Test Definition: WGSR

Whole Genome Sequencing Reanalysis, Varies

Overview

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

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
CULFB	Fibroblast Culture for Genetic Test	Yes	No

Genetics Test 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 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genome sequencing test will receive an amended report stating that the original whole genome sequencing results are no longer current.

Testing Algorithm

For cultured fibroblast, skin biopsy or tissue biopsy specimens, a fibroblast culture will be performed at an additional charge. If viable cells are not obtained, the client will be notified.

Special Instructions

- Muscle Biopsy Specimen Preparation Instructions
- Whole Genome Sequencing: Ordering Checklist
- Whole Exome and Genome Sequencing Information and Test Ordering Guide

Highlights

Additional information is available; see Whole Exome and Genome Sequencing Information and Test Ordering Guide.

Method Name

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

NY State Available

Yes

Specimen

Specimen Type

Varies

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:
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.

For more information see Whole Exome and Genome Sequencing Information and Test Ordering Guide.

Necessary Information

Whole Genome Sequencing: Ordering Checklist 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.

Specimen Required

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 hematopoietic stem cell transplant from an allogenic donor will interfere with testing. Call 800-533-1710 for instructions for testing patients who have received a hematopoietic stem cell transplant.

Submit only 1 of the following specimens for each family member.

Specimen Type: Whole blood
Container/Tube:
Preferred: Lavender top (EDTA) or yellow top (ACD)
Acceptable: Green top (Sodium heparin)
Specimen Volume: 3 mL
Collection Instructions:
1. Invert several times to mix blood.
2. Send whole blood specimen in original tube. **Do not aliquot**.
3. Whole blood collected postnatal from an umbilical cord is also acceptable. See Additional Information
Specimen Stability Information: Ambient (preferred) 4 days/Refrigerated 4 days/Frozen 4 days
Additional Information:
1. Specimens are preferred to be received within 4 days of collection. Extraction will be attempted for specimens received after 4 days, and DNA yield will be evaluated to determine if testing may proceed.
2. To ensure minimum volume and concentration of DNA is met, the requested volume must be submitted. Testing may be canceled if DNA requirements are inadequate.
3. For postnatal umbilical cord whole blood specimens, maternal cell contamination studies are recommended to ensure test results reflect that of the patient tested. A maternal blood specimen is required to complete maternal cell contamination studies. Order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on both the cord blood and maternal blood specimens under separate order numbers.

Specimen Type: Saliva

Patient Preparation: Patient should not eat, drink, smoke, or chew gum 30 minutes prior to collection.
Supplies:
DNA Saliva Kit High Yield (T1007)
Saliva Swab Collection Kit (T786)
Container/Tube:
Preferred: High-yield DNA saliva kit
Acceptable: Saliva swab
Specimen Volume: 1 Tube if using T1007 or 2 swabs if using T786
Collection Instructions: Collect and send specimen per kit instructions.
Specimen Stability Information: Ambient (preferred) 30 days/Refrigerated 30 days
Additional Information: Saliva specimens are acceptable but not recommended. Due to lower quantity/quality of DNA yielded from saliva, some aspects of the test may not perform as well as DNA extracted from a whole blood sample. When applicable, specific gene regions that were unable to be interrogated will be noted in the report. Alternatively, additional specimen may be required to complete testing.

Specimen Type: Cultured fibroblasts
Source: Skin
Container/Tube: T-25 Flask
Specimen Volume: 2 Flasks
Collection Instructions: Submit confluent cultured fibroblast cells from a skin biopsy. Cultured cells from a prenatal specimen will not be accepted.
Specimen Stability Information: Ambient (preferred) <24 hours/Refrigerated <24 hours
Additional Information:
1. Specimens are preferred to be received within 24 hours of collection. Culture and/or extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks are required to culture fibroblasts before genetic testing can occur.

Specimen Type: Tissue biopsy
Supplies: Hank's Solution (T132)
Container/Tube: Sterile container with sterile Hank's balanced salt solution, Ringer's solution, or normal saline
Specimen Volume: 0.5 to 3 cm(3) or larger
Specimen Stability Information: Ambient (preferred) <24 hours/Refrigerated <24 hours
Additional Information:
1. Specimens are preferred to be received within 24 hours of collection. Culture and/or extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks are required to culture fibroblasts before genetic testing can occur.

Specimen Type: Muscle tissue biopsy
Supplies: Muscle Biopsy Kit (T541)
Specimen Volume: 20 to 80 mg
Collection Instructions: Prepare and transport specimen per instructions in Muscle Biopsy Specimen Preparation Instructions.
Specimen Stability Information: Frozen (preferred) <24 hours/Ambient/Refrigerated <24 hours

Additional Information: Specimens are preferred to be received within 24 hours of collection. Extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.

Specimen Type: Extracted DNA
Container/Tube:
Preferred: Screw Cap Micro Tube, 2mL with skirted conical base
Acceptable: Matrix tube, 1 mL
Collection Instructions:
1. The preferred volume is at least 100 mcL at a concentration of 75 ng/mcL.
2. Include concentration and volume on tube.
Specimen Stability Information: Frozen (preferred) 1 year/Ambient/Refrigerated
Additional Information: DNA must be extracted in a CLIA-certified laboratory or equivalent and must be extracted from a specimen type listed as acceptable for this test (including applicable anticoagulants). Our laboratory has experience with Chemagic, Puregene, Autopure, MagnaPure, and EZ1 extraction platforms and cannot guarantee that all extraction methods are compatible with this test. If testing fails, one repeat will be attempted, and if unsuccessful, the test will be reported as failed and a charge will be applied. If applicable, specific gene regions that were unable to be interrogated due to DNA quality will be noted in the report.

Forms
1. **Whole Genome Sequencing: Ordering Checklist is required.**
2. If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:
-Neurology Specialty Testing Client Test Request (T732)
-Biochemical Genetics Test Request (T798)

Specimen Minimum Volume
Whole blood: 1 mL; Other specimen types: See Specimen Required

Reject Due To
All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

Clinical & Interpretive

Clinical Information
Note: Where applicable, verbiage refers to sex assigned at birth.

Whole genome sequencing utilizes next-generation sequencing technology to assess the genome of patients with suspected underlying genetic disorders. Based on a meta-analysis, the diagnostic utility of whole genome sequencing is approximately 41%.(2)

In patients who have had negative or inconclusive whole genome sequencing results, reanalysis of whole genome

sequencing data has been found to result in a new diagnosis in approximately 10% of cases.(3)

For more information, see Whole Exome Sequencing (WGS): Questions and Answers for Providers.

Reference Values

An interpretive report will be provided.

Interpretation

Variants of interest are evaluated according to American College of Medical Genetics and Genomics (ACMG) recommendations.(4,5) Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

Variants are reported in one of the following categories:
-Likely Causative: Variants with a high degree of suspicion for causing the patient's reported clinical features
-Possibly Relevant: Variants that may be related to the patient's clinical features or variants in genes of uncertain significance (GUS)
-Secondary Findings: Medically actionable variants unrelated to the indication for testing (see below for additional information)

It is possible that a variant may not be recognized as the underlying cause of disease due to incomplete scientific knowledge about the function of all genes in the human genome or the impact of variants in those genes.

Secondary Findings:
Patients are evaluated for medically actionable secondary findings and these findings are reported in accordance with the most current ACMG recommendations, including the most up-to-date gene list.(6) Variants in these genes will not be evaluated or reported if the patient opts out of this evaluation unless they overlap with the patient's reported clinical features.

The opt in or opt out selection from the original whole genome sequencing test will be maintained unless a new Informed Consent form is returned denoting a change in this selection. If the patient originally opted in to receive secondary findings and a secondary finding was originally reported, the status cannot be changed to opt out.

The presence of a secondary finding in family member comparator samples is stated on the patient's (proband's) report unless family members opt out of secondary findings. If the patient (proband) opts out, secondary findings will not be evaluated or reported in any family member comparators. Secondary findings that are present in family members comparators but absent from the patient (proband) are not evaluated or reported.

The absence of a reportable secondary finding does not guarantee that there are no disease-causing or likely disease-causing variants in these genes, as review is limited to known or highly suspected pathogenic findings, and not all regions of these genes are adequately evaluated by this technology.

Reanalysis and Raw Data Requests:
It is not guaranteed that patient data will be stored indefinitely. Requests for reanalysis or release of raw data may not be possible, and a new whole genome sequencing order may be required if the original patient data is no longer available or no longer compatible with current bioinformatics processes or analysis tools.

Requests for the raw data obtained from whole genome sequencing should be directed to the laboratory. A separate fee may apply. Raw data will be released for individuals who complete a Mayo Clinic release of information form. If raw data for family member comparators is requested, it will only be released with an accompanying request for the proband's raw data. Contact the laboratory for instructions on completing the release of information form. The laboratory is not responsible for providing software or other tools needed to visualize, filter, or interpret this data.

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 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 greater than 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 (STRs; also known as repeat expansions) in pathogenic ranges can be detected with this assay; however, comprehensive sensitivity/false negative rate is not established. Only STRs in genes overlapping the patient's (proband's) reported clinical features will be reported.

Short tandem repeat loci included in this assay: *AFF2, AR, ARX, ATN1, ATXN1, ATXN10, ATXN2, ATXN3, ATXN7, ATXN8OS, BEAN1, C9ORF72, CACNA1A, CBL, CNBP, COMP, CSTB, DAB1, DIP2B, DMD, DMPK, EIF4A3, FMR1, FOXL2, FXN, GIPC1, GLS, HOXA13, HOXD13, HTT, JPH3, LRP12, MARCHF6, NIPA1, NOP56, NOTCH2NLC, NUTM2B-AS1, PABPN1, PHOX2B, PPP2R2B, PRDM12, PRNP, RAPGEF2, RFC1, RUNX2, SAMD12, SOX3, STARD7, TBP, TBX1, TCF4, TNRC6A, VWA1, XYLT1, YEATS2, ZIC2*, and *ZIC3*.

Short tandem repeats identified in *ATN1, ATXN1, ATXN2, ATXN3, ATXN7, C9orf72, CACNA1A, CSTB, FMR1, FXN, HTT*, and *PABPN1* will be confirmed orthogonally by in-house validated clinical assays and reported accordingly. For the remaining loci, STRs will be reported as "expanded" and additional confirmatory clinical testing will be recommended to determine the exact repeat size.

Spinal Muscular Atrophy Variants:
SMN1 copy number and *SMN2* copy number are determined. This assay also ascertains whether the g.27134T>G alteration is present in patients found to have 2 copies of *SMN1*. 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 mutations 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.

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 professionals 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.(4,5) 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 as ClinVar or Matchmaker Exchange.

A genetic consultation is recommended for patients undergoing this test, both prior to testing and after results are available.

Clinical Reference

1. Deignan JL, Chung WK, Kearney HM, et al. 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. 2019;21(6):1267-1270
2. Clark MM, Stark Z, Farnaes L, et al. Meta-analysis of the diagnostic and clinical utility of genome and genome sequencing and chromosomal microarray in children with suspected genetic diseases. NPJ Genom Med. 2018;3:16. doi:10.1038/s41525-018-0053-8
3. Costain G, Jobling R, Walker S, et al. Periodic reanalysis of whole-genome sequencing data enhances the diagnostic advantage over standard clinical genetic testing. Eur J Hum Genet. 2018;26(5):740-744. doi:10.1038/s41431-018-0114-6
4. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-424
5. Riggs ER, Andersen EF, Cherry AM, et al. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen) [published correction appears in Genet Med. 2021 Nov;23(11):2230. Genet Med. 2020;22(2):245-257. doi:10.1038/s41436-019-0686-8
6. Miller DT, Lee K, Gordon AS, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2021 update: a policy statement of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2021;23(8):1391-1398. doi:10.1038/s41436-021-01171-4

Performance

Method Description

The human genome reference GRCh38/hg38 build is used for sequence read alignment. Variants are called using an optimized bioinformatics package. The average genomic coverage is at or above a read depth of 32X. Sensitivity is estimated at above 99% for single nucleotide variants, above 94% for deletion-insertions (delins) less than 50 base pairs (bp). This assay also detects greater than 99% copy number variants (deletions/duplications) at least 1000 bp in size. Confirmation of select reportable variants in the proband and submitted comparator samples may be performed by alternate methodologies based on internal laboratory criteria.

There may be regions of genes that cannot be effectively evaluated by sequencing as a result of technical limitations of the assay, including regions of homology, variable depth of coverage, and repetitive sequences.(Unpublished Mayo method)

PDF Report

Supplemental

Day(s) Performed

Varies

Report Available

84 days

Specimen Retention Time

Whole blood: 28 days (if available);; Saliva (if available): 30 days; Extracted DNA: 3 months

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Main Campus

Fees & Codes

Fees

- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

Test Classification

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information

First reanalysis: No charge

81427-For all subsequent reanalysis requests

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
WGSR	Whole Genome Sequencing Reanalysis	86206-0

Result ID	Test Result Name	Result LOINC® Value
618564	Interpretation	69047-9
618565	Specimen	31208-2
618566	Source	31208-2
618567	Released By	18771-6