UAB MEDICAL GENOMICS LABORATORY

Expanded *NF1*-RASopathy panel by Next-Gen Sequencing and Deletion/Duplication Analysis of *NF1* and *SPRED1* (RAS-NG)

Ordering Information

Acceptable specimen types:

- Blood (2-3ml EDTA; no time limitations associated with receipt)
- Saliva (OGR-575 DNA Genotek; kits are provided upon request)
- DNA (extracted from lymphocyte cells, a minimum of 3µg, O.D. value at 260:280nm ≥1.8)
- Flash frozen tumor sent on dry ice
- Fresh tumor or affected tissue biopsy, immersed in sterile culture media (PBS/RPMI)

Turnaround time:

30 working days for blood, saliva, or DNA; 40 working days for fresh/frozen tumor

Price, CPT codes, and Z code:

$1,500 (USD – institutional/self-pay price);

$2,500 for fresh/frozen tumor (USD – institutional/self-pay);

CPT: 81442 and 81479 (x3)

Z code: ZB6A6

Candidates for this test:

Patients with clinical features suggestive of either NS, NSML, CFC, NF1, Legius syndrome or Noonan-like syndrome; patients with a clinical diagnosis of any of these syndromes that

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previously tested negative in a subset of the genes included in this panel; patients with a diagnosis of Costello syndrome but no *HRAS* variant previously identified

Specimen shipping and handling:

- Please find acceptable specimen type above.
- All submitted specimens must be sent at room temperature. DO NOT ship on ice.
- Specimens must be packaged to prevent breakage and absorbent material must be included in the package to absorb liquids in the event that breakage occurs. Also, the package must be shipped in double watertight containers (e.g. a specimen pouch + the shipping company's diagnostic envelope).
- To request a sample collection kit, please visit the website or email medgenomics@uabmc.edu to complete the specimen request form.
- Please contact the MGL (via email at medgenomics@uabmc.edu, or via phone at 205-934-5562) prior to sample shipment and provide us with the date of shipment and tracking number of the package so that we can better ensure receipt of the samples.

Required forms:

- Test Requisition Form
- Form for Customs (for international shipments)

Note: Detailed and accurate completion of this document is necessary for reporting purposes. The Medical Genomics Laboratory issues its clinical reports based on the demographic data provided by the referring institution on the lab requisition form. It is the responsibility of the referring institution to provide accurate information. If an amended report is necessary due to inaccurate or illegible documentation, additional reports will be drafted with charge.

Requests for testing may not be accepted for the following reasons:

- No label (patients full name and date of collection) on the specimens
- No referring physician's or genetic counselor's names and addresses
- No billing information
- DNA samples must be extracted in a CLIA certified lab

For more information, test requisition forms, or sample collection and mailing kits, please call: 205-934-5562.

Disorder Background

The RASopathies are a genetically heterogeneous group of disorders caused by variants in the genes involved in the Ras-MAPK pathway. As a group, the RASopathies are one of the largest groups of malformation syndromes known, affecting ~1:1,000 and include Neurofibromatosis type 1, Legius syndrome, Noonan syndrome, cardio-facio-cutaneous (CFC) syndrome, Noonan Syndrome with Multiple Lentigines (NSML/LEOPARD) and Costello syndrome. Variants in *NF1* and *SPRED1* are typically loss-of-function variants and include the full spectrum of nonsense, missense, splice, frameshift, insertion-deletion, and copy number changes. Variants in the other RASopathy genes are typically missense variants or an in-frame deletion/insertion of an amino acid.

The genes within the Ras/MAPK pathway can have a profound deleterious effect on development due to their key role in differentiation, growth, senescence, and dysregulation. Clinical features of the RASopathies include short stature; cardiovascular defects; cutaneous and pigmentary findings; characteristic facies; skeletal and neurocognitive delays as well as a predisposition to neoplasia, both benign and malignant. The RASopathies are inherited in an autosomal dominant manner. A parent who carries a gene variant has a 50% chance of passing it on to every child, regardless of gender. The disorders have variable expressivity (individuals with the same disorder may show differing features and severity of symptoms, even within the

same family). Some of the genes/variants are not fully penetrant; therefore an individual may carry a variant and only show few to no signs of the syndrome. Moreover, features can change/progress with age, which makes it difficult to make an accurate clinical diagnosis.
An individual can carry a variant either:

a. Because (s)he inherited the variant from a parent (parent clinically affected or "non-penetrant"), or

b. Because the variant arose "de novo" in the egg or sperm from which the individual developed.

Sometimes, the variant occurred "post-zygotically", i.e. during development and in these individuals the variant may not be present in every cell of the body, typically resulting in a milder phenotype (mosaicism).

Noonan syndrome (NS), Noonan Syndrome with Multiple Lentigines (NSML, aka LEOPARD) and Noonan syndrome with "loose anagen hair" are autosomal dominant disorders affecting ~1:1,000-2,000 individuals. Patients present with craniofacial features and a variable clinical phenotype including congenital heart defects, reduced growth, bleeding disorders (NS), and variable degrees of neurocognitive delay. Patients with NSML also have multiple lentigines, genital abnormalities and sensorineural deafness. Patients with NS also have an increased cancer predisposition. Genes associated with NS and NSML are *PTPN11, LZTR1, KRAS, SOS1, RAF1, NRAS, BRAF, MAP2K1, CBL, RIT1, RASA2*, and *SOS2*. The *SHOC2* gene is associated with NS with "loose anagen hair" or sparse slow growing hair.

Cardio-Facio-Cutaneous syndrome (CFC) is a rare condition with genetic and phenotypic overlap with NS. Clinical features include craniofacial features similar to those found in NS, neurocognitive delay, failure to thrive, congenital heart defects, epilepsy and a wide range of ectodermal manifestations. Four genes have been associated with CFC: *BRAF, MAP2K1, MAP2K2 and KRAS*.

Costello syndrome (CS), caused by activating *HRAS* variants, is a very rare condition with the following key features: coarse facial features, severe feeding difficulty, mild to moderate intellectual disability, relative macrocephaly and short stature, high incidence of cardiac abnormalities and malignancy. Differentiation of CS from other RASopathies, particularly CFC may be difficult especially early in life.

Some individuals with a clinical diagnosis of one of the RASopathies have been found to carry a variant in a gene that was not considered to be consistent with their clinical diagnosis. Examples include *BRAF* variants reported in individuals with a clinical diagnosis of Noonan syndrome, a *SOS1* variant in an individual with CFC (Nystrom AM et al, 2008), *PTPN11* variants in individuals with paraspinal neurofibromas (Conboy E. et al, 2015), and an NF1 missense variant in patients with Noonan-like features and no neurofibromas (Rojnueangnit K et al, 2015). In addition, some genes are associated with more than one syndrome (*PTPN11, KRAS, BRAF, RAF1, and NF1*). Therefore, the comprehensive approach of simultaneously testing all 17 genes in some individuals eliminates the need to determine which genes to test based on an individual's clinical signs.

Test Description

The **Expanded *NF1*-Rasopathy panel by NGS** involves the simultaneous sequencing of **18 genes**: *NF1, SPRED1, LZTR1, PTPN11, PPP1CB, BRAF, CBL, HRAS, KRAS, NRAS, MAP2K1, MAP2K2, RAF1, RIT1, RASA2, SHOC2, SOS1* and *SOS2*. The test uses the same approach as detailed previously (see: *NF1*-only by NGS). **The average coverage is ~1600x with >98% of the coding region ≥350x and >99% ≥200x, allowing detection of very low-level mosaicism, down to 3-5% variant allele fraction with 95% confidence.** The minimum coverage for any additional areas is >30x. Variant and copy number calls are made using a unique bioinformatics pipeline detecting all types of variants including single nucleotide substitutions, indels, and frameshifts caused by deletion/ duplication up to 112bp. Deletion/duplication analysis for *NF1, SPRED1,* and *LZTR1* is included in this test, as such variants are a part of the variant spectrum for these conditions. Deletion/duplication analysis for the other 15 genes on this panel is not offered as current

empirical and biological evidence is not sufficient to allow the conclusion that an altered copy number of these genes is a mechanism critical for the phenotype associated with the Rasopathies.

Based on >15 years of experience with comprehensive RNA-based *NF1* testing, we designed the **customized and optimized NGS *NF1* assay** comprising **all regions** encountered through analysis of >15,000 unrelated individuals including >8,000 *NF1*-variant-positive individuals carrying 1 out of >3,100 different *unique NF1* variants identified in the UAB MGL cohort. Included in the NGS assay are the regions covering >65 different deep intronic splice variants (which reside beyond the +/-50 intronic base pairs that flank all exons). Validation of the full panel included, besides substitutions (missense, nonsense, splice variants), the most challenging variants such as insertions/deletions/duplications of 1-64 nucleotides (~25% of the UAB *NF1* cohort) and one-to-multiple exon deletions/duplications (~2.8% of the UAB *NF1* cohort). The analytical sensitivity of our NGS testing approach was 100% for substitutions as well as insertion/deletions up to 112bp. The panel has been validated for the detection of *germline* (heterozygous) *single*-exon deletions/duplications as well as multi-exon deletions/duplications. *Single* exon deletions/duplications are present in ~0.45% of *NF1*-positive patients from the UAB cohort with 9% of these individuals being mosaic (~0.045% of all in the UAB *NF1*-positive cohort).

With the **largest dataset of *NF1* genotypes matched with phenotypes**, any genotype-phenotype correlations identified will be reported in real time.

Confirmatory testing of reportable variants is performed by Sanger sequencing or other orthogonal methods.

For **novel *NF1* variants of unknown significance**, we offer free of charge targeted RNA-based testing to assess the effect of the variant on splicing and enhance the correct classification/ interpretation.

Relevant family members of a proband with any (novel or previously identified) variant of unknown significance are offered free of charge targeted analysis as long as accurate

phenotypic data are provided by a health care professional to enhance the interpretation. There is no limitation to the number of relatives that can be tested free of charge.

Mosaicism is often present in sporadic patients with an ***NF1* microdeletion** and has important repercussions for counseling. (Kehrer-Sawatzki H, Mautner VF, Cooper DN. 2017). Evaluation by **FISH analysis on 200 interphase chromosomes** can be offered in such cases.

REFERENCES available on website.

Other related testing options:

- Next-Gen Sequencing and Deletion/Duplication analysis of *NF1* only (NF1-NG)
- Next-Gen Sequencing and Deletion/Duplication analysis of *NF1* and *SPRED1* only (NFSP-NG)
- RNA-based *NF1* testing on blood (NF1-R)
- RNA-based *NF1* and DNA-based *SPRED1* testing on blood (NFSP-R)
- RNA-based *NF1/SPRED1* testing on affected tissues (NF14N/NF14C)