ZoomOut

Test Method: DNA extraction (if applicable) and ultrasonic fragmentation; targeted capture of the coding regions and intron/exon boundaries of protein coding RefSeq genes using a TWIST custom exome library capture; next generation sequencing (NGS) on an Illumina NovaSeq instrument; alignment to the human reference genome (GRCh37/hg19) using the Burrows-Wheeler Aligner (bwa); variant calling using GATK and detection of exonic deletions and duplications using ExomeDepth; mtDNA variant calling using GATK Mutect2 –mitomode and detection of mtDNA deletions using eKLIPse; Sanger sequencing to confirm low quality and/or complex indel variants related to the specified phenotype(s); Review of sequence and dosage data for the specified genes by multiple staff members; Variant classification following ACMG criteria (if applicable). Bioinformatic analysis was performed using DDL pipeline JHG_DDL_WES-d40e214.

Clinical Sensitivity: The clinical sensitivity of this assay is dependent on the phenotypic information provided to the laboratory. A causative genetic variant is identified in approximately 20-30% of affected individuals (Farwell et al., 2015, PMID 25356970; Retterer et al., 2016, PMID 26633542; Yang et al., 2013, PMID 24088041). A causative genetic variant in mtDNA is identified in approximately 50-75% of adults and 10-20% of children diagnosed with a primary mitochondrial disorder (Zeviani et al. 2004 PMID 15358637, Schaefer et al. 2008 PMID 17886296, Koenig 2008 PMID 18410845, Poulton et al. 2017 PMID 28536827). Disease-associated variants in the ACMG list of secondary findings genes are identified in approximately 3.4% of individuals (Johnston et al. 2022, PMID 34906458). This test is only validated for inherited gene alterations associated with the specified phenotype(s).

Analytical Sensitivity: Sequencing: >94% for single nucleotide and >76% for small insertion/deletion variants for the nucleotides evaluated. Exonic deletions/duplications: >97% for unique regions of the genome. mtDNA: >97% for single nucleotide variants and 75% for large deletions. The lower limit of detection for mtDNA SNV variants is 5% and for mtDNA large deletions is 10%. This test is not validated to identify small deletions/insertions of greater than 20bp, exonic deletions and duplications in pseudogenes or other repetitive regions of the genome (e.g. segmental duplications), nucleotide repeat expansions, mitochondrial copy number or mosaicism. Disease-associated variants in regions that are not captured and/or sufficiently sequenced will not be detected by this assay.

ACMG Secondary Findings Genes (v3.3): ABCD1, ACTA2, ACTC1, ACVRL1, APC, APOB, ATP7B, BAG3, BMPR1A, BRCA1, BRCA2, BTD, CACNA1S, CALM1, CALM2, CALM3, CASQ2, COL3A1, CYP27A1, DES, DSC2, DSG2, DSP, ENG, FBN1, FLNC, GAA, GLA, HFE, HNF1A, KCNH2, KCNQ1, LDLR, LMNA, MAX, MEN1, MLH1, MSH2, MSH6, MUTYH, MYBPC3, MYH11, MYH7, MYL2, MYL3, NF2, OTC, PALB2, PCSK9, PKP2, PLN, PMS2, PRKAG2, PTEN, RB1, RBM20, RET, RPE65, RYR1, RYR2, SCN5A, SDHAF2, SDHB, SDHC, SDHD, SMAD3, SMAD4, STK11, TGFBR1, TGFBR2, TMEM127, TMEM43, TNNC1, TNNI3, TNNT2, TP53, TPM1, TRDN, TSC1, TSC2, TTN (A-band truncating only), TTR, VHL, WT1