UAB MEDICAL GENOMICS LABORATORY

Next-Gen Sequencing and Deletion/Duplication Analysis of *NF1* Only (NF1-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 25µL at 3µg; O.D. of 260:280nm ≥1.8; must be extracted in a CLIA or equivalent certified lab)

Turnaround time:

30 working days

Price, CPT codes, and Z code:

$1,000 (USD – institutional/self-pay);

CPT: 81408 and 81479

Z code: ZB6A9

Candidates for this test:

Patients with classic NF1 including the presence of cutaneous neurofibromas or Lisch nodules, as no genetic heterogeneity has been demonstrated so far associated with this phenotype.

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 or equivalent certified lab

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

Disorder Background

The NF1 gene, cloned in 1990, was the first gene within the Ras-MAPK pathway shown to be associated with an autosomal dominant disorder, Neurofibromatosis type I (NF1). NF1 affects ~1/3000 individuals worldwide, with half of the patients resulting from a de novo event within their family (sporadic). NF1 is notorious for its phenotypic variability and is a progressive disorder with more signs developing with time. Although the NIH criteria enables clinicians to make a diagnosis in the majority of classically affected cases, diagnostic criteria are not met until a given age is reached. Atypical presentations also exist with patients not yet fulfilling NIH criteria by adulthood. The mutational spectrum of NF1 is very complex and includes a wealth of unusual splice variants affecting exonic sequences as well as deep intronic variants resulting in exonization of intronic sequences at the mRNA level.

Test Description

The ***NF1*-only by NGS** involves sequencing as well as **deletion/duplication analysis** of the entire coding *NF1* region plus the alternatively spliced exons 9br, 23a and 48a (60 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 are not included.

The average coverage is >1600x with >98% of the *NF1* coding region ≥350x and 99% ≥200x, allowing detection of very low level mosaicism, down to 3-5% variant allele fraction (regions covered by ≥350x and ≥200x respectively) with 95% confidence. 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.

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 >8,000 *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 1-112bp (~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. 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. 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* and *SPRED1* only (NFSP-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)