

## Noonan Syndrome: *RAF1* Gene Sequencing

**Test Code:** SRAF1

**Turnaround time:** 4 weeks

**CPT Codes:** 81406 x1

### Condition Description

Noonan syndrome (NS) is an autosomal dominant dysmorphology syndrome characterized by short stature, congenital heart defect, and developmental delay of variable degree. Other findings can include broad or webbed neck, unusual chest shape with superior pectus carinatum and inferior pectus excavatum, cryptorchidism, varied coagulation defects, lymphatic dysplasias, ocular abnormalities, and deafness. Characteristic facies include hypertelorism (74%), downward sloping palpebral apertures (38%), epicanthal folds (39%), ptosis (48%), and low-set posteriorly rotated ears. Early feeding difficulties such as poor suck or gastrointestinal dysfunction are also common. Although birth length is usually normal, final adult height approaches the lower limit of normal. Up to one-third of affected individuals have mild intellectual disability.

Congenital heart disease occurs in 50%-80% of individuals with NS. Pulmonary valve stenosis, often with dysplasia, is the most common heart defect and is found in 20%-50% of individuals. Hypertrophic cardiomyopathy is found in 20%-30% of individuals, and may be congenital or develop in infancy or childhood. Other structural defects include atrial and ventricular septal defects, branch pulmonary artery stenosis, and tetralogy of Fallot.

NS is clinically diagnosed. Affected individuals have normal chromosome studies. Molecular genetic testing identifies mutations in *PTPN11* in over 50% of affected individuals. Other genes known to be involved include *KRAS* in fewer than 5% of affected individuals, *SOS1* in approximately 13%, and *RAF1* in 3%-17%. Mutations in the *NRAS*, *BRAF*, and *MAP2K1* genes have been reported in less than 1% of cases.

Mutations in the *RAF1* gene (3p25) has been correlated with hypertrophic cardiomyopathy, with 95% of affected individuals with *RAF1* mutations showing this feature, in comparison with the overall prevalence in NS of 18%. This suggests that pathologic cardiomyocyte hypertrophy occurs because of increased Ras signaling.

Many affected individuals have de novo mutations; however, an affected parent is recognized in 30%-75% of families. When the parents are clinically unaffected, the risk to the sibs of a proband appears to be low (<1%). Noonan syndrome has an estimated incidence of 1 in 1,000 to 2,500 live births.

LEOPARD syndrome can also be caused by mutations in the *RAF1* gene. LEOPARD is an acronym for multiple lentiginos, electrocardiographic conduction abnormalities, ocular hypertelorism, pulmonic stenosis, abnormal genitalia, retardation of growth, and sensorineural deafness. LEOPARD syndrome is caused by gain-of-function mutations in *RAF1*. About one-third of families affected with LEOPARD syndrome without *PTPN11* mutations have a mutation in *RAF1*.

For patients with suspected *RAF1*-related Noonan syndrome, sequence analysis is recommended as the first step in mutation identification. For patients in whom mutations are not identified by full gene sequencing, deletion/duplication analysis is appropriate.

[Click here](#) for the GeneReviews Noonan syndrome summary.

[Click here](#) for the GeneReviews LEOPARD syndrome summary.

### Genes

[RAF1](#)

### Indications

This test is indicated for:

- Confirmation of a clinical diagnosis of Noonan syndrome

### Methodology

PCR amplification of 16 exons contained in the *RAF1* gene is performed on the patient's genomic DNA. Direct sequencing of amplification products is performed in both forward and reverse directions, using automated fluorescence dideoxy sequencing methods. The patient's gene sequences are then compared to a normal reference sequence. Sequence variations are classified as mutations, benign variants unrelated to disease, or variations of unknown clinical significance. Variants of unknown clinical significance may require further studies of the patient and/or family members. This assay does not interrogate the promoter region, deep intronic regions, or other regulatory elements, and does not detect large deletions.

### Detection

Clinical Sensitivity: *RAF1* mutations are found in 3%-17% of individuals clinically diagnosed with Noonan syndrome. Mutations in the promoter region, some mutations in the introns and other regulatory element mutations cannot be detected by this analysis. Large deletions will not be detected by this analysis. Results of molecular analysis should be interpreted in the context of the patient's biochemical phenotype.

Analytical Sensitivity: ~99%

### Specimen Requirements

**Submit only 1 of the following specimen types**

**Type: DNA, Isolated****Specimen Requirements:**

Microtainer

8µg

Isolation using the Perkin Elmer™ Chemagen™ Chemagen™ Automated Extraction method or Qiagen™ Puregene kit for DNA extraction is recommended.

**Specimen Collection and Shipping:**

Refrigerate until time of shipment in 100 ng/µL in TE buffer. Ship sample at room temperature with overnight delivery.

**Type: Whole Blood (EDTA)****Specimen Requirements:**

EDTA (Purple Top)

Infants and Young Children ( 2 years of age to 10 years old: 3-5 ml

Older Children &amp; Adults: 5-10 ml

Autopsy: 2-3 ml unclotted cord or cardiac blood

**Specimen Collection and Shipping:**

Ship sample at room temperature for receipt at EGL within 72 hours of collection. Do not freeze.

**Type: Saliva****Specimen Requirements:**

Oragene™ Saliva Collection Kit

Oragene™ Saliva Collection Kit used according to manufacturer instructions. Please contact EGL for a Saliva Collection Kit for patients that cannot provide a blood sample.

**Specimen Collection and Shipping:**

Please do not refrigerate or freeze saliva sample. Please store and ship at room temperature.

**Special Instructions**

Submit copies of diagnostic biochemical test results with the sample, if appropriate. Contact the laboratory if further information is needed.

Sequence analysis is required before deletion/duplication analysis by targeted CGH array. If sequencing is performed outside of EGL Genetics, please submit a copy of the sequencing report with the test requisition.

**Related Tests**

- Deletion/duplication analysis of the *RAF1* gene by CGH array is available for those individuals in whom sequence analysis is negative.
- Sequence and deletion/duplication analysis of the *PTPN11*, *RAF1*, *SOS1*, *NRAS* and *KRAS* genes are also available.
- [Custom diagnostic mutation analysis \(KM\)](#) is available to family members if mutations are identified by targeted mutation testing or sequencing analysis.
- Prenatal testing is available to adults who are confirmed carriers of mutations. Please contact the laboratory genetic counselor to discuss appropriate testing prior to collecting a prenatal specimen.