## Test Information

The benefits and risks of the Genomic Unity® test are described below. It is recommended that you receive genetic counseling from a licensed healthcare provider who can answer your questions about genetic testing and provide information about alternatives. Information about genetic counselors in your area is available at https://www.nsgc.org/.

## Background

The purpose of genetic testing is to identify changes in the DNA sequence that cause an affected individual's condition. This test uses a PCR-free protocol that produces comprehensive and consistent coverage of all exons and non-coding regions in an individual's genome. When applicable to familial samples, whole genome protocols are used for comparison to the proband. The resulting data is subjected to in-silico analyses optimized for small sequence changes (single nucleotide variants and deletion/insertions), structural variants in chromosomes (deletions, duplications, copy number variants, mobile element insertions, aneuploidy, runs of homozygosity and uniparental iso- and hetero-disomy, SMN1/SMN2 copy number), short tandem repeats (STRs) and mitochondrial variants (single nucleotide variants and small deletion/insertions and large deletions). The Genomic Unity® Whole Genome Analysis test considers mitochondrial variants from the mitochondrial genome as well as most variant types overlapping the exome traditionally reported using other methodologies, excluding technically challenging variants listed in the limitations of testing. The Genomic Unity® Exome Analysis test considers most variant types overlapping the exome traditionally reported using other methodologies, excluding technically challenging variants listed in the limitations of testing. The Genomic Unity® Constitutional Genome-Wide Copy Number Variant Analysis test considers structural variants only. The Genomic Unity® Mitochondrial Genome Analysis test considers mitochondrial variants from the mitochondrial genome only, and therefore does not include nuclear encoded genes. All other tests consider variants in or overlapping a subset of genes which are described in brief in the Targeted Analyses section of the test requisition form and in more detail on the individual test information web page indicated. When a Genomic Unity® Custom Analysis is specified, only variants in or overlapping the listed gene(s) specified are considered and only for small sequence changes, deletion/duplications and short tandem repeats as applicable to the gene. All tests are focused on rare variants. When noted for the specified analysis, this test uniquely assesses the tandem repeats in the following genes: *AFF2, AR, ARX, ATN1, ATXN1, ATXN2, ATXN3, ATXN7, ATXN8OS, ATXN10, C9ORF72, CACNA1A, CNBP, CSTB, DIP2B, DMPK, FGF14, FMR1, FOXL2, FXN, GIPC1, GLS, HTT, JPH3, LRP12, NOP56, NOTCH2NLC, PABPN1, PHOX2B, PPP2R2B, RFC1, SOX3, TBP, TCF4, VWA1, ZFHX3* and *ZIC2*. For Genomic Unity® 2.0 only: the following short tandem repeats are included: *BEAN1, HOXA13, PRDM12, PRNP, RILPL1, RUNX2,* and *SAMD12* with methylation patterns associated with Angelman, Prader-Willi and Fragile X syndromes.

Additional information about the Genomic Unity® test is available from your healthcare provider and on the Variantyx website at https://www.variantyx.com/. Adult-onset disorders not related to the indication for testing, and therefore representing predictive testing, are not reported with this test. Requests for predictive, carrier and other non-diagnostic genetic testing are available by ordering the Genomic Inform® test.

## Technical Limitations

Genetic testing is accurate, but may not always identify a genetic variant even though one exists. This test attempts to evaluate the entire DNA sequence (within the scope described for the test), but may not be able to detect all DNA changes due to limitations in current technology. Certain regions of the DNA may not be well covered. Certain variant types may not be detectable such as methylation abnormalities, other than those listed in Genomic Unity®2.0, variants in genes with highly homologous pseudogenes and variants in regions that are difficult to assay based on current technology. Unusual circumstances including bone marrow transplantation, blood transfusion, and variants that exist in only a small fraction of cells (mosaicism) may interfere with variant identification. The false negative rate for mitochondrial large deletions have not been determined. The false negative rate for repeat expansions has not been determined. ARX repeat expansions will be reported only in cases where the clinical symptoms of the patient include early onset seizures. For dominant repeat expansion disorders parental inheritance will not be reported on the initial report. Any additional test specific limitations are noted on the individual test information web page indicated. Additionally, interpretation of the results is limited by the current medical understanding of disease and available scientific information. This test requires high-quality DNA. In some cases, an additional sample may be needed if the volume, quality and/or condition of the initial sample is not sufficient. Samples submitted as genomic DNA will only be processed if the extraction was performed in a CLIA/CAP accredited laboratory. This test does not consider somatic variants. While the targeted average coverage is 15X for long read sequencing, a minimum coverage of 8X is required to determine methylation status. The regions interrogated for methylation status include chr15:23647361-23647992 (*MAGEL2* gene) and chr15:24954788-24955196 (*SNURF* gene), which does not include the *UBE3A* region, and chrX:147911779-147912310 (FMR1 gene). Mosaicism and biological variability of methylation patterns can affect clinical interpretation, therefore false positive and false negative results might be reported. For example, methylation of expansions in *FMR1* in females may be detected with a reduced sensitivity as a result of skewed X-inactivation.

## Possible Test Results

**Positive result** - A positive result indicates that one or more genetic variants were identified that either explain or partially explain the cause of the disorder or indicate an increased risk of developing the disorder in the future. Individuals with positive results may wish to consider further independent testing and/or consultation with their physician or genetic counselor.

**Negative result** - A negative result indicates that no genetic variant explaining the disorder was identified by this test. This reduces the likelihood of, but does not exclude the possibility of, the disorder being genetic in nature.

**Uncertain result** - A variant of uncertain significance was identified by this test. This means that a genetic variant was identified, but based on available information in the medical literature and research and scientific databases it is not certain whether the variant may cause the disorder. The variant could be a normal genetic difference that does not cause the disorder. Without further information, the effects of the variant cannot be known and an "uncertain/clinically inconclusive" result may be reported. The uncertainty may be resolved over time if additional information becomes available. Periodic reanalysis of the sequence data or further analysis, including testing of additional family members, may be recommended.

**Indeterminate result** - An indeterminate result indicates that there were relevant genetic variant(s) identified in the analysis, but that it is uncertain whether they are true variants or artifacts. Furthermore, it is considered that a repeat test will not resolve the technical uncertainty and orthogonal confirmation is necessary to resolve the result.

**Inconclusive result** - A technically inconclusive result indicates that there was an issue with the patient sample that resulted in data that the lab cannot

interpret. It is considered that a repeat test will likely resolve the technical uncertainty and therefore a repeat sample is recommended to complete the analysis.

## Reporting Standards

All reportable variants in the clinical report will be categorized as pathogenic, likely pathogenic or a variant of uncertain significance (VUS) utilizing the American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP) guidelines as published by Richards et al. 2015 (for more information see: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4544753/. Variants may have a strong phenotypic correlation with the reported patient phenotype(s) and be considered a strong causal candidate for the disorder or may have some phenotypic overlap with the reason for testing but not be considered the sole genetic cause for the phenotype(s) in the patient. Both types of variants may be reported. Even if this test finds DNA changes that are responsible for the reported symptoms, the testing may not completely predict the severity of the disorder, possible future problems, or response to treatment. Variants of uncertain clinical significance will only be reported if found to be associated with patient phenotype. Variants of uncertain clinical significance will not be reported in targeted analysis (phenotypic based analyses) unless sufficient clinical information was provided.

Variantyx reviews clinical notes provided with the test submission and may report results from other labs for: (a) detection of the variant on our platform, (b) variant classification, and (c) inheritance, if applicable. This is possible if there is detailed information in the notes provided with the test requisition. Information required includes (but is not limited to): reference genome, chromosome location/gene name, variant change (c./p. or breakpoints), and transcript. It is recommended to include previous test results containing the required information.

Variants in many disease-causing genes are evaluated in comprehensive testing, including variants involved with adult onset neurodegenerative disorders. These conditions affect the nervous system earlier or later in adulthood and each condition may present differently. Symptoms may be progressive and can shorten one's lifespan. Currently, there are no cures and there may be limited treatment or prevention options. Some examples of these conditions include Huntington disease, Huntington-like disease, amyotrophic lateral sclerosis (ALS), and familial prion disease. Variants in genes that cause these conditions are reported if there is specific phenotypic overlap between the clinical symptoms provided with the test request and the gene.

## Reporting of Unrelated Findings

Unrelated findings are findings obtained from genomic sequencing, usually whole genome or exome sequencing, and can be related to conditions that were not the primary reason for testing or findings that can allow one to deduce information as a result of testing that is not directly related to the test.

**ACMG Secondary Findings**
The American College of Medical Genetics and Genomics (ACMG) recommends reporting pathogenic and likely pathogenic variants in a list of genes in both a gene-specific and variant-specific manner. Variantyx evaluates the secondary findings list of genes, the version of which will be listed in the report and can be found on the Variantyx website, www.variantyx.com/acmg-secondary-findings. These variants are not typically reviewed during routine processing of patient samples, but are actively sought and reported to the patient. The ACMG recommends reviewing variants in the genes in their recommended list because the genes are related to conditions that are considered 'actionable', meaning that there are steps that can be taken to mitigate the onset or severity of the clinical outcome. It is important to understand that it is possible to have a pathogenic variant but to have it not detected by the assay. In addition, variants of uncertain significance are not reported in these genes. If a variant is of uncertain significance, and later is considered pathogenic, it cannot be determined without a reanalysis of the data.

**Actionable Findings**
Other actionable findings are likely pathogenic/pathogenic variants detected unexpectedly during routine processing of patient samples. These variants are in genes apparently unrelated to the patient's reported phenotype, but with some degree of clinical actionability. These genes are not restricted to a specific list (such as the ACMG Secondary Findings list), but are similar in that they could impact medical management and decision making. Other actionable findings are not actively sought and therefore all actionable variants may not be identified during processing. Variants of uncertain clinical significance are not reported in these genes. Some examples of these findings are pathogenic/likely pathogenic variants in high penetrance oncogenic related genes, polycystic kidney related genes with increased surveillance recommendations and/or genes associated with conditions for which possible treatment is available. Variants for late-onset conditions unrelated to the patient's phenotype, such as adult onset neurodegenerative diseases, will typically not be reported. The option for receiving other actionable findings should be discussed with the patient and family prior to testing.

**Unavoidable Incidental Findings (typically reported if present)**
Some incidental findings are unavoidable and can be deduced from testing, such as discovering non-paternity when testing the parents of a child in trio analysis or discovering that a parent is a carrier for the condition identified in the child. Other incidental findings are variants in genes that may fit the patient's clinical phenotype but are also related to clinical symptoms unrelated or with a later onset. For example, more than 450 different pathogenic variants have been identified in the *LMNA* gene, which can cause a wide variety of distinct and disparate diseases involving striated muscle (dilated cardiomyopathy, skeletal myopathies), adipose tissue (lipodystrophy syndromes), peripheral nerve (Charcot-Marie-Tooth neuropathy) or multiple systems with accelerated aging (progerias). These results would likely be reported because they are integral to testing. The possibility of receiving unavoidable incidental findings should be discussed with the patient and family prior to testing, so they are aware that these results, if present, are likely to be returned to them. If the patient does not wish to receive these results, they can decide not to continue with testing.

With this test related findings are reported, such as genetic findings useful for the current diagnosis of the disease that initially led to the analysis and any clinically relevant genetic findings, which may have immediate benefits for the patient related to present diseases or clinical conditions. However, some unrelated findings may be reported as an option to receive with the report, while others such as pharmacogenomic, high frequency risk alleles, and late onset neurodegenerative disorders (such as ALS or Huntington Disease), are outside the scope of testing and would not be typically reported. These different findings and options to receive results are described below.

ACMG Secondary Findings and Incidental findings are available for Genomic Unity® Whole Genome Analysis, Genomic Unity® Exome Plus Analysis and Genomic Unity® Exome Analysis and are not available to relatives, with the exception of the reported parental inheritance of the variants identified in the patient. No specific parental results are issued as a separate report under the family member's name. If the patient chooses to receive secondary findings, those findings will be included in a separate section of the clinical report.

## Testing of Family Samples

In the case of trio and/or larger cohort analysis, and for parental confirmation of singleton analysis, sequencing and analysis of family samples may be used to improve the interpretation of genetic variants identified in the patient's DNA. Accurate interpretation of test results requires accurate assignment of family relationships. Analysis of the sequenced DNA is performed with the assumption that correct family relationships have been provided. Parental samples that fail concordance with the patient (i.e. one parent does not share the expected number of variants with the child) will not be analyzed. Family samples are analyzed only with regard to the patient's condition. Parental inheritance is reported on variants if identifiable, this may include the inheritance of variants related to incidental or secondary findings. However for patients with repeat expansions, parental inheritance may not be reported. Additional counseling for the parents may be recommended prior to reporting parental inheritance of the repeat expansion.

## Genomic Unity® Pharmacogenomics Analysis

### Background

The Genomic Unity® Pharmacogenomics Analysis is a whole genome based test designed to identify common variants associated with drug metabolism and pharmacogenetic response. The test includes sequence analysis of known star alleles in 13 genes and copy number variant analysis of selected genes that were recommended by the FDA for predicted adverse drug reactions and drug response.

### Methods

Whole genome sequencing is performed on DNA isolated from blood samples using next generation sequencing methods. Analyses are performed to detect, analyze and report clinically relevant variants using the Variantyx Genomic Intelligence® platform.

### Report Standards

Test results will be issued as a separate clinical report for the patient to identify variants in genes consistent with current FDA guidance. Sequence variation is compared to reference data using genome build GRCh38. Genomic Unity® Pharmacogenomics analysis list of star alleles can be found at https://www.variantyx.com/pharmacogenomics.

### Limitations

The detection or absence of results does not replace the need for therapeutic monitoring by healthcare providers. The report is based on the genotype to phenotype mappings and FDA usage guidelines and includes a set of specific genes, star alleles, and select copy number variants as described in the gene list. This test will not detect all known variants that result in altered gene activity and drug metabolism. The patient's unique genotype is only one factor used in the evaluation in drug metabolism, concentration and response. In addition, this report is limited to certain pharmacogenetic associations only and does not include all of the information necessary for safe and effective use of a drug. For example drug–drug interactions may alter the metabolizer phenotype. This test was designed to provide gene-drug associations and was not designed to diagnose health conditions. The information provided in this report does not contain medication recommendations, and any dosage adjustments or other changes to medications should be evaluated by the ordering healthcare provider with consideration of current prescriptions, family and patient's history, presenting symptoms, and other factors.

Pharmacogenomics results and recommendations are based on current guidance and are not reviewed when guidelines are updated. Patients are not notified if changes impact their results. Research data evolves and amendments to the prescribing information of the drugs listed might change over time as more information becomes available.

### Patient Confidentiality

To maintain confidentiality, test results will only be released to the ordering healthcare provider or ordering laboratory, and upon your request, to additional healthcare provider(s) indicated on this test requisition form. Test results will only be disclosed to others by your written consent and/or if demanded by a court of competent jurisdiction. It is your responsibility to consider the possible impact of test results on insurance rates, the ability to obtain disability, life or long-term care insurance and employment. The Genetic Information Non-discrimination Act (GINA), enacted by the US Federal Government, provides some protection against discrimination by health insurance companies and employers based on genetic test results, but does not cover life, disability or long-term care insurance. Information about GINA is available at https://www.genome.gov/10002328.

Anonymized information obtained from the test may be included in variant and allele frequency databases used to help healthcare providers and scientists understand human disease, as well as in scientific publications. Names and personal identifying information will not be revealed. Separate from the above, if there are opportunities to participate in research relevant to your condition, and you have consented for recontact, Variantyx may contact you or your healthcare provider for research purposes.

### Turnaround Time

The turnaround time (TAT) of this test can be found on the Variantyx website, which begins at the time of sample receipt. For family testing, the timing starts when the last sample is received. Please note that the following scenarios will likely result in extension of the turnaround time (1) when the DNA sample fails QC and is determined to be insufficient for testing, requiring collection of a new sample; (2) when the test is sent for orthogonal confirmation at an external laboratory. In the second scenario, the turnaround time can be expected to be extended by the turn around time of the external laboratory plus 1 week.

### Sample Retention

DNA extracted from submitted samples may be stored for at least 3 months following completion of testing and may be discarded thereafter. Extracted DNA is not returned unless requested prior to testing (additional fees apply). After completion of testing, anonymized DNA may be used for test development and improvement, internal validation, quality assurance and training purposes before being discarded.

NY state residents: No other test shall be performed on this sample except the test ordered by the clinician, unless waived by the patient or authorized individual. In addition, the patient's biological sample will be destroyed within 60 days or upon the completion of testing, unless waived by the patient