GeneMate® Service Overview

GeneMate® is a gDNA Next Generation Sequencing (NGS) test that analyzes 41 genes associated with a predisposition for certain hereditary cancers. The GeneMate® test uses a saliva sample to analyze for variants of genes associated with hereditary cancer risk. The test result includes a genetic analysis, information about population-level risk, and potential clinical actions for prevention and surveillance. Genetic counseling is included in the service.

The 41-gene panel covers variants associated with well chparacterized cancer phenotypes that increase the lifetime risk of breast, ovarian, colorectal, endometrial, pancreatic, prostate, neuroendocrine, and/or other cancers. The panel has been selected based on the best practice recommendations from the American Society of Clinical Oncology (ASCO)[1], the American College of Medical Genetics and Genomics (ACMG)[2], the US center for disease control (CDC)[3], the US National Comprehensive Cancer Network (NCCN)[4], "Svenska Vårdprogram"[5], and the Swedish Association of Medical Genetics and Genomics (SFMG) guidelines[6].

At the time of test development, surveillance, management, and genetic counseling guidelines existed for disease-causing variants of the genes in the panel.

GENES ANALYZED

APC	*ATM*	*BAP1*	*BMPR1A*	*BRCA1*
BRCA2	*BRIP1*	*CDH1*	*CDKN2A*	*CHEK2*
DICER1	*EPCAM*	*MAX*	*MEN1*	*MLH1*
MSH2	*MSH6*	*MUTYH*	*NF1*	*NF2*
PALB2	*PMS2*	*PTEN*	*RAD51C*	*RAD51D*
RB1	*RET*	*SDHA*	*SDHAF2*	*SDHB*
SDHC	*SDHD*	*SMAD4*	*SMARCB1*	*STK11*
TMEM127	*TP53*	*TSC1*	*TSC2*	*VHL*
WT1				

Technical Specifications

DNA EXTRACTION/LIBRARY PREPARATION

Saliva samples are collected using DNA Oragene collection kits from DNA Genotek™. Total genomic DNA is extracted from submitted samples, using prepIT.L2P from DNA Genotek™. Purity and quantity of DNA are assessed with absorbance / fluorometric quantification prior to library preparation to ensure optimal performance. Qualified genomic DNA is then fragmented, end repaired, and A-tailed with a controlled multi-enzyme reaction, followed by ligation at 5' ends with sequencing adapters containing Unique Molecular Identifiers (UMIs) and sample indices. Following ligation, targeted PCR is performed using target-specific primers (across the 41 genes) and one

universal primer complementary to the adapter to enrich DNA molecules within region of interest (ROI). A final PCR is carried out to amplify the library, add platform-specific adapter sequences and additional sample indices. The amplified libraries are subjected to further quality assessments to ensure correct average size, quantity and effective removal of primer-dimers. The target-enriched libraries are then sequenced on an Illumina NextSeq550Dx platform with paired end sequencing mode (151 bp paired end reads).

DATA QUALITY ASSESSMENT AND BIOINFORMATIC ANALYSIS

The raw sequence data is assessed and secured for quality control parameters (Q30 scores and cluster density passing filter) before proceeding to gene-level bioinformatic analysis. iCellate has developed a proprietary germline DNA variant detection bioinformatic pipeline for identification and analysis of single nucleotide variations (SNVs), insertions and deletions (INDELS) and copy number variations (CNVs). Briefly, the sequencing reads are trimmed to remove adapters and then mapped to the reference genome (hg19). Mapped reads are sorted to create Unique Molecular Index (UMI) groups, which facilitate more accurate quantification and detection of variants in subsequent steps of analysis. The grouped reads, after local realignment and primer trimming, are subjected to variant calling. SNV and INDELS are called via a Fixed Ploidy Variant detection tool and CNVs by a Copy Number Variant Detection tool. Each tool uses dedicated models optimized for variants or CNV detection. Detected variants (SNV and INDELS) undergo a series of filtration steps to ensure they pass rigorous quality control thresholds (Average coverage, average quality, minimum quality, forward reverse balance, allele frequency and overlapping region of interest). The CNVs are assessed at the gene/regional level for loss/gain. The variant data for SNVs, INDELS and CNVs are then further processed in anticipation of clinical interpretation.

VARIANT CLASSIFICATION

The classification of genomic variants is performed in accordance with established guidelines[7].The following are taken into consideration: knowledge regarding well-established and/or predicted functional implication on the protein/transcript, frequency in the population, segregation data, allelic data, and other data obtained from reputable sources. To guarantee the highest standard of classification, an automated pre-assessment of genomic variants is followed by professional review. Sources for interpretation include, but are not limited to, the following: CADD, Allele Frequency Community, EVS, Refseq Gene Model, Clinical Trials, PolyPhen- 2, 1000 Genome Frequency (phase 3), ExAC, PhyloP hg18, PhyloP hg19, DbSNP, TargetScan, GENCODE, OMIM, gnomAD, BSIFT, TCGA, Clinvar, DGV, COSMIC, HGMD, SIFT4G. Variant classification is based on the assessment of the available lines of evidence that are weighted and combined following ACMG guidelines[7]. Variants are subsequently classified as one of the following: pathogenic, likely pathogenic, unknown significance, likely benign, or benign. Pathogenic and likely pathogenic variants are included in the report, benign and likely benign variants are not included. Variants of unknown significance are generally not reported unless otherwise recommended by the Clinical Geneticist.

ANALYTICAL VALIDATION

Selected reference DNA materials from three certified institutions (SeraCare, Coriell Institute and NIBSC) containing well-defined SNVs, INDELS, and CNVs were used to provide objective

references for performance characteristics of the GeneMate® NGS service.

The criteria included in the test specification are based on the guidelines published by American College of Medical Genetics and Genomics, The Association for Molecular Pathology and College of American Pathologists, US Food & Drug Administration, The Next-Generation Sequencing: Standardization of Clinical testing (Nex-StoCT) as well as the ISO 15189 guideline.

Performance characteristics for GeneMate® demonstrated in the validation study:

- Sensitivity: >99% for all variant types
- Specificity: >99% for all variant types
- Accuracy: >99,9% for all variant types
- Reproducibility: >99,9% for all variant types

CONFIRMATION

Reported variants may require confirmation with an orthogonal test, including but not exclusive to Sanger sequencing (for SNVs and INDELS), and/or qPCR (for CNVs).

REGULATION AND ACCREDITATION

This test has been developed and its performance characteristics determined by iCellate Medical AB, a clinical laboratory and approved care provider by the Swedish Health and Care Inspectorate (IVO).

LIMITATIONS

iCellate Medical AB only reports findings within the genes found on the panel (please see the list of genes covered by the test). There may exist clinically significant variants in the tested genes that the current technology is not designed to detect, and there may exist additional relevant genes that are not included in this test, based on the best practice requirement.

The GeneMate® test does not report chromosomal aneuploidies (i.e. an abnormal number of chromosomes), complex gene conversions, fusions, inversions, balanced translocations, certain repeat expansions, non-coding intronic variants deeper than 10 base pairs from exon-intron boundary and copy number variations spanning less than 6 exons/target region as defined by panel. The sensitivity/specificity to detect specific variants may vary. This variation includes deletions and insertions in the range of 40-150 bp, deletions and insertions of certain repetitive elements, deletions-duplication or copy number variations, variants in regions with low/high GC content and within or in the vicinity of homopolymers, variants in simple sequence repeats, and in pseudogene and /duplicated segments. Since we know that standard target enrichment protocols cannot reliably analyze some genomic regions (for example *PMS2* exons 12-15), variations from those areas will not be reported. In selected genes analysis is restricted to only positions known to impact cancer risk, for example 3' end of EPCAM gene.

Results of the current test may be inaccurate in patients receiving blood transfusion, bone marrow transplant(s), and in patients with certain hematological malignancies. Additional variants that are associated with hereditary cancer but not part of GeneMate® product panel and/or variants that associated with disease other than hereditary cancer will not be reported by iCellate.

DISCLAIMER

GeneMate® test results may not report some gene variants that current technology is not designed to detect and there may exist additional relevant genes that are not included in this test. A normal test report for an individual does not guarantee a complete cancer free and healthy life. Vice versa, this test is not a diagnostic test for cancer and an elevated cancer risk estimation in the report neither predicts development of cancer/disease in an individual, nor predicts when such cancer may be diagnosed. A follow up genetic counseling and/or physician consultation is advised to ensure complete understanding of GeneMate® test results.

While comprehensive efforts are taken by iCellate to avoid any analytical errors, iCellate is not responsible for errors in sample collection, transportation, and/or any other errors made prior to receipt of the sample at our laboratory. Laboratory and diagnostic errors may occur due to sample processing, DNA contamination, or operational procedures (including but not limited to equipment or reagent errors, or supplier errors) at any stage of the GeneMate® test. Any of the above errors may limit and or affect the sensitivity, specificity, and/or accuracy of the GeneMate® test results.

All classifications are based on review, interpretation, and/or analysis of evidence available at the time of reporting, including medical literature and scientific databases, and will change as new evidence becomes available. Standard risk models may be employed to report risk assessments if pathogenic or likely pathogenic variants were not identified by guidelines following risk identification for the GeneMate® test. The accuracy of the risk estimation for each individual depends in part on the accuracy of the personal and/or family history information, as well as exposures, provided by the tested individual such that iCellate is not responsible for inaccuracy of test results in case of discrepancy in information provided by the individual.

References

1. American Society of Clinical Oncology Expert Statement: Collection and Use of a Cancer family History for Oncology Providers. 2014, 32, 833-840.
2. Green, R. C. *et al.* ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genetics in medicine : official journal of the American College of Medical Genetics* **15**, 565-574, doi:10.1038/gim.2013.73 (2013).
3. CDC Tier 1 Genomic Applications: CDC (Center for disease control US). Genomics and Population Health Action Collaborative. *The National Academies of Sciences*. Published November 18, 2015.
4. **(a)** N.C.C.N. Clinical Practice Guidelines in Oncology (NCCN Guidelines®): Genetic/Familial High-Risk Assessment Colorectal, National Comprehensive Cancer Network, USA, 2018. **(b)** N.C.C.N. Clinical Practice Guidelines in Oncology (NCCN guidelines®) Gastric Cancer, 2018. **(c)** N.C.C.N. Clinical Practice Guidelines in Oncology (NCCN guidelines®): Genetic/Familial High-Risk Assessment: Breast and Ovarian, 2019. **(d)** N.C.C.N. Clinical Practice Guidelines in Oncology (NCCN guidelines®) prostate cancer, 2018.
5. Svenskt Vårdprogram.
6. SFMG guidelines for hereditary cancer - https://sfmg.se/riktlinjer/
7. Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W. W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K., & Rehm, H. L. (2015). 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. *Genetics in Medicine*, *17*(5), 405–424. https://doi.org/10.1038/gim.2015.30