

## ExomeDx™ Prenatal and ExomeDx™ Prenatal, targeted: Clinical Exome Sequencing for Fetal Anomalies

### Description

ExomeDx™ Prenatal and ExomeDx™ Prenatal, targeted tests use clinical exome sequencing (ES) to identify the underlying molecular basis of a genetic disorder in a pregnancy with fetal anomalies. Several organizations have published guidance on the use of prenatal clinical exome sequencing (PMID: 35583085, 31911674, 30134429). On average, studies have shown a positive diagnostic result in approximately 31% of fetuses with abnormal ultrasound anomalies (PMID: 35170059, 35041238), with higher diagnostic yields for cases with strict clinical inclusion criteria and no prior genetic testing. At GeneDx, our internal data show that ES identified a definitive molecular diagnosis in approximately 25% of fetuses presenting with ultrasound anomalies.

These tests target the protein-coding regions (exons) of ~20,000 genes, which account for approximately ~2% of the human genome (PMID: 21946919). These targeted regions are captured, sequenced using next-generation sequencing, filtered against published reference sequences, and compared to other sequenced family members, population databases, and control sequences to reveal potentially reportable variants. The fetal sequence is then evaluated using gene-phenotype associations, in conjunction with multiple resources including gnomAD, HGMD, OMIM, PubMed, and ClinVar (PMID: 26633542). Sequence changes of interest are interpreted according to the American College of Medical Genetics and Genomics guidelines (PMID: 25741868). Clinical exome sequencing is most effective when other family members (both biological parents, if available) are included in the analysis of the affected fetal exome.

The clinical records and results of previous fetal screening, fetal imaging, and/or genetic testing will be reviewed prior to analysis. The fetus's analysis includes evaluation of variants that are identified to be *de novo* (when both biological parents submitted), compound heterozygous (when both biological 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 comprehensive analysis of a very large number of genes; therefore, reported results are focused on pathogenic and likely pathogenic variants, along with clinically relevant variants of uncertain significance, in genes related to the provided clinical information. Rarely, pathogenic, likely pathogenic, and molecularly strong variants of uncertain significance in genes known to cause pediatric morbidity or mortality may be reported.

### Result Reporting

Exome sequence analysis is performed on the affected fetus (i.e., the proband) and, when submitted together for analysis, biological parental samples, and/or additional biological relatives as needed. A report will be issued only for the proband. A separate report will not be issued for parents or other family members who may also have submitted a specimen for the purpose of allowing better interpretation of the results for the affected fetus. If additional reports are requested for other affected family members, additional fees will apply.

The ExomeDx™ Prenatal and ExomeDx™ Prenatal, targeted reports will include medically relevant pathogenic or likely pathogenic variants in genes expected to be related to the reported fetal phenotype.

Variants of uncertain significance (VUS) may be reported when there is compelling evidence to suggest clinical significance. Pathogenic and likely pathogenic variants in genes known to cause significant childhood morbidity or mortality may be reported. For ExomeDx™ Prenatal variants in novel candidate genes may also be reported; candidate gene findings are **not** reported as part of the ExomeDx™ Prenatal, targeted test.

These tests have expedited turnaround times, designed for a current pregnancy with fetal anomalies. They are also appropriate for a deceased fetus or products of conception with ultrasound anomalies.

If results are non-diagnostic or do not fully explain new clinical features presenting in the proband after delivery, a reanalysis of the exome sequencing data can be ordered by the healthcare provider. For fetuses with new ultrasound findings identified after a report has been issued, ordering providers can contact [prenatal@genedx.com](mailto:prenatal@genedx.com) to determine if a targeted reanalysis can be conducted. GeneDx may contact the ordering healthcare provider to recommend a reanalysis if a proband's exome data suggests the presence of a clinically relevant variant in a newly identified gene implicated in human disease.

## ACMG Secondary Findings

The American College of Medical Genetics and Genomics (ACMG) recommends that secondary findings identified in a specific subset of genes associated with medically actionable, inherited disorders be reported for all probands undergoing exome or genome sequencing. Please refer to the latest version of the ACMG recommendations for reporting of secondary findings in clinical exome and genome sequencing for complete details of the genes and associated genetic disorders. Secondary findings will be included for all exome or genome sequencing reports, unless a family opts out of receiving this information on the Informed Consent as part of the test requisition form. The status of any secondary finding(s) reported for the affected fetus will be provided for all relatives included as part of the fetus's test; GeneDx does not conduct an independent evaluation of secondary findings in relatives. Relatives have the ability to opt out of receiving secondary findings. Secondary findings will be confirmed by an alternate test method when needed.

## 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 with CNV calling (NGS-CNV). 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. Data are filtered and analyzed to identify sequence variants and most deletions and duplications involving three or more coding exons. Smaller deletions or duplications may not be reliably identified. Reportable variants include pathogenic, likely pathogenic, and variants of uncertain significance; likely benign and benign variants, if present, are not routinely reported. Carrier status for the proband is not reported unless associated with the provided phenotype. Reported 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. Available evidence for variant classification may change over time and reported

variant(s) may be reclassified according to the ACMG/AMP Standards and Guidelines (PMID: 25741868), which may lead to issuing a revised report.

Maternal cell contamination (MCC) of the fetal specimen is evaluated from the next-generation sequencing (NGS) data; complete MCC cannot be excluded in a sample from a female fetus unless a maternal sample is included for analysis by NGS.

## Limitations

These 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 fetus's exome will be assessed with this test 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 ES, such as repeat expansions. Average read depth statistics for this test are as follows:

| Read Depth                     | 10x | 20x | 30x | 40x | 50x |
|--------------------------------|-----|-----|-----|-----|-----|
| Mean Percent of Target Covered | 98% | 98% | 97% | 95% | 91% |

Exome sequencing for fetal anomalies is a phenotype-informed analysis; specimens will not be accepted in the absence of abnormal ultrasound findings. The test is not a substitute for fetal cytogenetic analysis, newborn screening, or carrier screening. Carrier status in the fetus or parents is not reported unless associated with the presenting phenotype of the fetus.

The available scientific knowledge about the function of all genes in the human genome is incomplete at this time. This test may identify the presence of a genetic variant in the exome sequence of an affected fetus, but it will not be recognized as causative for the affected fetus'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 health care provider to incorporate updated clinical information and/or newly emerging gene and variant information. Even if this test identifies the underlying genetic cause of a disorder in an affected fetus, it is possible that such a diagnosis will not permit an accurate prediction of the prognosis or severity of the disease. While identifying the genetic cause may help direct management and treatment of the disease, it is also possible that this knowledge may not change management or treatment.