

Next-Gen Sequencing of *HRAS* Only for Costello Syndrome (CST-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)

Turnaround time:

25 working days

Price, CPT codes, and Z code:

\$700 (USD – institutional/self-pay);

CPT: 81404

Z code: ZB67V

Candidates for this test:

Patients with key CS features including coarse facial features, severe feeding difficulty, mild to moderate intellectual disability, relative macrocephaly and short stature, high incidence of cardiac abnormalities and malignancy. Differentiation of CS from other RASopathies, particularly CFC may be difficult especially early in life

Specimen shipping and handling:

- Please find acceptable specimen type above.

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- 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 RASopathies are a genetically heterogeneous group of disorders caused by variants in the genes involved in the Ras-MAPK pathway. As a group, the RASopathies are one of the largest groups of malformation syndromes known, affecting ~1:1,000 and include Neurofibromatosis type 1, Legius syndrome, Noonan syndrome, cardio-facio-cutaneous (CFC) syndrome, Noonan Syndrome with Multiple Lentigines (NSML/LEOPARD) and Costello syndrome. Variants in *NF1* and *SPRED1* are typically loss-of-function variants and include the full spectrum of nonsense, missense, splice, frameshift, insertion-deletion, and copy number changes. Variants in the other rasopathy genes are typically missense variants or an in-frame deletion/insertion of an amino acid.

The Ras/MAPK pathway can have a profound deleterious effect on development as it plays a key role in differentiation, growth, senescence, and dysregulation. Clinical features of the RASopathies include short stature; cardiovascular defects; cutaneous and pigmentary findings; characteristic facies; skeletal and neurocognitive delays as well as a predisposition to neoplasia, both benign and malignant. The disorders have variable expressivity (individuals with the same disorder may show differing features and severity of symptoms, even within the same family). Some of the genes/variants are not fully penetrant; therefore an individual may carry a variant but not show any or only few signs of the syndrome. Moreover, features can change/progress with age, which makes it difficult to make an accurate clinical diagnosis. The RASopathies are inherited in an autosomal dominant manner. A parent who carries a gene variant has a 50% chance of passing it on to every child, regardless of gender.

An individual can carry a variant either:

- a. Because (s)he inherited the variant from a parent (parent clinically affected or “non-penetrant”), or

- b. Because the variant arose “de novo” in the egg or sperm from which the individual developed.

Sometimes, the variant occurred “post-zygotically”, i.e. during development and in these individuals the variant may not be present in every cell of the body, typically resulting in a Costello syndrome (CS), caused by activating *HRAS* variants, is a very rare condition with the following key features: coarse facial features, severe feeding difficulty, mild to moderate intellectual disability, relative macrocephaly and short stature, high incidence of cardiac abnormalities and malignancy. Differentiation of CS from other rasopathies, particularly CFC may be difficult especially early in life.

Some individuals with a clinical diagnosis of one of the RASopathies have been found to carry a variant in a gene that was not considered to be consistent with their clinical diagnosis.

Examples include *BRAF* variants reported in individuals with a clinical diagnosis of Noonan syndrome, a *SOS1* variant in an individual with CFC (Nystrom AM et al, 2008), *PTPN11* variants in individuals with paraspinal neurofibromas (Conboy E. et al, 2015), and an NF1 missense variant in patients with Noonan-like features and no neurofibromas (Rojnueangnit K et al, 2015). In addition, some genes are associated with more than one syndrome (*PTPN11*, *KRAS*, *BRAF*, *RAF1*, and *NF1*). Therefore, the comprehensive approach of simultaneously testing all 16 genes in some individuals eliminates the need to determine which genes to test based on an individual’s clinical signs.

Test Description

The ***HRAS*-only by NGS** involves the sequencing of the entire *HRAS* coding region. **The average coverage is 1800x with 100% of the coding region covered at $\geq 350x$.** 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.

REFERENCES available on website.

Other related testing options:

- Non-NF1 Rasopathy panel by Next-Gen Sequencing and Deletion/Duplication Analysis of *SPRED1* (NNP-NG)
- Expanded *NF1*-Rasopathy panel by Next-Gen Sequencing (RAS-NG)