Medical College of Wisconsin | Genomic Sciences & Precision Medicine Center

Whole Genome Sequencing (WGS)

Turn Around Time:	**30 Days**
CPT Codes:	**Proband – 81425, Family Member – 81426**
Test Includes:	✓ **DNA Extraction** ✓ **Library Prep** ✓ **Library QC** ✓ **Illumina Platform Sequencing** ✓ **Data Analysis** ✓ **Sanger Variant Confirmation (if requested)** ✓ **Interpreted Clinical Report**

Expedited WGS testing is available.
Contact the lab for more information.

Test Description

Whole Genome Sequencing (WGS) is used to detect variants in a patient's genome in order to determine the role of genomic variants in disease outcomes. The patient's genome will be sequenced to an average depth of 40X with a minimum depth of coverage of 35X. Over 90% of the genome will be sequenced to a depth of 10X. The mitochondrial genome of the patient will be sequenced to a minimum depth of 200X.

Sample Requirements

Whole Blood: 2-4mL (4mL preferred) of whole blood in EDTA (purple top tube). For infants, a minimum of 1mL of blood is required. Ship blood tubes overnight at room temperature in an insulated container within 5 days of collection.

gDNA: 10µg of purified gDNA with a minimum concentration of 75ng/µL in a screw cap tube and a 260/280 purity ratio of 1.75-2.0. The tube must be labeled with at least two patient identifiers (patient name/submitter ID number and date of birth). Ship gDNA overnight at room temperature. Products of genome amplification or other amplification reactions not accepted.

Tissue: Contact lab for requirements.

Test Methodology

DNA will be extracted from whole blood. Extracted DNA is quantified and sheared to the correct size. The sample then undergoes library preparation. After quality assurance, the library is then subjected to next generation DNA sequencing on the Illumina platform. The reads from this sequencing are aligned to a reference sequence and variations from this reference are identified. The sequence variants are then loaded into a commercial software package that contains data sources and algorithms allowing for the evaluation of whole genome sequencing variants for evolutionary conservation, predicted impact on protein structure and function (including Polyphen2 (5) and SIFT (6)), ability to disrupt conserved splice sites, and presence in databases including OMIM, dbSNP, and HGMD (1,2,3). The software annotates variants with this data, taking into account both the reference gene model and any identified alternate transcripts (4). A second commercial software package is used to determine what parts of the genome are not sequenced. If parental/familial samples (maximum of 2) are submitted concurrently with the proband, segregation of alleles will be determined. This will enhance the interpretation of results. When a variant of interest is identified, if requested, the DNA sequence of a segment of the genome surrounding each variant is PCR amplified from purified genomic DNA followed by sequencing in the forward and reverse directions using automated fluorescent dideoxy (Sanger) sequencing or an alternate orthologous technique in the proband.

Limitations

Using current sequencing and alignment technology, it is only possible to sequence 90-95% of the human reference genome to the minimum 10X coverage estimated to be required to reliably detect heterozygous variants. In addition, certain types of sequence variations are difficult to identify and have not been validated to be reliably detected for current clinical use. These include insertions, deletions, copy number variations, triplet repeat expansions, and structural chromosomal rearrangements.

Contact & Submission

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Whole Genome Sequencing (WGS) | References

1. Online Mendelian Inheritance in Man, OMIM (TM). Johns Hopkins University, Baltimore, MD. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/ 2. Database of Single Nucleotide Polymorphisms (dbSNP). Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine. World Wide Web URL: http://www.ncbi.nlm.nih.gov/SNP/ 3. The Human Gene Mutation Database: 2008 update. Stenson PD, Mort M, Ball EV, Howells K, Phillips AD, Thomas NS, Cooper DN. Genome Med. 2009 Jan 22;1(1):13. World Wide Web URL: http://www.hgmd.org/ 4. The NCBI handbook [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2002 Oct. Chapter 18, The Reference Sequence (RefSeq) Project. World Wide Web URL: http://www.ncbi.nlm.nih.gov/RefSeq/ 5. Ramensky V, Bork P, Sunyaev S. Human non-synonymous SNPs: server and survey. Nucleic Acids Res 30(17):3894-3900 (2002). 6. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc. 2009;4(7):1073-81.