INFLECTION MEDICINE

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Inflection Genome Test Description

Test overview

The Inflection Genome is a diagnostic rapid whole-genome sequencing (rWGS) test developed to identify underlying genetic etiology for patients with previously unexplained phenotype. Sequencing of nuclear and mitochondrial genomes is conducted by hybrid short-read and long-read methods, with both methodologies performed in parallel to maximize sensitivity, yield, and accuracy.
A results report describing reportable variants in the nuclear and mitochondrial genomes will be returned to the ordering clinician within four calendar days of proband sample accession. In a small proportion of cases, this report will be preliminary, pending orthogonal confirmation, and a final report will be issued thereafter.

Ordering

The Inflection Genome is ordered through the Inflection Platform (EHR or Web App). The test supports proband, duo, and trio test configurations. Most partner accounts are configured for a proband-first model, in which the affected patient receives genome sequencing, and follow-up parental testing is recommended if comparator analysis has the potential to meaningfully impact the proband's results. This test is validated for EDTA whole blood and buccal specimens. Either sample type is accepted for proband and comparators.

The Inflection Genome is appropriate for patients presenting with signs and symptoms of unknown cause. Yield of rWGS is documented to be highest in patients with multiple congenital anomalies, epilepsy or other unexplained neurologic conditions, and/or suspected inborn error of metabolism. Professional societies recommend WGS as a first-line test in patients with congenital anomalies, developmental delay/intellectual disability, neonatal hypotonia, unexplained seizures, and/or critically ill neonates and infants with unexplained illness and suspected genetic etiology (PMID: 34211152, 34930662, 35254387, 36281494, 38409289).

Sequencing

This test evaluates the protein-coding and non-protein coding regions of the nuclear genome, as well as the entire mitochondrial genome. For sequencing, genomic DNA is extracted from each submitted blood or buccal specimen. PCR-free libraries are prepared for dual-platform sequencing: short-read sequencing is performed on an Illumina NovaSeq X Plus using 2×150 bp paired-end reads, and long-read sequencing is performed on an Oxford Nanopore Technologies PromethION P24 using super-accuracy basecalling. Sequencing data from both platforms are aligned to the human reference genome GRCh38 (no-alt) and variants are called through combined analysis of short-read and long-read evidence.

Baseline coverage thresholds for routine analysis require genome-wide coverage of at least 8× from short reads, at least 4× from long reads, and at least 14× combined, with a mean aligned long-read length of at least 1,000 bases. As detailed below in Test Specifications, actual median sequencing coverage exceeds these minimums.

Variant spectrum

The current Inflection Genome test offering evaluates the following variant types:

- Single-nucleotide variants (SNVs)
- Small insertions and deletions (up to 50 bp)

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- Structural variants: insertions, deletions, inversions and duplications (51 bp to whole chromosome)
- Copy-number variants (>5 kb)
- Whole chromosome aneuploidy
- Mitochondrial SNVs and small insertions and deletions
- SMN1 and SMN2 copy-number
- Repeat expansions in ARX, PHOX2B, DMPK, ATXN7, FXN, HOXD13, SOX3, and ZIC2 and contraction in SOX3 (screen only)
- Uniparental disomy (trios only)/regions of homozygosity (proband, duo) involving clinically relevant imprinting regions
- Non-coding variants with established clinical significance

Analysis and Interpretation

Genomic data analysis is phenotype-driven, prioritizing variants based on overlap with HPO terms describing the patient's phenotype, submitted at the time of test order. Prioritized variants are curated using various sources, including gnomAD, in-silico prediction tools, published literature, ClinVar, and internal case data. Variants are classified as pathogenic, likely pathogenic, variant of uncertain significance, likely benign, or benign, according to guidelines from the American College of Medical Genetics and Association of Molecular Pathologists (ACMG/AMP) and recommendations from ClinGen (PMID: 25741868, 31690835, 32906214; ClinGen Variant Classification Guidance).

Reporting Guidelines

The three broad categories of reportable variants include:

- Variants associated with patient phenotype: Pathogenic (P) variants, likely pathogenic (LP) variants, and certain variants of uncertain significance (VUS) with zygosity consistent with inheritance of the associated condition and which overlap the patient's reported phenotype will be reported.
    - VUS reporting: Reported VUSs may have pathogenic leaning, limited, or balanced (pathogenic and benign) evidence supporting their classification. Further, candidate variants in genes of uncertain significance (GUS), which are reported if no other variants explaining the phenotype are identified, will be classified as VUS due to evolving understanding of molecular mechanism and phenotypic spectrum.
- Additional findings: Likely pathogenic and pathogenic variants in RYR1, CACNA1S, and MT-RNR1 associated with malignant hyperthermia or aminoglycoside-induced ototoxicity will be reported if detected, even if not associated with the patient's reported phenotype.
- Incidental Findings: Sex chromosome aneuploidies identified by quality control (QC) assessment will be reported, even if not associated with the patient's reported phenotype.

The following findings are not reported on this test: Likely benign variants, benign variants, VUS-leaning benign variants, carrier status in absence of strong phenotype overlap, and ACMG secondary findings unrelated to patient phenotype

Results

Results will be delivered through the Inflection Platform (EHR or Web App). The results report will include variant details, transcript, zygosity in the proband, associated condition, classification, a

summary of the evidence supporting the classification, and a summary of the associated condition for all reported variants.

Reported variants are placed in one of two report sections:
- Variants Associated with Patient Phenotype: These are variants which may explain some or all of the phenotypes reported in the proband.
- Additional Findings: These variants are unrelated to the patient's phenotype. These include reportable variants in RYR1, CACNA1S, and MT-RNR1 (described above) and incidentally detected sex chromosome aneuploidies.

Each report is labeled with a high-level result, as follows:
- Positive Result- LP/P variant or variant combination associated with patient phenotype
- Inconclusive Result- VUS or VUS+LP/P combination associated with patient phenotype
- Variant Reported- Additional or incidental finding only
- Negative Result- No variants reported

Reports with multiple findings will be labeled based on their strongest variant classification.

Orthogonal confirmation

Reportable variants requiring confirmation under laboratory policy may undergo orthogonal testing at LSMC or another CLIA-certified laboratory using an appropriate assay for the variant class. Any associated technical limitations or recommendations will be documented on the report. To support urgent clinical decision-making, a preliminary report may be issued at the Laboratory Director's discretion while confirmatory testing is underway. A final report incorporating the confirmatory findings will be issued upon completion.

Technical Specifications

During test validation, analytical performance for single-nucleotide variants demonstrated sensitivity of 99.9% and positive predictive value (PPV) of 99.9%. For small insertions and deletions up to 50 bases, sensitivity was 99.8% and PPV was 99.9%. Structural-variant detection varies by event type, size and genomic region; for the insertions and deletions tested (at least 50 bp), precision was 91.1% and recall was 78.2%. Median short read sequencing coverage was 41x and median long read coverage was 13x. Mitochondrial genome coverage exceeded 10,000x.

Test Limitations

A negative or non-diagnostic result does not exclude the presence of a genetic disorder. Diagnostic sensitivity varies across the genome, and false-negative results may occur due to biological, technical, or interpretive limitations. Regions of high sequence homology, pseudogenes, extreme GC content, repetitive elements, and low complexity (e.g., centromeric and telomeric regions) may have reduced coverage or cannot be uniquely aligned and reliably evaluated.

A recommendation for follow-up testing will be included on the report if needed to confirm or further characterize a reported finding due to limitations of the WGS assay. Findings for which follow-up testing is indicated include but are not limited to:

Finding type	**Purpose of follow-up testing**
Repeat expansion/contraction	Determine precise repeat size

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Mitochondrial variant	Quantify % heteroplasmy
Suspected chromosomal rearrangement	Visualize/characterize chromosomal configuration
Suspected mosaicism	Quantify precise allele fraction, consider testing other tissue

This assay does not detect complex chromosomal rearrangements (such as balanced translocations or inversions lacking breakpoint coverage), mobile-element insertions, or low-level somatic or germline mosaicism. Structural variant and copy-number boundaries are approximate, and analytical sensitivity for single- or few-exon deletions and duplications has not been established. Mitochondrial variant analysis is qualitative and does not report heteroplasmy percentages; analytical sensitivity for low-level heteroplasmy has not been established, and large mitochondrial deletions and Y-chromosome haplogroup assignments are not evaluated. Tandem repeat analysis is a screening tool and does not provide diagnostic sizing. Quantitative performance has not been established for each screened locus and suspected repeat expansions/contractions require targeted confirmatory testing. SMN1 and SMN2 copy-number results may not identify all silent carriers or establish whether copies or changes are on the same chromosome or opposite chromosomes. Additional testing may be needed to clarify these results.

Specialized segmental-duplication analyses other than SMN1 and SMN2 copy-number analysis are outside the reporting scope. This limitation does not exclude otherwise reportable findings identified by additional bioinformatics analyses in the same genes. UPD and runs-of-homozygosity, when detected, support reporting; however, this variant class has not been individually validated, and therefore confirmation by an appropriate method is recommended.

Specimen related factors including poor quality, low DNA yield, contamination, specimen misidentification, or labeling errors, allogeneic DNA resulting from bone marrow transplantation, recent blood transfusion, or other sources of donor-derived hematopoiesis, may interfere with analysis and lead to false positive, false negative, or otherwise inaccurate results. Somatic genetic alterations, including variants associated with clonal hematopoiesis, may be detected and could be misinterpreted as germline findings. The possibility of false positive or false negative results arising from pre-analytic, analytic, or post-analytic errors cannot be completely excluded.

This test is designed to evaluate the contribution of rare variants in genes with an established relationship to human disease. It is not designed to assess common risk-association alleles or conditions with multigenic or complex inheritance. Interpretation reflects the clinical information supplied and the scientific knowledge available at the time of analysis; variant classifications and gene–disease relationships may change as evidence evolves, and reanalysis may be warranted.