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)

Turnaround time:

25 working days (RUSH option: 15 working days for additional $600 USD)

Price, CPT codes, and Z code:

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

CPT: 81442 and 81479 (x2)

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

720 Twentieth Street South, Suite 330 Phone: (205) 934-5562
Birmingham, Alabama 35294-0005 Fax: (205) 996-2929
www.uab.edu/medicine/genetics/medical-genomics-laboratory

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 mutations 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. Mutations in *NF1* and *SPRED1* are typically loss-of-function mutations and include the full spectrum of nonsense, missense, splice, frameshift, insertion-deletion, and copy number changes. Mutations in the other RASopathy genes are typically missense mutations or an in-frame deletion/insertion of an amino acid.

The Ras/MAPK pathway can have a profound deleterious effect on development as it plays a 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 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/mutations are not fully penetrant; therefore an individual may carry a mutation but not show any or only few signs of the syndrome. Moreover, features can change/progress with age, which makes it difficult to make an accurate clinical diagnosis. The RASopathies are inherited in an autosomal dominant manner. A parent who carries a mutated

gene has a 50% chance of passing it on to every child, regardless of gender.
An individual can carry a mutation either:

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

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

Sometimes, the mutation occurred "post-zygotically", i.e. during development and in these individuals the mutation may not be present in every cell of the body, typically resulting in a milder phenotype due to 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, 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* mutations, 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 mutation 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*mutations in individuals with paraspinal neurofibromas (Conboy E. et al, 2015), and an NF1 missense mutation 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 16 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 **17 genes**: *NF1, SPRED1, 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 >1800x with >99.8% of the *NF1* coding region ≥350x and 100% ≥200x, allowing detection of very low level mosaicism, down to 3-5% MAF respectively (regions covered by ≥350x respectively ≥200x) with 95% confidence.** For the remainder of the genes, the average coverage is 1800x with >99.4% of the coding region covered at ≥350x and 99.85% covered at 200x. 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 mutations including single nucleotide substitutions, indels, and frameshifts caused by deletion/ duplication up to 112bp. Deletion/duplication analysis for NF1/SPRED1 is included in this test, as such mutations are a part of the mutation 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*-component** of the assay to comprise **all regions** encountered through analysis of >15,000 unrelated individuals including >8,100 *NF1*-mutation-positive individuals carrying 1 out of >3,100 different *unique NF1* mutations identified in the UAB MGL cohort. Included in the NGS assay are the regions covering >65 different deep intronic splice mutations (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 mutations such as insertions/deletions/duplications of 1-112bp (~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. This panel has not yet been validated to identify deletions/duplications >112bp and <1 exon, but such mutations have not yet been found in the UAB cohort, and therefore are likely very rare. The panel has been validated for the detection of *germline* (heterozygous) *single*-exon deletions/duplications as well as multi-exon deletions/duplications, however *mosaic* single-exon deletion/duplications validation is still pending. *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). Detection of Alu/LINE insertions, identified in 0.25% of patients from the UAB *NF1*-positive cohort, has not yet been validated using the current NGS approach.

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. Free of charge evaluation by F**ISH analysis on 200 interphase chromosomes** is 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)