GenomeNext™ Sample Report

Secondary Findings Report

Ambry Genetics
A TEMPUS COMPANY

Patient
Legal Name **Sample Patient**
Sex Assigned at Birth **Male**
Birthdate **01/01/2000**
MRN **000000**
Indication **Screening**

Provider
Ordering Provider **Sample Doctor**
Client **Sample Facility**

Test Information
Accession # **00-000000**
Family # **000000**
Specimen **Blood EDTA (Purple top)**
Portal Order # **000000**
Collected **07/01/2026**
Received **07/01/2026**
Test Started **07/01/2026**

⊖ Negative

No Reportable Variant(s) Detected

Results and Interpretation

- No reportable variants were detected in the ACMG recommended minimum list of genes v3.3 (Lee, 2025). Only pathogenic and likely pathogenic alterations are reported.
- A negative secondary findings result does not rule out the possibility that the individual carries a pathogenic or likely pathogenic alteration in a gene on the ACMG list.

Considerations

- Alteration(s) related to this individual's phenotype are reported within the primary report (when applicable), and are not included in secondary findings reports.
- Reclassification reports will be issued for positive secondary findings that are downgraded to a variant of uncertain significance or lower after the time of the report. Results are not systematically reanalyzed for additional secondary findings after the time of the report.
- Genetic counseling is a recommended option for all patients undergoing genetic testing.

All content hereafter is supplemental information to the preceding report.

ACMG Recommended Secondary Findings List

Genes and associated phenotypes as recommended in Lee, 2025

ABCD1+, ACTA2, ACTC1, ACVRL1, APC, APOB, ATP7B* #, BAG3, BMPR1A, BRCA1, BRCA2, BTD*, CACNA1S#, CALM1, CALM2, CALM3, CASQ2*, COL3A1#, CYP27A1* #, DES, DSC2, DSG2, DSP, ENG, FBN1, FLNC#, GAA*, GLA, HFE (homozygous c.845G>A p.C282Y only)***, HNF1A, KCNH2, KCNQ1, LDLR, LMNA, MAX, MEN1, MLH1, MSH2, MSH6, MUTYH*, MYBPC3, MYH11, MYH7, MYL2, MYL3, NF2, OTC, PALB2, PCSK9, PKP2, PLN, PMS2#, PRKAG2, PTEN, RB1, RBM20, RET, RPE65* #, RYR1#, RYR2, SCN5A, SDHAF2, SDHB, SDHC, SDHD, SMAD3, SMAD4, STK11, TGFBR1, TGFBR2, TMEM127, TMEM43, TNNC1, TNNI3, TNNT2, TP53, TPM1, TRDN*, TSC1, TSC2, TTN^, TTR, VHL***, and ***WT1#.***

+only reported when hemizygous, homozygous, or 2 pathogenic or likely pathogenic variants present
**only reported when homozygous or 2 pathogenic or likely pathogenic variants present*
^only pathogenic or likely pathogenic frameshift and nonsense variants, and variants known to impact the splicing of TTN exons with high PSI
#sequencing analysis only

Assay Information

General Information	Ambry's GenomeNext™ is a comprehensive genome sequencing assay designed to increase diagnostic yield for suspected genetic disorders that have remained unsolved using traditional approaches. The genome includes both protein-coding and non-coding regions, and genome sequencing has been successfully applied to identify inherited and de novo variants across autosomal dominant, autosomal recessive, and X-linked disorders. This assay includes analysis of single nucleotide variants (SNVs); small insertions and deletions (indels); copy number variants (CNVs); mitochondrial genome SNVs and indels; repeat expansions in the *DMPK*, *FMR1*, and *PHOX2B* genes; *SMN1* deletions; uniparental disomy (UPD); and aneuploidy. In addition to the primary analysis focused on the patient's reported clinical presentation, genome testing may also detect secondary findings (pathogenic/likely pathogenic variants in select genes) unrelated to the indication for testing.
Methodology	Genomic DNA is isolated from the patient's specimen using standardized technology. PCR-free libraries are prepared and sequenced on Illumina platforms using paired-end sequencing chemistry with an average mean sequencing coverage of at least 30x. Initial data processing (base calling, alignment, and variant calling) is performed using validated bioinformatics tools and aligned to the GRCh38/hg38 human reference genome assembly. Variants are annotated and prioritized using the clinical and family information provided by the ordering provider, population databases, published literature, sequence level quality metrics, and established variant classification guidelines. Copy number changes are assessed using coverage and/or breakpoint evidence from NGS data. Reportable findings not satisfying established quality metrics are verified with an appropriate orthogonal method as needed. Screening for repeat expansions in *DMPK*, *FMR1*, and *PHOX2B* is performed via sequencing. For reported *DMPK* and *FMR1* expansions, repeat size is determined by repeat-primed PCR. *SMN1* deletions are assessed by sequencing and are confirmed by multiplex ligation-dependent probe amplification (MLPA) prior to reporting. Co-segregation studies may be performed when family members are available. If ordered, ribonucleic acid (RNA) is isolated from the patient's whole blood. RNA is converted to complementary DNA (cDNA) by reverse transcriptase polymerase chain reaction (RT-PCR). RNA analysis is performed for reportable germline DNA variants expected to affect splicing, provided such studies are likely to meaningfully inform variant classification.
Result Reports	A primary clinical report is generated only for the proband; family member information will be reported if applicable. Only variants relevant to the proband's current phenotype are included in the primary report. Relevant pathogenic and likely pathogenic variants are routinely reported; selected variants of uncertain significance may also be reported. Variants that increase a statistical risk for disease are not routinely reported. Incidental findings, as well as benign or likely benign variants, are not reported. Pathogenic and likely pathogenic secondary findings within the ACMG-recommended gene list are reported separately unless opted out.
Test Limitations and Disclaimers	This test was developed and its performance characteristics determined by Ambry Genetics. It has not been cleared or approved by the U.S. Food and Drug Administration (FDA). This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) to perform high-complexity clinical laboratory testing. Results should be interpreted in the context of the patient's clinical and family history and do not constitute medical advice; a negative result does not rule out a genetic condition. Detectable variant types include nucleotide substitutions and small insertions/deletions <50 bp, copy number variants and select structural variants, and mitochondrial variants with ≥5% heteroplasmy. Sensitivity may be reduced for copy number variants between 1kb and 10kb. Uniparental disomy of clinically relevant regions will only be reported when testing is

run as a trio analysis. Trinucleotide repeat screening by this assay is limited to the *DMPK*, *FMR1*, and *PHOX2B* genes. Repeat sizes are reported with an accuracy of +/- 2 repeats for alleles with 1-54 repeats and an accuracy of +/- 5 repeats for alleles with 55-200 repeats. Exact repeat size cannot be determined for alleles with >200 repeats.

The overall coverage of each genomic region varies and each individual may have slightly different coverage yield; regions with shared homology/repetitive sequence and other sequence-context limitations may reduce sensitivity and specificity (including for CNVs/structural variation). This test is not intended to analyze certain variant types, including but not limited to: gross/balanced rearrangements, portions of genes with highly homologous pseudogenes, mitochondrial genome deletions/duplications, epigenetic effects, oligogenic inheritance, X-linked recessive variants in females who manifest disease due to skewed X-inactivation, or variants involved in tri-allelic inheritance. Some variants may not be detected due to technical limitations such as but not limited to low coverage, repetitive/highly homologous regions, certain structural rearrangements, or low-level mosaicism. Although molecular tests are highly accurate, rare diagnostic errors may occur (e.g., sample mix-up, erroneous paternity identification, technical, clerical, or genotyping errors; trace contamination; rare variants that may interfere with analysis; or other sources of error).

Resources Used for Bioinformatics, Medical Review Filtering, and Reporting

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