



## Donor 5130

### Genetic Testing Summary

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

Last Updated: 09/16/19

Donor Reported Ancestry: German

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 149 mutations- in the CFTR gene	1/496
Spinal Muscular Atrophy (SMA) carrier screening	Negative for deletions of exon 7 in the SMN1 gene	1/632
Standard testing attached- 22 diseases by genotyping	Negative for mutations tested	
Special testing		
Smith-Lemli-Opitz Syndrome (DHCR7)	Negative by gene sequencing in the DHCR7 gene	1/750

\*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: 2016-03-25

5130

DOB: [REDACTED]  
Gender: Male  
Ethnicity: European  
Procedure ID: 47413  
Kit Barcode: [REDACTED]  
Method: Genotyping  
Specimen: Blood, #49758  
Specimen Received: 2016-03-21  
Specimen Analyzed: 2016-03-25

Partner Not Tested

## SUMMARY OF RESULTS

## NO MUTATIONS IDENTIFIED


5130 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: Fairfax Cryobank Panel V2 , Diseases Tested: 22, Mutations Tested: 452, Genes Tested: 22, 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.

**Spinal Muscular Atrophy:** Spinal Muscular Atrophy: Carrier status for SMA is assessed via genotyping and via copy number analysis by qPCR. 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 via genotyping. 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.

## Diseases & Mutations Assayed

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

H	T	X	M	Disease	#	Mutations
●	○	○	○	Alpha Thalassemia	9	♂ Genotyping   SEA deletion, c.207C>A (p.N69K), c.223G>C (p.D75G), c.2T>C (p.M1T), c.207C>G (p.N69K), c.340_351delCTCCCCGCCGAG (p.L114_E117del), c.377T>C (p.L126P), c.427T>C (p.X143Qext32), c.*+94A>G
●	●	○	○	Beta Thalassemia	84	♂ 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.34G>A (p.V12I), 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.27_28insG, c.92+1G>T, c.92+1G>C, 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_A129delQAinsP), 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_204delTG (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.46delT (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
●	○	○	○	Bloom Syndrome	24	♂ Genotyping   c.2207_2212delATCTGAinsTAGATTC (p.Y736Lfs), c.2407insT, c.557_559delCAA (p.S186X), c.1284G>A (p.W428X), c.1701G>A (p.W567X), 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.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_357delTA (p.C120Hfs), c.4076+1delG, c.3281C>A (p.S1094X)
●	○	○	○	Canavan Disease	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)

H	T	X	M	Disease	#	Mutations
				Cystic Fibrosis	149	<p>♂ 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&gt;A, c.164+12T&gt;C, c.1680-886A&gt;G, c.1680-1G&gt;A, c.1766+1G&gt;A, c.1766+1G&gt;T, c.1766+5G&gt;T, c.1818del84, c.1911delG, c.1923delCTCAAACTinsA, c.1973delGAAATTCATCTinsAGAAA, c.2052delA (p.K684fs), c.2052insA (p.Q685fs), c.2051_2052delAAinsG (p.K684SfsX38), c.2174insA, c.261delTT, c.2657+5G&gt;A, c.273+1G&gt;A, c.273+3A&gt;C, c.274-1G&gt;A, c.2988+1G&gt;A, c.3039delC, c.3140-26A&gt;G, c.325delTATinsG, c.3527delC, c.3535delACCA, c.3691delT, c.3717+12191C&gt;T, c.3744delA, c.3773_3774insT (p.L1258fs), c.442delA, c.489+1G&gt;T, c.531delT, c.579+1G&gt;T, c.579+5G&gt;A (IVS4+5G&gt;A), c.803delA (p.N268fs), c.805_806delAT (p.I269fs), c.933_935delCTT (p.311delF), c.946delT, c.1645A&gt;C (p.S549R), c.2128A&gt;T (p.K710X), c.1000C&gt;T (p.R334W), c.1013C&gt;T (p.T338I), c.1364C&gt;A (p.A455E), c.1477C&gt;T (p.Q493X), c.1572C&gt;A (p.C524X), c.1654C&gt;T (p.Q552X), c.1657C&gt;T (p.R553X), c.1721C&gt;A (p.P574H), c.2125C&gt;T (p.R709X), c.223C&gt;T (p.R75X), c.2668C&gt;T (p.Q890X), c.3196C&gt;T (p.R1066C), c.3276C&gt;G (p.Y1092X), c.3472C&gt;T (p.R1158X), c.3484C&gt;T (p.R1162X), c.349C&gt;T (p.R117C), c.3587C&gt;G (p.S1196X), c.3712C&gt;T (p.Q1238X), c.3764C&gt;A (p.S1255X), c.3909C&gt;G (p.N1303K), c.1040G&gt;A (p.R347H), c.1040G&gt;C (p.R347P), c.1438G&gt;T (p.G480C), c.1558G&gt;T (p.V520F), c.1624G&gt;T (p.G542X), c.1646G&gt;A (p.S549N), c.1646G&gt;T (p.S549I), c.1652G&gt;A (p.G551D), c.1675G&gt;A (p.A559T), c.1679G&gt;C (p.R560T), c.178G&gt;T (p.E60X), c.1865G&gt;A (p.G622D), c.254G&gt;A (p.G85E), c.271G&gt;A (p.G91R), c.274G&gt;T (p.E92X), c.3209G&gt;A (p.R1070Q), c.3266G&gt;A (p.W1089X), c.3454G&gt;C (p.D1152H), c.350G&gt;A (p.R117H), c.3611G&gt;A (p.W1204X), c.3752G&gt;A (p.S1251N), c.3846G&gt;A (p.W1282X), c.3848G&gt;T (p.R1283M), c.532G&gt;A (p.G178R), c.988G&gt;T (p.G330X), c.1090T&gt;C (p.S364P), c.3302T&gt;A (p.M1101K), c.617T&gt;G (p.L206W), c.14C&gt;T (p.P5L), c.19G&gt;T (p.E7X), c.171G&gt;A (p.W57X), c.313delA (p.I105fs), c.328G&gt;C (p.D110H), c.580-1G&gt;T, c.1055G&gt;A (p.R352Q), c.1075C&gt;A (p.Q359K), c.1079C&gt;A (p.T360K), c.1647T&gt;G (p.S549R), c.1976delA (p.N659fs), c.2290C&gt;T (p.R764X), c.2737_2738insG (p.Y913X), c.3067_3072delATAGTG (p.I1023_V1024delT), c.3536_3539delICCAA (p.T1179fs), c.3659delC (p.T1220fs), c.54-5940_273+10250del21080bp (p.S18fs), c.4056G&gt;C (p.Q1352H), c.4364C&gt;G (p.S1455X), c.4003C&gt;T (p.L1335F), c.2538G&gt;A (p.W846X), c.200C&gt;T (p.P67L), c.4426C&gt;T (p.Q1476X), c.1116+1G&gt;A, c.1986_1989delAACT (p.T663R), c.2089_2090insA (p.R697Kfs), c.2215delG (p.V739Y), c.263T&gt;G (p.L196X), c.3022delG (p.V1008S), c.3908dupA (p.N1303Kfs), c.658C&gt;T (p.Q220X), c.868C&gt;T (p.Q290X), c.1526delG (p.G509fs), c.2908+1085-3367+260del7201, c.11C&gt;A (p.S4X), c.3700A&gt;G (p.I1234V), c.416A&gt;T (p.H139L), c.366T&gt;A (p.Y122X), c.3767_3768insC (p.A1256fs), c.613C&gt;T (p.P205S), c.293A&gt;G (p.Q98R), c.3731G&gt;A (p.G1244E), c.535C&gt;A (p.Q179K), c.3368-2A&gt;G, c.455T&gt;G (p.M152R), c.1610_1611delAC (p.D537fs), c.3254A&gt;G (p.H1085R), c.496A&gt;G (p.K166E), c.1408_1417delGTGATTATGG (p.V470fs), c.1585-8G&gt;A, c.2909G&gt;A (p.G970D), c.653T&gt;A (p.L218X), c.1175T&gt;G (p.V392G), c.3139_3139+1delGG</p>
				Familial Dysautonomia	4	<p>♂ Genotyping   c.2204+6T&gt;C, c.2741C&gt;T (p.P914L), c.2087G&gt;C (p.R696P), c.2128C&gt;T (p.Q710X)</p>
				Familial Hyperinsulinism: Type 1: ABCC8 Related	10	<p>♂ Genotyping   c.3989-9G&gt;A, c.4159_4161delITC (p.1387delF), c.4258C&gt;T (p.R1420C), c.4477C&gt;T (p.R1493W), c.2147G&gt;T (p.G716V), c.4055G&gt;C (p.R1352P), c.560T&gt;A (p.V187D), c.4516G&gt;A (p.E1506K), c.2506C&gt;T (p.Q836X), c.579+2T&gt;A</p>
				Fanconi Anemia: Type C	8	<p>♂ Genotyping   c.456+4A&gt;T, c.67delG, c.37C&gt;T (p.Q13X), c.553C&gt;T (p.R185X), c.1661T&gt;C (p.L554P), c.1642C&gt;T (p.R548X), c.66G&gt;A (p.W22X), c.65G&gt;A (p.W22X)</p>
				Gaucher Disease	6	<p>♂ Genotyping   c.84_85insG, c.1226A&gt;G (p.N409S), c.1343A&gt;T (p.D448V), c.1504C&gt;T (p.R502C), c.1297G&gt;T (p.V433L), c.1604G&gt;A (p.R535H)</p>
				Glycogen Storage Disease: Type IA	13	<p>♂ Genotyping   c.376_377insTA, c.79delC, c.979_981delITC (p.327delF), c.1039C&gt;T (p.Q347X), c.247C&gt;T (p.R83C), c.724C&gt;T (p.Q242X), c.248G&gt;A (p.R83H), c.562G&gt;C (p.G188R), c.648G&gt;T, c.809G&gt;T (p.G270V), c.113A&gt;T (p.D38V), c.975delG (p.L326fs), c.724delC</p>

H	T	X	M	Disease	#	Mutations
●	○	○	○	Joubert Syndrome	2	♂ Genotyping   c.218G>T (p.R73L), c.218G>A (p.R73H)
●	●	○	○	Maple Syrup Urine Disease: Type 1B	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.E163X), c.853C>T (p.R285X)
●	●	○	○	Maple Syrup Urine Disease: Type 3	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)
●	○	○	○	Mucopolipidosis: Type IV	5	♂ Genotyping   c.-1015_788del6433, c.406-2A>G, c.1084G>T (p.D362Y), c.304C>T (p.R102X), c.244delC (p.L82fsX)
●	○	○	○	Nemaline Myopathy: NEB Related	1	♂ Genotyping   c.7434_7536del2502bp
●	○	○	○	Niemann-Pick Disease: Type A	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)
●	●	○	○	Sickle-Cell Anemia	1	♂ Genotyping   c.20A>T (p.E7V)
●	○	○	○	Spinal Muscular Atrophy: SMN1 Linked	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 qPCR   DEL EXON 7
●	○	○	○	Tay-Sachs Disease	76	♂ Genotyping   c.1073+1G>A, c.1277_1278insTATC, 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.533G>T (p.R178L), c.508C>T (p.R170W), c.409C>T (p.R137X), c.380T>G (p.L127R), c.346+1G>C, c.116T>G (p.L39R), c.78G>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_627delTCCT (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)
●	○	○	○	Usher Syndrome: Type 1F	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)
●	○	○	○	Usher Syndrome: Type 3	5	♂ Genotyping   c.144T>G (p.N48K), c.359T>A (p.M120K), c.300T>G (p.Y176X), c.634C>T (p.Q212X), c.221T>C (p.L74P)
●	○	○	○	Walker-Warburg Syndrome	1	♂ Genotyping   c.1167insA (p.F390fs)



#### Patient Information

Name: Donor 5130  
Date of Birth: [REDACTED]  
Sema4 ID: [REDACTED]  
Client ID: [REDACTED]  
Indication: Carrier Testing

#### Specimen Information

Specimen Type: Purified DNA  
Date Collected: 07/29/2019  
Date Received: 08/03/2019  
Final Report: 08/12/2019

#### Referring Provider

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

## Custom Carrier Screen (ECS)

Number of genes tested: 1

### SUMMARY OF RESULTS AND RECOMMENDATIONS

⊖ Negative

**Negative for all genes tested: *DHCR7***

To view a full list of genes and diseases tested  
please see Table 1 in this report

#### Recommendations

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

## Test description

This patient was tested for the genes listed above using one or more of the following methodologies: target capture and short-read sequencing, long-range PCR followed by short-read sequencing, targeted genotyping, and/or copy number analysis. Please note that negative results reduce but do not eliminate the possibility that this individual is a carrier for one or more of the disorders tested. Please view the Table of Residual Risks Based on Ethnicity at the end of this report or at [go.sema4.com/residualrisk](http://go.sema4.com/residualrisk) for gene transcripts, sequencing exceptions, specific detection rates, and residual risk estimates after a negative screening result. With individuals of mixed ethnicity, it is recommended to use the highest residual risk estimate. Only known pathogenic or likely pathogenic variants are reported. This carrier screening test does not report likely benign variants and variants of uncertain significance (VUS). If reporting of likely benign variants and VUS are desired in this patient, please contact the laboratory at 800-298-6470, option 2 to request an amended report.

**Anastasia Larmore, Ph.D., Assistant Laboratory Director**

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



## Genes and diseases tested

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

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

Disease	Gene	Inheritance Pattern	Status	Detailed Summary
⊖ Negative				
Smith-Lemli-Opitz Syndrome	DHCR7	AR	Reduced Risk (see table below)	

AR=Autosomal recessive; XL=X-linked

**Table 2: Residual Risk by ethnicity for negative results**

Disease (Inheritance)	Gene	Ethnicity	Carrier Frequency	Detection Rate	Residual Risk	Analytical Detection Rate
Smith-Lemli-Opitz Syndrome (AR) 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	

\* Carrier detection by HEXA enzyme analysis has a detection rate of approximately 98% (Applies to *HEXA* gene testing only).

† Carrier frequencies include milder and reduced penetrance forms of the disease. Therefore, carrier frequencies may appear higher than reported in the literature (Applies to *BTD*, *Fg*, *GJB2*, *GJB1*, *GLA* and *MEFV* gene testing only).

‡ Please note that *GJB2* testing includes testing for the two upstream deletions, del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854) (PMID:11807148 and 15994881) (Applies to *GJB2* gene testing only).

AR: Autosomal recessive; N/A: Not available; XL: X-linked

## Test methods and comments

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

### 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<sup>TM</sup>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<sup>®</sup> genotyping platform.



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

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

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

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

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

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

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

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

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

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

#### **Residual Risk Calculations**

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

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

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

#### **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.