UAB MEDICAL GENOMICS LABORATORY

Next-Gen Sequencing and Deletion/Duplication Analysis of *SPRED1* Only (SPD1-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:

$800 (USD – institutional/self-pay);

CPT: 81405, and 81479

Z code: ZB6AC

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) after comprehensive *NF1* variants analysis

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

Germline loss-of-function variants 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 pigmentary signs only will carry a variants 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, a *SPRED1* variants will be found 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 ***SPRED1*-only by NGS** involves sequencing as well as **deletion/duplication** analysis of the entire coding *SPRED1* regions. 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 of the *SPRED1*-only by NGS panel is >1600x with >98% of the coding region ≥350x and >99% ≥200X, allowing detection of very low level mosaicism, down to 3-5% variant allele fraction respectively (regions covered by ≥350x respectively ≥200x) 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.

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)