Department of Genetics

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NF1-RASopathy NGS panel or RNA-based *NF1* testing at UAB

Which test suits best your needs?

The *NF1*-RASopathy NGS panel includes 18 genes (*NF1, SPRED1, PPP1CB, PTPN11, BRAF, CBL, HRAS, KRAS, NRAS, MAP2K1, MAP2K2, LZTR1*, *RAF1, RIT1, RASA2, SHOC2, SOS1* and *SOS2*) implicated in RASopathy spectrum disorders, covers all the coding regions and ~50bp intronic sequences flanking all exons of the targeted genes. All regions carrying pathogenic *NF1* mutations, as identified between 2003-2016 in the UAB cohort of 8,000 mutation-positive patients, and >65 deep intronic mutations are included. In addition, through deep coverage (average coverage 1,600x and >98% of the NF1 coding regions at >350x and 99% at >200x, allowing detection of very low-level mosaicism, down to 3-5% mutant allele fraction (regions covered by >350x and >200x respectively) with 95% confidence.

Important Consideration	*NF1*-RASopathy NGS panel	RNA-based *NF1* testing
Indications of Testing	• Patients with clinical features of NF1, Legius, Noonan, Noonan with multiple lentigines (LEOPARD), Cardio-Facio-Cutaneous syndrome or Costello syndrome. • Patients with ambiguous phenotype including some of the characteristic features associated with a RASopathy (cardiac abnormalities, developmental delay, intellectual disability, short stature, pigmentary skin findings, characteristic facial features, etc.)	• Patients who have had negative testing elsewhere and with a strong suspicion that the phenotype is NF1 related, including individuals with a positive family history. • Sporadic patients who had a negative *NF1*-RASopathy panel on blood and who may have mosaic/segmental NF can consider Reflex RNA-based testing on cultured cells from fresh neurofibroma tissue (Schwann cells) or café-au-lait biopsy (melanocytes). Testing includes *NF1* and *SPRED1* for patients with only pigmentary signs.
Specimen Types	Blood, Saliva, DNA	Fresh EDTA blood sample (to arrive in lab <60-70 hours after blood draw), fresh biopsy of CALMs/neurofibromas
Detection of Mosaicism	Low level mosaic mutations in blood that affect as low as **3-5% of the alleles** in the sequenced regions	Most sensitive detection of mosaicism through analysis of cultured neural crest derived cells (Schwann cells from neurofibromas; melanocytes from CALMs) from affected tissues.
Detection of Complex Mutation	Validation was performed using rigorous criteria and included the most challenging mutations for an NGS platform such as **deep intronic splice mutations** (~2.5% of *NF1*-mutation-positive patients), **insertion/ deletion/duplication of 1-64 nucleotides** (~25%) and **one-to-multiple exon deletion/duplications** (~2.8%)	In blood, novel retrotransposon insertion and not previously identified deep intronic mutations affecting splicing or loss of expression can be found (conservatively estimated frequency ~ 0.25%; would be missed by the NGS platform)
Copy Number Analysis	Included in the NGS panel for the *NF1* and *SPRED1* genes	Provided via MLPA and Reverse Transcriptase Long-Range PCR fragment analysis
Tested Genes	Full panel includes **18** genes (*NF1, SPRED1, PTPN11, PPP1CB, BRAF, CBL, HARAS, KRAS, NRAS, MAP2K1, MAP2K2, LZTR1, RAF1, RIT1, RASA1, RASA2, SHOC2, SOS1* and *SOS2*); can also be ordered a "*NF1*-only", "*NF1-SPRED1*-only" and as "non-*NF1* RASopathy"	*NF1 +/- SPRED1*