Technical overview

PureTarget library preparation using PureTarget kit 2.0

Vega system ICS v1.1
Revio system ICS v13.3
SMRT Link v25.3

PN 103-742-600 Rev 01 | September 2025

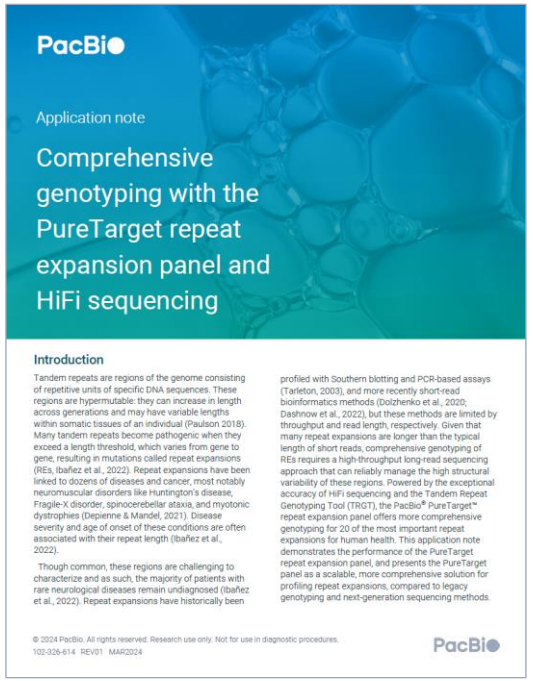
Technical overview

PureTarget library preparation using PureTarget kit 2.0

1. PureTarget method & products overview
2. Library preparation workflow details
3. Sequencing preparation workflow details
4. Data analysis workflow overview
5. Example sequencing performance data
6. Technical documentation & applications support resources
7. Appendix

PureTarget library preparation using PureTarget kit 2.0: Getting started

Application-specific literature → **Application-specific protocol** → **Application-specific technical overview** → **Library preparation, sequencing & analysis**


PacBio

Application note

Comprehensive genotyping with the PureTarget repeat expansion panel and HiFi sequencing

Introduction

Tandem repeats are regions of the genome consisting of repetitive units of specific DNA sequences. These regions are hypermutable: they can increase in length across generations and may have variable lengths within somatic tissues of an individual (Paulson 2018). Many tandem repeats become pathogenic when they exceed a length threshold, which varies from gene to gene, resulting in mutations called repeat expansions (REs, Ibañez et al., 2022). Repeat expansions have been linked to dozens of diseases and cancer, most notably neuromuscular disorders like Huntington's disease, Fragile-X disorder, spinocerebellar ataxia, and myotonic dystrophies (Depienne & Mandel, 2021). Disease severity and age of onset of these conditions are often associated with their repeat length (Ibañez et al., 2022).

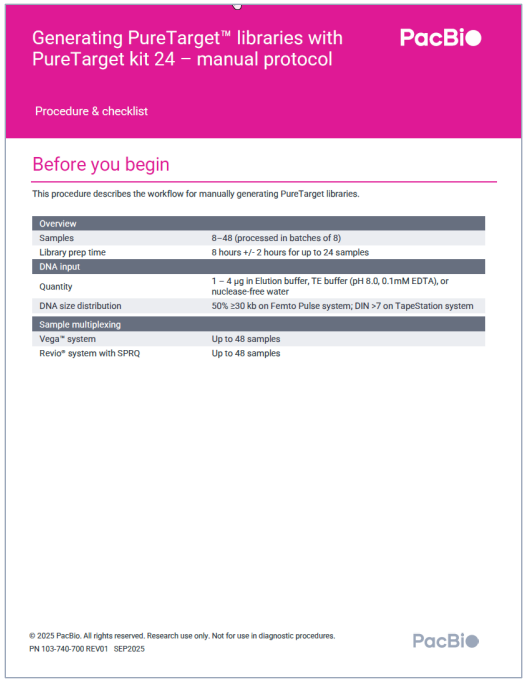
Though common, these regions are challenging to characterize and as such, the majority of patients with rare neurological diseases remain undiagnosed (Ibañez et al., 2022). Repeat expansions have historically been profiled with Southern blotting and PCR-based assays (Tarleton, 2003), and more recently short-read bioinformatics methods (Dolzhenko et al., 2020; Dashnow et al., 2022), but these methods are limited by throughput and read length, respectively. Given that many repeat expansions are longer than the typical length of short reads, comprehensive genotyping of REs requires a high-throughput long-read sequencing approach that can reliably manage the high structural variability of these regions. Powered by the exceptional accuracy of HiFi sequencing and the Tandem Repeat Genotyping Tool (TRGT), the PacBio® PureTarget™ repeat expansion panel offers more comprehensive genotyping for 20 of the most important repeat expansions for human health. This application note demonstrates the performance of the PureTarget repeat expansion panel, and presents the PureTarget panel as a scalable, more comprehensive solution for profiling repeat expansions, compared to legacy genotyping and next-generation sequencing methods.

102-326-614 REV01 MAR2024

Application note – Comprehensive genotyping with the PureTarget repeat expansion panel and HiFi sequencing (102-326-614)

Application note – Consolidate challenging genes with PureTarget carrier screen panel (102-326-653)

Technical note – A practical guide to amplification-free PureTarget custom panels (102-326-652)


Generating PureTarget™ libraries with PureTarget kit 24 – manual protocol

PacBio

Procedure & checklist

Before you begin

This procedure describes the workflow for manually generating PureTarget libraries.

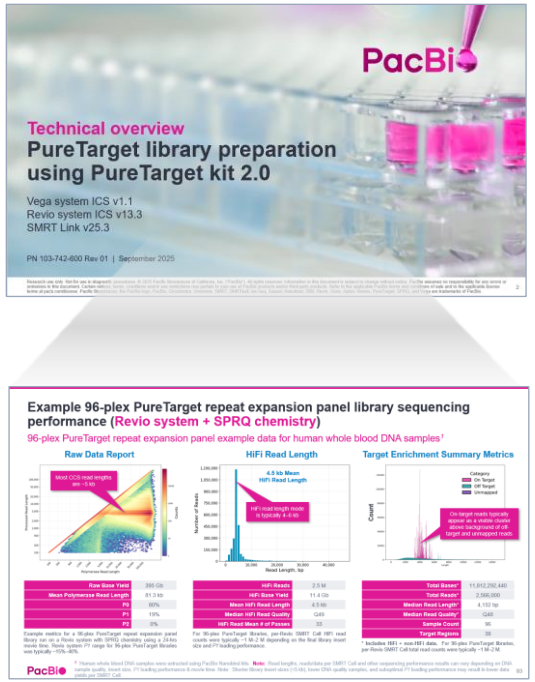
Overview	
Samples	8–48 (processed in batches of 8)
Library prep time	8 hours +/- 2 hours for up to 24 samples
DNA input	
Quantity	1 – 4 µg in Elution buffer, TE buffer (pH 8.0, 0.1mM EDTA), or nuclease-free water
DNA size distribution	50% ≥30 kb on Femto Pulse system; DIN >7 on TapeStation system
Sample multiplexing	
Vega® system	Up to 48 samples
Revio® system with SPRQ	Up to 48 samples

PN 103-740-700 REV01 SEP2025

Procedure & checklist – Generating PureTarget libraries with PureTarget kit 24 – Manual protocol (103-740-700)

Procedure & checklist – Generating PureTarget libraries with PureTarget kit 96 – Automation protocol (103-740-800)

Guide & overview – Automated PureTarget kit 96 for the Hamilton NGS STAR MOA system (103-740-900)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Technical overview

PureTarget library preparation using PureTarget kit 2.0

Vega system ICS v1.1
Revio system ICS v13.3
SMRT Link v25.3

PN 103-742-600 Rev 01 | September 2025

Example 96-plex PureTarget repeat expansion panel library sequencing performance (Revio system + SPRQ chemistry)

96-plex PureTarget repeat expansion panel example data for human whole blood DNA samples

Raw Data Report

HiFi Read Length

Target Enrichment Summary Metrics

Technical Overview – PureTarget library preparation using PureTarget kit 2.0 (103-742-600)

Technical overview presentations describe workflow details for constructing PacBio SMRTbell libraries for specific applications. Example sequencing performance data for a given application are also summarized.

gDNA sample extraction & QC
(Nanobind kit)

1 – 2 µg DNA per sample
Genome quality number (30 kb) ≥5.0
Use Nanobind PanDNA kit for blood extraction

PureTarget library preparation
(Manual or automated workflow)

PureTarget repeat expansion / carrier / custom panel options available
PureTarget kit 24 (manual)
PureTarget kit 96 (automated)

SMRT sequencing
(Vega & Revio systems)

Vega system: Up to 48-plex
Revio system + SPRQ: Up to 96-plex

Data analysis
(SMRT Link + PacBio variant calling tools)

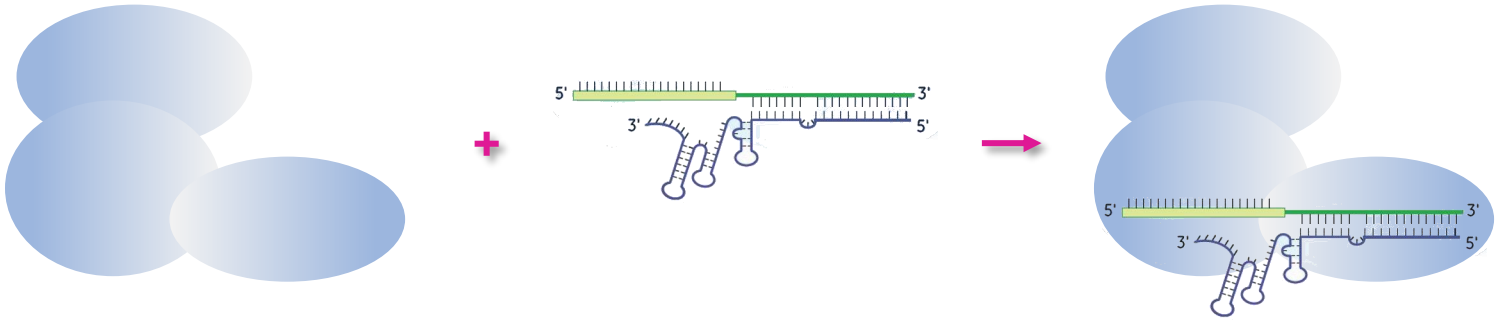
SMRT Link support for coverage QC analysis and repeat expansion genotyping

PacBio

PureTarget sequencing method overview

CRISPR-Cas9 technology overview

CRISPR-Cas9 system comprises a guide RNA (gRNA or sgRNA) and Cas9 nuclease, which together form a ribonucleoprotein (RNP) complex that can introduce a site-specific double-strand break in DNA[1,2]



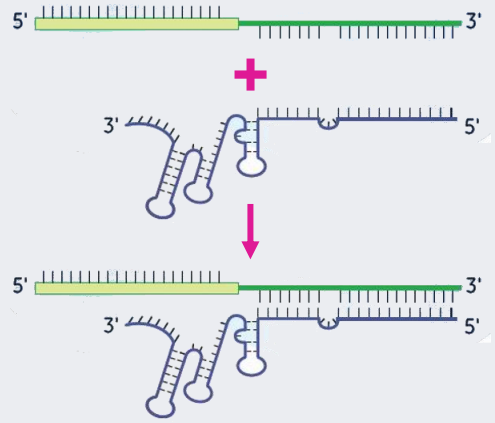
Cas9 nuclease | Guide RNA | Cas9-gRNA complex

PureTarget uses the **CRISPR-Cas9 system** to generate targeted **native DNA libraries** for HiFi sequencing. This amplification-free approach retains epigenetic signals and has no PCR artifacts or errors.

Guide RNA configurations

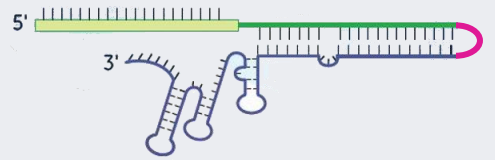
Two-component guide RNA (gRNA)

Annealed [crRNA : tracrRNA] complex



Single guide RNA (sgRNA)

Synthetic crRNA-tracrRNA single-molecule hybrid construct



sgRNA is used in PureTarget library prep workflow

1 *Image modified from:* https://horizondiscovery.com/en/applications/gene-editing

2 CRISPR (= *clustered regularly interspaced short palindromic repeats*) is a family of DNA sequences found in the genomes of prokaryotic organisms such as bacteria that play a role in the anti-viral defense system of these organisms.

CRISPR-Cas9 technology overview (cont.)

CRISPR-Cas9 system comprises a guide RNA (gRNA or sgRNA) and Cas9 nuclease, which together form a ribonucleoprotein (RNP) complex that can introduce a site-specific double-strand break in DNA[1,2]

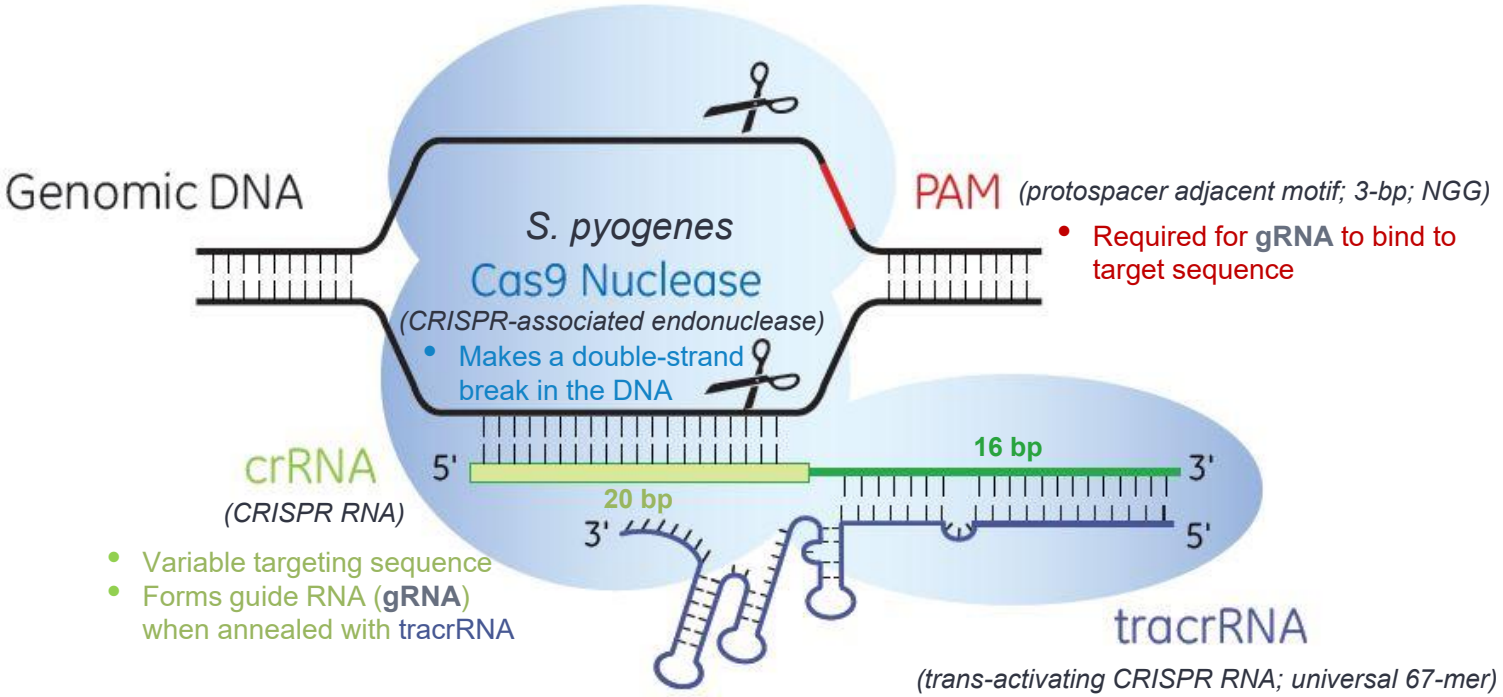
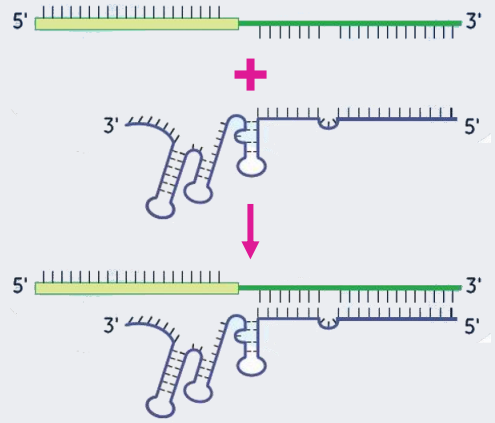


Illustration of **Cas9 nuclease** (blue), programmed by the **tracrRNA** (violet) : **crRNA** (olive) complex (= **guide RNA**) cutting both strands of genomic DNA 5' of the protospacer-adjacent motif (**PAM**) (red).

Guide RNA configurations

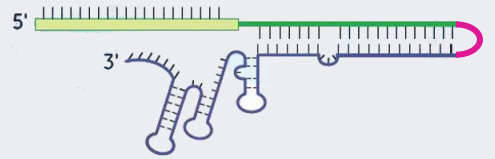
Two-component guide RNA (gRNA)

Annealed [crRNA : tracrRNA] complex



Single guide RNA (sgRNA)

Synthetic crRNA-tracrRNA single-molecule hybrid construct



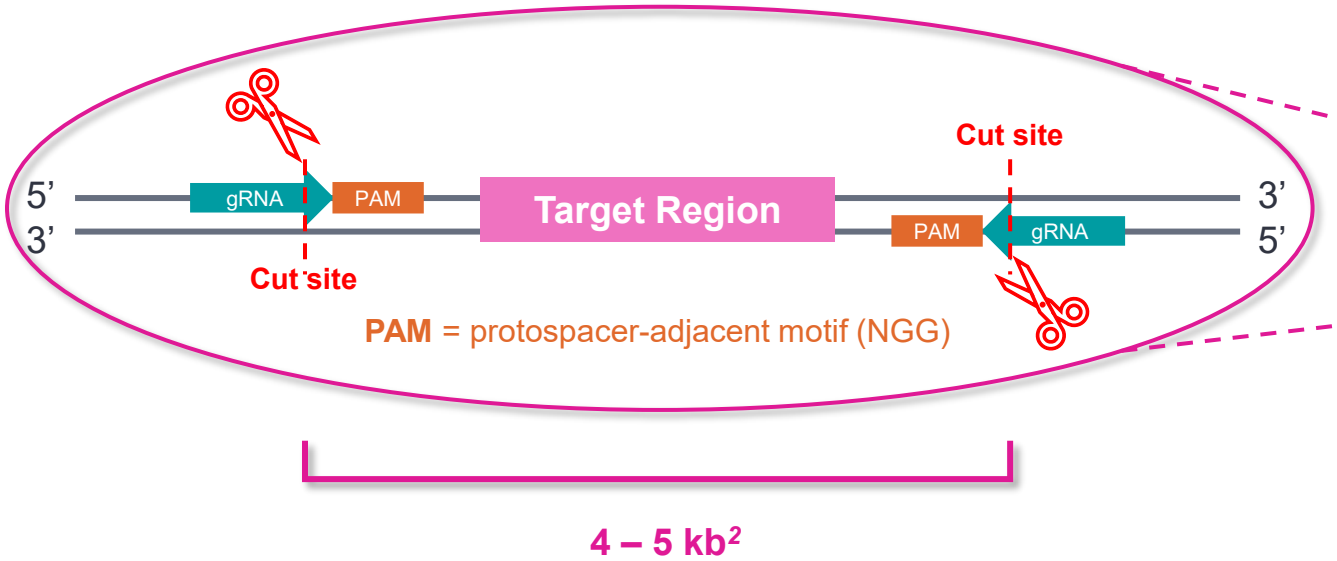
sgRNA is used in PureTarget library prep workflow

[1] *Image modified from:* https://horizondiscovery.com/en/applications/gene-editing

[2] CRISPR (= *clustered regularly interspaced short palindromic repeats*) is a family of DNA sequences found in the genomes of prokaryotic organisms such as bacteria that play a role in the anti-viral defense system of these organisms.

How CRISPR-Cas9 is used in PureTarget native DNA library prep workflow

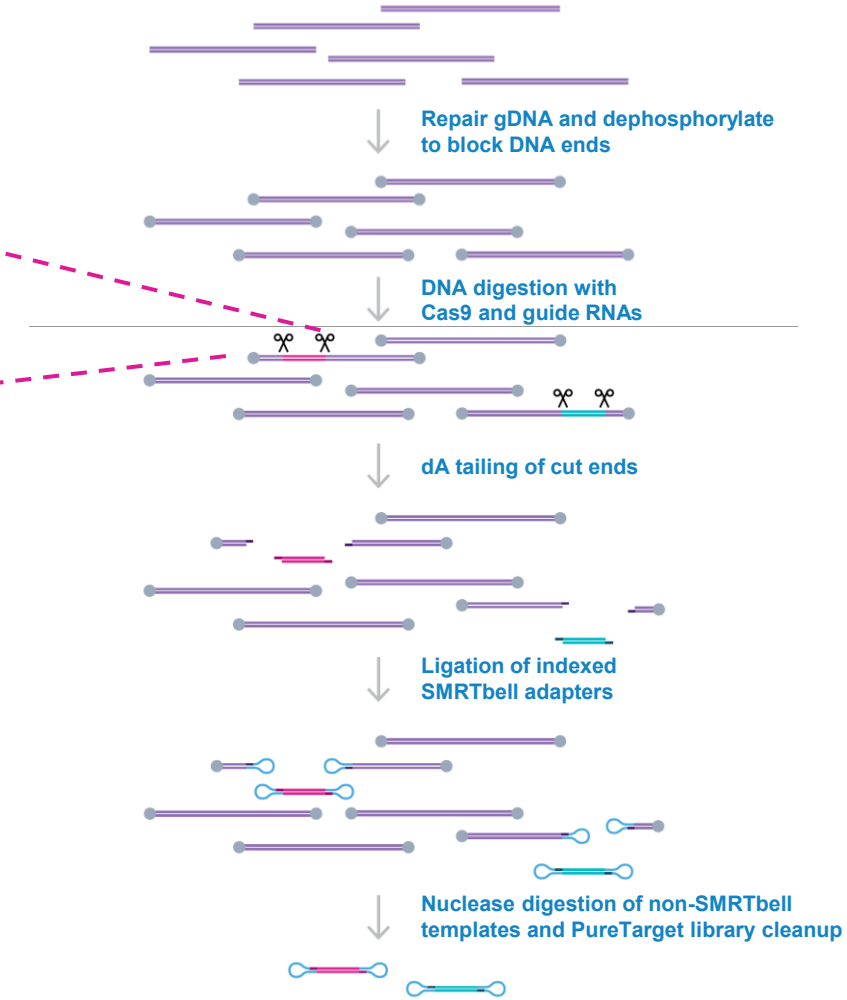
For PureTarget HiFi sequencing, CRISPR-Cas9 system is used to selectively enrich for regions of interest in a genomic sample prior to long-read sequencing of native DNA molecules[1]



Excising a target region with two flanking gRNA-Cas9 complexes

- Cas9 nuclease, in close association with appropriate guide RNA (gRNA) oligonucleotides, identifies and then cleaves a specific recognition site on each side of the target region
- Different regions of interest can be simultaneously targeted in a single CRISPR-Cas9 digestion reaction by using multiple sets of gRNA pairs

Target enrichment with CRISPR-Cas9



[1] Tsai, Y. C., et al. (2022). Multiplex CRISPR/Cas9-Guided No-Amp targeted sequencing panel for spinocerebellar ataxia repeat expansions. In Genomic Structural Variants in Nervous System Disorders (pp. 95-120). New York, NY: Springer US.

[2] **Note:** PureTarget guide RNAs are designed to cut a 5-kb region in the human reference genome; however, some DNA fragment sizes that are sequenced may be much larger with repeat expansion alleles.

PureTarget 2.0 consumables for manual and automated library prep workflows

PureTarget 2.0 panel products enable flexibility in content and scale

Automated PureTarget library prep

HiFi Sequencing

PureTarget kit 96 bundle (103-708-000)[1]	Panel gRNA reagents	Indexed SMRTbell adapters	PacBio long-read systems
 PureTarget kit 2.0 (103-632-900)  PureTarget cleanup kit (103-708-100)[2]  HiFi plex prep kit 96 (103-122-800) SMRTbell cleanup beads (102-158-300) [2 x 10 mL] Elution buffer (101-633-500) [1 x 50 mL]	 PureTarget repeat expansion panel 2.0 (103-633-100); *or* PureTarget carrier panel (103-633-200); *or* PureTarget control panel (103-633-300)	 SMRT adapter index plate 96A/B/C/D (102-009-200)	 Revio system + SPRQ 96-plex *With automation, a single Revio system can process 96,000 samples per year with full system utilization using PureTarget kit 96*

Manual PureTarget library prep

HiFi Sequencing

PureTarget kit 24 bundle (103-707-900)	Panel gRNA reagents	Indexed SMRTbell adapters	PacBio long-read systems
 PureTarget kit 2.0 (103-632-900)  PureTarget cleanup kit (103-708-100)[2]  SMRTbell prep kit 3.0 (102-141-700) SMRTbell cleanup beads (102-158-300) [1 x 10 mL] Elution buffer (101-633-500) [1 x 50 mL]	 PureTarget repeat expansion panel 2.0 (103-633-100); *or* PureTarget carrier panel (103-633-200); *or* PureTarget control panel (103-633-300)	 SMRT adapter index plate 96A/B/C/D (102-009-200)	 Revio system + SPRQ 8- to 48-plex  Vega system 8- to 48-plex *Vega system can process ~10,000 samples a year using manual workflow at 48-plex*

[1] PureTarget kit 96 bundle (103-708-000) is intended to support automated PureTarget library preparation workflows and is not intended to support manual workflows.

PureTarget 2.0 product portfolio & workflow overview

Updated product portfolio supports PureTarget repeat expansion panel 2.0, PureTarget carrier screening & PureTarget custom [manual + automated] workflows

PureTarget panel

PureTarget repeat expansion panel 2.0

38-gene panel covering a variety of commonly screened tandem repeats associated with neurological disorders

Sample prep

Supported DNA extraction kits

Nanobind PanDNA
Nanobind CBB

Supported sample types

Human blood
Lymphoblastoid cell lines
Coriell lymphoblastoid cell DNA
Human saliva (manual only)[1]

Refer to ***Application note – Comprehensive genotyping with the PureTarget repeat expansion panel and HiFi sequencing*** (102-326-614) for the most up-to-date list of supported DNA extraction kits and sample types for PureTarget sequencing applications

Library prep

Library preparation procedure & checklist and consumables

Generating PureTarget libraries using PureTarget kit 24 – Manual protocol (103-740-700)

Generating PureTarget libraries using PureTarget kit 96 – Automation protocol (103-740-800)

PureTarget kit 24 bundle (103-707-900)
PureTarget kit 2.0[2]; SMRTbell prep kit 3.0; SMRTbell cleanup beads; EB [manual]

PureTarget kit 96 bundle (103-708-000)
PureTarget kit 2.0[2]; HiFi plex prep kit 96; SMRTbell cleanup beads; EB [automated]

PureTarget repeat expansion panel 2.0 (103-633-100) gRNA mix to target 38-gene panel

PureTarget cleanup kit (103-708-100)

SMRTbell adapter index plate 96A/B/C/D (102-009-200)

Sequencing

Supported instruments and run design setup

Revio system + SPRQ
Up to 48-plex for manual
Up to 96-plex for automated

Vega system
Up to 48-plex

SMRT Link v25.3 run design
Select **PureTarget repeat expansion** application type option when using SMRT Link v25.3 on-premise software or SMRT Link Cloud v25.3 software

Analysis

Panel performance QC and variant calling

SMRT Link v25.3 SMRT Analysis
PureTarget repeat expansion analysis application produces target enrichment summary QC statistics and uses tandem repeat genotyping tool (TRGT 3.0) for variant calling and for visualization

[1] **Note:** Human saliva samples are only supported for **manual** library prep workflows using PureTarget kit 24.
[2] PureTarget kit 2.0 does not include any guide RNA mix.

PureTarget 2.0 product portfolio & workflow overview (cont.)

Updated product portfolio supports PureTarget repeat expansion panel 2.0, PureTarget carrier screening & PureTarget custom [manual + automated] workflows

PureTarget panel

PureTarget carrier panel

12-gene panel enables the consolidated analysis of several challenging genes associated with rare disease

Sample prep

Supported DNA extraction kits

Nanobind PanDNA
Nanobind CBB

Supported sample types

Human blood
Lymphoblastoid cell lines
Coriell lymphoblastoid cell DNA
Human saliva (manual only)[1]

Refer to ***Application note – Consolidate challenging genes with PureTarget carrier screen panel*** (102-326-653) for the most up-to-date list of supported DNA extraction kits and sample types for PureTarget sequencing applications

Library prep

Library preparation procedure & checklist and consumables

Generating PureTarget libraries using PureTarget kit 24 – Manual protocol (103-740-700)

Generating PureTarget libraries using PureTarget kit 96 – Automation protocol (103-740-800)

PureTarget kit 24 bundle (103-707-900)
PureTarget kit 2.0[2]; SMRTbell prep kit 3.0; SMRTbell cleanup beads; EB [manual]

PureTarget kit 96 bundle (103-708-000)
PureTarget kit 2.0[2]; HiFi plex prep kit 96; SMRTbell cleanup beads; EB [automated]

PureTarget carrier panel (103-633-200)
gRNA mix to target 12-gene panel

PureTarget cleanup kit (103-708-100)

SMRTbell adapter index plate 96A/B/C/D (102-009-200)

Sequencing

Supported instruments and run design setup

Revio system + SPRQ
Up to 48-plex for manual
Up to 96-plex for automated

Vega system
Up to 48-plex

SMRT Link

SMRT Link v25.3 run design
Select **PureTarget carrier** application type option when using SMRT Link v25.3 on-premise software or SMRT Link Cloud v25.3 software

Analysis

Panel performance QC, variant calling and variant interpretation

UPDATED SMRT Link

SMRT Link v25.3 SMRT Analysis
Target Enrichment analysis application produces target enrichment summary QC statistics using carrier panel target BED file

GitHub DNAnexus

PureTarget Carrier Pipeline (PTCP)
PureTarget Carrier Pipeline (PTCP) analysis workflow available through GitHub or PacBio Compatible partners enables variant calling for tandem repeat regions and hard genes using TRGT 3.0 & Paraphase 3.3

PacBio Compatible

PacBio compatible partner analysis tools
PacBio compatible partner analysis workflows enable variant interpretation and generation of reports

Golden Helix

[1] **Note:** Human saliva samples are only supported for **manual** library prep workflows using PureTarget kit 24.
[2] PureTarget kit 2.0 does not include any guide RNA mix.

PureTarget 2.0 product portfolio & workflow overview (cont.)

Updated product portfolio supports PureTarget repeat expansion panel v2, PureTarget carrier screening & PureTarget custom [manual + automated] workflows

PureTarget panel | **Sample prep** | **Library prep** | **Sequencing** | **Analysis**

PureTarget custom panel

PureTarget custom panel workflow supports custom panel designs for analysis of specific gene targets of interest

Supported DNA extraction kits

Nanobind PanDNA
Nanobind CBB

Supported sample types

Human blood
Lymphoblastoid cell lines
Coriell lymphoblastoid cell DNA
Human saliva (manual only)[1]

Refer to ***Technical note – A practical guide to amplification-free PureTarget custom panels*** (102-326-652) for the most up-to-date list of supported DNA extraction kits and sample types for PureTarget sequencing applications

Library preparation procedure & checklist and consumables

Generating PureTarget libraries using PureTarget kit 24 – Manual protocol (103-740-700)

Generating PureTarget libraries using PureTarget kit 96 – Automation protocol (103-740-800)

PureTarget kit 24 bundle (103-707-900)
PureTarget kit 2.0[2]; SMRTbell prep kit 3.0; SMRTbell cleanup beads; EB [manual]

PureTarget kit 96 bundle (103-708-000)
PureTarget kit 2.0[2]; HiFi plex prep kit 96; SMRTbell cleanup beads; EB [automated]

PureTarget control panel (103-633-300)
gRNA mix to target 3 positive control targets[3]

PureTarget cleanup kit (103-708-100)

SMRTbell adapter index plate 96A/B/C/D (102-009-200)

Supported instruments and run design setup

Revio system + SPRQ
Up to 48-plex for manual
Up to 96-plex for automated

Vega system
Up to 48-plex

SMRT Link

SMRT Link v25.3 run design
Select **PureTarget custom** application type option when using SMRT Link v25.3 on-premise software or SMRT Link Cloud v25.3 software

Panel performance QC and downstream genotyping analysis

SMRT Link

SMRT Link v25.3 SMRT Analysis
Target enrichment analysis application produces target enrichment summary QC statistics using a custom target BED file

GitHub

PacBio GitHub tools
Can use PacBio variant calling tools available on **GitHub** to perform downstream analysis

TRGT
Tandem repeat genotyping tool for HiFi sequencing data

Paraphase
HiFi-based caller for highly similar paralogous genes

Sawfish
Joint structural variant and copy number variant caller for HiFi sequencing data

[1] **Note:** Human saliva samples are only supported for **manual** library prep workflows using PureTarget kit 24.
[2] PureTarget kit 2.0 does not include any guide RNA mix.
[3] PureTarget custom control panel contains a gRNA mix to target 3 positive control genes (1 X-linked + 2 autosomal); customers may additionally spike in custom gRNA mixes for custom panel designs.

PureTarget manual library preparation procedure reference [For up to 48-plex]

Procedure & checklist – Generating PureTarget libraries with PureTarget kit 24 – Manual protocol (103-740-700) describes the manual workflow for generating up to 48-plex PureTarget libraries using the **PureTarget kit 24** reagent bundle for sequencing on PacBio long-read systems

Overview	
Samples	8–48 (processed in batches of 8)
Library prep time	8 hours +/- 2 hours for up to 24 samples
DNA input	
Quantity	1 – 4 µg in Elution buffer, TE buffer (pH 8.0, 0.1mM EDTA), or nuclease-free water
DNA size distribution	50% ≥30 kb on Femto Pulse system; DIN >7 on TapeStation system
Sample multiplexing	
Vega™ system	Up to 48 samples
Revio® system with SPRQ	Up to 48 samples

PureTarget kit 24 bundle
(103-707-900)

PureTarget cleanup kit
(103-708-100)

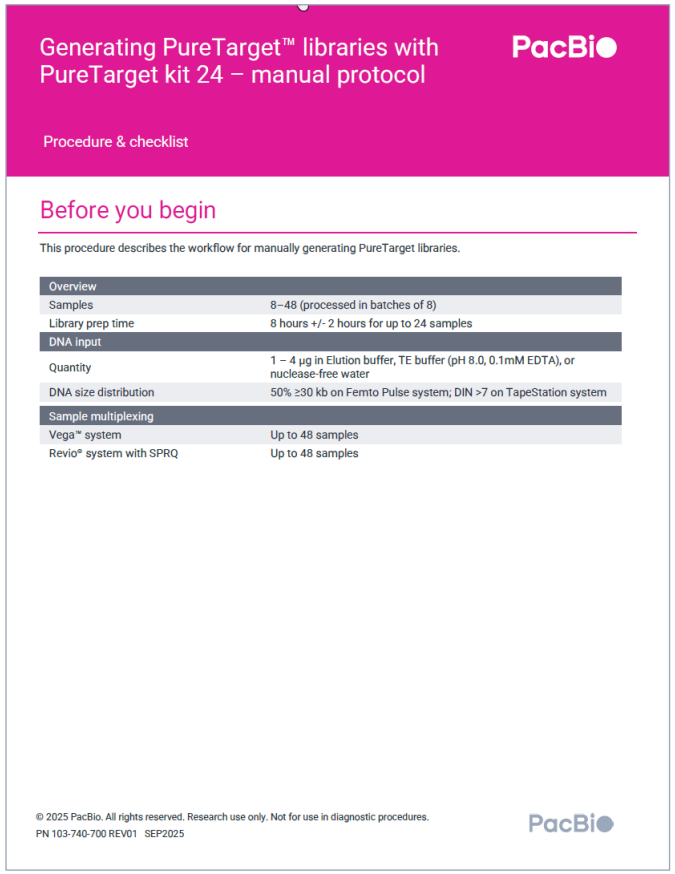
SMRTbell adapter index plate 96A/B/C/D
(102-009-200)

PureTarget repeat expansion panel 2.0
(103-633-100); *or*

PureTarget carrier panel
(103-633-200); *or*

PureTarget control panel
(103-633-300)

PureTarget library template (~4 – 5 kb)
Expanded alleles can be ≥20 kb[1]



Generating PureTarget™ libraries with PureTarget kit 24 – manual protocol

PacBio

Procedure & checklist

Before you begin

This procedure describes the workflow for manually generating PureTarget libraries.

Overview	
Samples	8–48 (processed in batches of 8)
Library prep time	8 hours +/- 2 hours for up to 24 samples
DNA input	
Quantity	1 – 4 µg in Elution buffer, TE buffer (pH 8.0, 0.1mM EDTA), or nuclease-free water
DNA size distribution	50% ≥30 kb on Femto Pulse system; DIN >7 on TapeStation system
Sample multiplexing	
Vega™ system	Up to 48 samples
Revio® system with SPRQ	Up to 48 samples

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Note: Procedure 103-740-700 includes instructions for manual **PureTarget library construction workflow *and* sequencing preparation (ABC[2]) workflow**

[1] For normal alleles, resulting sequences are ~4–5 kb in length but reads for expanded alleles may be longer. **Note:** Sequencing results for PureTarget samples with large expansions indicate that it is possible to span repeats up to 35 kb in length in a single HiFi read.

[2] For primer annealing, polymerase binding & complex cleanup (ABC) and final dilution steps, follow sample setup instructions for PureTarget libraries in ***Procedure & checklist – Generating PureTarget libraries with PureTarget kit 24 – Manual protocol*** (103-740-700) – Do not use SMRT Link Sample Setup.

PacBio

PureTarget automated library preparation procedure reference [For 96-plex]

Procedure & checklist – Generating PureTarget libraries with PureTarget kit 96 – Automation protocol (103-740-800) describes the automated workflow for generating 96-plex PureTarget libraries using the **PureTarget kit 96** reagent bundle[1] for sequencing on PacBio long-read systems

Overview	
Samples	96
Library prep time	16 hours with the Hamilton NGS STAR MOA system
DNA input	
Quantity	1 - 1.5 µg in Elution buffer, TE buffer (pH 8, 0.1mM EDTA), or nuclease-free water
DNA size distribution	50% ≥30 kb on Femto Pulse system; DIN >7 on TapeStation system
Sample multiplexing	
Revio® system with SPRQ™ chemistry	96 samples

PureTarget kit 96 bundle[1]
(103-708-000)

PureTarget cleanup kit
(103-708-100)

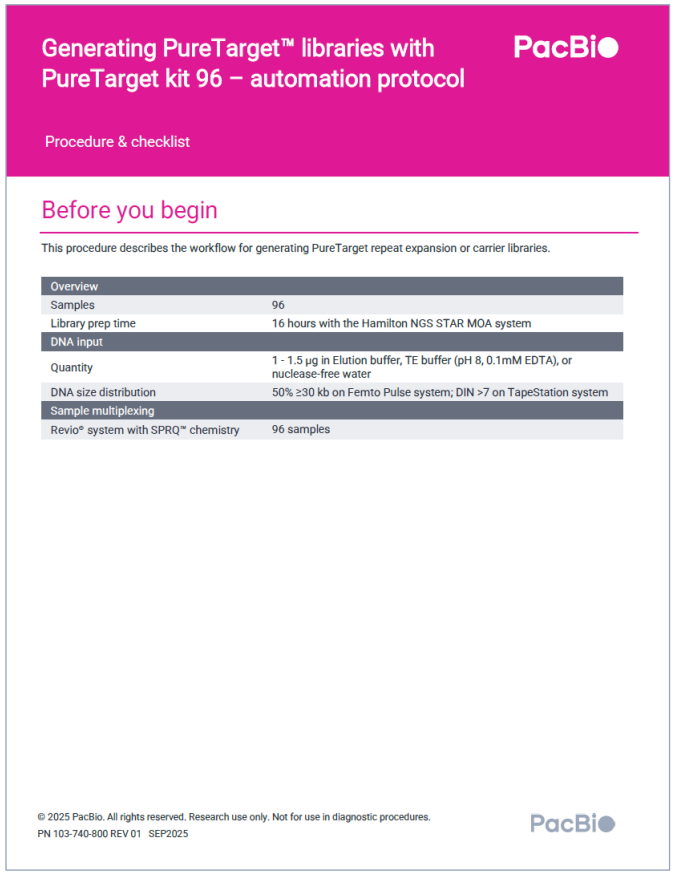
SMRTbell adapter index plate 96A/B/C/D
(102-009-200)

PureTarget repeat expansion panel 2.0
(103-633-100); *or*

PureTarget carrier panel
(103-633-200); *or*

PureTarget control panel
(103-633-300)

PureTarget library template (~4 – 5 kb)
Expanded alleles can be ≥20 kb[2]



Generating PureTarget™ libraries with PureTarget kit 96 – automation protocol

PacBio

Procedure & checklist

Before you begin

This procedure describes the workflow for generating PureTarget repeat expansion or carrier libraries.

Overview	
Samples	96
Library prep time	16 hours with the Hamilton NGS STAR MOA system
DNA input	
Quantity	1 - 1.5 µg in Elution buffer, TE buffer (pH 8, 0.1mM EDTA), or nuclease-free water
DNA size distribution	50% ≥30 kb on Femto Pulse system; DIN >7 on TapeStation system
Sample multiplexing	
Revio® system with SPRQ™ chemistry	96 samples

PN 103-740-800 REV 01 SEP2025

PacBio

Note: Procedure 103-740-800 includes instructions for automated **PureTarget library construction workflow *and* sequencing preparation (ABC[3]) workflow**

[1] PureTarget kit 96 bundle (103-708-000) is intended to support automated PureTarget library preparation workflows and is not intended to support manual workflows.
[2] For normal alleles, resulting sequences are ~4–5 kb in length but reads for expanded alleles may be longer. **Note:** Sequencing results for PureTarget samples with large expansions indicate that it is possible to span repeats up to 35 kb in length in a single HiFi read.
[3] For primer annealing, polymerase binding & complex cleanup (ABC) and final dilution steps, follow sample setup instructions for PureTarget libraries in ***Procedure & checklist – Generating PureTarget libraries with PureTarget kit 96 – Automation protocol*** (103-740-800) – Do not use SMRT Link Sample Setup.

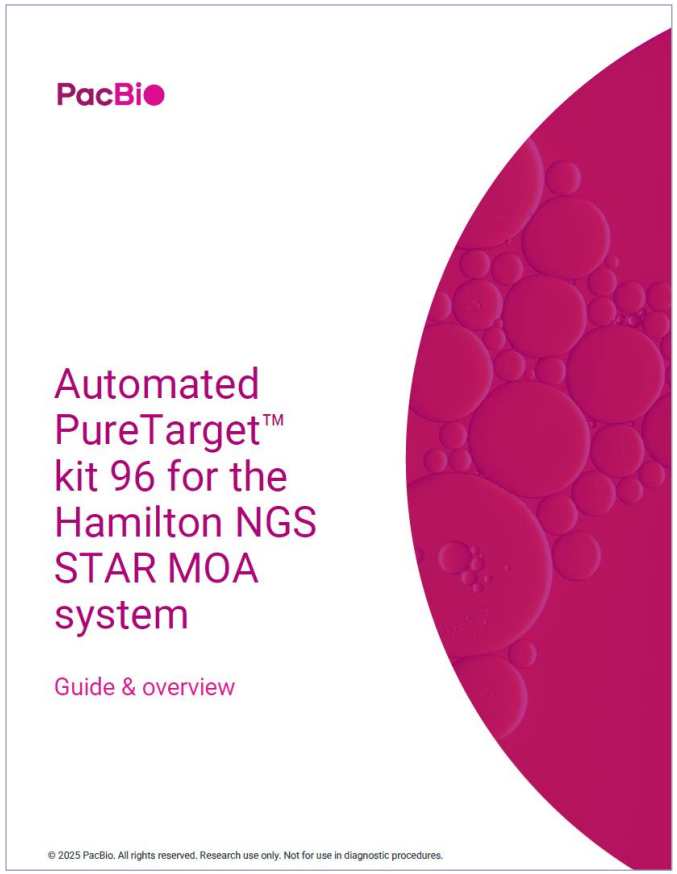
PacBio

PureTarget automated 96-plex library preparation guide & overview reference for Hamilton NGS STAR MOA system

Guide & overview – Automated PureTarget kit 96 for the Hamilton NGS STAR MOA system (103-740-900) describes the automated workflow for generating 96-plex PureTarget libraries using the **Hamilton NGS STAR MOA liquid handling system** for sequencing on PacBio long-read systems[1]

Overview	
Samples	96
Workflow step	**Automation time**
gDNA Repair & SMRTbell cleanup (Optional*)	2.5 hours
Dephosphorylation	1 hour
Cas9 digestion & SMRTbell cleanup	2 hours
dA-tail	0.5 hours
Adapter ligation, pool & SMRTbell cleanup	2 hours
Nuclease treatment 1, pool & SMRTbell cleanup	2 hours
Nuclease treatment 2 & SMRTbell cleanup	2 hours
PureTarget Library cleanup	1 hour
ABC	1 hour
Average total time	< 16 hours (including off-deck prep)
DNA input	
DNA input Quantity	1.0–1.5 µg in Elution buffer, TE buffer (pH 8, 0.1mM EDTA), or nuclease-free water
DNA size distribution	50% ≥30 kb on Femto Pulse system; DIN >7 on TapeStation system

Hamilton NGS STAR MOA system



Note: This Hamilton NGS STAR MOA system method supports automated PureTarget library preparation for a **single batch of 96 samples – batch sizes less than 96 are not supported**

* gDNA repair is required unless using Nanobind-extracted blood genomic DNA using RBC lysis method or whole blood extraction.

[1] For primer annealing, polymerase binding & complex cleanup (ABC) and final dilution steps, follow sample setup instructions for PureTarget libraries in ***Procedure & checklist – Generating PureTarget libraries with PureTarget kit 96 – Automation protocol*** (103-740-800) – Do not use SMRT Link Sample Setup.

PureTarget 2.0 consumables for manual and automated library prep workflows

PureTarget 2.0 panel products enable flexibility in content and scale

Automated PureTarget library prep

Sequencing

PureTarget kit 96 bundle (103-708-000)[1]	Panel gRNA reagents	Indexed SMRTbell adapters	PacBio long-read systems
 PureTarget kit 2.0 (103-632-900)	PureTarget repeat expansion panel 2.0 (103-633-100); *or*	 SMRT adapter index plate 96A/B/C/D (102-009-200)	 Revio system + SPRQ 96-plex
 PureTarget cleanup kit (103-708-100)	PureTarget carrier panel (103-633-200); *or*		
 HiFi plex prep kit 96 (103-122-800)	PureTarget control panel (103-633-300)		
SMRTbell cleanup beads (102-158-300) [2 x 10 mL]			
Elution buffer (101-633-500) [1 x 50 mL]			

Manual PureTarget library prep

Sequencing

PureTarget kit 24 bundle (103-707-900)	Panel gRNA reagents	Indexed SMRTbell adapters	PacBio long-read systems
 PureTarget kit 2.0 (103-632-900)	PureTarget repeat expansion panel 2.0 (103-633-100); *or*	 SMRT adapter index plate 96A/B/C/D (102-009-200)	 Revio system + SPRQ 8- to 48-plex
 PureTarget cleanup kit (103-708-100)	PureTarget carrier panel (103-633-200); *or*		 Vega system 8- to 48-plex
 SMRTbell prep kit 3.0 (102-141-700)	PureTarget control panel (103-633-300)		
SMRTbell cleanup beads (102-158-300) [1 x 10 mL]			
Elution buffer (101-633-500) [1 x 50 mL]			

PacBio

[1] PureTarget kit 96 bundle (103-708-000) is intended to support automated PureTarget library preparation workflows and is not intended to support manual workflows.

PureTarget kit 24 bundle[1,2] and PureTarget cleanup kit components

PureTarget kit 24 bundle includes PureTarget kit 2.0 and SPK 3.0 to support manual PureTarget library prep

PureTarget kit 2.0 (103-632-900)

- Reagents for PureTarget library preparation
- Note: This kit **does not** include guide RNA reagents[3]
- Supports 24 samples for manual PureTarget library prep workflows when bundled with SMRTbell prep kit 3.0

SMRTbell prep kit 3.0 (102-141-700) + auxilliary components

- Reagents for SMRTbell library construction + cleanup
- Supports 24 samples for manual PureTarget library prep workflows

PureTarget cleanup kit (103-708-100)

- Reagents for PureTarget library cleanup
- For use with PureTarget kit 24 (manual) or PureTarget kit 96 (automated) library prep workflows
- Supports cleanup of 24 PureTarget library pools

PureTarget kit 2.0 components

Component		Description
1		**Cas9 buffer** • For Cas 9 digestion of gDNA
2		**Phosphatase** • For blocking gDNA fragment ends
3		**Cas9 nuclease** • For Cas 9 digestion of gDNA
4		**PureTarget nuclease mix** • For nuclease Tx of SMRTbell libraries
5		**dA tail buffer** • For A-tailing reaction
6		**dATP** • For A-tailing reaction
7		**Taq DNA polymerase** • For A-tailing reaction

SMRTbell prep kit 3.0 + auxiliary components

Component		Description
1		**SMRTbell prep kit 3.0** • Contains core reagents for SMRTbell template construction
2		**Low TE buffer** • For DNA shearing and cleanup
3		**SMRTbell cleanup beads** • For DNA cleanup

PureTarget kit 24 bundle
103-707-900 (24 rxn)

PureTarget cleanup kit components

Component		Description
1		**PureTarget cleanup beads kit** • For performing bead-based cleanup of final PureTarget libraries
2		**PureTarget cleanup buffer kit** • Buffers needed for performing cleanup of final PureTarget libraries

PureTarget cleanup kit
103-708-100 (24 rxn)

[1] PureTarget kit 2.0 (103-632-900) can only be purchased as part of the PureTarget kit 24 bundle product (103-707-900) or the PureTarget kit 96 bundle product (103-708-000).
[2] PureTarget kit 24 bundle (103-707-900) reagent volumes are optimized for processing batches of 8 samples and reagent volumes may be insufficient to support batching of fewer than 8 samples.
[3] Guide RNA panel reagents [e.g., PureTarget repeat expansion panel 2.0 / PureTarget carrier panel / PureTarget control panel], PureTarget cleanup kit, and barcoded adapters are sold separately.

PureTarget kit 96 bundle[1,2] and PureTarget cleanup kit components

PureTarget kit 96 bundle includes PureTarget kit 2.0 and HPPK 96 to support automated PureTarget library prep

PureTarget kit 2.0 (103-632-900)

- Reagents for PureTarget library preparation
- Note: This kit **does not** include guide RNA reagents[3]
- Supports 96 samples for automated PureTarget library prep workflows when bundled with HiFi plex prep kit 96

HiFi plex prep kit 96 (103-122-800) + auxilliary components

- Reagents for SMRTbell library construction + cleanup
- Supports 96 samples for automated PureTarget library prep workflows

PureTarget cleanup kit (103-708-100)

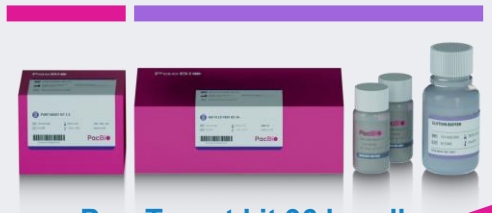
- Reagents for PureTarget library cleanup
- For use with PureTarget kit 24 (manual) or PureTarget kit 96 (automated) library prep workflows
- Supports cleanup of 24 PureTarget library pools

PureTarget kit 2.0 components

Component		Description
1		**Cas9 buffer** • For Cas 9 digestion of gDNA
2		**Phosphatase** • For blocking gDNA fragment ends
3		**Cas9 nuclease** • For Cas 9 digestion of gDNA
4		**PureTarget nuclease mix** • For nuclease Tx of SMRTbell libraries
5		**dA tail buffer** • For A-tailing reaction
6		**dATP** • For A-tailing reaction
7		**Taq DNA polymerase** • For A-tailing reaction

HiFi plex prep kit 96 + auxiliary components

Component		Description
1		**SMRTbell prep kit 3.0** • Contains core reagents for SMRTbell template construction
2		**Low TE buffer** • For DNA shearing and cleanup
3		**SMRTbell cleanup beads** • For DNA cleanup



PureTarget kit 96 bundle
103-708-000 (96 rxn)

Note: PureTarget kit 96 bundle is intended to support **automated** PureTarget library preparation workflows and is **not** intended to support manual workflows

PureTarget cleanup kit components

Component		Description
1		**PureTarget cleanup beads kit** • For performing bead-based cleanup of final PureTarget libraries
2		**PureTarget cleanup buffer kit** • Buffers needed for performing cleanup of final PureTarget libraries

PureTarget cleanup kit
103-708-100 (24 rxn)

[1] PureTarget kit 2.0 (103-632-900) can only be purchased as part of the PureTarget kit 24 bundle product (103-707-900) or the PureTarget kit 96 bundle product (103-708-000).
[2] PureTarget kit 96 bundle (103-707-900) reagent volumes support processing of batches of 96 samples in automated library prep workflows (batches <96 are not supported for automated workflows).
[3] Guide RNA panel reagents [e.g., PureTarget repeat expansion panel 2.0 / PureTarget carrier panel / PureTarget control panel], PureTarget cleanup kit, and barcoded adapters are sold separately.

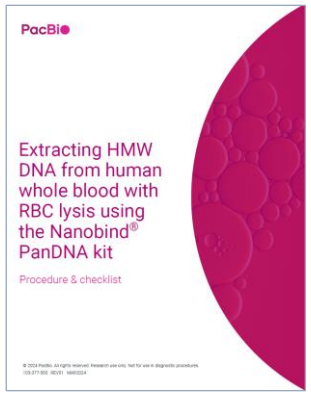
Other recommended kits & consumables for PureTarget sample DNA extraction, sample multiplexing and HiFi sequencing

Ancillary kits must be purchased separately from PureTarget kit bundle (103-390-400)

HMW DNA extraction

Nanobind PanDNA kit
(103-260-000)

Nanobind HT CBB kit
(102-762-700)



Procedure & checklist
(Documentation)

- Nanobind PanDNA kit [24 rxn] is recommended for manual DNA extraction from cultured cells, human blood and saliva for PureTarget panel applications[1]
- Nanobind HT CBB kit [96 rxn] is recommended for automated DNA extraction from blood and cells

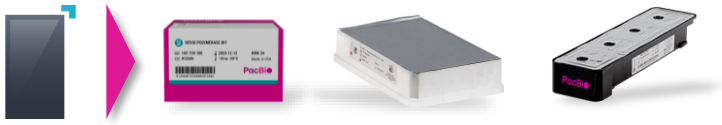
Sample multiplexing[2]

SMRTbell adapter index plate 96A/B/C/D
(102-009-200/102-547-800/102-547-900/102-548-000)

	1	2	3	4	5	6	7	8	9	10	11	12
A	bc2001	bc2009	bc2017	bc2025	bc2033	bc2041	bc2049	bc2057	bc2065	bc2073	bc2081	bc2089
B	bc2002	bc2010	bc2018	bc2026	bc2034	bc2042	bc2050	bc2058	bc2066	bc2074	bc2082	bc2090
C	bc2003	bc2011	bc2019	bc2027	bc2035	bc2043	bc2051	bc2059	bc2067	bc2075	bc2083	bc2091
D	bc2004	bc2012	bc2020	bc2028	bc2036	bc2044	bc2052	bc2060	bc2068	bc2076	bc2084	bc2092
E	bc2005	bc2013	bc2021	bc2029	bc2037	bc2045	bc2053	bc2061	bc2069	bc2077	bc2085	bc2093
F	bc2006	bc2014	bc2022	bc2030	bc2038	bc2046	bc2054	bc2062	bc2070	bc2078	bc2086	bc2094
G	bc2007	bc2015	bc2023	bc2031	bc2039	bc2047	bc2055	bc2063	bc2071	bc2079	bc2087	bc2095
H	bc2008	bc2016	bc2024	bc2032	bc2040	bc2048	bc2056	bc2064	bc2072	bc2080	bc2088	bc2096

- Each plate contains 96 indexed SMRTbell adapters in plate format (1 sample per index)
- E.g., plate 96A includes indexes bc2001–bc2096

HiFi sequencing



Revio system + SPRQ chemistry supports 8- to 96-plex PureTarget sample multiplexing

Vega system supports 8- to 48-plex PureTarget sample multiplexing

- Revio SPRQ polymerase kit supports up to **12 Revio SMRT Cells** for PureTarget libraries
- Vega polymerase kit supports up to **4 Vega SMRT Cells** for PureTarget libraries

[1] For genomic DNA extraction from blood, we recommend using the **red blood cell lysis protocol** described in ***Procedure & checklist – Extracting HMW DNA from human whole blood with RBC lysis using the Nanobind PanDNA kit*** (103-377-500) or using the whole blood extraction protocol described in ***Procedure & checklist – Extracting HMW DNA from human whole blood using Nanobind kits*** (102-573-500).

[2] **Note:** PureTarget automated library preparation procedure (103-740-800) supports up to 96-plex sample multiplexing through use of 96 different SMRTbell indexed adapters.

PureTarget supported use cases and experimental design considerations

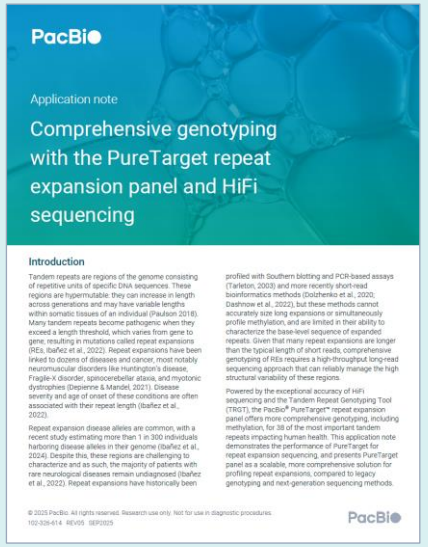
PureTarget repeat expansion panel 2.0 features 38 targets covering a variety of commonly screened tandem repeats associated with neurological disorders

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 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*

18 new targets

PureTarget repeat expansion panel 2.0 enables genotyping of critical pathogenic repeat expansion loci at scale

- Panel targets 38 repeat expansion loci and captures ~2 kb upstream and downstream of the repeat (total panel size = ~163 kb)
- For normal alleles, resulting sequences are 4–5 kb in length but reads for expanded alleles may be longer
- Sequencing results for samples with large expansions indicate that it is possible to span repeats up to 35 kb in length in a single read



PureTarget repeat expansion panel 2.0 (103-633-100)

Pooled guide RNAs for PureTarget repeat expansion panel 2.0 (38 genes). Supports 24 samples with manual PureTarget 24 kit and 96 samples with automated PureTarget 96 kit.

Application note – Comprehensive genotyping with the PureTarget repeat expansion panel and HiFi sequencing (102-326-614)[1]

[1] See ***Application note – Comprehensive genotyping with the PureTarget repeat expansion panel and HiFi sequencing*** (102-326-614) for recommendations about PureTarget repeat expansion panel analysis options. **Note:** PureTarget repeat expansion panel 2.0 kit does not support analysis of non-human samples as guide RNAs used in the panel are designed against the human genome.

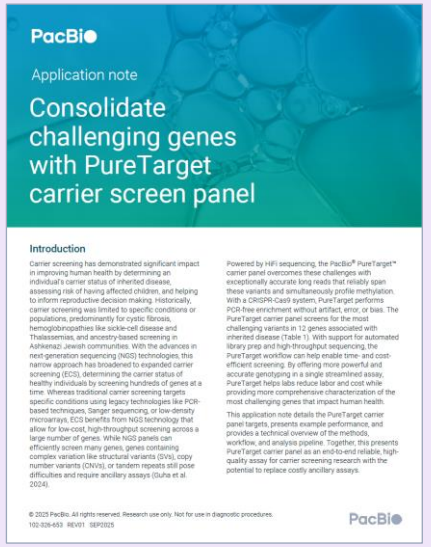
PureTarget supported use cases and experimental design considerations (cont.)

PureTarget carrier panel enables consolidated analysis of 12 challenging genes commonly genotyped with legacy non-NGS assays

Disease	Targets	Variant type
Hemophilia	*F8*	Inversions
Friedreich ataxia (FRDA)	*FXN*	Repeat Expansion
Fragile-X disease (FXS)	*FMR1*	Repeat Expansion
Congenital adrenal hyperplasia	*CYP21A2*	Small variants and copy number
Classical-like Ehlers-Danlos syndrome	*TNXB*	Small variants and copy number
Alpha thalassemia	*HBA1/2*	Deletions
Gaucher disease	*GBA*	Small variants and copy number
Spinal muscular atrophy	*SMN1/2*	Small variants and copy number
Early-infantile epileptic encephalopathy (EIEE1) and Partington syndrome (PRTS)	*ARX*	Repeat Expansion (N=2)
Beta thalassemia	*HBB*	Small variants
X-linked retinitis pigmentosa	*RPGR*	Small variants
Fragile X syndrome, FRAXE type	*AFF2*	Repeat Expansion

PureTarget carrier panel enables comprehensive, scalable screening of challenging carrier genes that are difficult to resolve with traditional technologies

- Panel targets 12 challenging genes and captures ~2 kb upstream and downstream of the gene (total panel size = ~141 kb)
- PureTarget carrier panel is a highly accurate and streamlined assay that can be run alongside routine short read panels to capture all expanded carrier screening targets.



PureTarget carrier Screening panel (103-633-200)

Pooled guide RNAs for PureTarget carrier panel (12 genes). Supports 24 samples with manual PureTarget 24 kit and 96 samples with automated PureTarget 96 kit.

Application note – Consolidate challenging genes with PureTarget carrier screen panel (102-326-653)[1]

1 See ***Application note – Consolidate challenging genes with PureTarget carrier screen panel*** (102-326-653) for recommendations about PureTarget carrier panel analysis options. **Note:** PureTarget carrier panel kit does not support analysis of non-human samples as guide RNAs used in the panel are designed against the human genome.

PureTarget supported use cases and experimental design considerations (cont.)

PureTarget custom panel option enables users to design target-specific panels to analyze biological targets of interest

Technical note – A practical guide to amplification-free custom PureTarget panels (102-326-652) provides guidance to customers on creating custom panels

Performance specifications
- Max and min panel size that has been tested
- Coverage for different fragment length

Types of custom designs
- Removing targets (from existing panel designs)
- Adding targets (by spiking in new guide RNA reagents to a control panel or other existing panel designs)
- Tiling and phasing

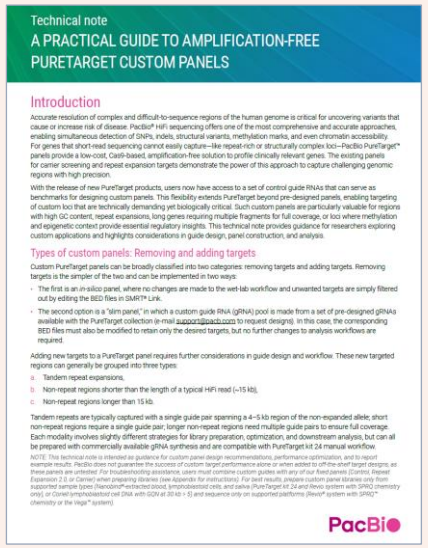
Designing guide RNAs
- Recommended design tools, vendors and products
- Success rate, common challenges

Protocol modifications
- Specific steps to modify in protocol, reagent concentrations etc.
- Spiking in custom guides to control panel
- Protocol modifications for tiled designs

Analysis recommendations
- SMRT Analysis target enrichment for coverage QC assessment
- Recommend PacBio GitHub tools for genotyping of different types of targets

Note about using custom panel designs

- PureTarget custom panels should include the PureTarget control panel, which contains a guide RNA mix to target 3 positive control genes (1 X-linked + 2 autosomal)
  - → Users may additionally spike in custom gRNA mixes for their own custom panel designs
- In all cases, we recommend first demonstrating success on the PureTarget repeat expansion panel or PureTarget carrier panel using supported sample types before designing your own custom panels


Technical note

A PRACTICAL GUIDE TO AMPLIFICATION-FREE PURETARGET CUSTOM PANELS

Introduction

Accurate resolution of complex and difficult-to-sequence regions of the human genome is critical for uncovering variants that cause or increase risk of disease. PacBio® HiFi sequencing offers one of the most comprehensive and accurate approaches, enabling simultaneous detection of SNPs, indels, structural variants, methylation marks, and even chromatin accessibility. For genes that short-read sequencing cannot easily capture—like repeat-rich or structurally complex loci—PacBio PureTarget™ panels provide a low-cost, Cas9-based, amplification-free solution to profile clinically relevant genes. The existing panels for carrier screening and repeat expansion targets demonstrate the power of this approach to capture challenging genomic regions with high precision.

With the release of new PureTarget products, users now have access to a set of control guide RNAs that can serve as benchmarks for designing custom panels. This flexibility extends PureTarget beyond pre-designed panels, enabling targeting of custom loci that are technically demanding yet biologically critical. Such custom panels are particularly valuable for regions with high GC content, repeat expansions, long genes requiring multiple fragments for full coverage, or loci where methylation and epigenetic context provide essential regulatory insights. This technical note provides guidance for researchers exploring custom applications and highlights considerations in guide design, panel construction, and analysis.

Types of custom panels: Removing and adding targets

Custom PureTarget panels can be broadly classified into two categories: removing targets and adding targets. Removing targets is the simpler of the two and can be implemented in two ways:

- The first is an *in-silico* panel, where no changes are made to the wet-lab workflow and unwanted targets are simply filtered out by editing the BED files in SMRT® Link.
- The second option is a "slim panel," in which a custom guide RNA (gRNA) pool is made from a set of pre-designed gRNAs available with the PureTarget collection (e-mail support@pacb.com to request designs). In this case, the corresponding BED files must also be modified to retain only the desired targets, but no further changes to analysis workflows are required.

Adding new targets to a PureTarget panel requires further considerations in guide design and workflow. These new targeted regions can generally be grouped into three types:

a. Tandem repeat expansions.
b. Non-repeat regions shorter than the length of a typical HiFi read (~15 kb).
c. Non-repeat regions longer than 15 kb.

Tandem repeats are typically captured with a single guide pair spanning a 4–5 kb region of the non-expanded allele; short non-repeat regions require a single guide pair; longer non-repeat regions need multiple guide pairs to ensure full coverage. Each modality involves slightly different strategies for library preparation, optimization, and downstream analysis, but can all be prepared with commercially available gRNA synthesis and are compatible with PureTarget kit 24 manual workflow.

NOTE: This technical note is intended as guidance for custom panel design recommendations, performance optimization, and to report example results. PacBio does not guarantee the success of custom target performance alone or when added to off-the-shelf target designs, as these panels are untested. For troubleshooting assistance, users must combine custom guides with any of our fixed panels (Control, Repeat Expansion 2.0, or Carrier) when preparing libraries (see Appendix for instructions). For best results, prepare custom panel libraries only from supported sample types (Revio/PromethION-extracted blood, lymphoblastoid cells, and saliva (PureTarget kit 24 and Revio system with SPRQ chemistry only); or Coriell lymphoblastoid cell DNA with GQN at 30 kb ± 5) and sequence only on supported platforms (Revio® system with SPRQ™ chemistry or the Vega™ system).

PacBio

PureTarget carrier Screening panel (103-633-200)

Pooled guide RNAs for PureTarget control panel (3 genes). Supports 24 samples with manual PureTarget 24 kit and 96 samples with automated PureTarget 96 kit.

Technical note – A practical guide to amplification-free PureTarget custom panels (102-326-652)[1]

[1] See ***Technical note – A practical guide to amplification-free PureTarget custom panels*** (102-326-652) for guidance surrounding designing guide RNAs for custom PureTarget panels.

PureTarget 2.0 performance specifications for supported sample types

PureTarget enables comprehensive characterization of repeat expansions and other difficult genes at scale[1]

Parameter	Specification	Notes
Target gene panel size	**PureTarget repeat expansion panel 2.0:** 38 genes (163 kb) **PureTarget carrier panel:** 12 genes (141 kb)	• See ***PureTarget Brochure*** (102-326-609) for list of target genes included in PureTarget repeat expansion panel 2.0 (103-633-100) and PureTarget carrier panel (103-633-200)
DNA input amount	**Automated library prep with PureTarget kit 96:** 1–1.5 µg per sample	• Max. total DNA input = 150 µg per Revio SMRT Cell
	Manual library prep with PureTarget kit 24: 1–4 µg per sample	• Max. total DNA input = 50 µg per Revio SMRT Cell or 100 µg per Vega SMRT Cell
DNA input quality	GQN30kb > 5	• 50% of mass of DNA molecules longer than 30 kb as measured on Femto Pulse (Agilent)
Target coverage	**Mean target coverage:** 100-fold or greater per 1 µg DNA per sample	• Mean coverage per 1 µg of input DNA from supported sample types[1] at max. sample multiplexing level (Revio SPRQ = 96, Vega = 48) • Higher coverage is possible with higher DNA input amounts and lower sample multiplexing
	Minimum target coverage: 20-fold per sample	• Minimum coverage per 1 µg of input DNA from supported sample types[1]
Sample multiplexing	**Vega system:** Up to 48-plex	• For up to 48-plex: PureTarget kit 24 bundle (103-707-900) supports smaller batches in multiples of 8 samples • For 96-plex: PureTarget kit 96 bundle (103-708-000) supports single batches of 96 samples
	Revio + SPRQ system: Up to 96-plex	
Library insert size	4 – 5 kb	• Inserts with expanded alleles will be longer
Methylation	5mC in CpG sites detected	• Methylation probabilities for CpG sites are encoded in BAM output file
PureTarget library prep protocol (Proc. & Checklist)	***Generating PureTarget libraries with PureTarget kit 24 – Manual protocol*** (103-740-700)	• Requires PureTarget kit 24 bundle (103-707-900)
	Generating PureTarget libraries with PureTarget kit 96 – Automation protocol (103-740-800)	• Requires PureTarget kit 96 bundle (103-708-000)
PureTarget data analysis workflow	**PureTarget repeat expansion panel 2.0** → SMRT Link PureTarget repeat expansion analysis application	• Produces target coverage summary QC stats and uses tandem repeat genotyping tool (TRGT) for variant calling and for visualization
	PureTarget carrier panel → SMRT Link Target enrichment analysis application & PureTarget carrier pipeline (PTCP) analysis	• SMRT Link Target enrichment analysis produces target coverage QC statistics • PTCP analysis workflow available through GitHub or PacBio Compatible partners enables variant calling for tandem repeat regions and hard genes
	PureTarget custom panel → SMRT Link Target enrichment analysis application & PacBio GitHub software tools for variant calling	• SMRT Link Target enrichment analysis produces target coverage QC statistics • Can use other PacBio GitHub software tools to perform variant calling

[1] Optimal performance is obtained using PacBio Nanobind extraction kits with **human blood or cell line samples.** Refer to ***Application note – Comprehensive genotyping with the PureTarget repeat expansion panel and HiFi sequencing*** (102-326-614) for the most up-to-date list of supported DNA extraction kits and sample types for PureTarget sequencing applications. Individual target coverage is lower for longer target regions or expanded alleles. The same library loaded on Revio SPRQ system will typically give higher coverage than Vega system.

PureTarget library preparation workflow details

Procedure & checklist – Generating PureTarget libraries with PureTarget kit 24 – Manual protocol (103-740-700)

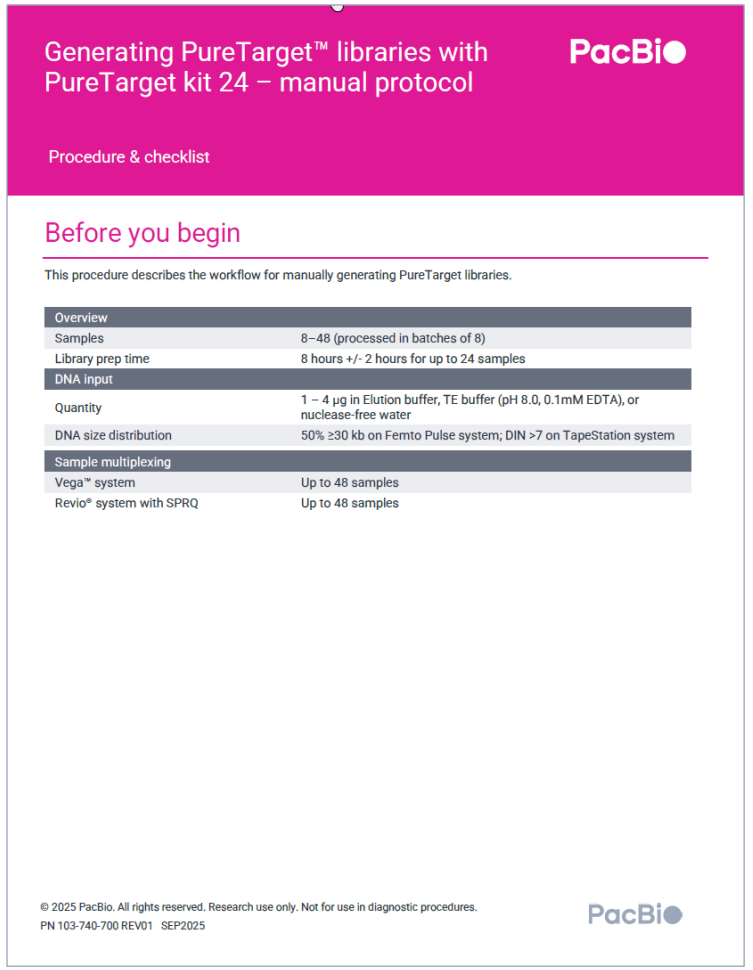
Procedure & checklist – Generating PureTarget libraries with PureTarget kit 24 – Manual protocol (103-740-700) describes the workflow for generating up to 48-plex PureTarget libraries using the **PureTarget kit 24** reagent bundle for sequencing on PacBio long-read systems

Procedure & checklist contents

1. DNA input QC recommendations and general best practices for reagent & sample handling.
2. Enzymatic workflow steps for performing targeted Cas9 digestion of input genomic DNA samples.
3. Enzymatic workflow steps for PureTarget SMRTbell library construction from Cas9-digested gDNA samples.
4. Workflow steps for final cleanup of PureTarget SMRTbell libraries using PureTarget cleanup beads
5. Workflow steps for sample setup ABC[1] and final dilution to prepare samples for sequencing

Note: Procedure 103-740-700 includes instructions for up to 48-plex PureTarget library construction using a **manual workflow**.

For instructions on high-throughput 96-plex PureTarget library construction using an **automated workflow**, refer to ***Procedure & checklist – Generating PureTarget libraries with PureTarget kit 96 – Automation protocol*** (103-740-800)



Generating PureTarget™ libraries with PureTarget kit 24 – manual protocol

PacBio

Procedure & checklist

Before you begin

This procedure describes the workflow for manually generating PureTarget libraries.

Overview	
Samples	8–48 (processed in batches of 8)
Library prep time	8 hours +/- 2 hours for up to 24 samples
DNA input	
Quantity	1 – 4 µg in Elution buffer, TE buffer (pH 8.0, 0.1mM EDTA), or nuclease-free water
DNA size distribution	50% ≥30 kb on Femto Pulse system; DIN >7 on TapeStation system
Sample multiplexing	
Vega™ system	Up to 48 samples
Revio® system with SPRQ	Up to 48 samples

PN 103-740-700 REV01 SEP2025

PacBio

PacBio Documentation (103-740-700)

[1] For primer annealing, polymerase binding & complex cleanup (ABC) and final dilution steps, follow sample setup instructions for PureTarget libraries in ***Procedure & checklist – Generating PureTarget libraries with PureTarget kit 24 – Manual protocol*** (103-740-700) – Do not use SMRT Link Sample Setup.

Key PureTarget library preparation and sequencing workflow steps [Manual]

Manual PureTarget library construction steps can be completed within 1 day[1]

Extraction & QC

DNA sample extraction — 2 – 2.5 hrs

Nanobind DNA extraction kit

Refer to Nanobind user guide documenation

Library construction

1 Input gDNA QC — 90 min

Qubit 4 fluorometer system (ThermoFisher Sci. Q33238)
DNA sizing QC tool (Femto pulse system or other tool)

2 gDNA repair[2] & SMRTbell bead cleanup — 2 hrs

Safe stop

PureTarget kit 2.0 (103-632-900)

3 Dephosphorylation — 30 min

PureTarget cleanup kit (103-708-100)

4 CAS-9 digestion & SMRTbell bead cleanup — 1.5 hrs

Safe stop

SMRTbell prep kit 3.0 (102-141-700)

5 dA tailing — 10 min

SMRTbell adapter index plate 96A/B/C/D
(102-009-200)

6 Adapter ligation & SMRTbell bead cleanup — 1 hr

Safe stop

PureTarget repeat expansion panel 2.0 (103-633-100) *or*
PureTarget carrier panel (103-633-200) *or*
PureTarget control panel(103-633-300)

7 Nuclease treatment & SMRTbell bead cleanup — 2 hrs

8 PureTarget library cleanup — 30 min

Safe stop

ABC

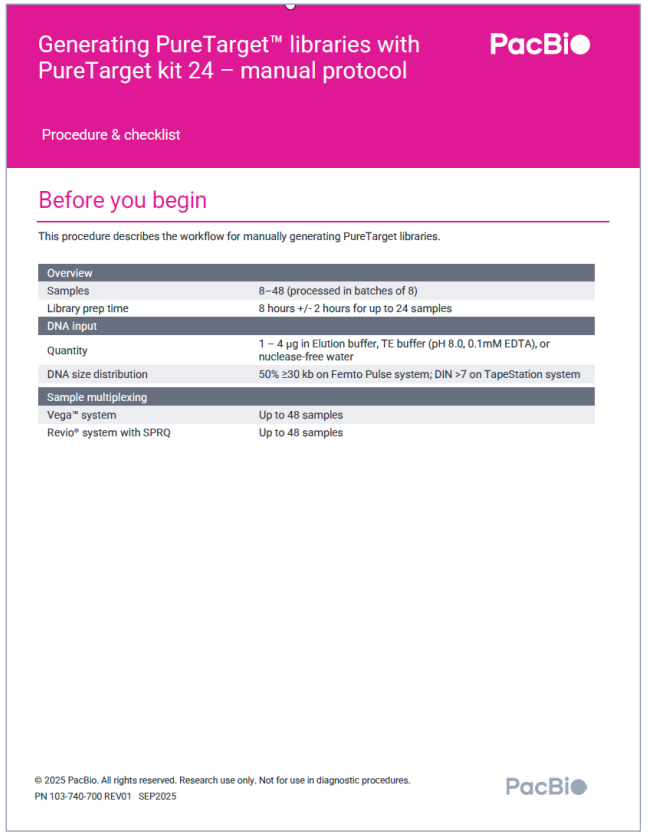
9 Annealing, binding & cleanup (ABC) — 1 hr

Revio SPRQ polymerase kit (103-496-900) *or*
Vega polymerase kit (103-426-500)

1 DAY

Manual PureTarget protocol reference

Procedure & checklist – Generating PureTarget libraries with PureTarget kit 24 – Manual protocol (103-740-700)


Generating PureTarget™ libraries with PureTarget kit 24 – manual protocol

PacBio

Procedure & checklist

Before you begin

This procedure describes the workflow for manually generating PureTarget libraries.

Overview	
Samples	8–48 (processed in batches of 8)
Library prep time	8 hours +/- 2 hours for up to 24 samples
DNA input	
Quantity	1 – 4 µg in Elution buffer, TE buffer (pH 8.0, 0.1mM EDTA), or nuclease-free water
DNA size distribution	50% ≥30 kb on Femto Pulse system; DIN >7 on TapeStation system
Sample multiplexing	
Vega™ system	Up to 48 samples
Revio® system with SPRQ	Up to 48 samples

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[1] Example workflow step times shown are for manually processing up 24 PureTarget samples using PureTarget kit 24 reagent bundle.
[2] This step repairs input genomic DNA, which increases target coverage when using lower quality gDNA. For Nanobind-extracted blood (whole blood or RBC lysis method) gDNA, this step *can* be skipped..

Key PureTarget library preparation and sequencing workflow steps [Automated]

Automated PureTarget library construction steps can be completed in 2 days[1]

Extraction & QC

DNA sample extraction — 2 – 2.5 hrs

Nanobind DNA extraction kit

Refer to Nanobind user guide documenation

Library construction

1 Input gDNA QC — 90 min

Qubit 4 fluorometer system (ThermoFisher Sci. Q33238)
DNA sizing QC tool (Femto pulse system or other tool)

Day 1

2 gDNA repair[2] & SMRTbell bead cleanup — 2.5 hrs

PureTarget kit 2.0 (103-632-900)

Safe stop

3 Dephosphorylation — 1.5 hrs

4 CAS-9 digestion & SMRTbell bead cleanup — 2 hrs

PureTarget cleanup kit (103-708-100)

Safe stop

Day 2

5 dA tailing — 30 min

HiFi plex prep kit 96 (103-122-800)

6 Adapter ligation & SMRTbell bead cleanup — 2 hrs

SMRTbell adapter index plate 96A/B/C/D
(102-009-200)

Safe stop

7 Nuclease treatment 1 & SMRTbell bead cleanup — 1.5 hrs

8 Nuclease treatment 2 & SMRTbell bead cleanup — 2 hrs

PureTarget repeat expansion panel 2.0 (103-633-100) *or*
PureTarget carrier panel (103-633-200) *or*
PureTarget control panel(103-633-300)

9 PureTarget library cleanup — 1 hr

Safe stop

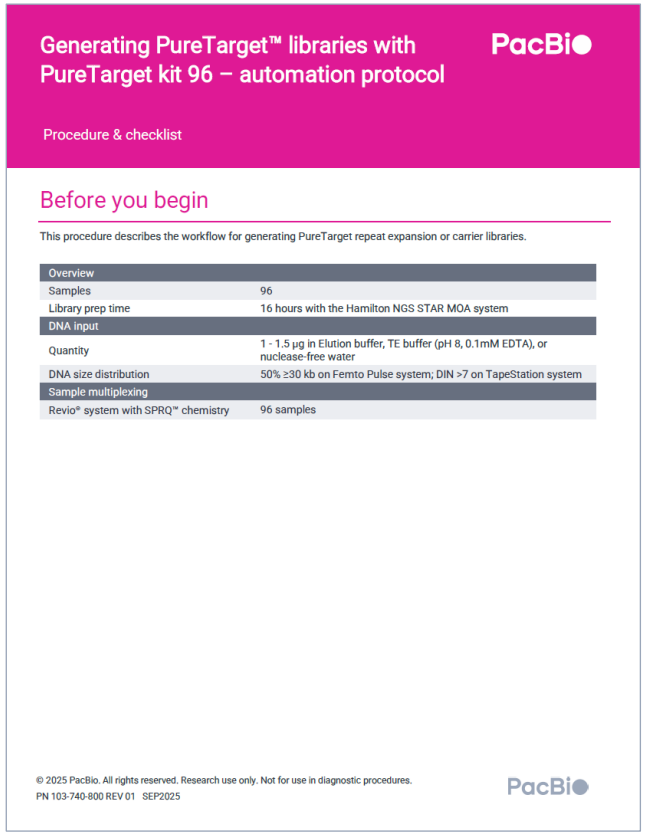
ABC

10 Annealing, binding & cleanup (ABC) — 1 hr

Revio SPRQ polymerase kit (103-496-900) *or*
Vega polymerase kit (103-426-500)

Automated PureTarget protocol reference

Procedure & checklist – Generating PureTarget libraries with PureTarget kit 96 – Automation protocol (103-740-800)



Generating PureTarget™ libraries with PureTarget kit 96 – automation protocol

PacBio

Procedure & checklist

Before you begin

This procedure describes the workflow for generating PureTarget repeat expansion or carrier libraries.

Overview	
Samples	96
Library prep time	16 hours with the Hamilton NGS STAR MOA system
DNA input	
Quantity	1 - 1.5 µg in Elution buffer, TE buffer (pH 8, 0.1mM EDTA), or nuclease-free water
DNA size distribution	50% ≥30 kb on Femto Pulse system; DIN >7 on TapeStation system
Sample multiplexing	
Revio® system with SPRQ™ chemistry	96 samples

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1 Example workflow step times shown are for automated processing 96 PureTarget samples using PureTarget kit 96 reagent bundle.
2 gDNA repair is required unless using Nanobind-extracted blood genomic DNA using the RBC lysis method or whole blood extraction..

General best practices recommendations for preparing PureTarget libraries

Supported input sample types and DNA extraction methods

PureTarget performance is sensitive to DNA quality, which can be impacted by DNA extraction method and sample type used

- It is recommended that users start with high-quality genomic DNA extracted with PacBio Nanobind extraction kits
- When using sample types and extraction methods other than the above, we recommend users:[1]
  - First, demonstrate success using supported sample types, starting with an 8-plex and increasing sample quantity thereafter
  - Introduce new sample types or extraction methods in limited numbers, for example, 3 or fewer new sample types in an 8-plex of otherwise controls

Table 1 (below). Officially supported samples types for PureTarget library preparation workflows.

Supported sample types	Supported DNA extraction methods
Human blood	Extracted by Nanobind RBC lysis PanDNA kit [103-377-500], Nanobind Whole blood manual CBB kit/PanDNA kit [102-573-500], Nanobind HT 200 µL protocol [103-028-100], or Nanobind HT 1 mL protocol [103-028-100]
Human B-lymphocyte cell lines	Extracted by Nanobind CBB kit [103-394-500 / 102-573-600], Nanobind PanDNA kit [103-394-500 / 102-573-600], or Nanobind CBB HT kit [103-028-100]; Coriell cell line genomic DNA (GQN30kb ≥5)
Human saliva (PureTarget kit 24 only)	Extracted by Nanobind PanDNA kit [103-544-000], Nanobind CBB kit [103-544-000], or Nanobind CBB HT kit **NOTE:** Saliva DNA samples are only supported for **manual** library prep workflows using PureTarget kit 24 [Up to 48-plex][2] • A diluted AMPure cleanup is **required** before the start of library prep for this sample type. Please refer to Appendix A1 in ***Procedure & checklist – Generating PureTarget libraries with PureTarget kit 24 – Manual protocol*** (103-740-700) for details.

Table 2 (right). Other demonstrated sample types and DNA extraction methods for PureTarget applications. Total sample multiplex level and total DNA input mass intro library prep (across all samples) is shown for unsupported sample types and extraction kits listed in table. Demonstrated sample configurations shown in table resulted in successful library prep and sequencing but have not been technically validated. Results may vary. All samples are human-derived.

Other sample types	Demonstrated DNA extraction method	Sample multiplexing level [Total DNA input]
Blood	QIAsymphony (QIAGEN)	Up to 96-plex [130 µg]
Blood	Puregene Blood kit (QIAGEN)	Up to 96 plex and [130 µg]
Blood	Biosystems MagMAX DNA Multi-Sample Ultra 2.0 Kit	Up to 32 plex and [40 µg]
Blood	Chemagic DNA blood kit (Revvity)	Up to 24 plex and [48 µg]
Blood	NucleoMag (Macherey-Nagel)	Up to 24-plex and [42 µg]
Blood	Monarch HMW DNA Extraction Kit for Cells & Blood (NEB)	Up to 8 plex and [20 µg]
Blood	QIAGEN Genomic-tips (QIAGEN)	Up to 8 plex and [16 µg]
Blood	FlexiGene DNA Whole Blood Kit (QIAGEN)	Up to 8 plex and [16 µg]
Blood	Bionano SP Blood and Cell Culture DNA Isolation Kit	Up to 8 plex and [16 µg]
Saliva	QIAsymphony (QIAGEN)	Up to 48 plex and [75 µg]
Brain tissue	Nanobind DNA extraction from animal tissue protocol	Up to 8-plex and [16 µg]
Skeletal muscle	Nanobind DNA extraction from animal tissue protocol	Less than 8-plex and [<16 µg]
iPSCs	Nanobind DNA extraction from cultured adherent cells protocol	Up to 16-plex and [36 µg]
Fibroblasts	Nanobind DNA extraction from cultured adherent cells protocol	Up to 8-plex and [16 µg]
Myoblasts	Nanobind DNA extraction from cultured adherent cells protocol	Less than 8-plex and [<16 µg]
iPSCs	QIAGEN Genomic-tips (QIAGEN)	Up 8-plex and [21 µg]
Fibroblasts	QIAGEN Genomic-tips (QIAGEN)	Up to 8-plex and [16 µg]
Corneal endothelial (CEC) cell culture	Bionano SP Blood and Cell Culture DNA Isolation Kit	Up to 8-plex and [16 µg]
Fibroblasts	Monarch HMW DNA Extraction Kit for Cells & Blood (NEB)	Up to 8-plex and [20 µg]
Brain tissue	Monarch HMW DNA Extraction Kit for Cells & Blood (NEB)	Less than 8-plex and [<16 µg]

[1] See ***Application note – Comprehensive genotyping with PureTarget repeat expansion panel and HiFi sequencing*** (102-326-614) for more information about samples that are officially supported or have been demonstrated to result in successful sequencing but have not been technically validated.

[2] Although 48-plex designs are supported for saliva samples, since saliva DNA is typically lower in quality and shorter in length, it is recommended to start at the gDNA repair step with 2 µg of (diluted AMPure-cleaned) saliva DNA per sample, with a maximum of 24-plex on Revio SPRQ or Vega chemistry for the best performance.

General best practices recommendations for preparing PureTarget libraries (cont.)

DNA sizing QC

- Agilent Femto Pulse system[1] is recommended for the accurate sizing of genomic DNA samples
  - Femto Pulse system enables simple, rapid sizing QC of genomic DNA and SMRTbell libraries, and conserves sample by using femtogram ranges of input DNA
    - Resolves fragments 1,300 bp to 165 kb using gDNA 165 kb Analysis kit (can resolve 100 – 6,000 bp using Ultra Sensitivity NGS kit)
    - Requires <1 ng of sample DNA
    - Can analyze up to 12 samples in <1.5 hrs
    - Outputs quality metrics such as Genomic Quality Number (GQN)[2] to quickly score integrity of HMW gDNA
- Alternative DNA sizing tools (e.g., TapeStation system) may be used if a Femto Pulse system is unavailable
  - However, caution should be used when interpreting results from other tools that employ constant-field electrophoresis technology
    - These technologies tend to inflate the true size of the gDNA (or library) and should only be used for qualitative assessment of whether an experiment was successful (e.g., intact library) rather than for accurate measurement of fragment size distributions

Femto Pulse system (Agilent Technologies)

DNA quantification QC

- For DNA quantification QC, we recommend using a quantification assay specific for double-stranded DNA (dsDNA) such as the Qubit 1X dsDNA high sensitivity assay kit[3] (Thermo Fisher Scientific)
  - **Note:** We do not recommend quantification with UV-Vis Spectrophotometers (e.g. NanoDrop) that measure all nucleic acids in a sample. For example, measuring all nucleic acid will inflate the true concentration of gDNA in samples.

Qubit 4 fluorometer (Thermo Fisher Scientific)

[1] See ***Product Note – HiFi WGS sequencing with the Agilent Femto Pulse system*** (102-326-561) for more details.
[2] See ***Application Note – Quality Metrics for Nucleic Acids with the Agilent Fragment Analyzer and Femto Pulse Systems*** (Agilent 5994-0521EN)
[3] Alternatively, for high-throughput applications DNA quantification QC may be performed with a microplate reader using the Quant-iT 1X dsDNA high sensitivity assay kit (Thermo Fisher Scientific).

General best practices recommendations for preparing PureTarget libraries (cont.)

Recommended genomic DNA input amount and quality

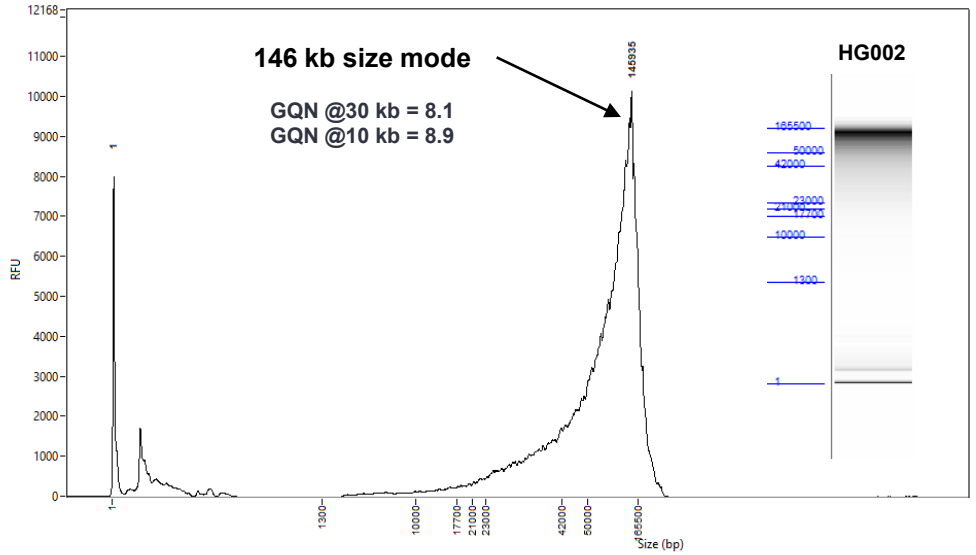
DNA input quality

- 50% or more of the DNA should be ≥30 kb for this PureTarget library prep protocol
  - → This corresponds to a genome quality number (GQN) of 5.0 or higher at 30 kb based on the Agilent Femto Pulse system

DNA quality	Femto Pulse genome quality score[1]	Notes
DNA size distribution (Femto pulse system)	50% ≥30 kb (GQN30kb ≥5.0)	• For low-quality human gDNA, it is still feasible to proceed but lower on-target coverage performance is expected[2]

- **Note:** Removal of RNA with RNAse is **required** for any DNA extraction method used
  - → Failure to remove RNA may result in sequencing inhibition

Any degradation present should be due to shearing from extraction process and **not** from poor sample handling/storage or biochemical processes



(Right figure.) Example DNA sizing QC analysis of a high-quality HG002 human genomic DNA sample using a Femto Pulse system with Genomic DNA 165 kb kit.

[1] If using an Agilent TapeStation for input gDNA sizing QC, then a DIN value >7 is recommended.

[2] **Important:** The HiFi yield and HiFi mean read length of a sequencing run are directly proportional to the quality of the genomic DNA input. To maximize yield and target coverage per SMRT Cell, start with high quality gDNA containing minimal DNA below 10 kb, and with >50% mass over 30 kb. High quality gDNA will typically have a higher percent library recovery and HiFi sequencing yield.

General best practices recommendations for preparing PureTarget libraries (cont.)

Recommended genomic DNA input amount and quality

Genomic DNA input amount required for manual library preparation using PureTarget kit 24

- Recommended mass of input gDNA is **2 µg per sample** to ensure there are sufficient gene copies to load and maximize sequencing coverage
  - Manual PureTarget library prep protocol supports input gDNA mass amounts in the range of 1–4 µg per sample.
- Recommend maximum total DNA mass per sequencing platform/chemistry is outlined in the following table across all multiplexed samples
  - For example, a multiplex of 48 samples on Revio (+SPRQ) should not exceed 1 µg per sample on average

Recommended genomic DNA input mass amounts across all multiplexed samples for manual PureTarget library preparation for Revio and Vega system sequencing platforms.

Minimum and maximum total gDNA mass for manual PureTarget library preparation using PureTarget kit 24		
Genomic DNA input for 1 SMRT Cell (8- to 48-plex)	**Vega system chemistry**	**Revio system with SPRQ chemistry**
Minimum	16 µg	16 µg
Maximum	100 µg	50 µg

Genomic DNA input amount required for automated library preparation using PureTarget kit 96

- Automated PureTarget library prep protocol supports **1 – 1.5 µg per sample**
  - 1.3 – 1.5 µg per sample is highly recommended for samples going through the gDNA repair step, to ensure sufficient mass to achieve optimal target coverage.
  - Maximum mass of 150 µg of gDNA input (across 96 samples) is recommended for a single Revio SPRQ SMRT cell.

Recommended genomic DNA input mass amounts across all multiplexed samples for automated PureTarget library preparation for Revio system sequencing platform.

Minimum and maximum total gDNA mass for automated PureTarget library preparation using PureTarget kit 96	
Genomic DNA input for 1 SMRT Cell (96-plex)	**Revio system with SPRQ chemistry**
Minimum	96 µg
Maximum	150 µg

General best practices recommendations for preparing PureTarget libraries (cont.)

Recommended genomic DNA input amount and quality

Expected PureTarget library construction yield for manual and automated workflows

- Overall PureTarget library construction yield is dependent on input gDNA quality and size
  - The recovery from input gDNA to completed SMRTbell library typically ranges between 0.02 – 0.2% for manual workflows and between 0.01 – 0.1% for automated workflows (includes PureTarget library construction using PureTarget kit 2.0 and PureTarget library cleanup using PureTarget cleanup kit)

PureTarget library construction step	Manual PureTarget library prep [Up to 48-plex][1]		Automated PureTarget library prep [96-plex][2]	
	DNA or SMRTbell step recovery	DNA or SMRTbell overall recovery	DNA or SMRTbell step recovery	DNA or SMRTbell overall recovery
Starting input genomic DNA	100%	**100%**	100%	**100%**
Post-gDNA repair & 1.2X SMRTbell bead cleanup	70 – 90%	**70 – 90%**	70 – 90%	**70 – 90%**
Post-CAS9 digestion & 1.2X SMRTbell bead cleanup	50 – 80%	**35 – 72%**	50 – 80%	**35 – 72%**
Post-adapter ligation & 1.0X SMRTbell bead cleanup	70 – 90%	**24 – 65%**	70 – 90%	**24 – 65%**
Post-nuclease treatment & 1.2X SMRTbell bead cleanup[3]	0.2 – 1%	**0.05 – 0.5%**	0.1 – 0.3%	**0.02 – 0.1%**
Post-PureTarget library cleanup using PureTarget cleanup kit	40 – 60%	**0.02 – 0.2%**	40 – 60%	**0.005 – 0.05%**

[1] Library prep recovery yields are shown for manual PureTarget library prep workflow using PureTarget kit 24 reagent bundle.
[2] Library prep recovery yields are shown for automated PureTarget library prep workflow using PureTarget kit 96 reagent bundle.
[3] **Note:** Automated PureTarget library prep workflow using PureTarget kit 96 employs **two** rounds of nuclease treatment.

General best practices recommendations for preparing PureTarget libraries (cont.)

Reagent and sample handling

- Room temperature is defined as any temperature in the range of 18–23°C for this protocol.
- Do not vortex enzymes.
- Bring SMRTbell cleanup beads to room temperature. Always vortex immediately prior to use.
- Bring Qubit reagents to room temperature prior to use.
- Thaw frozen reagents at room temperature. Place on ice after thawing.
- Keep master mixes involving temperature-sensitive reagents on ice until use.
- Quick-spin all reagents in a microcentrifuge to collect liquid at the bottom prior to use.
- Samples can be stored at the specified temperature at the safe stopping points listed in the protocol.

PureTarget library prep reagents

[Store kits at -20°C]

PureTarget kit 2.0		
Step used	**Tube color**	**Reagent**
Dephosphorylation	Blue	Phosphatase
Cas9 digestion	Green	Cas9 Nuclease
Dephosphorylation/Cas9 digestion	Red	Cas9 buffer
dA tailing	Orange	dA tail buffer
dA tailing	Violet	*Taq* DNA Polymerase
dA tailing	Yellow	dATP
Nuclease treatment	White	Pure Target Nuclease mix
PureTarget panel		
Cas9 digestion	White	Repeat expansion panel 2.0 *or*
	Purple	Carrier panel *or*
	Orange	Control panel
SMRTbell prep kit 3.0		
gDNA repair	Purple	Repair buffer
gDNA repair	Blue	End repair mix
gDNA repair	Green	DNA repair mix
Adapter ligation	Yellow	Ligation mix
Adapter ligation	Red	Ligation enhancer
Nuclease treatment	Light green	Nuclease mix
Nuclease treatment	Light purple	Nuclease buffer
SMRTbell adapter index plate		
Adapter ligation	White plate	SMRTbell adapter index plate 96A, 96B, 96C, or 96D

PureTarget cleanup beads kit[1]

[Store kit at room temperature]

Component	Tube color
PureTarget cleanup wash buffer*	Bottle, white
PureTarget cleanup binding buffer	Bottle, white
PureTarget cleanup beads	Clear

PureTarget cleanup buffer kit

[Store kit at room temperature]

Component	Tube color
PureTarget cleanup buffer 1	Red
PureTarget cleanup buffer 2	Blue
PureTarget cleanup buffer 3	Green

Polymerase kit[2]

[Store kit at -20°C]

Component	Tube color
Annealing buffer	Light blue
Standard sequencing primer	Light green
Polymerase buffer	Yellow
Loading buffer	Green
Dilution buffer	Blue
Sequencing polymerase	Purple
Sequencing control	Red

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[1] **Important:** Prior to the 1st use of the PureTarget cleanup beads kit, add 15mL of 200 Proof ethanol to PureTarget Cleanup Wash Buffer and mix well. Store bottles upright to prevent leakage.
[2] **Note:** Bring the Loading buffer to room temperature prior to use. The Loading buffer is light sensitive and should be protected from light when not in use.

Multiplexed sample processing workflow for manual library preparation method using PureTarget kit 24 [For up to 48-plex]

Procedure & checklist – Generating PureTarget libraries (103-329-400)

1. Input gDNA QC
2. gDNA repair & SMRTbell bead cleanup
   - Safe stop
3. Dephosphorylation
4. CAS-9 digestion & SMRTbell bead cleanup
   - Safe stop
5. dA tailing
6. Adapter ligation & SMRTbell bead cleanup
   - Safe stop
7. Nuclease treatment & SMRTbell bead cleanup
8. PureTarget library cleanup
   - Safe stop
9. Annealing, binding & cleanup (ABC)

To process 8, 16 or 24 samples

Steps 1 - 7 — **PureTarget library construction** (8/16/24 samples)

Step 8 — **PureTarget library cleanup** (8/16/24 samples)

Step 9 — **ABC** (8/16/24 samples)

Sequence pooled samples on one Revio or Vega SMRT Cell

Note: Each PureTarget kit 24 supports the preparation of **24** samples. Therefore, **two** PureTarget library prep kits are required to generate a Revio/Vega run of **32, 40, or 48 samples**.

To process 32, 40 or 48 samples

PureTarget library construction workflow 1 (24 samples)

Steps 1 - 7

PureTarget library construction workflow 2 (8/16/24 samples)

Pool up to 48 samples

Step 8 — **PureTarget library cleanup** (32/40/48 samples)

Step 9 — **ABC** (32/40/48 samples)

Sequence pooled samples on one Revio or Vega SMRT Cell

- With manual library prep workflows using PureTarget kit 24, up to 48 samples can be barcoded and sequenced per Revio or Vega SMRT Cell[1]
- Multiplexed samples should be processed in batches of 8/16/24/32/40/48 samples
- For runs with 8/16/24 samples (left figure), follow protocol steps 1 – 9 to process all samples in a **single** workflow

- For runs with 32/40/48 samples (right figure), process **two** workflows in parallel:
  - **Workflow 1:** Process 24 samples following protocol steps 1 – 7 (PureTarget library construction); and
  - **Workflow 2:** Process an additional 8, 16 or 24 samples following protocol steps 1 – 7
- At the end of step 7, pool 32/40/48 samples and proceed with PureTarget library cleanup (Step 8) and ABC (Step 9)

[1] For automated library prep workflows using PureTarget kit 96, 96 samples can be processed in a single workflow and pooled for sequencing on a single Revio SMRT Cell (see ***Procedure & checklist – Generating PureTarget libraries with PureTarget kit 96 – Automation protocol*** (103-740-800)).

Input DNA quality control

Before you begin, evaluate the size distribution of the input DNA using an Agilent Femto Pulse system or TapeStation system to determine whether it is suitable for the protocol

1 Input gDNA QC
↓
2 gDNA repair & SMRTbell bead cleanup
↓ Safe stop
3 Dephosphorylation
↓
4 CAS-9 digestion & SMRTbell bead cleanup
↓ Safe stop
5 dA tailing
↓
6 Adapter ligation & SMRTbell bead cleanup
↓ Safe stop
7 Nuclease treatment & SMRTbell bead cleanup
↓
8 PureTarget library cleanup
↓ Safe stop
9 Annealing, binding & cleanup (ABC)

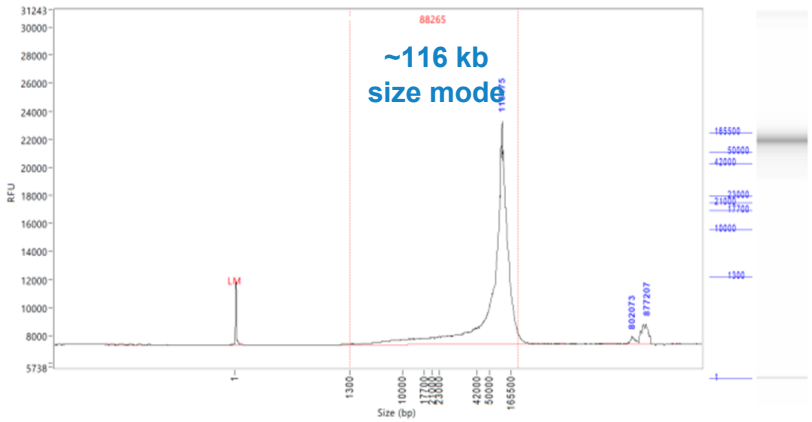
1. Input DNA quality control and dilution

✔	Step	Instructions
	1.1	Bring the Qubit 1X dsDNA HS working solution and standards to room temperature.
	1.2	Pulse vortex and/or pipette-mix each sample 5 times to homogenize the DNA in solution. Note: For viscous input DNA, it is important to homogenize the extracted DNA prior to start of the protocol. To homogenize the DNA, pulse-vortex and/or pipette-mix full sample volume with standard (not wide bore) tips. These steps will maintain HMW of your DNA but will improve accuracy of quantification and subsequent handling.
	1.3	Quick-spin each sample to collect liquid.
	1.4	Take a 1 µL aliquot from each sample and dilute with 9 µL of Elution buffer or water.
	1.5	Measure DNA concentration with a Qubit fluorometer using the 1X dsDNA HS kit.
	1.6	Measure DNA size distribution with a Femto Pulse system using the gDNA 165 kb analysis kit. Or Measure DNA size distribution with a TapeStation system using the Genomic DNA ScreenTape Analysis
	1.7	Aliquot or bring **1- 4 µg DNA** to a final volume of 43 µL per sample (24- 93 ng/µL) with nuclease-free water or Elution buffer.

SAFE STOPPING POINT - Store at 4°C

- For **viscous** input DNA, it is important to homogenize the extracted DNA prior to starting the protocol
- To homogenize the DNA, pulse-vortex and/or pipette-mix full sample volume with standard (not wide bore) tips.
- These steps will maintain HMW of your DNA but will improve accuracy of quantification and subsequent handling

- Protocol requires high-quality, high molecular weight (HMW) human gDNA with ≥50% of the mass of DNA in molecules of length ≥30 kb, or Genome quality number (GQN) at 30 kb of ≥5 based on Agilent Femto Pulse system (or DIN >7 based on Agilent TapeStation).
- For manual workflows, recommended input DNA amount is 2 µg per sample to ensure sufficient gene copies to load and maximize sequencing coverage. This protocol supports 1-4 µg input DNA per sample.[1]



Example Femto Pulse genomic DNA sizing QC analysis results for high-molecular weight genomic DNA extracted from a human whole blood sample using Nanobind PanDNA kit.

[1] For manual PureTarget library prep workflows, the standard input DNA mass recommended for PureTarget libraries is **2 µg**. Users who wish to increase coverage of a sample may use up to 4 µg of DNA for library prep. If higher coverage is needed, we recommend multiple preps of 4 µg for the sample and combining the data during analysis.

Genomic DNA repair and SMRTbell bead cleanup

This step repairs input genomic DNA, which increases target coverage when using lower quality gDNA.

1. Input gDNA QC
2. gDNA repair & SMRTbell bead cleanup
   - Safe stop
3. Dephosphorylation
4. CAS-9 digestion & SMRTbell bead cleanup
   - Safe stop
5. dA tailing
6. Adapter ligation & SMRTbell bead cleanup
   - Safe stop
7. Nuclease treatment & SMRTbell bead cleanup
8. PureTarget library cleanup
   - Safe stop
9. Annealing, binding & cleanup (ABC)

2.1 Genomic DNA repair

Step	Instructions
2.1	Add the following components to a microcentrifuge tube. Adjust component volumes for the number of samples being prepared, plus overage according to the table below.

gDNA repair master mix

Reagent	Component	Volume per sample	8 reactions*	16 reactions*	24 reactions*
Purple	Repair buffer	5 µL	48 µL	96 µl	144 µl
Green	DNA repair mix	1.8 µL	17.3 µL	34.6 µl	51.8 µl
Blue	End repair mix	0.2 µL	1.9 µL	3.8 µl	5.8 µl
	Total volume	7µL	67.3 µL	134.4 µl	201.6 µl

*20% overage included

2.2	Pipette-mix and quick-spin the gDNA repair master mix.
2.3	Add 7 µL of the gDNA repair master mix to each gDNA sample from step 1.7 for a total reaction volume of 50 µL.
2.4	Pipette-mix and quick-spin.
2.5	Run the DNA repair thermocycler program. Set the lid temperature to ≥65°C.

Step	Time	Temperature
1	60 min	37°C
2	30 min	52°C
3	Hold	4°C

1.2X SMRTbell bead cleanup
(Steps 2.6 – 2.21)

- ***IMPORTANT!*** For Nanobind extracted blood (whole blood or RBC lysis method) gDNA, this step can be skipped. For **other** sample types or extraction methods, the gDNA repair step is **required** to ensure sufficient coverage.
- ***IMPORTANT!*** for Nanobind extracted saliva gDNA, a 3.1X 35% AMPure bead cleanup is **required** before the start of library prep.[1]

- Prepare a reaction master mix by adding the required components in the order and volume listed to a new microcentrifuge tube
- Adjust component volumes for the number of samples being prepared (8-plex, 16-plex or 24-plex), plus 15% overage according to table
- ***IMPORTANT!*** Prepare the master mix immediately before use. Keep the DNA repair mix on ice and immediately return the DNA repair mix back to freezer (-20°C) after use.

- Run **DNA repair** thermocycler program
- Set lid temperature to ≥65°C (if lid temp. is not programmable, leave at 95–105°C)

[1] **Note:** For human saliva DNA samples extracted by Nanobind PanDNA or Nanobind CBB, a diluted (35%) AMPure bead cleanup is **required** to be performed **before** proceeding with the genomic DNA repair step. Please refer to Appendix A1 in ***Procedure & checklist – Generating PureTarget libraries with PureTarget kit 24 – Manual protocol*** (103-740-700) for details.

Genomic DNA repair and SMRTbell bead cleanup (cont.)

This step repairs input genomic DNA, which increases target coverage when using lower quality gDNA. [For Nanobind extracted blood (whole blood or RBC lysis method) gDNA, this step can be skipped.]

1. Input gDNA QC
2. gDNA repair & SMRTbell bead cleanup
   - Safe stop
3. Dephosphorylation
4. CAS-9 digestion & SMRTbell bead cleanup
   - Safe stop
5. dA tailing
6. Adapter ligation & SMRTbell bead cleanup
   - Safe stop
7. Nuclease treatment & SMRTbell bead cleanup
8. PureTarget library cleanup
   - Safe stop
9. Annealing, binding & cleanup (ABC)

2.6 1.2X SMRTbell bead cleanup

✔	Step	Instructions
	2.6	Add 60 µL of resuspended, room-temperature SMRTbell cleanup beads to each sample.
	2.7	Pipette-mix the beads until evenly distributed. Bead clumping may occur and is not a concern. Avoid over-pipetting as it may cause DNA/bead mixture to stick to the pipette tip.
	2.9	Incubate at room temperature for 10 minutes to allow DNA to bind beads.
	2.10	Place on a magnetic separation until beads separate fully from the solution.
	2.11	Slowly remove the cleared supernatant without disturbing the beads. Discard the supernatant.
	2.12	Slowly dispense 200 µL, or enough to cover the beads, of freshly prepared 80% ethanol into each well. After 30 seconds, remove the 80% ethanol and discard.
	2.13	Repeat the previous step.
	2.14	Remove residual 80% ethanol and discard.
	2.15	Remove sample from the magnetic rack. Immediately add 68 µL of **Elution buffer** to each well and resuspend the beads.
	2.17	Incubate at room temperature for 5 minutes to elute DNA.
	2.18	Place on a magnetic separation rack until beads separate fully from the so…
	2.19	Slowly transfer 68 µL cleared eluate without disturbing the beads to a new tube… Discard the old tube strip with beads.
	2.20	Optional QC step: Take a 1 µL each sample and measure DNA concentration with a Qu… …meter using the 1X dsDNA HS kit. Expect a step recovery of 70-90% per sample.
	2.21	Proceed to the next step of the protocol.

SAFE STOPPING POINT – Store at 4°C over… …20°C long term

- Add **60 µL** of SMRTbell cleanup beads to each sample and pipette-mix DNA/bead mixture until evenly distributed
  - **Note: Bead clumping may occur and is not a concern**
  - **Avoid over-pipetting** as it may cause DNA/bead mixture to stick to the pipette tip and clog the tip

- **Optional QC step:** Measure eluted DNA concentration using Qubit 1X dsDNA HS kit
- Expected step recovery is **70-90%** per sample

- Elute sample in **68 µL** of EB buffer
  - Pipette-mix DNA/bead mixture until beads are fully resuspended and no clumps appear

PacBio

Dephosphorylation

This step dephosphorylates the genomic DNA 5’ termini, which prevents adapter ligation to non-targeted genomic DNA.

1 Input gDNA QC
↓
2 gDNA repair & SMRTbell bead cleanup
↓ Safe stop
3 Dephosphorylation
↓
4 CAS-9 digestion & SMRTbell bead cleanup
↓ Safe stop
5 dA tailing
↓
6 Adapter ligation & SMRTbell bead cleanup
↓ Safe stop
7 Nuclease treatment & SMRTbell bead cleanup
↓
8 PureTarget library cleanup
↓ Safe stop
9 Annealing, binding & cleanup (ABC)

3. Dephosphorylation

✔	Step	Instructions
	3.1	Add the following components to a new microcentrifuge tube. Adjust component volumes for the number of samples being prepared, plus overage according to the table below.

Dephosphorylation master mix

✔	Tube color	Component	Volume per sample	8 reactions*	16 reactions*	24 reactions*
	Red	Cas9 buffer	8 µL	76.8 µl	153.6 µl	230.4 µl
	Blue	Phosphatase	5 µL	48 µL	96 µl	144 µl
		Total volume	13 µL	124.8 µL	249.6 µl	374.4 µl

*20% overage included

	3.2	Pipette-mix the Dephosphorylation master mix and quick-spin in a microcentrifuge to collect liquid.
	3.3	Add 13 µL of the Dephosphorylation master mix to each sample from step 2.19 for total reaction volume of 80 µL.
	3.4	Pipette-mix each sample.
	3.5	Run the Dephosphorylation thermocycler program. Set the lid temperature to ≥95°C.

Step	Time	Temperature
1	20 min	37°C
2	5 min	85°C
3	Hold	4°C

	3.6	Proceed to the next step of the protocol.

- Prepare a reaction master mix by adding the required components in the order and volume listed to a new microcentrifuge tube
- Adjust component volumes for the number of samples being prepared (8-plex, 16-plex or 24-plex), plus 15% overage according to table

- Run **dephosphorylation** thermocycler program
- Set the lid temperature to ≥95°C (if lid temp. is not programmable, leave at 95–105°C)

PacBio

Cas9 digestion and SMRTbell bead cleanup

This step enables digestion of double-stranded DNA at targeted regions mediated by Cas9-gRNA ribonucleoprotein complex formation

1. Input gDNA QC
2. gDNA repair & SMRTbell bead cleanup
   - Safe stop
3. Dephosphorylation
4. CAS-9 digestion & SMRTbell bead cleanup
   - Safe stop
5. dA tailing
6. Adapter ligation & SMRTbell bead cleanup
   - Safe stop
7. Nuclease treatment & SMRTbell bead cleanup
8. PureTarget library cleanup
   - Safe stop
9. Annealing, binding & cleanup (ABC)

4.1 Cas9 digestion

✔	Step	Instructions
	4.1	Add the following components to a new microcentrifuge tube. Adjust component volumes for the number of samples being prepared, plus overage according to the table below.

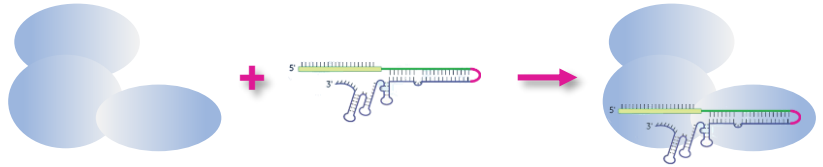
Cas9 digestion master mix

Reagent	Component	Volume per sample	8 reactions*	16 reactions*	24 reactions*
	Nuclease free water	2 µL	19.2 µL	38.4 µl	57.6 µl
Red	Cas9 buffer	0.5 µL	4.8 µL	9.6 µl	14.4 µl
Green	Cas9 Nuclease	0.5 µL	4.8 µL	9.6 µl	14.4 µl
variable	PureTarget panel**	2 µL	19.2 µL	38.4 µl	57.6 µl
	Total volume	5.0 µL	45.6 µL	91.2 µl	136.8 µl

*20% overage included
**Repeat expansion 2.0, Carrier, or Control panel with custom guide RNA

4.2	Pipette-mix Cas9 digestion master mix and quick-spin in a microcentrifuge to collect liquid.
4.3	Add 5 µL of the Cas9 digestion master mix to each sample for total reaction volume of 85 µL.
4.4	Pipette-mix each sample
4.5	Run the Cas-9 digestion thermocycler program. Set the lid temperature to ≥65°C.

Step	Time	Temperature
1	60 min	37°C
2	Hold	4°C



- Prepare a reaction master mix by adding the required components in the order and volume listed to a new microcentrifuge tube
- Adjust component volumes for the number of samples being prepared (8-plex, 16-plex or 24-plex), plus 15% overage according to table

- Run **Cas-9 digestion** thermocycler program
- Set the lid temperature to ≥65°C (if lid temp. is not programmable, leave at 95–105°C)

4.7 1.2X SMRTbell bead cleanup

✔	Step	Instructions
	4.7	Add 102 µL of resuspended, room-temperature SMRTbell cleanup beads to each sample.
	4.8	Pipette-mix the beads slowly until evenly distributed. Bead clumping may occur and is not a concern. Avoid over-pipetting as it may cause DNA/bead mixture to stick to the pipette tip.

- Perform **1.2X** SMRTbell bead cleanup
- **Optional QC step:** Measure eluted DNA concentration using Qubit 1X dsDNA HS kit
- Expected step recovery is **~50-80%** per sample

PacBio

dA tail

This step enables A-tailing of DNA 3’ ends after Cas9-gRNA digestion at targeted regions

1 Input gDNA QC
↓
2 gDNA repair & SMRTbell bead cleanup
↓ Safe stop
3 Dephosphorylation
↓
4 CAS-9 digestion & SMRTbell bead cleanup
↓ Safe stop
5 dA tailing
↓
6 Adapter ligation & SMRTbell bead cleanup
↓ Safe stop
7 Nuclease treatment & SMRTbell bead cleanup
↓
8 PureTarget library cleanup
↓ Safe stop
9 Annealing, binding & cleanup (ABC)

5. dA tail

✔	Step	Instructions

5.1 Add the following components to a new microcentrifuge tube. Adjust component volumes for the number of samples being prepared, plus overage according to the table below.

dA-tailing master mix

Reagent	Component	Volume per sample	8 reactions*	16 reactions*	24 reactions*
	Nuclease free water	3.5	34 µL	68 µl	102 µl
Orange	dA tail buffer	5 µL	48 µL	96 µl	144 µl
Yellow	dATP (100 mM)	0.2 µL	2 µL	4 µl	6 µl
Violet	*Taq* DNA polymerase	1.3 µL	12.5 µL	25 µl	37.5 µl
	Total volume	10 µL	96 µL	192 µl	288 µl

*20% overage included

5.2 Pipette-mix the dA-tailing master mix and quick-spin in a microcentrifuge to collect liquid.

5.3 Add 10 µL of the dA-tailing master mix to each sample for a total reaction volume of 50 µL.

5.4 Pipette-mix each sample.

5.5 Run the dA-tail thermocycler program. Set the lid temperature to ≥95°C.

Step	Time	Temperature
1	1 min	37°C
2	5 min	72°C
3	Hold	4°C

5.6 Proceed to the next step of the protocol.

- Prepare a reaction master mix by adding the required components in the order and volume listed to a new microcentrifuge tube
- Adjust component volumes for the number of samples being prepared (8-plex, 16-plex or 24-plex), plus 15% overage according to table

- Run **dA-tail** thermocycler program
- Set the lid temperature to ≥95°C (if lid temp. is not programmable, leave at 95–105°C)

PacBio

Adapter ligation and SMRTbell bead cleanup

This step ligates the indexed SMRTbell adapters to the ends of each targeted DNA fragment

1. Input gDNA QC
2. gDNA repair & SMRTbell bead cleanup
   - Safe stop
3. Dephosphorylation
4. CAS-9 digestion & SMRTbell bead cleanup
   - Safe stop
5. dA tailing
6. Adapter ligation & SMRTbell bead cleanup
   - Safe stop
7. Nuclease treatment & SMRTbell bead cleanup
8. PureTarget library cleanup
   - Safe stop
9. Annealing, binding & cleanup (ABC)

6.1 Adapter ligation

Step	Instructions
6.1	Add 4 µL SMRTbell adapter index to each sample from Section 5 and pipette mix.

- Add **4 µL SMRTbell adapter index plate 96A** to each sample and then tap-mix or pipette up and down 10 times (do not vortex)

6.2 Add the following to a new microcentrifuge tube. Adjust component volumes for the number of samples being prepared, plus overage according to the table below.

Note: Aspirate and dispense viscous ligation mix slowly to reduce liquid sticking to the exterior and interior wall of the pipette tip.

Ligation master mix

Reagent	Component	Volume per sample	8 reactions*	16 reactions*	24 reactions*
Yellow	Ligation mix	30 µL	264 µL	528 µl	792 µl
Red	Ligation enhancer	1 µL	8.8 µL	17.6 µl	26.4 µl
	Total volume	31 µL	272.8 µL	545.6 µl	818.4 µl

*10% overage included

- Prepare a reaction master mix by adding the required components in the order and volume listed to a new microcentrifuge tube
- Adjust component volumes for the number of samples being prepared (8-plex, 16-plex or 24-plex), plus 15% overage according to table

6.5 Pipette-mix each sample

6.6 Run the Adapter ligation thermocycler program. Heating of the lid is not necessary.

Step	Time	Temperature
1	30 min	20°C or RT
2	Hold	4°C

- Run **adapter ligation** thermocycler program
- Heating of the lid is not necessary

6.7 1X SMRTbell bead cleanup

Step	Instructions
6.7	Add 85 µL (1X) of resuspended, room-temperature SMRTbell cleanup beads to each sample.
6.8	Pipette-mix until evenly distributed.
6.9	Incubate at room temperature for 10 minutes to allow DNA to bind beads.

- Perform **1X** SMRTbell bead cleanup[1]
- **Optional QC step:** Measure eluted DNA concentration using Qubit 1X dsDNA HS kit
- Expected step recovery is **~70-90%** per sample

[1] **Note:** For **automated** PureTarget library prep workflow using PureTarget kit 96, the 96 ligation reactions are pooled after the addition of SMRTbell beads into 16 pools for the post-ligation clean-up step – refer to ***Procedure & checklist – Generating PureTarget libraries with PureTarget kit 96 – Automation protocol*** (103-740-800) for details regarding sample pooling procedure.

Nuclease treatment and SMRTbell bead cleanup

Nuclease treatment step removes unligated DNA fragments[1] that have not formed complete (intact) SMRTbell templates

1. Input gDNA QC
2. gDNA repair & SMRTbell bead cleanup
   - Safe stop
3. Dephosphorylation
4. CAS-9 digestion & SMRTbell bead cleanup
   - Safe stop
5. dA tailing
6. Adapter ligation & SMRTbell bead cleanup
   - Safe stop
7. **Nuclease treatment & SMRTbell bead cleanup**
   - Safe stop
8. PureTarget library cleanup
   - Safe stop
9. Annealing, binding & cleanup (ABC)

7.1 Nuclease treatment

✔	Step	Instructions
	7.1	Add the following components to a new microcentrifuge tube. Adjust component volumes for the number of samples being prepared, plus overage according to the table below.

Nuclease master mix

✔ Reagent	Component	Vol per sample	8 reactions*	16 reactions*	24 reactions*
Light purple	Nuclease buffer	5 µL	44 µl	88 µl	[illegible]
Light Green	Nuclease mix	5 µL	44 µl	88 µl	132 µl
White	PureTarget Nuclease	0.5 µL	4.4 µl	8.8 µl	13.2 µl
	Total volume	10.5 µL	92.4 µL	184.8 µl	277.2 µl

*10% overage included

7.2	Pipette-mix the Nuclease master mix and quick-spin in a microcentrifuge to collect liquid.
7.3	Add 10.5 µL of Nuclease master mix to 40 µL of each sample for a total volume of 50.5 µl.
7.4	Pipette-mix each sample.
7.5	Run the Nuclease treatment thermocycler program. Set the lid temperature to ≥65°C.

Step	Time	Temperature
1	60 min	37° C
2	Hold	4° C

- Prepare a reaction master mix by adding the required components in the order and volume listed to a new microcentrifuge tube
- Adjust component volumes for the number of samples being prepared (8-plex, 16-plex or 24-plex), plus 15% overage according to table

- Run **nuclease treatment** thermocycler program
- Set the lid temperature to ≥65°C (if lid temp. is not programmable, leave at 95–105°C)

Sample pooling and 1.2X SMRTbell bead cleanup
(Steps 7.6 – 7.19)

Concentrate samples with 1.2X SMRTbell bead cleanup
(Steps 7.20 – 7.33)

Note:

- **Manual** PureTarget library prep workflow using PureTarget kit 24 employs ***one*** **round of nuclease treatment**
- **Automated** PureTarget library prep workflow using PureTarget kit 96 employs ***two*** **rounds of nuclease treatment.**[2]

[1] Unligated DNA fragments comprise the majority of DNA fragments present prior to this step.

[2] Refer to ***Procedure & checklist – Generating PureTarget libraries with PureTarget kit 96 – Automation protocol*** (103-740-800) for details regarding nuclease treatment and post-nuclease cleanup steps for automated library prep workflows.

Nuclease treatment and SMRTbell bead cleanup (cont.)

Pool samples for post-nuclease cleanup and concentration steps using SMRTbell cleanup beads

1 Input gDNA QC
↓
2 gDNA repair & SMRTbell bead cleanup
↓ Safe stop
3 Dephosphorylation
↓
4 CAS-9 digestion & SMRTbell bead cleanup
↓ Safe stop
5 dA tailing
↓
6 Adapter ligation & SMRTbell bead cleanup
↓ Safe stop
7 Nuclease treatment & SMRTbell bead cleanup
↓
8 PureTarget library cleanup
↓ Safe stop
9 Annealing, binding & cleanup (ABC)

7.6 Sample pooling and 1.2X SMRTbell bead cleanup

✔	Step	Instructions
	7.6	In a 1.5 mL DNA LoBind tube combine nuclease treated libraries from step 7.5 in groups of 8 (8 x 50.5 µL) for a final sample volume of 404 µL.
	7.7	Add 485 µL of SMRTbell cleanup beads to each pooled nuclease-treated sample. Pipette-mix until the beads are evenly distributed.
	7.8	Quick-spin the tube in a microcentrifuge to collect all liquid from the sides of the tubes.
	7.15	Remove from the magnetic rack. Immediately add 55 µL of Elution buffer to each tube and resuspend the beads by pipette mixing until resuspended.
	7.16	Incubate at room temperature for 5 minutes to elute DNA.
	7.17	Place on a magnetic separation rack until beads separate fully from the solution.
	7.18	Slowly transfer 55 µL cleared eluate from each nuclease reaction and pool into a new 1.5 mL DNA LoBind tube.

Plex	Total pooled volume
8-plex	55 µl
16-plex	110 µl*
24-plex	165 µl*
48-plex	330 µl*

* requires concentration step if volume is >55 µl – proceed to step 7.20

	7.19	Proceed to the step 7.20 if the pooled volume is greater than **55 µL**; otherwise proceed to step 7.32 for QC.

7.20 Concentrate samples with 1.2X SMRTbell bead cleanup

✔	Step	Instructions
	7.20	Add **1.2X volume** of SMRTbell cleanup beads to the sample from step 7.18.

Plex	Total pooled volume	SMRTbell cleanup beads volume

	7.32	QC step: Take a **1 µL** aliquot from each sample and measure DNA concentration with a Qubit fluorometer using the **1X dsDNA HS kit.** **Expect an overall recovery of 0.05-0.5% relative to the starting gDNA total mass.**
	7.33	Proceed to the next step of the protocol.

- Pool nuclease-treated libraries from Step 7.5 in **groups of 8 (i.e., 8 x 50.5 µL)** for a final sample vol. of **404 µL**[1]
- Perform **1.2X** SMRTbell bead cleanup by adding **485 µL** of SMRTbell cleanup beads to each 8-plex pool

- Elute each 8-plex pooled sample in **55 µL** of EB buffer

- Slowly transfer **55 µL** of cleared eluate from **all** tubes into a **single** new 1.5 mL DNA LoBind tube
- Proceed to **Step 7.20** if total pooled sample volume **>55 µL**; otherwise proceed directly to **Step 7.32** for QC

- **If Step 7.18 total pooled volume >55 µL:** Concentrate samples by adding **1.2X** SMRTbell cleanup beads to pooled samples from Step 7.18

Plex	Total pooled volume	SMRTbell cleanup beads volume
16-plex	110 µl	132 µl
24-plex	165 µl	198 µl
48-plex	330 µl	396 µl

- Elute concentrated samples in **55 µL** of EB buffer

- **For manual workflows:** Expect an overall recovery of **0.05-0.5%** relative to the starting input gDNA total mass[1]
  - Higher-than-expected yields may indicate inefficient nuclease digestion, or sample incompatibility. Refer to Appendix A2 for recommendations to further digest sample

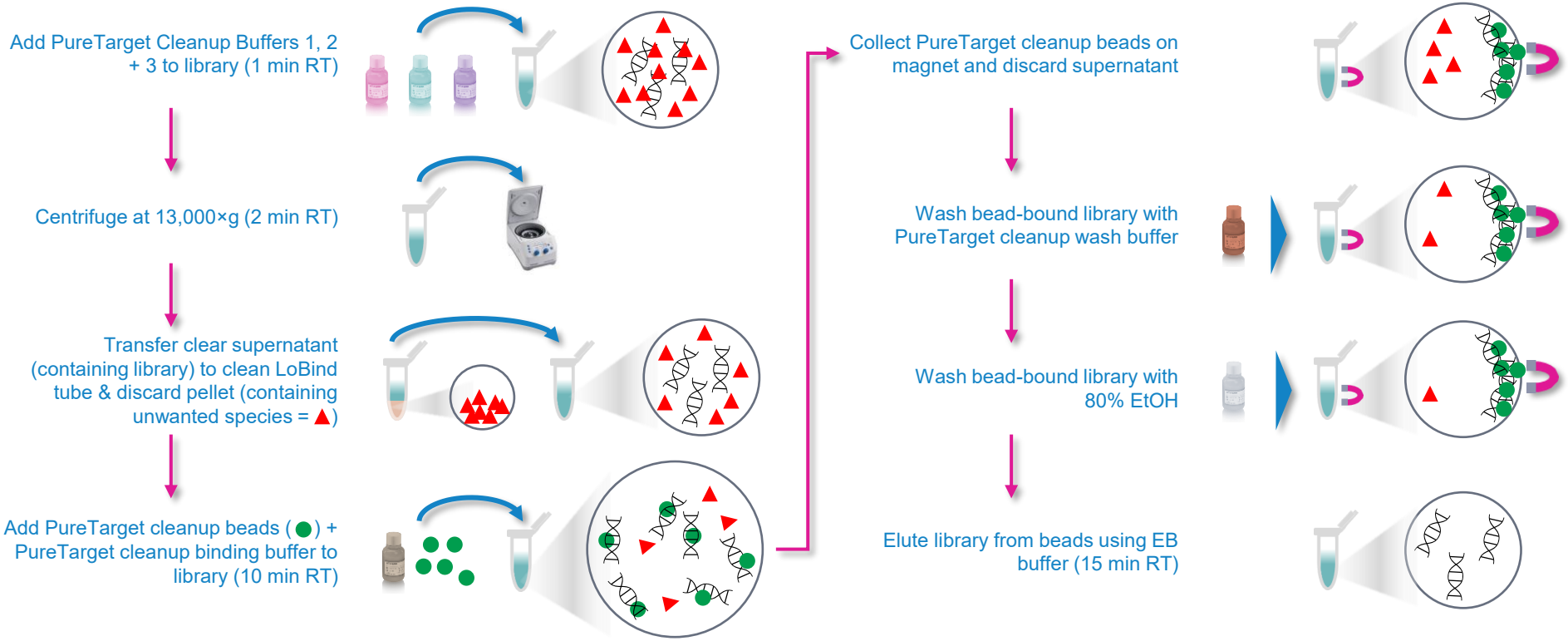
[1] Refer to ***Procedure & checklist – Generating PureTarget libraries with PureTarget kit 96 – Automation protocol*** (103-740-800) for details regarding post-nuclease cleanup procedure and expected recovery yields (~0.01-0.1%) for **automated** library prep workflows using PureTarget kit 96, which differ from those shown here for **manual** library prep workflows using PureTarget kit 24.

PureTarget library cleanup

Perform final library cleanup using PureTarget cleanup kit to prepare the SMRTbell library for sequencing

1. Input gDNA QC
2. gDNA repair & SMRTbell bead cleanup
   - Safe stop
3. Dephosphorylation
4. CAS-9 digestion & SMRTbell bead cleanup
   - Safe stop
5. dA tailing
6. Adapter ligation & SMRTbell bead cleanup
   - Safe stop
7. Nuclease treatment & SMRTbell bead cleanup
8. **PureTarget library cleanup**
   - Safe stop
9. Annealing, binding & cleanup (ABC)

PureTarget library cleanup procedure overview



- Perform one cleanup reaction per final pool of 8 – 48 PureTarget samples for manual library prep workflows using PureTarget kit 24[1]
- All reagents in the PureTarget cleanup kit should be stored at room-temperature; do not chill or freeze any reagent
- Vortex buffers in the PureTarget cleanup kit prior to use
- Prior to the first use of the PureTarget cleanup beads kit, add 15 mL of 200 Proof ethanol to PureTarget cleanup wash buffer and mix well

[1] Perform one cleanup reaction per final pool of **96** PureTarget samples for **automated** library prep workflows using PureTarget kit 96 (refer to ***Procedure & checklist – Generating PureTarget libraries with PureTarget kit 96 – Automation protocol*** (103-740-800) for further details regarding PureTarget library cleanup step).

PureTarget library cleanup (cont.)

Perform final library cleanup using PureTarget cleanup kit to prepare the SMRTbell library for sequencing

1 Input gDNA QC
↓
2 gDNA repair & SMRTbell bead cleanup
↓ Safe stop
3 Dephosphorylation
↓
4 CAS-9 digestion & SMRTbell bead cleanup
↓ Safe stop
5 dA tailing
↓
6 Adapter ligation & SMRTbell bead cleanup
↓ Safe stop
7 Nuclease treatment & SMRTbell bead cleanup
↓
8 PureTarget library cleanup
↓ Safe stop
9 Annealing, binding & cleanup (ABC)

8. PureTarget Cleanup of the SMRTbell library

✔	Step	Instructions
	8.1	Add **28 µL PureTarget cleanup buffer 1** (●) to **55 µL SMRTbell library** from step 7.31 and pipette-mix.
	8.2	Add the following components to a new microcentrifuge tube. **PureTarget cleanup buffer 2+3 mix** ✔ Reagent: Blue — PureTarget cleanup buffer 2 — 14 µL — 18 µl ✔ Reagent: Green — PureTarget cleanup buffer 3 — 14 µL — 18 µl Total volume — 28 µL — 36 µl Pulse vortex to mix and quick-spin. Important: PureTarget cleanup buffer 2 and buffer 3 must be pre-mixed prior to adding to sample. If added individually to sample, sample recovery may be low.
	8.3	Add 28 µL PureTarget cleanup buffer 2+3 mix to the mixture from step 8.1 and pipette-mix. The solution will turn opaque and cloudy after homogenous mixing. Incubate at room temperature for 1 minute.
	8.4	Centrifuge at 13,000 ×g for 2 minutes at room temperature.
	8.5	Taking care to avoid the pellet, transfer 95 µL of the clear supernatant to a clean 1.5 mL LoBind tube. Discard the tube with pellet.
	8.6	Thoroughly resuspend PureTarget cleanup beads by pulse-vortexing and quick spin. Add 5 µL of fully resuspended PureTarget cleanup beads to 95 µL of the sample from step 8.5.
	8.7	Add 100 µL of PureTarget cleanup binding buffer to the sample from step 8.6.
	8.8	Pipette-mix until evenly distributed. Incubate at room temperature for **15 mins**, with **periodic pipette-mixing to ensure that beads are resuspended during the entire incubation**.
	8.9	Quick-spin the tube in a microcentrifuge to collect all liquid from the sides of the tubes.
	8.10	Place the tube in a magnetic separation rack until beads separate fully from the solution.
	8.11	Slowly remove the cleared supernatant without disturbing the beads. Discard the supernatant.
	8.12	Slowly dispense 500 µL of PureTarget cleanup wash buffer (with ethanol added) into the tube. Remove the tube from the magnetic separation rack and gently resuspend the beads. Close the tube cap and invert the tube 5 times to wash off any residual PureTarget Cleanup Binding Buffer.
	8.13	Place the tube in a magnetic separation rack until beads separate fully from the solution.
	8.14	Slowly remove the cleared supernatant without disturbing the beads. Discard the supernatant.

- Add **PureTarget cleanup buffers 1, 2 and 3** to SMRTbell library sample (**55 µL**) in the order shown
  - → Prepare a master mix of PureTarget cleanup Buffer 2 and Buffer 3 by combining equal volumes of each (16 µL per buffer)
  - → Add PureTarget cleanup buffer 1 to SMRTbell library **before** adding the master mix containing PureTarget cleanup buffers 2 & 3
- **Note:** After adding the PureTarget cleanup buffer 2/3 mixture to the sample, the solution will turn **opaque and cloudy** after homogenous mixing; a white jelly-like pellet will form after centrifugation

- Prior to the first use of the PureTarget cleanup beads kit, **add 15 mL of 200 Proof ethanol to PureTarget cleanup wash buffer** and mix well

PacBio

PureTarget library cleanup (cont.)

Perform final library cleanup using PureTarget cleanup kit to prepare the SMRTbell library for sequencing

1. Input gDNA QC
2. gDNA repair & SMRTbell bead cleanup
   - Safe stop
3. Dephosphorylation
4. CAS-9 digestion & SMRTbell bead cleanup
   - Safe stop
5. dA tailing
6. Adapter ligation & SMRTbell bead cleanup
   - Safe stop
7. Nuclease treatment & SMRTbell bead cleanup
8. **PureTarget library cleanup**
   - Safe stop
9. Annealing, binding & cleanup (ABC)

8. PureTarget Cleanup of the SMRTbell library (cont.)

✔	Step	Instructions
	8.15	Slowly dispense 500 µL of fresh 80% ethanol into the tube without disturbing the beads.
	8.16	After 30 seconds, remove the 80% ethanol and discard.
	8.17	It is critical to remove residual 80% ethanol for efficient elution: • Remove the tube from the magnetic separation rack. • Quick-spin the tube in a microcentrifuge. • Place the tube back in a magnetic separation rack until beads separate fully from the solution. • Remove residual 80% ethanol and discard. • Air dry the bead pellet for 2 mins.
	8.18	Remove the tube from the magnetic rack. Add 26 µL of Elution buffer and resuspend the beads by gentle vortexing.
	8.19	Incubate at room temperature for 15 minutes to elute DNA, with gentle vortexing every 5 mins to fully resuspend the beads.
	8.20	Place the tube in a magnetic separation rack until beads separate fully from the solution.
	8.21	Slowly transfer 26 µL cleared supernatant without disturbing the beads to a new 1.5 mL DNA LoBind tube.
	8.22	QC step: Take a 1 µL aliquot from the sample and measure DNA concentration with a Qubit fluorometer using the 1X dsDNA HS kit. Expect a step recovery of 40-60%, and an overall recovery of 0.02-0.2% relative to starting gDNA total mass. For example, starting with 1.0 µg gDNA per sample for a 48-plex prep to load on Revio with SPRQ, the final mass of the pooled 48-plex library recovered at this step is expected to be in the range of 10 ng– 100 ng.

SAFE STOPPING POINT – Store at 4°C overnight or -20°C long term

Proceed to **Step 9** to perform sample setup (**ABC**) and final dilution (**Do not use** SMRT Link Sample Setup)

Elute cleaned library (containing up to 48-plex) into **26 µL** of EB

- **Take 1 µL** to perform DNA concentration QC using Qubit dsDNA HS kit
- Use remaining **25 µL** to proceed with sample setup (ABC[1])

- **QC step:** Measure DNA concentration using Qubit 1X dsDNA HS kit
- **For manual workflows:** Expected recovery is **~0.02 – 0.2%** relative to starting input gDNA mass **(e.g.,** ~10 ng – 100 ng of final library per 48-plex when starting with 1.0 µg per sample)[1]
- **Note:** Significantly higher recoveries could indicate a sample type incompatibility or an issue with the nuclease treatment step

For primer annealing, polymerase binding & complex cleanup (ABC) and final dilution steps, follow sample setup instructions provided in ***Procedure & checklist – Generating PureTarget libraries with PureTarget kit 24 – Manual protocol*** (103-740-700) [or ***Procedure & checklist – Generating PureTarget libraries with PureTarget kit 96 – Automation protocol*** (103-740-800) if using automation] – Do not use SMRT Link Sample Setup

[1] For **automated** library prep workflows using PureTarget kit 96: Expect an overall recovery of 0.005-0.05% relative to starting gDNA total mass, and a step recovery of 40-60%. For example, starting with 1.5 µg input per sample, the final mass of a pooled 96-plex library recovered at this step is expected to be in the range of 7.2 ng–72 ng.

PureTarget sequencing preparation workflow details

Sample setup and run design recommendations for PureTarget libraries

Key sample setup and run design setup parameters for Revio and Vega systems

Workflow	Key setup parameters	Vega system recommended settings for PureTarget libraries	Revio system recommended settings for PureTarget libraries
Sample setup	Library type	Standard	
	Primer	Standard sequencing primer	
	Polymerase kit	Vega polymerase kit	Revio SPRQ polymerase kit
Run design	Application type	PureTarget repeat expansion / PureTarget carrier / PureTarget custom	
	Library type	Standard	
	Insert size (bp)	5000[1]	
	Library concentration (pM)	<user-specified>[2]	
	Movie acquisition time	24 hours	
	Use adaptive loading	N/A	NO[3]
	Data options	Include base kinetics = NO Consensus Mode = MOLECULE	
	Analysis options[4]	**PureTarget repeat expansion panel 2.0:** Add Analysis = YES Analysis Workflow = PureTarget repeat expansion	
		PureTarget carrier panel: Add Analysis = YES Analysis Workflow = Target Enrichment[5]	
		PureTarget custom panel: Add Analysis = YES Analysis Workflow = Target Enrichment[5]	

For **PureTarget** samples, specify **Insert Size = 5000 bp**[1]

[1] **Note:** This '5000 bp' value only serves as a **placeholder** since the **actual average insert size distribution of PureTarget repeat expansion samples may vary**.

If you calculate your PureTarget library concentration, enter it – but if not, enter "**0**"[2]

[2] **Note:** For PureTarget samples, run conditions are not impacted by insert size or library concentration.

IMPORTANT! For Revio system, specify **Use Adaptive Loading = NO**[3]

[3] **Note:** In SMRT Link v25.3, Adaptive Loading is ON by default for all Revio system run designs. For PureTarget libraries, specify **Adaptive Loading = NO** to enable correct sample immobilization conditions to be used on the Revio system. PureTarget samples should not be included in the same run design as other sample types that require Adaptive Loading to be enabled (If an attempt is made to save a Revio run design with Adaptive Loading = YES and the run includes one or more PureTarget samples, then an error window will appear prompting the user to specify NO for Use Adaptive Loading.)

[4] Users who prefer to use command line tools may configure SMRT Link to perform automatic demultiplexing only. Demultiplexed BAM files may then be transferred for downstream command line analysis.

[5] SMRT Link Target Enrichment analysis application features a generalizable workflow that can be used to evaluate PureTarget panel performance QC (see SMRT Link user guide for further details.)

PacBio

Sample setup workflow overview for PureTarget libraries

Follow sample setup instructions for PureTarget libraries in ***Procedure & checklist*** library prep protocol – Do not use SMRT Link Sample Setup

1. Anneal sequencing primer
2. Bind sequencing polymerase
3. Clean up SMRTbell complexes
4. Internal control dilution
5. Final loading dilution

- Use entire volume (**25 µL**) of final PureTarget library per ABC reaction
- After complex cleanup, elute samples in Loading buffer and add specified volume of diluted sequencing control
  - → Do not use SMRT Link Loading Calculator

ABC procedure for PureTarget kit 24 manual workflows (8-, 16-, 24- or 48-plex)

P & C – Generating PureTarget libraries with PureTarget kit 24 – Manual protocol (103-740-700)

Perform **one** ABC reaction per final pool of 8 – 48 samples in 25 µL)

Step	Instructions
9.1	Prepare the appropriate volume of master mix with 10% overage using the per reaction volumes listed below.

Annealing mix

Tube	Component	Volume
Light blue	Annealing buffer	12.5 µL
Light green	Standard sequencing primer	12.5 µL
	Total volume	25 µL

9.2 Pipette-mix the **Annealing** mix and quick spin to collect liquid.

10.4 Prepare sample for sequencing. Add the following components for the appropriate sequencing platform.

Sequencing sample and control

Component	Revio SPRQ	Vega
Sample from step 9.22	23 µL	98 µl
Diluted internal sequencing control (Dilution 3)	1 µL	3 µl
Total volume	24 µL	101 µl

10.5 Load the Sequencing sample and control prepared in step 10.4. Load the exact volume indicated for the appropriate sequencing platform.

Revio SPRQ: 23 µL
Vega: 100 µL

Sequence pooled samples on one Revio or Vega SMRT Cell

ABC procedure for PureTarget kit 96 automated workflows (96-plex)

P & C – Generating PureTarget libraries with PureTarget kit 96 – Automation protocol (103-740-800)

Perform **one** ABC reaction per final pool of 96 samples in 25 µL)

Step	Instructions
9.1	Prepare the appropriate volume of master mix with 10% overage using the per reaction volumes listed below.

Annealing mix

Tube	Component	Volume	Volume (overage included)
Light blue	Annealing buffer	12.5 µL	15 µl
Light green	Standard sequencing primer	12.5 µL	15 µl
	Total volume	25 µL	30 µl

10.4 Prepare sample for sequencing. Add the following components to the Revio SPRQ sequencing reagent plate.

Sequencing reagent plate sample

Component	Volume
Sample from step 10.22	23 µL
Diluted internal sequencing control (Dilution 3)	1 µL
Total volume	24 µL

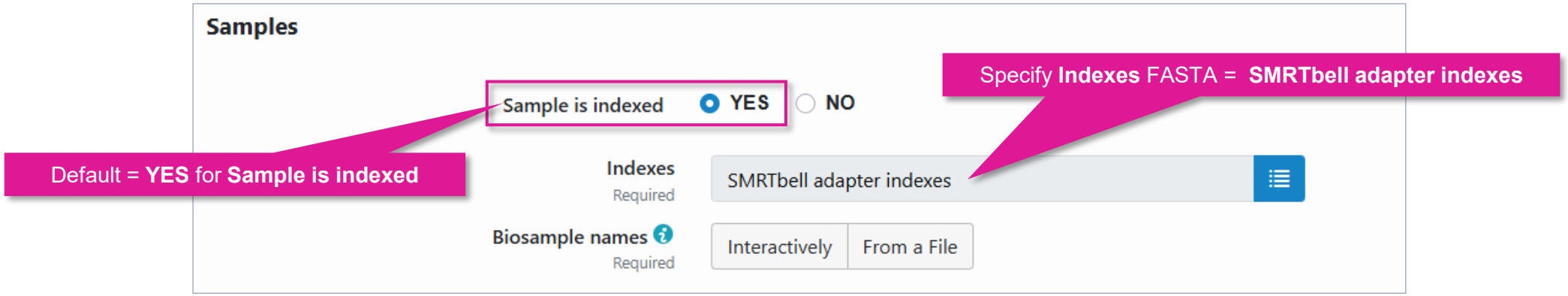
10.5 Load exactly 23 µL of sample prepared in 10.4 per sequencing well or store at 4°C for up to 24 hours before use. For long-term storage, store the sample at -20°C.

Sequence pooled samples on one Revio SMRT Cell

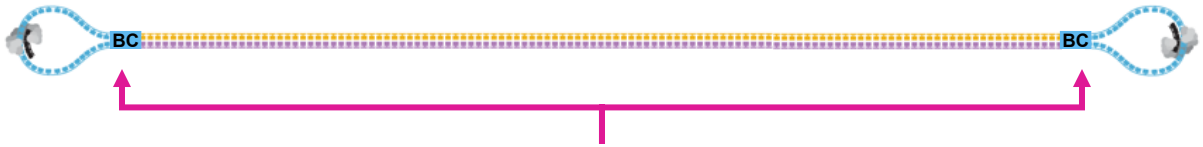
[1] For PureTarget samples, always use the fixed volumes shown in the tables of the procedure for each pooled batch – Do not adjust reagent volumes based on the measured sample concentration value or multiplex level of the batch.

SMRT Link Run Design options for PureTarget libraries – Sample indexing

Specifying sample indexing (barcoding) information for Revio and Vega systems



Example PureTarget library molecule containing SMRTbell indexed adapters[1] at both ends

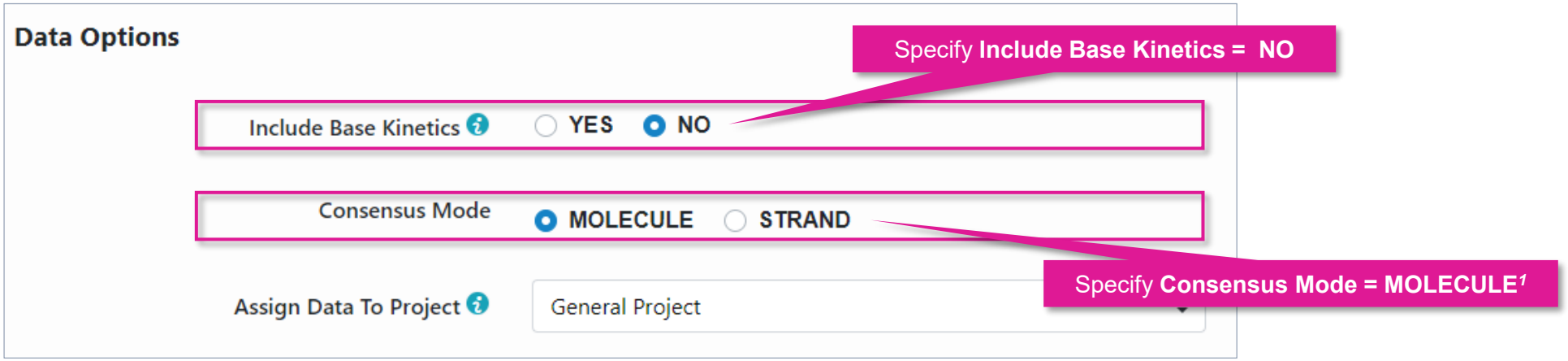


Example interactive biosample name specification for a multiplexed PureTarget library sample

[1] SMRTbell adapter index plate 96A (102-009-200) contains 96 indexed SMRTbell adapters in plate format (1 sample per index). The plate includes indexes bc2001–bc2096.

SMRT Link Run Design options for PureTarget libraries – Data options

Specifying data options for Revio and Vega systems



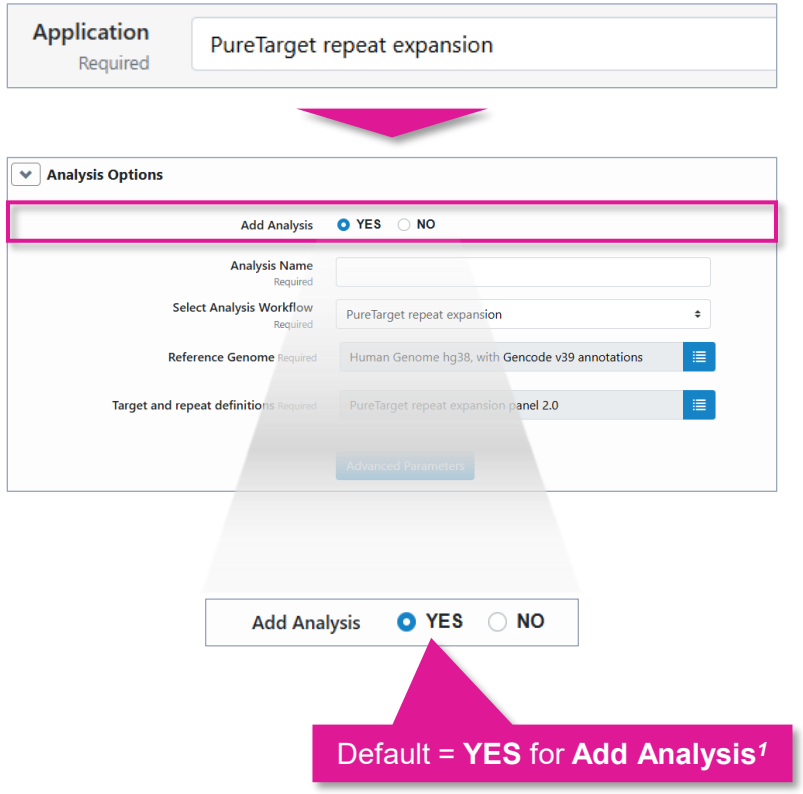
Can leave **Include Base Kinetics** and **Consensus Mode** fields at their **default settings** for PureTarget library samples

[1] **Molecule Consensus Mode** → generates a single circular consensus sequencing read for a given library insert molecule. **Strand Consensus Mode** → generates a consensus sequence for each strand (forward & reverse) of a given library insert molecule.

SMRT Link Run Design options for PureTarget libraries – Analysis options

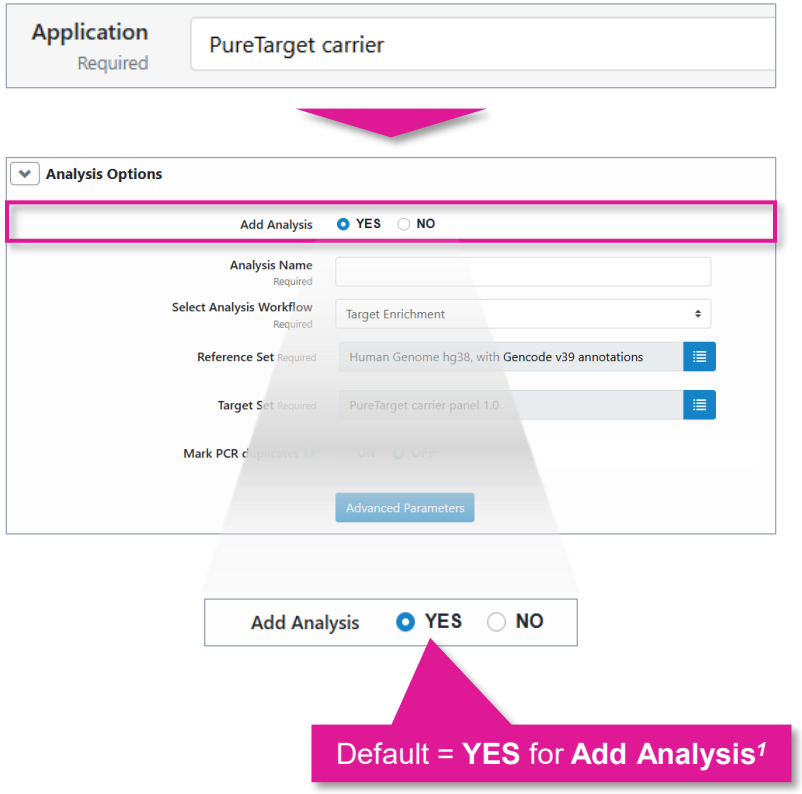
1. Specifying PureTarget analysis options for Revio and Vega systems – Add analysis

PureTarget repeat expansion panel



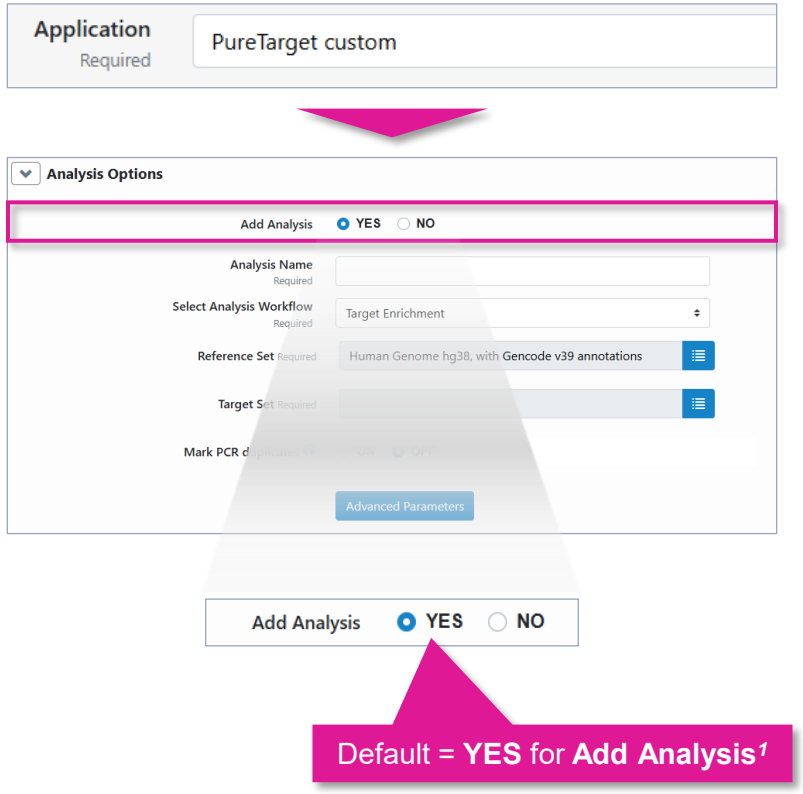
Default = **YES** for **Add Analysis**[1]

PureTarget carrier panel



Default = **YES** for **Add Analysis**[1]

PureTarget custom panel



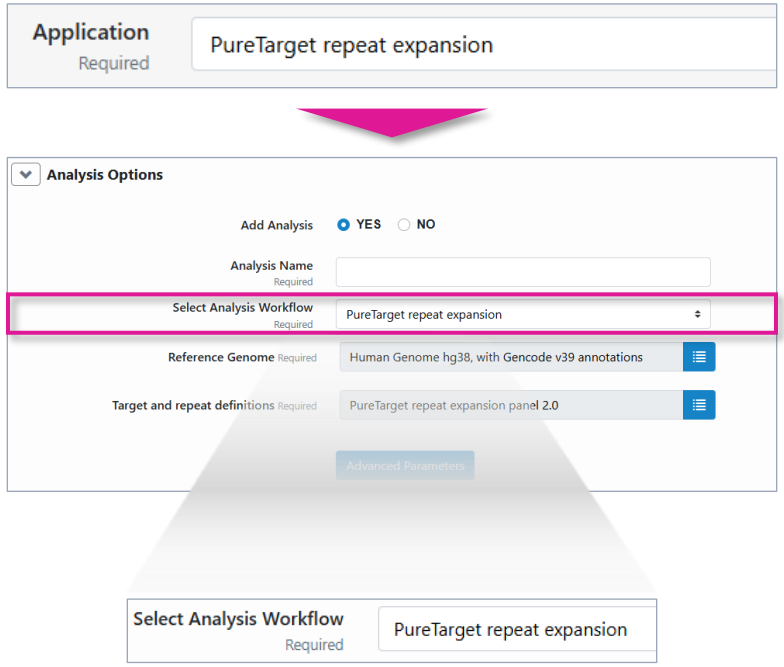
Default = **YES** for **Add Analysis**[1]

[1] Users have two options for analysis when setting up sequencing runs in SMRT Link. For the fastest turnaround time, users can specify to add the desired PureTarget analysis in their run design and analysis will be automatically performed when sequencing is complete. Alternatively, users who prefer command line analysis may configure SMRT Link to perform automatic demultiplexing only. Demultiplexed BAM files may then be transferred for downstream command line analysis using appropriate PacBio or PacBio Compatible software tools.

SMRT Link Run Design options for PureTarget libraries – Analysis options

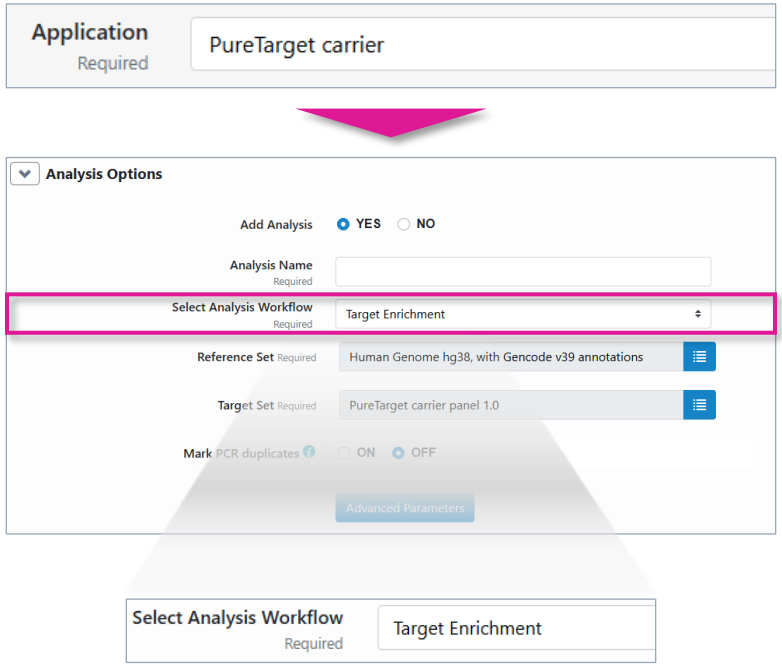
2. Specifying PureTarget analysis options for Revio and Vega systems – Analysis workflow selection[1]

PureTarget repeat expansion panel



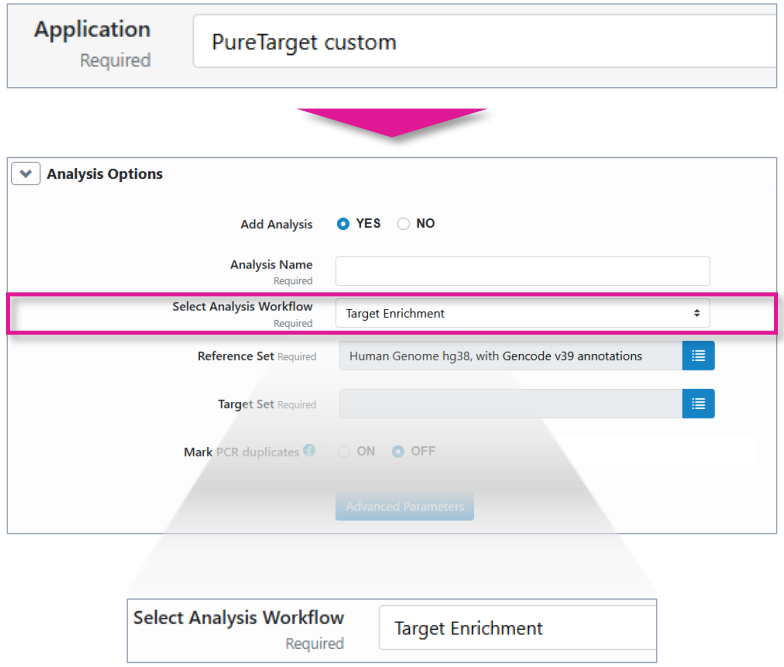
- SMRT Link PureTarget repeat expansion analysis application produces target enrichment summary QC statistics and uses tandem repeat genotyping tool (TRGT) for variant calling and for visualization

PureTarget carrier panel



- SMRT Link Target Enrichment analysis application produces target coverage summary QC statistics for PureTarget libraries

PureTarget custom panel



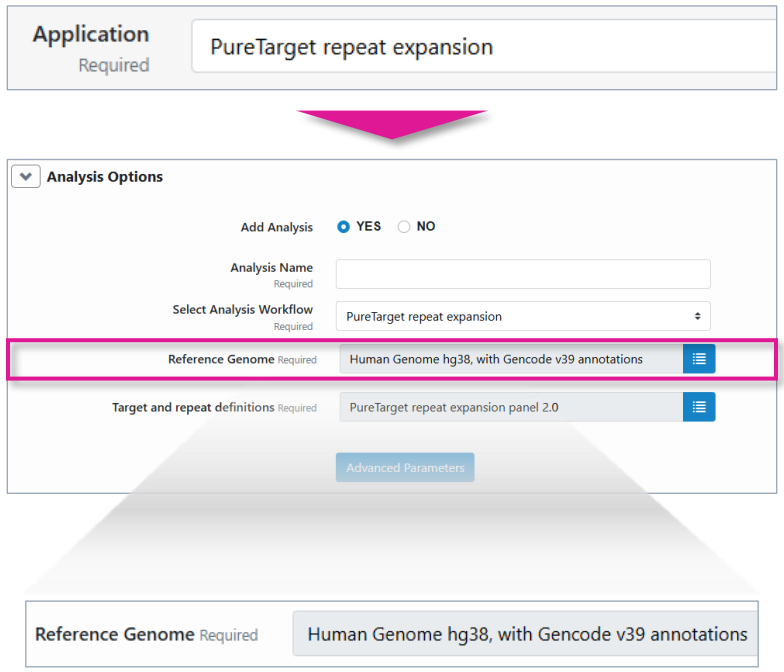
- SMRT Link Target Enrichment analysis application produces target coverage summary QC statistics for PureTarget libraries

[1] See ***SMRT Link User Guide*** (Documentation) for detailed descriptions of parameter settings for PureTarget analysis applications.

SMRT Link Run Design options for PureTarget libraries – Analysis options

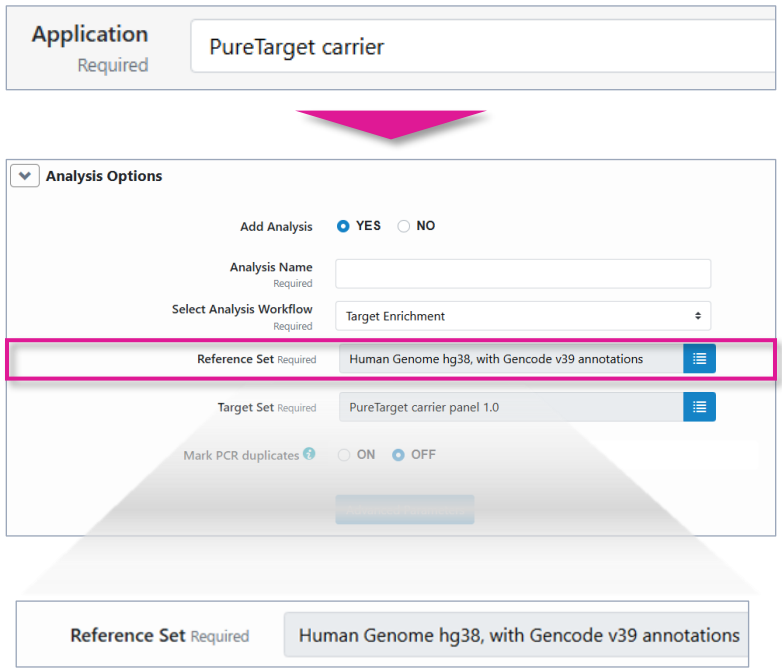
3. Specifying PureTarget analysis options for Revio and Vega systems – Reference set selection[1]

PureTarget repeat expansion panel



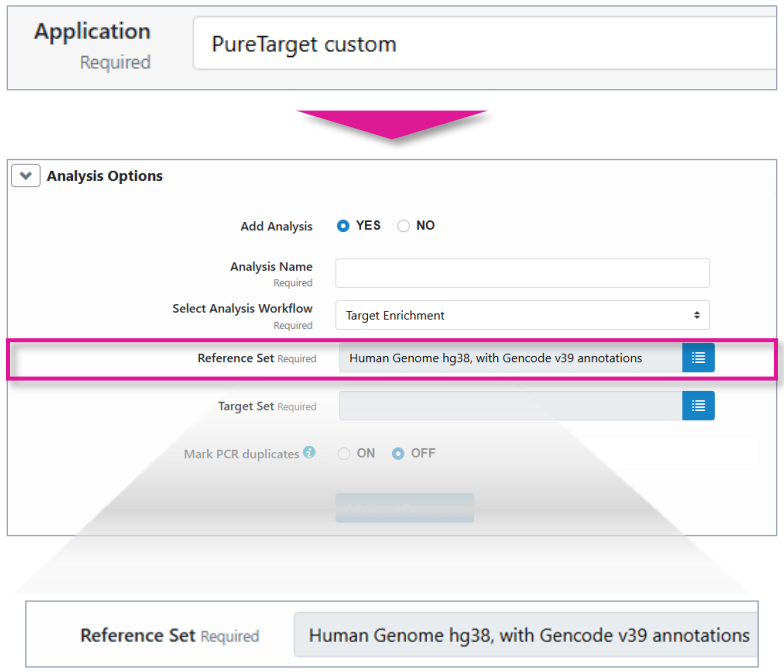
- Specify a reference genome against which to align the reads
- Default set = Human Genome hg38, with Gencode v39 annotations

PureTarget carrier panel



- Specify a reference set against which to align the reads
- Default set = Human Genome hg38, with Gencode v39 annotations

PureTarget custom panel



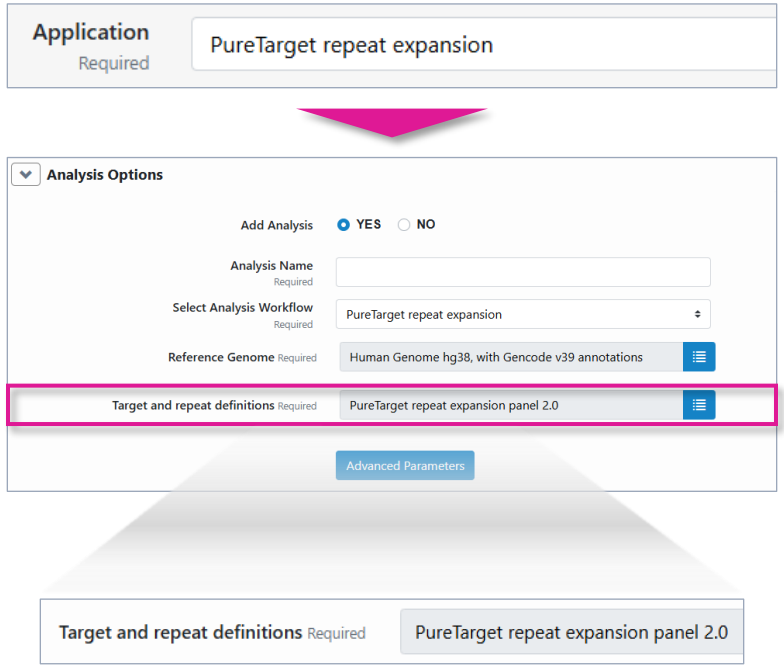
- Specify a reference set against which to align the reads
- Default set = Human Genome hg38, with Gencode v39 annotations

[1] See ***SMRT Link User Guide*** (Documentation) for detailed descriptions of parameter settings for PureTarget analysis applications.

SMRT Link Run Design options for PureTarget libraries – Analysis options

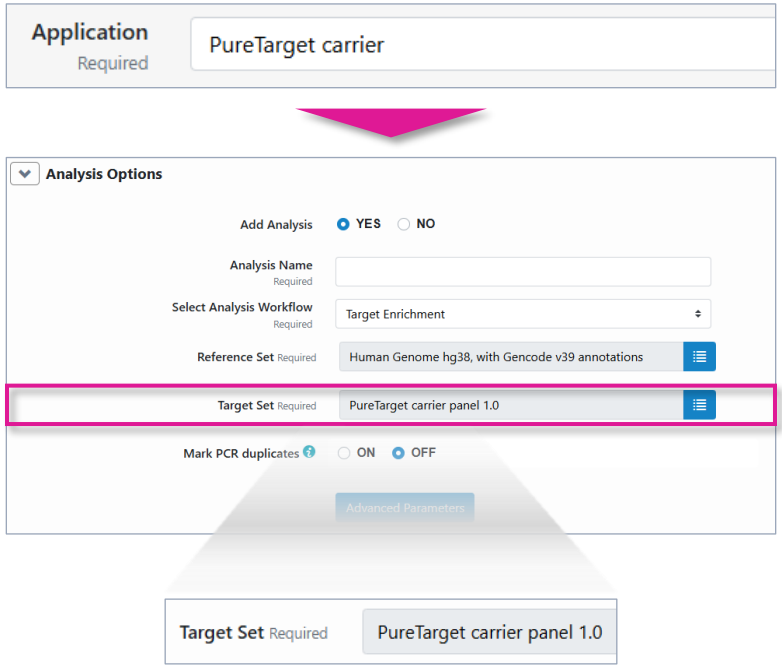
4. Specifying PureTarget analysis options for Revio and Vega systems – Target set selection[1]

PureTarget repeat expansion panel



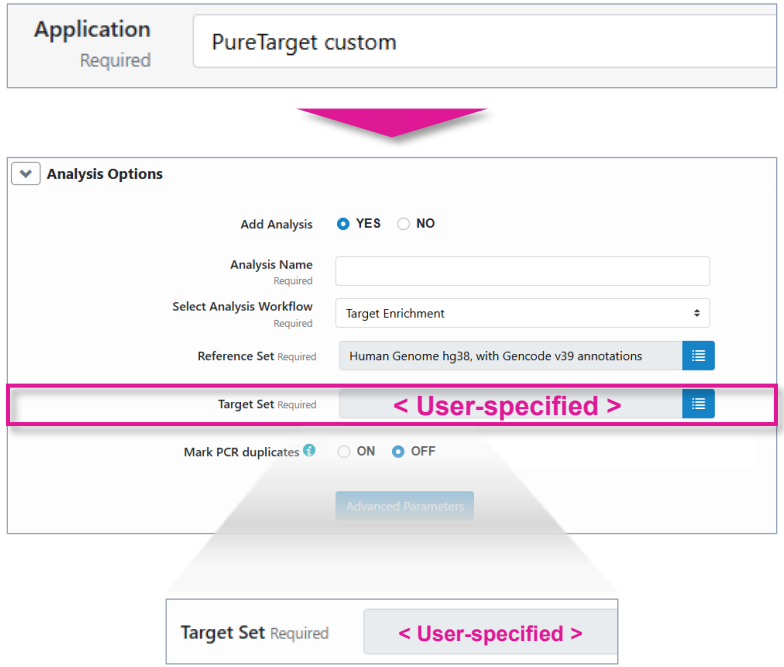
- Specify a target and repeat definition browser extensible data (BED[2]) dataset
  - Default set = PureTarget repeat expansion panel 2.0

PureTarget carrier panel



- Specify a target definition BED[2] dataset
  - Default set = PureTarget carrier panel 1.0

PureTarget custom panel



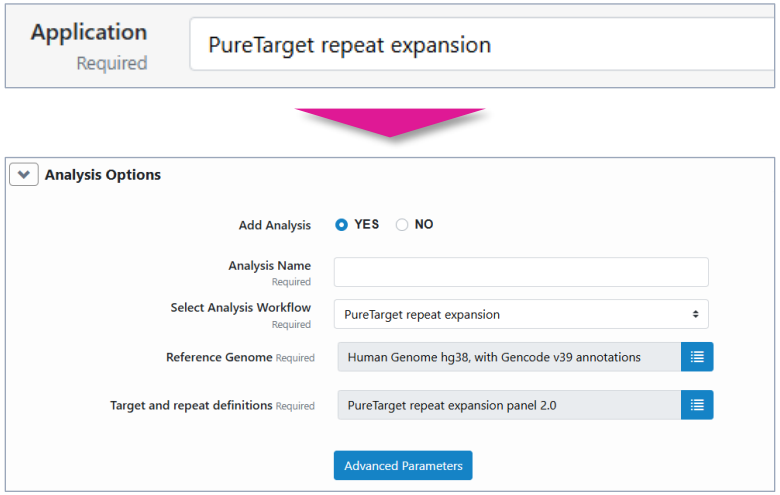
- Specify a target definition BED[2] dataset
  - User must manually specify the desired BED set (no default set is specified)
  - Recommend to include positive control target sequences corresponding to PureTarget control panel (103-633-300)

[1] See ***SMRT Link User Guide*** (Documentation) for detailed descriptions of parameter settings for PureTarget analysis applications.
[2] Only reads that map within the target regions in the BED file are included in the analysis. For more information about BED file format requirements, see ***SMRT Link User Guide*** (Documentation).

SMRT Link Run Design options for PureTarget libraries – Analysis options

5. Specifying PureTarget analysis options for Revio and Vega systems – Mark PCR duplicates[1]

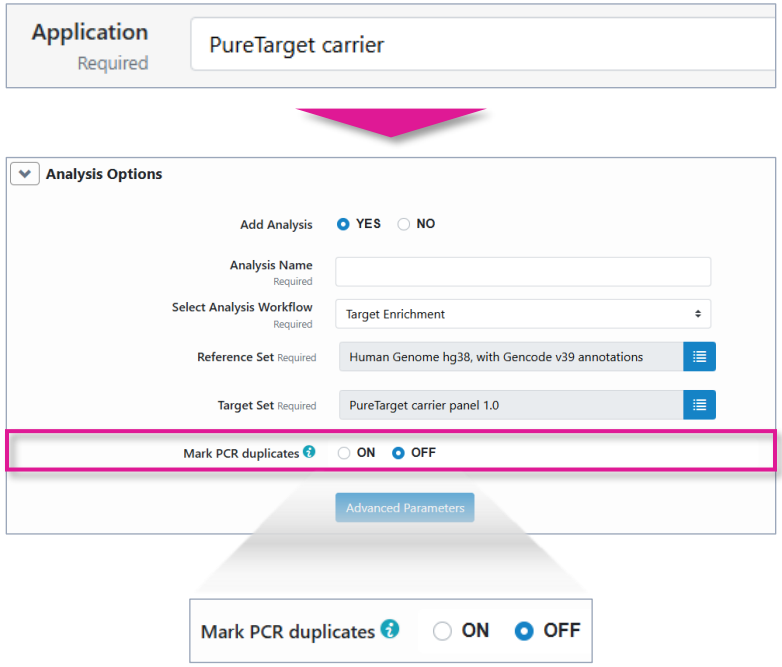
PureTarget repeat expansion panel



No **Mark PCR duplicates** option

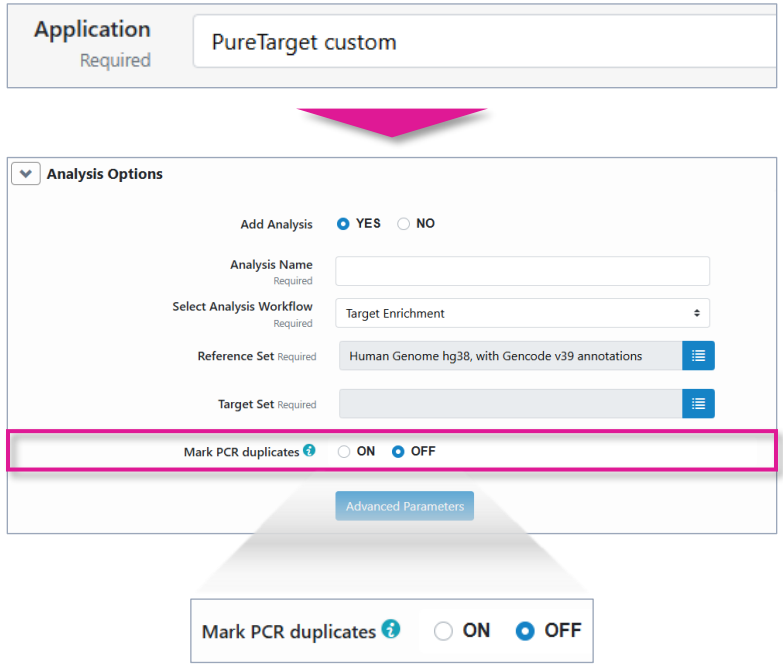
- Mark PCR duplicates option is not displayed
  - PureTarget repeat expansion analysis application workflow does not include mark PCR duplicates

PureTarget carrier panel



- Mark PCR duplicates option is displayed
  - For PureTarget carrier analysis application workflow, Mark PCR duplicates = OFF (default)

PureTarget custom panel



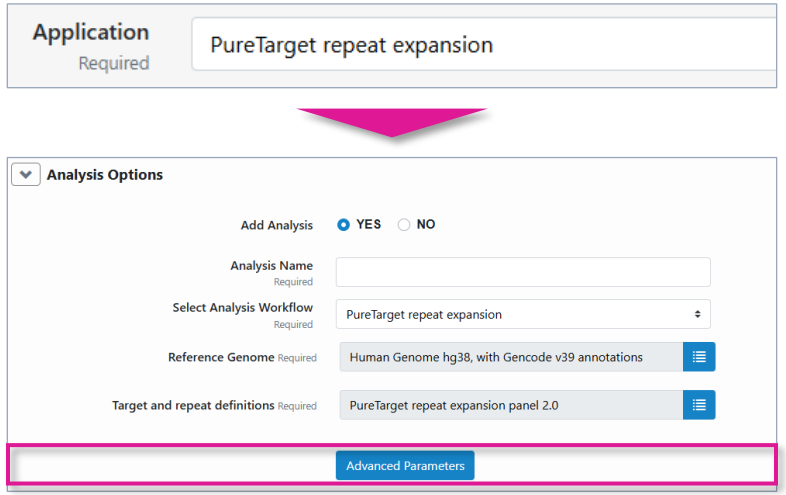
- Mark PCR duplicates option is displayed
  - For PureTarget custom analysis application workflow, Mark PCR duplicates = OFF (default)

[1] See ***SMRT Link User Guide*** (Documentation) for detailed descriptions of parameter settings for PureTarget analysis applications.

SMRT Link Run Design options for PureTarget libraries – Analysis options

6. Specifying PureTarget analysis options for Revio and Vega systems – Advanced Parameters[1]

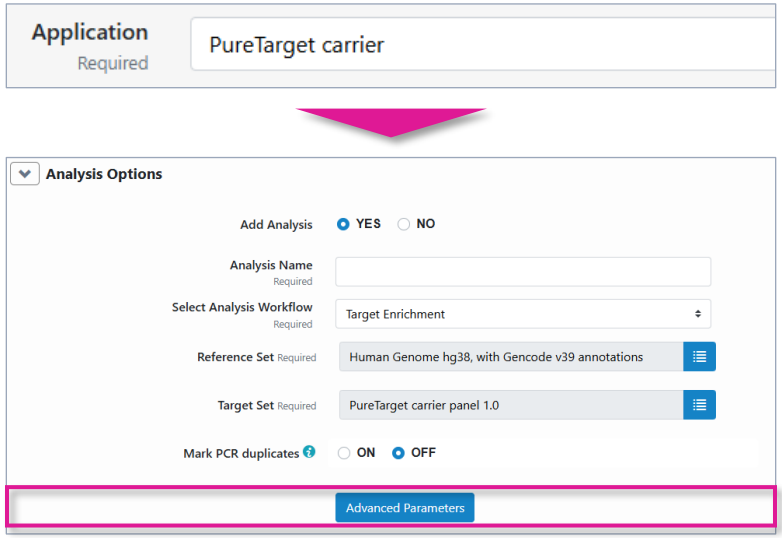
PureTarget repeat expansion panel



Optionally specify **Advanced Parameters**

- Example Advanced Parameters:
  - Optionally specify sample karyotypes (XX or XY; default = XX for unlisted samples)[2]

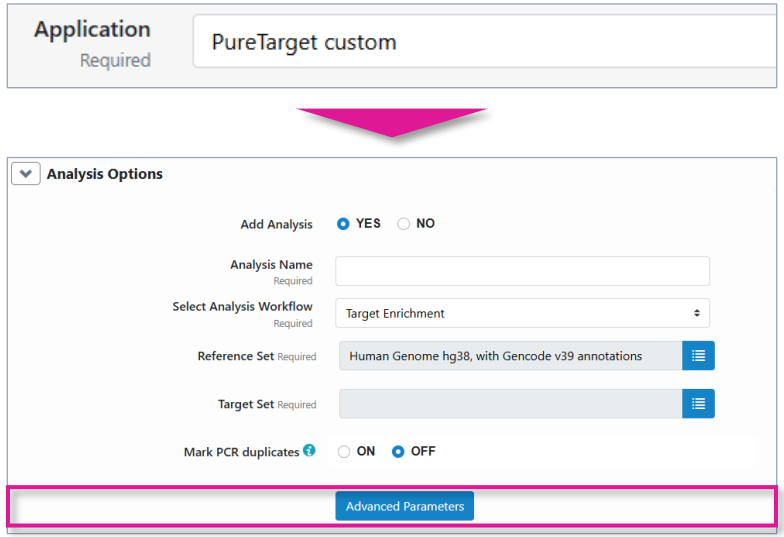
PureTarget carrier panel



Optionally specify **Advanced Parameters**

- Example Advanced Parameters:
  - Include Fail Reads (Leave default = ON)[3]
  - Perform variant calling (Leave default = OFF)[4]
  - Padding around regions (bp) (Leave default = 0)[5]

PureTarget custom panel



Optionally specify **Advanced Parameters**

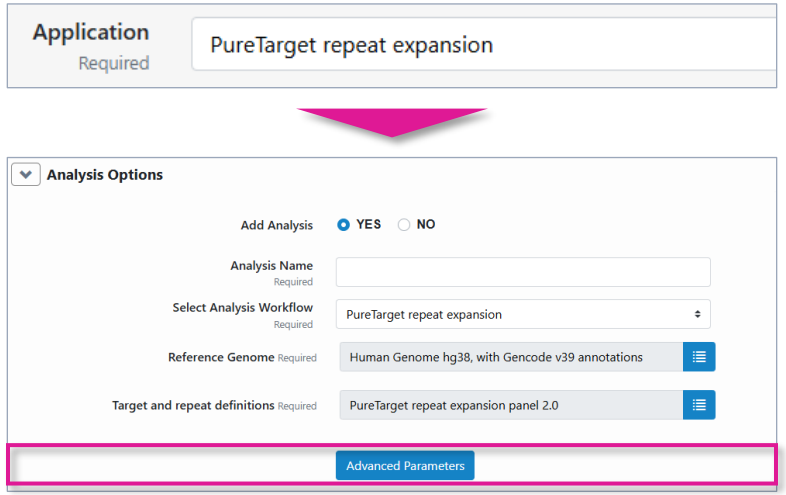
- Example Advanced Parameters:
  - Include Fail Reads (Set to = ON)[3]
  - Perform variant calling (Leave default = OFF)[4]
  - Padding around regions (bp) (Set to = 0)[5]

[1] See ***SMRT Link User Guide*** (Documentation) for detailed descriptions of parameter settings for PureTarget analysis applications.
[2] Optionally specify sample karyotypes since ploidy is considered when genotyping X-chromosome repeats. CSV header should be 'biosample, karyotype', followed by one sample and karyotype per line.
[3] Include Fail Reads should be set to ON for PureTarget applications. Use of fail reads increases sensitivity to detect long repeat expansions in PureTarget libraries.
[4] Perform variant calling should be set to OFF for PureTarget applications. For PureTarget carrier panel analysis, optionally call variants using the PureTarget Carrier Pipeline analysis workflow.
[5] Padding around regions should be set to 0 for PureTarget applications. This parameter adds padding on each side of regions specified in the target BED file.

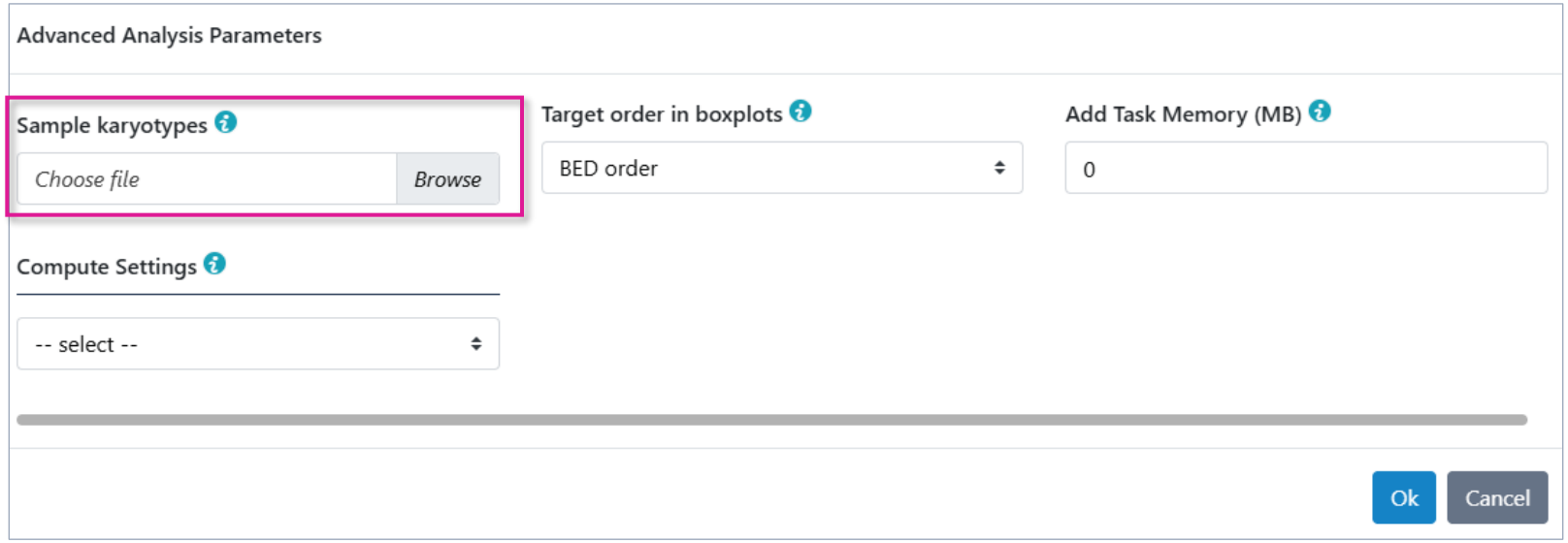
SMRT Link Run Design options for PureTarget libraries – Analysis options

6. Specifying PureTarget analysis options for Revio and Vega systems – Advanced Parameters[1]

PureTarget repeat expansion panel



Optionally specify **Advanced Parameters**



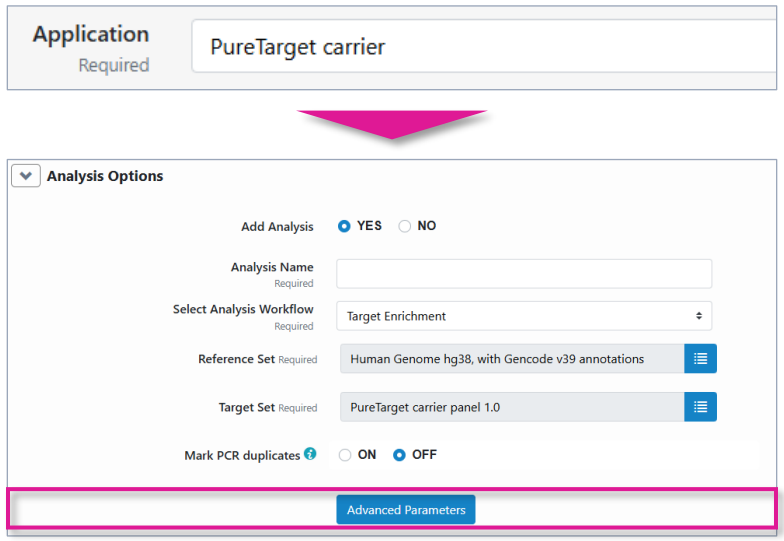
- Example Advanced Parameters:
  - Optionally specify sample karyotypes (XX or XY; default = XX for unlisted samples)[2]

[1] See ***SMRT Link User Guide*** (Documentation) for detailed descriptions of parameter settings for PureTarget analysis applications.
[2] Optionally specify sample karyotypes since ploidy is considered when genotyping X-chromosome repeats. CSV header should be 'biosample, karyotype', followed by one sample and karyotype per line.

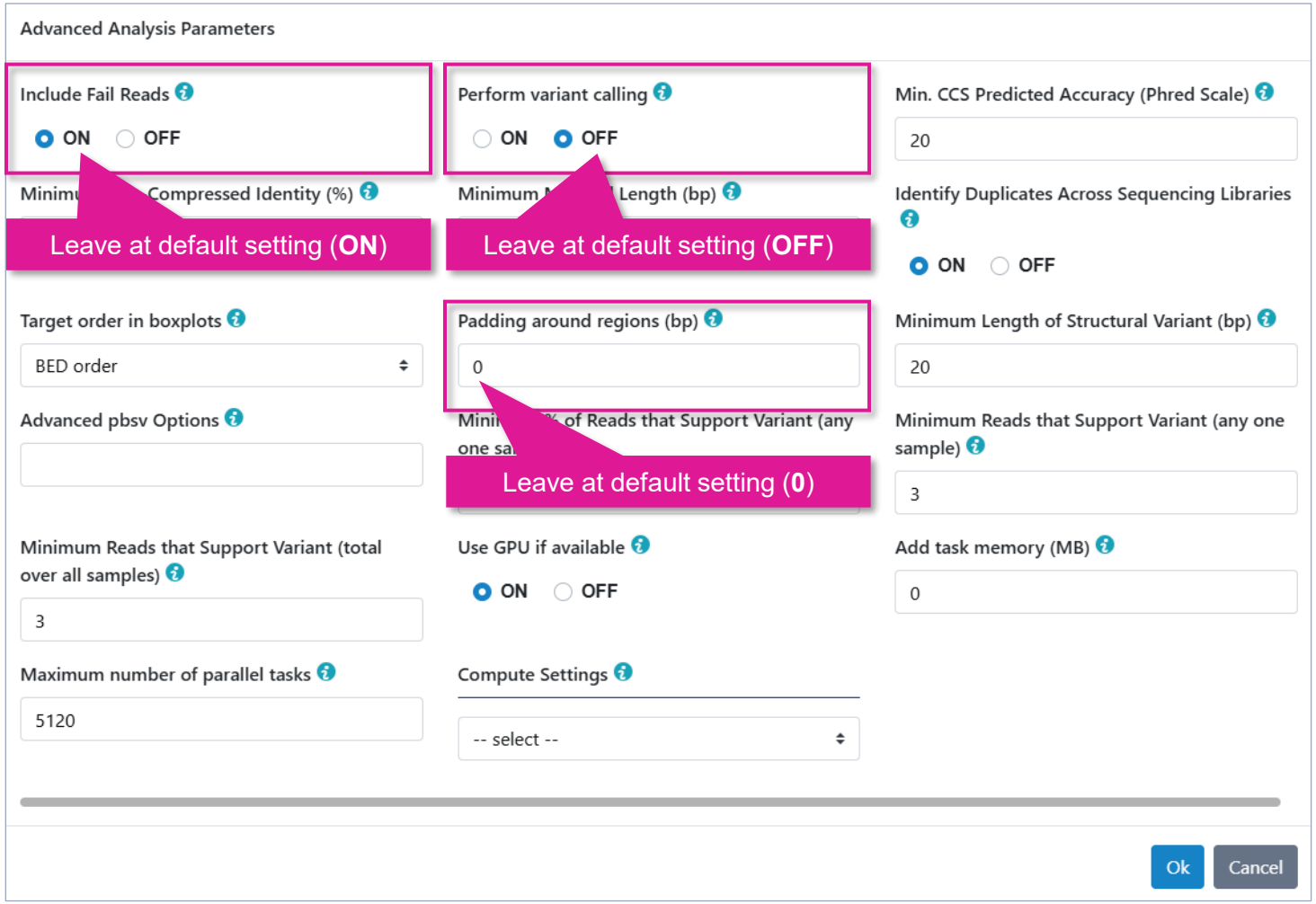
SMRT Link Run Design options for PureTarget libraries – Analysis options

6. Specifying PureTarget analysis options for Revio and Vega systems – Advanced Parameters[1]

PureTarget carrier panel



Optionally specify **Advanced Parameters**



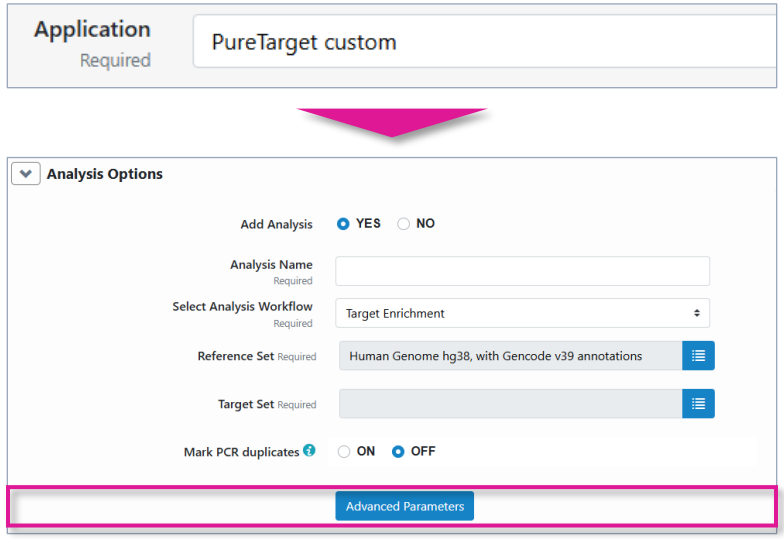
- Example Advanced Parameters:
  - Include Fail Reads (Leave default = ON)[2]
  - Perform variant calling (Leave default = OFF)[3]
  - Padding around regions (bp) (Leave default = 0)[4]

[1] See ***SMRT Link User Guide*** (Documentation) for detailed descriptions of parameter settings for PureTarget analysis applications.
[2] Include Fail Reads should be set to ON for PureTarget applications. Use of fail reads increases sensitivity to detect long repeat expansions in PureTarget libraries.
[3] Perform variant calling should be set to OFF for PureTarget applications. For PureTarget carrier panel analysis, optionally call variants using the PureTarget Carrier Pipeline analysis workflow.
[4] Padding around regions should be set to 0 for PureTarget applications. This parameter adds padding on each side of regions specified in the target BED file.

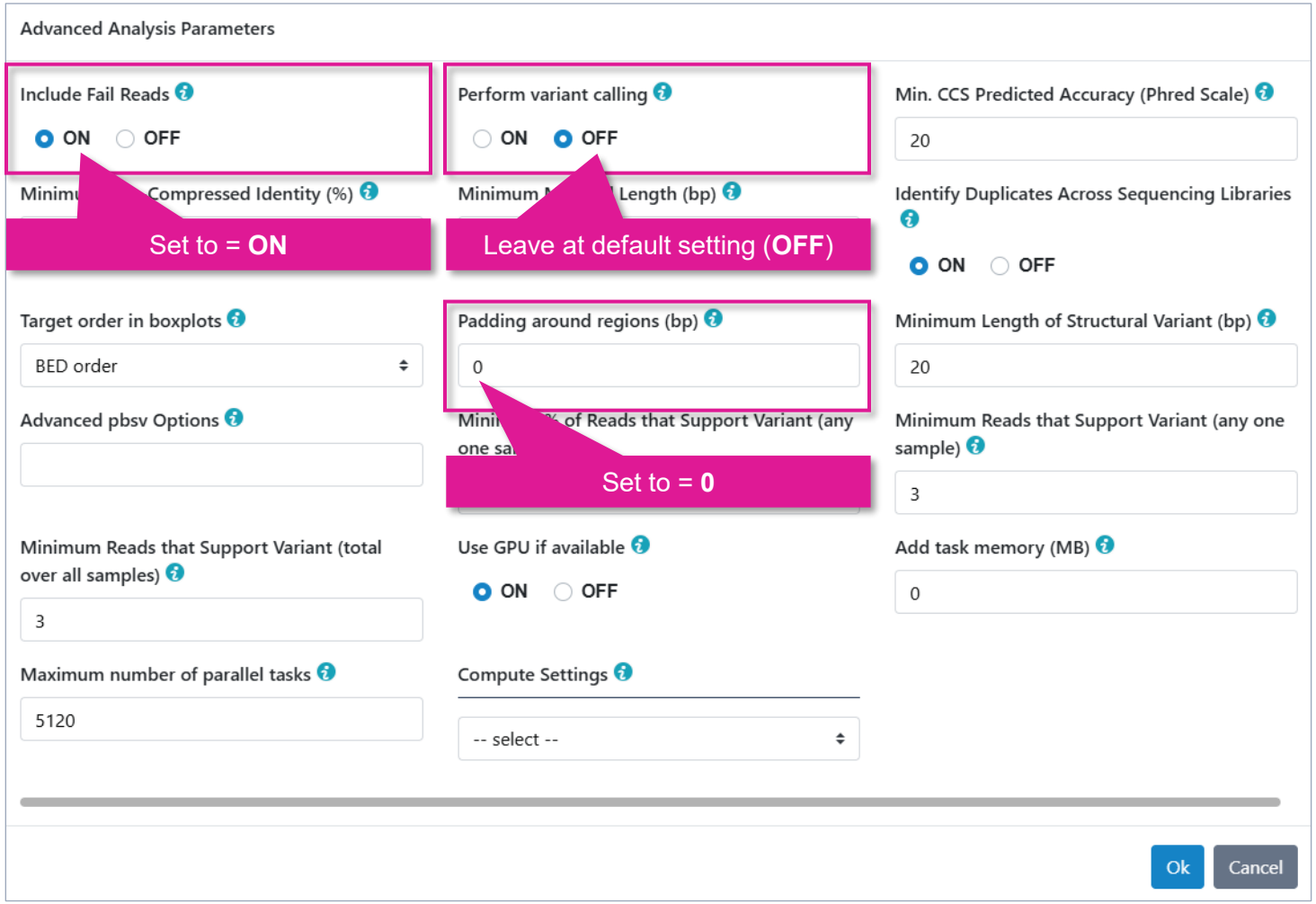
SMRT Link Run Design options for PureTarget libraries – Analysis options

6. Specifying PureTarget analysis options for Revio and Vega systems – Advanced Parameters[1]

PureTarget custom panel



Optionally specify **Advanced Parameters**



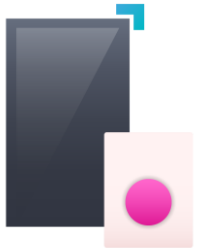
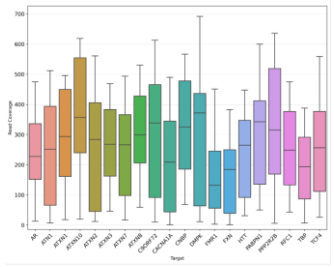
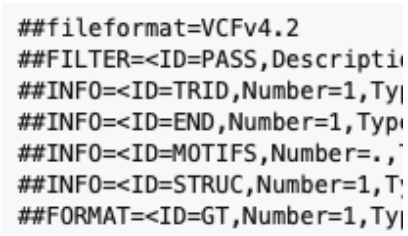
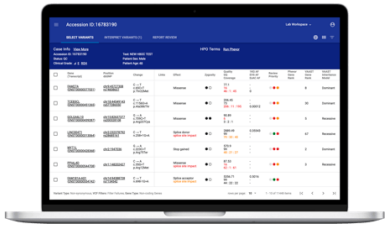
- Example Advanced Parameters:
  - Include Fail Reads (Set to = ON)[2]
  - Perform variant calling (Leave default = OFF)[3]
  - Padding around regions (bp) (Set to = 0)[4]

[1] See ***SMRT Link User Guide*** (Documentation) for detailed descriptions of parameter settings for PureTarget analysis applications.
[2] Include Fail Reads should be set to ON for PureTarget applications. Use of fail reads increases sensitivity to detect long repeat expansions in PureTarget libraries.
[3] Perform variant calling should be set to OFF for PureTarget applications. For PureTarget custom analysis, optionally perform call variants using other PacBio variant calling tools.
[4] Padding around regions should be set to 0 for PureTarget applications. This parameter adds padding on each side of regions specified in the target BED file.

PureTarget data analysis workflow overview

PureTarget data analysis software and workflow options

PureTarget applications leverage PacBio and PacBio Compatible Partner software for flexible secondary and tertiary analysis options

	Run Design & Monitoring	Panel performance	Variant calling	Variant interpretation
				
Repeat expansion panel[1]	SMRT Link run design[4]	SMRT Link PureTarget repeat expansion		
Carrier panel[2]	SMRT Link run design[4]	PureTarget carrier pipeline (PTCP) DNAnexus GitHub	Tertiary reporting Golden Helix varvis	
	SMRT Link run design[4]	SMRT Link Target Enrichment	For lab techs to quickly assess sequencing performance	
Custom panel[3]	SMRT Link run design[4]	SMRT Link Target Enrichment		

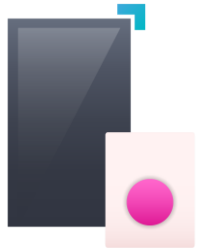
[1] See ***Application note – Comprehensive genotyping with the PureTarget repeat expansion panel and HiFi sequencing*** (102-326-614) for descriptions of analysis software and workflow options.
[2] See ***Application note – Consolidate challenging genes with PureTarget carrier screen panel*** (102-326-653) for descriptions of analysis software and workflow options.
[3] See ***Technical Note – A practical guide to amplification-free PureTarget custom panels*** (102-326-652) for descriptions of analysis software and workflow options.
[4] PureTarget run designs may be created using either SMRT Link (on-premise) or SMRT Link Cloud software.

PacBio

PureTarget repeat expansion panel 2.0 data analysis workflow overview

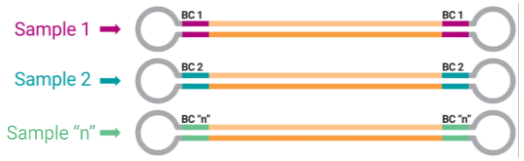
Analysis of PureTarget repeat expansion panel libraries can be performed in SMRT Link using the PureTarget repeat expansion analysis workflow or at the command line[1]

PureTarget repeat expansion panel SMRT Link analysis workflow[2]



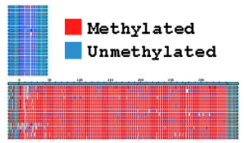
On-instrument

Demultiplex
`lima`



- Samples are demultiplexed on-instrument using `lima`

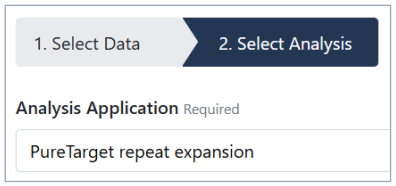
5mC probabilities



- 5mC methylation probabilities are detected on-instrument for CpG sites

SMRT Link v25.3

SMRT Analysis

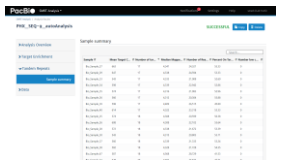


SMRT Link

Mapping
`pbmm2`

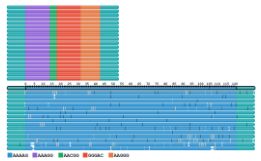
- Reads are mapped to hg38 reference genome with `pbmm2` to generate panel coverage QC statistics

Genotype
`trgt genotype`



- Repeat genotypes are called with TRGT

Visualize
`trgt plot`



- Visualizations of variant calling results are produced with TRGT

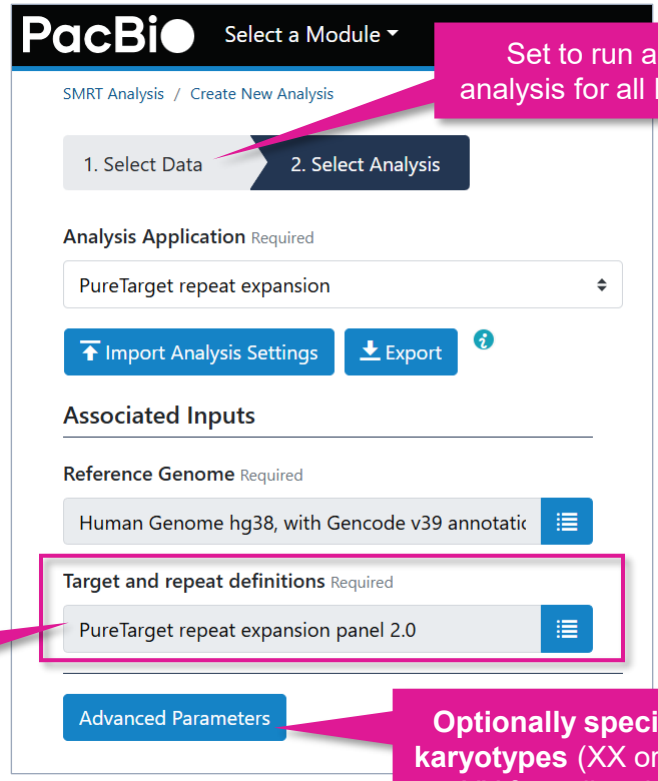
[1] See ***Application note – Analysis guide for PureTarget repeat expansion panel*** (102-326-616) for detailed descriptions of analysis workflow options PureTarget repeat expansion applications.
[2] See ***SMRT Link User Guide*** (Documentation) for detailed descriptions of parameter settings for PureTarget repeat expansion analysis application.

PureTarget repeat expansion panel 2.0 data analysis workflow overview (cont.)

Target and repeat definitions specification for SMRT Link PureTarget repeat expansion panel QC evaluation and variant calling analysis[1]

SMRT Link v25.3

SMRT Analysis



Set to run as "One analysis for all Data Sets"

Default target BED set is **PureTarget repeat expansion panel 2.0**

Optionally specify sample karyotypes (XX or XY; default = XX for unlisted samples)[3]

- Set to run as "One analysis for all Data Sets" to summarize across indexed samples
- Only reads that map within the target regions in the BED file are included in the analysis[2]

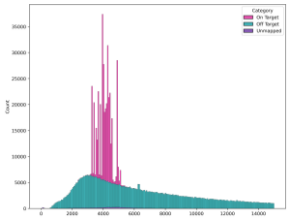
BED file target and repeat specification

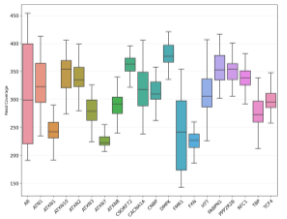
`PureTarget_repeat_expansion_panel_2.0.repeat_definition.GRCh38.bed`

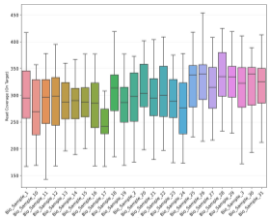
Example tandem repeat definition

```
chr4   3074876   3074966   ID=HTT,MOTIFS=CAG,CCG;STRUC=<TR>
```

- Repeat region has coordinates chr4:3074876-3074966
- Identifier is *HTT*
- **Note:** SMRT Link BED files must contain a fourth column with ID=NAME
- MOTIFS are the expected motifs the regions contain; CAG and CCG
- STRUC field can be always set to value <TR>



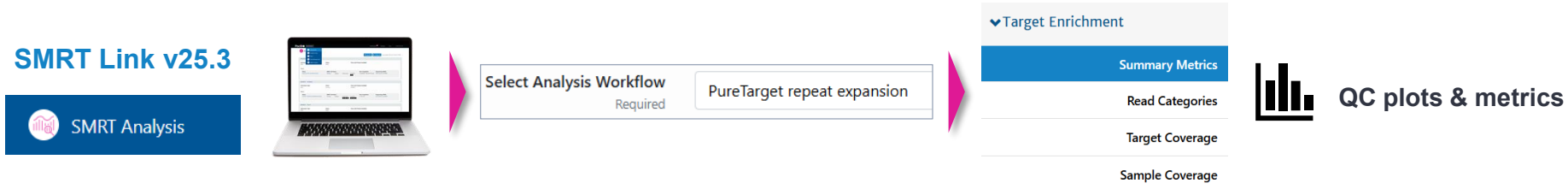




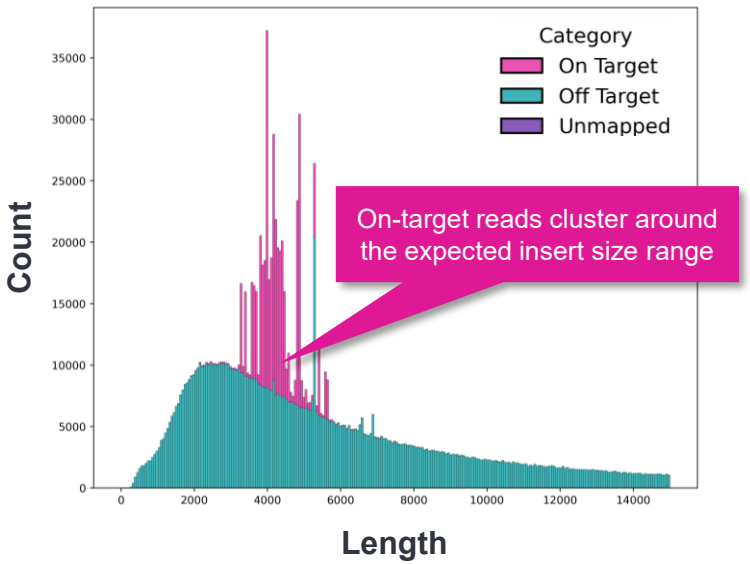
[1] See ***SMRT Link User Guide*** (Documentation) for detailed descriptions of parameter settings for PureTarget repeat expansion analysis application.
[2] To "in-silico" mask data from targets included in the 38-gene PureTarget repeat expansion panel 2.0, create a new BED dataset without that target.
[2] Optionally specify sample karyotypes since ploidy is considered when genotyping X-chromosome repeats. CSV header should be 'biosample, karyotype', followed by one sample and karyotype per line.

PureTarget repeat expansion panel 2.0 data analysis workflow overview (cont.)

SMRT Link PureTarget repeat expansion analysis outputs – Target coverage QC plots

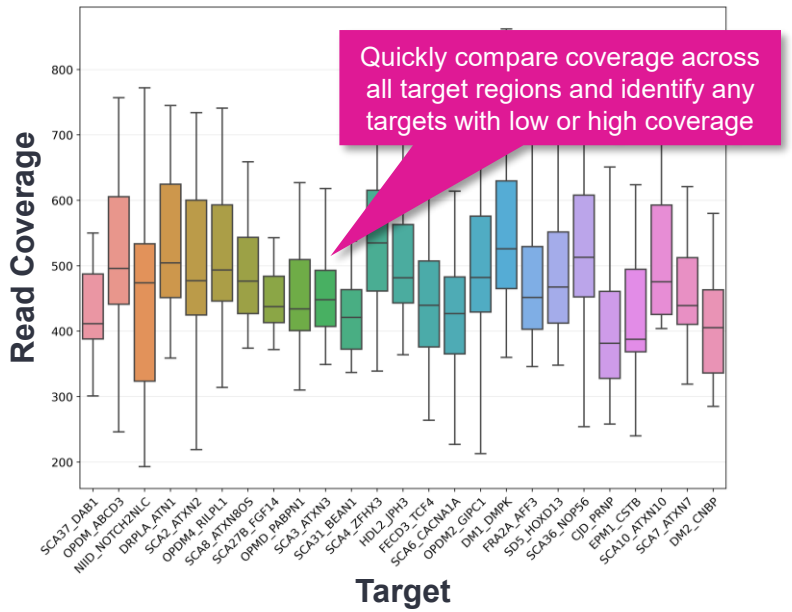


Read Categories[1]



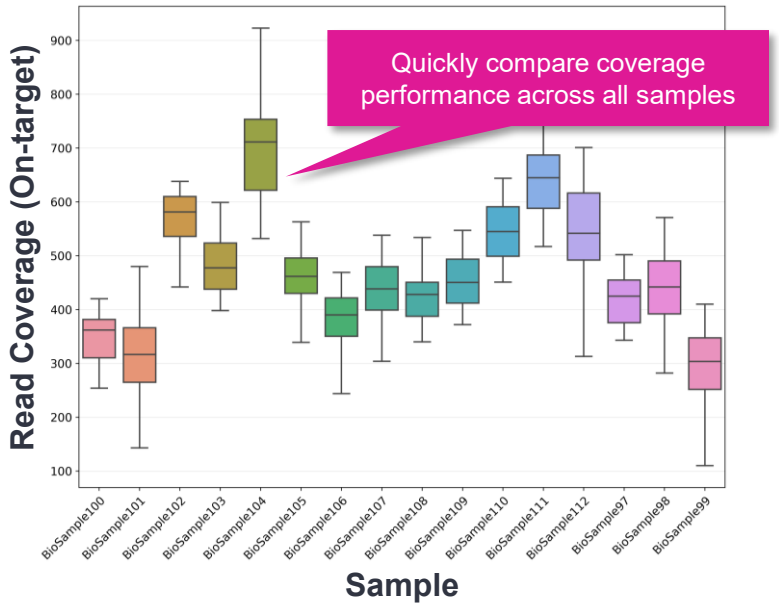
- Histogram mapping the length of On target, Off target, and Unmapped reads in the sample

Target Coverage[2]



- Box plot for each target regions of mean coverage across all samples analyzed

Sample Coverage[2]



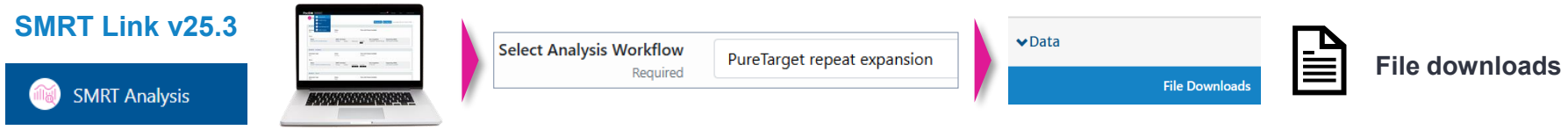
- Box plot for each sample of mean coverage across all target regions

[1] **Note:** A read is defined as being on-target if its alignment region in the reference genome has a non-empty overlap with any defined target in the input BED file. Histogram bars for the different categories are stacked.

[2] **Note:** PureTarget repeat expansion panel application reports mean read coverage for target coverage and sample coverage QC plots.

PureTarget repeat expansion panel 2.0 data analysis workflow overview (cont.)

SMRT Link PureTarget repeat expansion analysis outputs – File downloads



Edit Output File Name Prefix **Example:**analysis-GV-18-12105

File	Type	Size
Mapped BAM	zip	462 MB
Repeat expansion genotypes	csv	185 KB
SMRT Link Log	log	40 KB
Sample summary	csv	4 KB
TRGT plots meth-allele	zip	46 MB
TRGT plots meth-waterfall	zip	49 MB
TRGT plots motifs-allele	zip	24 MB
TRGT plots motifs-waterfall	zip	26 MB
TRGT spanning BAM	zip	22 MB

- **Repeat expansion genotypes**
  - CSV file containing repeat unit sequence, allele count, min/max/consensus repeat array length, motif count, and motif span information.

- **TRGT plots meth-allele**
  - Depicts consensus repeat alleles and reads aligning to them. Bases in repeats are colored by methylation levels.
- **TRGT plots meth-waterfall**
  - Depicts portions of reads spanning the repeat without aligning them, which is convenient for showing mosaicism. Bases in repeats are colored by methylation levels.
- **TRGT plots motif-allele**
  - Depicts consensus repeat alleles and reads aligning to them. Bases in repeats are colored by repeat motif.
- **TRGT plots motif-waterfall**
  - Depicts portions of reads spanning the repeat without aligning them, which is convenient for showing mosaicism. Bases in repeats are colored by repeat motif.

[1] See GitHub documentation for detailed instructions on how to interpret the read pileup plots generated by TRVZ.

PureTarget repeat expansion panel 2.0 data analysis workflow overview (cont.)

SMRT Link PureTarget repeat expansion analysis outputs – File downloads (cont.)

`trgt plot` enables visualizations with TRGT

- Create allele plots that depict repeat alleles and reads aligning to them
- Create waterfall plots that depict portions of HiFi reads spanning the repeat without aligning them; waterfall plots are especially convenient for visualizing mosaicism.

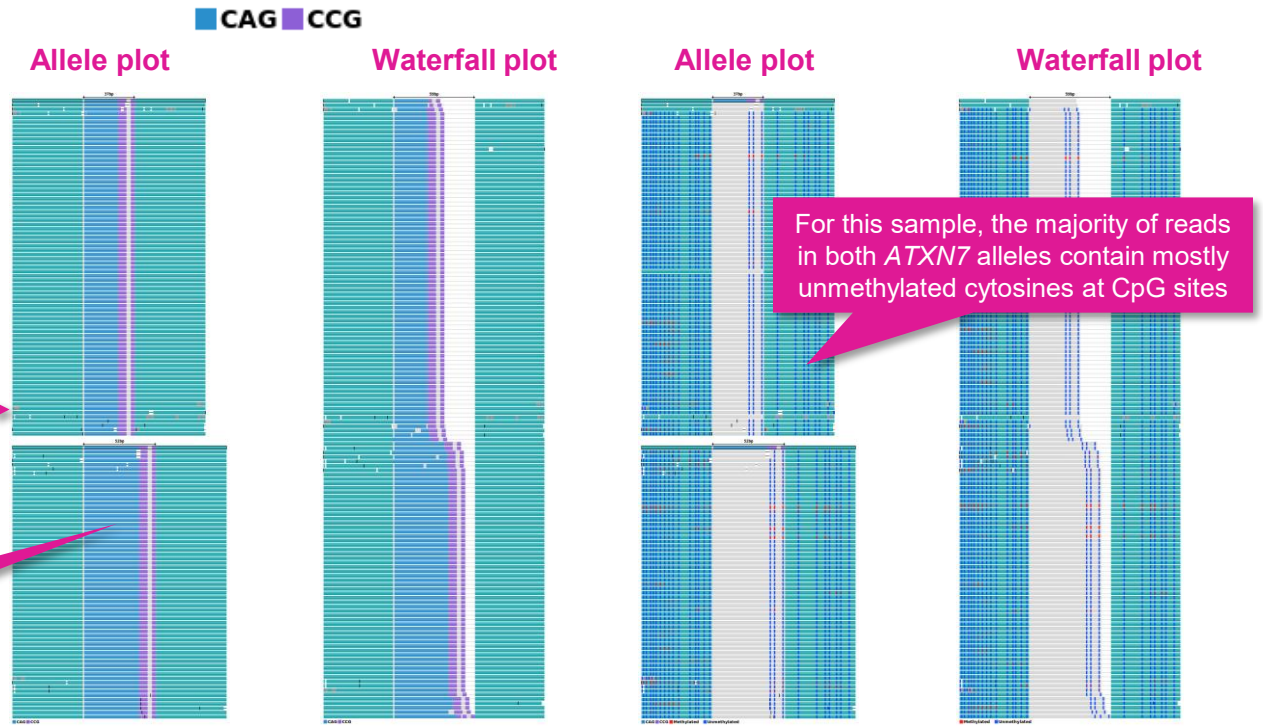
Edit Output File Name Prefix **Example:**analysis-GV-18-12105

File	Type	Size
Mapped BAM	zip	462 MB
Repeat expansion genotypes	csv	185 KB
SMRT Link Log	log	40 KB
Sample summary	csv	4 KB
TRGT plots meth-allele	zip	46 MB
TRGT plots meth-waterfall	zip	49 MB
TRGT plots motifs-allele	zip	24 MB
TRGT plots motifs-waterfall	zip	26 MB
TRGT spanning BAM	zip	22 MB

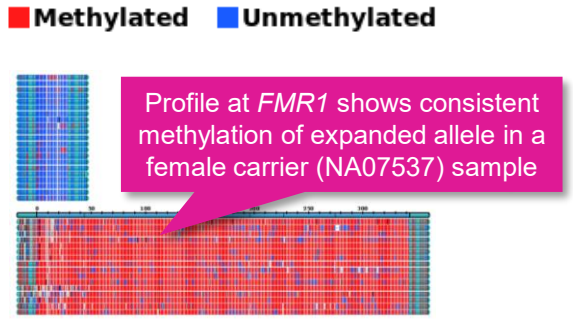
Repeat expansion genotypes

Sample	Bio_Sample_1
CNBP repeat unit	CAGG:CAGA:CA
CNBP read count allele 0	146
CNBP consensus size allele 0	139
CNBP min size allele 0	129
CNBP max size allele 0	317
CNBP motif counts allele 0	15_8_23
CNBP motif spans allele 0	0(0-61)_1(61-93)_2(93-139)

PureTarget motifs analysis



PureTarget methylation analysis

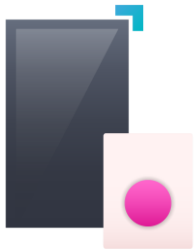


[1] See PacBio GitHub documentation for detailed instructions on how to interpret the read pileup plots generated by TRGT.

PureTarget carrier panel data analysis workflow overview

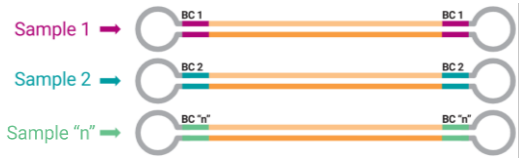
Evaluation of PureTarget carrier panel performance QC can be performed in SMRT Link using the Target Enrichment analysis workflow or at the command line[1]

PureTarget carrier panel SMRT Link QC analysis workflow[2]



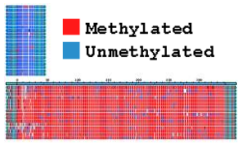
On-instrument

Demultiplex
`lima`



- Samples are demultiplexed on-instrument using `lima`

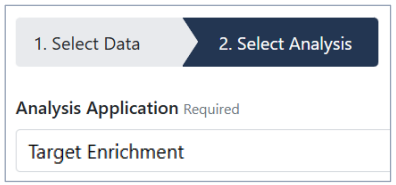
5mC probabilities



- 5mC methylation probabilities are detected on-instrument for CpG sites

SMRT Link v25.3

SMRT Analysis

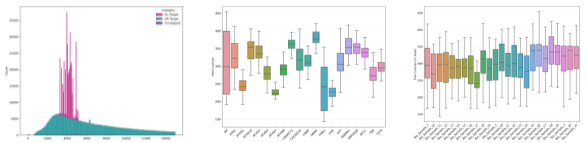


SMRT Link

Mapping
`pbmm2`

- Reads are mapped to hg38 reference genome with `pbmm2` to generate panel coverage QC statistics

Visualize



- Visualizations of PureTarget panel performance QC results are produced

[1] See ***Application note – Consolidate challenging genes with PureTarget carrier screening panel*** (102-326-653) for detailed descriptions of analysis workflow options for PureTarget carrier applications.

[2] See ***SMRT Link User Guide*** (Documentation) for detailed descriptions of parameter settings for Target Enrichment analysis application.

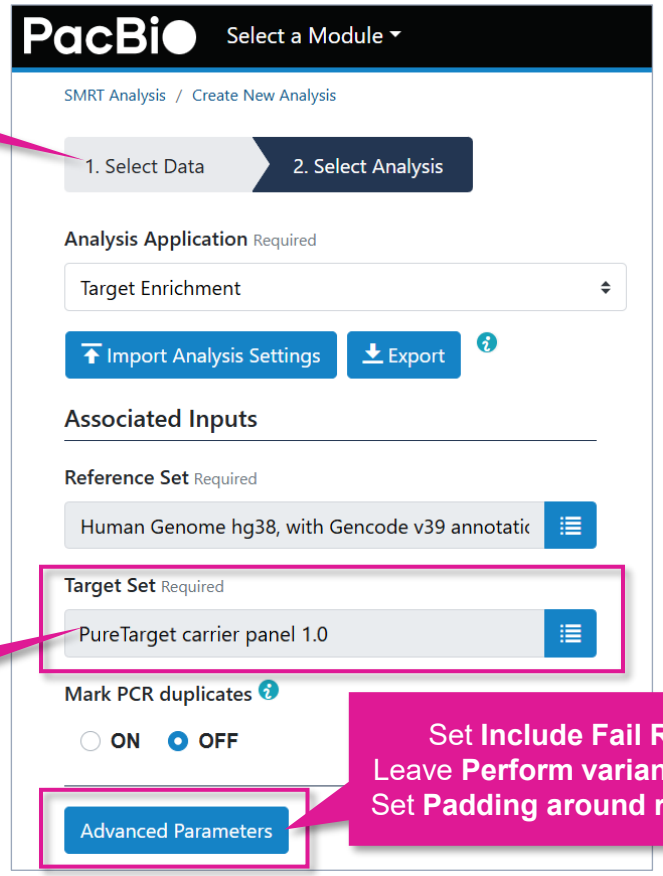
PureTarget carrier panel data analysis workflow overview (cont.)

Target definitions specification for SMRT Link PureTarget carrier panel QC evaluation[1]

Set to run as "One analysis for all Data Sets"

SMRT Link v25.3

SMRT Analysis



Select **PureTarget carrier panel 1.0** target BED set

Set **Include Fail Reads** = ON[2]
Leave **Perform variant calling** = OFF[3]
Set **Padding around regions (bp)** = 0[4]

- Set to run as "One analysis for all Data Sets" to summarize across indexed samples

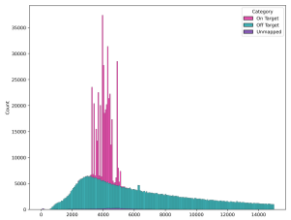
BED file target specification

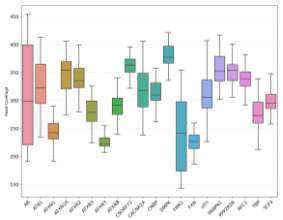
`PureTarget_carrier_panel_1.0.cut_site.GRCh38.bed`

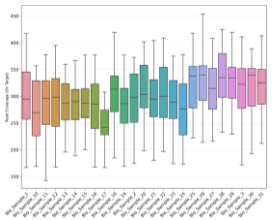
Example target cut site definition

```
chr4   3074876   3074966   ID=GBA
```

- Target cut site has coordinates chr4:3074876-3074966
- Identifier is *GBA*
- **Note:** SMRT Link BED files must contain a fourth column with ID=NAME
- See ***SMRT Link user guide*** for more details[1]



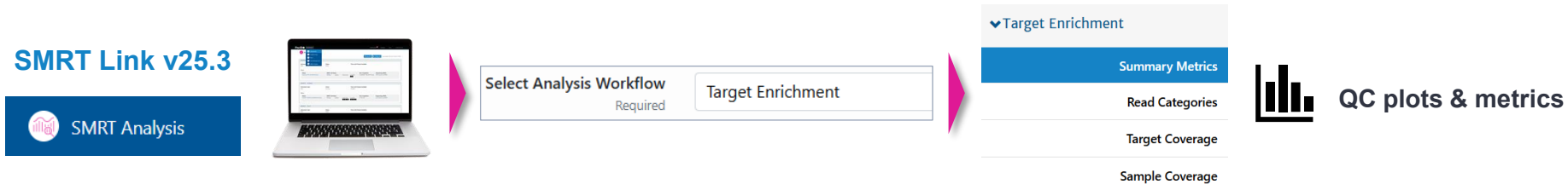




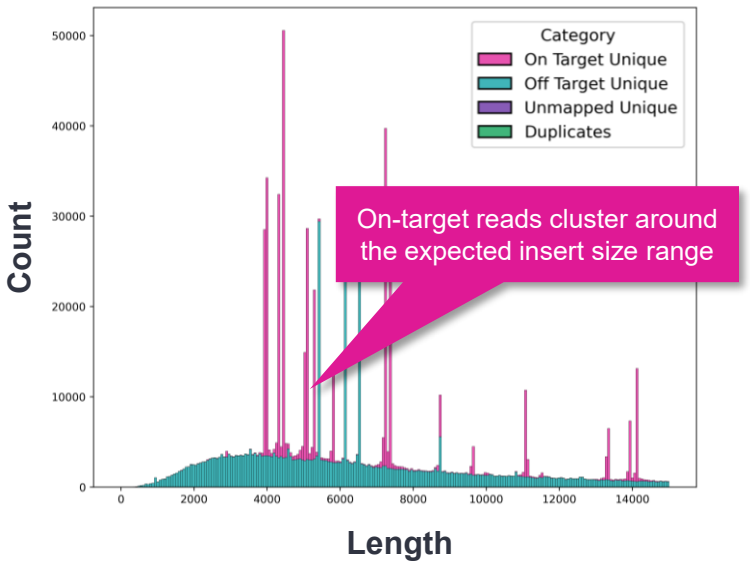
[1] See ***SMRT Link User Guide*** (Documentation) for detailed descriptions of parameter settings for PureTarget Target Enrichment analysis application.
[2] Include Fail Reads should be set to ON for PureTarget applications. Use of fail reads increases sensitivity to detect long repeat expansions in PureTarget libraries.
[3] Perform variant calling should be set to OFF for PureTarget applications. For PureTarget carrier panel analysis, optionally perform genotyping using the PureTarget carrier panel analysis workflow.
[4] Padding around regions should be set to 0 for PureTarget applications. This parameter adds padding on each side of regions specified in the target BED file.

PureTarget carrier panel data analysis workflow overview (cont.)

SMRT Link PureTarget carrier panel QC analysis outputs – Target coverage QC plots

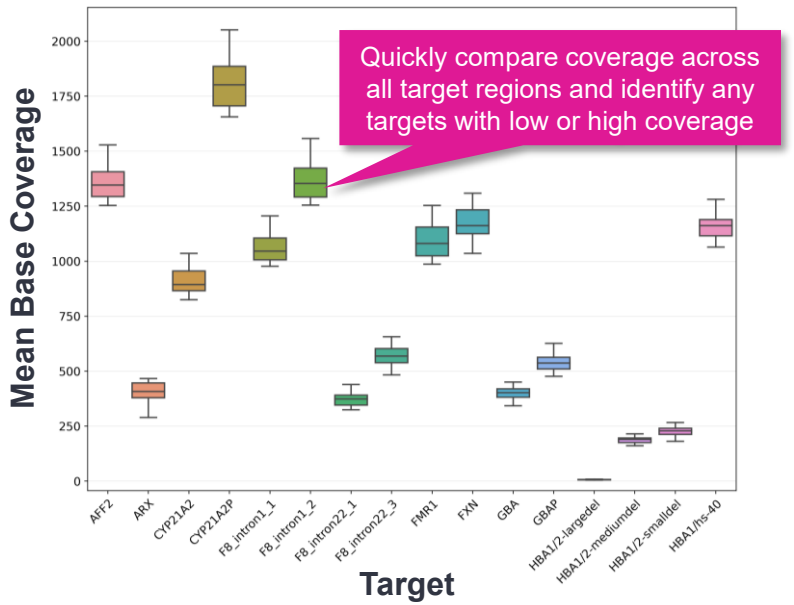


Read Categories[1]



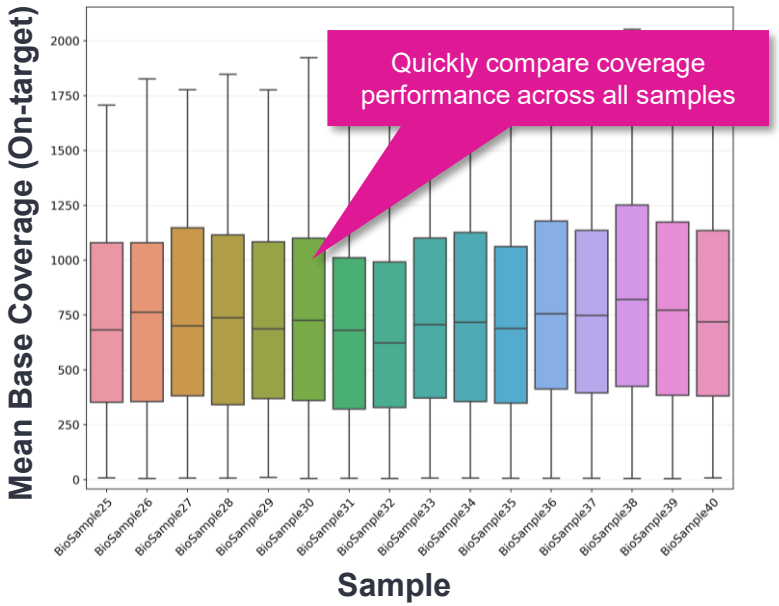
- Histogram mapping the length of On target, Off target, and Unmapped reads in the sample

Target Coverage[2]



- Box plot for each target regions of mean coverage across all samples analyzed

Sample Coverage[2]



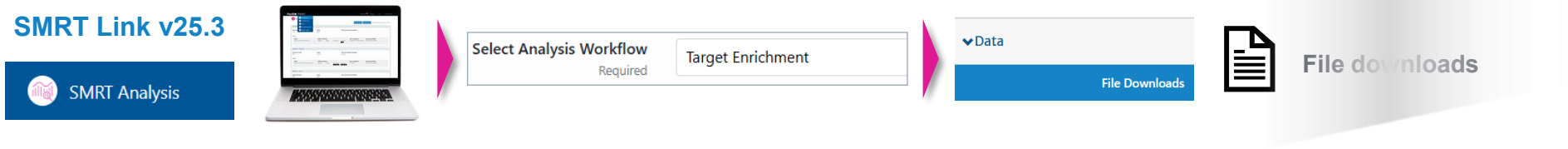
- Box plot for each sample of mean coverage across all target regions

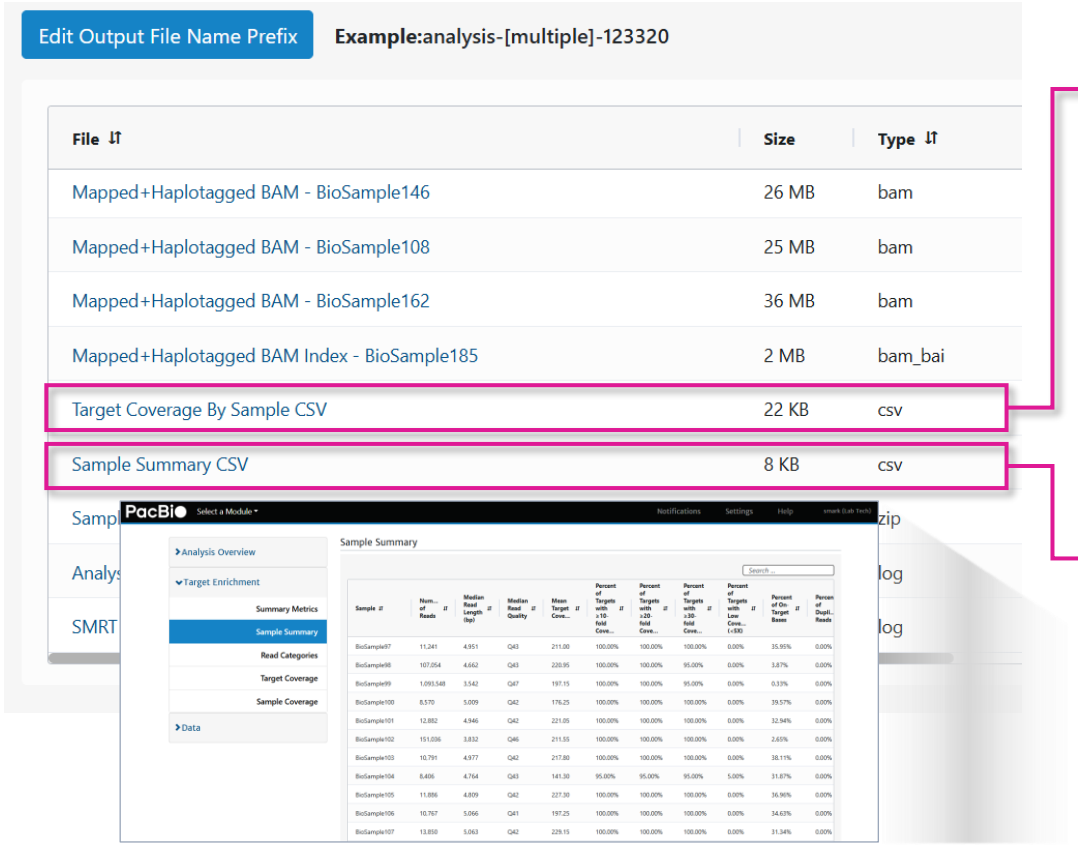
[1] **Note:** A read is defined as being on-target if its alignment region in the reference genome has a non-empty overlap with any defined target in the input BED file. Histogram bars for the different categories are stacked. For PureTarget samples, the number reads in the 'Duplicates' category is typically zero since PCR duplicates are not expected to be present in PureTarget libraries and Mark PCR Duplicates is therefore not performed..

[2] **Note:** For PureTarget carrier panels and PureTarget custom panels, SMRT Link Target Enrichment analysis workflow reports mean base coverage for target coverage and sample coverage QC plots.

PureTarget carrier panel data analysis workflow overview (cont.)

SMRT Link PureTarget carrier panel QC analysis outputs – File downloads





- **Target Coverage By Sample CSV**
  - Comma-delimited text file of the matrix of all mean coverage values for each target (rows) and sample (columns). The data in this file is used to generate the Target Coverage and Sample Coverage box plot.

	A	B	C	D	E	F	G	H	I	J	K
1	targetName	BioSample100	BioSample101	BioSample102	BioSample103	BioSample104	BioSample	BioSample	BioSample	BioSample	BioSample
2	GBAP	143.039226	235.618455	159.680491	205.643511	164.104372	187.5212	172.4631	201.5143	157.0218	179.8754
3	GBA	109.468538	183.412531	176.706672	152.876051	131.967724	185.0193	177.8427	140.387	122.852	102.0357
4	HBB	196.493551	295.325873	201.311077	282.075683	239.581563	258.0986	263.5472	273.1737	182.9837	230.0867
5	HBA1/hs-40	187.466172	257.382333	253.085188	192.052596	201.34637	248.0074	207.1164	244.3817	272.1106	253.6923
6	HBA1/2-smalldel	42.775765	50.690922	52.119821	86.559026	43.743181	37.81439	83.11514	80.20248	41.1167	78.1674
7	HBA1/2-mediumdel	14.634827	23.264869	35.464813	25.631649	18.096078	22.81532	20.14724	25.31156	31.40876	14.31733
8	HBA1/2-largedel	2.558176	2.955431	4.608815	3.48891	2.294498	5.938984	2.33284	4.378707	2.198251	3.434638
9	SMN2	46.470405	51.342962	29.593529	76.550765	0.248159	36.29135	50.87199	81.42325	26.48485	33.91766

- **Sample Summary CSV**
  - CSV version of the data displayed in the Target Enrichment Sample Summary table.

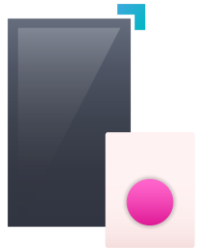
	A	B	C	D	E	F
1	Sample	Number of Reads	Median Read Length (bp)	Median Read Quality	Mean Target Coverage	Percent of Targets with â‰¥10-fold Coverage
2	BioSample97	11,241	4,951	Q43	211	100.00%
3	BioSample98	107,054	4,662	Q43	220.95	100.00%
4	BioSample99	1,093,548	3,542	Q47	197.15	100.00%
5	BioSample100	8,570	5,009	Q42	176.25	100.00%
6	BioSample101	12,882	4,946	Q42	221.05	100.00%
7	BioSample102	151,036	3,832	Q46	211.55	100.00%

[1] See ***SMRT Link User Guide*** (Documentation) for detailed descriptions of Target Enrichment analysis workflow output files available for download.

PureTarget carrier panel data analysis workflow overview (cont.)

PureTarget carrier panel variant calling analysis can be performed using PureTarget carrier pipeline (PTCP) analysis software available from GitHub and PacBio Compatible partners[1]

PureTarget carrier panel variant calling analysis workflow[2]



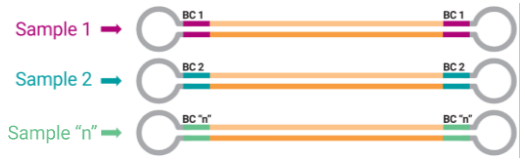
On-instrument

Customer or PacBio Compatible compute

GitHub
DNAnexus

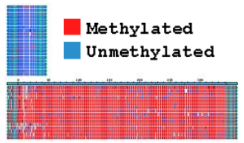
PTCP analysis workflow

Demultiplex `lima`



- Samples are demultiplexed on-instrument using `lima`

5mC probabilities



- 5mC methylation probabilities are detected on-instrument for CpG sites

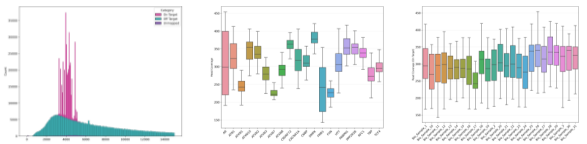
Mapping `pbmm2`

- Reads are mapped to hg38 reference genome with `pbmm2` to generate panel coverage QC statistics

Variant calling `trgt` `paraphase` `sawfish`

- Repeat genotypes called with TRGT
- Gene paralog variants called with Paraphase
- Structural variants are called with Sawfish

QC



- QC stats are aggregated using PTCP-QC

PacBio

[1] See ***Application note – Consolidate challenging genes with PureTarget carrier screen panel*** (102-326-653) for detailed descriptions of analysis workflow options for PureTarget carrier applications.
[2] DNAnexus offers a prebuilt GUI-based cloud workflow for PTCP analysis. GitHub PTCP pipeline is a command line-based, locally installed workflow maintained by customers.

PureTarget carrier panel data analysis workflow overview (cont.)

PureTarget carrier panel (PTCP) variant calling analysis **setup**

GitHub

Customer HPC

Deployment

DNAnexus

DNAnexus platform

Configure workflow

```
git clone \
https://github.com/PacificBiosciences/ptcp.git
cd ptcp
conda create -n ptcp \
python=3.12.11
conda activate ptcp python -m pip install \
-r requirements.txt
```

```
docker pull quay.io/pacbio/ptcp:latest
```

Create Sample Sheet

```
movie.bc2046.bam,movie.bc2046,F
movie.bc2047.bam,movie.bc2047,F
movie.bc2048.bam,movie.bc2048,F
movie.bc2049.bam,movie.bc2049,F
```

Create inputs JSON

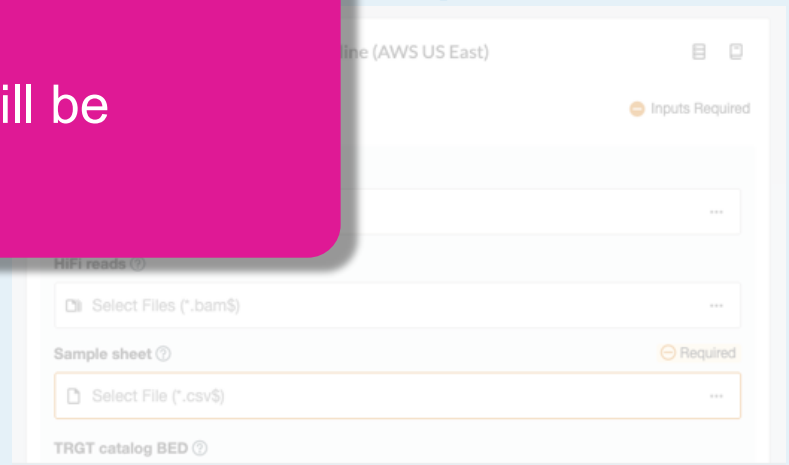
```
{
 "ptcp.sample_sheet": "inputs/sample_sheet.csv",
 "ptcp.ref_fasta": "data/reference/GRCh38.fa",
                                    GRCh38.fa.fai",
                                    nition.GRCh38.bed",
                                    ta/config.GRCh38.yaml",

                                    .GRCh38.bed",

                                    bc2042.bam"

                                    bc2042.bam"
```

Upload data

- Import from AWS S3
- Local import using Upload Agent

Select workflow

ct inputs



Minimally PTCP requires:

- HiFi (+ fail) reads BAM files
- A reference genome
- trgt BED file
- paraphase configuration JSON
- ptpc-qc configuration JSON

Configuration and setup will be deployment specific

PacBio

PTCP is pre-configured

PureTarget carrier panel data analysis workflow overview (cont.)

PureTarget carrier panel (PTCP) variant calling analysis **setup**

GitHub

Customer HPC

Deployment

DNAnexus

DNAnexus platform

Configure workflow

```
git clone \
https://github.com/PacificBiosciences/ptcp.git
cd ptcp
conda create -n ptcp \
python=3.12.11
conda activate ptcp python -m pip install \
-r requirements.txt
```

```
docker pull quay.io/pacbio/ptcp:latest
```

Create Sample Sheet

```
bam_name,bam_id,sex
movie.bc2042.bam,movie.bc2042,F
movie.bc2043.bam,movie.bc2043,F
movie.bc2044.bam,movie.bc2044,F
movie.bc2045.bam,movie.bc2045,F
movie.bc2046.bam,movie.bc2046,F
movie.bc2047.bam,movie.bc2047,F
movie.bc2048.bam,movie.bc2048,F
movie.bc2049.bam,movie.bc2049,F
```

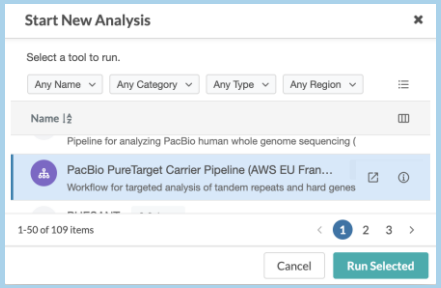
Create inputs JSON


```
{
 "ptcp.sample_sheet": "inputs/sample_sheet.csv",
 "ptcp.ref_fasta": "data/reference/GRCh38.fa",
 "ptcp.ref_index": "data/reference/GRCh38.fa.fai",
 "ptcp.trgt_bed": "meta/repeat_definition.GRCh38.bed",
 "ptcp.paraphase_config_yaml": "meta/config.GRCh38.yaml",
 "ptcp.genome_version": "38",
 "ptcp.ptcp_qc_bed": "meta/ptcp-qc.GRCh38.bed",
 "ptcp.hifi_reads": [
  "/data/reads/SAMPLE1.hifi_reads.bc2042.bam"
],
 "ptcp.fail_reads": [
  "/data/reads/SAMPLE1.fail_reads.bc2042.bam"
]
}
```


Upload data

- Import from AWS S3
- Local import using Upload Agent

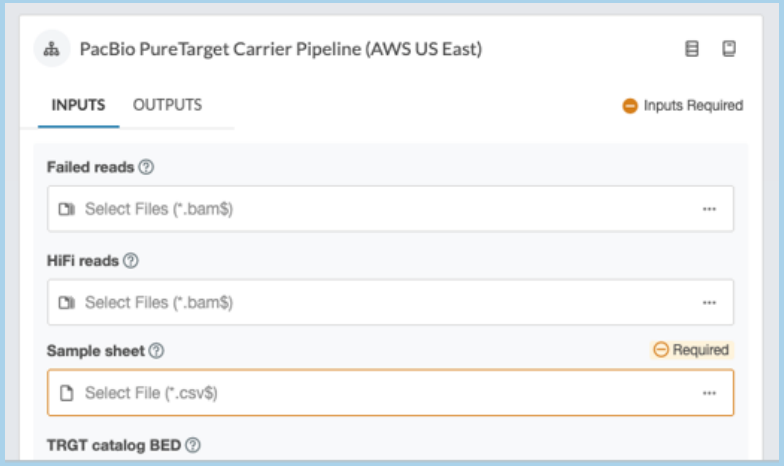
Select workflow



Create Sample Sheet

```
bam_name,bam_id,sex
movie.bc2042.bam,movie.bc2042,F
movie.bc2043.bam,movie.bc2043,F
movie.bc2044.bam,movie.bc2044,F
movie.bc2045.bam,movie.bc2045,F
movie.bc2046.bam,movie.bc2046,F
movie.bc2047.bam,movie.bc2047,F
movie.bc2048.bam,movie.bc2048,F
movie.bc2049.bam,movie.bc2049,F
```

Select inputs



PacBio

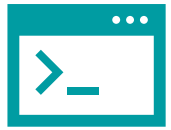
PTCP is pre-configured

PureTarget carrier panel data analysis workflow overview (cont.)

PureTarget carrier panel (PTCP) variant calling analysis **execution and outputs**

GitHub

Customer HPC



Deployment

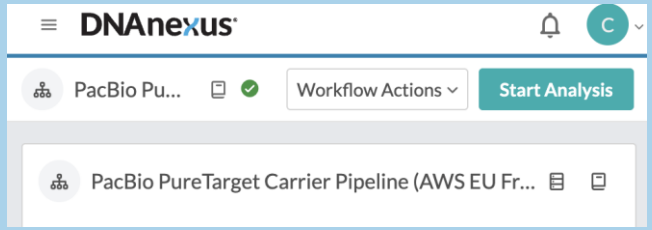
DNAnexus

DNAnexus platform

Run workflow

```
conda activate ptcp

cd /Path/to/ptcp

miniwdl run \
   --verbose \
   --dir /Path/to/output_dir \
   --cfg /Path/to/miniwdl.cfg \
   --input /Path/to/ptcp_inputs.json \
   main.wdl
```

Start analysis



Per sample outputs for tandem repeat analysis, gene phasing, and structural variant detection

```
bc2048.mapped.bam              <- pbmm2 mapped reads
bc2048.mapped.bam.bai
bc2048.repeats.bam             <- pbmm2 mapped reads intersecting the repeats
bc2048.repeats.bam.bai
bc2048.trgt.sorted.spanning.bam        <- TRGT BAM with spanning reads
bc2048.trgt.sorted.spanning.bam.bai
bc2048.trgt.vcf                        <- Tandem repeat genotypes generated by TRGT
bc2048.meth_allele.trgt_plots.zip      <- Tandem repeat plots generated by TRGT
bc2048.meth_waterfall.trgt_plots.zip   <- ...
bc2048.motifs_allele.trgt_plots.zip    <- ...
bc2048.motifs_waterfall.trgt_plots.zip <- ...
bc2048.f8inversion.json                <- F8 inversion calls with extended information
bc2048.f8inversion.vcf                 <- F8 inversion calls
bc2048.paraphase.bam                   <- Paraphase re-aligned BAM with annotations
bc2048.paraphase.bam.bai
bc2048.paraphase.json                  <- Paraphase results per target region
bc2048_cyp21.vcf  <- Paraphase VCF with small variant calls per target per haplotype
bc2048.havanno.json            <- Annotated small variants called by Paraphase
```

Coverage and quality metrics per sample and aggregated report across all sample

```
qc.aggregate.json   <- per target coverage
qc.bc2045.json      <- per sample per target coverage
qc.bc2046.json      <- ...
qc.bc2047.json      <- ...
qc.bc2048.json      <- ...
qc.bc2049.json      <- ...
```

PacBio

PureTarget carrier panel data analysis workflow overview (cont.)

PureTarget carrier panel (PTCP) variant calling analysis outputs **read count** as a measure of depth

Coverage and quality metrics per sample and aggregated report across all sample

qc.aggregate.json <- per target
qc.bc2045.json <- per sample+target
qc.bc2046.json <- ...
qc.bc2047.json <- ...
qc.bc2048.json <- ...
qc.bc2049.json <- ...

qc.aggregate.json

```
...
"hbb": {
      "samples_with_coverage": 16,
      "total_reads": 4388,
      "coverage_stats": {
        "min": 130.0,
        "q1": 234.5,
        "median": 271.0,
        "mean": 274.25,
        "q3": 318.0,
        "max": 421.0
      }
    },
...
```

qc.bc2045.json

```
{
  "sample_name": "bc2045",
  "genome_version": "38",
  "targets_bed": "ptcp-qc.GRCh38.bed",
  "ptcp-qc_version": "0.8.0",
  "timestamp": "2025-09-16T03:09:18.573904366+00:00",
  "stats": {
    "total_reads": 23230,
    "on_target": {
      "total": 2773,
      "hifi": 2445,
      "fail": 328
    },
    "off_target": {
      "total": 20402,
      "hifi": 19195,
      "fail": 1207
    },
    "unmapped": {
      "total": 55,
      "hifi": 5,
      "fail": 50
    },
    "loci": {
      "count": 24,
      "coverages": {
        "stats": {
          "min": 1.0,
          "q1": 2.0,
          "median": 47.0,
          "mean": 115.541664,
          "q3": 215.75,
          "max": 400.0
        }
      }
    },
...
```

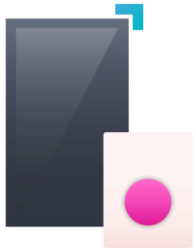
qc.bc2045.json

```
...
          }
        }
      }
    },
    "paraphase_results": {
      "gba": {
        "total_reads": 334,
        "unique_reads": 334,
        "total_cn": 4,
        "haplotypes": {
          "gba_hap1": {
            "total_count": 58,
            "unique_count": 58,
            "fractional_count": 58.0
          },
          "gba_hap2": {
            "total_count": 201,
            "unique_count": 201,
            "fractional_count": 201.0,
            "n_copy": 2
          },
          "gba_hap3": {
            "total_count": 75,
            "unique_count": 75,
            "fractional_count": 75.0
          }
        }
      }
    }
  },
...
```

PureTarget custom panel data analysis workflow overview

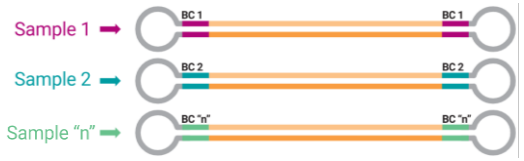
Evaluation of PureTarget custom panel performance QC can be performed in SMRT Link using the Target enrichment analysis workflow or at the command line[1]

PureTarget custom panel SMRT Link QC analysis workflow[2]



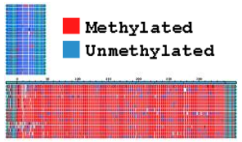
On-instrument

Demultiplex
`lima`



- Samples are demultiplexed on-instrument using `lima`

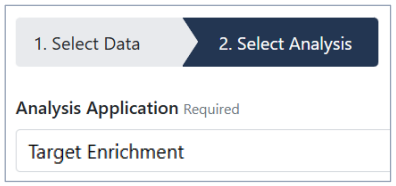
5mC probabilities



- 5mC methylation probabilities are detected on-instrument for CpG sites

SMRT Link v25.3

SMRT Analysis

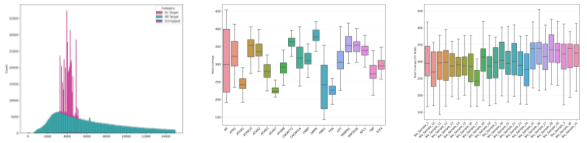


SMRT Link

Mapping
`Pbmm2`

- Reads are mapped to hg38 reference genome with `pbmm2` to generate panel coverage QC statistics

Visualize



- Visualizations of PureTarget panel performance QC results are produced

[1] See ***Technical note – A practical guide to amplification-free PureTarget panels*** (102-326-652) for detailed descriptions of analysis workflow options for custom PureTarget applications.
[2] See ***SMRT Link User Guide*** (Documentation) for detailed descriptions of parameter settings for Target Enrichment analysis application.

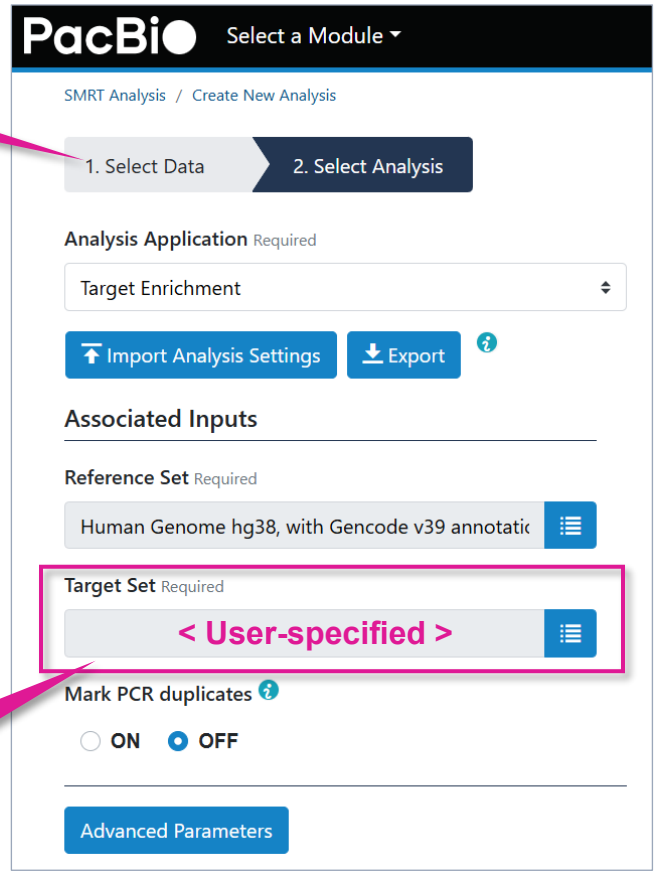
PureTarget custom panel data analysis workflow overview (cont.)

Target definitions specification for SMRT Link PureTarget custom panel QC evaluation[1]

Set to run as "One analysis for all Data Sets"

SMRT Link v25.3

SMRT Analysis



Specify to use the desired **user-created target BED** set

- Set to run as "One analysis for all Data Sets" to summarize across indexed samples
- To analyze data from a custom panel, create a new BED dataset containing positive control target sequences corresponding to PureTarget control panel (103-633-300) along with additional desired custom targets[2]

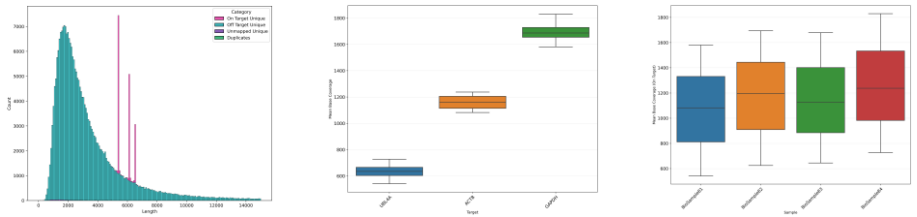
BED file target specification

`*. PureTarget_control_panel.cut_site.GRCh38.bed`

Example target cut site definitions for PureTarget control panel

```
chrX   154482054   154488569   ID=UBL4A
chr7   5525920     5532077     ID=ACTB
chr12  6534182     6539602     ID=GAPDH
```

- Target cut site are located on Chr X, Chr 7 and Chr 12
- Identifiers are *UBL4A*, *ACTB* and *GAPDH*
- Note: SMRT Link BED files must contain a fourth column with ID=NAME. See the user guide for more details[1]



[1] See ***SMRT Link User Guide*** (Documentation) for detailed descriptions of parameter settings for PureTarget Target Enrichment analysis application.
[2] See TRGT documentation in PacBio GitHub for more information on the required BED file format.

PureTarget custom panel data analysis workflow overview (cont.)

SMRT Link PureTarget custom panel QC analysis outputs

SMRT Link v25.3

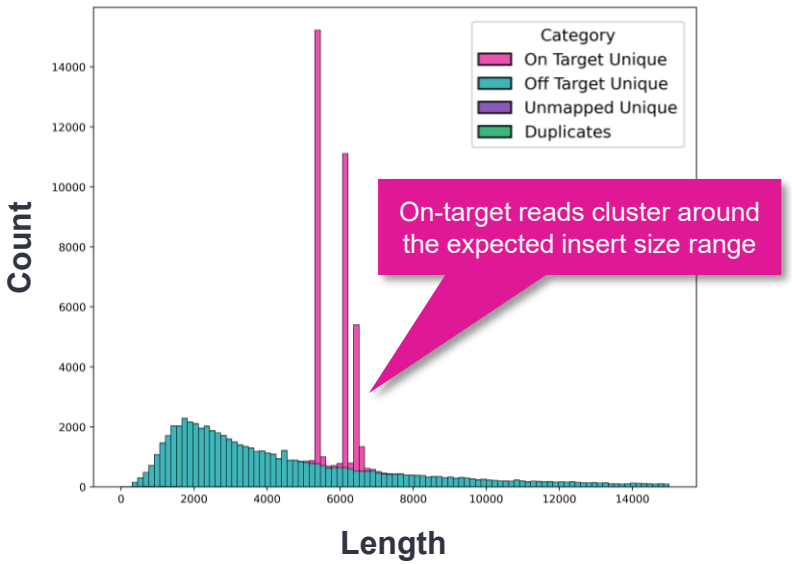
SMRT Analysis

Select Analysis Workflow (Required): Target Enrichment

- Target Enrichment
  - Summary Metrics
  - Read Categories
  - Target Coverage
  - Sample Coverage

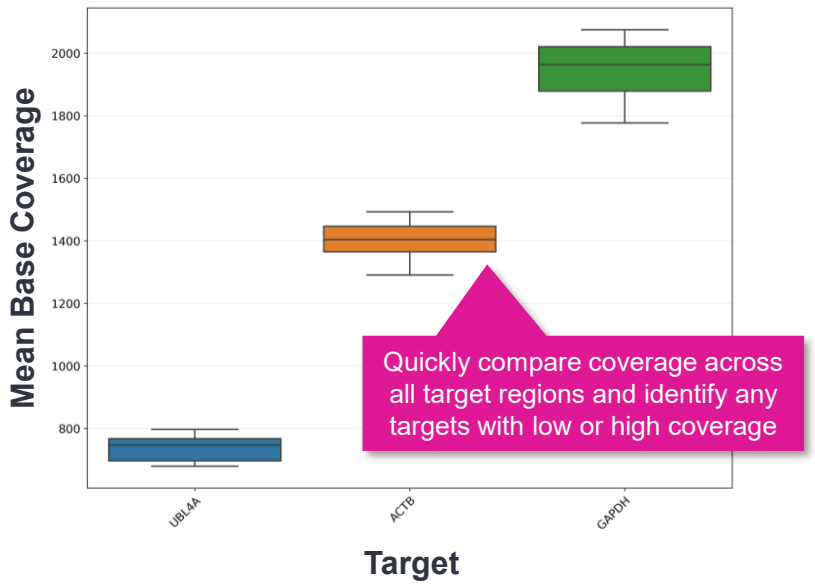
QC plots & metrics

Read Categories[1]



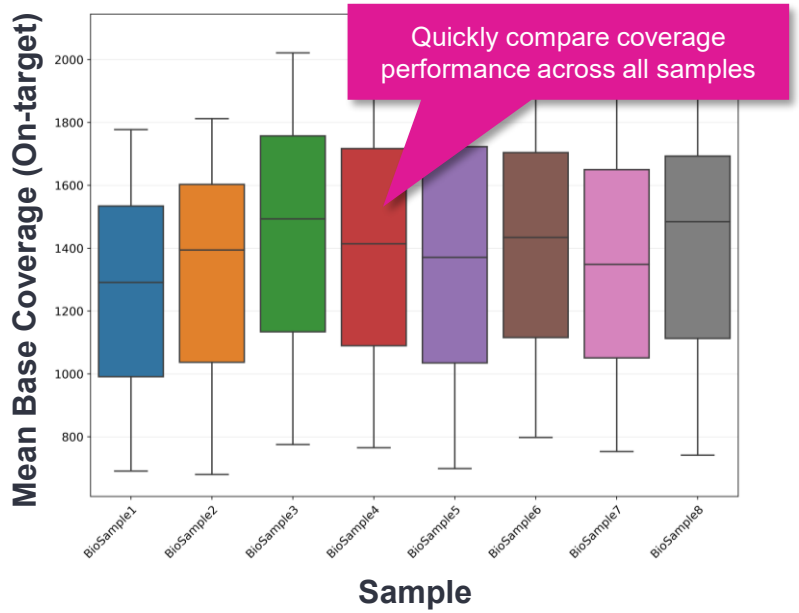
- Histogram mapping the length of On target, Off target, and Unmapped reads in the sample

Target Coverage[2]



- Box plot for each target regions of mean coverage across all samples analyzed

Sample Coverage[2]



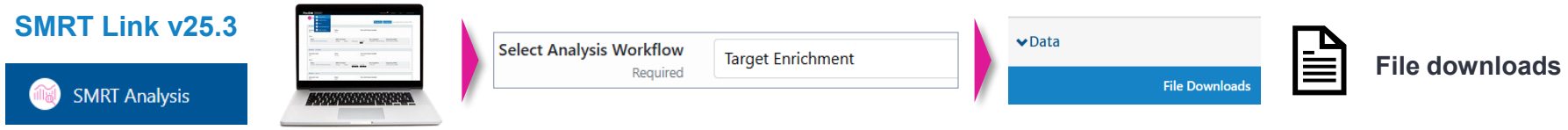
- Box plot for each sample of mean coverage across all target regions

[1] **Note:** A read is defined as being on-target if its alignment region in the reference genome has a non-empty overlap with any defined target in the input BED file. Histogram bars for the different categories are stacked. For PureTarget samples, the number reads in the 'Duplicates' category is typically zero since PCR duplicates are not expected to be present in PureTarget libraries and Mark PCR Duplicates is therefore not performed.

[2] **Note:** For PureTarget carrier panels and PureTarget custom panels, SMRT Link Target Enrichment analysis workflow reports mean base coverage for target coverage and sample coverage QC plots.

PureTarget custom panel data analysis workflow overview (cont.)

SMRT Link PureTarget custom panel QC analysis outputs – File downloads



Edit Output File Name Prefix **Example:**analysis-[multiple]-123320

File	Size	Type
Mapped+Haplotagged BAM - BioSample146	26 MB	bam
Mapped+Haplotagged BAM - BioSample108	25 MB	bam
Mapped+Haplotagged BAM - BioSample162	36 MB	bam
Mapped+Haplotagged BAM Index - BioSample185	2 MB	bam_bai
Target Coverage By Sample CSV	22 KB	csv
Sample Summary CSV	8 KB	csv
Sample Mapped BAMs	5 GB	zip
Analysis Log	441 KB	log
SMRT Link Log	28 KB	log

- **Target Coverage By Sample CSV**
  - Comma-delimited text file of the matrix of all mean coverage values for each target (rows) and sample (columns). The data in this file is used to generate the Target Coverage and Sample Coverage box plot.

	A	B	C	D	E	F	G	H	I
1	targetName	BioSample1	BioSample2	BioSample3	BioSample4	BioSample5	BioSample6	BioSample7	BioSample8
2	UBL4A	690.897467	679.900844	774.944129	765.051266	698.799233	797.569916	753.020107	741.452341
3	ACTB	1290.993503	1394.134319	1493.070814	1414.276108	1371.073412	1434.178983	1348.245737	1484.30242
4	GAPDH	1777.403321	1812.297048	2021.746494	2020.251476	2075.365129	1974.968635	1951.868081	1901.698708
5									
6									
7									
8									

- **Sample Summary CSV**
  - CSV version of the data displayed in the Target Enrichment Sample Summary table.

	A	B	C	D	E	F
1	Sample	Number of Reads	Median Read Length (bp)	Median Read Quality	Mean Target Coverage	Percent of Targets with â‰¥10-fold Coverage
2	BioSample1	12,576	5,419	Q41	1236.67	100.00%
3	BioSample2	12,188	5,419	Q41	1275.67	100.00%
4	BioSample3	12,580	5,419	Q41	1408	100.00%
5	BioSample4	12,592	5,419	Q40	1373	100.00%
6	BioSample5	12,428	5,419	Q41	1354.67	100.00%
7	BioSample6	13,134	5,419	Q40	1354	100.00%

[1] See ***SMRT Link User Guide*** (Documentation) for detailed descriptions of Target Enrichment analysis workflow output files available for download.

SMRT Link Target Enrichment analysis application is a generalizable workflow that can be used to evaluate targeted sequencing performance QC

Recommended Target Enrichment analysis settings for amplicon, hybrid capture, and PureTarget sequencing applications

Targeted sequencing application	Target Enrichment analysis options			
	Include Fail Reads	Padding around regions (bp)	Mark PCR Duplicates	Variant Calling[1]
Amplicon sequencing	OFF (default)	0	OFF (default)	Optional
Hybrid capture sequencing[2]	OFF (default)	3000 (default)	ON	Optional
PureTarget sequencing (for PureTarget carrier screening & custom panels)	ON	0	OFF (default)	OFF (default)

[1] Singularity is only required if Variant Calling is performed.

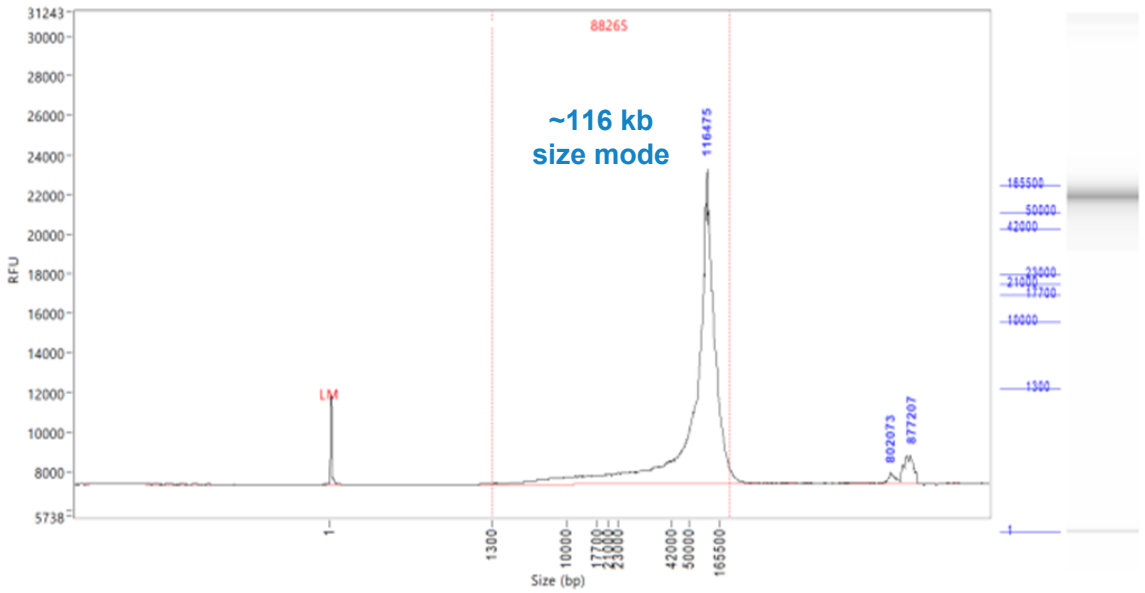
[2] **Note:** For QIAGEN hybrid capture long read panels, HiFi data analysis should be performed using QIAGEN software tools (e.g., QIAGEN GeneGlobe Data Analysis portal offers simplified downstream data analysis for HLA haplotyping and QIAGEN CLC Genomics Workbench is used to detect large structural variants in hereditary cancers).

PureTarget example sequencing performance data

Example PureTarget library preparation QC results

Example PureTarget repeat expansion panel library QC results for human blood gDNA samples

Input genomic DNA sizing QC



Example Femto Pulse genomic DNA sizing QC analysis results for high-molecular weight genomic DNA extracted from a human whole blood sample using Nanobind PanDNA kit.

PureTarget library preparation step yields

Library preparation step	DNA or SMRTbell overall recovery	
	Manual	Automated
Starting input genomic DNA	2,000 ng per sample	1,300 ng per sample
Post-gDNA repair & cleanup	1600 ng (80%) per sample	1040 ng (80%) per sample
Post-Cas9 digestion & cleanup	1200 ng (60%) per sample	780 ng (60%) per sample
Post-Adapter ligation & cleanup	1000 ng (50%) per sample	650 ng (50%) per sample
Post-nuclease treatment & cleanup[1]	288 ng (0.3%) per 48-plex	124.8 ng (0.1%) per 96-plex
Post-PureTarget library cleanup	96 ng (0.1%) per 48-plex	62.4 ng (0.05%) per 96-plex

Example library preparation yield results for PureTarget repeat expansion library prepared from a human whole blood sample using manual or automated workflows. For manual workflows, expected overall recovery is typically ~0.02-0.2% relative to starting gDNA total mass. For automated workflows, expected overall recovery is typically ~0.005-0.05%.[1,2]

Final PureTarget library yield is typically sufficient to load **1 SMRT Cell on Revio system + SPRQ chemistry or 1 SMRT Cell on Vega system**

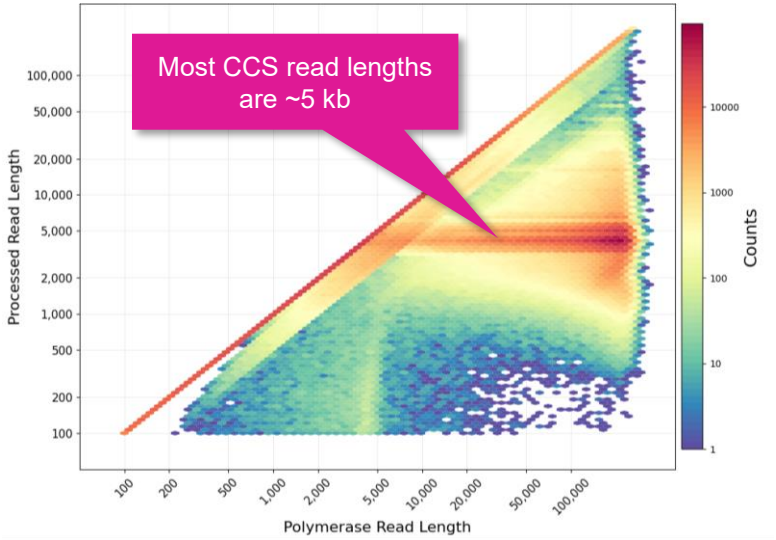
[1] **Note:** Manual PureTarget library prep workflow using PureTarget kit 24 employs **one** round of nuclease treatment. Automated PureTarget library prep workflow using PureTarget kit 96 employs **two** rounds of nuclease treatment.

[2] **Note:** It possible to observe PureTarget library prep recoveries outside of these expected ranges and still obtain acceptable sequencing yields and good target coverage performance.

Example 96-plex PureTarget repeat expansion panel library sequencing performance (Revio system + SPRQ chemistry)

96-plex PureTarget repeat expansion panel example data for human whole blood DNA samples[1]

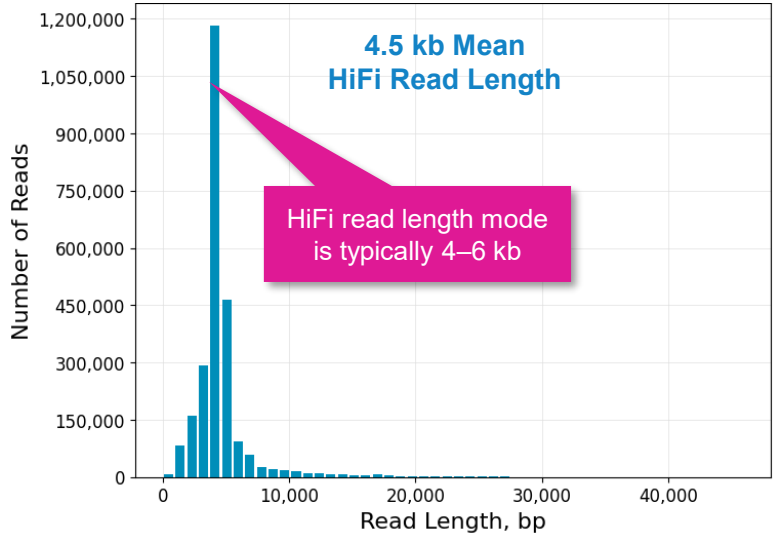
Raw Data Report



Raw Base Yield	395 Gb
Mean Polymerase Read Length	81.3 kb
P0	80%
P1	19%
P2	0%

Example metrics for a 96-plex PureTarget repeat expansion panel library run on a Revio system with SPRQ chemistry using a 24-hrs movie time. Revio system *P1* range for 96-plex PureTarget libraries was typically ~15%–40%.

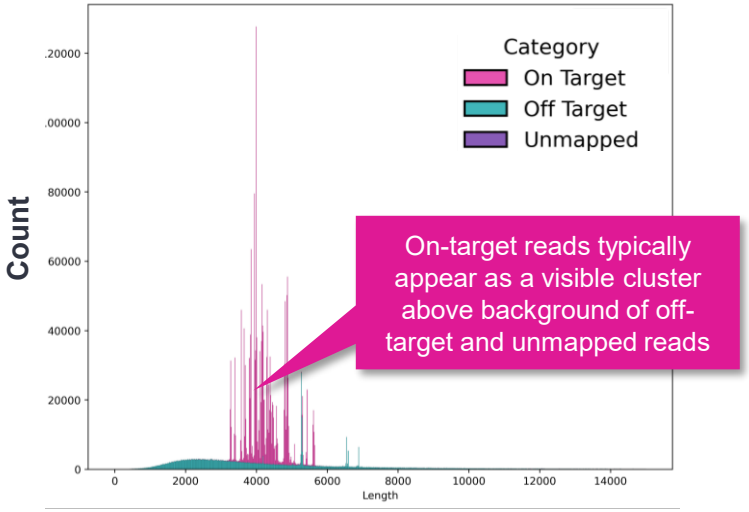
HiFi Read Length



HiFi Reads	2.5 M
HiFi Base Yield	11.4 Gb
Mean HiFi Read Length	4.5 kb
Median HiFi Read Quality	Q49
HiFi Read Mean # of Passes	33

For 96-plex PureTarget libraries, per-Revio SMRT Cell HiFi read counts were typically ~1 M–2 M depending on the final library insert size and *P1* loading performance.

Target Enrichment Summary Metrics



Total Bases*	11,812,292,440
Total Reads*	2,566,000
Median Read Length*	4,152 bp
Median Read Quality*	Q48
Sample Count	96
Target Regions	38

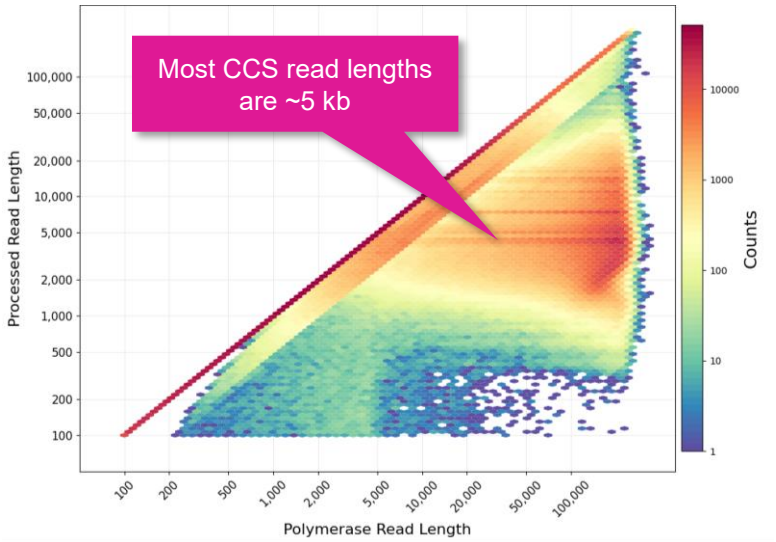
*** Includes HiFi + non-HiFi data.** For 96-plex PureTarget libraries, per-Revio SMRT Cell total read counts were typically ~1 M–2 M.

[1] Human whole blood DNA samples were extracted using PacBio Nanobind kits. **Note:** Read lengths, reads/data per SMRT Cell and other sequencing performance results can vary depending on DNA sample quality, insert size, *P1* loading performance & movie time. Note: Shorter library insert sizes (<5 kb), lower DNA quality samples, and suboptimal *P1* loading performance may result in lower data yields per SMRT Cell.

Example 96-plex PureTarget carrier panel library sequencing performance (Revio system + SPRQ chemistry)

96-plex PureTarget carrier panel example data for human whole blood DNA samples[1]

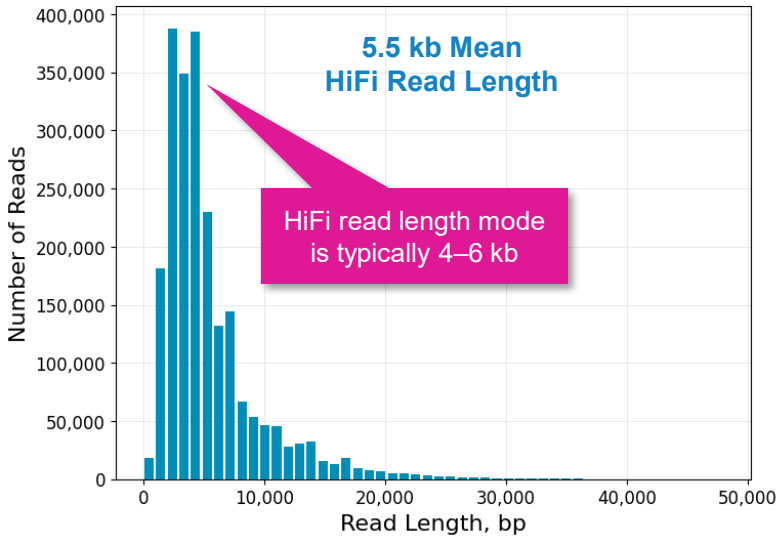
Raw Data Report



Raw Base Yield	362 Gb
Mean Polymerase Read Length	53.7 kb
P0	73%
P1	27%
P2	1%

Example metrics for a 96-plex PureTarget carrier panel library run on a Revio system with SPRQ chemistry using a 24-hrs movie time. Revio system *P1* range for 96-plex PureTarget libraries was typically ~15%–40%.

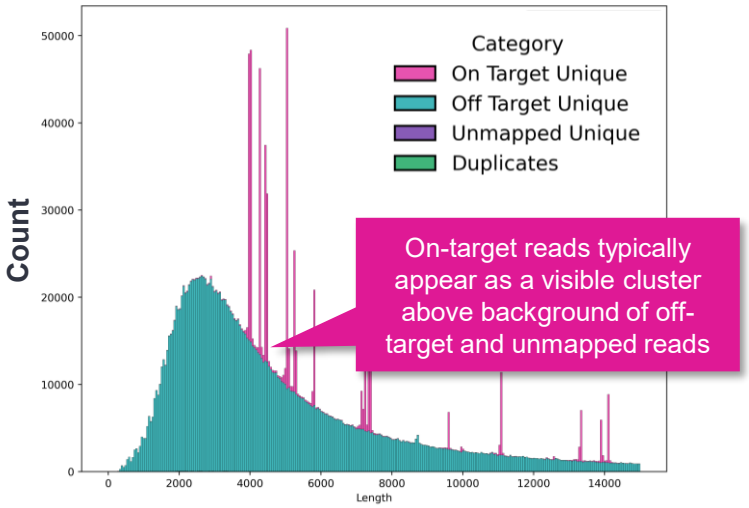
HiFi Read Length



HiFi Reads	2.2 M
HiFi Base Yield	12.2 Gb
Mean HiFi Read Length	5.5 kb
Median HiFi Read Quality	Q46
HiFi Read Mean # of Passes	31

For 96-plex PureTarget libraries, per-Revio SMRT Cell HiFi read counts were typically ~1 M–2 M depending on the final library insert size and *P1* loading performance.

Target Enrichment Summary Metrics



Total Bases*	13,043,277,783
Total Reads*	2,337,184
Median Read Length*	4,281 bp
Median Read Quality*	Q45
Sample Count	96
Target Regions	20

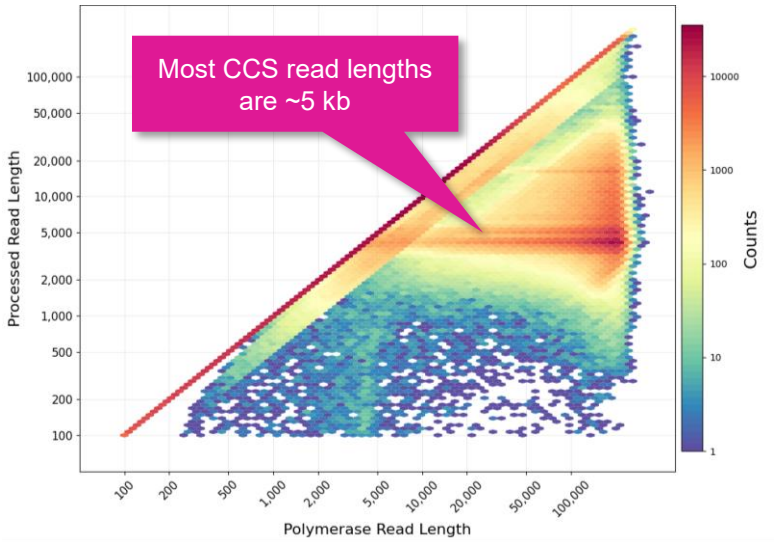
*** Includes HiFi + non-HiFi data.** For 96-plex PureTarget libraries, per-Revio SMRT Cell total read counts were typically ~1 M–2 M.

[1] Human whole blood DNA samples were extracted using a custom (non-Nanobind) third-party DNA extraction method. **Note:** Read lengths, reads/data per SMRT Cell and other sequencing performance results can vary depending on DNA sample quality, insert size, *P1* loading performance & movie time. Note: Shorter library insert sizes (<5 kb), lower DNA quality samples, and suboptimal *P1* loading performance may result in lower data yields per SMRT Cell.

Example 48-plex PureTarget repeat expansion panel library sequencing performance (Revio system + SPRQ chemistry)

48-plex PureTarget repeat expansion panel example data for human whole blood DNA samples[1]

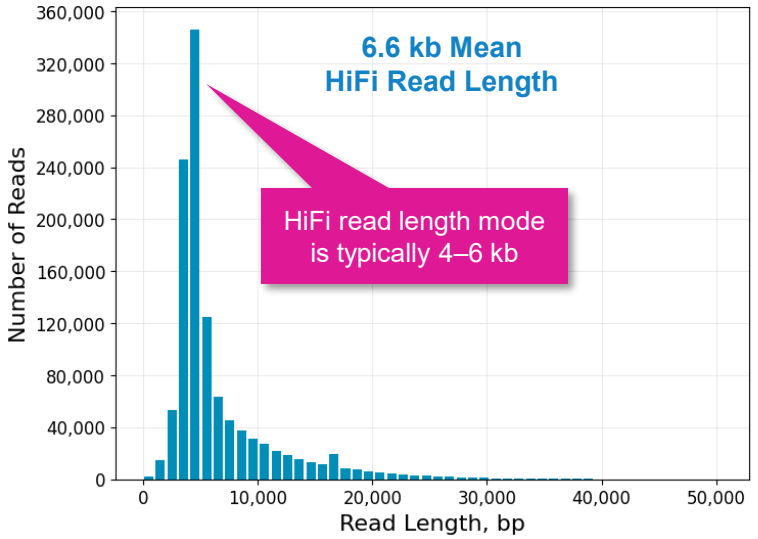
Raw Data Report



Raw Base Yield	202 Gb
Mean Polymerase Read Length	56.1 kb
P0	85%
P1	14%
P2	0%

Example metrics for a 48-plex PureTarget repeat expansion panel library run on a Revio system with SPRQ chemistry using a 24-hrs movie time. Revio system *P1* range for 48-plex PureTarget libraries was typically ~15%–50%.

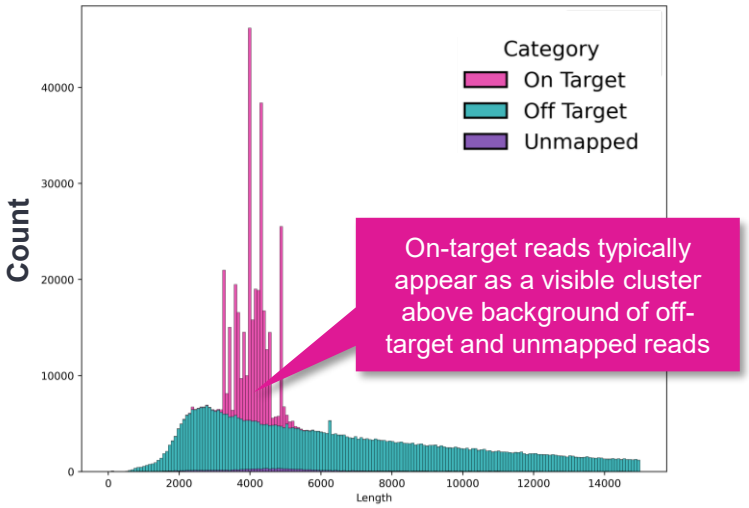
HiFi Read Length



HiFi Reads	1.1 M
HiFi Base Yield	7.5 Gb
Mean HiFi Read Length	6.6 kb
Median HiFi Read Quality	Q43
HiFi Read Mean # of Passes	26

For 48-plex PureTarget libraries, per-Revio SMRT Cell HiFi read counts were typically ~1 M–2 M depending on the final library insert size and *P1* loading performance.

Target Enrichment Summary Metrics



Total Bases*	7,692,021,081
Total Reads*	1,160,267
Median Read Length*	4,783 bp
Median Read Quality*	Q42
Sample Count	48
Target Regions	38

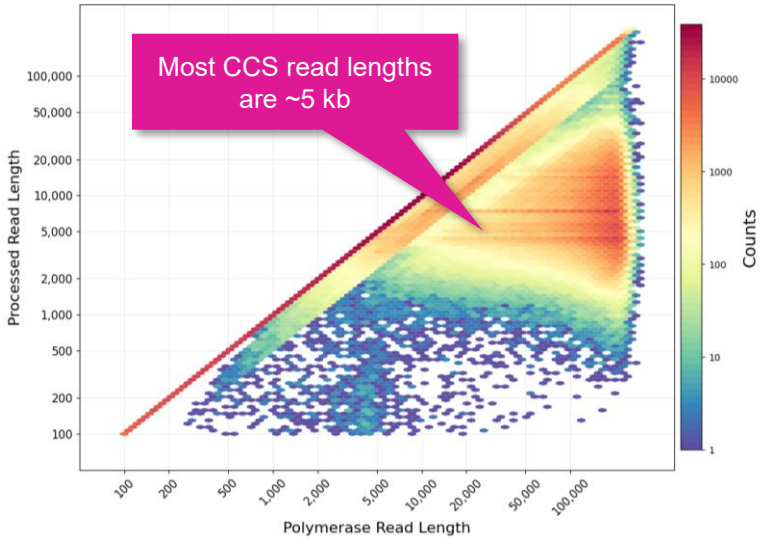
*** Includes HiFi + non-HiFi data.** For 48-plex PureTarget libraries, per-Revio SMRT Cell total read counts were typically ~1 M–2 M.

[1] Human whole blood DNA samples were extracted using PacBio Nanobind kits. 1 µg of input DNA per sample was used for PureTarget library preparation. **Note:** Read lengths, reads/data per SMRT Cell and other sequencing performance results can vary depending on DNA sample quality, insert size, *P1* loading performance & movie time. Note: Shorter library insert sizes (<5 kb), lower DNA quality samples, and suboptimal *P1* loading performance may result in lower data yields per SMRT Cell.

Example 48-plex PureTarget carrier panel library sequencing performance (Revio system + SPRQ chemistry)

48-plex PureTarget carrier panel example data for human whole blood DNA samples[1]

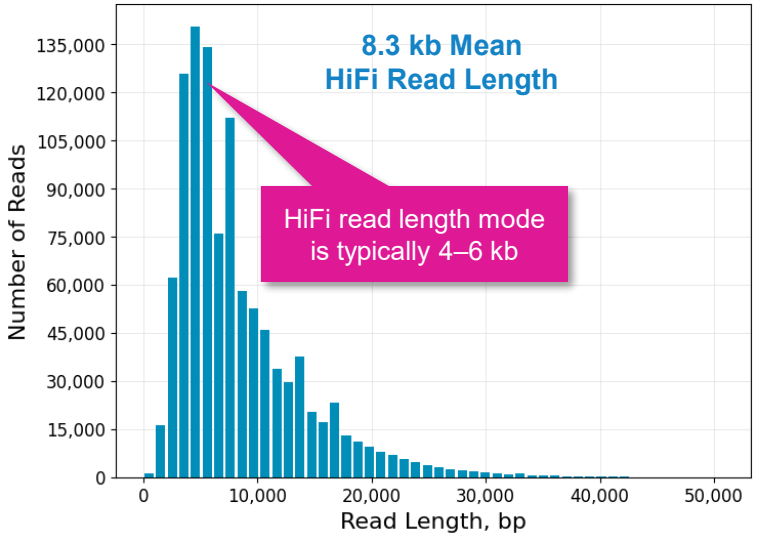
Raw Data Report



Raw Base Yield	194 Gb
Mean Polymerase Read Length	55.2 kb
P0	86%
P1	14%
P2	0%

Example metrics for a 48-plex PureTarget carrier panel library run on a Revio system with SPRQ chemistry using a 24-hrs movie time. Revio system *P1* range for 48-plex PureTarget libraries was typically ~15%–50%.

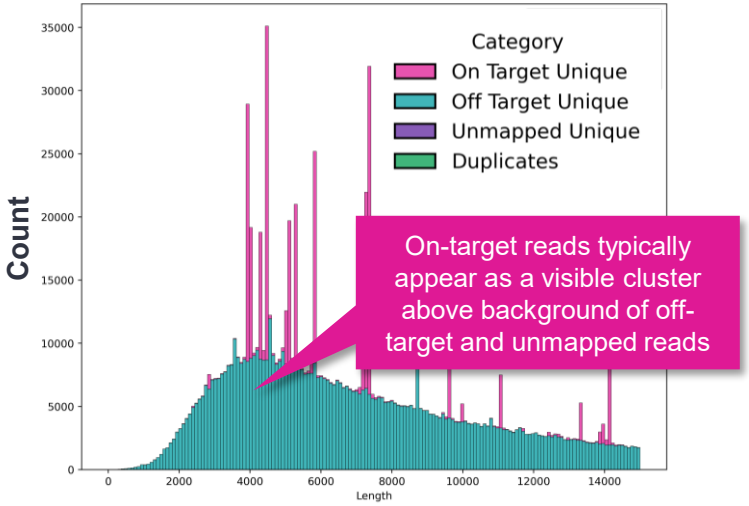
HiFi Read Length



HiFi Reads	1.1 M
HiFi Base Yield	8.8 Gb
Mean HiFi Read Length	8.3 kb
Median HiFi Read Quality	Q39
HiFi Read Mean # of Passes	22

For 48-plex PureTarget libraries, per-Revio SMRT Cell HiFi read counts were typically ~1 M–2 M depending on the final library insert size and *P1* loading performance.

Target Enrichment Summary Metrics



Total Bases*	9,099,180,991
Total Reads*	1,076,225
Median Read Length*	6,825 bp
Median Read Quality*	Q37
Sample Count	48
Target Regions	20

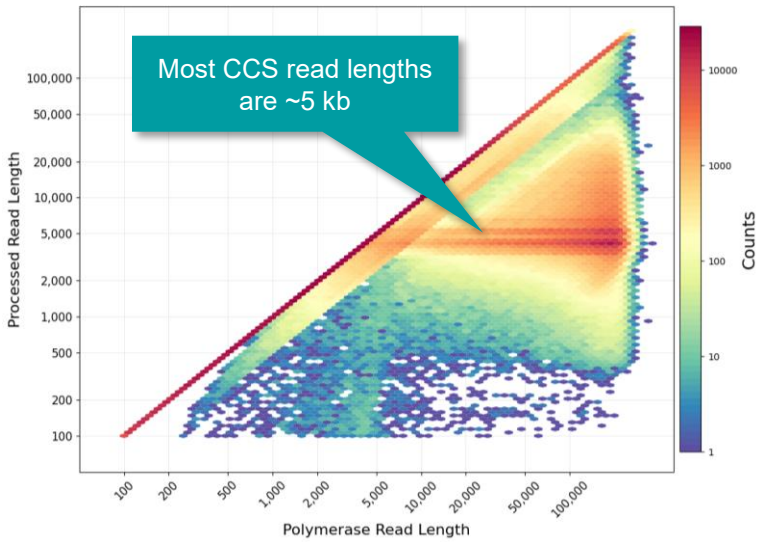
*** Includes HiFi + non-HiFi data.** For 48-plex PureTarget libraries, per-Revio SMRT Cell total read counts were typically ~1 M–2 M.

[1] Human whole blood DNA samples were extracted using PacBio Nanobind kits. 1 µg of input DNA per sample was used for PureTarget library preparation. **Note:** Read lengths, reads/data per SMRT Cell and other sequencing performance results can vary depending on DNA sample quality, insert size, *P1* loading performance & movie time. Note: Shorter library insert sizes (<5 kb), lower DNA quality samples, and suboptimal *P1* loading performance may result in lower data yields per SMRT Cell.

Example 48-plex PureTarget repeat expansion panel library sequencing performance (Vega system)

48-plex PureTarget repeat expansion panel example data for human blood & cell line DNA samples[1]

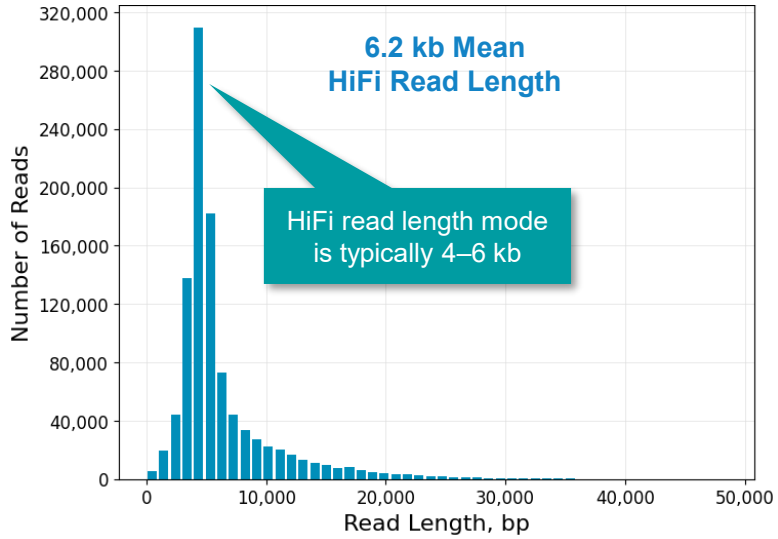
Raw Data Report



Raw Base Yield	167 Gb
Mean Polymerase Read Length	47.2 kb
Loading level	22%

Example metrics for a 16-plex PureTarget repeat expansion panel library run on a Vega system using a 24-hrs movie time. Vega system loading level range for 16-plex PureTarget libraries was typically ~20–40%.

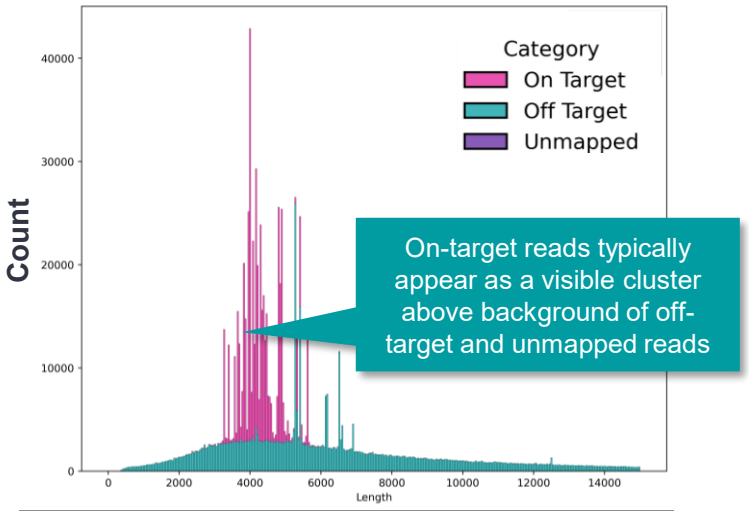
HiFi Read Length



HiFi Reads	1.0 M
HiFi Base Yield	6.4 Gb
Mean HiFi Read Length	6.2 kb
Median HiFi Read Quality	Q41
HiFi Read Mean # of Passes	24

For 16-plex PureTarget libraries, per-Vega SMRT Cell HiFi read counts were typically ~1 M–2 M depending on the final library insert size and loading level performance.

Target Enrichment Summary Metrics



Total Bases*	6,548,671,466
Total Reads*	1,042,657
Median Read Length*	4,799 bp
Median Read Quality*	Q40
Sample Count	48
Target Regions	38

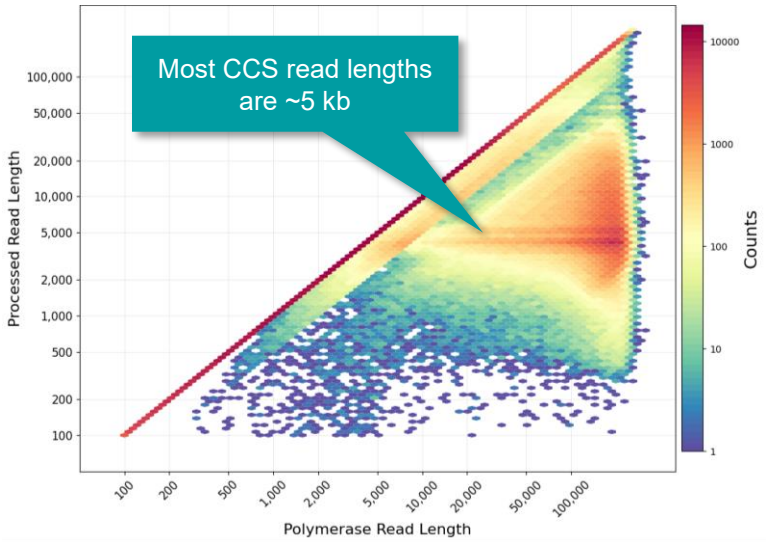
*** Includes HiFi + non-HiFi data.** For 16-plex PureTarget libraries, per-Vega SMRT Cell total read counts were typically ~1 M–2 M.

[1] Human blood DNA samples were extracted using PacBio Nanobind kits with or without RBC lysis. Cell line DNA samples were Coriell lymphoblastoid cell DNA with GQN30kb > 5. 2 µg of input DNA per sample was used for PureTarget library preparation. **Note:** Each sample was processed with both the PureTarget repeat expansion panel 2.0 (103-633-100) and the PureTarget control panel (103-633-300). Read lengths, reads/data per SMRT Cell and other sequencing performance results can vary depending on DNA sample quality, insert size, sample loading performance & movie time. Note: Shorter library insert sizes (<5 kb), lower DNA quality samples, and suboptimal sample loading performance may result in lower data yields per SMRT Cell.

Example 16-plex PureTarget repeat expansion panel library sequencing performance (Vega system)

16-plex PureTarget repeat expansion panel example data for human cell line DNA samples[1]

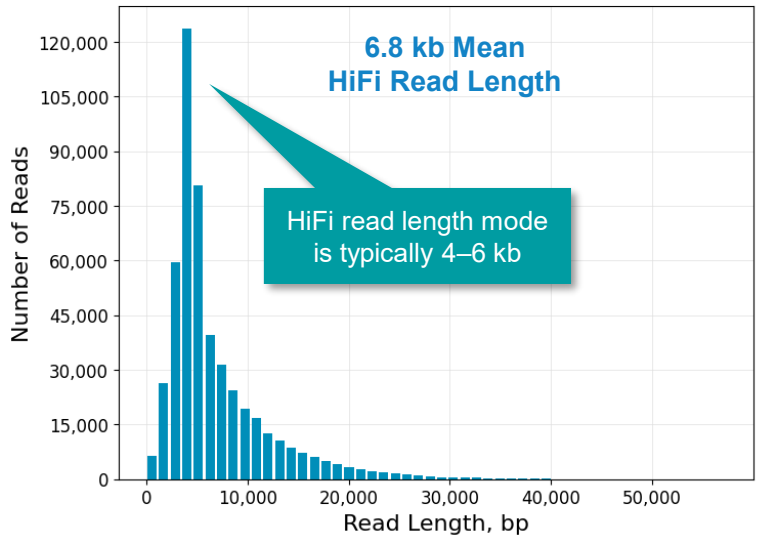
Raw Data Report



Raw Base Yield	93 Gb
Mean Polymerase Read Length	54.0 kb
Loading level	9%

Example metrics for a 16-plex PureTarget repeat expansion panel library run on a Vega system using a 24-hrs movie time. Vega system loading level range for 16-plex PureTarget libraries was typically ~10–15%.

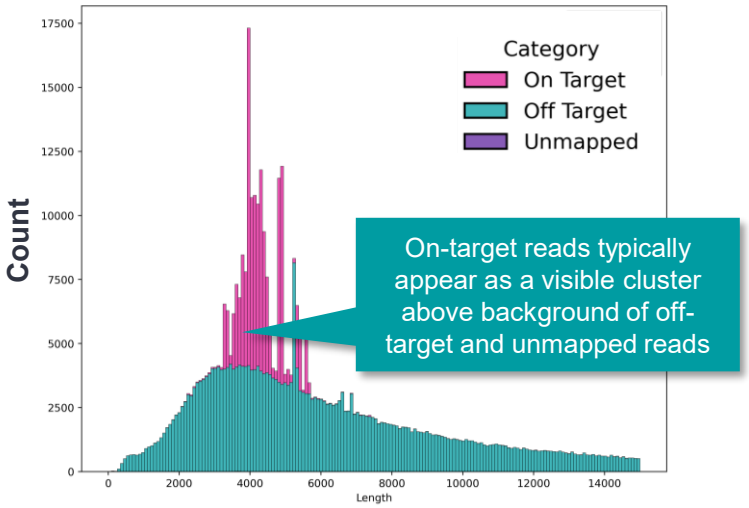
HiFi Read Length



HiFi Reads	0.5 M
HiFi Base Yield	3.4 Gb
Mean HiFi Read Length	6.8 kb
Median HiFi Read Quality	Q39
HiFi Read Mean # of Passes	27

For 16-plex PureTarget libraries, per-Vega SMRT Cell HiFi read counts were typically ~0.5 M–1 M depending on the final library insert size and loading level performance.

Target Enrichment Summary Metrics



Total Bases*	3,198,060,800
Total Reads*	469,669
Median Read Length*	4,880 bp
Median Read Quality*	Q39
Sample Count	16
Target Regions	38

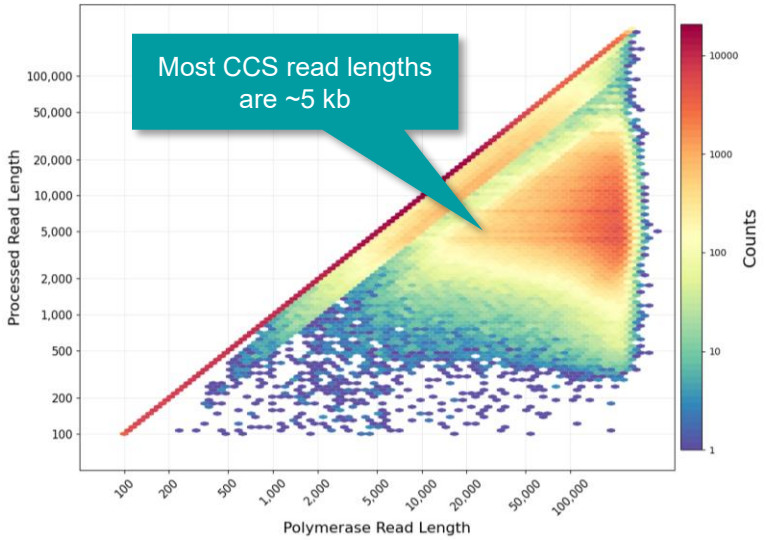
* **Includes HiFi + non-HiFi data.** For 16-plex PureTarget libraries, per-Vega SMRT Cell total read counts were typically ~0.5 M–1 M.

[1] Cell line DNA samples were Coriell lymphoblastoid cell DNA with GQN30kb > 5. 1 µg of input DNA per sample was used for PureTarget library preparation. Read lengths, reads/data per SMRT Cell and other sequencing performance results can vary depending on DNA sample quality, insert size, sample loading performance & movie time. Note: Shorter library insert sizes (<5 kb), lower DNA quality samples, and suboptimal sample loading performance may result in lower data yields per SMRT Cell.

Example 16-plex PureTarget carrier panel library sequencing performance (Vega system)

16-plex PureTarget carrier panel example data for human cell line DNA samples[1]

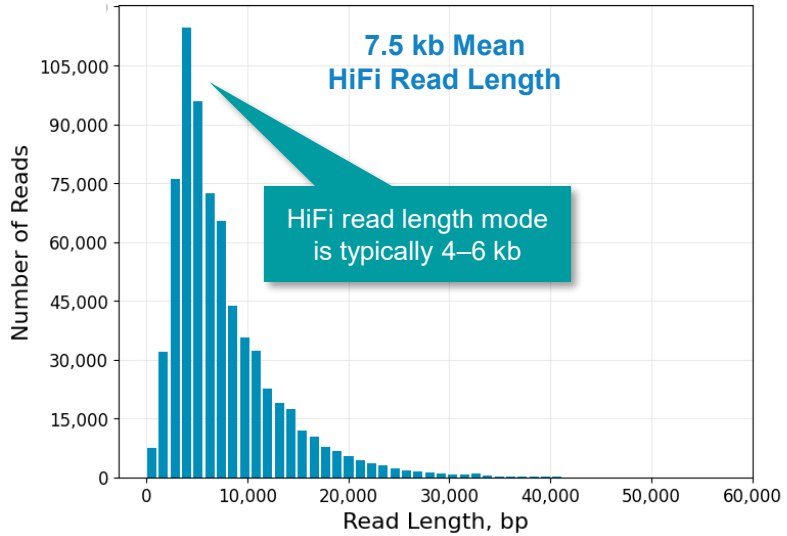
Raw Data Report



Raw Base Yield	126 Gb
Mean Polymerase Read Length	58.2 kb
Loading level	11%

Example metrics for a 16-plex PureTarget carrier panel library run on a Vega system using a 24-hrs movie time. Vega system loading level range for 16-plex PureTarget libraries was typically ~10–15%.

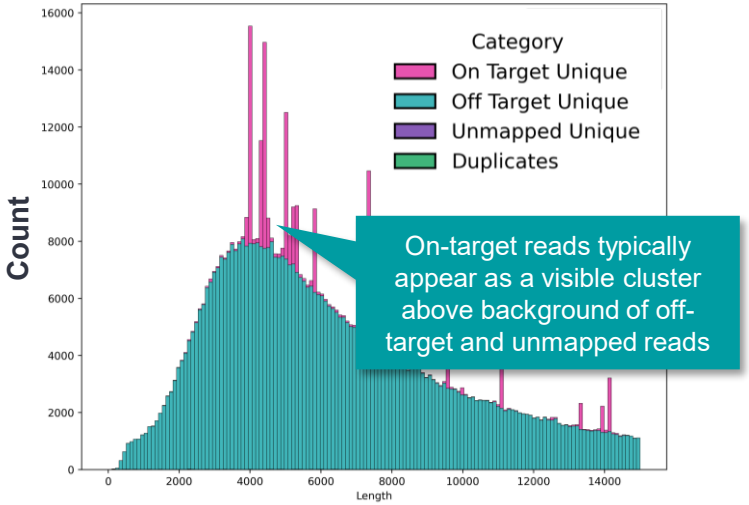
HiFi Read Length



HiFi Reads	0.7 M
HiFi Base Yield	5.2 Gb
Mean HiFi Read Length	7.5 kb
Median HiFi Read Quality	Q37
HiFi Read Mean # of Passes	23

For 16-plex PureTarget libraries, per-Vega SMRT Cell HiFi read counts were typically ~0.5 M–1 M depending on the final library insert size and loading level performance.

Target Enrichment Summary Metrics



Total Bases*	5,132,040,788
Total Reads*	666,609
Median Read Length*	6,000 bp
Median Read Quality*	Q36
Sample Count	16
Target Regions	20

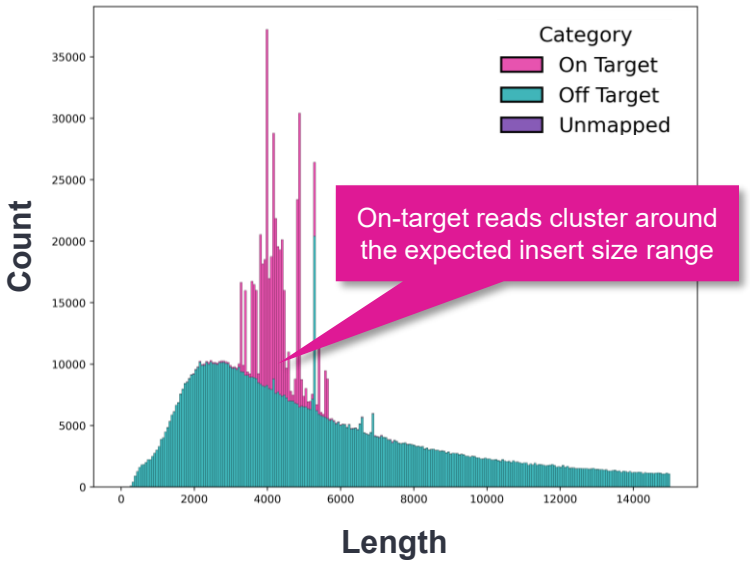
* **Includes HiFi + non-HiFi data.** For 16-plex PureTarget libraries, per-Vega SMRT Cell total read counts were typically ~0.5 M–1 M.

[1] 1.5 µg of input DNA per sample was used for PureTarget library preparation. Read lengths, reads/data per SMRT Cell and other sequencing performance results can vary depending on DNA sample quality, insert size, sample loading performance & movie time. Note: Shorter library insert sizes (<5 kb), lower DNA quality samples, and suboptimal sample loading performance may result in lower data yields per SMRT Cell.

Recommended guidance for evaluating PureTarget repeat expansion panel sequencing run performance

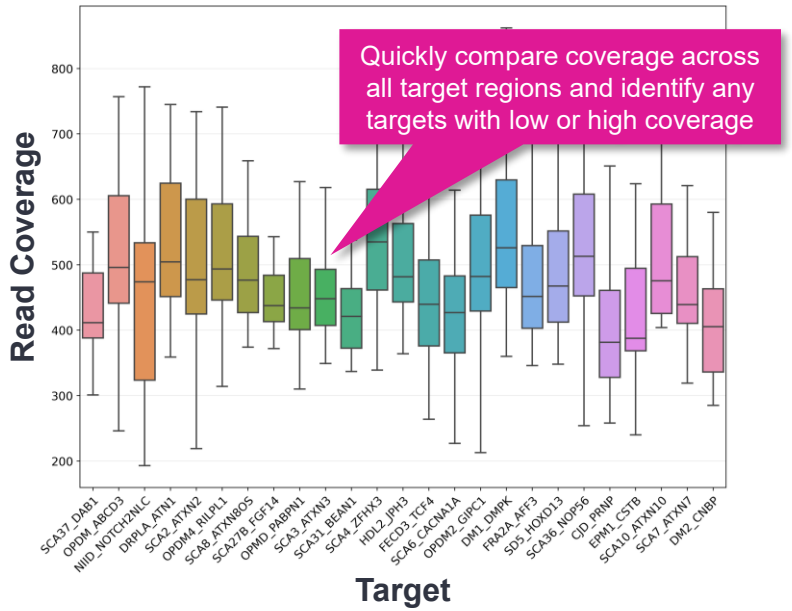
When evaluating PureTarget runs, it is generally more useful to examine the **secondary analysis results** (e.g., on-target coverage) since primary sequencing metrics like Productivity (*P0, P1, P2)* or Loading level are mostly dominated by 'background' non-targeted reads

Read Categories[1]



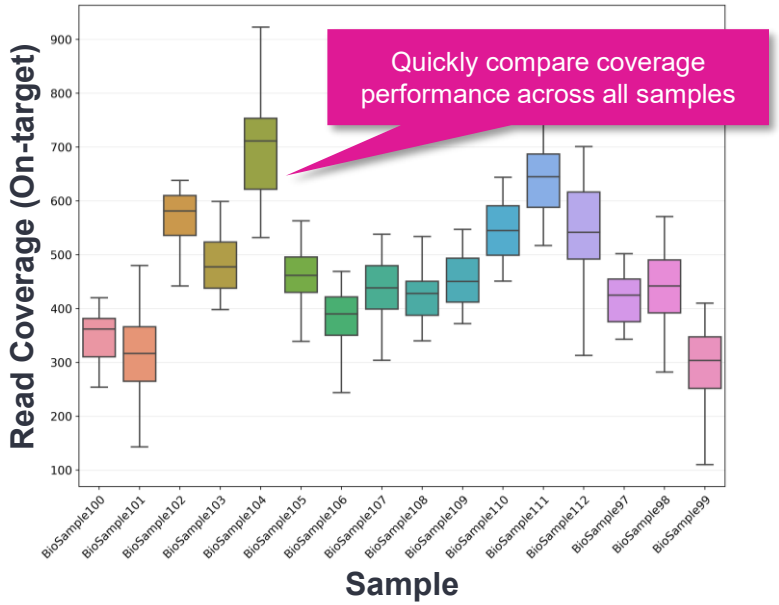
- Histogram mapping the length of On target, Off target, and Unmapped reads in the sample

Target Coverage[2]



- Box plot for each target regions of mean coverage across all samples analyzed

Sample Coverage[2]

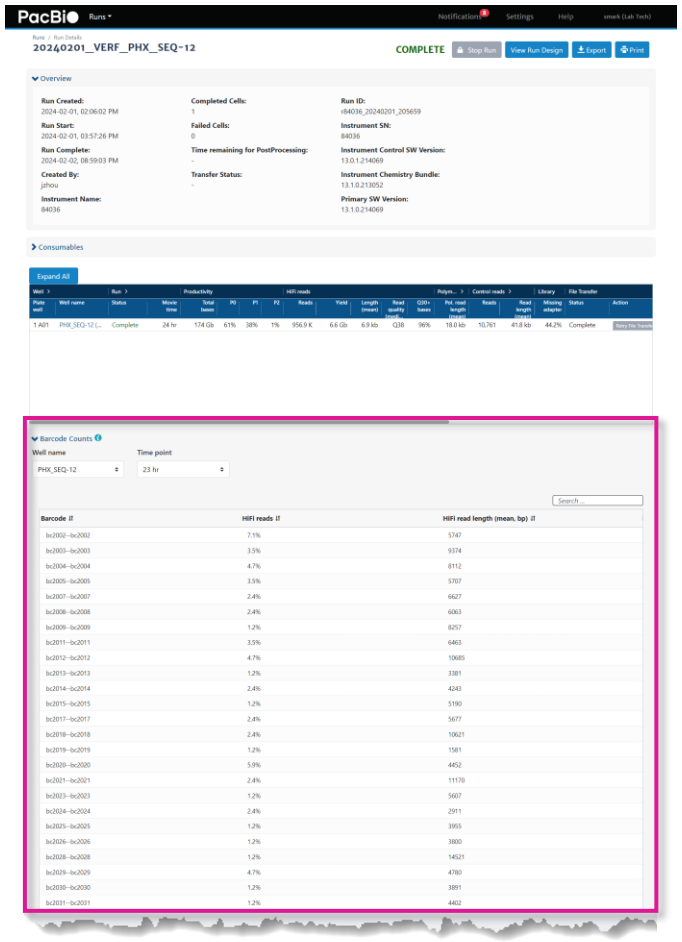


- Box plot for each sample of mean coverage across all target regions

[1] **Note:** A read is defined as being on-target if its alignment region in the reference genome has a non-empty overlap with any defined target in the input BED file.
[2] **Note:** PureTarget repeat expansion panel application reports mean read coverage for target coverage and sample coverage QC plots.

Recommended guidance for evaluating PureTarget repeat expansion panel sequencing run performance (cont.)

For Revio system, Barcode Counts preview metrics[1] in SMRT Link Run Details report are useful for early evaluation of PureTarget sample demultiplexing performance and per-sample mean HiFi read length



Barcode Counts

Well name: PHX_SEQ-12 | Time point: 23 hr

HiFi read length, mean: An estimate of the average HiFi read length for each barcode or for unbarcoded reads

Barcode: An individual barcode detected in the sample, as well as unbarcoded reads

HiFi reads: An estimate of the % of reads with each barcode, as well as the % of unbarcoded reads

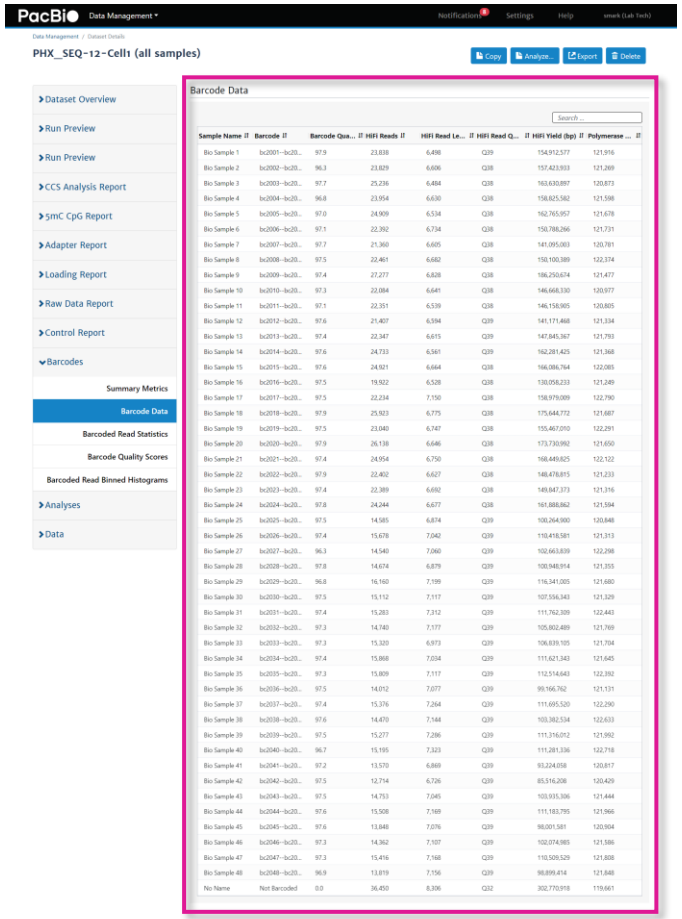
Search ...

Barcode	HiFi reads	HiFi read length (mean, bp)
bc2002--bc2002	7.1%	5747
bc2003--bc2003	3.5%	9374
bc2004--bc2004	4.7%	8112
bc2005--bc2005	3.5%	5707
bc2007--bc2007	2.4%	6627
bc2008--bc2008	2.4%	6063
bc2009--bc2009	1.2%	8257
bc2011--bc2011	3.5%	6463
bc2012--bc2012	4.7%	10685
bc2013--bc2013	1.2%	3381
bc2014--bc2014	2.4%	4243
bc2047--bc2047	3.5%	4884
bc2048--bc2048	1.2%	4294
Not Barcoded	5.9%	6508

[1] **Note:** The Barcode Counts preview values displayed may overestimate the number of unbarcoded reads. In addition, all estimates may be less accurate for barcodes at low frequency (<10%) due to sample size. Any barcodes below a 1% frequency are not displayed, and are grouped into the "Other" category

Recommended guidance for evaluating PureTarget repeat expansion panel sequencing run performance (cont.)

View Barcode demultiplexing results in SMRT Link SMRT Analysis to perform more detailed evaluation of PureTarget sample demultiplexing performance



PacBio Data Management Notifications 8 Settings Help smark (Lab Tech)

Data Management / Dataset Details

PHX_SEQ-12-Cell1 (all samples)

Copy Analyze... Export Delete

- Dataset Overview
- Run Preview
- Run Preview
- Barcodes
  - Summary Metrics
  - Barcode Data
  - Barcoded Read Statistics
  - Barcode Quality Scores
  - Barcoded Read Binned Histograms
- Analyses

Barcode Data

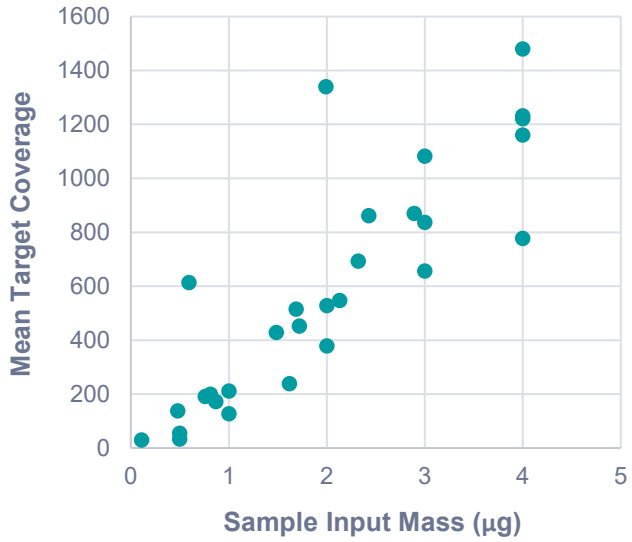
Search ...

Sample Name	Barcode	Barcode Qua...	HiFi Reads	HiFi Read Le...	HiFi Read Q...	HiFi Yield (bp)	Polymerase ...
Bio Sample 1	bc2001--bc20...	97.9	23,838	6,498	Q39	154,912,577	121,916
Bio Sample 2	bc2002--bc20...	96.3	23,829	6,606	Q38	157,423,933	121,269
Bio Sample 14	bc2014--bc20...	97.6	24,733	6,561	Q39	162,281,425	121,368
Bio Sample 15	bc2015--bc20...	97.6	24,921	6,664	Q38	166,086,764	122,085
Bio Sample 16	bc2016--bc20...	97.5	19,922	6,528	Q38	130,058,233	121,249
Bio Sample 17	bc2017--bc20...	97.5	22,234	7,150	Q38	158,979,009	122,790
Bio Sample 18	bc2018--bc20...	97.9	25,923	6,775	Q38	175,644,772	121,687
Bio Sample 19	bc2019--bc20...	97.5	23,040	6,747	Q38	155,467,010	122,291
Bio Sample 20	bc2020--bc20...	97.9	26,138	6,646	Q38	173,730,992	121,650
Bio Sample 21	bc2021--bc20...	97.4	24,954	6,750	Q38	168,449,825	122,122
Bio Sample 22	bc2022--bc20...	97.9	22,402	6,627	Q38	148,478,815	121,233
Bio Sample 23	bc2023--bc20...	97.4	22,389	6,692	Q38	149,847,373	121,316
Bio Sample 24	bc2024--bc20...	97.8	24,244	6,677	Q38	161,888,862	121,594
Bio Sample 48	bc2048--bc20...	96.9	13,819	7,156	Q39	98,899,414	121,848
No Name	Not Barcoded	0.0	36,450	8,306	Q32	302,770,918	119,661

Recommended guidance for evaluating PureTarget repeat expansion panel sequencing run performance (cont.)

On-target coverage is affected by input gDNA quantity, input gDNA quality and multiplex level[1]

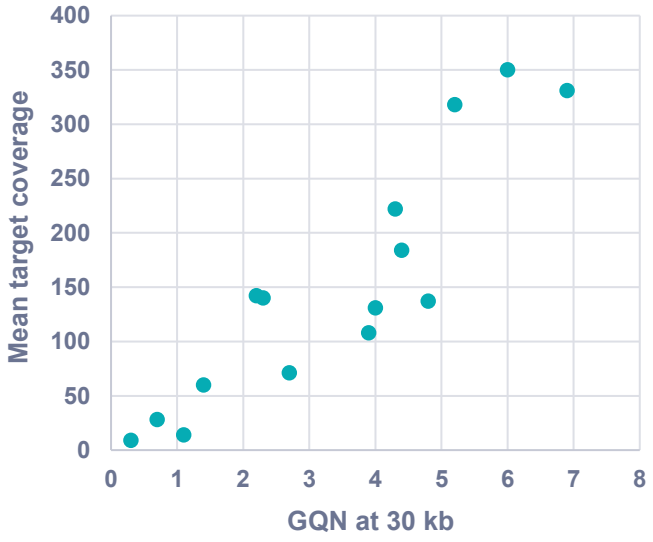
Input DNA quantity and read coverage[1]



DNA input quantity versus coverage. A total mass of 58 µg was prepared from 29 samples and sequenced on a Revio system with SPRQ chemistry.[2]

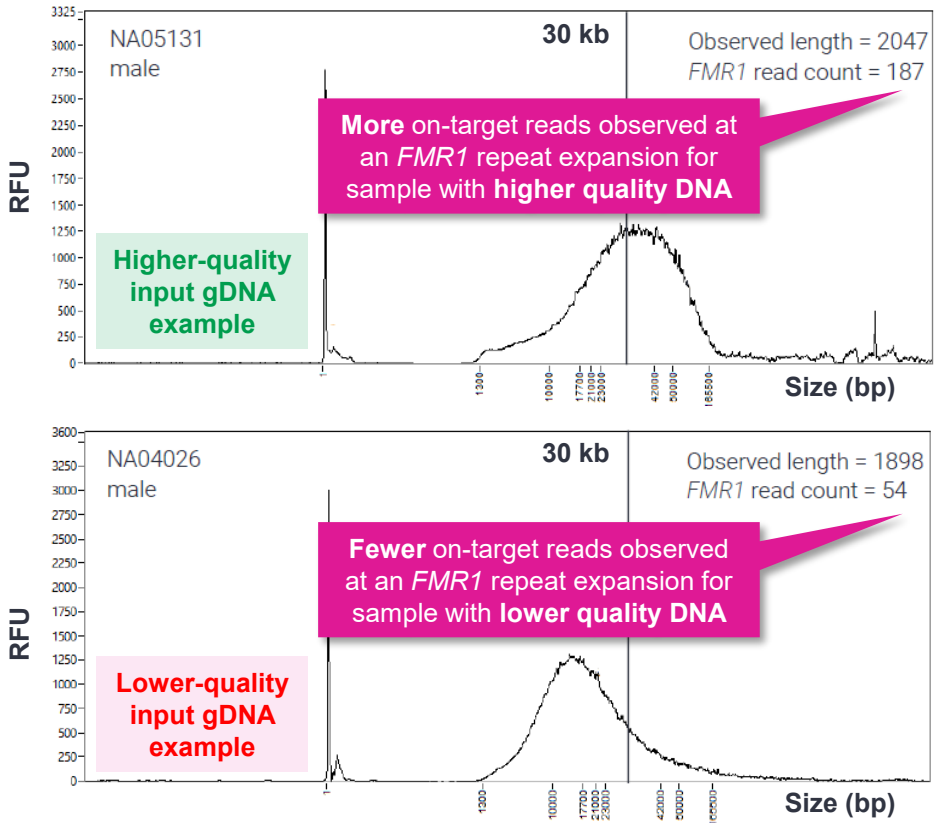
Sample coverage for target regions increases with quantity of DNA used in PureTarget library prep for a given multiplex level

Input DNA quality and read coverage[2]



Importance of DNA quality for target coverage. PureTarget repeat expansion panel 1.0 libraries were prepared with 2 µg DNA input each and sequenced in a 16-plex on a Revio system with v1 chemistry.[3]

Higher on-target coverage is obtained for samples of higher quality, measured as GQN at 30 kb with the Femto Pulse system



Comparison of a) high- and b) low-quality gDNA samples illustrates that more on-target reads are observed at an *FMR1* repeat expansion for the sample with higher molecular weight DNA

[1] See ***Application note — Comprehensive genotyping with the PureTarget repeat expansion panel and HiFi sequencing*** (102-326-614).
[2] DNA was extracted from iPSCs using Nanobind PanDNA kit and QIAGEN Genomic-Tip.
[1] DNA samples were extracted with the MadMAX kit from Applied Biosystems. Mean target coverage is calculated using SMRT Link v13.1 PureTarget repeat expansion analysis application.

Example coverage performance for PureTarget repeat expansion panel library (96-plex, Revio system + SPRQ chemistry, Nanobind whole blood DNA)

DNA sample preparation

- 1.2 µg DNA per sample
- DNA samples were extracted from human whole blood with Nanobind kits

PureTarget library preparation

- *Procedure & checklist – Generating PureTarget libraries with PureTarget kit 96 – Automation protocol* (103-740-800)
- PureTarget repeat expansion panel 2.0 (38 targets) (103-633-100)
- 96-plex

Sequencing run design

- Revio system with SPRQ chemistry
- 24 hrs movie time
- No adaptive loading

Genotyping analysis

- SMRT Link PureTarget repeat expansion analysis application (default settings)

Coverage (per target per sample)

- Mean = 457 (*N* = 96)

On-target coverage results for one representative human blood sample from a 96-plex PureTarget repeat expansion panel library.

Gene	Motif	Motif repeat number and PureTarget sequencing coverage[1]			
		Allele 1 motif count	Allele 1 read count	Allele 2 motif count	Allele 2 read count
ABCD3	GCC	7	224	7	228
AFF2	GCC	37	320	41	339
AFF3	GCC	8	290	22	302
AR	GCA	23	457	28	422
ATN1	CAG	17	328	20	299
ATXN1	CTG	27	333	27	286
ATXN10	ATTCT	14	328	13	335
ATXN2	CTG	20	200	21	218
ATXN3	CTG	20	306	23	256
ATXN7	CAG:CCG	10_3	350	10_3	346
ATXN8OS	CTA:CTG	8_10	314	10_18	303
BEAN1	TGGAA:TAGAA:AATAA	0_0_10	276	0_0_17	282
C9orf72	GGCCCC	2	312	2	300
CACNA1A	CTG	13	227	12	236
CNBP	CAGG:CAGA:CA	13_10_15	168	13_10_21	136
CSTB	CGCGGGGCGGGG	3	241	3	241
DAB1	AAAAT:GAAAT	12_0	301	22_0	290
DMPK	CAG	12	360	14	388
FGF14	GAA:GAAGGA:GAAGAAGAAGAAGCA:AAGGAG	17_0_0_0	252	105_1_2_0	205
FMR1	CGG:AGG	27_2	272	49_2	262
FXN	A:GAA	18_9	332	16_16	325
GIPC1	CCG	11	267	10	261
HOXD13	GCN	15	293	15	333
HTT	CAG:CCG	18_8	310	16_11	285
JPH3	CTG	15	279	15	270
LRP12	CGC	8	314	12	285
NOP56	GGCCTG:CGCCTG	6_2	273	8_2	295
NOTCH2NLC	GGC	10	262	15	262
PABPN1	GCN	10	358	10	327
PHOX2B	GCN	20	150	20	110
PPP2R2B	GCT	10	312	10	311
PRNP	CCTCAGGGCGGTGGTGGCTGGGGGCAG:CCTCATGGTGGTGGCTGGGGGCAG:GGTGGTGGCTGGGGGCAGCCTCAT	1_4_0	179	1_4_0	178
RFC1	AAGGG:ACAGG:AGGGC:AAGGC:AGAGG:AAAAG:AAAGG:AAGAG:AAAGGG	0_0_0_0_0_16_0_0_0	270	0_0_0_0_0_92_0_0_0	231
RILPL1	GGC	10	269	10	272
SAMD12	TAAAA:TGAAA	20_0	200	15_0	189
TBP	GCA	32	120	33	109
TCF4	CAG	11	245	23	254
ZFHX3	GCC	18	349	18	348

[1] Note: SMRT Link PureTarget repeat expansion analysis application can accurately detect expanded alleles containing >100 repeat expansions – See ***Application note — Comprehensive genotyping with the PureTarget repeat expansion panel and HiFi sequencing*** (102-326-614).

Example coverage performance for PureTarget repeat expansion panel library (48-plex, Revio system + SPRQ chemistry, Nanobind whole blood DNA)

DNA sample preparation

- 1.0 µg DNA per sample
- Blood DNA samples were extracted from human whole blood (with or without RBC lysis) using Nanobind kits
- Cell line DNA samples were Coriell lymphoblastoid cell DNA with GQN30kb > 5

PureTarget library preparation

- *Procedure & checklist – Generating PureTarget libraries with PureTarget kit 24 – Manual protocol* (103-740-700)
- PureTarget repeat expansion panel 2.0 (38 targets) (103-633-100)
- 48-plex

Sequencing run design

- Revio system with SPRQ chemistry
- 24 hrs movie time
- No adaptive loading

Genotyping analysis

- SMRT Link PureTarget repeat expansion analysis application (default settings)

Coverage (per target per sample)

- Mean = 267 (*N* = 48)

On-target coverage results for one representative human blood sample from a 48-plex PureTarget repeat expansion panel library.

Gene	Motif	Motif repeat number and PureTarget sequencing coverage[1]			
		Allele 1 motif count	Allele 1 read count	Allele 2 motif count	Allele 2 read count
ABCD3	GCC	7	241	7	240
AFF2	GCC	37	237	37	233
AFF3	GCC	8	196	10	164
AR	GCA	24	210	30	240
ATN1	CAG	17	207	17	207
ATXN1	CTG	25	201	28	182
ATXN10	ATTCT	14	214	16	198
ATXN2	CTG	20	197	21	225
ATXN3	CTG	21	200	24	209
ATXN7	CAG:CCG	10_3	225	10_3	193
ATXN8OS	CTA:CTG	8_11	239	9_16	229
BEAN1	TGGAA:TAGAA:AATAA	0_0_9	210	0_0_10	193
C9orf72	GGCCCC	4	203	5	200
CACNA1A	CTG	11	172	12	166
CNBP	CAGG:CAGA:CA	15_10_18	156	15_9_21	160
CSTB	CGCGGGGCGGGG	3	189	2	183
DAB1	AAAAT:GAAAT	15_0	215	13_0	241
DMPK	CAG	11	231	12	211
FGF14	GAA:GAAGGA:GAAGAAGAAGAAGCA:AAGGAG	9_0_0_0	206	23_0_1_0	228
FMR1	CGG:AGG	19_1	198	28_2	212
FXN	A:GAA	16_8	214	18_9	226
GIPC1	CCG	11	223	41	208
HOXD13	GCN	15	242	15	235
HTT	CAG:CCG	18_8	180	17_11	178
JPH3	CTG	15	217	14	190
LRP12	CGC	11	266	12	236
NOP56	GGCCTG:CGCCTG	4_2	217	6_2	250
NOTCH2NLC	GGC	11	220	16	214
PABPN1	GCN	10	203	10	206
PHOX2B	GCN	20	151	20	145
PPP2R2B	GCT	10	209	13	207
PRNP	CCTCAGGGCGGTGGTGGCTGGGGGCAG:CCTCATGGTGGTGGCTGGGGGCAG:GGTGGTGGCTGGGGGCAGCCTCAT	1_4_0	145	1_3_0	171
RFC1	AAGGG:ACAGG:AGGGC:AAGGC:AGAGG:AAAAG:AAAGG:AAGAG:AAAGGG	0_0_0_0_0_11_0_0_0	170	0_0_0_0_0_90_0_0_0	177
RILPL1	GGC	10	236	10	206
SAMD12	TAAAA:TGAAA	17_0	169	21_0	163
TBP	GCA	29	161	29	177
TCF4	CAG	16	179	26	188
ZFHX3	GCC	18	220	18	219

[1] Note: SMRT Link PureTarget repeat expansion analysis application can accurately detect expanded alleles containing >100 repeat expansions – See ***Application note — Comprehensive genotyping with the PureTarget repeat expansion panel and HiFi sequencing*** (102-326-614).

Example coverage performance for PureTarget repeat expansion panel library (48-plex, Vega system, Nanobind whole blood DNA)

DNA sample preparation

- 2.0 µg DNA per sample
- Blood DNA samples were extracted from human whole blood (with or without RBC lysis) using Nanobind kits
- Cell line DNA samples were Coriell lymphoblastoid cell DNA with GQN30kb > 5

PureTarget library preparation

- *Procedure & checklist – Generating PureTarget libraries with PureTarget kit 24 – Manual protocol* (103-740-700)
- PureTarget repeat expansion panel 2.0 (38 targets) (103-633-100)
- 48-plex

Sequencing run design

- Vega system
- 24 hrs movie time

Genotyping analysis

- SMRT Link PureTarget repeat expansion analysis application (default settings)

Coverage (per target per sample)

- Mean = 223 (*N* = 48)

On-target coverage results for one representative human blood sample from a 48-plex PureTarget repeat expansion panel library.

Gene	Motif	Motif repeat number and PureTarget sequencing coverage[1]			
		Allele 1 motif count	Allele 1 read count	Allele 2 motif count	Allele 2 read count
ABCD3	GCC	7	201	7	200
AFF2	GCC	37	170	37	191
AFF3	GCC	8	167	10	173
AR	GCA	24	168	30	183
ATN1	CAG	17	188	17	187
ATXN1	CTG	25	187	28	191
ATXN10	ATTCT	14	144	16	130
ATXN2	CTG	20	188	21	170
ATXN3	CTG	21	155	24	185
ATXN7	CAG:CCG	10_3	155	10_3	10_3
ATXN8OS	CTA:CTG	8_11	157	9_16	126
BEAN1	TGGAA:TAGAA:AATAA	0_0_9	177	0_0_10	184
C9orf72	GGCCCC	4	149	5	173
CACNA1A	CTG	11	186	12	171
CNBP	CAGG:CAGA:CA	15_10_18	141	15_9_21	119
CSTB	CGCGGGGCGGGG	3	158	2	153
DAB1	AAAAT:GAAAT	15_0	151	13_0	156
DMPK	CAG	11	181	12	162
FGF14	GAA:GAAGGA:GAAGAAGAAGAAGCA:AAGGAG	9_0_0_0	160	23_0_1_0	187
FMR1	CGG:AGG	19_1	169	28_2	186
FXN	A:GAA	16_8	195	18_9	191
GIPC1	CCG	11	185	41	191
HOXD13	GCN	15	185	15	172
HTT	CAG:CCG	18_8	140	17_11	142
JPH3	CTG	15	212	14	178
LRP12	CGC	11	198	12	216
NOP56	GGCCTG:CGCCTG	4_2	176	6_2	177
NOTCH2NLC	GGC	11	209	16	180
PABPN1	GCN	10	161	10	165
PHOX2B	GCN	20	127	20	138
PPP2R2B	GCT	10	151	13	168
PRNP	CCTCAGGGCGGTGGTGGCTGGGGGCAG:CCTCATGGTGGTGGCTGGGGGCAG:GGTGGTGGCTGGGGGCAGCCTCAT	1_4_0	156	1_3_0	156
RFC1	AAGGG:ACAGG:AGGGC:AAGGC:AGAGG:AAAAG:AAAGG:AAGAG:AAAGGG	0_0_0_0_0_11_0_0_0	157	0_0_0_0_0_83_0_0_0	138
RILPL1	GGC	10	242	10	207
SAMD12	TAAAA:TGAAA	17_0	144	21_0	132
TBP	GCA	29	123	29	128
TCF4	CAG	16	161	26	171
ZFHX3	GCC	18	185	18	184

[1] Note: SMRT Link PureTarget repeat expansion analysis application can accurately detect expanded alleles containing >100 repeat expansions – See ***Application note — Comprehensive genotyping with the PureTarget repeat expansion panel and HiFi sequencing*** (102-326-614).

Example coverage performance for PureTarget carrier panel library (96-plex, Revio system + SPRQ chemistry, Non-Nanobind whole blood DNA)

DNA sample preparation

- 1.2 µg DNA per sample
- DNA samples were extracted from human whole blood with a custom (non-Nanobind) third-party DNA extraction method

PureTarget library preparation

- *Procedure & checklist – Generating PureTarget libraries with PureTarget kit 96 – Automation protocol* (103-740-800)
- PureTarget carrier panel (12 genes) (103-633-200)
- 96-plex

Sequencing run design

- Revio system with SPRQ chemistry
- 24 hrs movie time
- No adaptive loading

Panel QC analysis

- SMRT Link Target Enrichment analysis application [Include Fail Reads = On; Padding around regions (bp) = 0]

Coverage (per target per sample)

- Mean = 294 (*N* = 96)

On-target coverage results for one representative human blood sample from a 96-plex PureTarget carrier panel library.

Target	Variant type(s) commonly detected[1,2]	PureTarget sequencing coverage
AFF2	Repeat expansion	627
ARX	Repeat expansion	130
CYP21A2	Small variants and copy number variants	483
CYP21A2P	Small variants and copy number variants	761
F8_intron1_1	Inversions	589
F8_intron1_2	Inversions	704
F8_intron22_1	Inversions	123
F8_intron22_3	Inversions	217
FMR1	Repeat expansion	629
FXN	Repeat expansion	641
GBA	Small variants and copy number variants	282
GBAP	Small variants and copy number variants	322
HBA1/2-largedel	Large deletions	5
HBA1/2-mediumdel	Medium deletions	29
HBA1/2-smalldel	Small deletions	101
HBA1/hs-40	Deletions	458
HBB	Small variants	460
RPGR	Small variants	571
SMN1	Small variants and copy number variants	83
SMN2	Small variants and copy number variants	91

[1] Example variant types listed in the table can be analyzed using the PureTarget Carrier Pipeline analysis workflow available in GitHub and DNAnexus.

[2] **Note:** *TNXB* and *CYP21A2* genes overlap and so sequencing coverage for this region is reported under the *CYP21A2* target in SMRT Link PureTarget carrier panel coverage reports. PureTarget carrier panel includes multiple guide RNA pairs to target *F8* introns 1 and 22 regions to detect inversion variant types (Inv1 and Inv22) resulting from intra-chromosomal translocation events occurring within *F8* intron 1 and intron 22 hotspots. Similarly, multiple guide RNAs are used (in a nested design spanning ~40 kb) to target the *HBA 1/2* region in order to enable detection of small, medium and large deletion events.

Example coverage performance for PureTarget carrier panel library (48-plex, Revio system + SPRQ chemistry, Nanobind saliva DNA)

DNA sample preparation

- 1 µg DNA per sample
- DNA samples were extracted from human saliva using Nanobind kits

PureTarget library preparation

- *Procedure & checklist – Generating PureTarget libraries with PureTarget kit 24 – Manual protocol* (103-329-400)
- PureTarget carrier panel (12 genes) (103-633-200)
- 48-plex

Sequencing run design

- Revio system with SPRQ chemistry
- 24 hrs movie time
- No adaptive loading

Panel QC analysis

- SMRT Link Target Enrichment analysis application [Include Fail Reads = On; Padding around regions (bp) = 0]

Coverage (per target per sample)

- Mean = 250 (*N* = 48)

On-target coverage results for one representative human blood sample from a 24-plex PureTarget carrier panel library.

Target	Variant type(s) commonly detected[1,2]	PureTarget sequencing coverage
AFF2	Repeat expansion	353
ARX	Repeat expansion	73
CYP21A2	Small variants and copy number variants	673
CYP21A2P	Small variants and copy number variants	483
F8_intron1_1	Inversions	252
F8_intron1_2	Inversions	318
F8_intron22_1	Inversions	36
F8_intron22_3	Inversions	104
FMR1	Repeat expansion	334
FXN	Repeat expansion	590
GBA	Small variants and copy number variants	262
GBAP	Small variants and copy number variants	313
HBA1/2-largedel	Large deletions	5
HBA1/2-mediumdel	Medium deletions	37
HBA1/2-smalldel	Small deletions	230
HBA1/hs-40	Deletions	579
HBB	Small variants	560
RPGR	Small variants	237
SMN1	Small variants and copy number variants	128
SMN2	Small variants and copy number variants	114

[1] Example variant types listed in the table can be analyzed using the PureTarget Carrier Pipeline analysis workflow available in GitHub and DNAnexus.

[2] **Note:** *TNXB* and *CYP21A2* genes overlap and so sequencing coverage for this region is reported under the *CYP21A2* target in SMRT Link PureTarget carrier panel coverage reports. PureTarget carrier panel includes multiple guide RNA pairs to target *F8* introns 1 and 22 regions to detect inversion variant types (Inv1 and Inv22) resulting from intra-chromosomal translocation events occurring within *F8* intron 1 and intron 22 hotspots. Similarly, multiple guide RNAs are used (in a nested design spanning ~40 kb) to target the *HBA 1/2* region in order to enable detection of small, medium and large deletion events.

Example coverage performance for PureTarget carrier panel library (24-plex, Revio system + SPRQ chemistry, Nanobind saliva DNA)

DNA sample preparation

- 2 µg DNA per sample
- DNA samples were extracted from human saliva using Nanobind kits

PureTarget library preparation

- *Procedure & checklist – Generating PureTarget libraries with PureTarget kit 24 – Manual protocol* (103-329-400)
- PureTarget carrier panel (12 genes) (103-633-200)
- 24-plex

Sequencing run design

- Revio system with SPRQ chemistry
- 24 hrs movie time
- No adaptive loading

Panel QC analysis

- SMRT Link Target Enrichment analysis application [Include Fail Reads = On; Padding around regions (bp) = 0]

Coverage (per target per sample)

- Mean = 281 (*N* = 24)

On-target coverage results for one representative human blood sample from a 24-plex PureTarget carrier panel library.

Target	Variant type(s) commonly detected[1,2]	PureTarget sequencing coverage
AFF2	Repeat expansion	541
ARX	Repeat expansion	129
CYP21A2	Small variants and copy number variants	321
CYP21A2P	Small variants and copy number variants	355
F8_intron1_1	Inversions	388
F8_intron1_2	Inversions	561
F8_intron22_1	Inversions	128
F8_intron22_3	Inversions	163
FMR1	Repeat expansion	502
FXN	Repeat expansion	539
GBA	Small variants and copy number variants	216
GBAP	Small variants and copy number variants	232
HBA1/2-largedel	Large deletions	7
HBA1/2-mediumdel	Medium deletions	40
HBA1/2-smalldel	Small deletions	193
HBA1/hs-40	Deletions	477
HBB	Small variants	449
RPGR	Small variants	431
SMN1	Small variants and copy number variants	122
SMN2	Small variants and copy number variants	61

[1] Example variant types listed in the table can be analyzed using the PureTarget Carrier Pipeline analysis workflow available in GitHub and DNAnexus.

[2] **Note:** *TNXB* and *CYP21A2* genes overlap and so sequencing coverage for this region is reported under the *CYP21A2* target in SMRT Link PureTarget carrier panel coverage reports. PureTarget carrier panel includes multiple guide RNA pairs to target *F8* introns 1 and 22 regions to detect inversion variant types (Inv1 and Inv22) resulting from intra-chromosomal translocation events occurring within *F8* intron 1 and intron 22 hotspots. Similarly, multiple guide RNAs are used (in a nested design spanning ~40 kb) to target the *HBA 1/2* region in order to enable detection of small, medium and large deletion events.

Example coverage performance for PureTarget carrier panel library (24-plex, Revio system + SPRQ chemistry, Nanobind whole blood DNA)

DNA sample preparation

- 1 µg DNA per sample
- DNA samples were extracted from human whole blood with RBC lysis using Nanobind kits

PureTarget library preparation

- *Procedure & checklist – Generating PureTarget libraries with PureTarget kit 24 – Manual protocol* (103-329-400)
- PureTarget carrier panel (12 genes) (103-633-200)
- 24-plex

Sequencing run design

- Revio system with SPRQ chemistry
- 24 hrs movie time
- No adaptive loading

Panel QC analysis

- SMRT Link Target Enrichment analysis application [Include Fail Reads = On; Padding around regions (bp) = 0]

Coverage (per target per sample)

- Mean = 747 (*N* = 24)

On-target coverage results for one representative human blood sample from a 24-plex PureTarget carrier panel library.

Target	Variant type(s) commonly detected[1,2]	PureTarget sequencing coverage
AFF2	Repeat expansion	1427
ARX	Repeat expansion	447
CYP21A2	Small variants and copy number variants	956
CYP21A2P	Small variants and copy number variants	1900
F8_intron1_1	Inversions	1101
F8_intron1_2	Inversions	1334
F8_intron22_1	Inversions	392
F8_intron22_3	Inversions	602
FMR1	Repeat expansion	1155
FXN	Repeat expansion	1230
GBA	Small variants and copy number variants	405
GBAP	Small variants and copy number variants	539
HBA1/2-largedel	Large deletions	5
HBA1/2-mediumdel	Medium deletions	208
HBA1/2-smalldel	Small deletions	240
HBA1/hs-40	Deletions	1281
HBB	Small variants	958
RPGR	Small variants	942
SMN1	Small variants and copy number variants	363
SMN2	Small variants and copy number variants	353

[1] Example variant types listed in the table can be analyzed using the PureTarget Carrier Pipeline analysis workflow available in GitHub and DNAnexus.

[2] **Note:** *TNXB* and *CYP21A2* genes overlap and so sequencing coverage for this region is reported under the *CYP21A2* target in SMRT Link PureTarget carrier panel coverage reports. PureTarget carrier panel includes multiple guide RNA pairs to target *F8* introns 1 and 22 regions to detect inversion variant types (Inv1 and Inv22) resulting from intra-chromosomal translocation events occurring within *F8* intron 1 and intron 22 hotspots. Similarly, multiple guide RNAs are used (in a nested design spanning ~40 kb) to target the *HBA 1/2* region in order to enable detection of small, medium and large deletion events.

Example coverage performance for PureTarget carrier panel library (24-plex, Vega system , Nanobind whole blood DNA)

DNA sample preparation

- 2 µg DNA per sample
- DNA samples were extracted from human whole blood with RBC lysis using Nanobind kits

PureTarget library preparation

- *Procedure & checklist – Generating PureTarget libraries with PureTarget kit 24 – Manual protocol* (103-329-400)
- PureTarget carrier panel (12 genes) (103-633-200)
- 24-plex

Sequencing run design

- Vega system
- 24 hrs movie time

Panel QC Analysis

- SMRT Link Target Enrichment analysis application [Include Fail Reads = On; Padding around regions (bp) = 0]

Coverage (per target per sample)

- Mean = 592 (*N* = 24)

On-target coverage results for one representative human blood sample from a 24-plex PureTarget carrier panel library.

Target	Variant type(s) commonly detected[1,2]	PureTarget sequencing coverage
AFF2	Repeat expansion	1064
ARX	Repeat expansion	329
CYP21A2	Small variants and copy number variants	919
CYP21A2P	Small variants and copy number variants	1662
F8_intron1_1	Inversions	860
F8_intron1_2	Inversions	977
F8_intron22_1	Inversions	288
F8_intron22_3	Inversions	426
FMR1	Repeat expansion	847
FXN	Repeat expansion	863
GBA	Small variants and copy number variants	365
GBAP	Small variants and copy number variants	496
HBA1/2-largedel	Large deletions	8
HBA1/2-mediumdel	Medium deletions	169
HBA1/2-smalldel	Small deletions	218
HBA1/hs-40	Deletions	925
HBB	Small variants	765
RPGR	Small variants	856
SMN1	Small variants and copy number variants	258
SMN2	Small variants and copy number variants	244

[1] Example variant types listed in the table can be analyzed using the PureTarget Carrier Pipeline analysis workflow available in GitHub and DNAnexus.

[2] **Note:** *TNXB* and *CYP21A2* genes overlap and so sequencing coverage for this region is reported under the *CYP21A2* target in SMRT Link PureTarget carrier panel coverage reports. PureTarget carrier panel includes multiple guide RNA pairs to target *F8* introns 1 and 22 regions to detect inversion variant types (Inv1 and Inv22) resulting from intra-chromosomal translocation events occurring within *F8* intron 1 and intron 22 hotspots. Similarly, multiple guide RNAs are used (in a nested design spanning ~40 kb) to target the *HBA 1/2* region in order to enable detection of small, medium and large deletion events.

PacBio

Technical documentation & applications support resources

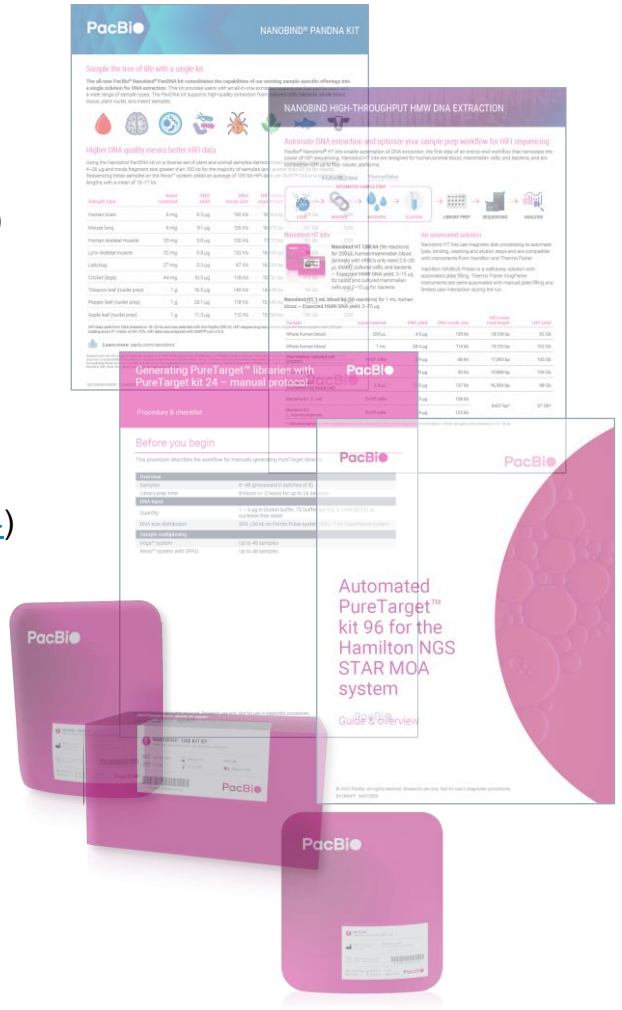
Technical resources for PureTarget library preparation, sequencing & data analysis

DNA sample preparation literature & other resources

- Brochure – Nanobind PanDNA kit (102-326-604)
- Procedure & checklist – Extracting HMW DNA from human whole blood using Nanobind kits (102-573-500)
- Procedure & checklist – Extracting HMW DNA from human whole blood with RBC lysis using Nanobind kits (103-377-500)
- Procedure & checklist – Extracting HMW DNA from cultured suspension cells using Nanobind kits (103-394-500)
- Procedure & checklist – Extracting HMW DNA from cultured adherent cells using Nanobind kits (102-573-600)

PureTarget library preparation literature & other resources

- Application note – Comprehensive genotyping with PureTarget repeat expansion panel and HiFi sequencing (102-326-614)
- Application note – Consolidate challenging genes with PureTarget carrier screen panel (102-326-653)
- Brochure – Comprehensive genotyping with PureTarget repeat expansion panel (102-326-609)
- Guide & overview – Automated PureTarget kit 96 for the Hamilton NGS STAR MOA system (103-740-900)
- Procedure & checklist – Generating PureTarget libraries with PureTarget kit 24 – Manual protocol (103-740-700)
- Procedure & checklist – Generating PureTarget libraries with PureTarget kit 96 – Automation protocol (103-740-800)
- Technical note – A practical guide to amplification-free PureTarget custom panels (102-326-652)
- Technical overview – PureTarget library preparation using PureTarget kit 2.0 (103-742-600)



Technical resources for PureTarget library preparation, sequencing & data analysis (cont.)

Data analysis resources

- Application note – Comprehensive genotyping with PureTarget repeat expansion panel and HiFi sequencing (102-326-614)
- Application note – Consolidate challenging genes with PureTarget carrier screen panel (102-326-653)
- PacBio GitHub Wiki – PureTarget Carrier Pipeline (PTCP) analysis workflow [Link]
- SMRT Link software installation guide [Link]
- SMRT Link user guide [Link]
- SMRT Tools reference guide [Link]

Publications and posters

- PacBio ESHG poster (2025) - Targeted long-read sequencing of native DNA for genetic disease diagnostic and screening research [Link]
- PacBio ACMG poster (2025) – Targeted long-read sequencing of native DNA for genetic disease diagnostic and screening research [Link]
- PacBio AMP poster (2024) – Targeted long-read sequencing of native DNA for comprehensive characterization of repeat expansions [Link]

Videos

- PacBio YouTube video (2025) – PureTarget in action: Scalable and accurate repeat expansion sequencing [Link]
- PacBio YouTube video (2025) – PureTarget: PCR-Free Enrichment with Methylation for Challenging Genes [Link]
- PacBio YouTube video (2025) – TRGT: profiling variation in and around tandem repeats [Link]

Technical resources for PureTarget library preparation, sequencing & data analysis (cont.)

Example PacBio data sets[1]

Application	Dataset	Data type	PacBio system
PureTarget 2.0	Carrier panel Nanobind 96plex [Link]	HiFi long read	Revio + SPRQ
	Carrier panel Coriell 16plex [Link]	HiFi long read	Revio + SPRQ
	Carrier panel Coriell 16plex [Link]	HiFi long read	Vega system
	Repeat Expansion 2.0 Nanobind Coriell 48plex [Link]	HiFi long read	Revio + SPRQ
	Repeat Expansion 2.0 Nanobind Coriell 48plex [Link]	HiFi long read	Vega system

[1] Visit PacBio Datasets website to explore publicly available datasets generated with PacBio sequencing.

www.pacb.com

Revision history (description)	Version	Date
Initial release	01	September 2025