

## Noonan Syndrome Panel

Test code: CA0501

Is a 35 gene panel that includes assessment of non-coding variants.

Is ideal for patients with a clinical suspicion of a RASopathy including Noonan syndrome with or without lentigines, cardio-facio-cutaneous (CFC) syndrome, Costello syndrome, Noonan-like syndromes or other syndromes causing differential diagnostic challenges such as Legius syndrome, Baraitser-Winter syndromes and neurofibromatosis.

### About Noonan Syndrome

Noonan syndrome is one of the most common syndromes with an estimated prevalence of 1 in 1,000 to 1 in 2,500 live births. It is clinically and genetically heterogeneous condition characterized by cardiovascular abnormalities, distinctive facial features, chest deformity, short stature, and other co-morbidities. Among the Noonan syndrome associated genes, many different genotype-phenotype correlations have been established although no phenotypic features are exclusively associated with one genotype. There are, however, significant differences in the risk of various Noonan syndrome manifestations based on the causative gene.

### Availability

4 weeks

### Gene Set Description

Genes in the Noonan Syndrome Panel and their clinical significance

Gene	Associated phenotypes	Inheritance	ClinVar	HGMD
<a href="#">ACTB*</a>	Baraitser-Winter syndrome	AD	55	60
<a href="#">ACTG1*</a>	Deafness, Baraitser-Winter syndrome	AD	27	47
<a href="#">BRAF*</a>	LEOPARD syndrome, Noonan syndrome, Cardiofaciocutaneous syndrome	AD	134	65
CBL	Noonan syndrome-like disorder with or without juvenile myelomonocytic leukemia	AD	24	43
CCNK	Intellectual disability	AD		
CDC42	Takenouchi-Kosaki syndrome, Noonan-syndrome like phenotype	AD	11	9
EPHB4	Hydrops fetalis, nonimmune, and/or atrial septal defect, Capillary malformation-arteriovenous malformation	AD	1	51
FGD1	Aarskog-Scott syndrome, Mental retardation, syndromic	XL	29	51
HRAS	Costello syndrome, Congenital myopathy with excess of muscle spindles	AD	43	31
KAT6B	Ohdo syndrome, SBBYS variant, Genitopatellar syndrome	AD	47	73
<a href="#">KRAS*</a>	Noonan syndrome, Cardiofaciocutaneous syndrome	AD	63	35
LZTR1	Schwannomatosis, Noonan syndrome	AD/AR	34	71
MAP2K1	Cardiofaciocutaneous syndrome	AD	45	23

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MAP2K2	Cardiofaciocutaneous syndrome	AD	21	35
MAP3K8	Noonan syndrome	AD		1
MRAS	Noonan syndrome	AD	1	2
<a href="#">NF1*</a>	Watson syndrome, Neurofibromatosis, Neurofibromatosis-Noonan syndrome	AD	1157	2901
NF2	Schwannomatosis, Neurofibromatosis	AD	66	433
NRAS	Noonan syndrome	AD	31	14
NSUN2	Dubowitz syndrome, Non-syndromic intellectual disability	AD/AR	8	7
<a href="#">PPP1CB#</a>	Noonan syndrome-like disorder with loose anagen hair 2	AD	8	11
PTPN11	Noonan syndrome, Metachondromatosis	AD	135	140
RAF1	LEOPARD syndrome, Noonan syndrome, Dilated cardiomyopathy (DCM)	AD	45	53
<a href="#">RASA1#</a>	Parkes Weber syndrome, Capillary malformation-arteriovenous malformation, Spinal arteriovenous anomalies	AD	55	132
<a href="#">RASA2#</a>	Noonan syndrome	AD	1	3
RIT1	Noonan syndrome	AD	23	26
RRAS	Noonan-syndrome like phenotype	AD/AR		2
SASH1	Dyschromatosis universalis hereditaria	AD	1	12
SHOC2	Noonan-like syndrome with loose anagen hair	AD	2	4
SMARCB1	Schwannomatosis, Rhabdoid tumor predisposition syndrome, Coffin-Siris syndrome 3	AD	36	118
SOS1	Noonan syndrome	AD	44	71
SOS2	Noonan syndrome 9	AD	4	6
SPRED1	Legius syndrome	AD	38	71
STAMBP	Microcephaly-capillary malformation syndrome	AR	15	19
SYNGAP1	Mental retardation	AD	102	83

\*Some regions of the gene are duplicated in the genome. [Read more.](#)

# 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
FGD1	ChrX:54476768	c.2016-35delA	NM_004463.2	
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	

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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
NSUN2	Chr5:6622224	c.538-11T>G	NM_017755.5
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
STAMBP	Chr2:74077998	c.1005+358A>G	NM_006463.4

## Test Strengths

This Panel covers many recently discovered rasopathy genes such as *RRAS*, *PPP1CB*, *NRAS*, and *RASA2* not included in most panels in the market and provides wider differential diagnostics (Baraitser-Winter syndrome, NF1 and Legius syndrome).

**The strengths of this test include:**

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- 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
- 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 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 (please see the Panel Content section)
- Repeat expansion disorders unless specifically mentioned
- Non-coding variants deeper than  $\pm 20$  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 and see our Analytic Validation.

## 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

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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%

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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 artificial mutations; n=1500)		
Homoplasmic (100%) 500 bp, 1kb, 5 kb	100.0%	100.0%



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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  $\pm 20$  base pairs from the exon-intron boundary. In addition, the panel includes non-coding 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. 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.

## 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 corner stone of clinical interpretation and resulting patient management decisions. Our classifications follow the [ACMG guideline 2015](#).

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 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



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population cohorts and detailed information about related phenotypes. We also provide links to the references used, congress abstracts and mutation variant databases used 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.

## Reference information

[Pandit, B. et al. Gain-of-function \*RAF1\* mutations cause Noonan and LEOPARD syndromes with hypertrophic cardiomyopathy. \*Nat Genet\* 2007, 39\(8\), 1007–1012.](#)

[Richards S et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. \*Genet Med\* 2015 Mar 5, in press.](#)

[Romano, A.A. et al. Noonan syndrome: clinical features, diagnosis, and management guidelines. \*Pediatrics\* 2010, 126\(4\), 746–759.](#)

[Tartaglia, M. et al. Gain-of-function \*SOS1\* mutations cause a distinctive form of Noonan syndrome. \*Nat Genet\* 2006, 39\(1\), 75–79.](#)

[Yoshida, R. et al. Protein-Tyrosine Phosphatase, Nonreceptor Type 11 Mutation Analysis and Clinical Assessment in 45 Patients with Noonan Syndrome. \*J Clin Endocr Metab\* 2004, 89\(7\), 3359–3364.](#)

[Zenker, M. et al. Genotype-phenotype correlations in Noonan syndrome. \*J Pediatr\* 2004, 144\(3\), 368–374.](#)

## 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

- [Cook DM et al. A review of guidelines for use of growth hormone in pediatric and transition patients. Pituitary. 2012 Sep;15\(3\):301-10.](#)
- [GeneReviews - Noonan Syndrome](#)
- [NORD - Noonan Syndrome](#)
- [Noonan Syndrome Association - UK](#)
- [Pierpont ME et al. Cardio-facio-cutaneous syndrome: clinical features, diagnosis, and management guidelines. Pediatrics. 2014 Oct;134\(4\):e1149-62.](#)
- [Romano AA et al. Noonan syndrome: clinical features, diagnosis, and management guidelines. Pediatrics. 2010 Oct;126\(4\):746-59.](#)
- [The Noonan Syndrome Foundation - USA](#)