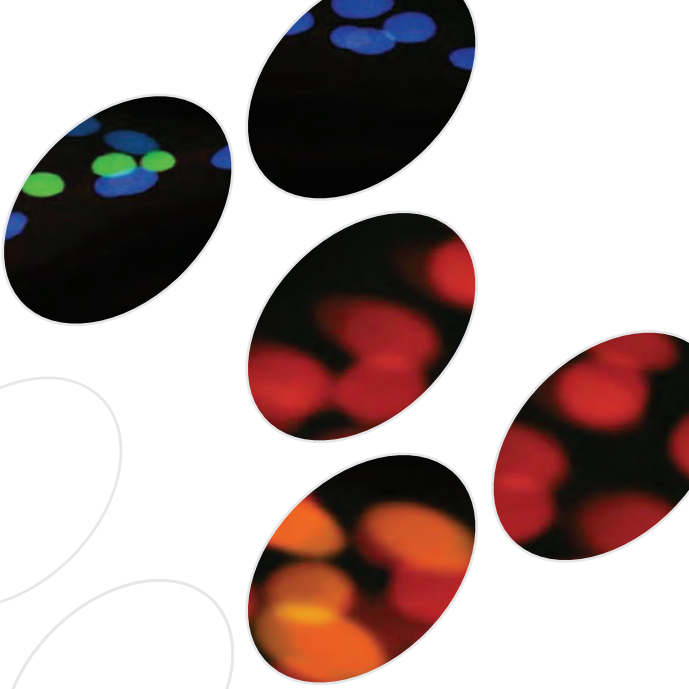
ExomeSeq

Exome Sequencing



Description:

The exome is comprised of all of the protein-encoding exons in the genome. Even though the exome accounts for only 1% of the entire genome, mutations in the exons account for many genetic disorders. Exome sequencing (ES) examines the majority of exons and exon/intron boundaries of most of the genes at one time. This test is different from most genetic tests that only analyze one gene or a set of genes at a time. Approximately 25% of individuals who have exome sequencing receive a diagnosis or a suspected diagnosis from the test.

Indications:

- The patient's symptoms or family history suggest a genetic etiology but does not correspond with a specific genetic disorder.
- The patient has symptoms of a well-defined genetic disorder that is caused by multiple genes (genetic heterogeneity) for which a multi-gene panel is not clinically available.
- The patient's clinical presentation is unclear/atypical and there are multiple genetic conditions in the differential diagnosis.

What is Reported:

Variants that will be discussed in detail in the report:

- Variants that are known to be pathogenic or for which the laboratory has sufficient evidence suggesting pathogenicity in a gene that is suspected to cause the patient's signs/symptoms.

Variants that will be listed in the report:

- Variants of uncertain clinical significance in genes related to the patient's phenotype
- Variants that are pathogenic or for which the laboratory has sufficient evidence suggesting pathogenicity in a gene that is medically significant but unrelated to the patient's presenting symptoms, unless the patient or parent/guardian declines this information. (See Incidental/Secondary Findings.)

What is Not Reported:

- Variants in genes not believed to be medically significant
- Variants currently believed to be unassociated with any disease and that are seen in healthy individuals
- Variants that predict an increased risk of diseases, but do not cause a disease by themselves.
- Variants identified in research studies and whose relationship with disease is unclear.

Note: Family members who submit samples for comparative analysis will not receive a separate written report.

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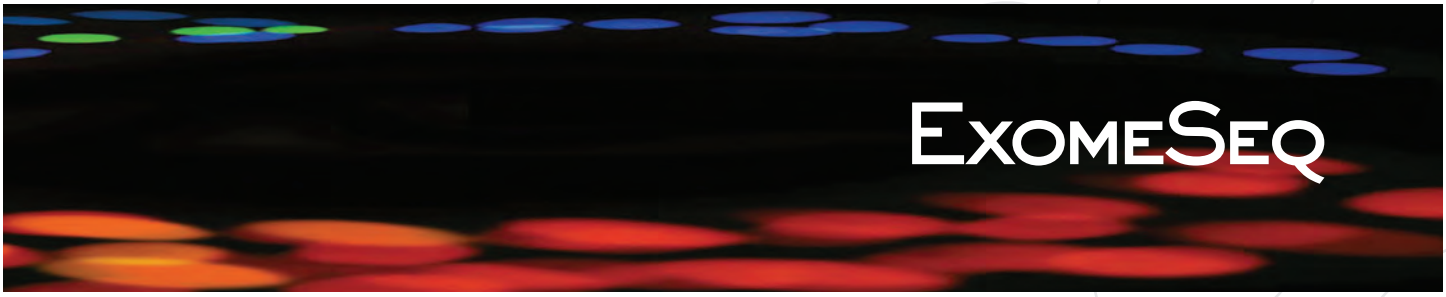


Incidental/Secondary Findings:

This laboratory will seek and report pathogenic variants deemed to be of medical value, including those recommended by the American College of Medical Genetics and Genomics (Miller et al. 2022).

Secondary findings will not be sought or reported if the patient or patient's representative chooses not to receive them. For families who choose to receive secondary findings, information on these disease-causing variants will be included on the patient's report. In addition, the report will note if these variants were found in family members who submitted samples.

Submission Requirements:

It is important to have reliable clinical information and an accurate family history in order to interpret data from ES correctly. ES testing is most likely to provide a genetic diagnosis when several family members are analyzed at one time. These items must be included in order to begin the ES process:

- Proband's sample
- Maternal sample (for trio testing)
- Paternal sample (for trio testing)
- Additional family members' samples (following discussion with laboratory)
- Test requisition (all billing and clinical information must be completed)
- Signed informed consent form
- Family history and pedigree
- Detailed patient clinical history/clinical summary or medical notes
- Summary of previous genetic test results and reports, if available

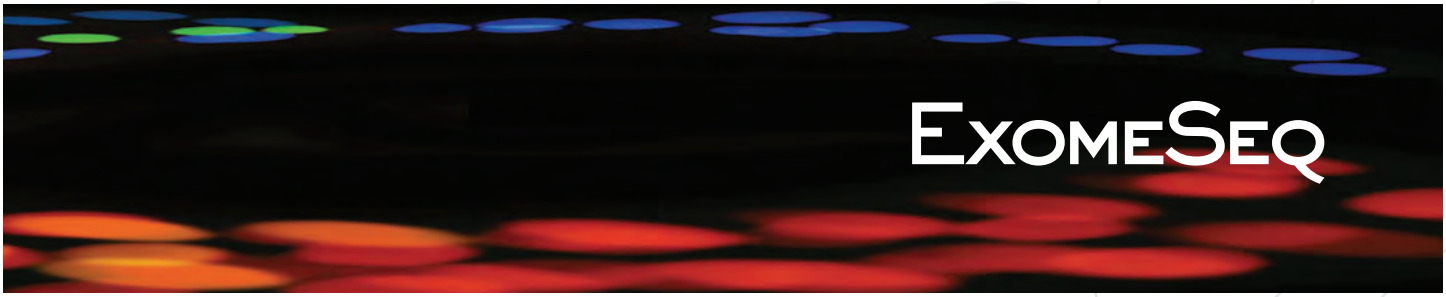
Methodology:

Procedure: ExomeSeq is performed on genomic DNA using the Human Comprehensive Exome kit from Twist Bioscience to enrich the whole exome. The exome is sequenced using an Illumina sequencing system with paired-end reads at a minimum coverage of 20X of 95% of the target regions. The exome DNA sequences are aligned to the human reference genome (build UCSC hg19) with BWA-mem. Variants are called using GATK and QC is performed as part of an in-house developed pipeline based on GATK best practices.

Data Analysis: Variants identified by the GATK-based bioinformatics pipeline are uploaded to the Fabric Genomics Analysis platform, which is used to annotate, analyze, and classify these identified variants. Exome analyses interrogate thousands of genetic variants in a proband and a subset of these is identified as potentially clinically relevant. Sanger confirmation is performed on all variants included in the report.

Technical Limitations:

- This exome sequencing test attempts to examine the important coding regions of approximately 20,000 genes in the genome, known as the exome. However, the technical ability to capture and sequence the exome is limited, and currently 85%-92% of the entire exome can be evaluated.
- Pathogenic variants may be present in a portion of the genes not covered by this test and therefore would not be identified. Thus, the absence of reportable findings for any gene does not mean there are no pathogenic variants in that gene.
- Certain types of mutations are not detected. Only single base pair changes or small insertions or deletions of DNA are detected. Large deletions, duplications, or rearrangements, mitochondrial genome mutations, nucleotide repeat expansions, genes with pseudogenes, and many epigenetic defects, may not be detected by this test.



- The clinical utility of ES depends on the accuracy of the clinical information provided by the referring physician and the predicted inheritance pattern. DNA sequencing from family members often improves the interpretation of test results.
- Genetic changes identified may not predict severity or age of onset of a particular condition.
- Our understanding of the human exome is incomplete at this time.

Genetic Counseling and Interpretation:

- It is highly recommended that patients have genetic counseling before the test is ordered, as they will have an important choice to make regarding which results they wish to know. Understanding the risks and benefits of this testing is important for the patient and his or her family. Genetic counseling after the test is likewise important to aid in the understanding of test results and their implications for the patient and his or her family members.
- It is the ordering physician's responsibility to interpret the results from this test within a clinical context.

Specimen: At least 3 mls whole blood in a lavender top (EDTA) tube, saliva in an Oragene saliva kit or amniotic fluid.* Label the tube with patient's name, birth date, and date of collection. Alternately, 10 mcg of DNA extracted in a CLIA lab may be submitted.

*Please call the lab at **513-636-4474** before sending **amniotic fluid** or for a free saliva collection kit.

Turn-Around Time: The time to complete the case preparation and obtain payor precertification is variable. Once payment precertification is obtained, the turnaround time for sequencing and analysis of the exome is 112 days.

CPT Codes:

Trio (proband and two family members): 81415, 81416 (x2)

Additional family members: 81416

Proband only: 81415

Shipping Instructions:

Please enclose **test requisition** with sample. **All information must be completed before sample can be processed.** Place samples in Styrofoam mailer and ship at room temperature by overnight Federal Express to arrive Monday through Saturday.

Ship to:

Laboratory of Genetics and Genomics
3333 Burnet Avenue NRB 1042
Cincinnati, OH 45229
513-636-4474

References:

Ayuso, C. et al. (2013). "Informed consent for whole-genome sequencing studies in the clinical setting. Proposed recommendations on essential content and process." Eur J Hum Genet 21(10):1054-9.

Deignan, J. L., Chung, W. K., et al. (2019). "Points to consider in the reevaluation and reanalysis of genomic test results: a statement of the American College of Medical Genetics and Genomics (ACMG)." Genet Med. 21(6):1267-1270.

Gahl, W. A., T. C. Markello, et al. (2012). "The National Institutes of Health Undiagnosed Diseases Program: insights into rare diseases." Genet Med 14(1): 51-59.

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