

Whole Exome Family Plus

Test code: WE0401

Whole Exome Family Plus includes high-quality Whole Exome sequence analysis of an index patient and parents (trio) or other family members, coupled with Whole Exome Deletion/Duplication analysis. Whole Exome Family Plus is essential tool for detecting *de novo* mutations and copy number variants, which underlie many of the 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, unspecific 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 Plus test is available with TAT of 8-10 weeks.

Test Strengths

The strengths of this test include:

- CAP accredited laboratory
- CLIA-certified personnel performing clinical testing in a CLIA-certified laboratory
- Powerful sequencing technologies, advanced target enrichment methods and precision bioinformatics pipelines ensure superior analytical performance
- Careful construction of clinically effective and scientifically justified gene panels
- Our Nucleus online portal providing transparent and easy access to quality and performance data at the patient level
- Our publicly available analytic validation demonstrating complete details of test performance
- ~2,000 non-coding disease causing variants in our clinical grade NGS assay for panels (please see 'Non-coding disease causing variants covered by this panel' in the Panel Content section)
- Our rigorous variant classification scheme
- Our systematic clinical interpretation workflow using proprietary software enabling accurate and traceable processing of NGS data
- Our comprehensive clinical statements

Test Limitations

This test does not detect the following:

- Complex inversions
- Gene conversions
- Balanced translocations
- Mitochondrial DNA variants
- Repeat expansion disorders unless specifically mentioned

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- Non-coding variants deeper than ± 20 base pairs from exon-intron boundary unless otherwise indicated (please see above Panel Content / non-coding variants covered by the panel).

This test may not reliably detect the following:

- Low level mosaicism (variant with a minor allele fraction of 14.6% is detected with 90% probability)
- Stretches of mononucleotide repeats
- Indels larger than 50bp
- Single exon deletions or duplications
- 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.

For additional information, please refer to the Test performance section and see our Analytic Validation.

Test performance

Our panels are sectioned from our high-quality, clinical grade NGS assay. Please see our sequencing and detection performance table for details regarding our ability to detect different types of alterations (Table).¹

Assays have been validated for various sample types including EDTA-blood, isolated DNA (excluding from formalin fixed paraffin embedded tissue), saliva and dry blood spots (filter cards). These sample types were selected in order to maximize the likelihood for high-quality DNA yield. The diagnostic yield varies depending on the assay used, referring healthcare professional, hospital and country. Plus analysis increases the likelihood of finding a genetic diagnosis for your patient, as large deletions and duplications cannot be detected using sequence analysis alone. Blueprint Genetics' Plus Analysis is a combination of both sequencing and deletion/duplication (copy number variant (CNV)) analysis.

Performance of Blueprint Genetics high-quality, clinical grade NGS sequencing assay for panels.

	Sensitivity % (TP/(TP+FN))	Specificity %
Single nucleotide variants	99.89% (99,153/99,266)	>99.9999%
Insertions, deletions and indels by sequence analysis		
1-10 bps	99.2% (7,745/7,806)	>99.9999%
11-50 bps	99.13% (2,524/2,546)	>99.9999%
Copy number variants (exon level dels/dups)		
1 exon level deletion (heterozygous)	100% (20/20)	NA
1 exon level deletion (homozygous)	100% (5/5)	NA
1 exon level deletion (het or homo)	100% (25/25)	NA
2-7 exon level deletion (het or homo)	100% (44/44)	NA
1-9 exon level duplication (het or homo)	75% (6/8)	NA
Simulated CNV detection		
5 exons level deletion/duplication	98.7%	100.00%

Size range (0.1-47 Mb)

100% (25/25)

The performance presented above reached by Blueprint Genetics high-quality, clinical grade NGS sequencing assay with the following coverage metrics

Mean sequencing depth

143X

Nucleotides with >20x sequencing coverage (%)

99.86%

Bioinformatics

The target region for each gene includes coding exons and ± 20 base pairs from the exon-intron boundary. In addition, the panel includes non-coding and regulatory 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 including, 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, ordering providers have access to the details of the analysis, including patient specific sequencing metrics, a gene level coverage plot and a list of regions with <20X sequencing depth if applicable. This reflects our mission to build fully transparent diagnostics where ordering providers can easily visualize the 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 is orthogonal confirmation. Sequence variants classified as pathogenic, likely pathogenic and variants of uncertain significance (VUS) are confirmed using bi-directional Sanger sequencing when they do not meet our stringent NGS quality metrics for a true positive call. □ Reported heterozygous and homo/hemizygous copy number variations with a size <10 and <3 target exons are confirmed by orthogonal methods such as qPCR if the specific CNV has been seen and confirmed 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, abstracts and variant databases used to help ordering providers 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.

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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. We do not recommend using variants of uncertain significance (VUS) for family member risk stratification or patient management. Genetic counseling is recommended.

Our interpretation team analyzes millions of variants from thousands of individuals with rare diseases. Our internal database and our understanding of variants and related phenotypes increases with every case analyzed. 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.

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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.