

NO-AMP TARGETED SEQUENCING ACCESS PREVIOUSLY INACCESSIBLE GENOMIC REGIONS

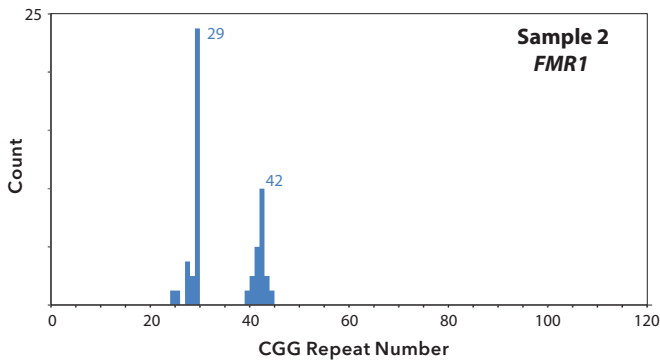
THE LEADER IN LONG-READ SEQUENCING



Due to technology limitations, repeat-expansion disorders have gone without the needed base-level resolution of the disease causative long repetitive elements^{1,2,3}. Enrichment of these hard-to-amplify genomic regions is now possible with our **no-amplification (No-Amp) targeted sequencing method utilizing the CRISPR/Cas9 system**. By combining CRISPR/Cas9 enrichment with **Single Molecule, Real-Time (SMRT®) Sequencing on the Sequel® System**, scientists are now able to:

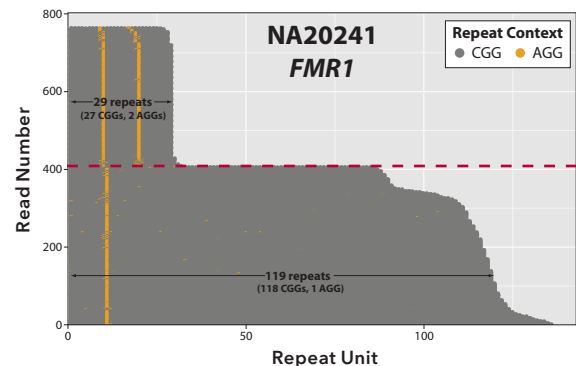
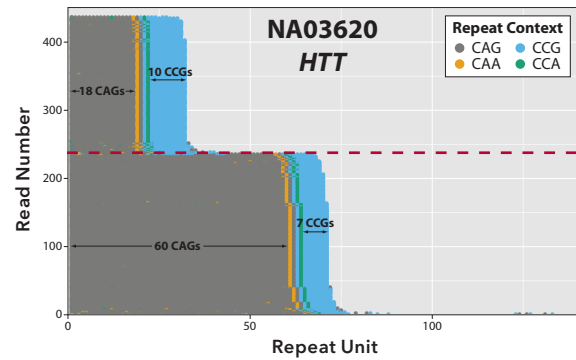
- Eliminate PCR bias and errors
- Sequence through entire repeat expansions with base-level resolution
- Quantify repeat numbers in normal- and mutant-expanded alleles
- Identify interruption sequences
- Characterize somatic mosaicism

ALLELE-SPECIFIC REPEAT COUNT IN FRAGILE-X PREMUTATION DISEASE SAMPLE



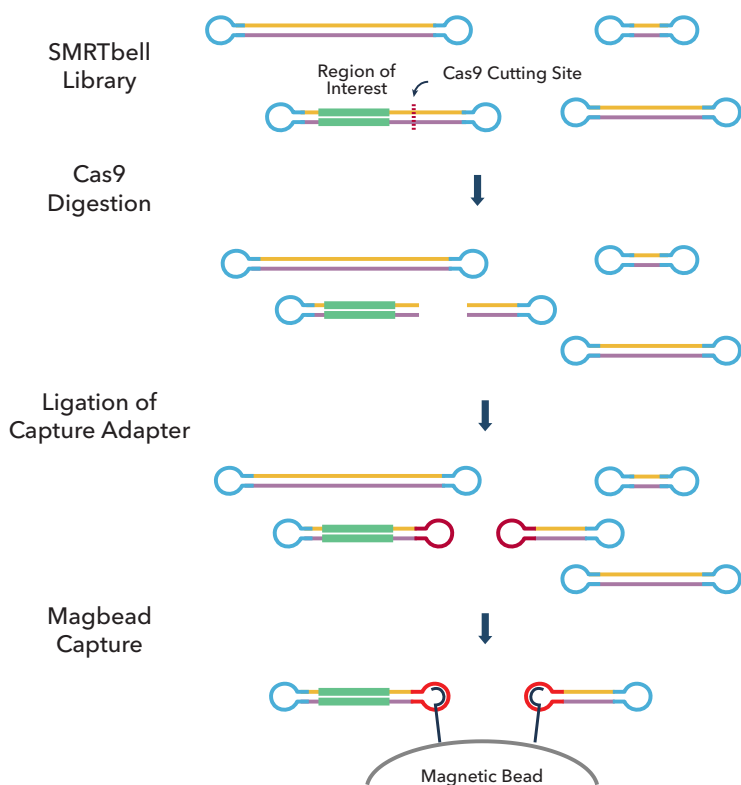
This graph outlines the number of CGG repeats that are present for each allele; normal healthy allele on the left and expanded mutated allele on the right for the fragile X mental retardation 1 (FMR1) gene^{4,5}. A widening of the repeat number distribution can be seen for the mutated allele which is a feature present in many repeat expansion disorders.

VISUALIZATION OF INTERNAL REPEAT STRUCTURE



Allele-specific repeat structure of the disease-causing expansions in the Huntingtin (HTT) and Fragile-X Mental Retardation 1 (FMR1) genes can be rendered through the repeat expansion sizes and the repeat context sequence^{4,6}.

FROM DNA TO BASE-LEVEL CHARACTERIZATION OF PREVIOUSLY INACCESSIBLE GENOMIC REGIONS



Sample Preparation

- Prepare a standard SMRTbell® library
- Enrich for target
 - Digest SMRTbell template using Cas9 and a target-specific guide RNA
 - Ligate capture adapter
 - Pull down templates containing region of interest using magbeads

SMRT Sequencing on the Sequel System

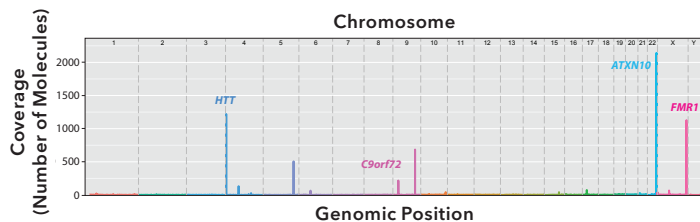
- Achieve exceptionally long sequencing reads - half the reads >20 kb
- Obtain consensus accuracies >99.999% by avoiding mapping and systematic errors
- Ability to capture and sequence hundreds to thousands of individual molecules per locus

Data Analysis

- Generate consensus sequence of each on-target molecule
- *De novo* construct the average repeat content for each allele
- Count repeat distributions, including interruption sequences
- Visualize repeats, including type, number, and location for each allele



Sequel System



KEY REFERENCES

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4. Tsai Y.C. et al. (2017) Amplification-free, CRISPR-Cas9 Targeted Enrichment and SMRT Sequencing of Repeat-Expansion Disease Causative Genomic Regions bioRxiv doi: <http://dx.doi.org/10.1101/203919>
5. Ekholm J. et al. (Oct, 2016) Enrichment of unamplified DNA and long-read SMRT Sequencing to unlock repeat expansion disorders. *66th Annual Meeting of the American Society of Human Genetics*. Vancouver, BC.
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