XomeDx*Xpress*®, Genome*Xpress* and XomeDx®*Priority*: Rapid Exome and Genome Sequencing

Description:

The **XomeDx*Xpress***® test is exome sequencing with an expedited turnaround time (TAT) of approximately 2 weeks. The **Genome*Xpress*** test is genome sequencing with an expedited TAT of approximately 2 weeks. For both tests, a verbal result is given within 7 calendar days after the start of testing. International clients will receive an email status update at 7 days. The verbal result will include pathogenic and/or expected pathogenic variants in known disease-causing genes. A written report including all potentially clinically relevant variants will be reported within approximately 2 weeks after the start of testing.

The **XomeDx®*Priority*** test is exome sequencing with an expedited TAT of approximately 4 weeks. A written report including all clinically relevant variants will be reported within approximately 4 weeks after the start of testing.

Because of the rapid TAT for XomeDx*Xpress*, Genome*Xpress*, and XomeDx*Priority*, samples from the proband and both biological parents should be submitted at the same time, along with clinical information. These tests are best suited for patients whose medical management may be altered by having a rapid molecular diagnosis. These tests require approval by GeneDx; please email **Xpress@GeneDx.com** to discuss prior to sending in samples.

Exome sequencing (ES) and genome sequencing (GS) can be used to identify the underlying molecular basis of a genetic disorder in an affected individual. These tests are different from other types of genetic diagnostic tests in terms of the number of genes that are sequenced simultaneously; genome sequencing also includes sequencing of non-coding regions of the genome. Exome and genome sequencing can be used to identify the molecular basis of a genetic disorder in individuals:

- With a genetically heterogeneous disease, as pathogenic findings could be present in many different genes
- With a long list of differential diagnoses
- With an atypical presentation of a genetic disorder

The XomeDx*Xpress* and XomeDx*Priority* tests target the protein-coding regions of the human genome, which represent ~20,000 genes and account 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 next generation sequencing.
In comparison, Genome*Xpress* includes both protein-coding and non-coding regions of the human genome, allowing for the potential detection of characterized/pathogenic variants in

regions that are not assessed by exome sequencing. The non-coding regions include promoter, intronic, and untranslated regions. While much of the data generated from sequencing the genome is not well-understood at this time, genome sequencing may provide more reliable coverage of the exonic regions (Lelieveld et al., 2015; Belkadi et al., 2015). GS has lower depth of coverage on average compared to ES, but more positions are covered to adequate depth for accurate variant calling than ES.

For XomeDx*Xpress*, XomeDx*Priority* and Genome*Xpress*, an individual's exome or genome sequence is compared to published reference sequences, other individuals from the affected individual's family and control individuals, and phenotype-driven reporting is performed using Human Phenotype Ontology and gene-phenotype associations. Additional resources such as GnomAD, OMIM, PubMed and ClinVar are used to evaluate genes and sequence changes of interest, which are then interpreted according to the American College of Medical Genetics and Genomics guidelines (Retterer et al., 2016; Richards et al., 2015). Exome and genome testing is most effective when other family members, specifically both biological parents (if available), are included in the analysis of the affected individual's exome or genome sequence.

The clinical sensitivity of exome and genome sequencing depends, in part, on the proband's clinical phenotype. Several large studies have demonstrated that exome sequencing identifies a causal variant in 25-58% of cases, with a higher yield for cases that specifically include other family members (Retterer et al., 2016; Farwell et al., 2015; Lee et al., 2014; Yang et al., 2014; Gubbels et al., 2019). The diagnostic utility of genome sequencing is approximately 20-68% with a higher yield for cases that specifically include other family members and have strict clinical inclusion criteria (Clark et al., 2018; Petrikin et al., 2018; Bick et al., 2017; Willig et al., 2015; Farnaes et al., 2018; Kingsmore et al., 2019; Scocchia et al., 2019).

Result Reporting:
Exome or genome sequence analysis is performed on the proband and parental samples, and/or additional relatives as needed, when submitted together for analysis. A single report will be issued for 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.

The report issued for the affected individual in the family may contain variations in genes previously implicated in a human disease similar to the clinical presentation of the affected individual or in genes hypothesized to be related to the cause of the disease (candidate genes), based upon the function, tissue of expression and phenotype of model organisms with alterations in the gene. Variants in candidate genes may also be reported based on internal

data, such as observations of previous cases with similar phenotypes and types of variations in the same gene.

ACMG Secondary Findings:
The American College of Medical Genetics and Genomics (ACMG) recommends that secondary findings, known and/or expected pathogenic variants, identified in a specific subset of genes associated with medically actionable, inherited disorders be reported for all probands undergoing exome and genome sequencing. Please refer to the latest version of the ACMG Recommendations for Reporting of Secondary Findings in Clinical Exome and Genome Sequencing Report for complete details of the genes and associated genetic disorders. Secondary findings will be included for all exome and genome sequencing reports, unless a family opts-out of receiving this information on the Informed Consent and Authorization Form as part of the Test Requisition Form. The status of any secondary finding(s) reported for the affected individual will be provided for all relatives tested by XomeDx*Xpress*, Genome*Xpress* and XomeDx*Priority*. GeneDx does not conduct an independent evaluation of secondary findings in relatives that are not present in the proband. Relatives have the ability to opt-out of receiving secondary findings.

Test Methods:
For XomeDx*Xpress* and XomeDx*Priority*, an affected individual's clinical records and prior genetic testing results will be reviewed prior to analysis. 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.

For Genome*Xpress*, an affected individual's clinical records and prior genetic testing results will be reviewed prior to analysis. Genomic DNA from the submitted specimen is sequenced with paired-end reads on an Illumina platform. On average, the mean depth of coverage across the genome is ≥40x. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19.

For XomeDx*Xpress*, Genome*Xpress* and XomeDx*Priority*, a custom-developed analysis tool (XomeAnalyzer), is used to filter and analyze data for the identification of sequence variants and copy number variants (Retterer et al., 2015). Reported, clinically significant variants, are confirmed by an appropriate orthogonal method in the proband and, if submitted, in selected

relatives as necessary. Sequence variants and copy number variants are reported according to the Human Genome Variation Society (HGVS) recommendations or International System for Human Cytogenetic Nomenclature (ISCN) guidelines, respectively. Reportable variants include pathogenic variants, likely pathogenic variants and variants of uncertain significance. Likely benign and benign variants, if present, are not routinely reported.

The analysis for the proband includes evaluation of variants that are identified to be *de novo* (when both parents submitted), compound heterozygous (when both parents submitted), homozygous, heterozygous and X-linked. In addition, analysis takes into consideration family structure, reported phenotype and provided clinical and/or differential diagnosis. This is a phenotype-driven test of a very large number of genes; therefore, reported results are focused on pathogenic and likely pathogenic variants in genes related to the clinical information provided.

Limitations:
The XomeDx*Xpress* and XomeDx*Priority* tests attempt 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 98% of the targeted region of an affected individual's exome will be assessed at a minimum of 10x coverage, the minimum read depth necessary to detect a variant. Across the exome, the average depth of coverage is 100-120x. The test report will include case-specific exome coverage. 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 exome sequencing, such as repeat expansions. Average read depth statistics for exome sequencing tests are as follows:

Read Depth	10x	20x	30x	40x	50x
Mean Percent of Target Covered	98%	97%	94%	90%	83%

The Genome*Xpress* test attempts to evaluate the complete coding and non-coding regions of the genome. However, it is not technically possible to uniquely resolve and align the entire genome at present due to homology and other structural complexities in some regions. It is anticipated that approximately 99% of the coding region of an affected individual's genome (i.e., the exome) will be assessed with the Genome*Xpress* test at 10x coverage.

The available scientific knowledge about the function of all genes in the human genome is incomplete at this time. It is possible that these tests may identify the presence of a genetic variant in the exome or genome sequence of 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. Reanalysis of the data is available upon request by the healthcare provider to incorporate updated clinical information and/or newly emerging gene and variant information. Updates to the classification of a sequence variant may be accessed through ClinVar (www.clinvar.com). Even if these tests identify 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.

References:

1. Bamshad et al. (2011) Nature Reviews. Genetics 12 (11):745-55 (PMID: 21946919)
2. Lelieveld et al. (2015) Hum. Mutat. 36 (8):815-22 (PMID: 25973577)
3. Belkadi et al. (2015) Proc. Natl. Acad. Sci. U.S.A. 112 (17):5473-8 (PMID: 25827230)
4. Retterer et al. (2016) Genet. Med. 18 (7):696-704 (PMID: 26633542)
5. Richards et al. (2015) Genetics In Medicine 17 (5):405-24 (PMID: 25741868)
6. Farwell et al. (2015) Genet. Med. 17 (7):578-86 (PMID: 25356970)
7. Lee et al. (2014) Jama 312 (18):1880-7 (PMID: 25326637)
8. Yang et al. (2014) JAMA 312 (18):1870-9 (PMID: 25326635)
9. Gubbels et al. (2019) Genet. Med. : (PMID: 31780822)
10. Clark et al. (2018) NPJ Genom Med 3 :16 (PMID: 30002876)
11. Petrikin et al. (2018) NPJ Genom Med 3 :6 (PMID: 29449963)
12. Bick et al. (2017) J Pediatr Genet 6 (2):61-76 (PMID: 28496993)
13. Willig et al. (2015) Lancet Respir Med 3 (5):377-87 (PMID: 25937001)
14. Farnaes et al. (2018) NPJ Genom Med 3 :10 (PMID: 29644095)
15. Kingsmore et al. (2019) Am. J. Hum. Genet. 105 (4):719-733 (PMID: 31564432)
16. Scocchia et al. (2019) NPJ Genom Med 4 :5 (PMID: 30792901)
17. Kalia et al. (2017) Genet. Med.19 (2):249-255 (PMID: 27854360)
18. Green et al. (2013) Genet. Med.15 (7):565-74 (PMID: 23788249)
19. Retterer et al. (2015) Genet. Med. 17 (8):623-9 (PMID: 25356966)