

# UAB MEDICAL GENOMICS LABORATORY

## Next-Gen Sequencing and Deletion/Duplication Analysis of *NF1* and *SPRED1* Only (NFSP-NG)

### Ordering Information

#### Acceptable specimen types:

- Fresh blood sample (3-6 ml EDTA; no time limitations associated with receipt)
- Saliva (OGR-575 DNA Genotek; kits are provided upon request)
- DNA (extracted from lymphocyte cells; a minimum volume of 25 $\mu$ L at 3 $\mu$ g; O.D. of 260:280nm  $\geq$ 1.8; must be extracted in a CLIA or equivalent certified lab)

#### Turnaround time:

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

#### Price, CPT codes, and Z code:

\$1,100 (USD – institutional/self-pay);

CPT: 81408, 81405, and 81479 (x2)

Z code: ZB6A8

#### Candidates for this test:

Patients with multiple CALMs with/without skinfold freckling and no other typical *NF1* features (Lisch nodules, bone abnormalities, neurofibromas, optic pathway gliomas)

#### Specimen shipping and handling:

- Please find acceptable specimen type above.
- All submitted specimens must be sent at room temperature. DO NOT ship on ice.

# UAB MEDICAL GENOMICS LABORATORY

- 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](mailto:medgenomics@uabmc.edu) to complete the specimen request form.
- Please contact the MGL (via email at [medgenomics@uabmc.edu](mailto: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 or equivalent certified lab

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

## Disorder Background

Germline loss-of-function mutations in *SPRED1*, a negative regulator of the RAS-MAPK pathway, cause a neurofibromatosis type 1-like phenotype, first described in 2007 (Legius syndrome). Patients present with multiple café-au-lait spots with or without skinfold freckling. Other typical NF1 associated features (Lisch nodules, bone abnormalities, neurofibromas, optic pathway gliomas) are systematically absent. However, in some individuals Noonan-like features have been reported.

In individuals with CALMs with or without freckling and no other specific distinguishing features, the NIH criteria cannot reliably distinguish NF1 from Legius syndrome. In such patients, a correct diagnosis has important implications for prognosis, counseling, and potential prenatal genetic diagnosis. Based on a cross-sectional study we estimate that patients presenting sporadically with these pigmented signs only will carry a mutation in the *NF1* gene in ~43% of cases and in the *SPRED1* gene in ~1.3% of cases. When such patients have a family history of CALMs with or without freckling and no additional NF1-related criteria, an *NF1* mutation will be found in ~73% of cases and in the *SPRED1* gene in ~19% of cases.

*SPRED1* is a member of the SPROUTY/SPRED family of proteins that act as negative regulators of RAS-RAF interaction and mitogen-activated protein kinase (MAPK) signaling.

## Test Description

The **DNA-based *NF1/SPRED1*-only by NGS** involves sequencing as well as **deletion/duplication analysis** of the entire coding *NF1/SPRED1* region plus the alternatively spliced exons 9br, 23a and 48a (67 exons total). The test uses an extensively customized and optimized set of Agilent HaloPlex capture probes, followed by sequencing of overlapping amplicons within the regions of interest using 300bp paired-end Illumina sequencing chemistry. Each coding exon plus ~50bp of flanking intronic sequence are simultaneously sequenced. 5' and 3' untranslated sequences

# UAB MEDICAL GENOMICS LABORATORY

are not included. **The average coverage is >1800x with >99.8% of the *NF1* coding region  $\geq 350x$  and 100%  $\geq 200x$ , allowing detection of very low level mosaicism, down to 3-5% MAF respectively (regions covered by  $\geq 350x$  respectively  $\geq 200x$ ) with 95% confidence.** 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.

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.

# UAB MEDICAL GENOMICS LABORATORY

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 **FISH 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)
- Expanded *NF1*-Rasopathy panel by Next-Gen Sequencing (RAS-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)