

## Noonan Syndrome and Related Disorders Panel: Sequencing and CNV Analysis

**Test Code:** MNOO1

**Turnaround time:** 6 weeks

**CPT Codes:** 81311 x1, 81405 x1, 81406 x1

### Condition Description

The Ras/mitogen activated protein kinase (MAPK) pathway is involved in the control of the cell cycle and differentiation. Because of this critical role, a disruption in the pathway results in congenital developmental conditions. A class of human genetic syndromes, called the rasopathies, are caused by germline mutations in the genes in this pathway. The rasopathies, while distinct syndromes, share some overlapping features such as craniofacial dysmorphism, varying degrees of neurocognitive impairments, cutaneous, ocular, and musculoskeletal abnormalities, and cardiac malformations. Some syndromes have an increased risk of cancer.

#### Noonan Syndrome

Noonan syndrome (NS) is an autosomal dominant disorder characterized by congenital heart defects, short stature, developmental delay, distinctive craniofacial features that change with age, broad or webbed neck, unusual chest shape, apparently low-set nipples and cryptorchidism. About one third of individuals with NS have mild intellectual disability. Individuals with NS have an increased risk for cancer.

There are seven genes known to be cause NS; *PTPN11*, *SOS1*, *RAF1*, *KRAS*, *NRAS*, *BRAF*, and *MAP2K1*. Approximately 50% of NS cases are caused by mutations in the *PTPN11* gene, 10-13% by the *SOS1* gene, 3-17% by the *RAF1* gene, and fewer than 5% by the *KRAS*. Fewer than 1% of cases are caused by mutations in the *NRAS*, *BRAF*, and *MAP2K1* genes.

#### LEOPARD Syndrome

LEOPARD syndrome (LS) is an autosomal dominant disorder characterized by features that make up the acronym LEOPARD. They are lentigens, EKG abnormalities, ocular hypertelorism, pulmonary valve stenosis, abnormal genitalia, retardation of growth, and sensorineural deafness. Like NS, up to one third of individuals with LS have mild intellectual disability.

Three genes are known to cause LS; *PTPN11*, *RAF1*, and *BRAF*. Approximately 90% of LS cases are caused by mutations in the *PTPN11* gene. Fewer than 5% of LS cases are caused by mutations in the *RAF1* or the *BRAF* gene.

#### Cardiofaciocutaneous Syndrome

Cardiofaciocutaneous (CFC) syndrome is an autosomal dominant disorder characterized by features in three primary systems: cardiac, craniofacial, and ectodermal; however, other systems may be involved as well. Cardiac abnormalities can include pulmonic stenosis and other valve dysplasias, septal defects, hypertrophic cardiomyopathy, and rhythm disturbances. Individuals with CFC syndrome have a distinctive craniofacial appearance. Ectodermal features include skin findings, such as xerosis, hyperkeratosis, ichthyosis, keratosis pilaris, ulerythema oophorogenes, eczema, pigmented moles, palmoplantar hyperkeratosis; hair findings such as sparse, curly, fine or thick, woolly, or brittle hair, and possible absent eyelashes and eyebrows; and the nails may be dystrophic or fast growing. Cognitive delay (ranging from mild to severe) is seen in all affected individuals. Neoplasias have been reported in some individuals with CFC.

There are four genes known to be associated with CFC; *BRAF*, *MAP2K1*, *MAP2K2*, and *KRAS*. Mutations in the *BRAF* gene account for ~75% of cases, *MAP2K1* and *MAP2K2* account for ~25% of cases, and *KRAS* accounts for <2% of cases.

#### Costello Syndrome

Costello syndrome is an autosomal dominant disorder characterized by developmental delay, intellectual disability, diffuse hypotonia, failure to thrive in infancy (due to severe postnatal feeding difficulties), short stature, coarse facial features (full lips, large mouth); curly or sparse, fine hair; loose, soft skin, and tight Achilles tendons. Cardiac features includes cardiac hypertrophy, congenital heart defect, and arrhythmias. Individuals with Costello syndrome have an approximately 15% lifetime risk for malignant tumors. The solid tumors rhabdomyosarcoma and neuroblastoma occur most frequently in young children. Adolescents and young adults are at risk for transitional cell carcinoma of the bladder.

The *HRAS* gene is the only gene currently known to be associated with Costello syndrome. In patients with a clinical diagnosis of Costello syndrome, 80-90% of mutations in the *HRAS* gene can be identified.

#### Noonan-Like Syndrome with Loose Anagen Hair

Noonan-like syndrome with loose anagen hair (NS/LAH) is an autosomal dominant disorder characterized by facial features similar to Noonan syndrome, reduced growth, intellectual disability, distinctive hyperactive behavior, and hair anomalies. The hair is typically sparse, thin, easily pluckable, and slow growing. Additional features include hairless skin that is darkly pigmented, eczema or ichthyosis, sparse eyebrows, thin or dystrophic nails, hypernasal or hoarse voice, and cardiac anomalies.

The *SHOC2* gene is the only gene known to be associated with NS/LAH.

#### References:

- GeneReviews
- Tidyman and Rauen (2009). *Curr Opin Genet Dev*, 19:230-236.
- Tartaglia *et al.* (2011). *Best Pract Res Clin Endocrinol Metab*, 25:161-179.

### Genes

[BRAF](#), [CBL](#), [HRAS](#), [KRAS](#), [MAP2K1](#), [MAP2K2](#), [NRAS](#), [PTPN11](#), [RAF1](#), [RIT1](#), [SHOC2](#), [SOS1](#), [SPRED1](#)

### Indications

Confirmation of a clinical diagnosis of Noonan syndrome or a related disorder.

## Methodology

**Next Generation Sequencing:** In-solution hybridization of all coding exons is performed on the patient's genomic DNA. Although some deep intronic regions may also be analyzed, this assay is not meant to interrogate most promoter regions, deep intronic regions, or other regulatory elements, and does not detect single or multi-exon deletions or duplications. Direct sequencing of the captured regions is performed using next generation sequencing. The patient's gene sequences are then compared to a standard reference sequence. Potentially causative variants and areas of low coverage are Sanger-sequenced. Sequence variations are classified as pathogenic, likely pathogenic, benign, likely benign, or variants of unknown significance. Variants of unknown significance may require further studies of the patient and/or family members.

**Copy Number Analysis:** Comparative analysis of the NGS read depth (coverage) of the targeted regions of genes on this panel was performed to detect copy number variants (CNV). The accuracy of the detected variants is highly dependent on the size of the event, the sequence context and the coverage obtained for the targeted region. Due to these variables and limitations a minimum validated CNV size cannot be determined; however, single exon deletions and duplications are expected to be below the detection limit of this analysis.

## Detection

**Clinical Sensitivity:** Noonan syndrome - 70-88%, Leopard syndrome – 95%, CFC - unknown, Costello syndrome – 80-90%, NS/LAH - unknown. Mutations in the promoter region, some mutations in the introns and other regulatory element mutations cannot be detected by this analysis. Results of molecular analysis should be interpreted in the context of the patient's clinical and/or biochemical phenotype.

Analytical sensitivity for sequence variant detection is ~99%.

**Copy Number Analysis:** The sensitivity and specificity of this method for CNV detection is highly dependent on the size of the event, sequence context and depth of coverage for the region involved. The assay is highly sensitive for CNVs of 500 base pairs or larger and those containing at least 3 exons. Smaller (< 500 base pairs) CNVs and those that involving only 1 or 2 exons may or may not be detected depending on the sequence context, size of exon(s) involved and depth of coverage.

## 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 & 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.

## Related Tests

- Single gene sequencing and deletion/duplication analysis are available for the *PTPN11*, *SOS1*, *RAF1*, *KRAS*, *HRAS*, *BRAF*, *MAP2K1*, *MAP2K2*, *SHOC2* and *NRAS* genes.
- A next generation sequencing panel is available for short stature.
- Custom diagnostic mutation analysis (KM) is available to family members if mutations are identified by targeted mutation testing or sequencing analysis.