

Next-Gen Sequencing and Deletion/Duplication Analysis of *NF2* Only (NF2-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 μ L at 3 μ g; O.D. of 260:280nm \geq 1.8; must be extracted in a CLIA or equivalent certified lab)
- A minimum of 2 anatomically distinct tumor samples is suggested, however, a single tumor can be provided
 - Flash frozen tumor sent on dry ice*
 - Fresh tumor or affected tissue biopsy, immersed in sterile culture media (PBS/RPMI)*
 - Tumor block should have a surface area \geq 5mm squared or the specimen contains at least 3-6 loose paraffin curls (no slides) that are 30-50 microns thick*

*Specimen should contain at least 70% pure tumor content and >80% nucleated cells

Turnaround time:

30 working days for blood, saliva, or DNA

40 working days for fresh/frozen tumor or tumor block

Price, CPT codes, and Z code:

\$800 for blood, saliva, or DNA (USD – institutional/self-pay);

\$1,500 for fresh/frozen tumor or tumor block (USD – institutional/self-pay);

CPT: 81406, 81405

UAB MEDICAL GENOMICS LABORATORY

Z code: ZB6AA

Candidates for this test:

Patients with bilateral vestibular schwannomas with or without other typical *NF2*-associated features (congenital cataracts, ependymoma, facial weakness, etc).

Tumor: Patients with one or more features associated with *NF2*-related schwannomatosis without a family history of the condition and/or no variant was identified by blood based testing.

Tumor: For sporadic patients with multiple schwannomas but without vestibular nerve involvement and in whom *NF2* variants are found in the tumor, we will only be able to differentiate between mosaic *NF2*-related schwannomatosis and *LZTR1*- or *SMARCB1*-schwannomatosis if material from 2 separate tumors is available and provided. There will be no additional cost associated with the analysis of more than one tumor.

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.

UAB MEDICAL GENOMICS LABORATORY

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

NF2-related schwannomatosis is an autosomal dominant disorder characterized by bilateral vestibular schwannomas with associated symptoms of tinnitus, hearing loss and balance dysfunction. Almost all affected individuals develop bilateral vestibular schwannomas by 30 years of age. Affected individuals may also develop schwannomas of other cranial and peripheral nerves, meningiomas, and ependymomas. *NF2*-related schwannomatosis is an autosomal dominant disorder with a frequency of 1:33-40,000 births in all populations. About 50% of patients are due to a de novo variant, where neither parent has signs of the disorder.

UAB MEDICAL GENOMICS LABORATORY

The offspring of an affected individual have a 50% risk of inheriting the altered *NF2* gene. *NF2* is the only gene in which pathogenic variants are known to cause *NF2*-related schwannomatosis.

Test Description

The ***NF2*-only by NGS** involves sequencing as well as **deletion/duplication analysis** of the entire coding *NF2* region using MLPA. 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 100% of the *NF2* coding region \geq 350x. This allows for detection of very low-level mosaicism by sequencing (as low as 3% of the alleles in 100% of the coding region 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. Deletion/duplication analysis for *NF2* using MLPA is included in this test, as such variants are a part of the variant spectrum for these conditions.

Variant detection rate in leukocytes is >90% in non-founder *NF2* patients. Variants detected include truncating variants (nonsense, frameshift, splicing variants), missense variants, multi-exon deletions or duplications and total gene deletions.

In about 25-30% of founders (simplex cases, patients with unaffected parents), variants are not detected in blood lymphocytes as a result of somatic mosaicism. Only variants with mosaicism levels greater than 10% can be detected in lymphocyte DNA (Evans et al, 2007). Identification of the majority of mosaic variants requires testing of tumor tissue (Evans et al, 2007). As RNA is most often degraded in available tumor material, a DNA-based comprehensive analysis is applied.

UAB MEDICAL GENOMICS LABORATORY

REFERENCES available on website.

Other related testing options:

- Meningiomatosis/Multiple Meningioma Panel by Next-Gen Sequencing (MEN-NG)
- Schwannomatosis/Multiple Schwannoma Panel by Next-Gen Sequencing (SCH-NG)