Integrated GENETICS
LabCorp Specialty Testing Group

Client/Sending Facility:
Phoenix Sperm Bank

1492 S Mill Ave Suite 306
Tempe, AZ 85281
Ph: (602)888-7255
AZB-45

LCLS Specimen Number:	**274-944-3299-0**	Account Number:	[redacted]
Patient Name:	**10083, DONOR**	Ordering Physician:	**J OLLIFFE**
Date of Birth:	[redacted]	Specimen Type:	**BLOOD**
Gender:	M	Client Reference:	
Patient ID:		Date Collected:	09/30/2016
Lab Number:	YU16-78080 L	Date Received:	10/02/2016
Indications:	NOT GIVEN	Date Reported:	**10/13/2016**

Test: **Chromosome, Blood, Routine**

Cells Counted:	20	Cells Karyotyped:	2
Cells Analyzed:	20	Band Resolution:	500

CYTOGENETIC RESULT: 46,XY

INTERPRETATION: NORMAL MALE KARYOTYPE

Cytogenetic analysis of PHA stimulated cultures has revealed a MALE karyotype with an apparently normal GTG banding pattern in all cells observed.

This result does not exclude the possibility of subtle rearrangements below the resolution of cytogenetics or congenital anomalies due to other etiologies.

Chromosome analysis performed by Laboratory Corporation of America Inc., CLIA 29D0539775. 1015 Telegraph St. Suite B, Reno, NV 89502. Laboratory Director, Roger S Ritzlin, M.D.

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Date Received: 10/02/2016

John E. Wiley, Ph.D., FACMG.
Board Certified Cytogeneticist

Arundhati Chatterjee, MD
Medical Director
Peter Papenhausen, PhD
National Director of Cytogenetics

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Professional Component performed by LabCorp CLIA 34D1008914, 1904 TW Alexander Dr, Research Triangle Park, NC 27709. Medical Director, Arundhati Chatterjee, MD.
Integrated Genetics is a brand used by Esoterix Genetic Laboratories, LLC, a wholly-owned subsidiary of Laboratory Corporation of America Holdings.

Counsyl

RESULTS RECIPIENT
SEATTLE SPERM BANK
Attn: Dr. Jeffrey Olliffe
4915 25th Ave NE, Suite 204W
Seattle, WA 98105
Phone: (206) 588-1484
Fax: (206) 588-1484
NPI: 1306838271
Report Date: 10/10/2016

MALE
DONOR 10083
DOB: [redacted]
Ethnicity: Mixed or Other Caucasian
Sample Type: EDTA Blood
Date of Collection:
Date Received: 10/01/2016
Date Tested: 10/10/2016
Barcode: 11200059725005
Indication: Egg or sperm donor

FEMALE
N/A

Family Prep Screen

NEGATIVE

ABOUT THIS TEST

The Counsyl Family Prep Screen (version 2.0) utilizes sequencing, maximizing coverage across all DNA regions tested, to help you learn about your chance to have a child with a genetic disease.

RESULTS SUMMARY

Risk Details	DONOR 10083	Partner
Panel Information	Family Prep Screen 2.0 Universal Panel Minus X-Linked **(102 conditions tested)**	N/A
All conditions tested A complete list of all conditions tested can be found on page 4.	⊖ **NEGATIVE** No disease-causing mutations were detected.	N/A

CLINICAL NOTES

- None

NEXT STEPS

- If necessary, patients can discuss residual risks with their physician or a genetic counselor.

Methods and Limitations

DONOR 10083 [Family Prep Screen 2.0]: sequencing, targeted genotyping, copy number analysis, and analysis of homologous regions.

Sequencing

High-throughput sequencing is used to analyze the listed exons, as well as selected intergenic and intronic regions, of the genes in the Conditions Tested section of the report. These regions are sequenced to high coverage and the sequences are compared to standards and references of normal variation. Mutations may not be detected in areas of lower sequence coverage. On average, more than 99% of all bases in the exons listed for each gene are sequenced at the minimum read depth. Variants discovered in other exons of these genes will also be reported if they meet quality control criteria. Triplet repeats and large deletions and duplications may not be detected. Small insertions and deletions may not be as accurately determined as single nucleotide variants. Genes that have closely related pseudogenes are not well analyzed by this method.

Detection rates are calculated by estimating from literature the fraction of disease alleles that the methodology is unable to detect.

All variants that are a recognized cause of the disease will be reported. In addition, variants that have not previously been established as a recognized cause of disease may be identified. In these cases, only variants classified as "predicted" or "likely" pathogenic are reported. Predicted/likely pathogenic variants are described elsewhere in the report as "predicted/likely to have a negative impact on gene function". In general, predicted pathogenic variants are those which are predicted to be pathogenic based on the nature of the sequence change, while likely pathogenic variants are evaluated by reviewing reports of allele frequencies in cases and controls, functional studies, variant annotation and effect prediction, and segregation studies. Benign variants, variants of uncertain significance, and variants not directly associated with the intended disease phenotype are not reported. Literature citations validating reported variants are available upon request.

Targeted genotyping

Targeted DNA mutation analysis is used to determine the genotypes of the listed variants in the Conditions Tested section of the report. The test is not validated for detection of homozygous mutations, and although rare, asymptomatic individuals affected by the disease may not be genotyped accurately.

Copy number analysis

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

Analysis of homologous regions

A combination of high-throughput sequencing, read depth-based copy number analysis, and targeted genotyping is used to determine the number of functional gene copies and/or the presence of selected loss of function mutations in certain genes that have homology to other regions. The precise breakpoints of large deletions in these regions cannot be determined, but are estimated from copy number analysis. Patients who have one or more additional copies of the *CYP21A2* gene and a loss of function mutation may not actually be a carrier of 21-hydroxylase-deficient congenital adrenal hyperplasia (CAH). In addition, some individuals with four alpha globin genes are carriers with three genes on one chromosome and a deletion on the other chromosome. This and similar carrier states, where complementary changes exist in both the gene and a pseudogene, may not be detected by the assay.

Because the true incidence of non-classic CAH is unknown, the residual carrier and reproductive risk numbers on the report are only based on published incidences for classic CAH. However, the published prevalence of non-classic CAH is highest in individuals of Ashkenazi Jewish, Hispanic, Italian, and Yugoslav descent. Therefore, the residual and reproductive risks are likely an underestimate of overall chances for 21-hydroxylase-deficient CAH, especially in the aforementioned populations, as they do not account for non-classic CAH.

Limitations

In an unknown number of cases, nearby genetic variants may interfere with mutation detection. Other possible sources of diagnostic error include sample mix-up, trace contamination, bone marrow transplantation, blood transfusions and technical errors. If more than one variant is detected in a gene, additional studies may be necessary to determine if those variants lie on the same chromosome or different chromosomes. The Family Prep Screen does not fully address all inherited forms of intellectual disability, birth defects and genetic disease. A family history of any of these conditions may warrant additional evaluation. Furthermore, not all mutations will be identified in the genes analyzed and additional testing may be beneficial for some patients. For example, individuals of African, Southeast Asian, and Mediterranean ancestry are at increased risk for being carriers for hemoglobinopathies, which can be identified by CBC and hemoglobin electrophoresis or HPLC (*ACOG Practice Bulletin No. 78. Obstet.Gynecol. 2007;109:229-37*), and additional Tay-Sachs disease testing can be performed using a biochemical assay (*Gross et al. Genet. Med. 2008:10(1):54-56*).

This test was developed and its performance characteristics determined by Counsyl, Inc. It has not been cleared or approved by the US Food and Drug Administration (FDA). The FDA does not require this test to go through premarket review. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical testing. These results are adjunctive to the ordering physician's evaluation. CLIA Number: **#05D1102604**.

LAB DIRECTORS

Hyunseok Kang

H. Peter Kang, MD, MS, FCAP

Conditions Tested

21-Hydroxylase-Deficient Congenital Adrenal Hyperplasia - **Gene:** CYP21A2. Autosomal Recessive. Analysis of Homologous Regions. **Variants (13):** CYP21A2 deletion, CYP21A2 duplication, CYP21A2 triplication, G111VfsX21, I173N, L308FfsX6, P31L, Q319*, Q319*+CYP21A2dup, R357W, V281L, [I237N;V238E;M240K], c.293-13C>G. **Detection Rate:** Mixed or Other Caucasian 96%.

ABCC8-related Hyperinsulinism - **Gene:** ABCC8. Autosomal Recessive. Sequencing. **Exons:** NM_000352:1-39. **Detection Rate:** Mixed or Other Caucasian >99%.

Achromatopsia - **Gene:** CNGB3. Autosomal Recessive. Sequencing. **Exons:** NM_019098:1-18. **Detection Rate:** Mixed or Other Caucasian >99%.

Alkaptonuria - **Gene:** HGD. Autosomal Recessive. Sequencing. **Exons:** NM_000187:1-14. **Detection Rate:** Mixed or Other Caucasian >99%.

Alpha Thalassemia - **Genes:** HBA1, HBA2. Autosomal Recessive. Analysis of Homologous Regions. **Variants (13):** -(alpha)20.5, --BRIT, --MEDI, --MEDII, --SEA, --THAI or --FIL, -alpha3.7, -alpha4.2, HBA1+HBA2 deletion, Hb Constant Spring, anti3.7, anti4.2, del HS-40. **Detection Rate:** Unknown due to rarity of disease.

Alpha-1 Antitrypsin Deficiency - **Gene:** SERPINA1. Autosomal Recessive. Sequencing. **Exons:** NM_000295:2-5. **Detection Rate:** Mixed or Other Caucasian >99%.

Alpha-Mannosidosis - **Gene:** MAN2B1. Autosomal Recessive. Sequencing. **Exons:** NM_000528:1-15,17-24. **Detection Rate:** Mixed or Other Caucasian >99%.

Andermann Syndrome - **Gene:** SLC12A6. Autosomal Recessive. Sequencing. **Exons:** NM_133647:1-25. **Detection Rate:** Mixed or Other Caucasian >99%.

ARSACS - **Gene:** SACS. Autosomal Recessive. Sequencing. **Exons:** NM_014363:2-10. **Detection Rate:** Mixed or Other Caucasian >99%.

Aspartylglycosaminuria - **Gene:** AGA. Autosomal Recessive. Sequencing. **Exons:** NM_000027:1-9. **Detection Rate:** Mixed or Other Caucasian >99%.

Ataxia With Vitamin E Deficiency - **Gene:** TTPA. Autosomal Recessive. Sequencing. **Exons:** NM_000370:1-5. **Detection Rate:** Mixed or Other Caucasian >99%.

Ataxia-Telangiectasia - **Gene:** ATM. Autosomal Recessive. Sequencing. **Exons:** NM_000051:2-63. **Detection Rate:** Mixed or Other Caucasian 92%.

Autosomal Recessive Polycystic Kidney Disease - **Gene:** PKHD1. Autosomal Recessive. Sequencing. **Exons:** NM_138694:2-67. **Detection Rate:** Mixed or Other Caucasian >99%.

Bardet-Biedl Syndrome, BBS1-related - **Gene:** BBS1. Autosomal Recessive. Sequencing. **Exons:** NM_024649:1-17. **Detection Rate:** Mixed or Other Caucasian >99%.

Bardet-Biedl Syndrome, BBS10-related - **Gene:** BBS10. Autosomal Recessive. Sequencing. **Exons:** NM_024685:1-2. **Detection Rate:** Mixed or Other Caucasian >99%.

Biotinidase Deficiency - **Gene:** BTD. Autosomal Recessive. Sequencing. **Exons:** NM_000060:1-4. **Detection Rate:** Mixed or Other Caucasian >99%.

Bloom Syndrome - **Gene:** BLM. Autosomal Recessive. Sequencing. **Exons:** NM_000057:2-22. **Detection Rate:** Mixed or Other Caucasian >99%.

Canavan Disease - **Gene:** ASPA. Autosomal Recessive. Sequencing. **Exons:** NM_000049:1-6. **Detection Rate:** Mixed or Other Caucasian 94%.

Carnitine Palmitoyltransferase IA Deficiency - **Gene:** CPT1A. Autosomal Recessive. Sequencing. **Exons:** NM_001876:2-19. **Detection Rate:** Mixed or Other Caucasian >99%.

Carnitine Palmitoyltransferase II Deficiency - **Gene:** CPT2. Autosomal Recessive. Sequencing. **Exons:** NM_000098:1-5. **Detection Rate:** Mixed or Other Caucasian >99%.

Cartilage-Hair Hypoplasia - **Gene:** RMRP. Autosomal Recessive. Sequencing. **Exon:** NR_003051:1. **Detection Rate:** Mixed or Other Caucasian >99%.

Citrullinemia Type 1 - **Gene:** ASS1. Autosomal Recessive. Sequencing. **Exons:** NM_000050:3-16. **Detection Rate:** Mixed or Other Caucasian >99%.

CLN3-related Neuronal Ceroid Lipofuscinosis - **Gene:** CLN3. Autosomal Recessive. Sequencing. **Exons:** NM_001042432:2-16. **Detection Rate:** Mixed or Other Caucasian >99%.

CLN5-related Neuronal Ceroid Lipofuscinosis - **Gene:** CLN5. Autosomal Recessive. Sequencing. **Exons:** NM_006493:1-4. **Detection Rate:** Mixed or Other Caucasian 98%.

Cohen Syndrome - **Gene:** VPS13B. Autosomal Recessive. Sequencing. **Exons:** NM_017890:2-62. **Detection Rate:** Mixed or Other Caucasian 83%.

Congenital Disorder of Glycosylation Type Ia - **Gene:** PMM2. Autosomal Recessive. Sequencing. **Exons:** NM_000303:1-8. **Detection Rate:** Mixed or Other Caucasian >99%.

Congenital Disorder of Glycosylation Type Ib - **Gene:** MPI. Autosomal Recessive. Sequencing. **Exons:** NM_002435:1-8. **Detection Rate:** Mixed or Other Caucasian >99%.

Congenital Finnish Nephrosis - **Gene:** NPHS1. Autosomal Recessive. Sequencing. **Exons:** NM_004646:2-23,26-27,29. **Detection Rate:** Mixed or Other Caucasian >99%.

Costeff Optic Atrophy Syndrome - **Gene:** OPA3. Autosomal Recessive. Sequencing. **Exons:** NM_025136:1-2. **Detection Rate:** Mixed or Other Caucasian >99%.

Cystic Fibrosis - **Gene:** CFTR. Autosomal Recessive. Sequencing. **Exons:** NM_000492:1-27. IVS8-5T allele analysis is only reported in the presence of the R117H mutation. **Detection Rate:** Mixed or Other Caucasian 97%.

Cystinosis - **Gene:** CTNS. Autosomal Recessive. Sequencing. **Exons:** NM_004937:3-12. **Detection Rate:** Mixed or Other Caucasian >99%.

D-Bifunctional Protein Deficiency - **Gene:** HSD17B4. Autosomal Recessive. Sequencing. **Exons:** NM_000414:1-24. **Detection Rate:** Mixed or Other Caucasian >99%.

Factor XI Deficiency - **Gene:** F11. Autosomal Recessive. Sequencing. **Exons:** NM_000128:2-15. **Detection Rate:** Mixed or Other Caucasian >99%.

Familial Dysautonomia - **Gene:** IKBKAP. Autosomal Recessive. Sequencing. **Exons:** NM_003640:19-20,26. **Detection Rate:** Mixed or Other Caucasian >99%.

Familial Mediterranean Fever - **Gene:** MEFV. Autosomal Recessive. Sequencing. **Exons:** NM_000243:1-10. **Detection Rate:** Mixed or Other Caucasian >99%.

Fanconi Anemia Type C - **Gene:** FANCC. Autosomal Recessive. Sequencing. **Exons:** NM_000136:2-15. **Detection Rate:** Mixed or Other Caucasian >99%.

Galactosemia - **Gene:** GALT. Autosomal Recessive. Sequencing. **Exons:** NM_000155:1-11. **Detection Rate:** Mixed or Other Caucasian >99%.

Gaucher Disease - **Gene:** GBA. Autosomal Recessive. Targeted Genotyping. **Variants (10):** D409V, D448H, IVS2+1G>A, L444P, N370S, R463C, R463H, R496H, V394L, p.L29Afs*18. **Detection Rate:** Mixed or Other Caucasian 60%.

GJB2-related DFNB1 Nonsyndromic Hearing Loss and Deafness - **Gene:** GJB2. Autosomal Recessive. Sequencing. **Exons:** NM_004004:1-2. **Detection Rate:** Mixed or Other Caucasian 98%.

Glutaric Acidemia Type 1 - **Gene:** GCDH. Autosomal Recessive. Sequencing. **Exons:** NM_000159:2-12. **Detection Rate:** Mixed or Other Caucasian >99%.

Glycogen Storage Disease Type Ia - **Gene:** G6PC. Autosomal Recessive. Sequencing. **Exons:** NM_000151:1-5. **Detection Rate:** Mixed or Other Caucasian >99%.

Glycogen Storage Disease Type Ib - **Gene:** SLC37A4. Autosomal Recessive. Sequencing. **Exons:** NM_001164277:3-11. **Detection Rate:** Mixed or Other Caucasian >99%.

Glycogen Storage Disease Type III - **Gene:** AGL. Autosomal Recessive. Sequencing. **Exons:** NM_000642:2-34. **Detection Rate:** Mixed or Other Caucasian >99%.

Glycogen Storage Disease Type V - **Gene:** PYGM. Autosomal Recessive. Sequencing. **Exons:** NM_005609:1-20. **Detection Rate:** Mixed or Other Caucasian >99%.

GRACILE Syndrome - **Gene:** BCS1L. Autosomal Recessive. Sequencing. **Exons:** NM_004328:3-9. **Detection Rate:** Mixed or Other Caucasian >99%.

Hb Beta Chain-Related Hemoglobinopathy (Including Beta Thalassemia and Sickle Cell Disease) - **Gene:** HBB. Autosomal Recessive. Sequencing. **Exons:** NM_000518:1-3. **Detection Rate:** Mixed or Other Caucasian 96%.

Hereditary Fructose Intolerance - **Gene:** ALDOB. Autosomal Recessive. Sequencing. **Exons:** NM_000035:2-9. **Detection Rate:** Mixed or Other Caucasian >99%.

Hereditary Thymine-Uraciluria - **Gene:** DPYD. Autosomal Recessive. Sequencing. **Exons:** NM_000110:1-23. **Detection Rate:** Mixed or Other Caucasian >99%.

Herlitz Junctional Epidermolysis Bullosa, LAMA3-related - **Gene:** LAMA3. Autosomal Recessive. Sequencing. **Exons:** NM_000227:1-16,18-38. **Detection Rate:** Mixed or Other Caucasian >99%.

Herlitz Junctional Epidermolysis Bullosa, LAMB3-related - **Gene:** LAMB3. Autosomal Recessive. Sequencing. **Exons:** NM_000228:2-23. **Detection Rate:** Mixed or Other Caucasian >99%.

Herlitz Junctional Epidermolysis Bullosa, LAMC2-related - **Gene:** LAMC2. Autosomal Recessive. Sequencing. **Exons:** NM_005562:1-23. **Detection Rate:** Mixed or Other Caucasian >99%.
Hexosaminidase A Deficiency (Including Tay-Sachs Disease) - **Gene:** HEXA. Autosomal Recessive. Sequencing. **Exons:** NM_000520:1-14. **Detection Rate:** Mixed or Other Caucasian >99%.
Homocystinuria Caused by Cystathionine Beta-Synthase Deficiency - **Gene:** CBS. Autosomal Recessive. Sequencing. **Exons:** NM_000071:3-17. **Detection Rate:** Mixed or Other Caucasian >99%.
Hurler Syndrome - **Gene:** IDUA. Autosomal Recessive. Targeted Genotyping. **Variants (2):** Q70*, W402*. **Detection Rate:** Mixed or Other Caucasian 67%.
Hypophosphatasia, Autosomal Recessive - **Gene:** ALPL. Autosomal Recessive. Sequencing. **Exons:** NM_000478:2-12. **Detection Rate:** Mixed or Other Caucasian >99%.
Inclusion Body Myopathy 2 - **Gene:** GNE. Autosomal Recessive. Sequencing. **Exons:** NM_001128227:3-12. **Detection Rate:** Mixed or Other Caucasian >99%.
Isovaleric Acidemia - **Gene:** IVD. Autosomal Recessive. Sequencing. **Exons:** NM_002225:1-12. **Detection Rate:** Mixed or Other Caucasian >99%.
Joubert Syndrome 2 - **Gene:** TMEM216. Autosomal Recessive. Sequencing. **Exons:** NM_001173990:1-5. **Detection Rate:** Mixed or Other Caucasian >99%.
Krabbe Disease - **Gene:** GALC. Autosomal Recessive. Sequencing. **Exons:** NM_000153:1-17. **Detection Rate:** Mixed or Other Caucasian >99%.
Limb-Girdle Muscular Dystrophy Type 2D - **Gene:** SGCA. Autosomal Recessive. Sequencing. **Exons:** NM_000023:1-9. **Detection Rate:** Mixed or Other Caucasian >99%.
Limb-Girdle Muscular Dystrophy Type 2E - **Gene:** SGCB. Autosomal Recessive. Sequencing. **Exons:** NM_000232:1-6. **Detection Rate:** Mixed or Other Caucasian >99%.
Lipoamide Dehydrogenase Deficiency - **Gene:** DLD. Autosomal Recessive. Sequencing. **Exons:** NM_000108:1-14. **Detection Rate:** Mixed or Other Caucasian >99%.
Long Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency - **Gene:** HADHA. Autosomal Recessive. Sequencing. **Exons:** NM_000182:1-20. **Detection Rate:** Mixed or Other Caucasian >99%.
Maple Syrup Urine Disease Type 1B - **Gene:** BCKDHB. Autosomal Recessive. Sequencing. **Exons:** NM_183050:1-10. **Detection Rate:** Mixed or Other Caucasian >99%.
Medium Chain Acyl-CoA Dehydrogenase Deficiency - **Gene:** ACADM. Autosomal Recessive. Sequencing. **Exons:** NM_000016:1-12. **Detection Rate:** Mixed or Other Caucasian >99%.
Megalencephalic Leukoencephalopathy With Subcortical Cysts - **Gene:** MLC1. Autosomal Recessive. Sequencing. **Exons:** NM_015166:2-12. **Detection Rate:** Mixed or Other Caucasian >99%.
Metachromatic Leukodystrophy - **Gene:** ARSA. Autosomal Recessive. Sequencing. **Exons:** NM_000487:1-8. **Detection Rate:** Mixed or Other Caucasian >99%.
Mucolipidosis IV - **Gene:** MCOLN1. Autosomal Recessive. Sequencing. **Exons:** NM_020533:1-14. **Detection Rate:** Mixed or Other Caucasian >99%.
Muscle-Eye-Brain Disease - **Gene:** POMGNT1. Autosomal Recessive. Sequencing. **Exons:** NM_017739:2-22. **Detection Rate:** Mixed or Other Caucasian >99%.
NEB-related Nemaline Myopathy - **Gene:** NEB. Autosomal Recessive. Sequencing. **Exons:** NM_004543:7-8,18,25,28,33,36,45,48,54-55,58,61,71,73-74,91,94,101,111-112, 114,118-119,122-123,127,129,132-135,138,140,143,146-147. **Detection Rate:** Mixed or Other Caucasian >99%.
Niemann-Pick Disease Type C - **Gene:** NPC1. Autosomal Recessive. Sequencing. **Exons:** NM_000271:1-25. **Detection Rate:** Mixed or Other Caucasian 96%.
Niemann-Pick Disease, SMPD1-associated - **Gene:** SMPD1. Autosomal Recessive. Sequencing. **Exons:** NM_000543:1-6. **Detection Rate:** Mixed or Other Caucasian >99%.
Nijmegen Breakage Syndrome - **Gene:** NBN. Autosomal Recessive. Sequencing. **Exons:** NM_002485:1-16. **Detection Rate:** Mixed or Other Caucasian >99%.
Northern Epilepsy - **Gene:** CLN8. Autosomal Recessive. Sequencing. **Exons:** NM_018941:2-3. **Detection Rate:** Mixed or Other Caucasian >99%.
Pendred Syndrome - **Gene:** SLC26A4. Autosomal Recessive. Sequencing. **Exons:** NM_000441:2-21. **Detection Rate:** Mixed or Other Caucasian >99%.
PEX1-related Zellweger Syndrome Spectrum - **Gene:** PEX1. Autosomal Recessive. Sequencing. **Exons:** NM_000466:1-24. **Detection Rate:** Mixed or Other Caucasian >99%.
Phenylalanine Hydroxylase Deficiency - **Gene:** PAH. Autosomal Recessive. Sequencing. **Exons:** NM_000277:1-13. **Detection Rate:** Mixed or Other Caucasian 98%.
Polyglandular Autoimmune Syndrome Type 1 - **Gene:** AIRE. Autosomal Recessive. Sequencing. **Exons:** NM_000383:1-14. **Detection Rate:** Mixed or Other Caucasian >99%.
Pompe Disease - **Gene:** GAA. Autosomal Recessive. Sequencing. **Exons:** NM_000152:2-20. **Detection Rate:** Mixed or Other Caucasian 90%.
PPT1-related Neuronal Ceroid Lipofuscinosis - **Gene:** PPT1. Autosomal Recessive. Sequencing. **Exons:** NM_000310:1-9. **Detection Rate:** Mixed or Other Caucasian >99%.
Primary Carnitine Deficiency - **Gene:** SLC22A5. Autosomal Recessive. Sequencing. **Exons:** NM_003060:1-10. **Detection Rate:** Mixed or Other Caucasian >99%.
Primary Hyperoxaluria Type 1 - **Gene:** AGXT. Autosomal Recessive. Sequencing. **Exons:** NM_000030:1-11. **Detection Rate:** Mixed or Other Caucasian >99%.
Primary Hyperoxaluria Type 2 - **Gene:** GRHPR. Autosomal Recessive. Sequencing. **Exons:** NM_012203:1-9. **Detection Rate:** Mixed or Other Caucasian >99%.
PROP1-related Combined Pituitary Hormone Deficiency - **Gene:** PROP1. Autosomal Recessive. Sequencing. **Exons:** NM_006261:1-3. **Detection Rate:** Mixed or Other Caucasian >99%.
Pseudocholinesterase Deficiency - **Gene:** BCHE. Autosomal Recessive. Sequencing. **Exons:** NM_000055:2-4. **Detection Rate:** Mixed or Other Caucasian >99%.
Pycnodysostosis - **Gene:** CTSK. Autosomal Recessive. Sequencing. **Exons:** NM_000396:2-8. **Detection Rate:** Mixed or Other Caucasian >99%.
Rhizomelic Chondrodysplasia Punctata Type 1 - **Gene:** PEX7. Autosomal Recessive. Sequencing. **Exons:** NM_000288:1-10. **Detection Rate:** Mixed or Other Caucasian >99%.
Salla Disease - **Gene:** SLC17A5. Autosomal Recessive. Sequencing. **Exons:** NM_012434:1-11. **Detection Rate:** Mixed or Other Caucasian >99%.
Segawa Syndrome - **Gene:** TH. Autosomal Recessive. Sequencing. **Exons:** NM_000360:1-13. **Detection Rate:** Mixed or Other Caucasian >99%.
Short Chain Acyl-CoA Dehydrogenase Deficiency - **Gene:** ACADS. Autosomal Recessive. Sequencing. **Exons:** NM_000017:1-10. **Detection Rate:** Mixed or Other Caucasian >99%.
Sjogren-Larsson Syndrome - **Gene:** ALDH3A2. Autosomal Recessive. Sequencing. **Exons:** NM_000382:1-10. **Detection Rate:** Mixed or Other Caucasian >99%.
Smith-Lemli-Opitz Syndrome - **Gene:** DHCR7. Autosomal Recessive. Sequencing. **Exons:** NM_001360:3-9. **Detection Rate:** Mixed or Other Caucasian >99%.
Spinal Muscular Atrophy - **Gene:** SMN1. Autosomal Recessive. Copy Number Analysis. **Variant (1):** SMN1 copy number. **Detection Rate:** Mixed or Other Caucasian 95%.
Steroid-Resistant Nephrotic Syndrome - **Gene:** NPHS2. Autosomal Recessive. Sequencing. **Exons:** NM_014625:1-8. **Detection Rate:** Mixed or Other Caucasian >99%.
Sulfate Transporter-Related Osteochondrodysplasia - **Gene:** SLC26A2. Autosomal Recessive. Sequencing. **Exons:** NM_000112:2-3. **Detection Rate:** Mixed or Other Caucasian >99%.
TPP1-related Neuronal Ceroid Lipofuscinosis - **Gene:** TPP1. Autosomal Recessive. Sequencing. **Exons:** NM_000391:1-13. **Detection Rate:** Mixed or Other Caucasian >99%.
Tyrosinemia Type I - **Gene:** FAH. Autosomal Recessive. Sequencing. **Exons:** NM_000137:1-14. **Detection Rate:** Mixed or Other Caucasian >99%.
Usher Syndrome Type 1F - **Gene:** PCDH15. Autosomal Recessive. Sequencing. **Exons:** NM_033056:2-33. **Detection Rate:** Mixed or Other Caucasian 97%.
Usher Syndrome Type 3 - **Gene:** CLRN1. Autosomal Recessive. Sequencing. **Exons:** NM_174878:1-3. **Detection Rate:** Mixed or Other Caucasian >99%.
Very Long Chain Acyl-CoA Dehydrogenase Deficiency - **Gene:** ACADVL. Autosomal Recessive. Sequencing. **Exons:** NM_000018:1-20. **Detection Rate:** Mixed or Other Caucasian >99%.
Walker-Warburg Syndrome - **Gene:** FKTN. Autosomal Recessive. Sequencing. **Exons:** NM_001079802:3-11. **Detection Rate:** Mixed or Other Caucasian >99%.
Wilson Disease - **Gene:** ATP7B. Autosomal Recessive. Sequencing. **Exons:** NM_000053:1-21. **Detection Rate:** Mixed or Other Caucasian >99%.

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RESULTS RECIPIENT
SEATTLE SPERM BANK
Attn: Dr. Jeffrey Olliffe
NPI: 1306838271
Report Date: 10/10/2016

MALE
DONOR 10083
DOB: [redacted]
Ethnicity: Mixed or Other Caucasian
Barcode: 11200059725005

FEMALE
N/A

Risk Calculations

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

Disease	DONOR 10083 Residual Risk	Reproductive Risk
21-Hydroxylase-Deficient Congenital Adrenal Hyperplasia	1 in 1,400	1 in 310,000
ABCC8-related Hyperinsulinism	1 in 11,000	< 1 in 1,000,000
Achromatopsia	1 in 8,600	< 1 in 1,000,000
Alkaptonuria	< 1 in 500	< 1 in 1,000,000
Alpha Thalassemia	Alpha globin status: aa/aa.	Not calculated
Alpha-1 Antitrypsin Deficiency	1 in 3,400	1 in 460,000
Alpha-Mannosidosis	1 in 35,000	< 1 in 1,000,000
Andermann Syndrome	< 1 in 500	< 1 in 1,000,000
ARSACS	< 1 in 500	< 1 in 1,000,000
Aspartylglycosaminuria	< 1 in 500	< 1 in 1,000,000
Ataxia With Vitamin E Deficiency	< 1 in 500	< 1 in 1,000,000
Ataxia-Telangiectasia	1 in 2,100	< 1 in 1,000,000
Autosomal Recessive Polycystic Kidney Disease	1 in 6,100	< 1 in 1,000,000
Bardet-Biedl Syndrome, BBS1-related	1 in 16,000	< 1 in 1,000,000
Bardet-Biedl Syndrome, BBS10-related	1 in 16,000	< 1 in 1,000,000
Biotinidase Deficiency	1 in 12,000	< 1 in 1,000,000
Bloom Syndrome	< 1 in 500	< 1 in 1,000,000
Canavan Disease	< 1 in 500	< 1 in 1,000,000
Carnitine Palmitoyltransferase IA Deficiency	< 1 in 500	< 1 in 1,000,000
Carnitine Palmitoyltransferase II Deficiency	< 1 in 500	< 1 in 1,000,000
Cartilage-Hair Hypoplasia	< 1 in 500	< 1 in 1,000,000
Citrullinemia Type 1	1 in 12,000	< 1 in 1,000,000
CLN3-related Neuronal Ceroid Lipofuscinosis	1 in 22,000	< 1 in 1,000,000
CLN5-related Neuronal Ceroid Lipofuscinosis	< 1 in 500	< 1 in 1,000,000
Cohen Syndrome	< 1 in 500	< 1 in 1,000,000
Congenital Disorder of Glycosylation Type Ia	1 in 16,000	< 1 in 1,000,000
Congenital Disorder of Glycosylation Type Ib	< 1 in 500	< 1 in 1,000,000
Congenital Finnish Nephrosis	< 1 in 500	< 1 in 1,000,000
Costeff Optic Atrophy Syndrome	< 1 in 500	< 1 in 1,000,000
Cystic Fibrosis	1 in 910	1 in 99,000
Cystinosis	1 in 22,000	< 1 in 1,000,000
D-Bifunctional Protein Deficiency	< 1 in 500	< 1 in 1,000,000
Factor XI Deficiency	< 1 in 500	< 1 in 1,000,000
Familial Dysautonomia	< 1 in 500	< 1 in 1,000,000
Familial Mediterranean Fever	< 1 in 500	< 1 in 1,000,000
Fanconi Anemia Type C	1 in 16,000	< 1 in 1,000,000
Galactosemia	1 in 8,600	< 1 in 1,000,000
Gaucher Disease	1 in 280	1 in 120,000
GJB2-related DFNB1 Nonsyndromic Hearing Loss and Deafness	1 in 1,700	1 in 220,000
Glutaric Acidemia Type 1	1 in 10,000	< 1 in 1,000,000
Glycogen Storage Disease Type Ia	1 in 18,000	< 1 in 1,000,000
Glycogen Storage Disease Type Ib	1 in 35,000	< 1 in 1,000,000
Glycogen Storage Disease Type III	1 in 16,000	< 1 in 1,000,000
Glycogen Storage Disease Type V	1 in 16,000	< 1 in 1,000,000
GRACILE Syndrome	< 1 in 500	< 1 in 1,000,000
Hb Beta Chain-Related Hemoglobinopathy (Including Beta Thalassemia and Sickle Cell Disease)	1 in 1,200	1 in 240,000
Hereditary Fructose Intolerance	1 in 8,000	< 1 in 1,000,000
Hereditary Thymine-Uraciluria	1 in 10,000	< 1 in 1,000,000
Herlitz Junctional Epidermolysis Bullosa, LAMA3-related	< 1 in 500	< 1 in 1,000,000

Counsyl

RESULTS RECIPIENT
SEATTLE SPERM BANK
Attn: Dr. Jeffrey Olliffe
NPI: 1306838271
Report Date: 10/10/2016

MALE
DONOR 10083
DOB: [redacted]
Ethnicity: Mixed or Other Caucasian
Barcode: 11200059725005

FEMALE
N/A

Disease	DONOR 10083 Residual Risk	Reproductive Risk
Herlitz Junctional Epidermolysis Bullosa, LAMB3-related	< 1 in 500	< 1 in 1,000,000
Herlitz Junctional Epidermolysis Bullosa, LAMC2-related	< 1 in 500	< 1 in 1,000,000
Hexosaminidase A Deficiency (Including Tay-Sachs Disease)	1 in 30,000	< 1 in 1,000,000
Homocystinuria Caused by Cystathionine Beta-Synthase Deficiency	1 in 25,000	< 1 in 1,000,000
Hurler Syndrome	1 in 480	1 in 300,000
Hypophosphatasia, Autosomal Recessive	1 in 16,000	< 1 in 1,000,000
Inclusion Body Myopathy 2	< 1 in 500	< 1 in 1,000,000
Isovaleric Acidemia	1 in 25,000	< 1 in 1,000,000
Joubert Syndrome 2	< 1 in 500	< 1 in 1,000,000
Krabbe Disease	1 in 15,000	< 1 in 1,000,000
Limb-Girdle Muscular Dystrophy Type 2D	1 in 45,000	< 1 in 1,000,000
Limb-Girdle Muscular Dystrophy Type 2E	< 1 in 500	< 1 in 1,000,000
Lipoamide Dehydrogenase Deficiency	< 1 in 500	< 1 in 1,000,000
Long Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency	1 in 15,000	< 1 in 1,000,000
Maple Syrup Urine Disease Type 1B	1 in 25,000	< 1 in 1,000,000
Medium Chain Acyl-CoA Dehydrogenase Deficiency	1 in 5,900	< 1 in 1,000,000
Megalencephalic Leukoencephalopathy With Subcortical Cysts	< 1 in 500	< 1 in 1,000,000
Metachromatic Leukodystrophy	1 in 20,000	< 1 in 1,000,000
Mucolipidosis IV	< 1 in 500	< 1 in 1,000,000
Muscle-Eye-Brain Disease	< 1 in 500	< 1 in 1,000,000
NEB-related Nemaline Myopathy	< 1 in 500	< 1 in 1,000,000
Niemann-Pick Disease Type C	1 in 5,400	< 1 in 1,000,000
Niemann-Pick Disease, SMPD1-associated	1 in 25,000	< 1 in 1,000,000
Nijmegen Breakage Syndrome	1 in 16,000	< 1 in 1,000,000
Northern Epilepsy	< 1 in 500	< 1 in 1,000,000
Pendred Syndrome	1 in 7,000	< 1 in 1,000,000
PEX1-related Zellweger Syndrome Spectrum	1 in 11,000	< 1 in 1,000,000
Phenylalanine Hydroxylase Deficiency	1 in 3,000	1 in 600,000
Polyglandular Autoimmune Syndrome Type 1	1 in 14,000	< 1 in 1,000,000
Pompe Disease	1 in 1,600	< 1 in 1,000,000
PPT1-related Neuronal Ceroid Lipofuscinosis	< 1 in 500	< 1 in 1,000,000
Primary Carnitine Deficiency	< 1 in 500	< 1 in 1,000,000
Primary Hyperoxaluria Type 1	1 in 35,000	< 1 in 1,000,000
Primary Hyperoxaluria Type 2	< 1 in 500	< 1 in 1,000,000
PROP1-related Combined Pituitary Hormone Deficiency	1 in 11,000	< 1 in 1,000,000
Pseudocholinesterase Deficiency	1 in 2,700	1 in 300,000
Pycnodysostosis	< 1 in 500	< 1 in 1,000,000
Rhizomelic Chondrodysplasia Punctata Type 1	1 in 16,000	< 1 in 1,000,000
Salla Disease	< 1 in 500	< 1 in 1,000,000
Segawa Syndrome	< 1 in 500	< 1 in 1,000,000
Short Chain Acyl-CoA Dehydrogenase Deficiency	1 in 16,000	< 1 in 1,000,000
Sjogren-Larsson Syndrome	1 in 25,000	< 1 in 1,000,000
Smith-Lemli-Opitz Syndrome	1 in 4,900	1 in 970,000
Spinal Muscular Atrophy	SMN1: 3+ copies 1 in 4,800	1 in 670,000
Steroid-Resistant Nephrotic Syndrome	1 in 40,000	< 1 in 1,000,000
Sulfate Transporter-Related Osteochondrodysplasia	1 in 11,000	< 1 in 1,000,000
TPP1-related Neuronal Ceroid Lipofuscinosis	1 in 30,000	< 1 in 1,000,000
Tyrosinemia Type I	1 in 17,000	< 1 in 1,000,000
Usher Syndrome Type 1F	1 in 6,600	< 1 in 1,000,000
Usher Syndrome Type 3	< 1 in 500	< 1 in 1,000,000
Very Long Chain Acyl-CoA Dehydrogenase Deficiency	1 in 8,800	< 1 in 1,000,000
Walker-Warburg Syndrome	< 1 in 500	< 1 in 1,000,000
Wilson Disease	1 in 8,600	< 1 in 1,000,000

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CARRIER SCREENING REPORT

Patient	Sample	Referring Doctor
Patient Name: Donor 10083 **Date of Birth:** [redacted] **Reference #:** SEATSB-S410083 **Indication:** Carrier Testing **Test Type:** Custom Carrier Screen (ECS)	**Specimen Type:** Blood **Lab #:** 18096308CS **Date Collected:** 11/7/2018 **Date Received:** 11/8/2018 **Final Report:** 11/23/2018	**Jeffrey Olliffe, M.D.** **Seattle Sperm Bank** **4915 25th Avenue NE** **Suite 204W** **Seattle, WA 98105** Fax: **206-466-4696**

Results

Negative: No clinically significant variant(s) detected

Gene(s) analyzed: *GLB1*

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 *GLB1* gene which is associated with mucopolysaccharidosis type IVb / GM1 gangliosidosis was performed by next generation sequencing and possibly genotyping for select variants on DNA extracted from this patient's sample. No clinically significant variants were detected during this analysis.

Please note that negative results reduce but do not eliminate the possibility that this individual is a carrier for the disorder(s) tested. Please see table of residual risks for specific detection rates and residual risk by ethnicity. With individuals of mixed ethnicity, it is recommended to use the highest residual risk estimate. Only variants determined to be pathogenic or likely pathogenic are reported in this carrier screening test.

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

Please note these tests were developed and their performance characteristics were determined by Mount Sinai Genomics, Inc. They have not been cleared or approved by the FDA. These analyses generally provide highly accurate information regarding the patient's carrier or affected status. Despite this high level of accuracy, it should be kept in mind that there are many potential sources of diagnostic error, including misidentification of samples, polymorphisms, or other rare genetic variants that interfere with analysis. Families should understand that rare diagnostic errors may occur for these reasons.

Patient:	Donor 10083	DOB:	[redacted]	Lab #:	18096308CS

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
Mucopolysaccharidosis, Type IVb / GM1 Gangliosidosis (AR) NM_000404.2	*GLB1*	African	1 in 356	76%	1 in 1,500	99%
		East Asian	1 in 305	75%	1 in 1,200	
		Finnish	1 in 246	97%	1 in 7,700	
		Caucasian	1 in 277	83%	1 in 1,700	
		Latino	1 in 431	81%	1 in 2,300	
		South Asian	1 in 285	77%	1 in 1,200	
		Worldwide	1 in 297	83%	1 in 1,800	
		Roma	1 in 50	99%	1 in 4,900	
		South Brazilian	1 in 58	99%	1 in 5,700	

AR: Autosomal Recessive

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

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

Patient:	Donor 10083	DOB:	[redacted]	Lab #:	18096308CS

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

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

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

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

For spinal muscular atrophy (SMA), the copy numbers of the *SMN1* and *SMN2* genes were analyzed. The individual dosage of exons 7 and 8 as well as the combined dosage of exons 1, 4, 6 and 8 of *SMN1* and *SMN2* were assessed. Copy number gains and losses can be detected with this assay. Depending on ethnicity, 6 - 29 % of carriers will not be identified by dosage sensitive methods as this testing cannot detect individuals with two copies (duplication) of the *SMN1* gene on one chromosome and loss of *SMN1* (deletion) on the other chromosome (silent 2+0 carrier) or individuals that carry an intragenic mutation in *SMN1*. Please also note that 2% of individuals with SMA have an *SMN1* mutation that occurred *de novo*. Typically in these cases, only one parent is an SMA carrier.

The presence of the c.*3+80T>G (chr5:70,247,901T>G) variant allele in an individual with Ashkenazi Jewish or Asian ancestry is typically indicative of a duplication of *SMN1*. When present in an Ashkenazi Jewish or Asian individual with two copies of *SMN1*, c.*3+80T>G is likely indicative of a silent (2+0) carrier. In individuals with two copies of *SMN1* with African American, Hispanic or Caucasian ancestry, the presence or absence of c.*3+80T>G significantly increases or decreases, respectively, the likelihood of being a silent 2+0 silent carrier.

Pathogenic or likely pathogenic sequence variants in exon 7 may be detected during testing for the c.*3+80T>G variant allele; these will be reported if confirmed to be located in SMN1 using locus-specific Sanger primers

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

Patient:	Donor 10083	DOB:	[redacted]	Lab #:	18096308CS

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 ΔΔCt 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.

Tay-Sachs Disease (TSD) Enzyme Analysis (Analytical Detection Rate ≥98%)
Hexosaminidase activity and Hex A% activity were measured by a standard heat-inactivation, fluorometric method using artificial 4-MU-β-N-acetyl glucosaminide (4-MUG) substrate. This assay is highly sensitive and accurate in detecting Tay-Sachs carriers and individuals affected with TSD. Normal ranges of Hex A% activity are 55.0-72.0 for white blood cells and 58.0-72.0 for plasma. It is estimated that less than 0.5% of Tay-Sachs carriers have non-carrier levels of percent Hex A activity, and therefore may not be identified by this assay. In addition, this assay may detect individuals that are carriers of or are affected with Sandhoff disease. False positive results may occur if benign variants, such as pseudodeficiency alleles, interfere with the enzymatic assay. False negative results may occur if both *HEXA* and *HEXB* pathogenic or pseudodeficiency variants are present in the same individual.

Please note these tests were developed and their performance characteristics were determined by Mount Sinai Genomics, Inc. They have not been cleared or approved by the FDA. These analyses generally provide highly accurate information regarding the patient's carrier or affected status. Despite this high level of accuracy, it should be kept in mind that there are many potential sources of diagnostic error, including misidentification of samples, polymorphisms, or other rare genetic variants that interfere with analysis. Families should understand that rare diagnostic errors may occur for these reasons.

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Additional disease-specific references available upon request.

Patient:	Donor 10083	DOB:	[redacted]	Lab #:	18096308CS