



PCR-free, targeted HiFi sequencing at scale streamlines testing of repeat expansion and difficult inherited disease genes

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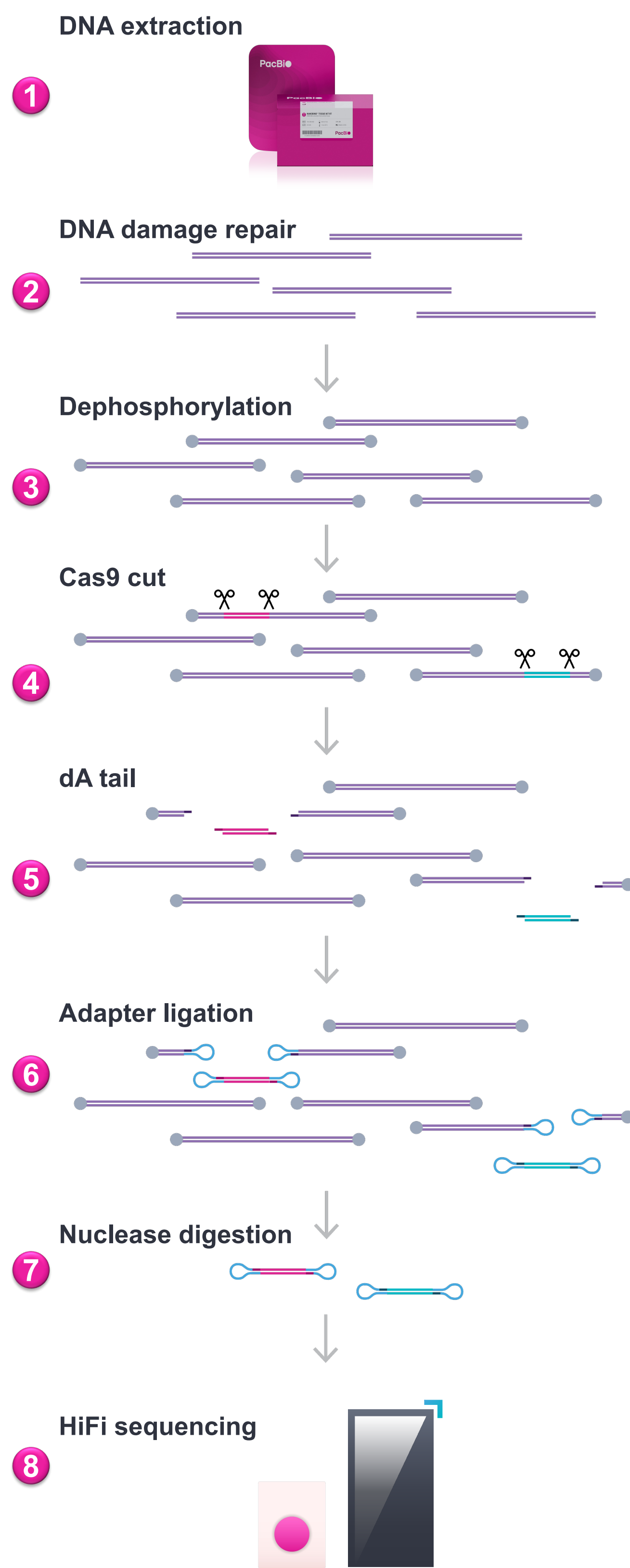
Platform & workflow

Introduction

Genetic testing often requires multiple assays to detect diverse variant types, including repeat expansions, single nucleotide variants (SNVs), structural variants (SV), and epigenetic modifications. PureTarget enrichment combined with PacBio HiFi sequencing enables comprehensive characterization of clinically relevant genomic regions within a single workflow.

PureTarget library prep workflows

- Starting with 1-4 µg of HMW DNA from blood, cell, or saliva
- Manual (24 samples) or HT (96 samples automated) kits



Analysis workflow

PTCP: an integrated workflow for tandem repeats and hard genes developed for ready-to-use PureTarget panels.

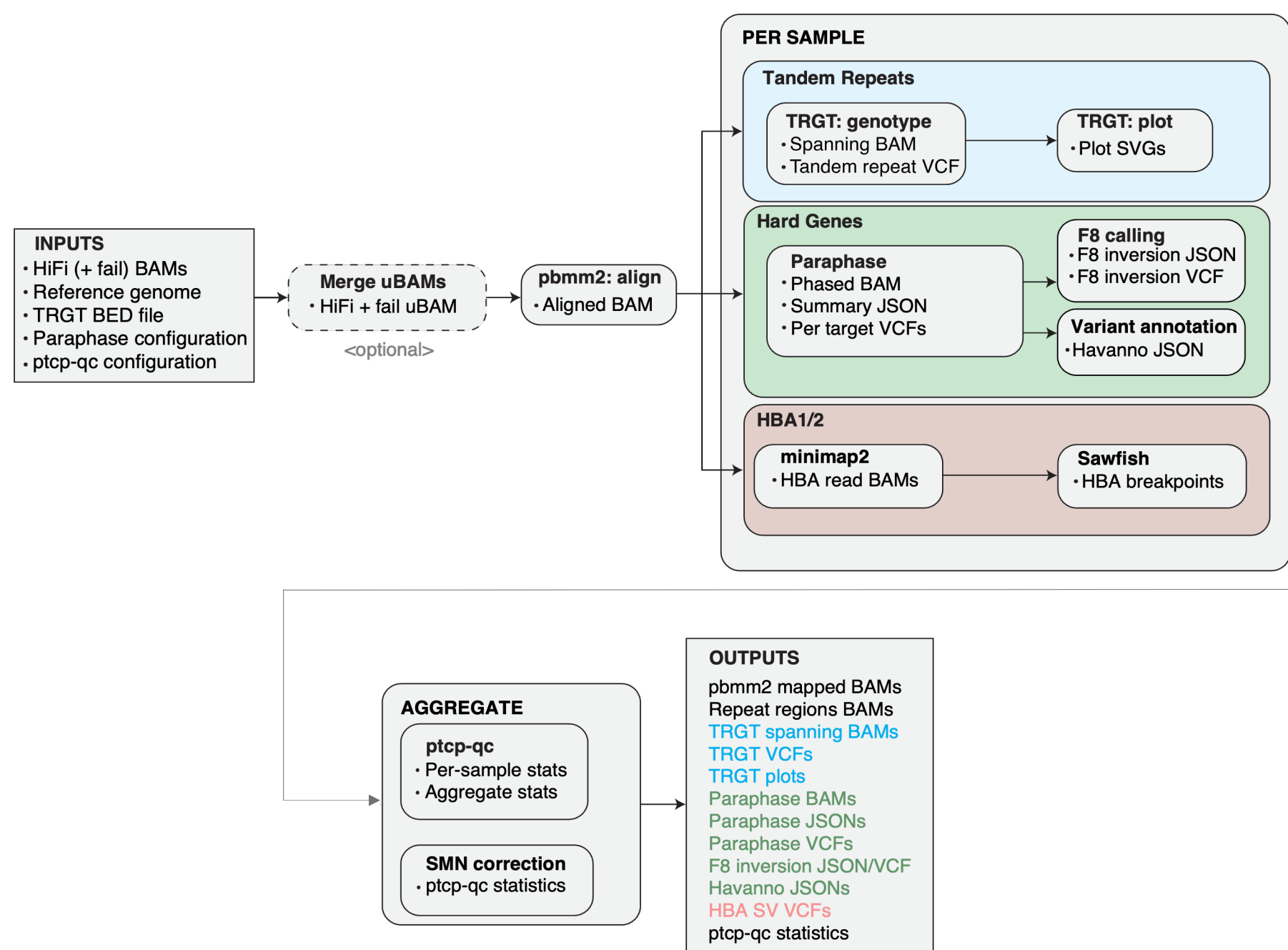


Figure 1. PureTarget Carrier Pipeline (PTCP): a portable, containerized WDL workflow for end-to-end analysis of PureTarget sequencing data, with per-sample tandem repeat genotyping (TRGT), homologous gene analysis (Paraphrase), and structural variant calling (Sawfish), followed by cohort-level QC aggregation.

Flexible PureTarget designs

Custom *MUC1* assay

MUC1 pathogenic variants cause ADTKD-*MUC1*, a hereditary kidney disease associated with a complex variable number tandem repeat (VNTR) region. Custom PureTarget guides were designed to target the entire 12 kb *MUC1* locus. Analysis of four blood-derived DNA samples generated HiFi reads spanning the full gene and revealed structural variation across donors.

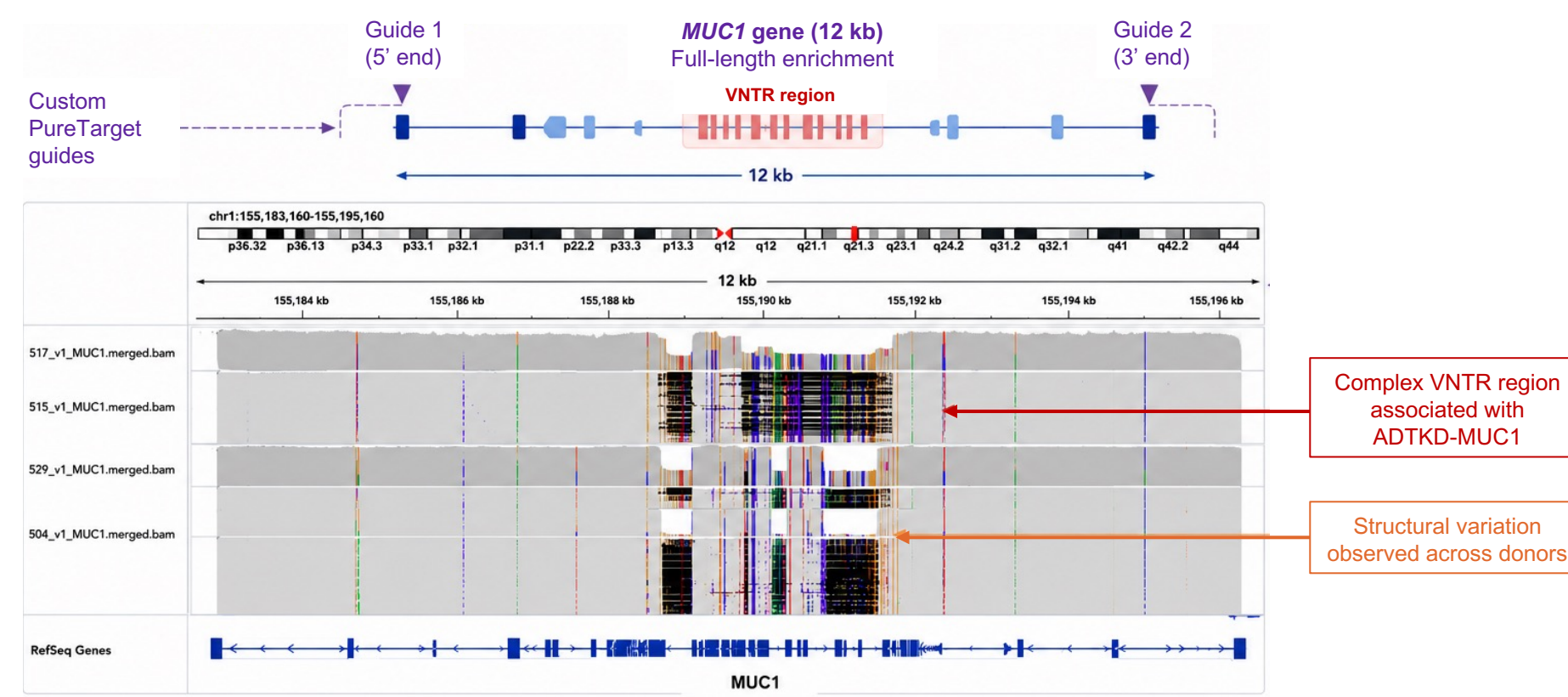


Figure 2. Complete *MUC1* locus coverage in four donor samples. PureTarget enrichment generated HiFi reads spanning the full 12 kb gene, including the complex VNTR region, enabling characterization of structural variation.

CFTR GeneSpan design

CFTR is associated with cystic fibrosis and spans a 189 kb genomic locus. The GeneSpan approach targets clinically relevant regions using non-overlapping 6–10 kb fragments designed around American College of Medical Genetics and Genomics (ACMG) recommended variants and diagnostic panel loci. This strategy enables focused analysis while maintaining compatibility with the standard PureTarget workflow.

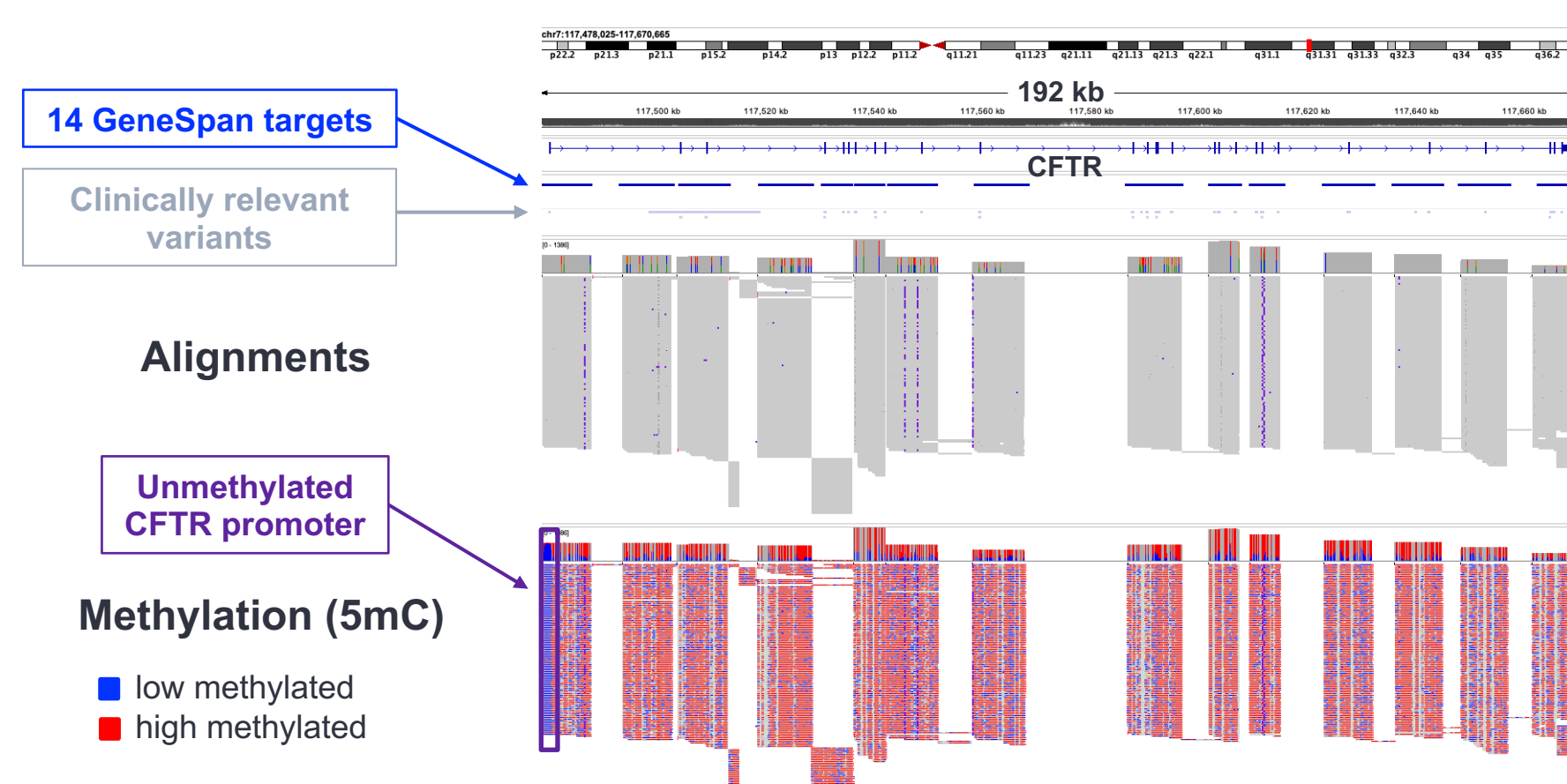


Figure 3. IGV visualization of the *CFTR* GeneSpan design. GeneSpan targets capture clinically relevant regions across the 189 kb *CFTR* locus while preserving compatibility with the standard PureTarget workflow. Methylation information is simultaneously captured, including the unmethylated *CFTR* promoter region.

189 kb Full-Genes *CFTR* tiling

Twenty-eight overlapping tiles were designed to provide continuous coverage across the entire 189 kb *CFTR* locus. Adjacent tile overlap enables extension of phased haplotype block across long genomic distances while simultaneously capturing methylation information.

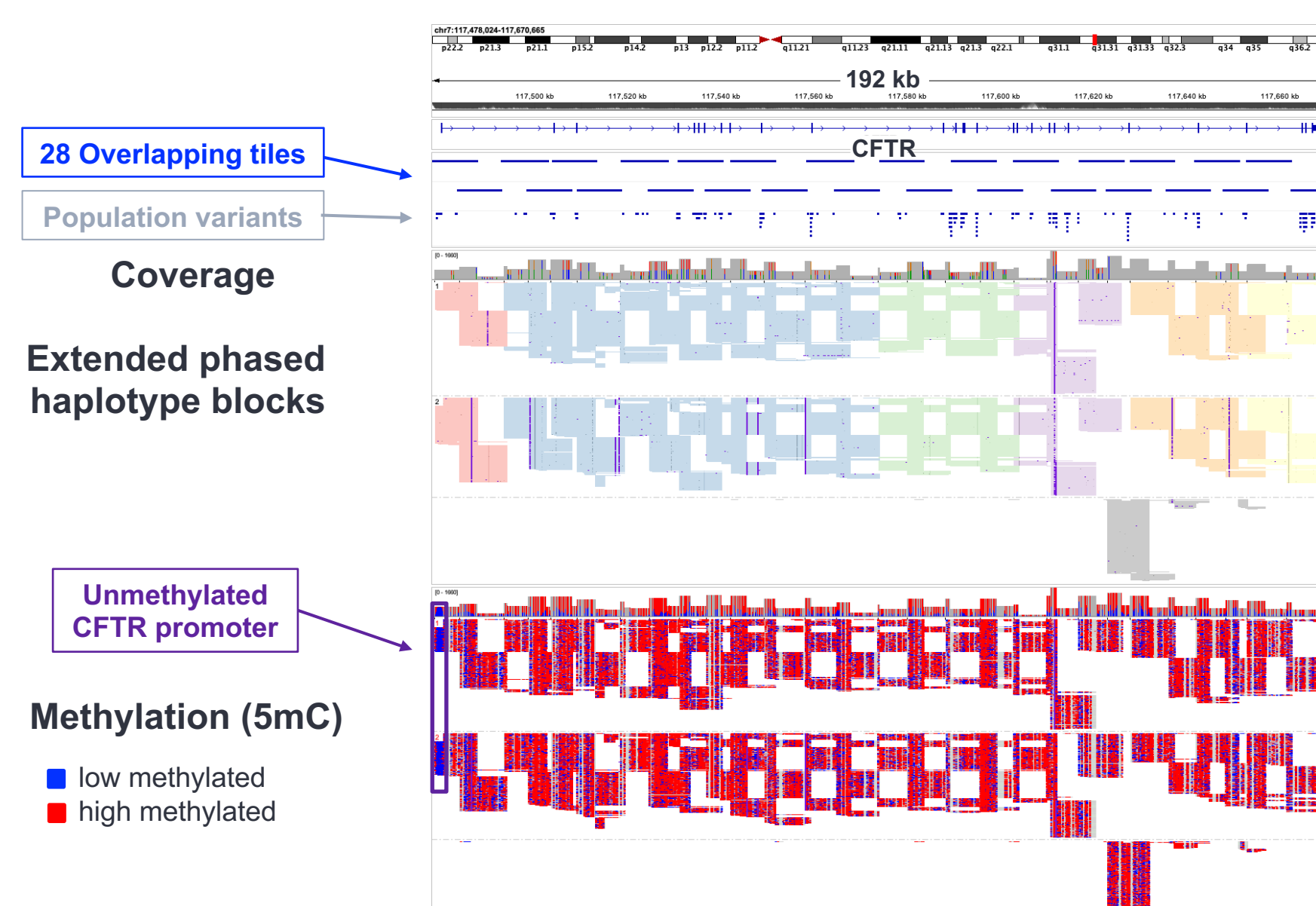


Figure 4. IGV visualization of full-gene *CFTR* tiling across the 189 kb locus. Overlapping tiles generated continuous coverage and enabled extension of phased haplotype blocks across the locus while simultaneously capturing methylation patterns, including an unmethylated *CFTR* promoter region.

Ready-to-use PureTarget panels

Repeat Expansion Panel

The PureTarget Repeat Expansion Panel targets 38 clinically relevant repeat loci associated with neurological and neuromuscular disorders. All panel targets were successfully enriched, enabling repeat sizing, repeat structure characterization, and methylation analysis using PacBio HiFi sequencing and TRGT.

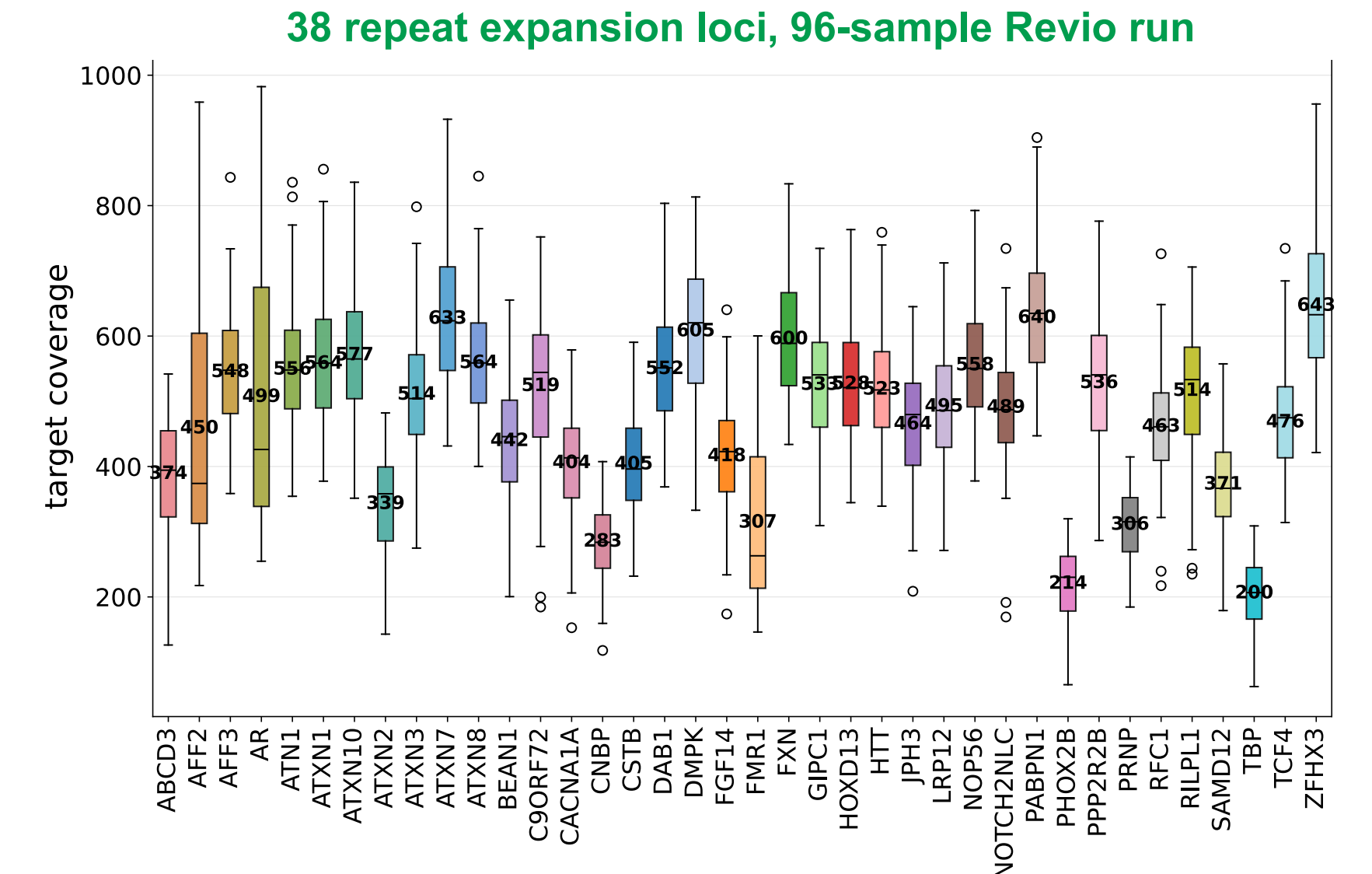


Figure 5. Coverage distribution across the 38 loci included in the PureTarget Repeat Expansion Panel from a 96-sample multiplexed Revio run. All targets were successfully enriched in a single assay, providing sufficient coverage for repeat characterization using PacBio HiFi sequencing and TRGT.

Difficult inherited disease genes panel

The PureTarget carrier panel consolidates clinically relevant and challenging genes into a single targeted assay. PacBio HiFi sequencing enables comprehensive characterization of genes with pseudogenes, segmental duplications, and complex genomic architectures within a unified workflow.

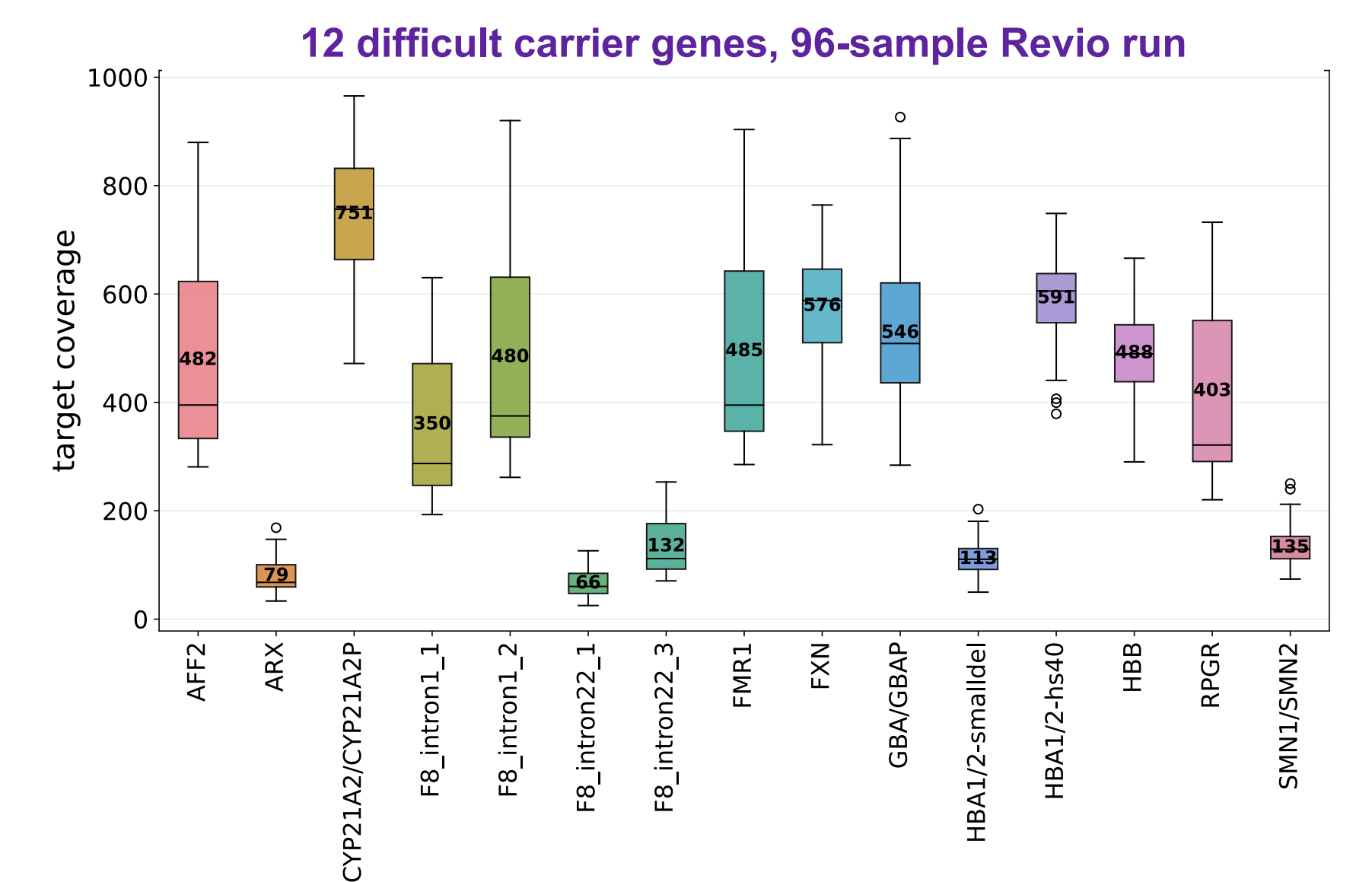


Figure 6. Coverage distribution across genes included in the PureTarget Difficult Inherited Disease Genes Panel from a 96-sample multiplexed Revio run. Successful enrichment was achieved across clinically relevant targets, including challenging genes with complex genomic architectures.

Key Takeaways

- ✓ Flexible targeting of challenging genomic regions.
- ✓ One workflow, multiple design strategies.
- ✓ Off-the-shelf and custom PureTarget designs.
- ✓ Variant detection, phasing, and methylation profiling in a single assay.

References:

- Tsai YC et al. (2022). Multiplex CRISPR/Cas9-guided No-Amp targeted sequencing. In *Genomic Structural Variants in Nervous System Disorders*, pp. 95–120.
- ACMG (2023). Recommendations for carrier screening in reproductive medicine. *Genet Med*.