Ambry Genetics
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SAMPLE REPORT

Ordered By Contact ID:1374148 Org ID:1	Patient Name: **Last, First**
Physician: Unknown, Unknown, MD	Accession #: **00-055938** Specimen #: 1234
Ph:N/A Fx:N/A	AP2 Order #: 633474 Specimen: Blood EDTA (Purple top)
Client: Ambry	Birthdate: 01/01/1976 Age: 43y 7m
CA US	Gender: M
Additional Authorized Recipient:	MRN #: N/A Collected: 08/26/2019
Thai, Julia Ph:(949) 900-5500 Fx:(949) 900-5501	Indication: Diagnostic Received: 08/27/2019
	Ethnicity: Caucasian

CancerNext-Expanded +RNAinsight™: Analyses of 67 Genes Associated with Hereditary Cancer

RESULTS

Pathogenic Mutation(s): None Detected
Variant(s) of Unknown Significance: None Detected
Gross Deletion(s)/Duplication(s): None Detected

SUMMARY

NEGATIVE: No Clinically Significant Variants Detected

INTERPRETATION

- No pathogenic mutations, variants of unknown significance, or gross deletions or duplications were detected.
- No clinically relevant aberrant RNA transcripts were detected in select analyzed genes.*
- **Risk Estimate:** low likelihood of variants in the genes analyzed contributing to this individual's clinical history.
- Genetic counseling is a recommended option for all individuals undergoing genetic testing.

Genes Analyzed (67 total): ***AIP, ALK, APC*, ATM*, BAP1, BARD1, BLM, BMPR1A, BRCA1*, BRCA2*, BRIP1*, CDH1*, CDK4, CDKN1B, CDKN2A, CHEK2*, DICER1, FANCC, FH, FLCN, GALNT12, HOXB13, MAX, MEN1, MET, MLH1*, MRE11A, MSH2*, MSH6*, MUTYH*, NBN, NF1*, NF2, PALB2*, PHOX2B, PMS2*, POLD1, POLE, POT1, PRKAR1A, PTCH1, PTEN*, RAD50, RAD51C*, RAD51D*, RB1, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, SMARCA4, SMARCB1, SMARCE1, STK11, SUFU, TMEM127, TP53*, TSC1, TSC2, VHL*** **and** ***XRCC2*** **(sequencing and deletion/duplication);** ***MITF*** **(sequencing only);** ***EPCAM*** **and** ***GREM1*** **(deletion/duplication only)**. DNA and RNA analyses performed for * genes.

Order Summary: The following products were included in the test order for this individual. Please note: tests on hold and those that have been cancelled (including reflex testing steps cancelled due to a positive result in a preceding test) are excluded. For additional information, please contact Ambry Genetics.

- CancerNext-Expanded +RNAinsight™ (Product Code 8874-R)

ASSAY INFORMATION

Methodology: The **CancerNext-*Expanded* +RNAinsight™** test is a comprehensive screen of 67 genes associated with hereditary cancer predisposition. Genomic deoxyribonucleic acid (gDNA) and ribonucleic acid (RNA) are isolated from the patient's specimen using standardized methodology and quantified. RNA is converted to complementary DNA (cDNA) by reverse transcriptase polymerase chain reaction (RT-PCR). Sequence enrichment of the targeted coding exons and adjacent intronic nucleotides is carried out by a bait-capture methodology using long biotinylated oligonucleotide probes followed by polymerase chain reaction (PCR) and Next-Generation sequencing. Additional DNA analyses include Sanger sequencing for any regions missing or with insufficient read depth coverage for reliable heterozygous variant detection. Reportable small insertions and deletions, potentially homozygous variants, variants in regions complicated by pseudogene interference, and single nucleotide variant calls not satisfying 100x depth of coverage and 40% het ratio thresholds are verified by Sanger sequencing (Mu W et al. *J Mol Diagn*. 2016 Oct 4. PubMed PMID: 27720647). For *BRCA2* and *MSH2*, the Portuguese founder mutation, c.156_157insAlu (also known as 384insAlu), and the coding exons 1-7 inversion, respectively, are detected by next generation sequencing and confirmed by multiplex ligation-dependent probe amplification (MLPA) or PCR and agarose gel electrophoresis. Gross deletion/duplication analysis for 65 of the genes (excluding *MITF* and *PMS2*) is performed using a custom pipeline based on read-depth from NGS data and/or targeted chromosomal microarray with confirmatory MLPA when applicable. Gross deletion/duplication analysis of *PMS2* is performed using MLPA kit P008-B1. If a deletion is detected in exons 13, 14, or 15 of *PMS2*, double stranded sequencing of the appropriate exon(s) of the pseudogene *PMS2CL* will be performed to determine if the deletion is located in the *PMS2* gene or pseudogene. All sequence analysis is based on the following NCBI reference sequences: *AIP*- NM_003977.2, *ALK*- NM_004304.4, *APC*- NM_000038.5 & NM_001127511.2, *ATM*- NM_000051.3, *BAP1*- NM_004656.2, *BARD1*- NM_000465.2, *BLM*- NM_000057.2, *BMPR1A*- NM_004329.2, *BRCA1*- NM_007294.3, *BRCA2*- NM_000059.3, *BRIP1*- NM_032043.2, *CDH1*- NM_004360.3, *CDK4*- NM_000075.3, *CDKN1B*- NM_004064.3, *CDKN2A*- NM_000077.4 and NM_058195.3 (p14ARF), *CHEK2*- NM_007194.3, *DICER1*-NM_177438.2, *FANCC*- NM_000136.2, *FH*- NM_000143.3, *FLCN*- NM_144997.5, *GALNT12*- NM_024642.4, *HOXB13*- NM_006361.5, *MAX*- NM_002382.3, *MEN1*- NM_130799.2, *MET*- NM_000245.1, *MITF*- NM_000248.3, *MUTYH*- NM_001128425.1, *MRE11A*- NM_005591.3, *MLH1*- NM_000249.3, *MSH2*- NM_000251.1, *MSH6*- NM_000179.2, *NBN*- NM_002485.4, *NF1*-NM_000267.3, *NF2*- NM_000268.3, *PALB2*- NM_024675.3, *PHOX2B*- NM_003924.3, *PMS2*- NM_000535.5, *POLD1*- NM_002691.2, *POLE*- NM_006231.2, *POT1*-NM_015450.2, *PRKAR1A*- NM_002734.3, *PTCH1*- NM_000264.3, *PTEN*- NM_000314.4, *RAD50*- NM_005732.3, *RAD51C*- NM_058216.1, *RAD51D*- NM_002878.3, *RB1*- NM_000321.2, *RET*- NM_020975.4, *SDHA*- NM_004168.2, *SDHAF2*- NM_017841.2, *SDHB*- NM_003000.2, *SDHC*- NM_003001.3, *SDHD*- NM_003002.2, *SMAD4*- NM_005359.5, *SMARCA4*- NM_001128849.1, *SMARCB1*- NM_003073.3, *SMARCE1*- NM_002079.4, *STK11*- NM_000455.4, *SUFU*- NM_016169.3, *TMEM127*- NM_017849.3, *TP53*- NM_000546.4, *TSC1*- NM_000368.4, *TSC2*- NM_000548.3, *VHL*- NM_000551.3, *XRCC2*- NM_005431.1.

Analytical Range: The **CancerNext-*Expanded* +RNAinsight™** test targets detection of DNA sequence mutations in 65 genes by either Next-Generation or Sanger sequencing of all coding domains and well into the flanking 5' and 3' ends of all the introns and untranslated regions. In addition, sequencing of the promoter region is performed for the following genes: *PTEN* (c.-1300 to c.-745), *MLH1* (c.-337 to c.-194), and *MSH2* (c.-318 to c.-65). For *MITF*, only the status of the c.952G>A (p.E318K) alteration is analyzed and reported. For *POLD1* and *POLE,* missense variants located outside of the exonuclease domains (codons 311-541 and 269-485, respectively) are not routinely reported. For *ALK*, only variants located within the kinase domain (c.3286-c.4149) are reported. For *PHOX2B*, the polyalanine repeat region is excluded from analysis. Gross deletion/duplication analysis determines gene copy number for the covered exons and untranslated regions for 64 of the 65 sequenced genes (excluding *MITF*), *EPCAM,* and *GREM1*. For *GREM1,* only the status of the 40kb 5'UTR gross duplication is analyzed and reported. For *EPCAM*, only gross deletions encompassing the 3' end of the gene are reported. For *APC*, all promoter 1B gross deletions as well as single nucleotide substitutions within the promoter 1B YY1 binding motif (NM_001127511 c.-196_-186) are analyzed and reported. RNA transcripts are screened for 18 genes (*APC, ATM, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, MLH1, MSH2, MSH6, MUTYH, NF1, PALB2, PMS2* exons 1-10, *PTEN, RAD51C, RAD51D*, and *TP53*) and compared to a human reference pool. The absence or presence of RNA transcripts meeting quality thresholds are incorporated as evidence towards assessment and classification of DNA variants. Any regions not meeting RNA quality thresholds are excluded from analysis. Regions routinely excluded due to chronically low expression in human peripheral lymphocytes include: *BRCA2* (exon 1), *BRIP1* (exons 18, 20), *CDH1* (Exons 1, 2, 16), and *CHEK2* (exons 1, 7, 8).

Result Reports: In result reports, DNA alterations in the following classifications are always reported, and are based on the following definitions:

- **Pathogenic Mutation**: alterations with sufficient evidence to classify as pathogenic (capable of causing disease). Previously described pathogenic mutations, including intronic mutations at any position, are always reported when detected.
- **Variant, Likely Pathogenic (VLP)**: alterations with strong evidence in favor of pathogenicity. Previously described likely pathogenic variants, including intronic VLPs at any position, are always reported when detected.
- **Variant, Unknown Significance (VUS)**: alterations with limited and/or conflicting evidence regarding pathogenicity. Intronic VUSs are always reported out to 5 basepairs from the splice junction when detected.

Alterations of unlikely clinical significance (those with strong/very strong evidence to argue against pathogenicity) are not routinely included on results reports. These include findings classified as "likely benign" and "benign" alterations.

Assay Information Continued on Next Page

ASSAY INFORMATION *(Supplement to Test Results - Continued)*

Resources: The following references are used in variant analysis and classification when applicable for observed genetic alterations.

1. The 1000 Genomes Project Consortium. An integrated map of genetic variation from 1092 human genomes. *Nature*. 2012;491:56-65.
2. ACMG Standards and guidelines for the interpretation of sequence variants. *Genet Med.* 2015 May;17(5):405-23.
3. Ambry Genetics Variant Classification Scheme. http://www.ambrygen.com/variant-classification.
4. Berkeley Drosophila Genome Project [Internet]. Reese MG et al. *J Comp Biol.* 1997;4:311-23. http://www.fruitfly.org/seq_tools/splice.html.
5. Database of Single Nucleotide Polymorphisms (dbSNP) [Internet]. Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine (dbSNP Build ID:135) Available from: www.ncbi.nlm.nih.gov/SNP. Accessed Jan 2012).
6. ESEfinder [Internet]. Smith PJ, et al. (2006) *Hum Mol Genet.* 15(16):2490-2508 and Cartegni L, et al. *Nucleic Acid Research.* 2003;31(13):3568-3571. http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi?process=home.
7. Exome Variant Server, NHLBI Exome Sequencing Project (ESP) [Internet], Seattle WA. Available from: evs.gs.washington.edu/EVS.
8. Grantham R. Amino acid difference formula to help explain protein evolution. *Science.* 1974;185(4151):862-864.
9. HGMD® [Internet]: Stenson PD et al. *Genome Med.* 2009;1(1):13. www.hgmd.cf.ac.uk.
10. Landrum MJ et al. ClinVar: public archive of relationships among sequence variation and human phenotype. *Nucleic Acids Res*. 2014 Jan 1;42(1):D980-5. doi: 10.1093/nar/gkt1113. PubMed PMID: 24234437.
11. Online Mendelian Inheritance in Man, OMIM®. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD), Copyright® 1966-2012. World Wide Web URL: http://omim.org.
12. Feng BJ. PERCH: A Unified Framework for Disease Gene Prioritization. *Hum Mutat.* 2017 Mar;38(3):243-251.
13. Exome Aggregation Consortium (ExAC) [Internet], Cambridge, MA. Available from: http://exac.broadinstitute.org.
14. Genome Aggregation Database (gnomAD) [Internet], Cambridge, MA. Available from: http://gnomad.broadinstitute.org.
15. Lek M et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016 Aug 17;536(7616):285-91. PMID: 27535533

Disclaimer: This test was developed and its performance characteristics were determined by Ambry Genetics Corporation. It has not been cleared or approved by the US Food and Drug Administration. The FDA does not require this test to go through premarket FDA review. It should not be regarded as investigational or for research. This test should be interpreted in context with other clinical findings. This report does not represent medical advice. Any questions, suggestions, or concerns regarding interpretation of results should be forwarded to a genetic counselor, medical geneticist, or physician skilled in interpretation of the relevant medical literature. This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing. This test analyzes the following types of mutations: nucleotide substitutions, small deletions (up to 25 bp), small insertions (up to 10 bp), small indels and gross deletions/duplications. Unless otherwise noted in the methodology section above, it is not intended to analyze the following types of alterations: gross rearrangements, deep intronic variations, Alu element insertions, and other unknown abnormalities. The pattern of mutation types varies with the gene tested and this test detects a high but variable percentage of known and unknown mutants of the classes stated. A negative result from the analysis cannot rule out the possibility that the tested individual carries a rare unexamined mutation or mutation in the undetectable group. This test is designed and validated to be capable of detecting ~99% of described mutations in the 67 genes represented on the test (analytical sensitivity). The clinical sensitivity of this test may vary widely according to the specific clinical and family history. Cancer is a complex clinical disorder. Mutations in other genes or the regions not analyzed by this test can also give rise to similar clinical conditions. Although molecular tests are highly accurate, rare diagnostic errors may occur. Possible diagnostic errors include sample mix-up, erroneous paternity identification, technical errors, clerical errors, and genotyping errors. Genotyping errors can result from trace contamination of PCR reactions, from maternal cell contamination in fetal samples, from rare genetic variants that interfere with analysis, low-level mosaicism, presence of pseudogenes, technical difficulties in regions with high GC content or homopolymer tracts, presence of pre-malignant or malignant cells in the sample, or from other sources. Rare variants present in the human genome reference sequence (GRCh37.p5/hg19) or rare misalignment due to presence of pseudogenes can lead to misinterpretation of patient sequence data.

Ambry Genetics®

Understanding Your Negative Hereditary Cancer Genetic Test Result

INFORMATION FOR PATIENTS

Result	**NEGATIVE**	Your testing did not find any disease-causing mutations (changes, like spelling mistakes) in the genes tested.
Cancer Risks	**VARIES**	Even though no mutation was found, you may still have an increased risk of developing cancer based on other possible factors, including the following: • Your medical and/or family history • You could have a mutation in the genes tested that cannot be found with current testing methods • You could have a mutation in a gene that has not yet been linked to cancer or was not tested Your healthcare provider can help you learn more about this.
Risk Management	**VARIES**	Risk management decisions are very personal, and depend on many factors. Talk to your healthcare provider about which, if any, options may be right for you.
Family Members	**VARIABLE RISKS**	Depending on your medical and/or family history, your relatives may still have an increased risk of developing cancer and may be eligible for genetic testing and/or increased cancer screening. They should discuss this with a healthcare provider.
Next Steps	**DISCUSS**	Please share this with family members so they can talk with their healthcare providers and learn more. Stay in contact with your healthcare provider for any relevant updates in genetic testing and/or cancer screening. Also, remember to update him/her with any new information about your family history, especially new cancer diagnoses, as this may change how they determine your cancer risks.
Reach Out	**RESOURCES**	• Ambry's Hereditary Cancer Site for Families patients.ambrygen.com/cancer • American Cancer Society cancer.org • Genetic Information Nondiscrimination Act (GINA) ginahelp.org • National Society of Genetic Counselors nsgc.org • Canadian Association of Genetic Counsellors cagc-accg.ca

Please discuss this information with your healthcare provider. The cancer genetics field is continuously evolving, so updates related to your genetic test result, medical recommendations, genetic testing options, and/or potential treatments may be available over time. This information is not meant to replace a discussion with a healthcare provider, and should not be considered or interpreted as medical advice.

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