sema4

Carrier screening report
5678 Donor
Date of Birth: 05/[redacted]/1993
Sema4 ID: 19178651CF

**Patient Information**
Name: 5678 Donor
Date of Birth: 05/[redacted]/1993
Sema4 ID: 19178651CF
Client ID: TSBCA-S4DONOR5678
Indication: Carrier Testing

**Specimen Information**
Specimen Type: Saliva
Date Collected: 07/17/2019
Date Received: 07/18/2019
Final Report: 07/29/2019

**Referring Provider**
Lorraine Bonner, M.D.
The Sperm Bank of California
2115 Milvia Street
Berkeley, CA, 94704
Fax: 510-841-0332

# Custom Carrier Screen (ECS)

Number of genes tested: 2

## SUMMARY OF RESULTS AND RECOMMENDATIONS

**Negative**

**Negative for all genes tested: *CFTR, and SMN1***
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** 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.

JUL 31 2019

TS 7-29-19
(See JM signature attached)

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# Genes and diseases tested

For specific detection rates and residual risk by ethnicity, please visit **go.sema4.com/residualrisk**

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

| Disease | Gene | Inheritance Pattern | Status | Detailed Summary |
|---|---|---|---|---|
| Negative | | | | |
| Cystic Fibrosis | *CFTR* | AR | Reduced Risk (see table below) | |
| Spinal Muscular Atrophy | *SMN1* | AR | Reduced Risk (see table below) | *SMN1* copy number: 2<br>*SMN2* copy number: 1<br>c.*3+80T>G: Negative |

*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 |
|---|---|---|---|---|---|---|
| Cystic Fibrosis (AR)<br>NM_000492.3 | *CFTR* | African | 1 in 58 | 91% | 1 in 630 | 99% |
| | | Ashkenazi Jewish | 1 in 24 | 98% | 1 in 1,200 | |
| | | East Asian | 1 in 277 | 80% | 1 in 1,400 | |
| | | Finnish | 1 in 75 | 93% | 1 in 1,100 | |
| | | Caucasian | 1 in 23 | 95% | 1 in 440 | |
| | | Latino | 1 in 40 | 96% | 1 in 1,000 | |
| | | South Asian | 1 in 73 | 91% | 1 in 800 | |
| | | Worldwide | 1 in 33 | 94% | 1 in 500 | |
| *Exception:* Exon 10 | | | | | | |

**Spinal Muscular Atrophy (AR)**
NM_000344.3 *SMN1*

| Ethnicity | Carrier Frequency | Detection rate | Residual risk after negative result* | Detection rate with *SMN1* c.*3+80T>G | Residual risk c.*3+80T>G negative | Residual risk c.*3+80T>G positive | Residual Risk with ≥3 Copies of *SMN1* |
|---|---|---|---|---|---|---|---|
| African American | 1 in 85 | 71% | 1 in 160 | 91% | 1 in 455 | 1 in 49 | 1 in 4,300 |
| Ashkenazi Jewish | 1 in 76 | 90% | 1 in 672 | 93% | 1 in 978 | 1 in 10 | 1 in 4,800 |
| East Asian | 1 in 53 | 94% | 1 in 864 | 95% | 1 in 901 | 1 in 12 | 1 in 4,900 |
| Caucasian | 1 in 48 | 95% | 1 in 803 | 95% | 1 in 894 | 1 in 23 | 1 in 4,900 |
| Latino | 1 in 63 | 91% | 1 in 609 | 94% | 1 in 930 | 1 in 47 | 1 in 4,800 |
| South Asian | 1 in 103 | 87% | 1 in 637 | 87% | 1 in 637 | 1 in 608 | 1 in 4,700 |
| Sephardic Jewish | 1 in 34 | 96% | 1 in 696 | 97% | 1 in 884 | 1 in 12 | 1 in 4,900 |

*Residual risk with two copies *SMN1* detected using dosage sensitive methods. The presence of three or more copies of *SMN1* reduces the risk of being an *SMN1* carrier between 5-10 fold, depending on ethnicity.

* Carrier detection by HEXA enzyme analysis has a detection rate of approximately 98% (Applies to *HEXA* gene testing only).
ǂ 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, F9, GJB2, GJB1, GLA,* and *MEFV* gene testing only).
ǂ 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*

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# Test methods and comments

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

**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$^{TM}$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.

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**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.

SAN FRANCISCO GENETIC COUNSELING

Reproductive Technologies, Inc.
**THE SPERM BANK OF CALIFORNIA**

Janine Mash, LCGC
Licensed, Certified Genetic Counselor
San Francisco Genetic Counseling
genetics@tsbca.org
June 26, 2019

# DIS 5678
# CARRIER SCREENING

Donor in screening (DIS) 5678 is of **Portuguese/Spanish & Native American descent**.
Based on his ethnicity, the diseases it is recommended he be screened for: **cystic fibrosis and spinal muscular atrophy.**

## PREVIOUS SCREENING COMPLETED:

DIS 5678 had a complete blood count (CBC) on 6/4/2019. From this result his mean corpuscular volume (MCV) was 86.4, and his hemoglobin electrophoresis concluded "consistent with a normal hemoglobin phenotype".
I reviewed this report on 06/26/19.
No further DNA screening is indicated.

## CARRIER SCREENING CONSENT

On 06/26/19, I provided genetic counseling for carrier screening and genetic testing. Donor 5678 provided verbal consent to test for cystic fibrosis and spinal muscular atrophy.

Sincerely,

Janine Mash

JUL 31 2019

Janine Mash, LCGC
Certified Genetic Counselor
San Francisco Genetic Counseling