenazi Jewish: 1/101	>99%	<1/10,100
	♂ General: Unknown	30.00%	Unknown
Gaucher Disease	♂ Ashkenazi Jewish: 1/15	87.16%	1/117
	♂ General: 1/112	31.60%	1/164
	♂ Spaniard: Unknown	44.29%	Unknown
	♂ Turkish: 1/236	59.38%	1/581
Glycogen Storage Disease: Type IA	♂ Ashkenazi Jewish: 1/71	>99%	<1/7,100
	♂ Chinese: 1/159	80.00%	1/795
	♂ European: 1/177	76.88%	1/765
	♂ Hispanic American: 1/177	27.78%	1/245
	♂ Japanese: 1/177	89.22%	1/1,641
Joubert Syndrome	♂ Ashkenazi Jewish: 1/92	>99%	<1/9,200
Maple Syrup Urine Disease: Type 1B	♂ Ashkenazi Jewish: 1/97	>99%	<1/9,700
Maple Syrup Urine Disease: Type 3	♂ Ashkenazi Jewish: 1/94	>99%	<1/9,400
	♂ General: Unknown	68.75%	Unknown
Mucopolidosis: Type IV	♂ Ashkenazi Jewish: 1/97	96.15%	1/2,522
Nemaline Myopathy: NEB Related	♂ Ashkenazi Jewish: 1/108	>99%	<1/10,800

Disease	Carrier Rate	Detection Rate	Residual Risk
Niemann-Pick Disease: Type A	♂ Ashkenazi Jewish: 1/101	95.00%	1/2,020
Sickle-Cell Anemia	♂ African American: 1/10	>99%	<1/1,000
	♂ Hispanic American: 1/95	>99%	<1/9,500
Tay-Sachs Disease	♂ Argentinian: 1/280	82.35%	1/1,587
	♂ Ashkenazi Jewish: 1/29	99.53%	1/6,177
	♂ Cajun: 1/30	>99%	<1/3,000
	♂ European: 1/280	25.35%	1/375
	♂ General: 1/280	32.09%	1/412
	♂ Indian: Unknown	85.71%	Unknown
	♂ Iraqi Jewish: 1/140	56.25%	1/320
	♂ Japanese: 1/127	82.81%	1/739
	♂ Moroccan Jewish: 1/110	22.22%	1/141
	♂ Portuguese: 1/280	92.31%	1/3,640
	♂ Spaniard: 1/280	67.65%	1/865
	♂ United Kingdom: 1/161	71.43%	1/564
Usher Syndrome: Type 1F	♂ Ashkenazi Jewish: 1/126	93.75%	1/2,016
Usher Syndrome: Type 3	♂ Ashkenazi Jewish: 1/120	>99%	<1/12,000
	♂ Finnish: 1/134	>99%	<1/13,400
Walker-Warburg Syndrome	♂ Ashkenazi Jewish: 1/150	>99%	<1/15,000



Patient	Sample	Referring Doctor
<b>Patient Name:</b> Donor 5447 <b>Date of Birth:</b> [REDACTED] <b>Reference #:</b> [REDACTED] <b>Indication:</b> Carrier Testing <b>Test Type:</b> Congenital Adrenal Hyperplasia (CYP21A2) Carrier Screen	<b>Specimen Type:</b> Blood <b>Lab #:</b> [REDACTED] <b>Date Collected:</b> 6/26/2018 <b>Date Received:</b> 6/27/2018 <b>Final Report:</b> 7/11/2018	[REDACTED] [REDACTED] [REDACTED] [REDACTED] [REDACTED] Fax: [REDACTED]

## RESULTS

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

*CYP21A2* copy number: 2

No pathogenic copy number variants detected

No pathogenic sequence variants detected in *CYP21A2*

Reduced risk of being a congenital adrenal hyperplasia carrier

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

**Inheritance:** Autosomal Recessive

### Recommendations

Consideration of residual risk by ethnicity (see below) after a negative carrier screen is recommended, especially in the case of a positive family history of congenital adrenal hyperplasia.

### Interpretation

This individual was negative for all pathogenic *CYP21A2* copy number variants that were tested, and no pathogenic or likely pathogenic variants were identified by sequence analysis. 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 Risk Based On Ethnicity - Classic Congenital Adrenal Hyperplasia Due to 21-Hydroxylase Deficiency

Ethnicity	Carrier Frequency	Detection Rate	Residual Risk
Ashkenazi Jewish	1 in 40	>95%	1 in 781
Caucasian	1 in 67	>95%	1 in 1321
Worldwide	1 in 60	>95%	1 in 1181

**Patient:** Donor 5447

**DOB:** [REDACTED]

**Lab #:** [REDACTED]

## Table of Residual Risk Based On Ethnicity - Non-Classic Congenital Adrenal Hyperplasia Due to 21-Hydroxylase Deficiency

Ethnicity	Carrier Frequency	Detection Rate	Residual Risk
Ashkenazi Jewish	1 in 7	>95%	1 in 121
Caucasian	1 in 11	>95%	1 in 201
Worldwide	1 in 16	>95%	1 in 301

## Test Methods and Comments

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

### 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 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.

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

A long-range PCR was performed to generate a locus-specific amplicon for *CYP21A2*. The PCR product was then prepared for short-read NGS sequencing as described below and sequenced. Sequenced reads were mapped back to the original genomic loci and converted to VCF files as described below.

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.

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

Samples 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 tested genes were assessed for the average depth of coverage (minimum of 20X) and data quality threshold values. Exons not meeting a minimum of >20X read depth across the exon are further analyzed by Sanger sequencing.

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. 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.

**Patient:** Donor 5447

**DOB:** [REDACTED]

**Lab #:** [REDACTED]

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.

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.

**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.

**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.

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

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

## Ordering Practice

Practice Code: [REDACTED]  
Fairfax Cryobank - [REDACTED]  
[REDACTED]  
Physician: [REDACTED]  
Report Generated: 2018-07-23

5447 [REDACTED]

DOB: [REDACTED]  
Gender: Male  
Ethnicity: European  
Procedure ID: 100,775  
Kit Barcode: [REDACTED]  
Specimen: Blood, #102,156  
Specimen Collection: 2017-07-31  
Specimen Received: 2017-08-01  
Specimen Analyzed: 2018-07-23

Partner Not Tested

## TEST INFORMATION

Test: Carriermap<sup>SEO</sup> (Genotyping & Sequencing)  
Panel: Custom Panel  
Diseases Tested: 1  
Genes Tested: 1  
Genes Sequenced: 1

## SUMMARY OF RESULTS: NO MUTATIONS IDENTIFIED

5447 [REDACTED] was not identified to carry any pathogenic mutations in the gene(s) tested

No pathogenic mutations were identified in the genes tested, reducing but not eliminating the chance to be a carrier for the associated genetic diseases. CarrierMap assesses carrier status for genetic disease via molecular methods including targeted mutation analysis and/ or next-generation sequencing; other methodologies such as CBC and hemoglobin electrophoresis for hemoglobinopathies and enzyme analysis for Tay-Sachs disease may further refine risks for these conditions. Results should be interpreted in the context of clinical findings, family history, and/or other testing. A list of all the diseases and mutations screened for is included at the end of the report. This test does not screen for every possible genetic disease.

For additional disease information, please visit [www.coopergenomics.com/diseases](http://www.coopergenomics.com/diseases) . To speak with a genetic counselor, call 855.687.4363 .

## Methods and Limitations

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

**Sequencing** : Sequencing is performed using a custom next-generation sequencing (NGS) platform. Only the described exons for each gene listed are sequenced. Variants outside of these regions may not be identified. Some splicing mutations may not be identified. Triplet repeat expansions, intronic mutations, and large insertions and deletions may not be detected. All identified variants are curated, and determination of the likelihood of their pathogenicity is made based on examining allele frequency, segregation studies, predicted effect, functional studies, case/control studies, and other analyses. All variants identified via sequencing that are reported to cause disease in the primary scientific literature will be reported. Variants considered to be benign and variants of unknown significance (VUS) are NOT reported. VUS reporting can be requested and will be assessed on a case-by-case basis. Variants may be re-curated over time due to emerging literature or other information. In the sequencing process, interval drop-out may occur, leading to intervals of insufficient coverage. Intervals of insufficient coverage will be reported if they occur.

**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 existing mutations within the genes assayed may not be detected, and additional testing may be appropriate for some individuals.

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 (FDA). The FDA does not currently regulate laboratory developed tests (LDTs).

## Diseases & Mutations Assayed

**Niemann-Pick Disease: Type B (SMPD1):** Mutation(s) (3): ♂ Genotyping | c.1280A>G (p.H427R), c.1829\_1831delGCC (p.610delR), c.880C>A (p.Q294K) | Sequencing | NM\_000543:1-6

## Residual Risk Information

Detection rates are calculated from the primary literature and may not be available for all ethnic populations. The values listed below are for genotyping. Sequencing provides higher detection rates and lower residual risks for each disease. More precise values for sequencing may become available in the future.

Disease	Carrier Rate	Detection Rate	Residual Risk
Niemann-Pick Disease: Type B	♂ Czech: 1/276	83.33%	1/1656
	♂ General: Unknown	19.82%	Unknown
	♂ North African: Unknown	86.67%	Unknown
	♂ Spaniard: Unknown	38.10%	Unknown

Patient	Sample	Referring Doctor
<b>Patient Name:</b> Donor 5447 <b>Date of Birth:</b> [REDACTED] <b>Reference #:</b> FFAXCB-S45447 <b>Indication:</b> Carrier Testing <b>Test Type:</b> Unmask Additional Gene(s)	<b>Specimen Type:</b> Blood <b>Lab #:</b> [REDACTED] <b>Date Collected:</b> 6/26/2018 <b>Date Received:</b> 10/31/2018 <b>Final Report:</b> 11/4/2018	<b>Fairfax Cryobank, Inc.</b> [REDACTED] [REDACTED] [REDACTED] [REDACTED] <b>Fax:</b> [REDACTED]

## RESULTS

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

**Gene(s) analyzed:** *DHCR7, GBA*

### 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 *DHCR7* and *GBA* genes which are associated with Smith-Lemli-Opitz syndrome and Gaucher disease 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.



<b>Patient:</b> Donor 5447	<b>DOB:</b> [REDACTED]	<b>Lab #:</b> [REDACTED]
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## 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
<b>Gaucher Disease (AR)</b> NM_000157.3	GBA	Caucasian	1 in 164	87%	1 in 1,300	95%
		Ashkenazi Jewish	1 in 15	95%	1 in 280	
		Worldwide	1 in 158	86%	1 in 1,100	
<b>Smith-Lemli-Opitz Syndrome (AR)</b> NM_001360.2	DHCR7	African	1 in 51	98%	1 in 2,400	99%
		Ashkenazi Jewish	1 in 39	97%	1 in 1,100	
		East Asian	1 in 357	91%	1 in 3,800	
		Finnish	1 in 141	94%	1 in 2,500	
		Caucasian	1 in 46	94%	1 in 750	
		Latino	1 in 118	93%	1 in 1,800	
		South Asian	1 in 334	71%	1 in 1,200	
		Worldwide	1 in 57	94%	1 in 970	

AR: Autosomal Recessive

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

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

<b>Patient:</b> Donor 5447	<b>DOB:</b> [REDACTED]	<b>Lab #:</b> [REDACTED]
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## 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

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 are further analyzed by Southern blot analysis to assess the size and methylation status of the *FMR1* CGG repeat.

### Genotyping

Multiplex PCR amplification and allele specific primer extension analyses using the MassARRAY® System or Luminex® xMAP® technology were used to identify variants that are complex in nature or are present in low copy repeat regions and are, therefore, not amenable to Next Generation Sequencing technologies. Rare sequence variants may interfere with assay performance.

### Multiplex Ligation-Dependent Probe Amplification (MLPA)

MLPA® probe sets and reagents, MRC-Holland, were used for the analysis of copy number of specific targets versus known control samples. Each target region was assayed with two adjacent oligonucleotide probes which following hybridization were ligated and used as template for subsequent rounds of amplification. Each complete probe within the assay has a unique length and amplicons are separated and identified by capillary electrophoresis. False positive or negative results may also 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. However, it does not detect all known alpha-thalassemia mutations such as point mutations. In addition, carriers of alpha-thalassemia with three or more *HBA* copies on one chromosome, and one or no copies on the other chromosome, will not be detected. This test detects most alpha-globin gene deletions, triplications, and the Constant Spring (CS) mutation using Multiplex Ligation-Dependent Probe Amplification (MLPA). It is expected to detect approximately 90% of all alpha-thalassemia mutations, varying by ethnicity. Therefore, this result reduces, but does not eliminate, the chance that this patient is a carrier of alpha-thalassemia. With the exception of triplications, other benign alpha-globin gene polymorphisms will not be reported.

For Duchenne Muscular Dystrophy, the copy numbers of all *DMD* exons were analyzed. Please note that single-exon deletions and duplications will not be reported unless they are confirmed by NGS data (for example, if breakpoints occurring in an exon can be visualized).

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.

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The presence of the g.27134T>G variant allele in an individual with Ashkenazi Jewish or Asian ancestry is indicative of a duplication of *SMN1*. When present in an Ashkenazi Jewish or Asian individual with two copies of *SMN1*, g.27134T>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 g.27134T>G significantly increases or decreases, respectively, the likelihood of being a silent 2+0 silent carrier.

### Next Generation Sequencing (NGS)

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 custom capture library to target the guaranteed list of mutations and exonic regions of the relevant genes. These targeted regions were sequenced using the Illumina HiSeq2500 system with 100 bp paired-end reads. The DNA sequences were mapped to and analyzed in comparison with the published human genome build UCSC hg19 reference sequence. The targeted coding exons and splice junctions of the known protein-coding RefSeq genes were assessed for the average depth of coverage and data quality threshold values. This technology may not detect all small insertion/deletions and is not diagnostic for large duplications/deletions, repeat expansions, and structural genomic variation. This test will detect variants within the exons and the intron-exon boundaries of the target regions. Variants outside these regions will either not be detected or are not guaranteed to be detected. These regions include, but are not limited to, UTRs, promoters, and deep intronic areas or regions that fall within low copy repeat segments. In addition, a mutation(s) in a gene not included on the panel could be present in this patient. 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 were not reported.

### Sanger Sequencing

Sanger sequencing, as indicated, was performed in both directions 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

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. 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, such as pseudodeficiency alleles, may occur if benign variants interfere with the enzymatic assay. False negative results may occur if both *HEXA* and *HEXB* pathogenic 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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### Alpha-thalassemia:

Galanello R et al. Gene test review: Alpha-thalassemia. *Genet Med.* 2011 13:83-8.

Waye JS et al. Diagnostic testing for  $\alpha$ -globin gene disorders in a heterogeneous North American population. *Int J Lab Hematol.* 2013 35:306-13.

### Cystic Fibrosis:

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### 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:

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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:

Aartsma-Rus A et al. Entries in the Leiden Duchenne muscular dystrophy mutation database: an overview of mutation types and paradoxical cases that confirm the reading-frame rule. *Muscle Nerve.* 2006b 34:135-44.

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.

### Beta Globin-related Disorders:

Cao A et al. Beta-Thalassemia. GeneReviews (<http://www.ncbi.nlm.nih.gov/books/NBK1426/>)

Modell B et al. Epidemiology of haemoglobin disorders in Europe: an overview. *Scand J Clin Lab Invest.* 2007 67:39-69.

### For further reading:

Orphanet: <http://www.orpha.net/consor/cgi-bin/index.php>

GeneReviews: <http://www.ncbi.nlm.nih.gov/books/NBK1116/>

### For Disease Specific Standards and Guidelines:

<https://www.acmg.net/>

Additional disease-specific references available upon request.

Patient	Sample	Referring Doctor
<b>Patient Name:</b> Donor 5447 <b>Date of Birth:</b> [REDACTED] <b>Reference #:</b> FFAXCB-S45447 <b>Indication:</b> Carrier Testing <b>Test Type:</b> Unmask Additional Gene(s) V1E	<b>Specimen Type:</b> Blood <b>Lab #:</b> [REDACTED] <b>Date Collected:</b> 6/26/2018 <b>Date Received:</b> 5/22/2019 <b>Final Report:</b> 6/7/2019	<b>Fairfax Cryobank, Inc.</b> [REDACTED] [REDACTED] [REDACTED] [REDACTED]

## RESULTS

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

**Gene(s) analyzed:** *MMAA* and *MEFV*

### 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 *MMAA* and *MEFV* genes which are associated with methylmalonic acidemia (*MMAA*-related) and familial Mediterranean fever, respectively, 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.

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## 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
<b>Familial Mediterranean Fever (AR)</b> NM_000243.2	<i>MEFV</i> †	African	1 in 230	74%	1 in 870	99%
		Ashkenazi Jewish	1 in 8	99%	1 in 720	
		East Asian	1 in 141	96%	1 in 3,400	
		Finnish	1 in 29	99%	1 in 2,800	
		Caucasian	1 in 40	97%	1 in 1,200	
		Latino	1 in 74	95%	1 in 1,500	
		South Asian	1 in 56	95%	1 in 1,000	
		Worldwide	1 in 40	97%	1 in 1,200	
		Sepharic Jewish	1 in 14	99%	1 in 1,300	
		Armenian	1 in 5	99%	1 in 400	
		Turkish	1 in 5	75%	1 in 17	
<b>Methylmalonic Acidemia (MMAA-Related) (AR)</b> NM_172250.2	<i>MMAA</i>	East Asian	1 in 2156	99%	1 in 216,000	99%
		Finnish	1 in 3890	99%	1 in 389,000	
		Caucasian	1 in 677	95%	1 in 14,600	
		Latino	1 in 2098	86%	1 in 15,500	
		South Asian	1 in 1167	91%	1 in 13,700	
		Worldwide	1 in 1082	95%	1 in 20,100	

† Carrier frequencies include milder and reduced penetrance forms of the disease; therefore, carrier frequencies may appear higher than reported in the literature.

AR: Autosomal Recessive

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

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

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**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).



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#### 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  $\Delta\Delta Ct$  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



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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.

#### 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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##### Ashkenazi Jewish Disorders:

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

##### Variant Classification:

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

Additional disease-specific references available upon request.

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