Test Definition: WESR

Whole Exome 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 exome sequencing test

Reanalyzing whole exome sequencing data when a patient (proband) presents with new clinical features

Reanalyzing whole exome 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 exome sequencing utilizes next-generation sequencing to detect variants within the protein-coding regions of approximately 20,000 genes. In patients who have had negative or inconclusive whole exome sequencing results, reanalysis of previously generated whole exome 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 one of the following tests performed by Mayo Clinic Laboratories and would like reanalysis of the results.
Currently available assays:
WESDX / Whole Exome Sequencing for Hereditary Disorders, Varies
WESMT / Whole Exome and Mitochondrial Genome Sequencing, Varies
Previously available assays:
WES / Whole Exome Sequencing, Varies
WESPP / Whole Exome Sequencing Plus Pharmacogenomics
WESPM / Whole Exome Sequencing plus Whole Mitochondrial Genome Sequencing, Varies

It is recommended to wait at least one year after the original whole exome 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 exome 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whole exome sequencing test will receive an amended report stating that the original whole exome sequencing results are no longer current.

Testing Algorithm

For skin biopsy or cultured fibroblast specimens, fibroblast culture testing will be performed at an additional charge. If

viable cells are not obtained, the client will be notified.

Special Instructions

- Whole Exome Sequencing: Ordering Checklist
- Blood Spot Collection Card-Spanish Instructions
- Blood Spot Collection Card-Chinese Instructions
- Blood Spot Collection Instructions
- 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 Exome Next-Generation Sequencing with Orthogonal Confirmation

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

This test is only appropriate for patients who have previously had one of the following whole exome sequencing tests performed by Mayo Clinic Laboratories:
WES / Whole Exome Sequencing, Varies
WESDX / Whole Exome Sequencing for Hereditary Disorders, Varies
WESPP / Whole Exome Sequencing Plus Pharmacogenomics
WESPM / Whole Exome Sequencing plus Whole Mitochondrial Genome Sequencing, Varies
WESMT / Whole Exome and Mitochondrial Genome Sequencing, Varies

If the patient has not had one of the above tests performed previously, consider either WESDX / Whole Exome Sequencing for Hereditary Disorders, Varies or WESMT / Whole Exome and Mitochondrial Genome Sequencing, Varies.

This test is for patients (probands) only. This test does not need to be ordered for family member comparators (CMPRE / Family Member Comparator Specimen for Exome Sequencing, Varies).

Additional Testing Requirements

DNA specimens from the patient (proband) and all family member comparators included in the original whole exome 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 exome sequencing test should be available for this testing.**

To use stored DNA for this test:
Order WESR / Whole Exome Sequencing Reanalysis, Varies by calling Mayo Clinic Laboratories at 800-533-1710 and requesting 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 WESR 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 WESR 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 exome sequencing test but do not have sufficient stored DNA, collect new comparator specimens and order CMPRE / Family Member Comparator Specimen for Exome Sequencing, Varies for the new specimens.

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

Shipping Instructions
Specimens are preferred to arrive within 96 hours of collection.

Necessary Information
From the Whole Exome Sequencing: Ordering Checklist, Patient Information is required for all patients.
Complete the following sections on pages 2 through 4:
Patient (Proband) Information
Provide reason for reanalysis request in Reason for Testing
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: WES 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 exome 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.

Submit only 1 of the following specimens:

Specimen Type: Whole blood
Container/Tube:
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.**
Specimen Stability Information: Ambient (preferred)/Refrigerated

Specimen Type: Skin biopsy
Supplies: Fibroblast Biopsy Transport Media (T115)
Container/Tube: Sterile container with any standard cell culture media (eg, minimal essential media, RPMI 1640). The solution should be supplemented with 1% penicillin and streptomycin.
Specimen Volume: 4-mm punch
Specimen Stability Information: Refrigerated (preferred)/Ambient
Additional Information: A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks is required to culture fibroblasts before genetic testing can occur.

Specimen Type: Cultured fibroblasts
Container/Tube: T-25 flask
Specimen Volume: 2 Flasks
Collection Instructions: Submit confluent cultured fibroblast cells from a skin biopsy from another laboratory. Cultured cells from a prenatal specimen will not be accepted.
Specimen Stability Information: Ambient (preferred)/Refrigerated (<24 hours)
Additional Information: A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks is required to culture fibroblasts before genetic testing can occur.

Specimen Type: Blood spot
Supplies: Card-Blood Spot Collection (Filtration Paper) (T493)
Container/Tube:
Preferred: Collection card (Whatman Protein Saver 903 Paper)
Acceptable: PerkinElmer 226 (formerly Ahlstrom 226) filter paper or blood spot collection card
Specimen Volume: 5 Blood spots
Collection Instructions:
1. An alternative blood collection option for a patient older than 1 year is a fingerstick. For detailed instructions, see How to Collect Dried Blood Spot Samples.
2. Let blood dry on the filter paper at ambient temperature in a horizontal position for a minimum of 3 hours.
3. Do not expose specimen to heat or direct sunlight.
4. Do not stack wet specimens.
5. Keep specimen dry
Specimen Stability Information: Ambient (preferred)/Refrigerated
Additional Information:
1. Due to lower concentration of DNA yielded from blood spot, it is possible that additional specimen may be required to complete testing.
2. For collection instructions, see Blood Spot Collection Instructions
3. For collection instructions in Spanish, see Blood Spot Collection Card-Spanish Instructions (T777)
4. For collection instructions in Chinese, see Blood Spot Collection Card-Chinese Instructions (T800)

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.

Forms

1**. Whole Exome Sequencing: Ordering Checklist, Patient Information 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)

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	Ambient (preferred)		
	Refrigerated		
	Frozen		

Clinical & Interpretive

Clinical Information

Whole exome sequencing utilizes next-generation sequencing to assess patients with suspected underlying genetic disorders for variants within the protein-coding regions (exons and splice junctions) of approximately 20,000 genes simultaneously. Based on a meta-analysis, the diagnostic utility of whole exome sequencing is approximately 36%.(2)

In patients who have had negative or inconclusive whole exome sequencing results, reanalysis of whole exome sequencing data has been found to result in a new diagnosis in approximately 15% of cases.(3)

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

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) 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
-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.(5) 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 phenotype.

The opt in or opt out selection from the original whole exome 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 variant in family member comparator specimens 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 reported for any family member.

Variants that are present in family members comparators but absent from the patient (proband) are not evaluated.

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 portions of the genes may not be adequately covered by this testing methodology.

Reanalysis and Raw Data Requests
Patient data is not guaranteed to be stored indefinitely. Requests for reanalysis or release of raw data may not be possible, and a new whole exome 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 exome 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 exome sequencing may not detect all types of genomic variants. In rare cases, false-negative or false-positive results may occur. The depth of coverage may be variable for some target regions. 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.

There may be regions of genes that cannot be effectively evaluated by sequencing or deletion and duplication analysis (as applicable) as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences. Confirmation of select reportable variants will be performed by alternate methodologies based on internal laboratory criteria.

This test is not designed to detect low levels of mosaicism or differentiate between somatic and germline variants. If there is a possibility that any detected variant is somatic, additional testing may be necessary to clarify the significance of results.

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) and the Association for Molecular Pathology recommendations as a guideline.(4) 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.

Multiple in silico evaluation tools may be used to assist in the interpretation of these results. The accuracy of predictions

made by in silico evaluation tools is highly dependent upon the data available for a given gene, and periodic updates to these tools may cause predictions to change over time. Results from in silico evaluation tools should be interpreted with caution and professional clinical judgment.

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 GeneMatcher or ClinVar.

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 June;21(6):1267-1270
2. Clark MM, Stark Z, Farnaes L, et al: Meta-analysis of the diagnostic and clinical utility of genome and exome sequencing and chromosomal microarray in children with suspected genetic diseases. NPJ Genom Med. 2018 Jul 9;3:16. doi: 10.1038/s41525-018-0053-8
3. Tan NB, Stapleton R, Stark Z, et al: Evaluating systematic reanalysis of clinical genomic data in rare disease from single center experience and literature review. Mol Genet Genomic Med. 2020;8(11):e1508
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 May;17(5):405-424
5. 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 Aug;23(8):1391-1398. doi: 10.1038/s41436-021-01171-4

Performance

Method Description

The human genome reference GRCh37/hg19 build is used for sequence read alignment. Variants are called using an optimized bioinformatics package. Resulting variants are filtered and annotated using public and proprietary resources and presented for analysis and interpretation using a vended interpretation tool. Confirmation of select reportable variants in the proband and submitted comparator specimens may be performed by alternate methodologies based on internal laboratory criteria. There may be regions of genes that cannot be effectively evaluated as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences.(Unpublished Mayo method)

If the original test was WES / Whole Exome Sequencing, Varies; WESPP / Whole Exome Sequencing Plus Pharmacogenomics; WESPM / Whole Exome Sequencing plus Whole Mitochondrial Genome Sequencing, Varies: Reanalysis includes reevaluation of previously recognized variants (both reported and unreported single nucleotide variants and small deletions/insertions). No new variant types will be detected; refer to the original WES/WESPP/WESPM report for additional information.

If the original test was WESDX / Whole Exome Sequencing for Hereditary Disorders, Varies or WESMT / Whole Exome and Mitochondrial Genome Sequencing, Varies: Reanalysis includes reevaluation of previously recognized variants (both reported and unreported single nucleotide variants, small deletions/insertions, and copy number variants) and may also involve bioinformatically reprocessing the data (sequence alignment and variant calling, annotation, and/or filtering). No new variant types will be detected; refer to the original WESDX/WESMT report for additional information.

PDF Report
Supplemental

Day(s) Performed
Varies

Report Available
84 days

Specimen Retention Time
Whole blood: 2 weeks (if available); Extracted DNA: 3 months; Blood spots, saliva, cultured fibroblasts, skin biopsy, cord blood: 1 month

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
The first reanalysis: No charge
For all subsequent reanalysis requests: 81417

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
WESR	Whole Exome Sequencing Reanalysis	86205-2

Result ID	Test Result Name	Result LOINC® Value
616885	Interpretation	69047-9

616886	Specimen	31208-2
616887	Source	31208-2
616888	Released By	18771-6