MAYO CLINIC LABORATORIES

NEW TEST

Notification Date: December 15, 2022
Effective Date: December 20, 2022

Whole Genome Sequencing Reanalysis, Varies

Test ID: WGSR

Useful for:

- Identifying a diagnosis or additional variants associated with the phenotype in patients who previously have had a negative or inconclusive whole genome sequencing test
- Reanalyzing whole genome sequencing data when a patient (proband) presents with new clinical features
- Reanalyzing whole genome sequencing data to pick up newly discovered gene-disease associations, changes to variant classification, and bioinformatics pipeline upgrades

Genetics Information:

- Whole genome sequencing utilizes next-generation sequencing to detect single nucleotide variants, small insertions and deletions, copy number variants, mitochondrial genome variants, and select repeat expansion variants throughout the genome. In patients who have had negative or inconclusive whole genome sequencing results, reanalysis of previously generated whole genome sequencing data has the potential to identify additional variants associated with the patient's phenotype and increase the diagnostic yield of this testing.
- This test is available for patients who have previously had WGSDX / Whole Genome Sequencing for Hereditary Disorders, Varies performed by Mayo Clinic Laboratories and would like reanalysis of the results.
- It is recommended to wait at least 1 year after the original whole genome sequencing test results were released to request reanalysis, unless there are substantial changes to the patient's phenotype.(1)
- This test may be ordered by the provider who ordered the original whole genome sequencing test or by a new provider if the patient is currently under their care. If this test is ordered by a new provider, results will be sent only to the new provider. The provider who ordered the original whole genome sequencing test will receive an amended report stating that the original whole genome sequencing results are no longer current.

Methods:

Reanalysis of Whole Genome Next-Generation Sequencing followed by Sanger Sequencing, Quantitative Polymerase Chain Reaction (qPCR), or other methods, as needed

Reference Values:

An interpretive report will be provided.

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Specimen Requirements:

- **For most patients, a new specimen submission will not be required. Testing can be performed using stored DNA from the original whole genome sequencing test. To order testing on the stored specimen, see Additional Testing Requirements.**
- **Patient Preparation:** A previous bone marrow transplant from an allogenic donor will interfere with testing. Call 800-533-1710 for instructions for testing patients who have received a bone marrow transplant.

Specimen Type:	**Whole blood**
Preferred:	Lavender top (EDTA) or yellow top (ACD)
Acceptable:	Any anticoagulant
Specimen Volume:	3 mL
Collection Instructions:	1. Invert several times to mix blood 2. Send whole blood specimen in original tube. **Do not aliquot.**
Minimum Volume:	1 mL

Note:

Specimen preferred to arrive within 96 hours of collection.

Forms:

Whole Genome Sequencing: Ordering Checklist, Patient Information is required

Specimen Stability Information:

Specimen Type	Temperature	Time	Special Container
Varies	Ambient (preferred)		
	Frozen		
	Refrigerated		

Ordering Guidance:

- This test is only appropriate for patients who have previously had WGSDX / Whole Genome Sequencing for Hereditary Disorders, Varies performed by Mayo Clinic Laboratories.
- This test is for affected patients (probands) only. This test does not need to be ordered for family member comparators (CMPRG / Family Member Comparator Specimen for Genome Sequencing, Varies).

Additional Testing Requirements:

DNA specimens from the patient (proband) and all family member comparators included in the original whole genome sequencing test are required to allow for confirmation of any new reportable variants, based on internal laboratory criteria. **For most patients, stored DNA from the original whole genome sequencing test should be available for this testing.**

To use stored DNA for this test:

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- Order WGSR / Whole Genome Sequencing Reanalysis, Varies. By calling Mayo Clinic Laboratories at 800-533-1710 to request that this test be added on to the remaining DNA specimen for the patient (proband). The laboratory will determine if there is sufficient DNA remaining for the proband and all comparators to perform confirmation of any new results. If there is sufficient DNA, the order will proceed.
- **If the patient and/or family member comparators are found to have an insufficient quantity of stored DNA, follow the instructions below:**
    1. **If there is not sufficient DNA remaining for the patient (proband)**: If an order for WGSR was already placed in the steps above, the order will be canceled and the client notified of the test cancellation. Collect a new proband specimen and order WGSR for the new specimen.
    2. **If there is not sufficient DNA remaining for one or more family member comparators:** For the family members who were included as comparators in the original whole genome sequencing test but do not have sufficient stored DNA, collect new comparator specimens and order CMPRG / Family Member Comparator Specimen for Genome Sequencing, Varies for the new specimens.

Necessary Information:

Whole Genome Sequencing: Ordering Checklist **Patient Information is required for all patients**.
Complete the following sections on pages 2 through 4:

- Patient (Proband) Information
- Reason for Testing: provide reason for reanalysis request
- Provide **new** information in:
    - Patient (Proband) Suspected Diagnoses
    - Patient (Proband) Clinical Evaluations
    - Patient (Proband) Clinical Features
- Attach clinic notes and pedigree with any relevant new clinical or family history information.
- Fax the paperwork, clinic notes, and pedigree to 507-284-1759, Attn: WGS Genetic Counselors.

Cautions:

Clinical Correlations:

- Test results should be interpreted in the context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.
- To discuss the availability of additional testing options or for assistance in the interpretation of these results, contact Mayo Clinic Laboratories genetic counselors at 800-533-1710.
- **Technical Limitations:**
- Whole genome sequencing may not detect all types of genomic variants; therefore, false-negative results are possible. There may be regions of genes that cannot be effectively evaluated by whole genome sequencing as a result of technical limitations of the assay, including variable depth of coverage, regions of homology, and repetitive sequences. Given these limitations, negative results do not rule out the diagnosis of a genetic disorder. If a specific clinical disorder is suspected, evaluation by alternative methods can be considered. In addition, in rare cases false-positive results may occur; however, false-positive events should be exceedingly rare as confirmation of reportable variants will be performed by alternate methodologies based on internal laboratory criteria.

Single Nucleotide Variants:

- Genome-wide sensitivity for single nucleotide variants (SNV) is greater than 98% and in noncomplex regions sensitivity is greater than 99.9%.

Deletions and Insertions (less than 1000 base pairs):

- This test is validated to detect greater than 99% of deletions and insertions (delins) up to 50 base pairs (bp) for noncomplex regions. Performance in complex regions is slightly reduced with sensitivities of 92% to

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99% depending on the size of the delins event. Although detected by this assay, performance for larger delins (51-999 bp) is not comprehensively established.

Copy Number Variants (greater than or equal to 1000 base pairs):

- Genome-wide sensitivity for copy number variants (CNV) detectable by chromosomal microarray is >99.9% as established by a comprehensive comparison with clinically validated nonmosaic CNV detected by chromosomal microarray.

Additional Variant Classes:

- A variety of additional variant classes can be detected by this test, including mitochondrial variants, repeat expansions, select spinal muscular atrophy (SMA)-associated variants, balanced structural rearrangements, and mosaic variants of all classes. A limited validation of each of these additional variant classes was conducted before inclusion in this assay; however, comprehensive assessment of sensitivity and false-negative rate has not been established. These variants will be evaluated per laboratory protocol, and findings of clinical relevance will be reported following confirmation with validated laboratory methods. Importantly, the sensitivity and performance for these variant classes is not expected to meet that of current gold-standard testing methodologies; therefore, additional testing may be indicated if there is clinical suspicion for a disorder involving these variant classes. Specific technical limitations for each class are described below.

Mitochondrial Genome Variants:

- This assay can detect mitochondrial genome SNV and small delins with heteroplasmy levels above 5%; however, comprehensive sensitivity/false-negative rate is not established. Detection of large deletion and duplication events involving the mitochondrial genome is not available with the current analysis. Any clinically relevant and reportable mitochondrial variants detected will be confirmed with standard validated methods prior to reporting.

Repeat Expansion Variants:

- Select short tandem repeats (STR; also known as repeat expansions) in pathogenic ranges can be detected with this assay; however, comprehensive sensitivity/false negative rate is not established. STR loci included in this assay are: *C9orf72, CSTB, ATN1, FXN, FMR1, HTT, AR, ATXN1, ATXN2, ATXN3, CACNA1A,* and *ATXN7*. Only loci overlapping the patient's (proband's) clinical features will be evaluated and reported. All repeat expansions meeting laboratory reporting criteria will be confirmed and further characterized by standard validated methods prior to reporting.

SMA Variants:

- Absence of the definitive C nucleotide in exon 7 of *SMN1* (NM_000344.3:c.840C) indicating the homozygous loss of *SMN1* exon 7 is detectable by this assay; however, comprehensive sensitivity/false-negative rate is not established. This assay does not identify *SMN1* or *SMN2* variants outside of this specific single nucleotide change. This assay does not detect *SMN1* carrier status or phase of *SMN1/SMN2* alleles. All variants will be confirmed by validated laboratory methods before reporting.

Balanced Structural Rearrangements:

- This assay does not involve comprehensive evaluation of balanced structural rearrangements (eg, translocations and inversions). However, select genomic regions may be evaluated and balanced events reported when there is a directed clinical focus (eg, known family history or specific locus of high clinical suspicion communicated to the laboratory). Comprehensive sensitivity/false-negative rate is not established. All reported rearrangements will be confirmed by validated laboratory methods before reporting (additional specimen may be required for further characterization).

Mosaicism:

- This assay is not designed to detect mosaicism or to differentiate between somatic and germline variants. Mosaic variants may be detected; however, comprehensive limits of detection for mosaic events are not established. All mosaic variants meeting laboratory reporting criteria will be confirmed by validated laboratory methods before reporting.

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- If the patient has had an allogeneic hematopoietic stem cell transplant or a recent blood transfusion, results may be inaccurate due to the presence of donor DNA. Call Mayo Clinic Laboratories for instructions for testing patients who have received a bone marrow transplant.

Reclassification of Variants:

- All previously reported variants will be reclassified at the time of reanalysis. However, it is not currently standard practice for the laboratory to systematically review previously classified variants on a regular basis. The laboratory encourages healthcare providers to contact the laboratory at any time to learn how the classification of a particular variant may have changed over time. Due to broadening genetic knowledge, it is possible that the laboratory may discover new information of relevance to the patient. Should that occur, the laboratory may issue an amended report.

Variant Evaluation:

- Evaluation and categorization of variants are performed using published American College of Medical Genetics and Genomics (ACMG) recommendations as a guideline.(9,10) Other gene-specific guidelines may also be considered. Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance. Variants classified as benign or likely benign are not reported.
- Rarely, incidental or secondary findings outside of the genes recommended by the ACMG may implicate another predisposition or presence of active disease. These findings will be carefully reviewed to determine whether they will be reported.

Data Sharing:

- Deidentified variant information may be shared in public genetic databases, such ClinVar and Matchmaker Exchange.
- A genetic consultation is recommended for patients undergoing this test, both prior to testing and after results are available.

CPT Code:

First reanalysis: No charge
81247-For all subsequent reanalysis requests

Day(s) Performed: Varies

Report Available: 12 weeks

Questions:

Contact Michelle Raths, Laboratory Resource Coordinator at 800-533-1710.

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