Donor 4203

Genetic Testing Summary

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

Last Updated: 12/12/22

Donor Reported Ancestry: English, Norwegian

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 108 mutations in the CFTR gene	1/260
Spinal Muscular Atrophy (SMA) carrier screening	Negative for deletions of exon 7 in the SMN1 gene	<1/500
Hb Beta Chain-Related Hemoglobinopathy (including Beta Thalassemia and Sickle Cell Disease) by genotyping	Negative for 37 mutations tested in the HBB gene	<1/500 for Beta-Thalassemia <1/500 for Sickle Cell
Special Testing		
Genes: HAX1, NPHS2	Negative by gene sequencing	See attached for residual risks

*No single test can screen for all genetic disorders. A negative screening result significantly reduces, but cannot eliminate, the risk for these conditions in a pregnancy.

**Donor residual risk is the chance the donor is still a carrier after testing negative.

Quest Diagnostics®

Patient Information	Specimen Information	Client Information
DONOR, 4203 **DOB: Not Given AGE: Not Given** Gender: M Fasting: U Phone: NG Patient ID: ███████	Specimen: ███████ Requisition: ███████ Collected: 12/02/2010 / 13:15 CST Received: 12/03/2010 / 02:36 CST Reported: 12/04/2010 / 09:34 CST	████████ ███████ ████ ███████████ ███████████████ ████████████

Test Name	In Range	Out Of Range	Reference Range	Lab
HEMOGLOBINOPATHY EVALUATION				
RED BLOOD CELL COUNT	4.96		4.20-5.80 Million/uL	IG
HEMOGLOBIN	15.1		13.2-17.1 g/dL	
HEMATOCRIT	44.4		38.5-50.0 %	
MCV	89.5		80.0-100.0 fL	
MCH	30.5		27.0-33.0 pg	
RDW	13.7		11.0-15.0 %	
* HEMOGLOBIN A	97.5		>96.0 %	IG
* HEMOGLOBIN F	<1.0		<2.0 %	
* HEMOGLOBIN A2 (QUANT)	2.5		1.8-3.5 %	
* INTERPRETATION				
Normal phenotype.				
CHOLESTEROL, TOTAL	152		125-200 mg/dL	IG
AST	19		10-35 U/L	IG
ALT	14		9-60 U/L	IG
CBC (INCLUDES DIFF/PLT)				IG
WHITE BLOOD CELL COUNT	8.2		3.8-10.8 Thousand/uL	
RED BLOOD CELL COUNT	4.96		4.20-5.80 Million/uL	
HEMOGLOBIN	15.1		13.2-17.1 g/dL	
HEMATOCRIT	44.4		38.5-50.0 %	
MCV	89.5		80.0-100.0 fL	
MCH	30.5		27.0-33.0 pg	
MCHC	34.1		32.0-36.0 g/dL	
RDW	13.7		11.0-15.0 %	
PLATELET COUNT	285		140-400 Thousand/uL	
ABSOLUTE NEUTROPHILS	5437		1500-7800 cells/uL	
ABSOLUTE LYMPHOCYTES	2148		850-3900 cells/uL	
ABSOLUTE MONOCYTES	508		200-950 cells/uL	
ABSOLUTE EOSINOPHILS	66		15-500 cells/uL	
ABSOLUTE BASOPHILS	41		0-200 cells/uL	
NEUTROPHILS	66.3		%	
LYMPHOCYTES	26.2		%	
MONOCYTES	6.2		%	
EOSINOPHILS	0.8		%	
BASOPHILS	0.5		%	
ABO GROUP AND RH TYPE				IG
ABO GROUP	O			
RH TYPE	RH(D) POSITIVE			

PERFORMING SITE:

IG   QUEST DIAGNOSTICS-IRVING, 4770 REGENT BLVD., IRVING, TX 75063 Laboratory Director: SUZANNE H. KREISBERG, MD, CLIA: 45D0697943

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Results Recipient	Ordering Healthcare Professional	Male Details	Female Details
Fairfax Cryobank - ▮▮▮ Attn: ▮▮▮▮▮ ▮▮▮▮▮▮▮▮ ▮▮▮▮▮▮▮▮ ▮▮▮▮▮ Report Date: 12/14/2010	Fairfax Cryobank - ▮▮▮ ▮▮▮▮▮▮▮ ▮▮▮▮▮▮▮ ▮▮▮▮▮▮▮	Name: Donor 4203 DOB: ▮▮▮▮ Ethnicity: Northern European Sample Type: Saliva (OG-300) Date of Collection: 12/02/2010 Barcode: ▮▮▮▮▮ Indication: Egg or Sperm Donor	Not tested

Universal Genetic Test (Egg or Sperm Donor)

The Universal Genetic Test uses targeted DNA mutation analysis to simultaneously determine the carrier status of an individual for a number of Mendelian diseases. This report indicates which mutations, if any, were detected for each mutation panel. Because only select mutations are tested, the percentage of carriers detected varies by ethnicity. A negative test result does not eliminate the possibility that the individual is a carrier. Interpretation is given as an estimate of the risk of conceiving a child affected with a disease, which is based on reported ethnicity, the test results, and an assumption of no family history.*

Donor 4203

Donor 4203's DNA test shows that he is not a carrier of any disease-causing mutation tested.

Partner

The child risk presented is based on a hypothetical pairing with a partner of the same ethnic group.

Child Risk Summary

Your Universal Genetic Test indicates that your future children have a reduced risk for the diseases tested, including those listed below which are common in your ethnicity.

Cystic Fibrosis   Spinal Muscular Atrophy

*Limitations: In an unknown number of cases, nearby genetic variants may interfere with mutation detection. The child risk summary is provided as an aid to genetic counseling. Inaccurate reporting of ethnicity may cause errors in risk calculation. Individuals of African, Asian, and Mediterranean ancestry are at increased risk for being carriers for hemoglobinopathies and should also be offered carrier testing by CBC and hemoglobin electrophoresis or HPLC.

Full Results

Below are the full test results for all diseases on the panel. Noted are the specific genetic mutations for which the patient tested positive or negative. If there was insufficient data to determine the genotype for any variant, this will be noted as "no call." Also listed in this section is the patient's post-test risk of being a carrier of each disease as well as the odds that his future children could inherit each disease.

Beta Thalassemia

Your child's risk:	Risk before testing:	
Less than 1 in 1,000,000	1 in 250,000	Reduced risk

Donor 4203: No mutations detected. No call for Hb C and Pro5fs. This does not rule out the possibility of being a carrier of untested mutations. The post-test risk of being a carrier, assuming a negative family history, is < 1 in 500. 80% detection rate.

Gene: HBB. **Variants (35):** K17X, Q39X, 619 bp deletion, Phe41fs, Ser9fs, IVS-II-654, IVS-II-745, IVS-II-850, IVS-I-6, IVS-I-110, IVS-I-5, IVS-I-1(G>A), IVS-I-1(G>T), -88C>T, -28A>G, -29A>G, Lys8fs, Phe71fs, IVS-II-849(A>C), IVS-II-849(A>G), Gly24 T>A, -87C>G, Hb C, Poly A: AATAAA->AATGAA, Poly A: AATAAA->AATAAG, W15X, Pro5fs, Gly16fs, Glu6fs, IVS-II-705, IVS-II-844, -30T>A, CAP+1 A>C, Hb E, Hb O-Arab.

Cystic Fibrosis

Your child's risk:	Risk before testing:	
1 in 29,000	1 in 3,100	Reduced risk

Donor 4203: No mutations detected. No call for R1066C, S1251N, and 3199del6. This does not rule out the possibility of being a carrier of untested mutations. The post-test risk of being a carrier, assuming a negative family history, is 1 in 260. 90% detection rate.

Gene: CFTR. **Variants (108):** G85E, R117H, R334W, R347P, A455E, G542X, G551D, R553X, R560T, R1162X, W1282X, N1303K, F508del, I507del, 2184delA, 3659delC, 621+1G>T, 711+1G>T, 1717-1G>A, 1898+1G>A, 2789+5G>A, 3120+1G>A, 3849+10kbC>T, E60X, R75X, E92X, Y122X, G178R, R347H, Q493X, V520F, S549N, P574H, M1101K, D1152H, S1235R, 394delTT, 1078delT, 3876delA, 3905insT, 1812-1G>A, 3272-26A>G, 2183AA>G, S549R(A>C), G91R, R117C, I148T, L206W, G330X, T338I, R352Q, S364P, G480C, I506V, F508C, C524X, S549I, S549R(T>G), Q552X, A559T, G622D, R709X, K710X, Q890X, R1066C, R1070Q, W1089X, Y1092X, R1158X, S1196X, W1204X(c.3611G>A), Q1238X, S1251N, S1255X, R1283M, dele2-3 21kb, 3199del6, F311del, 574delA, 663delT, 935delA, 936delTA, 1161delC, 1609delCA, 1677delTA, 1949del84, 2043delG, 2055del9>A, 2105-2117del13insAGAAA, 3171delC, 3667del4, 3821delT, 1288insTA, 2184insA, 2307insA, 2869insG, 296+12T>C, 405+1G>A, 405+3A>C, 406-1G>A, 711+5G>A, 712-1G>T, 1811+1.6kbA>G, 1898+1G>T, 1898+5G>T, 3120G>A, 457TAT>G, W1204X(c.3612G>A).

Sickle Cell Disease

Your child's risk:	Risk before testing:	
Less than 1 in 1,000,000	less than 1 in 1,000,000	Reduced risk

Donor 4203: No mutations detected. No call for Hb C and Pro5fs. This does not rule out the possibility of being a carrier of untested mutations. The post-test risk of being a carrier, assuming a negative family history, is < 1 in 500. >99% detection rate.

Gene: HBB. **Variants (37):** Hb S, K17X, Q39X, 619 bp deletion, Phe41fs, Ser9fs, IVS-II-654, IVS-II-745, IVS-II-850, IVS-I-6, IVS-I-110, IVS-I-5, IVS-I-1(G>A), IVS-I-1(G>T), -88C>T, -28A>G, -29A>G, Lys8fs, Phe71fs, IVS-II-849(A>C), IVS-II-849(A>G), Gly24 T>A, -87C>G, Hb C, Poly A: AATAAA->AATGAA, Poly A: AATAAA->AATAAG, W15X, Pro5fs, Gly16fs, Glu6fs, IVS-II-705, IVS-II-844, -30T>A, CAP+1 A>C, Hb E, Hb D-Punjab, Hb O-Arab.

Spinal Muscular Atrophy

Your child's risk:	Risk before testing:	
1 in 97,000	1 in 4,800	Reduced risk

Donor 4203: No mutations detected. This does not rule out the possibility of being a carrier of untested mutations. The post-test risk of being a carrier, assuming a negative family history, is < 1 in 500. 95% detection rate.

Gene: SMN1. **Variants (1):** Exon 7 deletion.

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Version: 1.0.55

GENETICS & IVF
Institute

Cytogenetic Report

Client	Fairfax Cryobank - ███████
Address	████████████████████
	████████████

Reporting Phone #	███████	**Fax #**	████████	**Email**	N/A

Patient name/Donor Alias	Donor #4203	**Patient DOB**	N/A
Donor #	4203-101202	**Specimen type**	Peripheral Blood
Collection Date	12/02/2010	**Accession #**	10-117CG
Date Received	12/03/2010		

RESULTS

CYTOGENETIC ANALYSIS

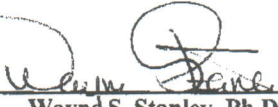|					
Cells counted	25	**Type of banding**	GTG	**Probe(s)**	N/A
Cells analyzed	5	**Band resolution**	550	**Nuclei scored**	N/A
Cells karyotyped	2				
Modal chromosome #	46				

FISH

KARYOTYPE   46,XY

INTERPRETATION

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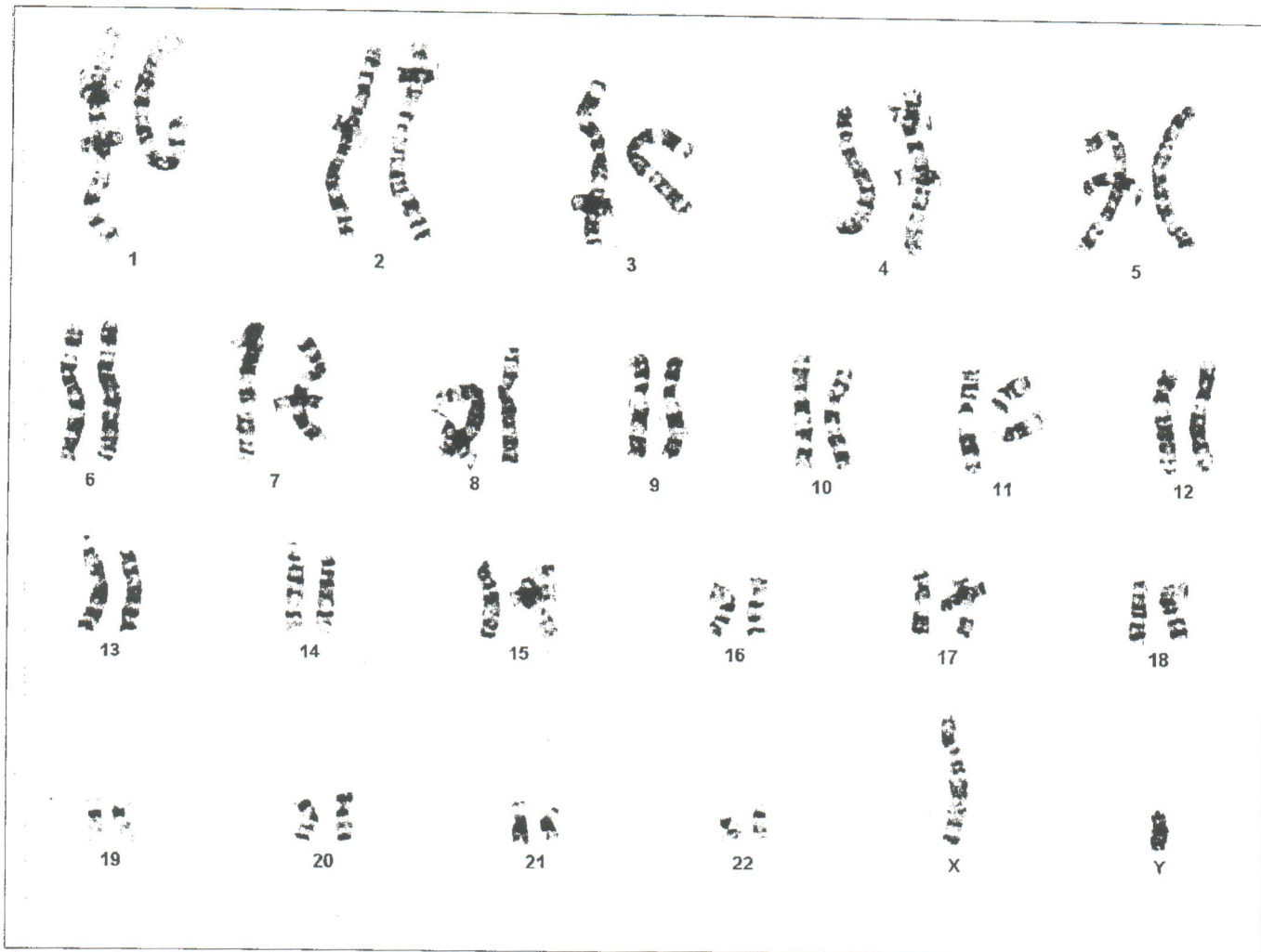
Comments

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

12/21/10
Date

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3015 Williams Drive | Fairfax, Virginia 22031 | 703-698-7355 | 800-552-4363 | givf@givf.com | www.givf.com

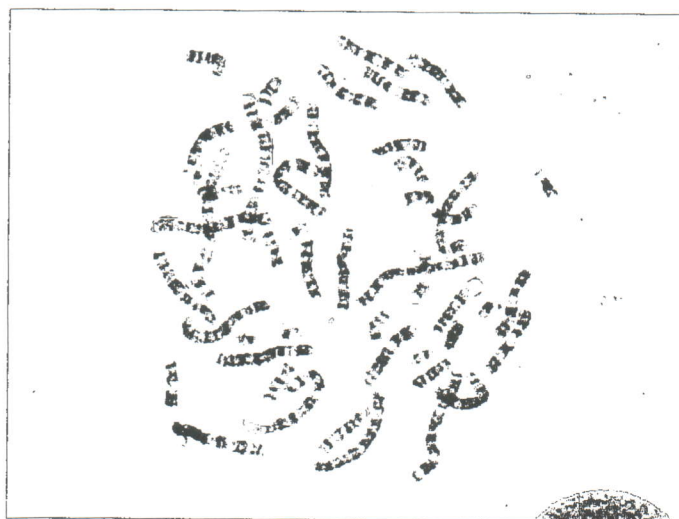## Genetics a   IVF Preimplantation Genetics Lab atory

Patient name: Donor#4203

Case name: 10-117CG

46,XY

Case: 10-117CG   Slide: B1   Cell: 2

sema4

Patient Information

Name: Donor 4203

Date of Birth: ▮▮▮▮▮

Sema4 ID: ▮▮▮▮▮

Client ID: ▮▮▮▮▮

Indication: Carrier Screening

Specimen Information

Specimen Type: Purified DNA

Date Collected: 10/10/2022

Date Received: 10/25/2022

Final Report: 11/06/2022

Referring Provider

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Fairfax Cryobank, Inc.

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Custom Carrier Screen (2 genes)

with Personalized Residual Risk

SUMMARY OF RESULTS AND RECOMMENDATIONS

⊖ **Negative**

Negative for all genes tested: *HAX1, and NPHS2*
To view a full list of genes and diseases tested
please see Table 1 in this report

AR=Autosomal recessive; XL=X-linked

Recommendations

- Consideration of residual risk by ethnicity after a negative carrier screen is recommended for the other diseases on the panel, especially in the case of a positive family history for a specific disorder. Please note that residual risks for X-linked diseases (including full repeat expansions for Fragile X syndrome) may not be accurate for males and the actual residual risk is likely to be lower.
- As genetic technologies may improve and variant classifications may change over time, it is recommended to obtain a new carrier screening test or reanalysis when a new pregnancy is being considered.

Test description

This patient was tested for the genes listed above using one or more of the following methodologies: target capture and short-read sequencing, long-range PCR followed by short-read sequencing, targeted genotyping, and/or copy number analysis. Please note that negative results reduce but do not eliminate the possibility that this individual is a carrier for one or more of the disorders tested. Please see Table 1 for a list of genes and diseases tested with the patient's personalized residual risk. If personalized residual risk is not provided, please see the complete residual risk table at **go.sema4.com/residualrisk.** 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., Associate Laboratory Director

Address: 62 Southfield Avenue, Stamford, CT 06902
CLIA # 07D2180805
CT LIC # CL-1016

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Genes and diseases tested

The personalized residual risks listed below are specific to this individual. The complete residual risk table is available at
go.sema4.com/residualrisk

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

Disease	Gene	Inheritance Pattern	Status	Detailed Summary
⊖ **Negative**				
Congenital Neutropenia (*HAX1*-Related)	*HAX1*	AR	Reduced Risk	**Personalized Residual Risk:** 1 in 82,000
Nephrotic Syndrome (*NPHS2*-Related) / Steroid-Resistant Nephrotic Syndrome	*NPHS2*	AR	Reduced Risk	**Personalized Residual Risk:** 1 in 780

AR=Autosomal recessive; XL=X-linked

Test methods and comments

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

Fragile X CGG Repeat Analysis (Analytical Detection Rate >99%)

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

Genotyping (Analytical Detection Rate >99%)

Multiplex PCR amplification and allele specific primer extension analyses using the MassARRAY® System were used to identify certain recurrent variants that are complex in nature or are present in low copy repeats. Rare sequence variants may interfere with assay performance.

Multiplex Ligation-Dependent Probe Amplification (MLPA) (Analytical Detection Rate >99%)

MLPA® probe sets and reagents from MRC-Holland were used for copy number analysis of specific targets versus known control samples. False positive or negative results may occur due to rare sequence variants in target regions detected by MLPA probes. Analytical sensitivity and specificity of the MLPA method are both 99%.

For alpha thalassemia, the copy numbers of the *HBA1* and *HBA2* genes were analyzed. Alpha-globin gene deletions, triplications, and the Constant Spring (CS) mutation are assessed. This test is expected to detect approximately 90% of all alpha-thalassemia mutations, varying by ethnicity. Carriers of alpha-thalassemia with three or more *HBA* copies on one chromosome, and one or no copies on the other chromosome, may not be detected. With the exception of triplications, other benign alpha-globin gene polymorphisms will not be reported. Analyses of *HBA1* and *HBA2* are performed in association with long-range PCR of the coding regions followed by short-read sequencing.

For Duchenne muscular dystrophy, the copy numbers of all *DMD* exons were analyzed. Potentially pathogenic single exon deletions and duplications are confirmed by a second method. Analysis of *DMD* is performed in association with sequencing of the coding regions.

For congenital adrenal hyperplasia, the copy number of the *CYP21A2* gene was analyzed. This analysis can detect large deletions typically due to unequal meiotic crossing-over between *CYP21A2* and the pseudogene *CYP21A1P*. Classic 30-kb deletions make up approximately 20% of *CYP21A2* pathogenic alleles. This test may also identify certain point mutations in *CYP21A2* caused by gene conversion events between *CYP21A2* and *CYP21A1P*. Some carriers may not be identified by dosage sensitive methods as this testing cannot detect individuals with two copies (duplication) of the *CYP21A2* gene on one chromosome and loss of *CYP21A2* (deletion) on the other chromosome. Analysis of *CYP21A2* is performed in association with long-range PCR of the coding regions followed by short-read sequencing.

For spinal muscular atrophy (SMA), the copy numbers of the *SMN1* and *SMN2* genes were analyzed. The individual dosage of exons 7 and 8 as well as the combined dosage of exons 1, 4, 6 and 8 of *SMN1* and *SMN2* were assessed. Copy number gains and losses can be detected with this assay. Depending on ethnicity, 6 - 29 % of carriers will not be identified by dosage sensitive methods as this testing cannot detect individuals with two copies (duplication) of the *SMN1* gene on one chromosome and loss of *SMN1* (deletion) on the other chromosome (silent

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2+0 carrier) or individuals that carry an intragenic mutation in *SMN1*. Please also note that 2% of individuals diagnosed with SMA have a causative *SMN1* variant that occurred de novo, and therefore cannot be picked up by carrier screening in the parents. Analysis of *SMN1* is performed in association with short-read sequencing of exons 2a-7, followed by confirmation using long-range PCR (described below).

In individuals with two copies of *SMN1* with Ashkenazi Jewish, East Asian, African American, Native American or Caucasian ancestry, the presence or absence of c.*3+80T>G significantly increases or decreases, respectively, the likelihood of being a silent 2+0 silent carrier.

MLPA for Gaucher disease *(GBA)*, cystic fibrosis *(CFTR)*, and non-syndromic hearing loss *(GJB2/GJB6)* will only be performed if indicated for confirmation of detected CNVs. If *GBA* analysis was performed, the copy numbers of exons 1, 3, 4, and 6 - 10 of the GBA gene (of 11 exons total) were analyzed. If *CFTR* analysis was performed, the copy numbers of all 27 *CFTR* exons were analyzed. If *GJB2/GJB6* analysis was performed, the copy number of the two *GJB2* exons were analyzed, as well as the presence or absence of the two upstream deletions of the *GJB2* regulatory region, del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854).

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

NGS was performed on a panel of genes for the purpose of identifying pathogenic or likely pathogenic variants.

Agilent SureSelect^{TM}XT Low Input technology was used with a custom capture library to target the exonic regions and intron/exon splice junctions of the relevant genes, as well as a number of UTR, intronic or promoter regions that contain previously reported mutations. Libraries were pooled and sequenced on the Illumina NovaSeq 6000 platform, using paired-end 100 bp reads. The sequencing data was analyzed using a custom bioinformatics algorithm designed and validated in house.

The coding exons and splice junctions of the known protein-coding RefSeq genes were assessed for the average depth of coverage (minimum of 20X) and data quality threshold values. Most exons not meeting a minimum of >20X read depth across the exon are further analyzed by Sanger sequencing. Please note that several genomic regions present difficulties in mapping or obtaining read depth >20X. These regions, which are described below, will not be reflexed to Sanger sequencing if the mapping quality or coverage is poor. Any variants identified during testing in these regions are confirmed by a second method and reported if determined to be pathogenic or likely pathogenic. However, as there is a possibility of false negative results within these regions, detection rates and residual risks for these genes have been calculated with the presumption that variants in these exons will not be detected, unless included in the MassARRAY$^{®}$ genotyping platform.

Exceptions: *ABCD1* (NM_000033.3) exons 8 and 9; *ACADSB* (NM_ 001609.3) chr10:124,810,695-124,810,707 (partial exon 9); *ADA* (NM_000022.2) exon 1; *ADAMTS2* (NM_014244.4) exon 1; *AGPS* (NM_003659.3) chr2:178,257,512-178,257,649 (partial exon 1); *ALDH7A1* (NM_001182.4) chr5:125,911,150-125,911,163 (partial exon 7) and chr5:125,896,807-125,896,821 (partial exon 10); *ALMS1* (NM_015120.4) chr2:73,612,990-73,613,041 (partial exon 1); *APOPT1* (NM_ 032374.4) chr14:104,040,437-104,040,455 (partial exon 3); *CDAN1* (NM_138477.2) exon 2; *CEP152* (NM_014985.3) chr15:49,061,146-49,061,165 (partial exon 14) and exon 22; *CEP290* (NM_025114.3) exon 5, exon 7, chr12:88,519,017-88,519,039 (partial exon 13), chr12:88,514,049-88,514,058 (partial exon 15), chr12:88,502,837-88,502,841 (partial exon 23), chr12:88,481,551-88,481,589 (partial exon 32), chr12:88,471,605-88,471,700 (partial exon 40); *CFTR* (NM_000492.3) exon 10; *COL4A4* (NM_000092.4) chr2:227,942,604-227,942,619 (partial exon 25); *COX10* (NM_001303.3) exon 6; *CYP11B1* (NM_000497.3) exons 3-7; *CYP11B2* (NM_000498.3) exons 3-7; *DNAI2* (NM_023036.4) chr17:72,308,136-72,308,147 (partial exon 12); *DOK7* (NM_173660.4) chr4:3,465,131-3,465,161 (partial exon 1) and exon 2; *DUOX2* (NM_014080.4) exons 6-8; *EIF2AK3* (NM_004836.5 exon 8; *EVC* (NM_153717.2) exon 1; *F5*(NM_000130.4) chr1:169,551,662-169,551,679 (partial exon 2); *FH* (NM_000143.3) exon 1; *GAMT* (NM_000156.5 exon 1; *GLDC*(NM_000170.2) exon 1; *GNPTAB* (NM_024312.4) chr17:4,837,000-4,837,400 (partial exon 2); *GNPTG* (NM_032520.4) exon 1; *GHR* (NM_000163.4) exon 3; *GYS2* (NM_021957.3) chr12:21,699,370-21,699,409 (partial exon 12); *HGSNAT* (NM_152419.2) exon 1; *IDS* (NM_000202.6 exon 3; *ITGB4* (NM_000213.4) chr17:73,749,976-73,750,060 (partial exon 33); *JAK3* (NM_000215.3) chr19:17,950,462-17,950,483 (partial exon 10); *LIFR* (NM_002310.5 exon 19; *LMBRD1* (NM_018368.3) chr6:70,459,226-70,459,257 (partial exon 5), chr6:70,447,828-70,447,836 (partial exon 7) and exon 12; *LYST* (NM_000081.3) chr1:235,944,158-235,944,176 (partial exon 16) and chr1:235,875,350-235,875,362 (partial exon 43); *MLYCD* (NM_012213.2) chr16:83,933,242-83,933,282 (partial exon 1); *MTR* (NM_000254.2) chr1 237,024,418-237,024,439 (partial exon 20) and chr1:237,038,019-237,038,029 (partial exon 24); *NBEAL2* (NM_015175.2) chr3 47,021,385-47,021,407 (partial exon 1); *NEB* (NM_001271208.1 exons 82-105; *NPC1* (NM_000271.4) chr18:21,123,519-21,123,538 (partial exon 14); *NPHP1* (NM_000272.3)chr2:110,937,251-110,937,263 (partial exon 3); *OCRL* (NM_000276.3) chrX:128,674,450-128,674,460 (partial exon 1); *PHKB* (NM_000293.2) exon 1 and chr16:47,732,498-47,732,504 (partial exon 30); *PIGN* (NM_176787.4) chr18:59,815,547-59,815,576 (partial exon 8); *PIP5K1C* (NM_012398.2) exon 1 and chr19:3637602-3637616 (partial exon 17); *POU1F1* (NM_000306.3) exon 5; *PTPRC* (NM_002838.4) exons 11 and 23; *PUS1* (NM_025215.5 chr12:132,414,446-132,414,532 (partial exon 2); *RPGRIP1L* (NM_015272.2) exon 23; *SGSH* (NM_000199.3) chr17:78,194,022-78,194,072 (partial exon 1); *SLC6A8* (NM_005629.3) exons 3 and 4; *ST3GAL5* (NM_003896.3) exon 1; *SURF1* (NM_003172.3) chr9:136,223,269-136,223,307 (partial exon 1); *TRPM6* (NM_017662.4) chr9:77,362,800-77,362,811 (partial exon 31); *TSEN54* (NM_207346.2) exon 1; *TYR* (NM_000372.4) exon 5; *VWF* (NM_000552.3) exons 24-26, chr12:6,125,675-6,125,684 (partial exon 30), chr12:6,121,244-6,121,265 (partial exon 33), and exon 34.

This test will detect variants within the exons and the intron-exon boundaries of the target regions. Variants outside these regions may not be detected, including, but not limited to, UTRs, promoters, and deep intronic areas, or regions that fall into the Exceptions mentioned above. This

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technology may not detect all small insertion/deletions and is not diagnostic for repeat expansions and structural genomic variation. In addition, a mutation(s) in a gene not included on the panel could be present in this patient.

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

Next Generation Sequencing for *SMN1*

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

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

Large duplications and deletions were called from the relative read depths on an exon-by-exon basis using a custom exome hidden Markov model (XHMM) algorithm. Deletions or duplications determined to be pathogenic or likely pathogenic were confirmed by either a custom arrayCGH platform, quantitative PCR, or MLPA (depending on CNV size and gene content). While this algorithm is designed to pick up deletions and duplications of 2 or more exons in length, potentially pathogenic single-exon CNVs will be confirmed and reported, if detected. Deletions and duplications near the lower limit of detection may not be detected due to run variability.

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

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

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

The relative quantification PCR is utilized on a Roche Universal Library Probe (UPL) system, which relates the PCR signal of the target region in one group to another. To test for genomic imbalances, both sample DNA and reference DNA is amplified with primer/probe sets that specific to the target region and a control region with known genomic copy number. Relative genomic copy numbers are calculated based on the standard $\Delta\Delta Ct$ 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. Please note that in rare cases, allele drop-out may occur, which has the potential to lead to false negative results. For *CYP21A2*, a certain percentage of healthy individuals carry a duplication of the *CYP21A2* gene, which has no clinical consequences. In cases where multiple copies of *CYP21A2* are located on the same chromosome in tandem, only the last copy will be amplified and assessed for potentially pathogenic variants, due to size limitations of the PCR reaction. However, because these alleles contain at least two copies of the *CYP21A2* gene in tandem, it is expected that this patient has at least one functional gene in the tandem allele and this patient is therefore less likely to be a carrier. A *CYP21A1P/CYP21A2* hybrid gene detected only by MLPA but not by long-range PCR will not be reported when the long-range PCR indicates the presence of two full *CYP21A2* gene copies (one on each chromosome), as the additional hybrid gene is nonfunctional. Classic 30-kb deletions are identified by MLPA and are also identified by the presence of multiple common pathogenic *CYP21A2* variants by long-range PCR. Since multiple pseudogene-derived variants are detected in all cases with the classic 30kb deletion, we cannot rule out the possibility that some variant(s) detected could be present in trans with the chimeric *CYP21A1P/CYP21A2* gene created by the 30kb deletion. When an individual carries both a duplication allele and a pathogenic variant, or multiple pathogenic variants, the current analysis may not be able to determine the phase (cis/trans configuration) of the *CYP21A2* alleles identified. Family studies may be required in certain scenarios where phasing is required to determine the carrier status.

Residual Risk Calculations

Carrier frequencies and detection rates for each ethnicity were calculated through the combination of internal curations of >30,000 variants and genomic frequency data from >138,000 individuals across seven ethnic groups in the gnomAD database. Additional variants in HGMD and novel deleterious variants were also incorporated into the calculation. Residual risk values are calculated using a Bayesian analysis combining the a *priori* risk of being a pathogenic mutation carrier (carrier frequency) and the detection rate. They are provided only as a guide for assessing approximate risk given a negative result, and values will vary based on the exact ethnic background of an individual. This report does

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not represent medical advice but should be interpreted by a genetic counselor, medical geneticist or physician skilled in genetic result interpretation and the relevant medical literature.

Personalized Residual Risk Calculations

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

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

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

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

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

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

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

This test was developed, and its performance characteristics determined by Sema4 Opco, Inc. It has not been cleared or approved by the US Food and Drug Administration. FDA does not require this test to go through premarket FDA review. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing. These analyses generally provide highly accurate information regarding the patient's carrier or affected status. Despite this high level of accuracy, it should be kept in mind that there are many potential sources of diagnostic error, including misidentification of samples, polymorphisms, or other rare genetic variants that interfere with analysis. Families should understand that rare diagnostic errors may occur for these reasons.

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Additional disease-specific references available upon request.

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