# Blueprint Genetics

# Hereditary Breast Cancer High Risk Panel Plus

## REFERRING HEALTHCARE PROFESSIONAL

**NAME**                         **HOSPITAL**

## PATIENT

| **NAME** | **DOB** | **AGE** | **GENDER** | **ORDER ID** |
|---|---|---|---|---|
| | | | Female | |

**PRIMARY SAMPLE TYPE**          **SAMPLE COLLECTION DATE**          **CUSTOMER SAMPLE ID**

## SUMMARY OF RESULTS

### PRIMARY FINDINGS
Negative

### SEQUENCING PERFORMANCE METRICS - NUCLEAR GENOME

| PANEL | GENES | EXONS / REGIONS | BASES | BASES > 20X | MEDIAN COVERAGE | PERCENT > 20X |
|---|---|---|---|---|---|---|
| Hereditary Breast Cancer High Risk Panel | 8 | 134 | 34801 | 34801 | 313 | 100 |

### TARGET REGION AND GENE LIST

The Blueprint Genetics Hereditary Breast Cancer High Risk Panel (version 3, Oct 19, 2019) Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: *BRCA1\*, BRCA2, CDH1, PALB2, PTEN\*, RECQL\*, STK11* and *TP53*. This panel targets protein coding exons, exon-intron boundaries (± 20 bps) and selected non-coding, deep intronic variants (listed in the SUMMARY OF THE TEST section). This panel should be used to detect single nucleotide variants and small insertions deletions (INDELs) and copy number variations defined as single exon or larger deletions and duplications. This panel should not be used for the detection of repeat expansion disorders or diseases caused by mitochondrial DNA (mtDNA) mutations. The test does not detect balanced translocations or complex rearrangements, and it may not detect low-level mosaicism.

\*Some, or all, of the gene is duplicated in the genome. Read more: https://blueprintgenetics.com/pseudogene/
The sensitivity to detect variants may be limited in genes marked with an asterisk (\*).

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## STATEMENT

**CLINICAL HISTORY**

Patient is a female with breast cancer.

**CLINICAL REPORT**

Sequence and Del/Dup (CNV) analysis using the Blueprint Genetics (BpG) Hereditary Breast Cancer High Risk Panel did not detect any known disease-causing or rare variants that could explain the patient's phenotype as described to the laboratory at the time of interpretation.

| STEP | DATE |
|---|---|
| Order date | |
| Sample received | |
| Sample in analysis | |
| Reported | |

(This statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results.)
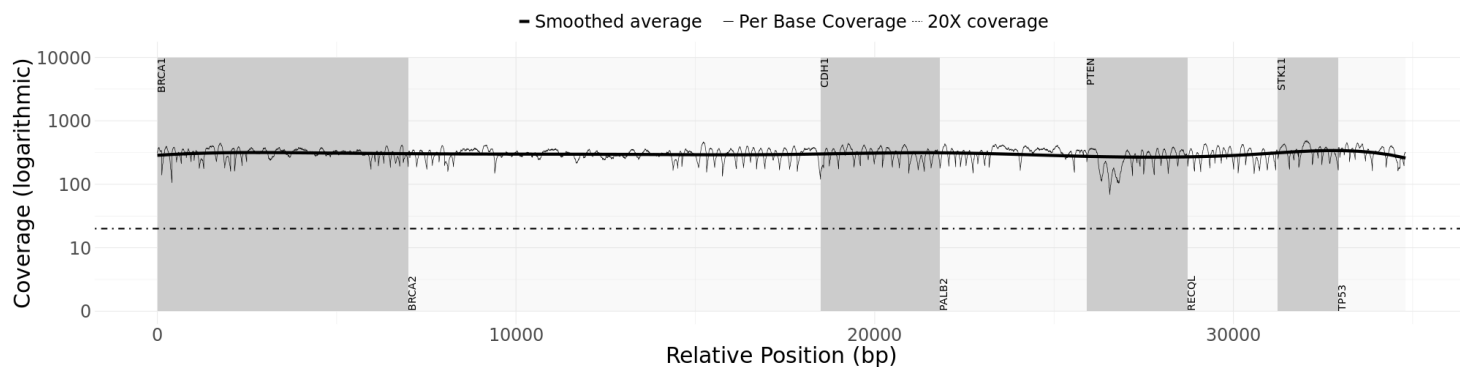
*Signature*

Name

Title

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# COVERAGE PLOT - NUCLEAR GENES

Readability of the coverage plot may be hindered by faxing. A high quality coverage plot can be found with the full report on nucleus.blueprintgenetics.com.



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## APPENDIX 5: SUMMARY OF THE TEST

**PLUS ANALYSIS**

**Laboratory process:** When required, the total genomic DNA was extracted from the biological sample using bead-based method. Quantity of DNA was assessed using fluorometric method. After assessment of DNA quantity, qualified genomic DNA sample was randomly fragmented using non-contact, isothermal sonochemistry processing. Sequencing library was prepared by ligating sequencing adapters to both ends of DNA fragments. Sequencing libraries were size-selected with bead-based method to ensure optimal template size and amplified by polymerase chain reaction (PCR). Regions of interest (exons and intronic targets) were targeted using hybridization-based target capture method. The quality of the completed sequencing library was controlled by ensuring the correct template size and quantity and to eliminate the presence of leftover primers and adapter-adapter dimers. Ready sequencing libraries that passed the quality control were sequenced using the Illumina's sequencing-by-synthesis method using paired-end sequencing (150 by 150 bases). Primary data analysis converting images into base calls and associated quality scores was carried out by the sequencing instrument using Illumina's proprietary software, generating CBCL files as the final output.

**Bioinformatics and quality control:** Base called raw sequencing data was transformed into FASTQ format using Illumina's software (bcl2fastq). Sequence reads of each sample were mapped to the human reference genome (GRCh37/hg19). Burrows-Wheeler Aligner (BWA-MEM) software was used for read alignment. Duplicate read marking, local realignment around indels, base quality score recalibration and variant calling were performed using GATK algorithms (Sentieon) for nDNA. Variant data for was annotated using a collection of tools (VcfAnno and VEP) with a variety of public variant databases including but not limited to gnomAD, ClinVar and HGMD. The median sequencing depth and coverage across the target regions for the tested sample were calculated based on MQ0 aligned reads. The sequencing run included in-process reference sample(s) for quality control, which passed our thresholds for sensitivity and specificity. The patient's sample was subjected to thorough quality control measures including assessments for contamination and sample mix-up. Copy number variations (CNVs), defined as single exon or larger deletions or duplications (Del/Dups), were detected from the sequence analysis data using a proprietary bioinformatics pipeline. The difference between observed and expected sequencing depth at the targeted genomic regions was calculated and regions were divided into segments with variable DNA copy number. The expected sequencing depth was obtained by using other samples processed in the same sequence analysis as a guiding reference. The sequence data was adjusted to account for the effects of varying guanine and cytosine content.

**Interpretation:** The clinical interpretation team assessed the pathogenicity of the identified variants by evaluating the information in the patient requisition, reviewing the relevant scientific literature and manually inspecting the sequencing data if needed. All available evidence of the identified variants was compared to classification criteria. Reporting was carried out using HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines. Likely benign and benign variants were not reported.

**Variant classification:** Our variant classification follows the Blueprint Genetics Variant Classification Schemes modified from the ACMG guideline 2015. Minor modifications were made to increase reproducibility of the variant classification and improve the clinical validity of the report. The classification and interpretation of the variant(s) identified reflect the current state of Blueprint Genetics' understanding at the time of this report. Variant classification and interpretation are subject to professional judgment, and may change for a variety of reasons, including but not limited to, updates in classification guidelines and availability of additional scientific and clinical information. This test result should be used in conjunction with the health care provider's clinical evaluation. Inquiries regarding potential changes to the classification of the variant is strongly recommended prior to making any future clinical decisions. For questions regarding variant classification updates, please contact our customer support (Global: support@blueprintgenetics.com, US: support.us@blueprintgenetics.com, Canada: support.ca@blueprintgenetics.com).

**Databases:** The pathogenicity potential of the identified variants were assessed by considering the predicted consequence of the change, the degree of evolutionary conservation as well as the number of reference population databases and mutation databases such as, but not limited to, the gnomAD, ClinVar, HGMD Professional and Alamut Visual. In addition, the clinical relevance of any identified CNVs was evaluated by reviewing the relevant literature and databases such as Database of Genomic Variants and DECIPHER. For interpretation of mtDNA variants specific databases including e.g. Mitomap, HmtVar and 1000G were used.

**Confirmation of sequence alterations:** Sequence variants classified as pathogenic, likely pathogenic and variants of uncertain significance (VUS) were confirmed using bi-directional Sanger sequencing when they did not meet our stringent NGS quality metrics for a true positive call. In addition, prenatal case with diagnostic findings were confirmed.

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**Confirmation of copy number variants:** CNVs (Deletions/Duplications) were confirmed using a digital PCR assay if they covered less than 10 exons (heterozygous), less than 3 exons (homo/hemizygous) or were not confirmed at least three times previously at our laboratory. Furthermore, CNVs of any size were not confirmed when the breakpoints of the call could be determined.

**Analytic validation:** The detection performance of this panel is expected to be in the same range as our high-quality, clinical grade NGS sequencing assay used to generate the panel data (nuclear DNA: sensitivity for SNVs 99.89%, indels 1-50 bps 99.2%, one-exon deletion 100% and five exons CNV 98.7%, and specificity >99.9% for most variant types). It does not detect very low level mosaicism as a variant with minor allele fraction of 14.6% can be detected in 90% of the cases. Detection performance for mtDNA variants (analytic and clinical validation): sensitivity for SNVs and INDELs 100.0% (10-100% heteroplasmy level), 94.7% (5-10% heteroplasmy level), 87.3% (<5% heteroplasmy level) and for gross deletions 100.0%. Specificity is >99.9% for all.

**Test restrictions:** A normal result does not rule out the diagnosis of a genetic disorder since some DNA abnormalities may be undetectable by the applied technology. Test results should always be interpreted in the context of clinical findings, family history, and other relevant data. Inaccurate, or incomplete information may lead to misinterpretation of the results.

**Technical limitations:** This test does not detect the following: complex inversions, gene conversions, balanced translocations, repeat expansion disorders unless specifically mentioned, non-coding variants deeper than ±20 base pairs from exon-intron boundary unless otherwise indicated (please see the list of non-coding variants covered by the test). Additionally, this test may not reliably detect the following: low level mosaicism, stretches of mononucleotide repeats, indels larger than 50bp, single exon deletions or duplications, and variants within pseudogene regions/duplicated segments. The sensitivity of this test may be reduced if DNA is extracted by a laboratory other than Blueprint Genetics. Laboratory error is also possible. Please see the Analytic validation above.

**Regulation and accreditations:** This test was developed and its performance characteristics determined by Blueprint Genetics (see Analytic validation). It has not been cleared or approved by the US Food and Drug Administration. This analysis has been performed in a CLIA-certified laboratory (#99D2092375), accredited by the College of American Pathologists (CAP #9257331) and by FINAS Finnish Accreditation Service, (laboratory no. T292), accreditation requirement SFS-EN ISO 15189:2013. All the tests are under the scope of the ISO 15189 accreditation.

## PERFORMING SITE:

BLUEPRINT GENETICS OY, KEILARANTA 16 A-B, 02150 ESPOO, FINLAND Laboratory Director: JUHA KOSKENVUO, MD, PHD, CLIA: 99D2092375

- DNA extraction and QC
- Next-generation sequencing
- Bioinformatic analysis
- Confirmation of sequence alterations
- Confirmation of copy number variants
- Interpretation

## NON-CODING VARIANTS COVERED BY THE PANEL:

chr10:g.89622883-89623482
NM_000314.6(*PTEN*):c.-1239A>G
NM_000314.6(*PTEN*):c.-1178C>T
NM_000314.6(*PTEN*):c.-1171C>T
NM_000314.6(*PTEN*):c.-1111A>G
NM_000314.4(*PTEN*):c.-1001T>C
NM_000314.4(*PTEN*):c.-931G>A
NM_000314.4(*PTEN*):c.-921G>T
NM_000314.4(*PTEN*):c.-896T>C
NM_000314.4(*PTEN*):c.-862G>T
NM_000314.4(*PTEN*):c.-854C>G
NM_000314.4(*PTEN*):c.-835C>T
NM_000314.4(*PTEN*):c.-799G>C
NM_000314.4(*PTEN*):c.-765G>A

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NM_000314.4(*PTEN*):c.210-8dupT
NM_000314.4(*PTEN*):c.254-21G>C
NM_000314.4(*PTEN*):c.\*65T>A
NM_000314.4(*PTEN*):c.\*75_\*92delTAATGGCAATAGGACATTinsCTATGGCAATAGGACATTG
NM_000059.3(*BRCA2*):c.-40+1G>A
NM_000059.3(*BRCA2*):c.-39-89delC
NM_000059.3(*BRCA2*):c.-39-1_-39delGA
NM_000059.3(*BRCA2*):c.-39-1G>A
NM_000059.3(*BRCA2*):c.426-12_426-8delGTTTT
NM_000059.3(*BRCA2*):c.8488-14A>G
NM_000059.3(*BRCA2*):c.8954-15T>G
NM_000059.3(*BRCA2*):c.9502-28A>G
NM_000059.3(*BRCA2*):c.9502-12T>G
NM_024675.3(*PALB2*):c.109-12T>A
NM_004360.3(*CDH1*):c.687+92T>A
chr17:g.7571520-7571520
NM_000546.5(*TP53*):c.673-39G>A
NM_000546.5(*TP53*):c.97-11C>G
NM_000546.5(*TP53*):c.-29+1G>T
NM_007294.3(*BRCA1*):c.\*1340_\*1342delTGT
NM_007294.3(*BRCA1*):c.\*1271T>C
NM_007294.3(*BRCA1*):c.\*528G>C
NM_007294.3(*BRCA1*):c.\*103_\*106delTGTC
NM_007294.3(*BRCA1*):c.\*58C>T
NM_007294.3(*BRCA1*):c.5468-40T>A
NM_007294.3(*BRCA1*):c.5407-25T>A
NM_007294.3(*BRCA1*):c.5333-36_5333-22delTACTGCAGTGATTTT
NM_007294.3(*BRCA1*):c.5277+2916_5277+2946delAAATTCTAGTGCTTTGGATTTTTTCCTCCATinsGG
NM_007294.3(*BRCA1*):c.5194-12G>A
NM_007294.3(*BRCA1*):c.5075-27delA
NM_007294.3(*BRCA1*):c.442-22_442-13delTGTTCTTTAC
NM_007294.3(*BRCA1*):c.213-11T>G
NM_007294.3(*BRCA1*):c.213-12A>G
NM_007294.3(*BRCA1*):c.213-15A>G
NM_007294.3(*BRCA1*):c.-19-2A>G
NM_000455.4(*STK11*):c.597+16_597+33delGGGGGGCCCTGGGGCGCCinsTG
NM_000455.4(*STK11*):c.598-32_597+31delGCCCCCTCCCGGGC


**GLOSSARY OF USED ABBREVIATIONS:**


**AD** = autosomal dominant
**AF** = allele fraction (proportion of reads with mutated DNA / all reads)
**AR** = autosomal recessive
**CNV** = Copy Number Variation e.g. one exon or multiexon deletion or duplication
**gnomAD** = genome Aggregation Database (reference population database; >138,600 individuals)
**gnomAD AC/AN** = allele count/allele number in the genome Aggregation Database (gnomAD)
**HEM** = hemizygous
**HET** = heterozygous
**HOM** = homozygous
**ID** = rsID in dbSNP
**MT** = Mitochondria
**MutationTaster** = *in silico* prediction tools used to evaluate the significance of identified amino acid changes.
**Nomenclature** = HGVS nomenclature for a variant in the nucleotide and the predicted effect of a variant in the protein level
**OMIM** = Online Mendelian Inheritance in Man®

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**PolyPhen** = *in silico* prediction tool used to evaluate the significance of amino acid changes.

**POS** = genomic position of the variant in the format of chromosome:position

**SIFT** = *in silico* prediction tool used to evaluate the significance of amino acid changes.

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