Whole Genome Sequencing

Last Literature Review: May 2023 Last Update: June 2024

Whole genome sequencing is used as a comprehensive first line test if a Mendelian genetic condition is suspected:

- **Rapid whole genome sequencing (3005935)** should be ordered in cases of **acute** clinical presentation. Parental control samples are **required** and are used to aid in interpretation of the proband's genome sequencing data.
- **Nonrapid whole genome sequencing (3016493)** should be ordered when clinical presentation is **not acute**. Parental controls samples are recommended, but not required, to aid in interpretation of the proband's genome sequencing data.

For both rapid and nonrapid whole genome sequencing, the analyzed genome includes all exons from all known human nuclear genes and all intronic variants that are suspected to influence splicing. Whole genome reanalysis is available for any sample originally sequenced at ARUP and may be considered 12-18 months after the initial test if a causative variant was not identified.

The results of genome sequencing may or may not:

- Identify the etiology of the patient's symptoms
- Determine prognosis
- Predict the severity of the patient's condition
- Guide medical management

Testing Summary

Test	Turnaround Time for Final Report	Use to Submit Samples for:	Primary Findings[a]	Secondary Findings
Rapid Whole Genome Sequencing (3005935)	≤7 days	Patient (proband) for RWGS	Reported	Reported if opted in
Rapid Whole Genome Sequencing, Familial Control (3005928)	≤7 days	Parental comparator for RWGS (if reporting of secondary findings for parent is not desired)	Not applicable	Not reported
Rapid Whole Genome Sequencing, Familial Control with Report (3005933)	≤7 days	Parental comparator for RWGS (if reporting of secondary findings for parent is desired)	Not applicable	Reported
Whole Genome Sequencing (3016493)	14-21 days	Patient (proband) for WGS	Reported	Reported if opted in
Whole Genome Sequencing, Familial Control with Report (3016497)	14-21 days	Parental comparator for WGS	Not applicable	Reported if opted in (charges will apply)
Whole Genome Reanalysis (Originally Tested at ARUP - No Specimen Required) (3005939)	≤21 days	No samples required; reanalysis of proband and parental genome sequencing data (only offered for samples originally sequenced at ARUP) for both RWGS and WGS	Reported	Reanalyzed for proband only (using the current ACMG gene list)

[a]Variants in genes of unknown function/significance are not reported for any test.

Featured ARUP Testing

Rapid Whole Genome Sequencing 3005935

Method: Massively Parallel Sequencing

- Preferred test to determine etiology of a patient's symptoms when a Mendelian genetic condition is suspected in cases of **acute** clinical presentation
- Parental control specimens are **required** for this test; order Rapid Whole Genome Sequencing, Familial Control (3005928) or Rapid Whole Genome Sequencing, Familial Control with Report (3005933).
- Submission of a completed Rapid Whole Genome Sequencing Intake Form is required for the proband.

Rapid Whole Genome Sequencing, Familial Control 3005928

Method: Massively Parallel Sequencing

- Use to submit required parental control samples for Rapid Whole Genome Sequencing (3005935) if reporting of secondary findings for parental samples is **not** desired.
- Secondary findings, including pathogenic variants in the American College of Medical Genetics and Genomics (ACMG) recommended genes, will **not** be reported.
- If reporting of ACMG-recommended secondary findings for parental samples is desired, refer to Rapid Whole Genome Sequencing, Familial Control with Report (3005933).

Rapid Whole Genome Sequencing, Familial Control with Report 3005933

Method: Massively Parallel Sequencing

- Use to submit required parental control samples for Rapid Whole Genome Sequencing (3005935) if reporting of secondary findings for parental samples is desired.
- Pathogenic variants in the American College of Medical Genetics and Genomics (ACMG) recommended genes will be reported.
- If reporting of ACMG-recommended secondary findings for parental samples is **not** desired, refer to Rapid Whole Genome Sequencing, Familial Control (3005928).

Whole Genome Sequencing 3016493

Method: Massively Parallel Sequencing

- Preferred test to determine etiology of a patient's symptoms when a Mendelian genetic condition is suspected in **nonacute** clinical presentation.
- Parental control specimens are recommended for this test; order Whole Genome Sequencing, Familial Control with Report (3016497).

Test Requirements

- **Parental samples**:
  - **Rapid whole genome sequencing** is not intended for a proband without parental samples. Parental specimens (familial controls) are necessary to identify de novo variants and the chromosomal phase of variants, and aid in the optimal interpretation of the patient's results.
  - **Whole genome sequencing** can be performed without parental samples, but parental specimens are recommended.
  - Submit parental samples within 7 days of the proband's sample.
- **Medical records**: Medical records detailing the patient's clinical findings, relevant previous testing results, and family history are required for optimal interpretation of the patient's results. The ability to identify causative variant(s) for the patient's presentation is influenced by the quality of the clinical information provided.
- **Informed consent**: Healthcare provider attestation of informed consent is required. Reporting of secondary findings is available for the proband and each parent if desired.

- Submission of a completed Genome Sequencing Intake Form is required for the proband.

Whole Genome Sequencing, Familial Control 3016497

Method: Massively Parallel Sequencing

- Use to submit parental control samples for Whole Genome Sequencing (3016493).
- Secondary findings, including pathogenic variants in the American College of Medical Genetics and Genomics (ACMG) recommended genes, will be reported if opted in (charges will apply).

Whole Genome Reanalysis 3005939

Method: Bioinformatic Processing and Variant Analysis

- Consider 12-18 months after performance of Rapid Whole Genome Sequencing (3005935) if a causative variant that explains the proband's condition was **not** identified.
- Whole Genome Reanalysis (3005939) is only offered for samples originally sequenced at ARUP Laboratories.

Test Description

Methodology

Genome sequencing is performed using the following sequence of steps:

- Genomic DNA is extracted from whole blood, prepared into libraries, and submitted to five cycles of PCR.
- Genome-wide libraries are sequenced by massively parallel sequencing (MPS; also known as next-generation sequencing [NGS]) followed by paired-end read alignment and variant calling using a custom bioinformatics pipeline for single nucleotide variants (SNVs) and insertions/deletions (indels). The pipeline includes an algorithm that assigns scores to variants with likely association to phenotypic indication(s) for testing.

Methodology notes:

- Human genome build 19 (Hg 19) is used for data analysis.
- The regions of interest (ROI) include approximately one-third of the human genome, including targeted deep intronic variants.
  - The ROI comprises all Human Gene Mutation Database (HGMD) variant positions and all coding transcripts from Ensembl and RefSeq.
  - Additional noncoding transcripts from Ensembl and RefSeq are also included if they intersect with HGMD variants.
- The overall proband sample mean coverage over the ROI will be >30X, with >94% of bases in the genome ROI >20X.

Clinical Sensitivity

Varies based on clinical symptoms, family history, inheritance pattern, and previous clinical evaluations.

Reporting

Primary Findings

- Variants in genes suspected to be causative for, or potentially related to, the patient's condition are reported.
- Variants identified in genes of unknown function/significance are **not** reported.

Secondary Findings

Secondary findings refer to medically actionable disease-associated variants that are not associated with the patient's clinical phenotype. The American College of Medical Genetics and Genomics (ACMG) recommends analysis of certain genes for secondary findings in all individuals undergoing genome sequencing.[1] Please refer to ACMG Secondary Findings Gene List for an up-to-date list of genes analyzed. ACMG genes are only analyzed to the extent that routine genome analysis allows.

- The Genome Sequencing Intake Form allows providers the option to opt-in to receive secondary findings for each individual sequenced (proband and each parent).
  - Secondary findings will **not** be reported unless each individual sequenced opts in.
  - Secondary findings will be reported for parents who elect to receive this information regardless of whether the finding was also identified in the proband.
- Single disease-causing variants in autosomal recessive ACMG genes are not reported.
- Additional medically actionable variants in non-ACMG genes may be reported at ARUP's discretion.

Interpretation, Storage, Reanalysis, and Data Sharing

- Accurate representation of biological relationships among family members is imperative for correct test interpretation.
- Test interpretation is based on information available at the time of testing and may change in the future.

- Whole genome sequencing data will be stored for a minimum of 5 years in compliance with ARUP's data retention policy.
- Many samples are discarded after testing is complete, however, samples may be stored indefinitely for test validation or education purposes after personal identifiers are removed.
  - Individuals may request disposal of their sample by calling ARUP Laboratories at 800-242-2787 ext.3301.
- Data reanalysis is available; an additional fee applies.
  - Order Whole Genome Reanalysis (Originally Tested at ARUP – No Specimen Required) (3005939)
  - Data reanalysis is only offered for samples originally sequenced at ARUP Laboratories.
- Deidentified information about genetic variants and clinical findings may be published in public international databases.
  - Individuals may request that their test result not be shared with public databases by calling ARUP Laboratories at 801-242-2787 ext. 3301.
- Patients have the opportunity to participate in patient registries and research.
  - For more information, refer to www.aruplab.com/genetics.
- Raw genome sequencing data may be requested by the ordering healthcare provider and hospital that submitted the test to ARUP.

Analytic Sensitivity and Specificity

Variant Class	Analytic Sensitivity (PPA) Estimate (%) and 95% Credibility Region	Analytic Specificity (NPA) Estimate (%) and 95% Credibility Region	PPV Estimate (%) and 95% Credibility Region
SNVs	98.6 (98.6-98.6)	>99.9 (1.00-1.00)	>99 (1.00-1.00)
Deletions 1-5 bp	99.3 (99.2-99.3)	>99 (1.00-1.00)	96.9 (96.9-97.0)
Deletions 6-15 bp	98.5 (98.4-98.6)	>99.9 (1.00-1.00)	95.6 (95.5-95.9)
Deletions ≥16 bp	96.9 (96.5-97.2)	>99.9 (1.00-1.00)	97.8 (97.5-98.0)
Insertions 1-5 bp	99.0 (99.0-99.0	>99 (1.00-1.00)	98.2 (98.2-98.3)
Insertions 6-15 bp	97.4 (97.2-97.5)	>99.9 (1.00-1.00)	98.3 (98.3-98.5)
Insertions ≥16 bp	92.0 (91.5-92.5)	>99.9 (1.00-1.00)	98.8 (98.7-99.1)

bp, base pairs; NPA, negative percent agreement; PPA, positive percent agreement; PPV, positive predictive value

Limitations

- A negative result does not exclude a genetic cause for the patient's condition.
- The human genome cannot be completely analyzed.
  - Some genes have not been identified.
  - Some genes cannot be sequenced or interpreted due to technical limitations.
- The current iteration of this assay is designed to detect only the following variant types:
  - SNVs and small deletions/insertions of <50 bp
  - Homozygous, heterozygous, and hemizygous variants
- The current iteration of this assay is **not** designed to detect the following variant classes or regions:
  - Large structural variants
  - Copy number variants
  - Triplet repeat expansions
  - High homology regions
  - Low coverage regions
  - Mosaicism or somatic variants
  - Indels >50 bp
  - Aneuploidy
  - Uniparental disomy
- Pathogenic variants may occur outside the regions analyzed by this assay.
- Mitochondrial DNA (mtDNA) is not analyzed by this assay.
- Result interpretation may be impacted if this individual has had an allogeneic stem cell transplant.
- Diagnostic errors can occur due to rare sequence variations.

References

1. Miller DT, Lee K, Abul-Husn NS, et al. ACMG SF v3.1 list for reporting of secondary findings in clinical exome and genome sequencing: A policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2022;24(7):1407-1414.