CFTR Whole Gene Sequencing

Test Method: DNA extraction (if applicable) and ultrasonic fragmentation; targeted capture of the *CFTR* gene (including 10kb upstream of the gene, the 5' and 3' untranslated regions, all introns, all exons and 5kb downstream of the gene) using a TWIST custom capture; next generation sequencing (NGS) on an Illumina NovaSeq 6000 instrument; alignment to the human reference genome (GRCh37/hg19) using the Burrows-Wheeler Aligner (bwa); variant calling using GATK; dosage analysis using Manta; Sanger sequencing to confirm variants that do not meet internal QC standards; and review of sequence data by multiple staff members. Bioinformatic analysis was performed using DDL pipeline JHU_DDL-CFTR_Full_Gene_Sequencing_Pipeline-56bd5f8.

Clinical Utility: Identification of causative variants for cystic fibrosis and *CFTR*-related disorder; facilitation of targeted carrier testing of relatives of proband and/or predictive prenatal testing.

Clinical Sensitivity: Whole gene *CFTR* sequencing and deletion/duplication analysis will detect two disease-associated *CFTR* variants in up to 98.1% of individuals with cystic fibrosis, but sensitivity may vary based upon ethnicity and clinical severity (Strom et al., 2003 PMID 12544470; Schrijver et al., 2008 PMID 18556774; Dequeker et al, 2009, PMID 18685558; Raraigh et al., 2022 PMID 34782259). This test is only validated for inherited gene alterations associated with cystic fibrosis.

Analytical Sensitivity: >99% for inherited single nucleotide variants, small insertion/deletion variants and exonic deletions/duplications not associated with an inversion; 50% for exonic deletions/duplications associated with an inversion. Disease-associated variants in regions that are not captured and/or sufficiently sequenced will not be detected by this assay. Non-coding variants of uncertain clinical significance that are not predicted to affect splicing and likely benign/benign variants will not be reported.