Custom Confirmation Testing for Variants Identified by a Research or Outside Laboratory

Services Provided:

- Confirmation of Variants Identified in a Research Lab
- Custom Variant-Specific Carrier or Pre-symptomatic Testing
- Custom Prenatal Diagnosis

Clinical Utility:

GeneDx provides custom targeted testing for specific variant(s) in any gene for a variety of indications. A common indication for this service is confirmation in a CLIA-certified diagnostic laboratory for results that were previously obtained in a research study, which allows the results to be included in a patient's medical records and used in medical management. Results may also be used in genetic counseling and for prenatal, carrier, and pre-symptomatic testing of at-risk family members. This testing can be ordered for any genetic disease with Mendelian inheritance and for maternally inherited variants in the mitochondrial genome (mtDNA). Test accuracy is dependent on having unambiguous variant descriptions as detailed below.

Autosomal recessive, autosomal dominant, X-linked recessive, X-linked dominant, and maternally inherited disorders may all be tested using this approach. Once the presence of the variant is confirmed in an affected individual, then carrier testing and pre-symptomatic testing can be performed on family members who are at risk to harbor the variant. Prenatal diagnosis may be available for nuclear gene variants with prior approval but is not available for mtDNA variants. In some cases, particularly those with time constraints such as prenatal diagnosis, confirmation in an affected family member can be run concurrently with other samples from the same family.

NOTE: When submitting family members other than the proband, a positive control is required for nuclear gene variants and may be required for mtDNA variants, depending upon what testing method** is used to analyze for the mtDNA variant.

Required Information:

Information about the variant should be provided to GeneDx in advance, in the form of a publication, lab report, or other communication from the laboratory that initially detected the variant.

To clearly identify nuclear gene variants, please provide:

1) The name of the gene
2) The variant in cDNA-level notation (e.g. residue c.123 G to T, where c.1 is the A of the initiator ATG)
3) One of the following:
    a. The variant given in protein-level notation (e.g. p.Ala12Gly or A12G)
    b. The variant given in gDNA-level notation, with reference to a specific public reference sequence
    c. A DNA sequence at least 30 bases long with the specific location of the variant clearly denoted

To clearly identify mtDNA variants, please provide:

1) The name of the gene
2) The position of the variant in the mitochondrial genome (m.) using reference sequence NC_012920.

Test Methods:

Using genomic DNA obtained from buccal (cheek) swabs or 1-5 mL blood in EDTA, GeneDx performs the analysis using information provided by the referring clinician or laboratory. PCR and sequencing-based methods can identify the majority of molecular variants that are associated with genetic disease, but certain variants such as deletions spanning one or more exons, complete gene deletions, gene rearrangements, or duplications may not be detected. To evaluate for gene deletions or duplications, GeneDx offers quantitative gene copy number analysis via ExonArrayDx, MLPA, or other methods via a separate service.

For variants that are detectable by sequence-based methods, primers are designed and ordered by GeneDx based on the information provided. If prenatal or carrier testing on unaffected family members is desired, a sample from a related individual known to have the variant(s) of interest must also be tested. PCR is used to amplify the region of interest in the gene and variant-specific testing on the index case is performed by sequencing. Prenatal diagnosis or testing of other family members may be done by sequencing, restriction digest analysis, or other molecular techniques known to have the ability to identify the specific variant.

**For mtDNA variants if ordering test #9017 or #9020, genomic DNA obtained from buccal, blood in EDTA, muscle or liver is analyzed using the information provided by the referring clinician or laboratory. Primers are designed and ordered by GeneDx based on the information provided. For these tests, levels of mutant heteroplasmy 25% or lower may not be detected and levels of mutant heteroplasmy 75% or higher may appear to be homoplasmic. If ordering test #453, the entire mitochondrial genome is amplified using a solid state sequencing

by-synthesis process and the relevant position is analyzed. Please note, buccal specimens are not accepted for test #453.

For prenatal diagnosis, we perform duplicate testing on a single specimen, which can be amniotic fluid cells, cultured amniocytes, or chorionic villus samples (CVS). Cultured CVS specimens are discouraged due to the increased risk of maternal cell contamination, but will be accepted. On all types of fetal specimens, we perform concurrent testing as needed to check for maternal cell contamination. *For this reason, a maternal sample must accompany all prenatal samples.* In specific circumstances, a paternal sample may also be required. One final report will be issued. Confirmation on cultured cells is not required following analysis of a direct sample. *Prenatal testing is NOT AVAILABLE for mtDNA variants.*