Hereditary Colorectal Cancer Panel

Test code: ON0201

The Blueprint Genetics Hereditary Colorectal Cancer Panel analyzes 17 genes associated with inherited susceptibility to colorectal cancer.

This panel covers genes associated with hereditary colorectal cancer syndromes, such as Lynch syndrome, familial adenomatous polyposis, MUTYH-associated polyposis, Peutz-Jeghers syndrome, juvenile polyposis, and Cowden syndrome. The inheritance pattern is autosomal dominant with the exception of MUTYH-associated polyposis that is inherited in an autosomal recessive manner. The Hereditary Colorectal Cancer Panel is suited for detecting heritable germline mutations and may not be used for the detection of somatic mutations in tumor tissue. This panel is included in the Hereditary Gastrointestinal Cancer Panel and Comprehensive Hereditary Cancer Panel.

About Hereditary Colorectal Cancer

Colorectal cancer (CRC) is one of the most common cancers worldwide with marked hereditary component. Hereditary CRC syndromes can be divided into non-polyposis syndromes and polyposis syndromes (PMID: 25582351). The most common form of hereditary CRC is Lynch syndrome (also known as hereditary non-polyposis colorectal cancer, HNPCC) that is estimated to account for 5% of all CRCs. Lynch syndrome is an autosomal dominant syndrome caused by mutations in mismatch repair genes, mainly \textit{MLH1}, \textit{MSH2}, \textit{PMS2} and \textit{MSH6}. Patients have a 78% lifetime risk of developing CRC and women have approximately 43% risk of endometrial cancer. Familial adenomatous polyposis (FAP) is characterized by the development of hundreds to thousands of adenomas throughout the large bowel. If untreated, patients with FAP have nearly 100% chance of developing CRC by the age of 35-40 years. FAP is caused by germline mutations in the \textit{APC} gene. FAP affects approximately 1 in 10,000 individuals and it accounts for 0.5-1% of all CRC cases. FAP patients might display some extracolonic features, such as papillary thyroid carcinoma and hepatoblastomas. Other rarer CRC predisposition syndromes include MUTYH-associated polyposis (MAP), Peutz-Jeghers syndrome (PJS), juvenile polyposis (JPS), and Cowden syndrome. The phenotype in MAP resembles that in FAP, but patients tend to develop fewer adenomas (5-100) and obtain diagnosis at an older age. MAP is caused by biallelic germline mutations in the \textit{MUTYH} gene. PJS is characterized by intestinal hamartomatous polyps and mucocutaneous pigmentation. The polyps in PJS are most commonly located in the small bowel but may also occur anywhere along the gastrointestinal tract. Patients have increased risk of developing extraintestinal cancers. PJS is caused by germline mutations in the \textit{STK11} gene. The prevalence of PJS is approximately 1 in 200,000. The features in JPS are multiple hamartomatous polyps in the colon and rectum and an increased risk of colon, gastric, small intestine, and pancreatic cancers. The causative genes of JPS are \textit{SMAD4} and \textit{BMPR1A}. Prevalence is estimated at 1:100,000. Cowden syndrome is characterized by multiple hamartomatous tumors that most commonly manifest in the skin, intestine, breast and thyroid gland. Patients have a particularly high risk of breast and thyroid cancers. Germline mutations in \textit{PTEN} have been described in 80% of Cowden syndrome patients. More recently, germline mutations in \textit{POLE}, \textit{POLD1} and \textit{GREM1} have been associated with hereditary CRC predisposition (PMID: 23263490, 26493165).

Availability

Results in 3-4 weeks. We do not offer a maternal cell contamination (MCC) test at the moment. We offer prenatal testing only for cases where the maternal cell contamination studies (MCC) are done by a local genetic laboratory. Read more: http://blueprintgenetics.com/faqs/#prenatal

Gene set description

Genes in the Hereditary Colorectal Cancer Panel and their clinical significance

<table>
<thead>
<tr>
<th>Gene</th>
<th>Associated phenotypes</th>
<th>Inheritance</th>
<th>ClinVar</th>
<th>HGMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC</td>
<td>Gardner syndrome, Desmoid disease, hereditary, Familial adenomatous polyposis</td>
<td>AD</td>
<td>294</td>
<td>1780</td>
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<tr>
<td>AXIN2</td>
<td>Oligodontia-colorectal cancer syndrome</td>
<td>AD</td>
<td>6</td>
<td>15</td>
</tr>
</tbody>
</table>

https://blueprintgenetics.com/
BLM  Bloom syndrome  AR  53  92
BMPR1A*  Polyposis, juvenile intestinal  AD  38  108
EPCAM  Diarrhea 5, with tufting enteropathy, congenital, Colorectal cancer, hereditary nonpolyposis  AD/AR  15  63
GREM1  Hereditary mixed polyposis syndrome  AD/AR  8
MLH1  Muir-Torre syndrome, Endometrial cancer, Mismatch repair cancer syndrome, Colorectal cancer, hereditary nonpolyposis  AD/AR  670  1084
MSH2  Muir-Torre syndrome, Endometrial cancer, Colorectal cancer, hereditary nonpolyposis, Mismatch repair cancer syndrome  AD/AR  646  1089
MSH6  Endometrial cancer, Mismatch repair cancer syndrome, Colorectal cancer, hereditary nonpolyposis  AD/AR  308  426
MUTYH  Familial adenomatous polyposis, Colorectal adenomatous polyposis, with pilomatricomas  AR  69  129
PMS2*  Mismatch repair cancer syndrome, Colorectal cancer, hereditary nonpolyposis  AD/AR  151  266
POLD1  Colorectal cancer  AD  1  17
POLE  Colorectal cancer, Facial dysmorphism, immunodeficiency, livedo, and short stature syndrome (FILS syndrome)  AD/AR  1  23
PTEN*  Bannayan-Riley-Ruvalcaba syndrome, Lhermitte-Duclos syndrome, Cowden syndrome  AD  192  564
SMAD4  Juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome, Polyposis, juvenile intestinal, Myhre dysplasia, Hereditary hemorrhagic telangiectasia  AD  119  128
STK11  Peutz-Jeghers syndrome  AD  69  399
TP53  Colorectal cancer, Li-Fraumeni syndrome, Ependymoma, intracranial, Choroid plexus papilloma, Breast cancer, familial, Adrenocortical carcinoma, Osteogenic sarcoma, Hepatoblastoma, Non-Hodgkin lymphoma  AD  148  391

*Some regions of the gene are duplicated in the genome leading to limited sensitivity within the regions. Thus, low-quality variants are filtered out from the duplicated regions and only high-quality variants confirmed by other methods are reported out. Read more.

Gene, refers to HGNC approved gene symbol; Inheritance to inheritance patterns such as autosomal dominant (AD), autosomal recessive (AR) and X-linked (XL); ClinVar, refers to a number of variants in the gene classified as pathogenic or likely pathogenic in ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/); HGMD, refers to a number of variants with possible disease association in the gene listed in Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/). The list of associated (gene specific) phenotypes are generated from CDG (http://research.nhgri.nih.gov/CGD/) or Orphanet (http://www.orpha.net/) databases.

Non-coding disease causing variants covered by the panel

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genomic location HG19</th>
<th>HGVS</th>
<th>RefSeq</th>
<th>RS-number</th>
<th>Comment</th>
<th>Reference</th>
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<tbody>
<tr>
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<td>c.-125delA</td>
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<tr>
<td>Gene</td>
<td>Chromosome:Position</td>
<td>Variant Description</td>
<td>Reference Transcript</td>
<td>Reference Variant ID</td>
<td>Mutation ID</td>
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<td>MLH1</td>
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<td>c.212-478T&gt;G</td>
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<td>NM_000314.4</td>
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</table>

**Test performance**

Blueprint Genetics offers a comprehensive Hereditary Colorectal Cancer Panel that covers classical genes associated with APC-related attenuated familial adenomatous polyposis, colorectal cancer, Cowden syndrome, familial adenomatous polyposis, generalized juvenile polyposis/juvenile polyposis coli, Lynch syndrome, MUTYH-related attenuated familial adenomatous polyposis and Peutz-Jeghers syndrome. The genes are carefully selected based on the existing scientific evidence, our experience and most current mutation databases. Candidate genes are excluded from this first-line diagnostic test. The test does not recognise balanced translocations or complex inversions, and it may not detect low-level mosaicism. The test should not be used for analysis of sequence repeats or for diagnosis of disorders caused by mutations in the mitochondrial DNA.

Analytical validation is a continuous process at Blueprint Genetics. Our mission is to improve the quality of the sequencing process and each modification is followed by our standardized validation process. Average sensitivity and specificity in Blueprint NGS Panels is 99.3% and 99.9% for detecting SNPs. Sensitivity to for indels vary depending on the size of the alteration: 1-10bps (96.0%), 11-20 bps (88.4%) and 21-30 bps (66.7%). The longest detected indel was 46 bps by sequence analysis. Detection limit for Del/Dup (CNV) analysis varies through the genome depending on exon size, sequencing coverage and sequence content. The sensitivity is 71.5% for single exon deletions and duplications and 99% for three exons' deletions and duplications. We have validated the assays for different starting materials including EDTA-blood, isolated DNA (no FFPE) and saliva that all provide high-quality results. The diagnostic yield varies substantially depending on the used assay, referring healthcare professional, hospital and country. Blueprint Genetics' Plus Analysis (Seq+Del/Dup) maximizes the chance to find molecular genetic diagnosis for your patient although Sequence Analysis or Del/Dup Analysis may be cost-effective first line test if your patient's phenotype is suggestive for a specific mutation profile.

**Bioinformatics**

The sequencing data generated in our laboratory is analyzed with our proprietary data analysis and annotation pipeline, integrating state-of-the-art algorithms and industry-standard software solutions. Incorporation of rigorous quality control steps throughout the workflow of the pipeline ensures the consistency, validity and accuracy of results. The highest relevance in the reported variants is achieved through elimination of false positive findings based on variability data for thousands of publicly available human reference sequences and validation against our in-house curated mutation database as well as the most current and relevant human mutation databases. Reference databases currently used are the 1000 Genomes Project (http://www.1000genomes.org), the NHLBI GO Exome Sequencing Project (ESP; http://evs.gs.washington.edu/EVS), the Exome Aggregation Consortium (ExAC; http://exac.broadinstitute.org), ClinVar database of genotype-phenotype associations (http://www.ncbi.nlm.nih.gov/clinvar) and the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk). The consequence of variants in coding and splice regions are estimated using the following in silico variant prediction tools: SIFT (http://sift.jcvi.org), Polyphen (http://genetics.bwh.harvard.edu/pph2/), and Mutation Taster (http://www.mutationtaster.org).

Through our online ordering and statement reporting system, Nucleus, the customer can access specific details of the analysis of the patient. This includes coverage and quality specifications and other relevant information on the analysis. This represents our mission to build fully transparent diagnostics where the customer gains easy access to crucial details of the analysis process.

**Clinical interpretation**

In addition to our cutting-edge patented sequencing technology and proprietary bioinformatics pipeline, we also provide the customers with the best-informed clinical report on the market. Clinical interpretation requires fundamental clinical and
genetic understanding. At Blueprint Genetics our geneticists and clinicians, who together evaluate the results from the sequence analysis pipeline in the context of phenotype information provided in the requisition form, prepare the clinical statement. Our goal is to provide clinically meaningful statements that are understandable for all medical professionals, even without training in genetics.

Variants reported in the statement are always classified using the Blueprint Genetics Variant Classification Scheme modified from the ACMG guidelines (Richards et al. 2015), which has been developed by evaluating existing literature, databases and with thousands of clinical cases analyzed in our laboratory. Variant classification forms the corner stone of clinical interpretation and following patient management decisions. Our statement also includes allele frequencies in reference populations and in silico predictions. We also provide PubMed IDs to the articles or submission numbers to public databases that have been used in the interpretation of the detected variants. In our conclusion, we summarize all the existing information and provide our rationale for the classification of the variant.

A final component of the analysis is the Sanger confirmation of the variants classified as likely pathogenic or pathogenic. This does not only bring confidence to the results obtained by our NGS solution but establishes the mutation specific test for family members. Sanger sequencing is also used occasionally with other variants reported in the statement. In the case of variant of uncertain significance (VUS) we do not recommend risk stratification based on the genetic finding. Furthermore, in the case VUS we do not recommend use of genetic information in patient management or genetic counseling. For some cases Blueprint Genetics offers a special free of charge service to investigate the role of identified VUS.

We constantly follow genetic literature adapting new relevant information and findings to our diagnostics. Relevant novel discoveries can be rapidly translated and adopted into our diagnostics without delay. These processes ensure that our diagnostic panels and clinical statements remain the most up-to-date on the market.

**CPT codes**

SEQ  81435

DEL/DUP  81436

**Accepted sample types**

- EDTA blood, min. 1 ml
- Purified DNA, min. 5μg
- Saliva (Oragene DNA OG-500 kit)

Label the sample tube with your patient’s name, date of birth and the date of sample collection.

Note that we do not accept DNA samples isolated from formalin-fixed paraffin-embedded (FFPE) tissue.

**Resources**

- American Cancer Society
- Canadian Cancer Society
- Live Strong
- Cancer.Net - Attenuated Familial Adenomatous Polyposis
- Cancer.Net - Juvenile Polyposis Syndrome
- Smart Patients
- Lynch Syndrome International
- Hereditary Colon Cancer Foundation
- Cancer.Net - MYH-Associated Polyposis
- Cancer.Net - Peutz-Jeghers Syndrome
- NORD - Familial Adenomatous Polyposis
- NORD - Peutz Jeghers Syndrome
- Gene Reviews - MUTYH-Associated Polyposis
- Gene Reviews - Lynch Syndrome
• Gene Reviews - APC-Associated Polyposis Conditions
• Gene Reviews - Juvenile Polyposis Syndrome
• Gene Reviews - Peutz-Jeghers Syndrome