Blueprint Genetics

CIZ1 single gene test

Test code: S02894

Phenotype information

- Primary cervical dystonia, adult-onset

Alternative gene names

LSFR1, ZNF356

Test Strengths

The strengths of this test include:

- CAP accredited laboratory
- CLIA-certified personnel performing clinical testing in a CLIA-certified laboratory
- Powerful sequencing technologies, advanced target enrichment methods and precision bioinformatics pipelines ensure superior analytical performance
- Careful construction of clinically effective and scientifically justified gene panels
- Our Nucleus online portal providing transparent and easy access to quality and performance data at the patient level
- Our publicly available analytic validation demonstrating complete details of test performance
- ~2,000 non-coding disease causing variants in our clinical grade NGS assay for panels (please see 'Non-coding disease causing variants covered by this test')
- Our rigorous variant classification scheme
- Our systematic clinical interpretation workflow using proprietary software enabling accurate and traceable processing of NGS data
- Our comprehensive clinical statements

Test Limitations

This test does not detect the following:

- Complex inversions
- Gene conversions
- Balanced translocations
- Mitochondrial DNA variants
- Repeat expansion disorders unless specifically mentioned
- Non-coding variants deeper than ±20 base pairs from exon-intron boundary unless otherwise indicated (please see above non-coding variants covered by the test).

This test may not reliably detect the following:

- Low level mosaicism (variant with a minor allele fraction of 14.6% is detected with 90% probability)
- Stretches of mononucleotide repeats
- Indels larger than 50bp
- Single exon deletions or duplications
- Variants within pseudogene regions/duplicated segments

The sensitivity of this test may be reduced if DNA is extracted by a laboratory other than Blueprint Genetics.

For additional information, please refer to the Test performance section and see our Analytic Validation.

Test performance

Our single gene tests are sectioned from our high-quality, clinical grade NGS assay. Please see our sequencing and detection performance table for details regarding our ability to detect different types of alterations (Table).

⬛⬛Assays have been validated for various sample types including EDTA-blood, isolated DNA (excluding from formalin fixed paraffin embedded tissue), saliva and dry blood spots (filter cards). These sample types were selected in order to maximize the likelihood for high-quality DNA yield. The diagnostic yield varies depending on the assay used, referring healthcare professional, hospital and country. Plus analysis increases the likelihood of finding a genetic diagnosis for your patient, as large deletions and duplications cannot be detected using sequence analysis alone. Blueprint Genetics' Plus Analysis is a combination of both sequencing and deletion/duplication (copy number variant (CNV)) analysis.

	Sensitivity % (TP/(TP+FN)	Specificity %
Single nucleotide variants	99.89% (99,153/99,266)	>99.9999%
Insertions, deletions and indels by sequence analysis		
1-10 bps	96.9% (7,563/7,806)	>99.9999%
11-50 bps	99.13% (2,524/2,546)	>99.9999%
Copy number variants (exon level dels/dups)		
1 exon level deletion (heterozygous)	100% (20/20)	NA
1 exon level deletion (homozygous)	100% (5/5)	NA
1 exon level deletion (het or homo)	100% (25/25)	NA
2-7 exon level deletion (het or homo)	100% (44/44)	NA
1-9 exon level duplication (het or homo)	75% (6/8)	NA
Simulated CNV detection		
5 exons level deletion/duplication	98.7%	100.00%
Microdeletion/-duplication sdrs (large CNVs, n=37))		
Size range (0.1-47 Mb)	100% (37/37)	

The performance presented above reached by Blueprint Genetics high-quality, clinical grade NGS sequencing assay with the following coverage metrics

Mean sequencing depth	143X
Nucleotides with >20x sequencing coverage (%)	99.86%

Performance of Blueprint Genetics Mitochondrial Sequencing Assay.

	Sensitivity % (TP/(TP+FN)	Specificity
ANALYTIC VALIDATION (NA samples; n=4)		

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Single nucleotide variants		
Heteroplasmic (45-100%)	100.0% (50/50)	100.0%
Heteroplasmic (35-45%)	100.0% (87/87)	100.0%
Heteroplasmic (25-35%)	100.0% (73/73)	100.0%
Heteroplasmic (15-25%)	100.0% (77/77)	100.0%
Heteroplasmic (10-15%)	100.0% (74/74)	100.0%
Heteroplasmic (5-10%)	100.0% (3/3)	100.0%
Heteroplasmic (<5%)	50.0% (2/4)	100.0%
CLINICAL VALIDATION (n=76 samples)		
All types		
Single nucleotide variants n=2084 SNVs		
Heteroplasmic (45-100%)	100.0% (1940/1940)	100.0%
Heteroplasmic (35-45%)	100.0% (4/4)	100.0%
Heteroplasmic (25-35%)	100.0% (3/3)	100.0%
Heteroplasmic (15-25%)	100.0% (3/3)	100.0%
Heteroplasmic (10-15%)	100.0% (9/9)	100.0%
Heteroplasmic (5-10%)	92.3%(12/13)	99.98%
Heteroplasmic (<5%)	88.7% (47/53)	99.93%
Insertions and deletions by sequence analysis n=42 indels		
Heteroplasmic (45-100%) 1-10bp	100.0% (32/32)	100.0%
Heteroplasmic (5-45%) 1-10bp	100.0% (3/3)	100.0%
Heteroplasmic (<5%) 1-10bp	100.0% (5/5)	>0.9999
SIMULATION DATA /(mitomap mutations)		
Insertions, and deletions 1-24 bps by sequence analysis; n=17		
Homoplasmic (100%) 1-24bp	100.0% (17/17)	99.98%
Heteroplasmic (50%)	100.0% (17/17)	99.99%
Heteroplasmic (25%)	100.0% (17/17)	100.0%
Heteroplasmic (20%)	100.0% (17/17)	100.0%
Heteroplasmic (15%)	100.0% (17/17)	100.0%
Heteroplasmic (10%)	94.1% (16/17)	100.0%
Heteroplasmic (5%)	94.1% (16/17)	100.0%

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Copy number variants (separate artifical mutations; n=1500)		
Homoplasmic (100%) 500 bp, 1kb, 5 kb	100.0%	100.0%
Heteroplasmic (50%) 500 bp, 1kb, 5 kb	100.0%	100.0%
Heteroplasmic (30%) 500 bp, 1kb, 5 kb	100.0%	100.0%
Heteroplasmic (20%) 500 bp, 1kb, 5 kb	99.7%	100.0%
Heteroplasmic (10%) 500 bp, 1kb, 5 kb	99.0%	100.0%

The performance presented above reached by following coverage metrics at assay level (n=66)		
	Mean of medians	Median of medians
Mean sequencing depth MQ0 (clinical)	18224X	17366X
Nucleotides with >1000x MQ0 sequencing coverage (%) (clinical)	100%	
rho zero cell line (=no mtDNA), mean sequencing depth	12X	

Bioinformatics

The target region for each gene includes coding exons and ±20 base pairs from the exon-intron boundary. In addition, the single gene test includes non-coding deep intronic variants if a tab for non-coding variants is displayed above. The sequencing data generated in our laboratory is analyzed with our proprietary data analysis and annotation pipeline, integrating state-of-the art algorithms and industry-standard software solutions. Incorporation of rigorous quality control steps throughout the workflow of the pipeline ensures the consistency, validity and accuracy of results. Our pipeline is streamlined to maximize sensitivity without sacrificing specificity. We have incorporated a number of reference population databases and mutation databases such as, but not limited, to 1000 Genomes Project, gnomAD, ClinVar and HGMD into our clinical interpretation software to make the process effective and efficient. For missense variants, *in silico* variant prediction tools such as SIFT, PolyPhen, MutationTaster are used to assist with variant classification. Through our online ordering and statement reporting system, Nucleus, the customer has an access to details of the analysis, including patient specific sequencing metrics, a gene level coverage plot and a list of regions with inadequate coverage if present. This reflects our mission to build fully transparent diagnostics where customers have easy access to crucial details of the analysis process.

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Clinical interpretation

We provide customers with the most comprehensive clinical report available on the market. Clinical interpretation requires a fundamental understanding of clinical genetics and genetic principles. At Blueprint Genetics, team of PhD molecular geneticists, medical geneticists and clinical consultants are preparing clinical statements by evaluating the identified variants in the context of the phenotypic information provided in the requisition form. Our goal is to provide clinically meaningful statements that are understandable for all medical professionals regardless of whether they have formal training in genetics.

Variant classification is the corner stone of clinical interpretation and resulting patient management decisions. Our classifications follow the Blueprint Genetics Variant Classification Schemes based on the ACMG guideline 2015. Minor modifications were made to increase reproducibility of the variant classification and improve the clinical validity of the report. Our experience with tens of thousands of clinical cases analyzed at our laboratory allowed us to further develop the industry standard.

The final step in the analysis of sequence variants is confirmation of variants classified as pathogenic or likely pathogenic using bi-directional Sanger sequencing. Variant(s) fulfilling the following criteria are not Sanger confirmed: the variant quality score is above the internal threshold for a true positive call and 2) visual check-up of the variant at IGV is in-line with the

variant call. Reported variants of uncertain significance are confirmed with bi-directional Sanger sequencing only if the quality score is below our internally defined quality score for true positive call. Reported copy number variations with a size <10 exons are confirmed by orthogonal methods such as qPCR if the specific CNV has been seen less than three times at Blueprint Genetics.

Our clinical statement includes tables for sequencing and copy number variants that include basic variant information (genomic coordinates, HGVS nomenclature, zygosity, allele frequencies, in silico predictions, OMIM phenotypes and classification of the variant). In addition, the statement includes detailed descriptions of the variant, gene and phenotype(s) including the role of the specific gene in human disease, the mutation profile, information about the gene's variation in population cohorts and detailed information about related phenotypes. We also provide links to the references used, congress abstracts and mutation databases to help our customers further evaluate the reported findings if desired. The conclusion summarizes all of the existing information and provides our rationale for the classification of the variant.

Identification of pathogenic or likely pathogenic variants in dominant disorders or their combinations in different alleles in recessive disorders are considered molecular confirmation of the clinical diagnosis. In these cases, family member testing can be used for risk stratification within the family. In the case of variants of uncertain significance (VUS), we do not recommend family member risk stratification based on the VUS result. Furthermore, in the case of VUS, we do not recommend the use of genetic information in patient management or genetic counseling.

Our interpretation team analyzes millions of variants from thousands of individuals with rare diseases. Thus, our database, and our understanding of variants and related phenotypes, is growing by leaps and bounds. Our laboratory is therefore well positioned to re-classify previously reported variants as new information becomes available. If a variant previously reported by Blueprint Genetics is re-classified, our laboratory will issue a follow-up statement to the original ordering health care provider at no additional cost.