AMPLIFICATION-FREE TARGETED SEQUENCING

ACCESS PREVIOUSLY INACCESSIBLE GENOMIC REGIONS

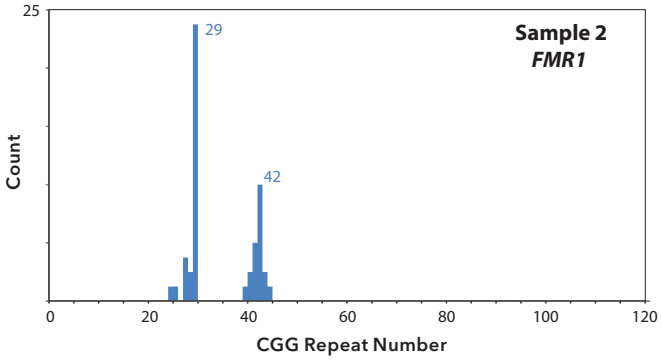
THE LEADER IN LONG-READ SEQUENCING

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 **amplification-free 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:

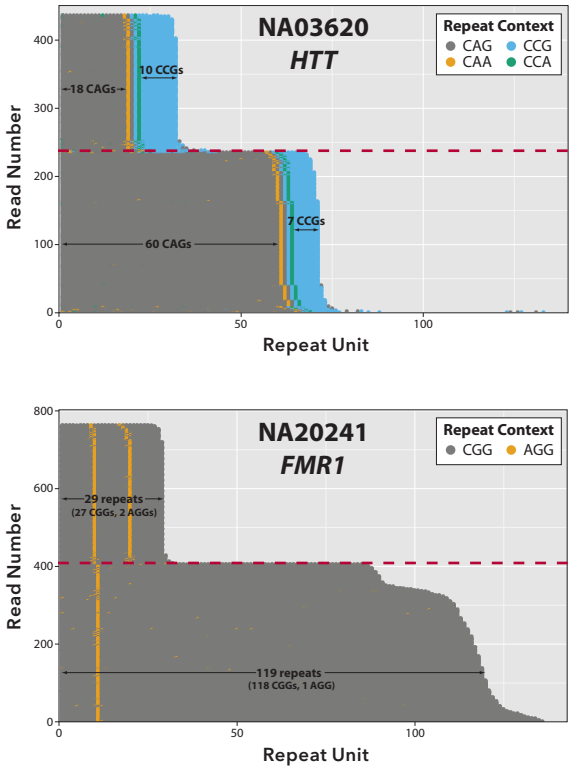
- Eliminate PCR bias and errors
- Sequence through entire repeat expansions with base-level resolution
- Quantify repeat numbers in normal- and mutant-expanded alleles
- Identify interruption sequences
- Characterize somatic mosaicism

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



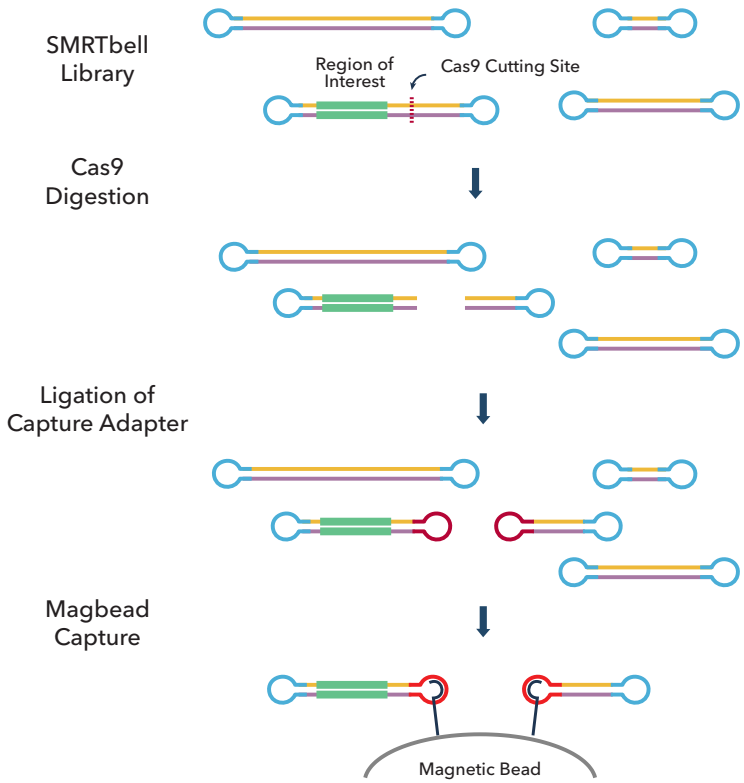
This graph outlines the number of CGG repeats that are present for each allele; normal healthy allele on the left and expanded mutated allele on the right for the fragile X mental retardation 1 (FMR1) gene[4]. A widening of the repeat number distribution can be seen for the mutated allele which is a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

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

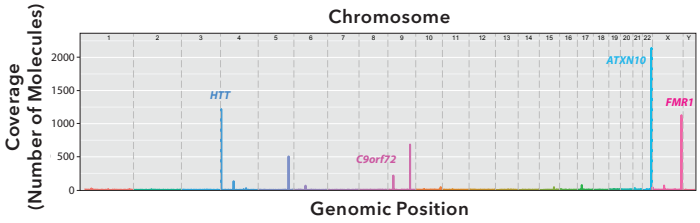
SMRT Sequencing on the Sequel System

- 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 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



KEY REFERENCES

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2. PacBio. (September 6, 2017) CRISPR/Cas9 and SMRT Sequencing yield new phenotype association for SCA10 repeat expansion disorder. [Blog post]. Retrieved from http://www.pacb.com/blog/crisprcas9-smrt-sequencing-yield-new-phenotype-association-sca10-repeat-expansion-disorder/
3. Ashizawa T. (October 13, 2016) CRISPR-Cas9-targeted SMRT Sequencing of large pentanucleotide repeats of Spinocerebellar Ataxia Type 10. [Webinar]. In *Springer Nature webinar series*. Retrieved from http://www.nature.com/webcasts/event/reveal-hidden-genetic-variation-by-combining-long-read-target-capture-with-smrt-sequencing/
4. 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.
5. Clark, T.A. et al. (Feb, 2017) Targeted SMRT Sequencing of difficult regions of the genome using a Cas9, non-amplification based method. In *Advances in Genome Biology and Technology*. Hollywood, FL.

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