Medical College of Wisconsin

Linda T. and John A. Mellowes Center for Genomic Sciences and Precision Medicine

Whole Exome Sequencing (WES)

Test Description

Whole Exome Sequencing (WES) enriches the human genome and focuses sequencing to ~2% of the human genome that includes protein-coding regions, proximal untranslated regions (UTRs), and splice sites that encompass a large percentage of currently described disease variants. By focusing the genomic targets, sequencing can be obtained at a greater depth and minimize costs while maintaining the ability to understand and discover the role of variants in disease phenotypes. Variants are compared to clinical and research databases that annotate the role of base pair changes as pathogenic, benign, or unknown significance.

Sample Types and Requirements

Whole Blood: 2-4mL in an EDTA tube (purple top)
Genomic DNA: 5-10µg with a minimum concentration of 75ng/µL, and 260/280 ratio of 1.75-2.0
Saliva, Cell Pellets, FFPE, Tissue: Consultation with the lab is requested, quality and quantity of DNA will be verified at the Mellowes Center prior to initiation of library preparation

Recommended Sequencing Depth

Equal distribution of total reads among all samples in the pool, aiming for ~100x depth of coverage, paired end, 2x150 base pair sequencing

Submission Requirements

Sample Intake Form and iLabs request. Contact lab for drop off or shipping requirements.

Turnaround Time

4-6 weeks for fastq files only
2-3 additional weeks for bioinformatics analysis

Deliverables

DNA-seq report (html with linked documents)
Variant call files (VCFs) with annotation and population frequency, BAM, fastq.gz files
All NGS data files will be delivered via Mellowes Center portal

Test Methodology

Isolated genomic DNA is fluorescently quantified using the BioTek Synergy LX or Qubit and, if necessary, quality assessed using the Agilent Fragment Analyzer. WES libraries are then prepared according to the Illumina Exome panel. The quality and quantity of the DNA library is checked by fragment analysis and qPCR respectively. The pooled library is sequenced on the Illumina NovaSeq.

Bioinformatic Core Analysis

WES report includes:

- Quality control and sequencing metrics (FastQC)
- Filtering to focus on variants
- Comprehensive identification of alterations including single nucleotide variants (SNV), insertions and deletions (Indels)
- Annotation with germline and somatic variant resources
- Classification workflow that separates variant calls into pathogenic or unknown significance (VUS) groups
- Comparison among genetically or disease related samples to identify shared or novel variants in genes and pathways
- Additional analysis can evaluate the potential for the variant to alter protein structure, function, dynamics, or expression

Contact & Submission

mellowescenterinfo@mcw.edu | 414-955-4887