Custom Targeted Exome Sequencing Tests: Single Gene Slice, Multi-Gene Slice, & Slice *Xpanded*®

Description:

The GeneDx Slice tests capture and sequence the entire exome, but analysis is limited to a custom-built gene list. Slice tests are best suited for individuals with a clearly defined, oligogenic phenotype where a comprehensive gene panel is not available, or the patient has a single gene disorder for which clinical testing is not currently available. ACMG secondary findings reported in comprehensive exome analysis (*XomeDx*® and *XomeDx*® Plus) are not reported as part of Slice tests unless these genes are on the custom gene list; the analytic pipeline used in Slice tests will only present data on the requested gene list and will not identify secondary findings.

Single Gene Slice (test TG70) for 1 gene; Multi-Gene Slice (test 706) for 2-150 genes:

These tests include generation of exome sequence (ES) data for the proband only and does not use family members' samples for analysis.

Slice *Xpanded*® (test J757) for greater than 150 genes:

This test utilizes a proband-only or a trio approach that includes concurrent generation of ES data and analysis of the affected proband with both biological parents, if available. Depending on the family structure, family history, and the availability of both parents, other family members of the affected proband may be included or substituted for the parents; contact GeneDx for approval of submission for alternate family members. Analysis and reporting are phenotype-driven and may not include all variants detected. Relevant clinical records are required to aid in the analysis and reporting of variants.

Gene List Instructions:

Prior to submitting the patient's specimen for testing, the gene list must be submitted by the ordering provider using the online Slice Tool (https://providers.genedx.com/xomedx-slice-tool). The submitted gene list will be reviewed and approved or denied by the GeneDx clinical staff within 3 business days. The approved gene list will be emailed to the ordering provider with the average percent covered at a minimum read depth of 10X. This email will contain a unique tracking number that must be submitted with the patient's sample, and for non-portal orders, it will also include a link to a reusable Custom Slice requisition form. The ordering provider may request assistance from GeneDx in selecting an appropriate list of genes; however, the provider remains solely responsible for the selection of the appropriate genes and the ordering of the genetic testing.

Result Reporting:

The Slice and Slice *Xpanded*® tests are performed on an affected proband. When submitted concurrently, parental samples may be included for analysis of Slice *Xpanded*®. A single report will be issued on the affected proband in the family. A separate report will not be issued for parents or other relatives who may have submitted a specimen for the purpose of allowing better interpretation of the results from the affected individual. If reports are requested for other affected family members, additional fees will apply.

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Single Gene Slice and Multi-Gene Slice (2-150 genes):

The report issued for the affected proband will include all variants in the selected gene list that are classified as pathogenic, likely pathogenic, or variant of uncertain significance (VUS). Single heterozygous variants of uncertain significance in genes associated with autosomal recessive disease may be reported as unconfirmed findings in a separate table. Variants that are considered to be benign or likely benign will not be reported.

Slice *Xpanded*®:

The report issued for the affected proband will include reportable variants in genes that are associated with the provided phenotype. The report will include clinically relevant pathogenic or likely pathogenic variants in the selected gene list. In some instances, the report may include variants of uncertain significance (VUS) in genes that are possibly associated with the patient's phenotype. Variants that are considered to be benign or likely benign will not be reported.

Reasons for Referral:

1. Confirmation of a clinical diagnosis
2. Genetic counseling and recurrence risk assessment

Test Methods:

Using genomic DNA from the submitted specimen(s), DNA is enriched for the complete coding regions and splice site junctions for most genes of the human genome using a proprietary capture system developed by GeneDx for next-generation sequencing. The enriched targets are simultaneously sequenced with paired-end reads on an Illumina platform. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. Using a custom-developed analysis tool, data are filtered and analyzed to identify sequence variants and copy number variants (CNVs) involving three or more exons, only for the genes selected by the ordering clinician prior to the start of testing. Reported clinically significant variants are confirmed, if necessary, by an appropriate orthogonal method in the proband and, if submitted, in selected relatives. Sequence variants are reported according to the Human Genome Variation Society (HGVS) guidelines. Copy number variants are reported based on the probe coordinates, the coordinates of the exons involved, or precise breakpoints when known.

Limitations:

Only the genes selected and included in the approved gene list will be analyzed. Changes can only be made to the gene list by contacting GeneDx directly at SliceGC@genedx.com. Genes that have poor coverage by exome sequencing, are significantly affected by homology to other regions of the genome, have other technical issues with sequencing, or are offered by single gene or panel testing at GeneDx or an outside laboratory may not be appropriate for Slice. Genes in the mitochondrial genome, non-coding genes, and regulatory or deep intronic regions are not captured by this technology and are therefore not analyzed by any Slice tests.

The coverage data in the online Slice Tool provides an average estimate of gene coverage, but the actual coverage of genes on a requested gene list may vary and will be provided in the test report for each

patient. For Slice *Xpanded*®, the average coverage for the entire exome may be reported instead. Complete sequencing coverage or NGS-CNV calling for the genes selected may not be available. There may be some genes or portions of genes that are not amenable to capture, sequencing, and alignment. Additionally, certain types of sequence variations are difficult to identify by this technology, including repeat expansions. The available scientific knowledge about the function of all genes in the human genome is incomplete at this time. It is possible that the Slice test may identify the presence of a genetic variant in an affected individual, but it will not be recognized as causative for the affected individual's disorder due to insufficient knowledge about the variant or the gene and its function.

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