Article:

WHOLE EXOME SEQUENCING AT MEDPACE

Offering CE-IVDR Compliant Twist Precision Exome Dx Assay

INTRODUCTION

Clinical laboratories worldwide have been implementing next-generation sequencing (NGS) to identify genetic variants associated with hereditary disease. Whole exome sequencing (WES) is a test that evaluates patients with suspected genetic disorders for variants within the protein-coding regions (exons) of their genes as well intron/exon boundaries. Because of its broad coverage, WES enables a comprehensive approach for identifying genomic variants that may contribute to a diagnosis or guide treatment decisions. Due to its broad coverage of protein-coding sequences and relatively higher efficiency compared to whole genome sequencing (WGS), WES is frequently employed to provide a comprehensive assessment of potentially pathogenic variants.

Medpace has validated a CE-IVDR germline whole exome sequencing assay using the Twist Precision Exome Dx Kit[1], starting from genomic DNA (gDNA) from whole blood collected in K2 EDTA tubes. The Twist Precision Dx Products are designed to maximize coverage of protein-coding regions from the consensus coding sequence (CCDS) project, GenCode, RefSeq, Ensembl, and ClinVar databases while also maximizing coverage of the mitochondrial genome[1]. The Twist Precision Prep and Enrichment Dx Kit, Twist Precision Exome Dx Panel, and Twist Precision Exome Dx Kit comply with the requirements of the EU Regulation: In-Vitro Diagnostic Regulation (2017/746). The Precision Dx workflow enables comprehensive coverage with more than 98% of target bases covered at 30x as well as reliable performance with uniform enrichment and consistent yield from high-complexity libraries.

METHODOLOGY

The procedure of whole exome sequencing starts from DNA extraction performed on the automated instrument Qiasymphony (Qiagen), and DNA quantification using a spectrophotometric method.

Library preparation involves an enzymatic fragmentation step that randomly shears the gDNA into appropriately sized fragments. Next, universal adapters are ligated to the fragmented DNA to enable downstream amplification and sequencing. Polymerase chain reaction (PCR) amplification is then carried out using Unique Dual Indexes (UDIs), which serve two major purposes: (1) uniquely tagging individual libraries to allow for multiplexed sequencing, and (2) minimizing the risk of index misassignment (index hopping). Following this initial amplification, targeted baits (exome capture probes) are hybridized to the library fragments that represent exonic regions, thereby enriching for coding sequences of interest. A subsequent post-capture PCR step further amplifies the enriched library, which is then subjected to quality control (QC), typically including concentration measurement and fragment size distribution analysis. NGS of the enriched libraries is performed on the Illumina NextSeq 2000 instrument.

Medpace's central laboratories can support single gene, small gene panel or large gene panels, as well as whole exome sequencing using the Twist Precision Exome Dx assay. The analytical sensitivities are 97%, 90%, 80% for SNVs, InDels, and CNVs respectively. All CNVs detected using the Twist Precision Exome Dx assay are confirmed by MPLA when a probemix is available. The specificity of the Twist Exome Precision Dx kit was tested by comparison to the results of analytical specificity of Roche KAPA Hyper Exome probes previously validated at Medpace's central laboratories. The analytical specificity is >99% for all variant types.

BIOINFORMATIC PIPELINE

Medpace uses bioinformatics pipelines for the analysis of NGS data, including DRAGEN BCL Convert v4.2.7 to create sequencing data in the FASTQ file format as well as Illumina BaseSpace and the Illumina Sequencing Analysis Viewer (SAV) version v2.5.12 to evaluate overall sequencing run quality. CLC Genomics Workbench 24.0.1 and CLC LightSpeed Module 24.1 are used to build a bioinformatics analysis pipeline and process all FASTQ files from the NextSeq 2000 instrument for exome data analysis. VarSeq software is used to perform variant analysis and interpretation. Medpace provides an end-to-end solution including DNA extraction, library preparation, next generation sequencing, bioinformatics data analysis and reporting.

REPORTABLE OUTCOMES

Detected variants are interpreted following the American College of Medical Genetics and Genomics (ACMG) guidelines, which recommends that the clinical pathogenicity of a variant be evaluated using multiple lines of evidence from available literature, structural/functional data, population frequencies, and statistical analysis of clinical data, with the possibility to report pathogenic, likely pathogenic, and variants of uncertain significance; variants determined to be benign or likely benign are only reported upon request.

SUMMARY

WES can be used for several clinical applications including identification of disease-causing genetic mutations, leading to more accurate diagnosis and personalized treatment plans. WES can also help in the discovery of new therapeutic targets and in the development of more effective therapies. In cases where diagnosis is not available, WES can help identify the underlying genetic cause. The assay available at Medpace is CE-marked and compliant with EU IVDR 2017/746 regulations, a requirement that is necessary to test any clinical samples in the European Union countries.

REFERENCES

1. Twist Precision Dx Products Instructions for Use, DOC-001311 REV 2.0, February 2024.