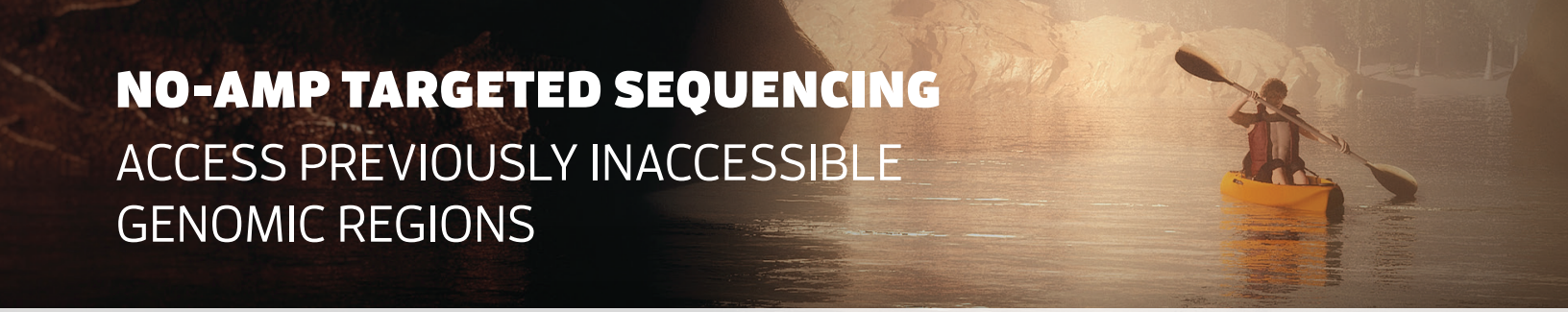


NO-AMP TARGETED SEQUENCING

ACCESS PREVIOUSLY INACCESSIBLE GENOMIC REGIONS

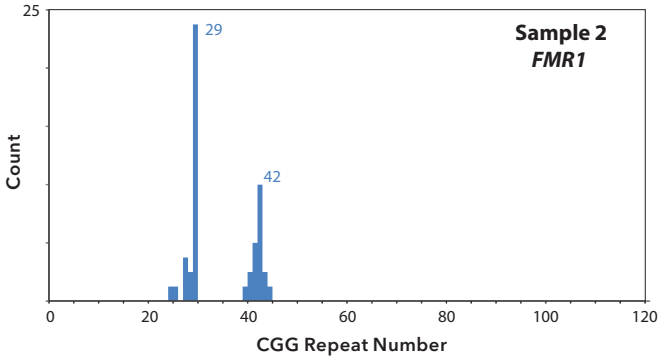
THE LEADER IN LONG-READ SEQUENCING

PACBIO®

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:

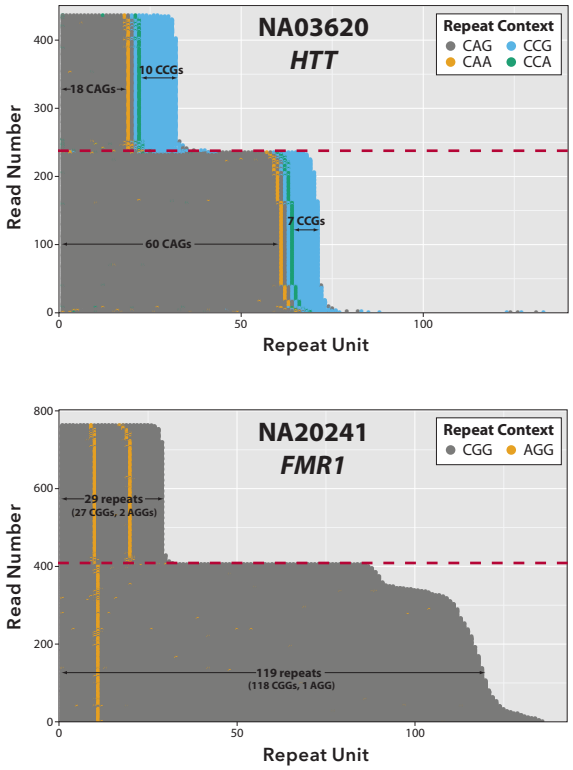
- Eliminate PCR bias and errors
- Sequence through entire repeat expansions with base-level resolution
- Quantify repeat numbers in normal- and mutant-expanded alleles
- Identify interruption sequences
- Characterize somatic mosaicism

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



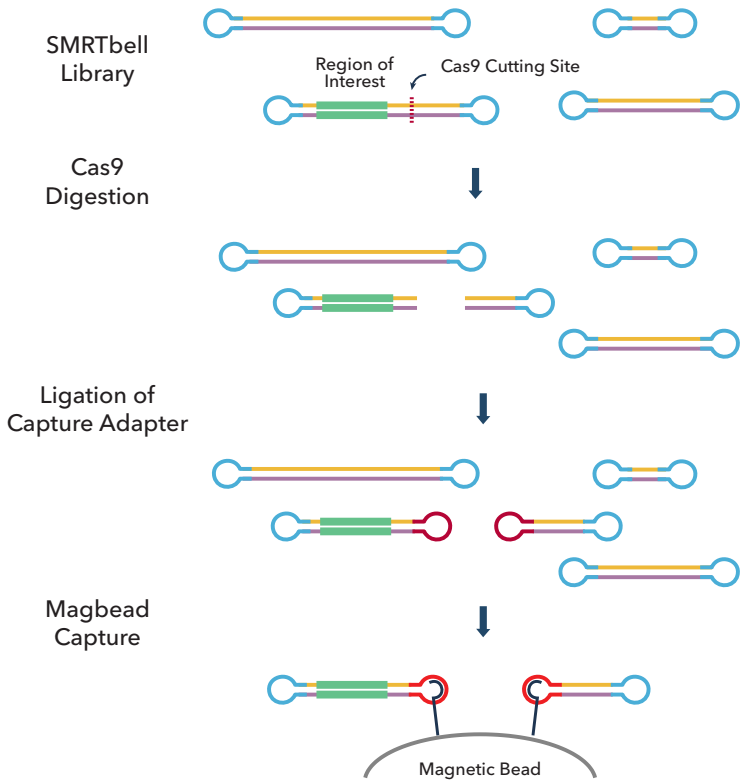
This graph outlines the number of CGG repeats that are present for each allele; normal healthy allele on the left and expanded mutated allele on the right for the fragile X mental retardation 1 (FMR1) gene[4,5]. A widening of the repeat number distribution can be seen for the mutated allele which is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

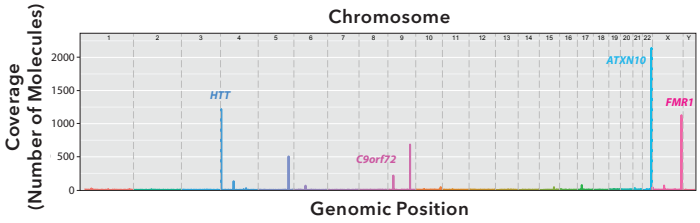
SMRT Sequencing on the Sequel System

- 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

Sequel System

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



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PN: AP107-020718