Shine a light on dark regions of the genome

Comprehensive and scalable characterization of challenging regions with PureTarget

PureTarget technology enables scalable HiFi sequencing of native DNA libraries that maintain methylation and are free of PCR artifacts, errors, and bias. With off-the-shelf panels of targets for repeat expansion neurological disease and carrier screening research, labs can retire their cumbersome legacy genotyping assays like qPCR, triplet-primed PCR, capillary electrophoresis, southern blots and more. With PureTarget, labs can consolidate with superior performance: run fewer assays, return results faster, and get more reliable answers.

PacBio

Off-the-shelf panels

PureTarget repeat expansion panel 2.0

The updated repeat expansion panel features 38 targets covering a variety of commonly screened tandem repeats associated with neurological disorders. Targets that have been added to the new version of the panel are ***highlighted.***

Disease	Targets
Spinocerebellar ataxia (SCA)	*ATN1, ATXN1, ATXN2, ATXN3, ATXN7, ATXN8, ATXN10, CACNA1A, PPP2R2B, TBP* ***BEAN1, DAB1, FGF14, NOP56, ZFHX3***
Fragile-X disease (FXS)	*FMR1*
Fragile X syndrome, FRAXE type	***AFF2***
Intellectual disability associated with fragile site FRA2A	***AFF3***
Frontotemporal dementia (FTD), amyotrophic lateral sclerosis (ALS)	*C9orf72*
Friedreich's ataxia (FRDA)	*FXN*
Cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS)	*RFC1*
Neuronal intranuclear inclusion disease, Alzheimer disease and parkinsonism phenotype (NIID)	***NOTCH2NLC***
Myotonic dystrophy (DM)	*DMPK, CNBP*
Huntington disease (HD)	*HTT*
Huntington's disease-like type2 (HDL2)	***JPH3***
Fuchs endothelial corneal dystrophy 3 (FECD3)	*TCF4*
Kennedy Disease, Spinal and bulbar muscular atrophy, (SBMA)	*AR*
Oculopharyngeal muscular dystrophy (OPMD)	*PABPN1*
Oculopharyngodistal myopathy (OPDM)	***ABCD3, GIPC1, LRP12, RILPL1***
Syndactyly (SD5)	***HOXD13***
Congenital central hypoventilation syndrome (CCHS)	***PHOX2B***
Creutzfeldt-Jakob disease (CJD)	***PRNP***
Progressive Myoclonic Epilepsy Type 1 (EPM1) Unverricht-Lundborg Disease (ULD)	***CSTB***
Familial adult myoclonic epilepsy type 1 (FAME)	***SAMD12***

PureTarget carrier panel

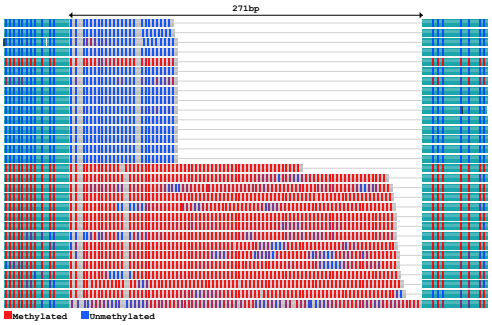
The carrier screening panel enables the consolidation of 12 genes that contain recessive disease-causing variants and require non-NGS ancillary methods for genotyping.[1] PureTarget is an attractive and streamlined assay that can be run alongside routine short read panels to capture all expanded carrier screening targets.

Disease	Targets
Hemophilia A	*F8*
Friedreich ataxia (FRDA)	*FXN*
Fragile-X disease (FXS)	*FMR1*
Congenital adrenal hyperplasia	*CYP21A2*
Classical-like Ehlers-Danlos syndrome	*TNXB*
Alpha thalassemia	*HBA1/2*
Gaucher disease	*GBA*
Spinal muscular atrophy	*SMN1/2*
Early-infantile epileptic encephalopathy (EIEE1) and Partington syndrome (PRTS)	*ARX*
Sickle Cell Anemia and Beta thalassemia	*HBB*
X-linked retinitis pigmentosa	*RPGR*
Fragile X syndrome, FRAXE type	*AFF2*

What can PureTarget do?

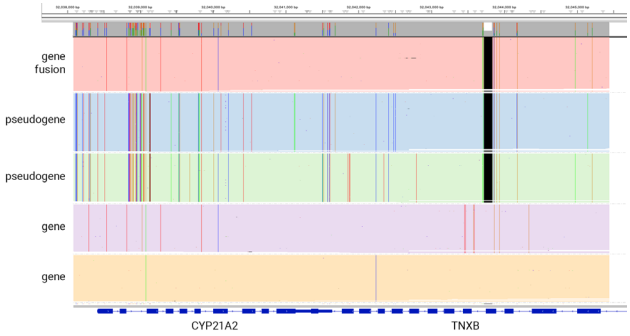
Powered by the *Tandem Repeat Genotyping Tool* (TRGT)[2] and *paraphase*[3], analysis of PureTarget data enables methylation detection, accurate sizing and motif analysis of repeat expansions like *FMR1* (Fragile-X disease) or *FGF14* (SCA27B), copy number and fusion allele detection at challenging genes like *SMN1/2* and *CYP21A2*, and capture of large structural variants in HBA1/2.

Allele-specific methylation in *FMR1* for carrier female



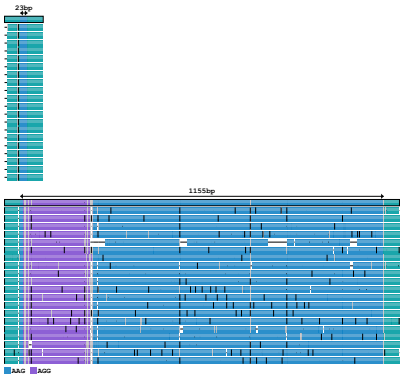
Carrier female (Coriell sample NA06905) with hypermethylated premutation. Expected genotype is 23 and 70 CGG copies based on Southern blot and PCR analysis. Sample was prepared with PureTarget repeat expansion panel using 1 µg of DNA and sequenced on the Vega™ system. Spanning read count for allele 1 is 121 and for allele 2 is 139. Observed genotype from trgt 2.0.0[2] is 23 and 79 CGG motifs with 1 AGG interruption motif observed on each allele (not shown). Discrepancy between observed and expected long allele length may be attributable to mosaicism in length as observe in the waterfall plot.

Copy number variation in *CYP21A2*



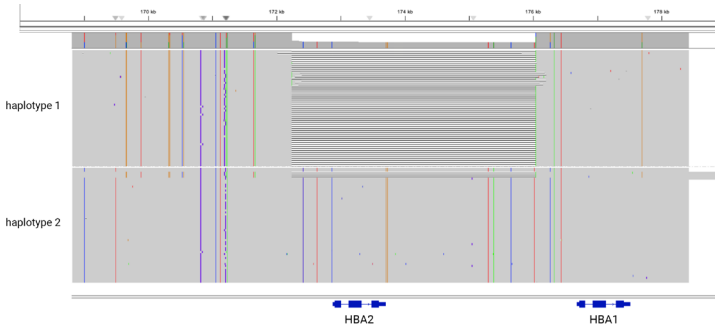
HG03540 reference sample with copy number variation in *CYP21A2*. A pair of gRNAs capture ~7.4 kb spanning the full *CYP21A2* gene and 13 exons of *TNXB* on the 3' end. The gRNAs also cut the segmental duplication containing *CYP21A1P* and *TNXA*. Sample was sequenced on the Revio® system + SPRQ™ chemistry, reads for gene and pseudogene are mapped to the gene and analyzed with paraphase v3.3.1 in the PureTarget Carrier Pipeline (PTCP). Five haplotypes are captured showing 2 gene copies, 2 pseudogene copies, and a fusion allele.

Heterozygous expansion in *FGF14* (SCA27B)



Sample with pathogenic heterozygous expansion in *FGF14* associated with SCA27B. DNA (2 µg) extracted from blood with QIAsymphony, was manually prepared with PureTarget repeat expansion panel 2.0, sequenced on the Revio system + SPRQ chemistry in an 8plex, and analyzed with TRGT v3.0.0. Expected genotype from rpPCR is 10 +/- 2 and approximately 350 repeats and is concordant with observed genotype: 8 and 390 repeats. HiFi sequencing reveals that the long alelle has with 66 AGG and 307 AAG motifs with consensus length 1155 bp.

Large deletion spanning *HBA2*



Sample was sequenced on Revio + SPRQ and analyzed with paraphase v3.3.1 in the PureTarget Carrier Pipeline (PTCP). A pair of gRNAs capture ~9.5 kb spanning *HBA1* and *HBA2*. The 3p7 deletion is neatly captured showing one copy of *HBA2* and 2 copies of *HBA1*.

Product specs

Spec	Metric
DNA Input per sample	1–1.5 µg – Automated prep with PureTarget kit 96 1–4 µg - Manual prep with PureTarget kit 24
DNA quality*	GQN at 30 kb ≥ 5
Mean target coverage	100-fold or greater per 1 ug DNA per sample[1]
Minimum target coverage	20-fold per target
Sample multiplexing	96 samples per SMRT® Cell on Revio + SPRQ with PureTarget kit 96 8–48 samples per SMRT Cell on Revio + SRPQ and Vega with PureTarget kit 24

* 50% of mass of DNA molecules longer than 30 kb as measured on Femto Pulse (Agilent).

1. Coverage per 1µg input at max plex (Revio + SPRQ 96, Vega 48) for Nanobind-extracted blood, lymphoblastoid cells, and saliva (manual prep only with PureTarget kit 24 on Revio + SPRQ), and Coriell lymphoblastoid cell DNA with GQN at 30 kb > 5. Higher coverage is possible with higher DNA input mass and lower plex. Individual target coverage is lower for longer target regions. The same library loaded on Revio + SPRQ will typically give higher coverage than Vega.

An end-to-end targeted solution

PureTarget libraries are scalable and flexible. For high-throughput labs, the PureTarget kit 96 sequenced on Revio + SPRQ can process 100,000 samples a year*. The PureTarget kit 24 on the Vega system enables flexible batching and fast turnaround time. PureTarget panels are pre-pooled guide RNAs that can be paired with either manual or automated prep. A panel of 3 control guides can be supplemented with your own custom designs.

Sample prep

Nanobind® HT CBB kit for automated extraction of blood and cells (102-762-700)

Nanobind PanDNA for manual extraction of blood and cells (103-260-000)

Curious how your samples will perform with PureTarget? See Table 4 in the repeat expansion app note or contact techsupport@pacb.com

Library prep

Automated prep with the **PureTarget kit 96** (103-708-000). Scripts available for Hamilton NGS STAR MOA

Manual prep with the **PureTarget kit 24** (103-707-900)

Library cleanup with the **PureTarget cleanup kit** (103-708-100). Use with manual and automated kits for best sequencing performance

Barcodes—**SMRTbell® adapter index plates 96A-D** (102-009-200, 102-547-800, 102-547-900, 102-548-000)

gDNA panels

Carrier panel (103-633-200)

Repeat expansion panel 2.0 (103-633-100)

Control panel—3 human genes intended to be used with additional custom guide RNA designs (103-633-300)

Sequencing

Revio + SPRQ

- 96 samples per SMRT Cell for the PureTarget 96 kit
- 8–48 samples per SMRT Cell for the PureTarget 24 kit

Vega

- 8–48 samples per SMRT Cell for the PureTarget 24 kit

* Assumes 96 samples per SMRT Cell, 4 SMRT Cells per run, 5 runs a week, 50 weeks a year.

Software

Use SMRT® Link version 25.3 for the latest PureTarget features: updated run designs, quick assessment of panel performance with SMRT® Analysis, and genotyping of the new 38 gene repeat expansion panel with TRGT v3.0. Variant calling for the PureTarget carrier panel is available through our partners at DNA Nexus and Golden Helix or deployed to on-prem servers from GitHub.

Run design and monitoring

Use PureTarget run designs in **SMRT Link Cloud** or **SMRT Link** v25.3 for optimized sequencing conditions.

Variant calling

The **PureTarget carrier pipeline (PTCP)** is a best-practice variant calling pipeline powered by **TRGT** and **paraphase**. PTCP is available in the **DNA Nexus marketplace**, **Golden Helix VarSeq**, and deployable to on-prem servers from GitHub.

SMRT Link PureTarget repeat expansion analysis offers genotyping with TRGT v3.0 and panel performance.

Panel performance

With **SMRT Link Target Enrichment analysis**, quickly assess panel performance with per-sample and per-target coverage stats and plots.

SMRT Link PureTarget repeat expansion analysis offers panel performance coverage analysis plus genotyping with **TRGT v3.0**.

Variant interpretation

Our partners at **Golden Helix** offer variant interpretation reports for PureTarget carrier panel.

KEY REFERENCES

1. Guha S, et al. (2024). Laboratory testing for preconception/prenatal carrier screening: A technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2024 Jul;26(7):101137. doi: 10.1016/j.gim.2024.101137.
2. Dolzhenko E, English A, Dashnow H, et al., (2024). Characterization and visualization of tandem repeats at genome scale. *Nature Biotechnology*, 1–9.
3. Chen X, Baker D, Dolzhenko E, et al. Genome-wide profiling of highly similar paralogous genes using HiFi sequencing. *Nature Communications*. 2025. doi:10.1038/s41467-025-57505-2

Learn more: pacb.com/technology/puretarget/

102-326-609 REV08 18SEP2025