Peripheral Nerve Sheath Tumor Panel by Next-Gen Sequencing (PNT-NG)

Ordering Information

Acceptable specimen types:

- Blood (3-6ml 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)
- Fresh or Frozen Tumor (3-5mm-cubed, >70% pure tumor material)

Turnaround time:

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

Price, CPT codes, and Z code:

$1,500 for blood, saliva, or DNA (USD – institutional/self-pay);
$2,500 for fresh/frozen tumor (USD – institutional/self-pay);
CPT: 81408, 81406, 81405, and 81479 (x4)
Z code: ZB6AE

Candidates for this test:

Patients presenting with both neurofibromas and schwannomas or peripheral nerve sheath tumors with mixed or unconfirmed cellularity with minimal additional findings meeting diagnostic criteria for any specific condition
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
Disorder Background

Peripheral nerve sheath tumors develop from the Schwann cells, i.e. cells covering the peripheral nerves, and can be divided into benign and malignant tumors, although ~90% of such tumors are benign. Nerve sheath tumors include neurofibromas and schwannomas. They can occur as solitary lesions; however, if multiple develop, this may indicate the presence of a hereditary predisposition. Neurofibromas are common, benign tumors, composed of a complex heterogeneous mixture of cells, and the presence of 2 or more such tumors (cutaneous, subcutaneous or plexiform) is a diagnostic sign of neurofibromatosis type 1, with two NF1 hits found specifically in the Schwann cells only. Whereas cutaneous neurofibromas never undergo malignant transformation, subcutaneous and plexiform neurofibromas may undergo malignant transformation (Evans DG, J Med Genet. 2002 May;39(5):311-4.).

Schwannomas are more homogeneously composed benign tumors and if multiple such tumors are present, they may indicate the presence of neurofibromatosis type 2 (NF2) or schwannomatosis. The location of the schwannomas differs between NF2 and SMARCB1- or LZTR1-related schwannomatosis with (bilateral) vestibular schwannomas typically present in classic NF2, where intradermal and non-intradermal schwannomas are also frequently found. Schwannomatosis-associated schwannomas are usually non-intradermal and non-vestibular, however overlap clearly exists and genetic characterization can be an important tool to distinguish between both disorders.
Some types of schwannomas mimic neurofibromas and vice versa. They are called myxoid schwannomas and hybrid neurofibromas/schwannomas.

In addition, one patient with a \textit{KRAS} germline variant \texttt{p.K5E} and multiple diffuse schwannomas was described and a \textit{KRAS} variant \texttt{p.G12S} was found in 1/40 sporadic schwannomas.


\textbf{Test Description}

The \textbf{Peripheral Nerve Sheath Tumor Panel by NGS} involves the simultaneous sequencing of 6 genes: \textit{NF1}, \textit{NF2}, \textit{KRAS}, \textit{LZTR1}, \textit{PTPN11}, and \textit{SMARCB1}. 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 \textasciitilde{}50bp of flanking intronic sequence are simultaneously sequenced. 5’ and 3’ untranslated sequences are not included. \textbf{The average coverage is >2000x with >98.7\% of the coding region \textasciitilde{}350x and >99.7\% at 200x.} The minimum coverage for any additional areas is \textasciitilde{}30x. This allows for \textbf{detection of very low-level mosaicism} by sequencing (as low as 3\% of the alleles in >98.7\% of coding regions with coverage \textasciitilde{}350x). 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 \textit{NF1}, \textit{NF2}, \textit{SMARCB1}, and \textit{LZTR1} is included in this test,
as such variants are a part of the variant spectrum for these conditions. Deletion/duplication analysis for PTPN11 and KRAS not offered as current empirical and biological evidence is not sufficient to allow the conclusion that an altered copy number of PTPN11 and KRAS is a mechanism critical for the phenotype associated with these conditions.

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. This panel has not yet been validated to identify deletions/duplications >112bp and <1 exon, but such variants 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.

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 healthcare professional to enhance the interpretation. There is no limitation to the number of relatives that can be tested free of charge.

For patients presenting with phenotypes that may overlap with these disorders, genetic analysis of the associated tumors may be beneficial in determining a diagnosis. Based on the genetic pattern seen in tumors, tumor based analysis may be able to confirm a diagnosis of schwannomatosis, chromosome 22 involvement, or mosaic NF2 based on the variants identified in the tumor specimen. For patients of specific concern for mosaic/segmental NF1, we suggest starting with our RNA-based testing options for NF1. Please see website for more information.

Tumor-based analysis can be performed on fresh or frozen tumor via next-generation sequencing. If the tumor specimen has been formalin-fixed paraffin embedded (FFPE) tumor,
please review our Sanger sequencing.

REFERENCES available on website.

Other related testing options:

- Schwannomatosis/Multiple Schwannomas panel by NGS (SCH-NG)
- Meningiomatosis/Multiple Meningiomas panel by NGS (MEN-NG)
- Neurofibromatosis type 2 by NGS (NF2-NG)
- Non-NF1 Rasopathy panel by Next-Gen Sequencing (NNP-NG)
- RNA-based NF1/SPRED1 testing on affected tissues (NF14N/NF14C)