GeneDx
DNA DIAGNOSTIC EXPERTS
WHERE *Rare* is *Common*

Test Information Sheet

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XomeDx: Whole Exome Sequencing
XomeDx*Plus*: Whole Exome Sequencing with Mitochondrial Genome Sequencing / Deletion Testing
XomeDx*Slice*: A Phenotype-Driven Targeted Exome Test

Description:
XomeDx, or whole exome sequencing (WES), can be used to identify the underlying molecular basis of a genetic disorder in an affected individual. The XomeDx test is different from other types of genetic diagnostic tests in terms of the number of genes that are sequenced simultaneously. The XomeDx test targets the protein-coding regions of the human genome, which represents ~20,000 genes and accounts for approximately ~2% of all human genetic material (Bamshad et al., 2011). These targeted regions of an individual's genes, called exons, are captured and sequenced using massively parallel sequencing. An individual's sequence is then compared to published reference sequences, other individuals from the affected individual's family and control individuals to identify causal variants that could explain the disorder in the affected patient. The XomeDx test is most efficient when other family members are included in the analysis of the affected individual's exome sequence. Depending on the family structure and the availability of other affected individuals within a family, both parents and/or other family members of the affected individual may be evaluated simultaneously in order to maximize the chance of identifying the cause of the disorder in the family. The XomeDx test is best suited for patients who have a familial condition that routine genetic testing has not been able to elucidate.

XomeDx*Plus* is a combined test including whole exome sequencing with mitochondrial genome sequencing and deletion testing. For more information on the mitochondrial genome sequencing and deletion component of the XomeDx*Plus* testing, please visit our neurology/mitochondrial genetics page on our website: http://www.genedx.com/test-catalog/genetic-testing-for-neurological-disorders/.XomeDxPlus is best suited for individuals with clinical features suggesting a mitochondrial disorder.

XomeDx*Slice* captures and sequences the whole exome, but analysis is targeted to a limited and specific phenotype-driven gene list. The gene list will be discussed and selected by the ordering provider and GeneDx medical specialists prior to the start of testing. Testing is only performed on the proband and does not use family members' samples for analysis. XomeDx*Slice* is 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. Please call GeneDx to discuss prior to sending a patient's sample for XomeDx*Slice*.

***XomeDxTest method*:**
An affected individual's clinical records and prior genetic testing results will be reviewed prior to analysis.Using genomic DNA from the submitted specimen and additional familial specimens, the Agilent SureSelect XT2 All Exon V4 kit is used to target the exon regions of their genomes. The targeted coding exons and splice junctions of the known protein-coding RefSeq genes are sequenced using the IlluminaHiSeq 2000 sequencing system with 100bp paired-end reads. The DNA sequence is mapped to and analyzed in comparison with the published human genome build UCSC hg19 reference sequence. Any mutations/copy number changes found in the affected individual and thought to cause the clinical condition will be confirmed by a second independent method such as dideoxy sequence analysis, or another appropriate method.
For **XomeDx*Plus***, whole exome sequencing (as described above) is performed concurrent with mitochondrial genome sequencing and deletion testing. The mitochondrial genome is amplified by long-range PCR and sequenced using massive parallel sequencing on the IlluminaMiSeq. DNA sequences are assembled and compared to the published mitochondrial genome reference sequences for analysis. The presence of any disease-associated sequence variant is confirmed by conventional dideoxy sequence analysis or other methods. A reference library of more than 6000 samples from different ethnic groups and online databases for mtDNA variations will be used to evaluate variants of unknown clinical significance identified in the mitochondrial genome. Full mitochondrial genome sequencing will be performed on the proband only. If a maternal sample is provided at the time of the proband's sample submission, maternal samples

will undergo Sanger sequencing to identify if reported mitochondrial findings are inherited, however maternal level of heteroplasmy will not be evaluated. Carrier testing for maternal relatives can be ordered separately.

XomeDx*Slice* uses whole exome sequencing paired with an analytic pipeline that presents data on only the genes that were pre-selected by the clinician prior to starting the test. Any mutations/copy number changes found in the affected individual and thought to cause the clinical condition will be confirmed by a second independent method such as dideoxy sequence analysis, or another appropriate method.

***Limitations*:**
The XomeDx test attempts to evaluate the most important regions of the majority of the ~20,000 genes in the human genome. However, it is not technically possible to capture and sequence the entire exome at present. It is anticipated that approximately 90-95% of the targeted region of an affected individual's exome will be assessed with the XomeDx test at 10x coverage, while >98% of the target region will be covered at a minimum of 1x. 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 using whole exome sequencing; however, GeneDx can utilize other types of diagnostic tests in conjunction with the XomeDx test to increase the likelihood of identifying a disease-causing variant in an affected individual's exome.

The scientific knowledge available about the function of all genes in the human genome is incomplete at this time. It is possible that the XomeDx test may identify the presence of a variant in the exome sequence of an affected individual, but that we will not recognize it as the cause of their disease due to insufficient knowledge about the gene and its function. Even if the XomeDx test identifies the underlying genetic cause of a disorder in an affected individual, it is possible that such a diagnosis will not permit an accurate prediction of the prognosis or severity of the disease. While there is a possibility that identifying the genetic cause may help direct management and treatment of the disease, it is also possible that this knowledge will not change management or treatment.

Result Reporting:
Whole exome sequence analysis is performed on the proband and parental samples, and/or additional relatives as needed, when submitted together for analysis. A single XomeDx, XomeDx*Plus* or XomeDx*Slice* report will be issued on the affected individual in the family. A separate report will not be issued for unaffected parents or other unaffected family members who may also have submitted a specimen for the purpose of allowing better interpretation of the results from the affected individual. If additional reports are requested for other affected family members, additional fees will apply. Targeted testing of family members for sequence changes identified in the affected individual is available for a fee of $350- $500, depending on the type of mutation to be tested (sequence-based or copy number variant).

The XomeDx or XomeDx*Plus* report issued for the affected individual in the family will contain variations in genes previously implicated in a human disease similar to the affected individual or in genes hypothesized to be related to the cause of the disease based upon the function, tissue of expression, and phenotype of model organisms with mutation in the gene.

Mutations in genes unrelated to the individual's reported phenotype are considered incidental findings. The American College of Medical Genetics (ACMG) recommends that incidental findings identified in 56 genes associated with various inherited disorders be reported for all probands undergoing whole exome sequencing. Please refer to the ACMG Recommendations for Reporting of Incidental Findings in Clinical Exome and Genome Sequencing Report for complete details of the genes and associated genetic disorders (Green et al., 2013). Incidental findings will be automatically included for all XomeDx and XomeDx*Plus* reports. Incidental findings will include all known pathogenic variants identified in the coding exons (for which a minimum of 10X coverage was achieved by the XomeDx test) of all 56 genes and expected pathogenic variants identified in the coding exons in 41 of the 56 genes, as recommended by the ACMG. The status for any incidental findings reported for the proband will be provided for all relatives tested by XomeDx. Incidental findings will be confirmed by an alternate test method. Relatives submitted for targeted segregation analysis only will not receive their status for the proband's incidental findings. Patients have the choice to opt-out of receiving incidental findings (please see page 6 of the XomeDx test submission form).

Reasons for Referral:

- Determination of a clinical diagnosis
- Identification of a gene implicated in genetic disease
- Genetic counseling and recurrence risk

Information and Familial Sample Requirements:

- A completed XomeDx consent form must be received for each individual submitting a specimen.
- If the patient chooses to opt-out of receiving incidental findings, page 6 of the XomeDx test submission form must be submitted and signed for the proband.
- Detailed clinical records and prior genetic testing results for the affected individual must be submitted prior to or at the same time as the biological specimens.
- In order to best make an assessment of the mode of inheritance for a disorder within a family, a detailed pedigree will be required with submission for specimens.
- Specimens on family members are recommended for XomeDx and XomeDx*Plus* testing; in most cases this includes both parents. Additional affected and unaffected family members may be requested. Please call to discuss any complex cases.

Specimen Requirements and Shipping/Handling:

- *Blood*: A single tube with 2-5 mL whole blood in EDTA. Ship overnight at ambient temperature, using a cool pack in hot weather. Specimens may be refrigerated for 7 days prior to shipping.
- *Purified DNA:* High quality extracted DNA can be accepted. At least 15ug is requested (with a minimum concentration of 50ng/ul).
- *Other Tissue Types:* Tissue biopsies are preferable for mtDNAanalysis, therefore, sending a blood sample together with a tissue biopsy from the same patient is recommended. Please call to discuss.
- *BuccalBrushes*:Cannot be accepted

Required Forms:

- XomeDx Consent Form and Consent to Opt-Out of Incidental Findings (if desired) – initial and sign all pages
- XomeDx*Slice* Consent Form (if applicable)
- Sample Submission (Requisition) Form – complete all pages
- Payment Options Form or Institutional Billing Instructions

For test codes, CPT codes, and turn-around-times, please refer to the "XomeDx", "XomeDx*Plus*" or "XomeDx*Slice*" page on our website: www.genedx.com

References: **Bamshad et al. (2011) Nature Reviews Genetics. 12:745-755; Green et al. (2013) Genet Med 15:565-574**