

Whole Exome Sequencing

Maximizing the diagnostic yield in various clinical indications.

Blueprint Genetics



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Reaching a correct genetic diagnosis in a timely manner allows for appropriate disease management and can significantly improve a patient's quality of life. 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, ie, 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. Indeed, WES has not only been successful in identification of new disease genes it is also a powerful method in clinical setting to identify the molecular basis of genetic disorders across medical specialties.

The diagnostic yield of WES is higher than some traditional gene diagnostic methods. A definite diagnosis is typically obtained in 20%-60% of cases, depending on the medical specialty, with severe, early-onset disorders having the highest diagnostic rates.^[1]

Who benefits the most from WES?

WES is most suitable for individuals with

- Complex, unspecific genetic disorders with multiple differential diagnoses
- Genetically heterogeneous disorders
- Suspected genetic disorders for which a specific genetic test is not available
- Unsuccessful previous genetic testing.

A powerful clinical tool to identify the molecular basis of genetic disorders across various medical specialties

Genetic diagnoses efficiently and reliably

WES generates a lot of genetic information, which requires thorough and high-quality procedures in data analysis and interpretation in order to be able to provide reliable, actionable genetic diagnoses.

Blueprint Genetics Whole Exome products have been developed to maximize diagnostic yields, first of all, by generating high-quality and uniform sequencing data. The WES data are analyzed for single-nucleotide and indel variants using our 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. Altogether, the high quality in all steps, leading from DNA sample arrival to clinical statement, means a quicker and more confident diagnosis, less time and money wasted cycling through various diagnostic screening methods, and, ultimately, faster paths to treatment for your patients.

Blueprint Genetics comprehensive genetic service - from sample to clinical statement

Sample

Ordering a test from us is quick and simple. The test requisition can be done online using our secure portal, Nucleus, or by sending us a paper form by mail. We ask for a detailed medical history of the patient to help with clinical interpretation of the results. After our laboratory receives the patient's blood, saliva, or DNA sample, we prepare it in our accredited laboratory.

Sequencing

We utilize high-quality exome capture technology and next-generation sequencing methods to obtain deep and uniform, clinical-grade WES data.

Analysis and Interpretation

We use our own proprietary automated bioinformatics process to quickly and reliably produce clinically relevant information from the sequencing data, including detailed test results, the pathogenicity evaluation, and the quality assessment of the test.

Clinical Statement

Our team of geneticists and specialized clinicians interpret the results and produce a comprehensive clinical statement. The clinical statement supports the diagnosis of the patient, and helps physicians to make more confident decisions concerning their patient's care and treatment.

WES in family setting facilitates the diagnostic process

Blueprint Genetics offers proband-only WES (Whole Exome [product ID WE0101]), and in addition, WES for both the index patient and parents (Whole Exome Family [WE0201]) to further improve the diagnostic rate.^{[1][2]} Both options are also available as Plus products, where Whole Genome Deletion/Duplication analysis is performed for the index patient (Whole Exome Plus [WE0301] and Whole Exome Family Plus [WE0401]).

Given the wealth of variants generated in WES, performing WES for first-degree family members (typically biological parents) in addition to the index patient facilitates significantly better exome variant analysis. Blueprint Genetics Whole Exome Family products include WES for the index patient and parents but other family members can also be analyzed upon request. When Whole Exome Family or Whole Exome Family Plus product is ordered, we require samples from first-degree relatives of the index patient at the start of testing.

Co-segregation analysis of variants using Sanger sequencing can be performed for all candidate alterations for the entire family as part of Family Member Testing. Also, for individuals whose sample are not to be subject to WES, we encourage families to send samples for all members at the start of testing to allow timely segregation analysis.

Including parents or other family members in WES leads to a smaller a number of candidate variants in data analysis, and consequently, facilitates obtaining a correct genetic diagnosis. Moreover, it reduces unnecessary segregation analyses of candidate variants by Sanger sequencing. Most importantly, performing WES in trio setting (index patient + parents) is a necessity for direct detection of *de novo* mutations, ie, new mutations that are not present in parents but occur either in the formation of eggs or sperm cells or early in the development. Notably, *de novo* changes account for the majority of severe developmental disorders. Family-based WES greatly improves the likelihood to obtain diagnosis in these disorders in particular.^[1]

High-quality sequencing in Blueprint Genetics Whole Exome products

We use a custom whole exome sequencing assay and Illumina next-generation sequencing methods to obtain deep and uniform sequencing data from coding exons, exon-intron boundary (+/-20 bps) and 1,501 selected noncoding deep intronic variants.

We have performed an extensive assay validation and determined the coverage, accuracy and precision of our WES data. The sequencing and variant calling specifications of our Whole Exome products are the following:

Highly uniform sequencing depth across all protein-coding genes of the genome

- Mean sequencing coverage on average 174x at guaranteed 100M sequencing reads
- 99.4 % of CCDS genes' coding regions and

selected intronic variants covered at least 20x

Highly sensitive and specific detection of single-nucleotide variants and indels

- 99.7% sensitivity and >99.99% specificity for single-nucleotide variant detection within coding regions of genes and selected intronic variants
- 97.0% sensitivity and >99.99% specificity for indel detection (1–50bp) within coding regions of genes and selected intronic variants

Assay performs with high precision

- Within-run precision (repeatability) 99.7%

Blueprint Whole Exome maximizes coverage of clinically relevant genes

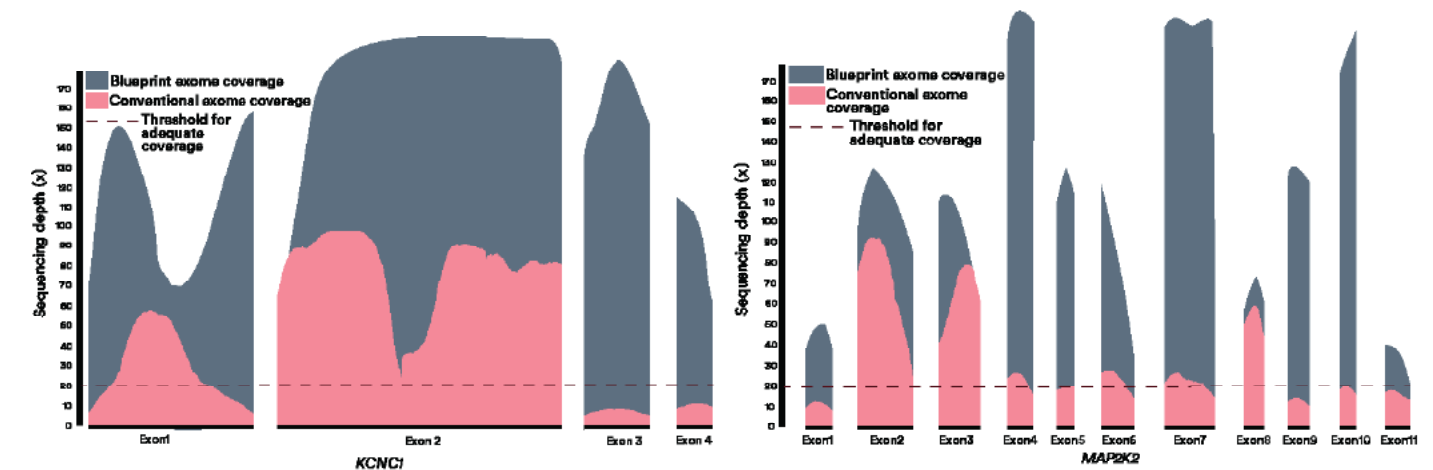


Figure 1. Comparison between the coverage of Blueprint and conventional exome sequencing, in genes *KCNC1* (left) and *MAP2K2* (right).

Deep and uniform sequencing depth is required for reliable detection of disease-causing mutations across all clinically relevant genes. The above figure illustrates how Blueprint Whole Exome products offer deep, clinical-grade sequencing coverage, minimizing gaps in the clinically relevant genes such as *KCNC1* and *MAP2K2*. On the contrary, conventional exomes suffer from low coverage, risking detection of disease-causing mutations.

Coverage data for 'Blueprint exome' represent data from our assay validation and data for 'Conventional exome' represent mean coverages of the corresponding genes in the ExAC database.

With improved sequencing coverage and sensitivity to detect clinically relevant mutations, we can provide higher diagnostic yield with our Whole Exome products.

Blueprint exomes are processed and analyzed utilizing **state-of-art algorithms** and procedures

Analysis of WES data is a complex process, imposing challenging requirements both in terms of computing resources and software. The proprietary automated bioinformatics pipeline developed and employed at Blueprint Genetics enables fast, reliable, and highly accurate results.

We utilize state-of-the-art algorithms for quality control and processing of sequence reads as well as for detection of single-nucleotide and small indel variants from WES data (large copy-number variants are analyzed by low-coverage whole-genome sequencing in Whole Exome Plus or Whole Exome Family Plus products). Furthermore, the pipeline employs accurate methods to determine consequence of variants as well as filtering steps to remove common variants based on allele frequencies in population cohorts.

WES data are primarily analyzed for changes in genes that are known to be associated with conditions showing overlap with the one observed in the patient. We monitor recent literature and up-to-date databases to link genes observed in patients with up-to-date information regarding the genes' association with relevant diseases. To further aid the process of variant interpretation, observed variants are matched against a comprehensive set of databases of disease-related mutations, collected and curated in-house, and accessed from the public domain or licensed from commercial sources.

WES data analysis needs to be tailored on an individual basis. In the analysis process, we consider the clinical and family history of the patient, including symptoms, age of onset, and prevalence and inheritance pattern of the disease. The applied filters for variant analysis are then selected based on this information to achieve an accurate diagnosis. Therefore, it is important that the clinical and family history information is delivered to us in as much as



A comprehensive and accurate clinical statement

The final phase of Blueprint Genetics' all-inclusive process is the geneticist's statement, supported by the insight of a specialized clinician. The Blueprint Genetics clinical statement provided for the customer is a thorough report of the whole diagnostic process and complies with ISO 15189 quality requirements. For positive results, the report generally includes valuable insights into potential treatments while highlighting any risks the disease presents to the patient. Our clinical statement is accessible through our online portal, Nucleus, or upon request a printed pdf version of the statement is sent to the customer via regular mail or fax.

A team of geneticists and clinicians prepares a comprehensive clinical statement from the sequence analysis, assisted by our Clinical Interpretation Platform. The platform allows our team to instantly query millions of genetic and medical sources. The result is actionable clinical statements from our genetic and medical experts, complete with the patient history, all the relevant test findings, interpretation of the results, and gene-specific information and research citations.

The patient's clinical and family history provided by the customer is recapped in the beginning of the statement. This information is extremely valuable in the interpretation process of highly complex WES variant data.

Most important is the careful literature review presenting all the evidence gathered for the variant classification such as number of previously reported patients with the same variant, their phenotype, available segregation data, citations (publications/mutation databases), and a list of possible additional analyses. The literature and databases accessed are included with references. For clarity, the review of literature first focuses specifically on the variant in question. Following the variant-specific

literature review, more information on the gene, associated disease(s) and other additional/supporting information is offered. Comprehensive assessment of the literature is essential in the interpretation process and to explain the rationale behind variant classification.

Reporting of variants follows the American College of Medical Genetics and Genomics (ACMG) guidelines and mutation nomenclature is based on the Human Genome Variation Society guidelines. Details on our variant classification scheme can be found on our website at blueprintgenetics.com. In the statement, we also report sequencing performance metrics of patient's (and family members') analysis.

Concluding remarks tie and recap the evidence presented in the clinical report and clarify the rationale for the classification of the identified variant(s). The utility of the genetic test results and possible recommendations for further actions are given. The conclusion also includes recommendations for genetic counseling and offers recommendations for family member testing, if relevant family members were not already included in the WES analysis. Test results with benign and likely benign variants are considered negative and therefore, these variants are not generally reported.

Pathogenic and likely pathogenic variants are confirmed with bi-directional Sanger sequencing. Other variants are confirmed upon judgment. Variants are not Sanger confirmed when 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 VUS's 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 3 times at Blueprint Genetics.

Customers are distinctly informed about the variants that were confirmed using Sanger sequencing.

Every report is signed by the clinical evaluation team, geneticists, and physicians, who have together evaluated the sequencing results.

Analysis and reporting of 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 selected 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 document and reported variants are confirmed using Sanger sequencing.

Extending variant analysis beyond current knowledge

One of the strengths of WES is that it covers all genes, not only those that are currently known to be associated with diseases. In time, a gene variant may turn out to be relevant to the patient's condition as new gene discoveries are made through research. Currently, new gene-disease links are established at the rate of hundreds of new discoveries annually.

To fully utilize information residing in the WES data, Blueprint Genetics does not discard variant information beyond currently established disease genes, but lists rare variants in all genes as candidate variants. Upon request, we provide ordering physicians filtered variants lists that contain these variants, which may allow establishment of a genetic diagnostic once new gene-disease associations are made. In addition, for research-oriented customers with appropriate tools for data analysis, we also offer the possibility to download the raw sequencing and variant data.

Re-evaluation of WES Variants

Blueprint Genetics WES re-evaluation service reviews all available scientific information in an attempt to re-classify variants originally classified as variants of uncertain significance (VUS) or as Likely Pathogenic (LP). Upon request, we review the relevant literature, available mutation/variant databases, and the latest research for new classifications or information regarding the previously reported VUS or LP variant and evaluate whether the new evidence will impact the original variant classification.

For results received after Spring 2018, a more extensive WES reanalysis is available with bioinformatics analysis and a full interpretation. In light of new phenotypic information from the patient or other developments, new clinically relevant variants may be discovered.

Our transparency helps to drive future developments

Finally, we at Blueprint Genetics are committed to be part of the medical genetics community which aims to share genetic data and work together to improve the clinical interpretation of disease-causing mutations. Therefore, we share our findings in public databases such as ClinVar. We hope that our transparent contributions will empower clinicians and geneticists around the world with the most accurate diagnostics possible, helping them to better serve their patients.

We are continuously developing our services and offering. We may amend service descriptions from time to time by posting new versions on our website. For up-to-date information, please visit blueprintgenetics.com.

References:

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