Blueprint Genetics

Neurofibromatosis Panel

Test code: MA1501

Is a 9 gene panel that includes assessment of non-coding variants.

Is ideal for patients with a clinical suspicion of neurofibromatosis type I and related disorders.

About Neurofibromatosis

Neurofibromatosis type 1 and 2 are autosomal dominant conditions. The panel is efficient in differential diagnosis of neurofibromatosis and related disorders, such as Legius syndrome (*SPRED1*), Noonan with multiple lentigines syndrome (aka LEOPARD syndrome; *PTPN11* and *RAF1*), and familial schwannomatosis (*SMARCB1*).
Neurofibromatosis type 1 (NF1) is clinically characterized by multiple café-au-lait spots, axillary and inguinal freckling, multiple cutaneous neurofibromas, and iris Lisch nodules. Learning disabilities are present in at least 50% of patients. Less common but potentially more serious manifestations include plexiform neurofibromas, optic nerve and other central nervous system gliomas, malignant peripheral nerve sheath tumors, scoliosis, tibial dysplasia, and vasculopathy. The clinical features are highly variable, even within the same family. NF1 is caused by heterozygous mutations in the *NF1* gene, encoding neurofibromin. About half of the affected individuals have a *de novo* mutation. NF1 is relatively common inherited disorder, the prevalence is 1:3,000. Neurofibromatosis type 2 (NF2) is a tumour-prone disorder characterised by the development of multiple schwannomas and meningiomas. The hallmark of NF2 is the development of bilateral vestibular schwannomas leading to hearing loss, tinnitus or imbalance or a combination of the three symptoms. The other main tumors are schwannomas of the other cranial, spinal and peripheral nerves; meningiomas both intracranial (including optic nerve meningiomas) and intraspinal, and some low-grade central nervous system malignancies (ependymomas). Ophthalmic features, including reduced visual acuity and cataract are also prominent. Cutaneous features in NF2 are much more subtle than in NF1. About 70% of NF2 patients have skin tumours. NF2 is caused by mutations in the *NF2* gene, and more than 50% of patients represent new mutations and as many as one-third are mosaic for the underlying disease-causing mutation. The prevalence of NF2 is 1:60,000. Some syndromes share similar clinical features with NF1 and NF2. Legius syndrome is a very rare condition charaterized by multiple café-au-lait macules with or without axillary or inguinal freckling. About 2% of patients fulfilling diagnostic criteria for NF1 are found to have the genetic mutation underlying Legius syndrome (*SPRED1*). Familial schwannomatosis is characterized by the development of multiple spinal, peripheral, and cranial-nerve schwannomas in the absence of vestibular schwannomas. It is inherited in an autosomal dominant manner and caused by mutations in *SMARCB1*.
LEOPARD syndrome is a multisystem disorder involving the skin, skeletal and cardiovascular systems. The characteristic feature is brown skin spots called lentigines that are similar to freckles.

Availability

4 weeks

Gene Set Description

Genes in the Neurofibromatosis Panel and their clinical significance

Gene	Associated phenotypes	Inheritance	ClinVar	HGMD
KIT	Gastrointestinal stromal tumor, Piebaldism	AD	79	116
KITLG	Hyperpigmentation with or without hypopigementation, familial progressive, Skin/hair/eye pigmentation, variation in, 7	AD	6	10
LZTR1	Schwannomatosis, Noonan syndrome	AD/AR	34	71
NF1*	Watson syndrome, Neurofibromatosis, Neurofibromatosis-Noonan syndrome	AD	1157	2901

NF2	Schwannomatosis, Neurofibromatosis	AD	66	433
PTPN11	Noonan syndrome, Metachondromatosis	AD	135	140
RAF1	LEOPARD syndrome, Noonan syndrome, Dilated cardiomyopathy (DCM)	AD	45	53
SMARCB1	Schwannomatosis, Rhabdoid tumor predisposition syndrome, Coffin-Siris syndrome 3	AD	36	118
SPRED1	Legius syndrome	AD	38	71

*

Some, or all, of the gene is duplicated in the genome. Read more.

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The gene has suboptimal coverage (means <90% of the gene's target nucleotides are covered at >20x with mapping quality score (MQ>20) reads), and/or the gene has exons listed under Test limitations section that are not included in the panel as they are not sufficiently covered with high quality sequence reads.

The sensitivity to detect variants may be limited in genes marked with an asterisk (*) or number sign (#). Due to possible limitations these genes may not be available as single gene tests.

Gene refers to the HGNC approved gene symbol; Inheritance refers to inheritance patterns such as autosomal dominant (AD), autosomal recessive (AR), mitochondrial (mi), X-linked (XL), X-linked dominant (XLD) and X-linked recessive (XLR); ClinVar refers to the number of variants in the gene classified as pathogenic or likely pathogenic in this database (ClinVar); HGMD refers to the number of variants with possible disease association in the gene listed in Human Gene Mutation Database (HGMD). The list of associated, gene specific phenotypes are generated from CGD or Mitomap databases.

Non-coding disease causing variants covered by the panel

Gene	Genomic location HG19	HGVS	RefSeq	RS-number
LZTR1	Chr22:21336623	c.-38T>A	NM_006767.3	
LZTR1	Chr22:21350968	c.2220-17C>A	NM_006767.3	rs1249726034
NF1	Chr17:29422055	c.-273A>C	NM_001042492.2	
NF1	Chr17:29422056	c.-272G>A	NM_001042492.2	
NF1	Chr17:29431417	c.60+9031_60+9035delAAGTT	NM_001042492.2	
NF1	Chr17:29475515	c.61-7486G>T	NM_001042492.2	
NF1	Chr17:29488136	c.288+2025T>G	NM_001042492.2	
NF1	Chr17:29508426	c.587-14T>A	NM_001042492.2	
NF1	Chr17:29508428	c.587-12T>A	NM_001042492.2	

NF1	Chr17:29510334	c.888+651T>A	NM_001042492.2	
NF1	Chr17:29510427	c.888+744A>G	NM_001042492.2	
NF1	Chr17:29510472	c.888+789A>G	NM_001042492.2	
NF1	Chr17:29527428	c.889-12T>A	NM_001042492.2	
NF1	Chr17:29530107	c.1260+1604A>G	NM_001042492.2	
NF1	Chr17:29533239	c.1261-19G>A	NM_001042492.2	
NF1	Chr17:29534143	c.1392+754T>G	NM_001042492.2	
NF1	Chr17:29540877	c.1393-592A>G	NM_001042492.2	
NF1	Chr17:29542762	c.1527+1159C>T	NM_001042492.2	
NF1	Chr17:29548419	c.1642-449A>G	NM_001042492.2	rs863224655
NF1	Chr17:29549489	c.*481A>G	NM_001128147.2	
NF1	Chr17:29553439	c.2002-14C>G	NM_001042492.2	
NF1	Chr17:29554225	c.2252-11T>G	NM_001042492.2	
NF1	Chr17:29556025	c.2410-18C>G	NM_001042492.2	
NF1	Chr17:29556027	c.2410-16A>G	NM_001042492.2	
NF1	Chr17:29556028	c.2410-15A>G	NM_001042492.2	
NF1	Chr17:29556031	c.2410-12T>G	NM_001042492.2	
NF1	Chr17:29556839	c.2851-14_2851-13insA	NM_001042492.2	
NF1	Chr17:29557267	c.2991-11T>G	NM_001042492.2	
NF1	Chr17:29558777	c.3198-314G>A	NM_001042492.2	
NF1	Chr17:29563299	c.3974+260T>G	NM_001042492.2	
NF1	Chr17:29577082	c.4110+945A>G	NM_001042492.2	
NF1	Chr17:29580296	c.4173+278A>G	NM_001042492.2	
NF1	Chr17:29588708	c.4578-20_4578-18delAAG	NM_001042492.2	
NF1	Chr17:29588715	c.4578-14T>G	NM_001042492.2	
NF1	Chr17:29654479	c.5269-38A>G	NM_001042492.2	
NF1	Chr17:29656858	c.5610-456G>T	NM_001042492.2	
NF1	Chr17:29657848	c.5812+332A>G	NM_001042492.2	rs863224491
NF1	Chr17:29661577	c.5813-279A>G	NM_001042492.2	
NF1	Chr17:29664375	c.6428-11T>G	NM_001042492.2	
NF1	Chr17:29664618	c.6642+18A>G	NM_001042492.2	

NF1	Chr17:29676126	c.7190-12T>A	NM_001042492.2
NF1	Chr17:29676127	c.7190-11_7190-10insGTTT	NM_001042492.2
NF1	Chr17:29685177	c.7971-321C>G	NM_001042492.2
NF1	Chr17:29685481	c.7971-17C>G	NM_001042492.2
NF1	Chr17:29685665	c.8113+25A>T	NM_001042492.2
NF2	Chr22:30050946	c.516+232G>A	NM_000268.3
PTPN11	Chr12:112915602	c.934-59T>A	NM_002834.3
SMARCB1	Chr22:24130008	c.93+559A>G	NM_003073.3
SMARCB1	Chr22:24176316	c.1119-12C>G	NM_003073.3
SMARCB1	Chr22:24176437	c.*70C>T	NM_003073.3
SMARCB1	Chr22:24176449	c.*82C>T	NM_003073.3

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
- Some of the panels include the whole mitochondrial genome (please see the Panel Content section)
- Our Nucleus online portal providing transparent and easy access to quality and performance data at the patient level
- ~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 panel' in the Panel Content section)
- 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

Genes with partial, or whole gene, segmental duplications in the human genome are marked with an asterisk (*) if they overlap with the UCSC pseudogene regions. The technology may have limited sensitivity to detect variants in genes marked with these symbols (please see the Panel content table above).

This test does not detect the following:

- Complex inversions
- Gene conversions
- Balanced translocations
- Some of the panels include the whole mitochondrial genome but not all (please see the Panel Content section)
- Repeat expansion disorders unless specifically mentioned
- Non-coding variants deeper than ±20 base pairs from exon-intron boundary unless otherwise indicated (please see

above Panel Content / non-coding variants covered by the panel).

This test may not reliably detect the following:

- Low level mosaicism in nuclear genes (variant with a minor allele fraction of 14.6% is detected with 90% probability)
- Stretches of mononucleotide repeats
- Low level heteroplasmy in mtDNA (>90% are detected at 5% level)
- Indels larger than 50bp
- Single exon deletions or duplications
- Variants within pseudogene regions/duplicated segments
- Some disease causing variants present in mtDNA are not detectable from blood, thus post-mitotic tissue such as skeletal muscle may be required for establishing molecular diagnosis.

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.

Test Performance

The genes on the panel have been carefully selected based on scientific literature, mutation databases and our experience.

Our panels 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.

The performance metrics listed below are from an initial validation performed at our main laboratory in Finland. The performance metrics of our laboratory in Seattle, WA, are equivalent.

Performance of Blueprint Genetics high-quality, clinical grade NGS sequencing assay for panels.

	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	99.2% (7,745/7,806)	>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% (25/25)	
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 %	**Specificity %**
ANALYTIC VALIDATION (NA samples; n=4)		
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=2026 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%)	88.9% (48/54)	99.93%
Insertions and deletions by sequence analysis n=40 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)	99,997%
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%
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 panel includes non-coding and regulatory variants if listed above (Non-coding variants covered by the panel). Some regions of the gene(s) may be removed from the panel if specifically mentioned in the 'Test limitations" section above. If the test includes the mitochondrial genome the target region gene list contains the mitochondrial genes. 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 including, 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, ordering providers have access to the details of the analysis, including patient specific sequencing metrics, a gene level coverage plot and a list of regions with suboptimal coverage (<20X for nuclear genes and <1000X for mtDNA) if applicable. This reflects our mission to build fully transparent diagnostics where ordering providers can easily visualize the crucial details of the analysis process.

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, our PhD molecular geneticists, medical geneticists, and clinical consultants prepare the clinical statement together 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 cornerstone of clinical interpretation and resulting patient management decisions. Our classifications follow the ACMG guideline 2015.

The final step in the analysis is orthogonal confirmation. Sequence and copy number variants classified as pathogenic, likely pathogenic, and variants of uncertain significance (VUS) are confirmed using bi-directional Sanger sequencing or by orthogonal methods such as qPCR/ddPCR when they do not meet our stringent NGS quality metrics for a true positive call.

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, abstracts, and variant databases used to help ordering providers 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. We do not recommend using variants of uncertain significance (VUS) for family member risk stratification or patient management. Genetic counseling is recommended.

Our interpretation team analyzes millions of variants from thousands of individuals with rare diseases. Our internal database and our understanding of variants and related phenotypes increases with every case analyzed. 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 healthcare provider at no additional cost, according to our latest follow-up reporting policy.

CPT code(s) *

81405(2), 81406(3), 81408, 81479

* The CPT codes provided are based on AMA guidelines and are for informational purposes only. CPT coding is the sole responsibility of the billing party. Please direct any questions regarding coding to the payer being billed.

ICD Codes

Refer to the most current version of ICD-10-CM manual for a complete list of ICD-10 codes.

Sample Requirements

- Blood (min. 1ml) in an EDTA tube
- Extracted DNA, min. 2 µg in TE buffer or equivalent
- Saliva (Please see Sample Requirements for accepted saliva kits)

Label the sample tube with your patient's name, date of birth and the date of sample collection.

We do not accept DNA samples isolated from formalin-fixed paraffin-embedded (FFPE) tissue. In addition, if the patient is affected with a hematological malignancy, DNA extracted from a non-hematological source (e.g. skin fibroblasts) is strongly recommended.

Please note that, in rare cases, mitochondrial genome (mtDNA) variants may not be detectable in blood or saliva in which case DNA extracted from post-mitotic tissue such as skeletal muscle may be a better option.

Read more about our sample requirements here.

For Patients

Other

- Children's Tumor Foundation
- Children's Tumor Foundation of Australia
- Eichenfield, LF. et al. Guidelines of care forneurofibromatosistype 1. American Academy of Dermatology Guidelines/Outcomes Committee. J Am Acad Dermatol. 1997 Oct;37(4):625-30.
- GeneReviews - LEOPARD Syndrome
- GeneReviews - Legius Syndrome
- GeneReviews - Neurofibromatosis 1
- GeneReviews - Neurofibromatosis 2
- Health supervision for children withneurofibromatosis. American Academy of Pediatrics Committee on Genetics. Pediatrics. 1995 Aug;96
- NORD - LEOPARD Syndrome
- Neurofibromatosis Network
- Radtke, HB. et al. Neurofibromatosistype 1 in genetic counseling practice: recommendations of the National Society of Genetic Counselors. J Genet Couns. 2007 Aug;16(4):387-407.
- Texas Neurofibromatosis Foundation
- The British Columbia Neurofibromatosis Foundation
- The Neuro Foundation
- The Neurofibromatosis Foundation