

# TruGenome™ Undiagnosed Disease Test

## Test Description

### Test indication

The TruGenome™ Undiagnosed Disease Test is intended to provide information to physicians to aid in the diagnosis of inherited diseases with high penetrance. The analysis and interpretation are designed to detect and report on single nucleotide variants (SNV), small insertion/deletion events, copy number variants and mitochondrial DNA SNVs that impact genes that have established association to genetic disease. This is typically done as a family-based analysis (e.g. a “trio” of the proband and his or her biological parents), but may be performed on just the proband. Family-based analyses may be comprised of a duo (parent and child), trio, or other higher order family structure. The analysis considers inheritance patterns consistent with the reported family history. In addition, the analysis considers clinical presentation and peer-reviewed literature to contextualize resulting variants from the analyses.

### Reasons for referral

This test is appropriate for situations where there are a large number of candidate genes to evaluate, the evaluation of the genome may clarify or refine the diagnosis because the presenting set of signs, symptoms, imaging and laboratory tests are inconclusive, or the phenotype might indicate multiple genetic conditions.

Examples of conditions for which this test is not appropriate include those that are caused by multiple genes, each with small effect or gene-environment interactions, such as diabetes or immune disorders. To assess if a patient's disorder is likely to have a genetic etiology, the referring physician should consider other lines of evidence such as increased severity, earlier than expected age of onset, multiple affected close family members, and unexpected phenotypic complexity.

Physicians ordering this testing should understand the intended use of and the performance characteristics of this test. Physicians should provide pre-test counseling to their patients and the family members being tested to review the potential benefits, risks, limitations and alternatives to this testing. Physicians ordering this test are responsible for obtaining informed consent from the persons being tested.

### Optional secondary finding analysis

A secondary findings analysis is available for each individual being tested as part of the TruGenome™ Undiagnosed Disease Test. This includes a targeted screen of variants that meet the current test definition in genes recommended for reporting of secondary findings by the American College of Medical Genetics and Genomics (ACMG). The list of genes included in this analysis is below.

*ACTA2, ACTC1, APC, APOB, ATP7B, BMPR1A, BRCA1, BRCA2, CACNA1S, COL3A1, DSC2, DSG2, DSP, FBN1, GLA, KCNH2, KCNQ1, LDLR, LMNA, MEN1, MLH1, MSH2, MSH6, MUTYH, MYBPC3, MYH11, MYH7, MYL2, MYL3, NF2, OTC, PCSK9, PKP2, PMS2, PRKAG2, PTEN, RB1, RET, RYR1, RYR2, SCN5A, SDHAF2, SDHB, SDHC, SDHD, SMAD3, SMAD4, STK11, TGFB1, TGFB2, TMEM43, TNNI3, TNNT2, TP53, TPM1, TSC1, TSC2, VHL, WT1*

Each family member tested through the TruGenome™ Undiagnosed Disease Test has the option to opt in or opt out of this analysis. In the instance where a family member opts out of the secondary findings analysis, please note the following:

- Opting out of the secondary findings analysis means that a targeted search for variants in the list of genes recommended by the ACMG will not be performed.
- Incidental findings (variants classified as pathogenic or likely pathogenic in genes that are unrelated to the patient's primary indication for testing and deemed reportable by the clinical laboratory director) will still be returned, if identified.
- If an individual opts out of the analysis, incidental findings related to the ACMG guidelines may still be reported if the finding lies within a large reportable copy number variant (CNV) that contains multiple genes including those on the ACMG list.
- In the case of a family-based analysis (e.g. the TruGenome™ Undiagnosed Disease Trio Test), identification of secondary findings in family members who opt in for the analysis may inform carrier status of other members of the family, even those who choose to opt out of the analysis.

## Deliverables

- A Clinical Report of genomic findings deemed clinically significant based on the patient's reported phenotype, including variant interpretation according to the ACMG guidelines. Literature references used to support the classifications will be provided.
- A Secondary Findings Report including variants classified as likely pathogenic or pathogenic within the 59 genes recommended by the ACMG for secondary findings.
- A Pharmacogenomics Report including 11 medically actionable genes associated with response to 16 different drugs (as specified by the FDA or the Clinical Pharmacogenomics Implementation Consortia (CPIC)).
- Clinical Appendices:
  - A Gene List Appendix including a list of genes generated by searching the Online Mendelian Inheritance In Man (OMIM) database for genes that have been associated with the phenotype. In the case of a proband-only analysis, this gene list is utilized to perform a targeted search for variants in these genes. In the case of a family-based analysis, this list is used to prioritize resulting variants from the family-based analysis and to guide secondary analyses of only the patient's genome.
  - An Exon Callability Appendix including a list of all RefSeq exons, miRNAs and snoRNAs for which callability is not 100%. The appendix also provides the fraction of bases called for these regions.
- A gVCF file that contains all SNVs and indels identified in the genome.

For family-based testing, technical data files, Secondary Findings Reports and Pharmacogenomics Reports are made available for each family member tested.

Technical data in BAM file format (sequence information provided in a standard open source binary format (Li et al., 2009, <http://samtools.sourceforge.net/>) is available for return with a fee to clinical researchers operating under an approved IRB protocol, through a CLIA-certified laboratory for clinical use, or by a physician or patient who signs a release.

## **Criteria for variant classification of single nucleotide variants (SNVs), small deletions and small insertions**

We follow the ACMG guidelines for variant classification and reporting (Richards et al., 2015). The guidelines take into account the variant consequence, location and inheritance, presence or absence of functional data supportive of a damaging effect on the gene or gene product, prevalence of the variant in cases and controls, segregation data, computational evidence and patient phenotype and family history to classify variants into one of four categories: pathogenic, likely pathogenic, likely benign or benign. Variants that do not meet the criteria for one of these four categories, or for which the criteria for benign and pathogenic classifications are contradictory, are classified as being of uncertain significance.

## **Criteria for classification for copy number variants (CNVs)**

We follow the ACMG guidelines for interpretation and reporting of postnatal copy number variants (Kearney et al., 2011; South et al., 2013).

- Pathogenic: Documented as clinically significant in multiple publications, even if penetrance and expressivity are variable. Includes large CNVs which may not be described in the literature as the same size, but overlap with an interval with established clinical significance.
- Uncertain clinical significance-likely pathogenic: CNV described in a single case report but with well-defined breakpoints and phenotype that overlaps with the patient, and/or a gene within the CNV has a very compelling function relevant to phenotype.
- Uncertain clinical significance: CNV contains genes but unknown if genes are dose sensitive, and/or CNV is described in multiple contradictory publications or databases.
- Uncertain clinical significance-likely benign: CNV has no genes in the interval but is identified because of size, and/or CNV is described in small number of cases in databases for the general population but does not represent a common polymorphism.
- Benign: CNV has been reported in publications or curated databases as a benign variant. CNV is documented to represent a common polymorphism.

## **Methods and performance characteristics of test**

Human whole genome sequencing is performed on DNA extracted from whole blood using sequencing-by-synthesis (SBS) next generation sequencing (NGS). The data are aligned and reported according to build 37.1 of the Human Reference Genome (<https://www.ncbi.nlm.nih.gov/grc/human/data?asm=GRCh37>). We sequence to an average of  $\geq 30$  fold coverage. Over 99% of the genome is covered at 10 fold coverage or more and 97% of the genome is callable (passes all quality filters). Based on the quality filters and through the analysis of an extended, multi-generation family set (Platinum Genomes) (Eberle et al., 2017), for SNVs, sensitivity is 98.9% and the analytical Positive Predictive Value (PPV), i.e.  $TP/[TP+FP]$  is 99.9%. Small insertion and deletion events are detected and reported for this test. Insertions up to 31 bases and deletions up to 27 bases have a sensitivity and analytical PPV of approximately 80-85%, determined through Platinum Genomes. This test has the capability to detect copy number events greater than 10 kb, however sensitivity was only assessed for events greater than 20 kb and was found to be approximately 85%. Boundaries of the CNVs reported cannot be assessed with complete accuracy, and the boundaries are estimated to lie within +/- 1 kb of the event, unless otherwise noted. For SNVs and small insertion and deletion events, we limit interpretation to variant positions that overlap an exon and its 15 base pair flanking sequence. For CNVs, we limit interpretation to events that either overlap an exon or have a boundary that lies within 1 kb upstream or downstream of an exon. Mitochondrial SNVs detected at an allele fraction greater than or equal to 3% will be interpreted for pathogenicity. However, percentage of heteroplasmy is not reported. Mitochondrial CNVs and small insertions and deletions are not reported.

Some regions of the human genome not covered by this test, including stretches of the human reference genome that have not been completely resolved, or regions where it is difficult to align fragments accurately. Additionally, genes that are associated with regions of high homology are difficult for this test to resolve. These include, but are not limited to, some immunoglobulin (HLA) genes, SMN associated with Spinal Muscular Atrophy, and telomeres. Please contact the laboratory regarding ability to make calls in regions of specific interest.

## Limitations

It is not technically possible to sequence the entire human genome at present. Only known bases of the human reference genome will be assessed. Single nucleotide substitutions, small insertion and deletion events, and copy number variants greater than 10 kb are reported for this test. Other types of genetic variants that may also lead to genetic disease are not detected or reported for this test (e.g. trinucleotide repeat variants). If clinically indicated, additional testing and analyses may be appropriate. Clinical sensitivity is unknown and may be dependent on the patient's phenotype.

## Lab Statement

TruGenome Undiagnosed Disease Test is a Laboratory Developed Test. It is developed and its performance characteristics determined by the Illumina Clinical Services Laboratory (CLIA #05D1092911). It has not been cleared or approved by the U.S. Food and Drug Administration. Pursuant to the requirements of CLIA '88, this laboratory test has established and verified the test's accuracy and precision. The laboratory is regulated under CLIA as qualified to perform high-complexity testing. This test is used for clinical purposes. It should not be regarded as investigational or for research. We cannot accept orders from the state of New York at this time.

## References

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