Whole Exome Family

Test code: WE0201

Whole Exome Family includes high-quality Whole Exome sequence analysis of an index patient and parents (trio) or other family members. Trio approach in WES improves diagnostic rate by facilitating sequence variant analysis and by enabling detection of \textit{de novo} mutations, which underlie many of the severe early-onset diseases.

Whole-exome sequencing (WES) is a robust and one of the most comprehensive genetic tests to identify the disease-causing changes in a large variety of genetic disorders. In WES, protein-coding regions of all genes (~20,000) of the human genome, i.e. exome, are sequenced using next-generation sequencing technologies. While the exome constitutes only \(~1\)% of the whole genome, 85\% of all disease-causing mutations are located there.

WES is most suitable for individuals with

- a complex, unspecified genetic disorder with multiple differential diagnoses.
- a genetically heterogeneous disorder.
- a suspected genetic disorder where a specific genetic test is not available.
- unsuccessful previous genetic testing.

Blueprint Genetics Whole Exome tests have been developed to maximize diagnostic yields, first of all, by generating high-quality and uniform sequencing data. The sequencing data are analyzed using in-house, state-of-the-art bioinformatics pipeline. Furthermore, the genetic information of patients is carefully interpreted by our team of geneticists and clinicians, utilizing information from latest publications and up-to-date databases.

Availability

Whole Exome Family test is available with TAT of 8-10 weeks.

Test performance

We utilize whole exome capture technology and Next-Generation Sequencing methods to obtain clinical-grade WES data, maximizing coverage of clinically relevant genes.

- Highly uniform sequencing depth across all protein-coding genes of the genome
  - Mean sequencing coverage on average \textbf{174x} at guaranteed \textbf{100M sequencing reads}
  - On average, \textbf{99.4\%} of base pairs in genes' coding regions and \textbf{selected intronic variants} covered at least \textbf{20x}
- Highly sensitive and specific detection of single-nucleotide variants and indels
  - \textbf{99.7\%} sensitivity and \textbf{>99.99\%} specificity for single-nucleotide variant detection within coding regions of genes and \textbf{selected intronic variants}.
  - \textbf{97.0\%} sensitivity and \textbf{>99.99\%} specificity for indel detection within coding regions of genes and \textbf{selected intronic variants}.
  - Deletions up to \textbf{220bp} detected, insertions up to \textbf{221bp}
- Assay performs with high precision
  - Within-run precision (repeatability) \textbf{99.7\%}, intermediate precision (reproducibility) \textbf{99.7\%}

Bioinformatics

The target region for each gene includes coding exons and \pm 20 base pairs from the exon-intron boundary. In addition, the panel includes non-coding variants if listed above (Non-coding variants covered by the panel). Some regions of the gene(s) may be removed from the panel if specifically mentioned in the “Test limitations” section above. 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. Our pipeline is streamlined to maximize sensitivity without sacrificing specificity. We have incorporated a number of reference population databases and mutation databases
such as, but not limited, to 1000 Genomes Project, gnomAD, ClinVar and HGMD into our clinical interpretation software to make the process effective and efficient. For missense variants, in silico variant prediction tools such as SIFT, PolyPhen, MutationTaster are used to assist with variant classification. Through our online ordering and statement reporting system, Nucleus, the customer has an access to details of the analysis, including patient specific sequencing metrics, a gene level coverage plot and a list of regions with inadequate coverage if present. This reflects our mission to build fully transparent diagnostics where customers have easy access to crucial details of the analysis process.

**Clinical interpretation**

We provide customers with the most comprehensive clinical report available on the market. Clinical interpretation requires a fundamental understanding of clinical genetics and genetic principles. At Blueprint Genetics, our PhD molecular geneticists, medical geneticists and clinical consultants prepare the clinical statement together by evaluating the identified variants in the context of the phenotypic information provided in the requisition form. Our goal is to provide clinically meaningful statements that are understandable for all medical professionals regardless of whether they have formal training in genetics.

Variant classification is the corner stone of clinical interpretation and resulting patient management decisions. Our classifications follow the Blueprint Genetics Variant Classification Schemes based on the ACMG guideline 2015. Minor modifications were made to increase reproducibility of the variant classification and improve the clinical validity of the report. Our experience with tens of thousands of clinical cases analyzed at our laboratory allowed us to further develop the industry standard.

The final step in the analysis of sequence variants is confirmation of variants classified as pathogenic or likely pathogenic using bi-directional Sanger sequencing. Variant(s) fulfilling the following criteria are not Sanger confirmed: the variant quality score is above the internal threshold for a true positive call, and visual check-up of the variant at IGV is in-line with the variant call. Reported variants of uncertain significance are confirmed with bi-directional Sanger sequencing only if the quality score is below our internally defined quality score for true positive call. Reported copy number variations with a size <10 exons are confirmed by orthogonal methods such as qPCR if the specific CNV has been seen less than three times at Blueprint Genetics.

Our clinical statement includes tables for sequencing and copy number variants that include basic variant information (genomic coordinates, HGVS nomenclature, zygosity, allele frequencies, in silico predictions, OMIM phenotypes and classification of the variant). In addition, the statement includes detailed descriptions of the variant, gene and phenotype(s) including the role of the specific gene in human disease, the mutation profile, information about the gene’s variation in population cohorts and detailed information about related phenotypes. We also provide links to the references used, congress abstracts and mutation databases to help our customers further evaluate the reported findings if desired. The conclusion summarizes all of the existing information and provides our rationale for the classification of the variant.

Identification of pathogenic or likely pathogenic variants in dominant disorders or their combinations in different alleles in recessive disorders are considered molecular confirmation of the clinical diagnosis. In these cases, family member testing can be used for risk stratification within the family. In the case of variants of uncertain significance (VUS), we do not recommend family member risk stratification based on the VUS result. Furthermore, in the case of VUS, we do not recommend the use of genetic information in patient management or genetic counseling.

Our interpretation team analyzes millions of variants from thousands of individuals with rare diseases. Thus, our database, and our understanding of variants and related phenotypes, is growing by leaps and bounds. Our laboratory is therefore well positioned to re-classify previously reported variants as new information becomes available. If a variant previously reported by Blueprint Genetics is re-classified, our laboratory will issue a follow-up statement to the original ordering health care provider at no additional cost.

**Secondary Findings**

As WES covers all protein-coding genes of the genome, it enables detection of variants that are not associated with the indication for ordering the sequencing but are of medical value for patient care. These kind of findings are called secondary or incidental findings. We follow the ACMG Recommendations for Reporting Incidental Findings in Clinical Exome and Genome Sequencing to seek and report clinically actionable mutations of specified types in 59 genes determined by ACMG, if the patient or the caregiver has opted-in for analysis and reporting of secondary findings. If parents or other family members are
also subjected to WES, they also have the possibility to opt-in for analysis and reporting of secondary findings. Secondary findings are reported in a separate statement. All reported secondary findings variants are based on high-quality variant calls in NGS data but these variants do not go through Sanger confirmation, which is in-line with the ACMG policy. Secondary findings are not analyzed or reported for deceased individuals or fetal samples.

**Accepted sample types**

For Whole Exome tests, sample requirements are:

- EDTA blood, min. 1 ml
- Purified DNA, min. 3 μg* in TE buffer or equivalent
- Saliva (Oragene DNA OG-500 kit)

When Whole Exome Family or Whole Exome Family Plus product is ordered, we require to send samples from first-degree relatives of the index patient at the start of testing.