# Genomic Unity® Testing

## Detecting tandem repeat expansions using WGS

Genomic Unity® testing detects and reports tandem repeat expansions in selected, characterized regions with known pathogenic associations.

### What role do tandem repeat expansions play in genetic disease?

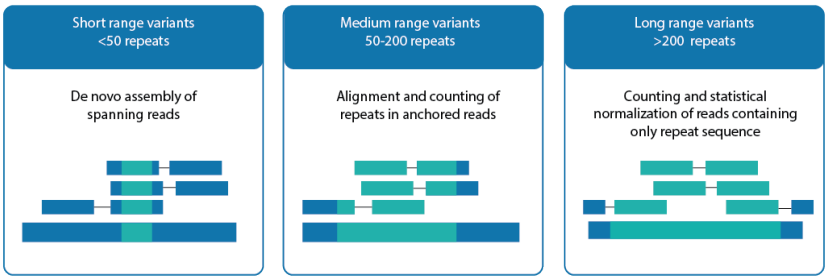
There are dozens of tandem repeat expansions that are known to be pathogenic. Some, like the CTG repeat expansion in the DMPK gene, lead to predominantly adult-onset neurological disorders like myotonic dystrophy type I. Others, like the CGG repeat expansion within the 5' UTR of the *FMR1* gene which causes Fragile X syndrome, affect the individual early in life and are routinely tested for as part of the rare disease diagnostic odyssey.

At each pathogenic locus, alleles may be classified as normal, intermediate, premutation or full mutation based on the number of repeats, with the ranges varying for each locus. For example, in the case of *HTT* which causes Huntington disease, alleles <27 repeats in length are considered normal. Alleles between 27 and 35 repeats in length are considered premutation and are at a high risk of having the number of repeats further expand into the full mutation range of >39 repeats when transmitted from a parent to child. Until recently, detecting repeat expansions has required the use of PCR or southern blot analysis, usually employed to interrogate a single targeted gene. Panel and exome-based tests will miss these genetic changes.

### How does Variantyx detect tandem repeats?

Variantyx uses whole genome sequencing (WGS) technology to provide consistent, comprehensive coverage of the entire genome. To analyze tandem repeats in known pathogenic loci, three separate paired-end read strategies are used, all within a single assay.

The first strategy focuses on short range variants that are less than 50 repeats in length. Here de novo assembly of spanning reads is used as the full length of the repeat is contained within either the R1 or R2 read, with uniquely mappable flanking sequences.




The second strategy focuses on medium range variants that span from 50 to 200 repeats in length, with the upper limit determined by the sequencing insert size of 550 bp. Here alignment and counting of repeats in anchored reads is used. With anchored reads, one member of the pair, either the R1 or R2 read, contains only repeat sequence while the other member contains partial repeat sequence and partial uniquely mappable sequence.

The final strategy focuses on long range variants that are >200 repeats in length. Here counting and statistical normalization of reads containing only repeat sequence is used to estimate the repeat length. Combing the three different methods, repeat length is calculated with good specificity up 200 repeats. Alleles with repeat lengths exceeding this threshold represent high-confidence estimates that should be independently confirmed by an orthogonal technology.

## What tandem repeats are analyzed?

The following table shows analysis results from a 50 year old male patient with suspected myotonic dystrophy type II. Testing confirmed the myotonic dystrophy type II diagnosis.

| Gene<br>Repeat: Allele count | Disorder<br>Allele interpretation | Gene<br>Repeat: Allele count | Disorder<br>Allele interpretation |
|---|---|---|---|
| *AFF2*<br>CCG: 12,- | Fragile XE syndrome<br>Normal | *CNBP*<br>CCTG: 110, 127 | Myotonic dystrophy type II<br>Full mutation |
| *AR*<br>CAG: 23, - | Spinal and bulbar muscular atrophy<br>Normal | *CSTB*<br>CCCCGCCCCGCG: 3, 2 | Myoclonus epilepsy<br>Normal |
| *ATN1*<br>CAG: 19, 12 | Dentatorubral-pallidoluysian atrophy<br>Normal | *DIP2B*<br>CGG: 12, 12 | FRA12A fragile site<br>Normal |
| *ATXN1*<br>CAG: 31, 32 | Spinocerebellar ataxia<br>Normal | *DMPK*<br>CTG: 5, 11 | Myotonic dystrophy type I<br>Normal |
| *ATXN10*<br>ATTCT: 12, 15 | Spinocerebellar ataxia<br>Normal | *FMR1*<br>CGG: 30,- | Fragile X syndrome<br>Normal |
| *ATXN2*<br>CAG: 22, 23 | Spinocerebellar ataxia<br>Normal | *FXN*<br>GAA: 9, 19 | Friedreich's ataxia<br>Normal |
| *ATXN3*<br>CAG: 20, 24 | Spinocerebellar ataxia<br>Normal | *HTT*<br>CAG: 17, 20 | Huntington disease<br>Normal |
| *ATXN7*<br>CAG: 10, 10 | Spinocerebellar ataxia<br>Normal | *JPH3*<br>CTG: 14, 16 | Huntington disease-like 2 syndrome<br>Normal |
| *ATXN80S*<br>CTG: 15, 16 | Spinocerebellar ataxia<br>Normal | *PABPN1*<br>GCN: 10, 10 | Oculopharyngeal muscular dystrophy |
| *C9ORF72*<br>GGGGC: 2, 5 | FTDALS1<br>Normal | *PPP2R2B*<br>CAG: 10, 17 | Spinocerebellar ataxia<br>Normal |
| *CACNA1A*<br>CAG: 11, 12 | Spinocerebellar ataxia<br>Normal | | |

Sensitivity and reporting policies differ by loci. The false negative rate for repeat expansions has not been determined for the following genes: *AFF2, ATXN10, CNBP, CSTB, DIP2B, NOTCH2NLC, PHOX2B, TBP.*

## What are the benefits of Genomic Unity® tandem repeat analysis?

With Genomic Unity® testing there is no need for a separate sample and separate assay.

Because WGS provides comprehensive coverage of the entire genome, all sequence data necessary for detection of tandem repeat expansions is present. Variantyx's custom-built, validated computer algorithms analyze the data, identifying repeat expansions for more than 25 different loci.