



# NO-AMP TARGETED SEQUENCING BEST PRACTICES



With the PacBio® no-amplification (No-Amp) targeted sequencing method, you can now sequence through previously inaccessible regions of the genome to provide base-level resolution of disease-causing repeat expansions. By combining the CRISPR/Cas9 enrichment method with Single Molecule, Real-Time (SMRT®) Sequencing on the Sequel® Systems you are no longer limited by hard-to-amplify targets.

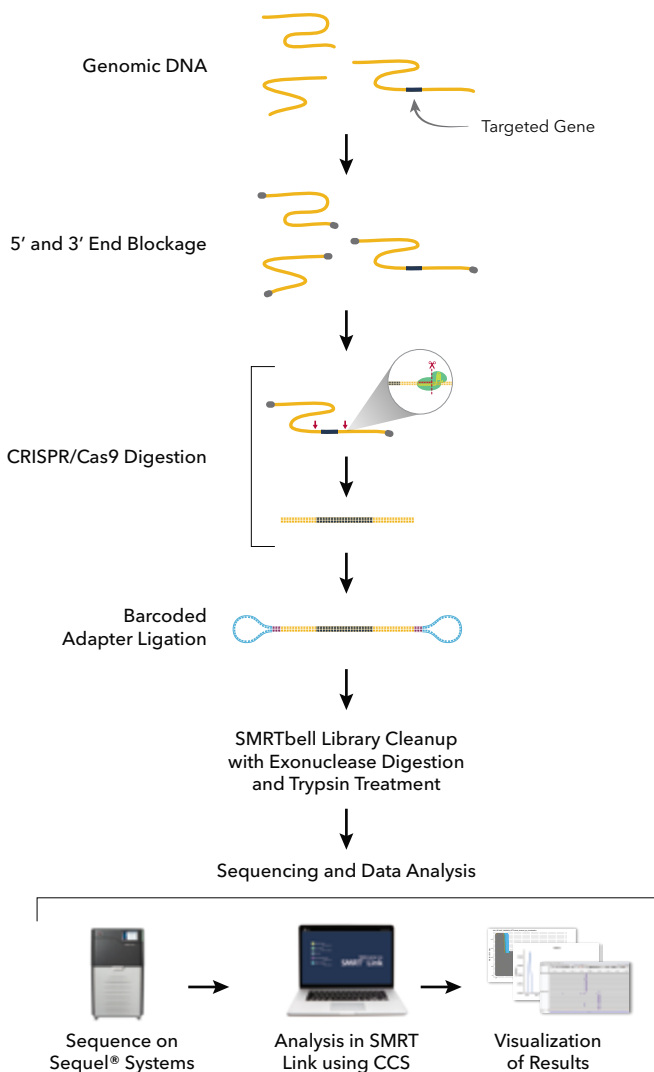
## WORKFLOW RECOMMENDATIONS

- Start with high-quality genomic DNA (~5-10 µg / SMRT Cell)
- Prepare SMRTbell® libraries in 2-days with stream-lined protocol<sup>1,2</sup>
  - Block 5' & 3' ends to prevent off-target ligation
  - Use custom-design guide RNAs to enrich for regions-of-interest<sup>3</sup>
  - Multiplex samples using barcoded adapters
  - Remove off-target products with trypsin exonuclease digestion
- Sequence multiplexed targets and/or samples on the Sequel Systems using the latest chemistry<sup>†</sup>
  - Adjust movie time parameters based on repeat expansion size<sup>2</sup>
  - Run up to 10 samples per SMRT Cell at ~\$120/sample on the Sequel II System<sup>†</sup>

\* Read lengths, reads/data per SMRT Cell type and other sequencing performance results vary based on sample quality/type and insert size.

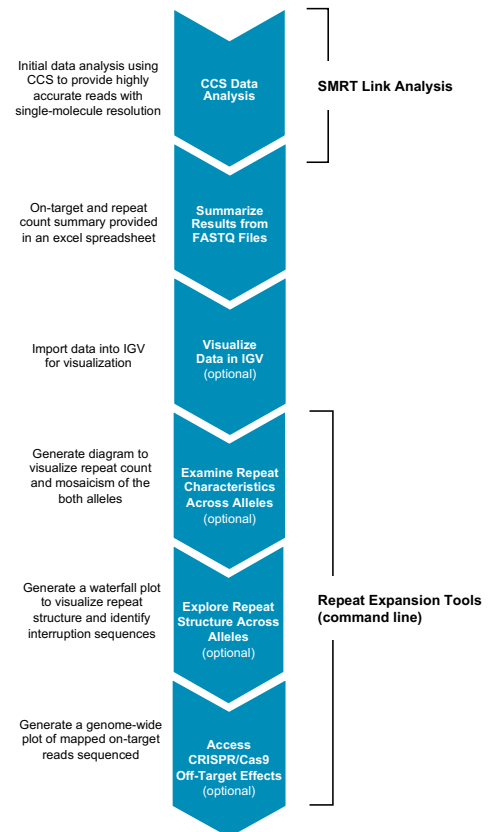
† Prices, listed in USD, are approximate and may vary by region. Pricing includes library and sequencing reagents run on a Sequel II System and does not include instrument amortization or other reagents.

## FROM gDNA TO COMPLETE REPEAT EXPANSION SEQUENCE

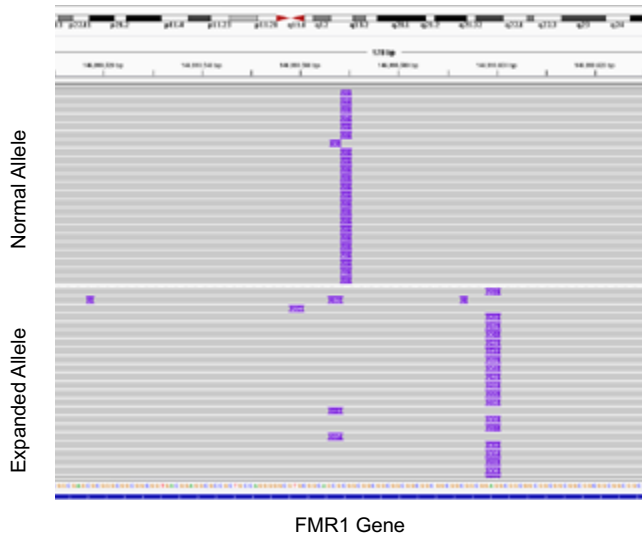


## DATA ANALYSIS SOLUTIONS

- Use SMRT Link to recall asymmetric SMRTbell adapters for demultiplexing of samples and perform circular consensus sequencing (CCS) to generate highly accurate long reads (HiFi reads)<sup>4</sup>
- Output data in FASTQ format for results summary reporting on repeat counts and on-target rates
- Visualize results with the IGV<sup>5</sup> and command-line scripts<sup>6</sup> for easy review of repeat count of both alleles, mosaic characterization, identification of interruption sequences and CRISPR/Cas9 off-targets

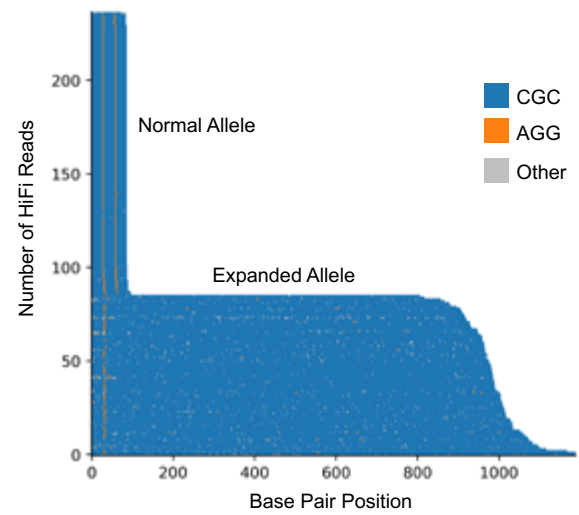


## VISUALIZE REPEATS WITH IGV



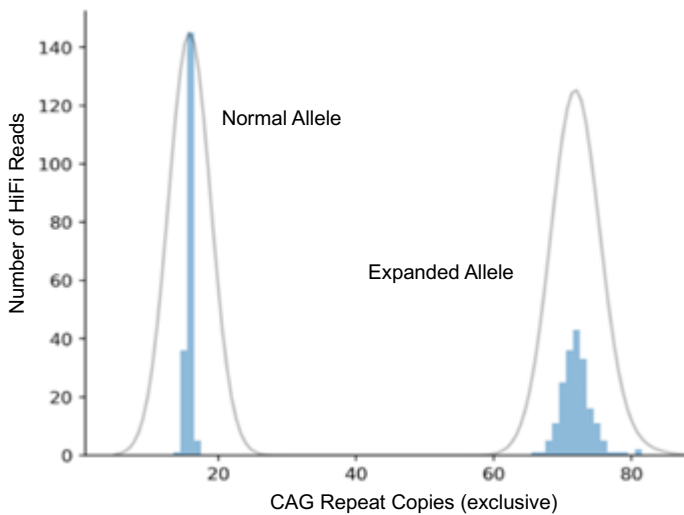
IGV allows you to easily visualize SMRT Sequencing data including all on-target reads for both alleles. Shown above are two alleles for the Fragile X Mental Retardation 1 (FMR1) gene, the normal allele has 27 repeats present and the expanded allele has a repeat expansion ranging from 218-353 repeats.

## EXPLORE REPEAT STRUCTURE



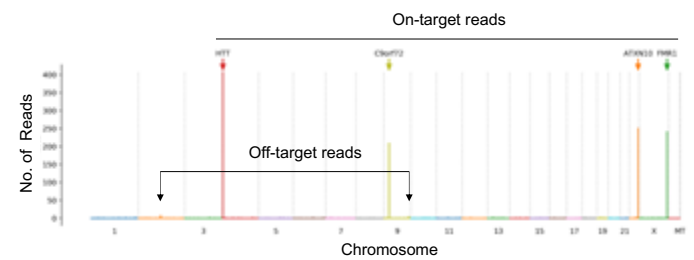
Waterfall plots outline the repeat structure of the two alleles. The Y-axis shows the number of HiFi reads whereas the X-axis shows the length of the repeat expansions in base pairs. Here, the repeat structure of a sample with a FMR1 expansion is shown including a normal allele with two interruption sequences (AGG) and an expanded allele with one interruption.

## EXAMINE REPEAT CHARACTERISTICS



Repeat counting allows the easy confirmation of allele sizes for both normal and expanded alleles as well as shows the extent of mosaicism of the expanded allele. Shown above is the repeat expansion range and peaks for a sample with a Huntington's disease (HTT) repeat expansion. The normal non-expanded allele shows a peak at 16 CAG repeat expansions, whereas the expanded allele ranges from 66-81 CAG repeats, peaking at 72.

## DISCOVER CRISPR/CAS9 OFF-TARGET EFFECTS



Readily identify CRISPR/Cas9 on- and off-target effects. The graph above shows four on-target loci (HTT, C9orf72, ATXN10 and FMR1) that have been enriched and sequenced and two off-target loci on chromosome 2 and 9 that are present due to a lack of specificity of the guide RNA design.

## KEY REFERENCES

1. Procedure & Checklist – No-Amp targeted sequencing utilizing the CRISPR Cas9 system. PacBio Documentation.
2. Overview – Sequel Systems application options and sequencing recommendations. PacBio Documentation.
3. Reference Guide – Designing CRISPR-Cas9 RNA Oligonucleotides for the No-Amp Targeted Sequencing Procedure. PacBio Documentation.
4. Analysis Procedure – No-Amp Data Preparation and Repeat Analysis. PacBio Documentation.
5. Robinson, J. et al. (2018) The Integrative Genomics Viewer (IGV).
6. Repeat Analysis Tools on PacBio Github.