

The background of the slide features a close-up, shallow depth-of-field photograph of a multi-well microplate. A pipette tip is positioned above one of the wells, dispensing a bright pink liquid. The other wells in the plate also contain a similar pink liquid. The lighting is soft and clinical, typical of a laboratory setting.

**PacBio**

# Technical overview – PureTarget repeat expansion panel library preparation using PureTarget kit

Sequel II and IIe systems ICS v11.0

Revo system ICS v13.1

SMRT Link v13.1

PN 103-418-100 Rev 01 | March 2024

# PureTarget repeat expansion panel library preparation using PureTarget kit



## Technical Overview

1. PureTarget repeat expansion panel sequencing method overview
2. PureTarget repeat expansion panel library preparation workflow overview
3. PureTarget repeat expansion panel sequencing preparation workflow overview
4. PureTarget repeat expansion panel example sequencing performance data
5. PureTarget repeat expansion panel data analysis workflow overview
6. Technical documentation & applications support resources
7. Appendix

# PureTarget repeat expansion panel library preparation using PureTarget kit: Getting started



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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, Bafiez 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 (DePierre & Mandel, 2021). Disease severity and age of onset of these conditions are often associated with their repeat length (Bafiez et al., 2022).

Though common, these regions are challenging to characterize and as such, the majority of patients with rare neurological diseases remain undiagnosed (Bafiez 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.

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

Summary overview of application-specific library preparation and data analysis workflow recommendations.

**PacBio**

## Generating PureTarget™ repeat expansion panel libraries

Procedure & checklist

**Before you begin**

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

Overview	
Samples	8-24 (processed in batches of 8)
Library prep time	8 hours +/- 2 hours for up to 24 samples
Annealing binding clean up time	1 hour +/- 10 minutes

DNA Input	
Quantity	2 µg in Buffer EB, TE buffer (pH 8), or nuclease-free water
DNA size distribution	50% >30 kb

Sample multiplexing	
Sequel® II systems	Up to 24 samples
Revio™ system	Up to 48 samples

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## Procedure & checklist – Generating PureTarget repeat expansion panel libraries (103-329-400)

Technical documentation containing application-specific library preparation protocol details.

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## Technical overview – PureTarget repeat expansion panel library preparation using PureTarget kit

Sequel II and IIe systems ICS v11.0  
Revio system ICS v13.1  
SMRT Link v13.1

PN 103-418-100 Rev 01 | March 2024

**Example sequencing performance for 48-plex PureTarget repeat expansion panel library prepared from human blood samples (Revio system)**

48-plex PureTarget repeat expansion panel Revio system example data\*

Raw Data Report	HiFi Read Length	Target Enrichment Summary Metrics
<p>Mean CCS read length: 10.1 kb</p>	<p>Mean HiFi read length: 15.1 kb</p>	<p>Target Enrichment: 100%</p>

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## Technical Overview – PureTarget repeat expansion panel library preparation using PureTarget kit (103-418-100)

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)

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

↓

**PureTarget library preparation**  
(PureTarget repeat expansion panel)

Use CRISPR/Cas9 & gRNAs to target repeats  
SMRTbell prep kit 3.0 for library construction  
Supports sample multiplexing up to 48-plex

↓

**SMRT sequencing**  
(Sequel II/Ie & Revio systems)

Perform ABC\* and sequence PureTarget libraries on PacBio long-read systems

↓

**Data analysis**  
(SMRT Link)

Use SMRT Link PureTarget repeat expansion analysis application to characterize normal & expanded alleles in a 20-gene target panel

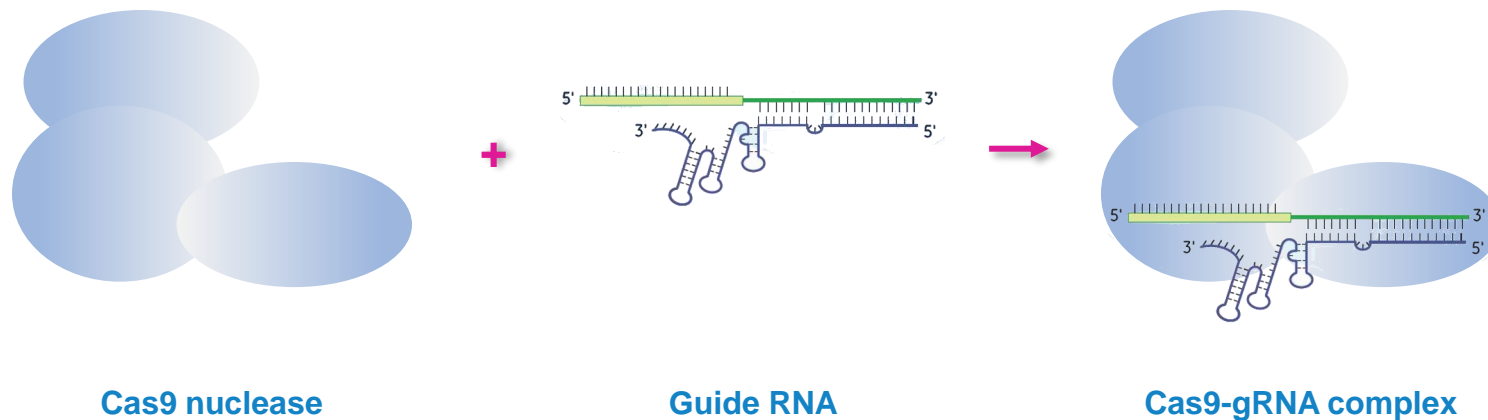


# PureTarget sequencing method overview



# CRISPR-Cas9 technology overview

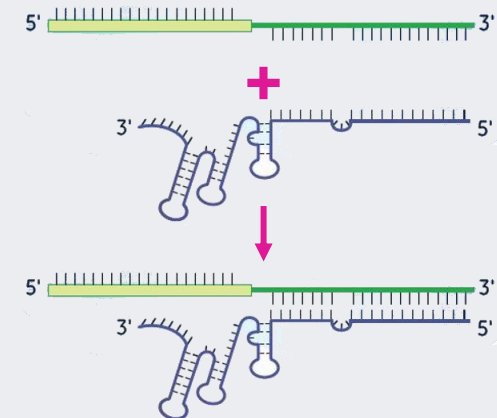
CRISPR-Cas9 system comprises a guide RNA (gRNA or sgRNA) and Cas9 nuclease, which together form a ribonucleoprotein (RNP) complex that can introduce a site-specific double-strand break in DNA<sup>1,2</sup>



## Guide RNA configurations

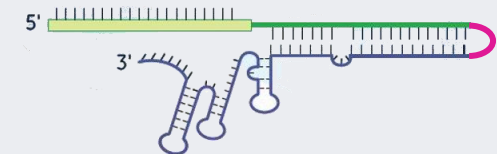
### Two-component guide RNA (gRNA)

Annealed [crRNA : tracrRNA] complex



### Single guide RNA (sgRNA)

Synthetic crRNA-tracrRNA single-molecule hybrid construct



sgRNA is used in PureTarget library prep workflow

<sup>1</sup> Image modified from: <https://horizondiscovery.com/en/applications/gene-editing>

<sup>2</sup> 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<sup>1,2</sup>

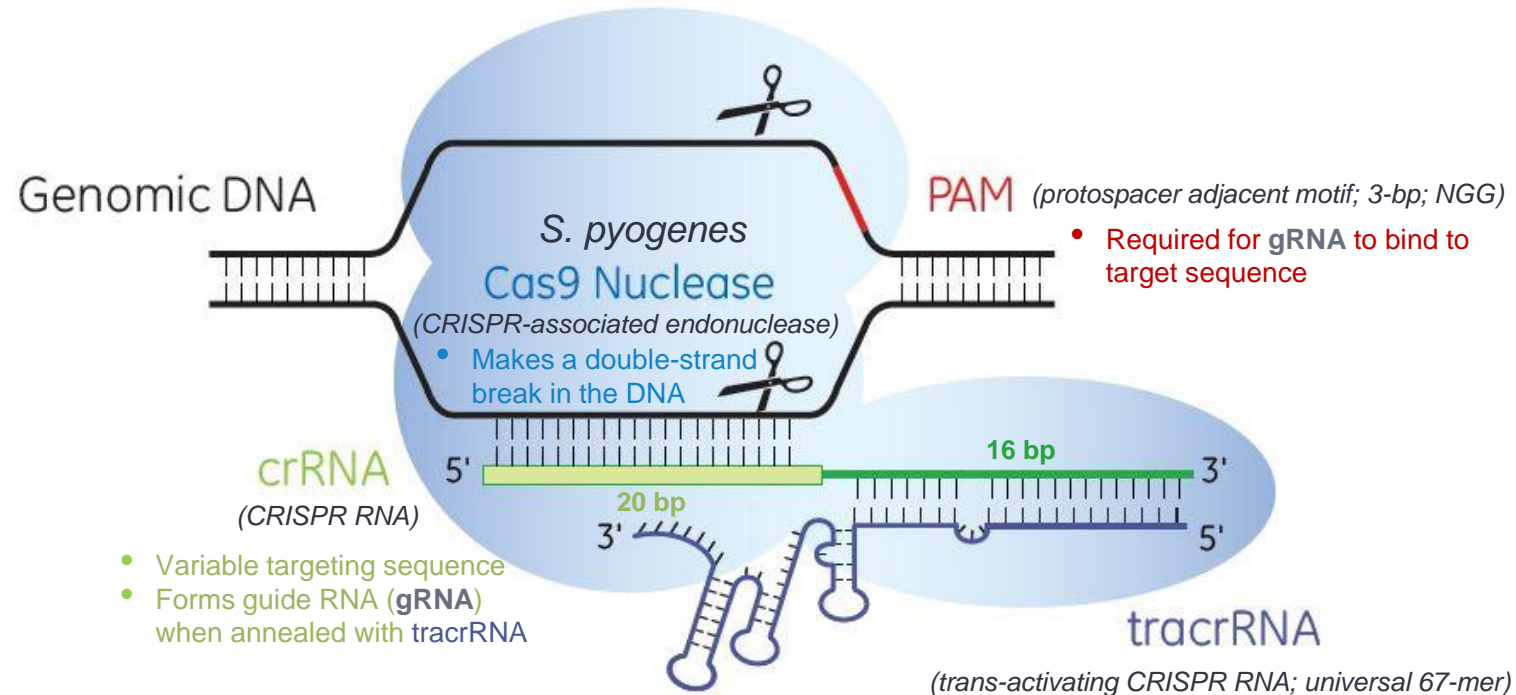
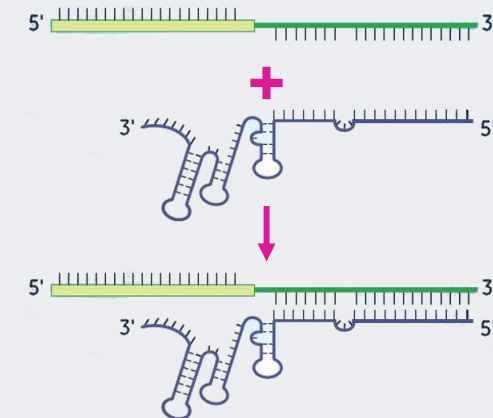


Illustration of **Cas9 nuclease** (blue), programmed by the **tracrRNA** (violet) : **crRNA** (olive) complex (= **guide RNA**) cutting both strands of genomic DNA 5' of the protospacer-adjacent motif (**PAM**) (red).

## Guide RNA configurations

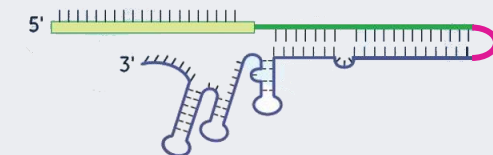
### Two-component guide RNA (gRNA)

Annealed [crRNA : tracrRNA] complex



### Single guide RNA (sgRNA)

Synthetic crRNA-tracrRNA single-molecule hybrid construct



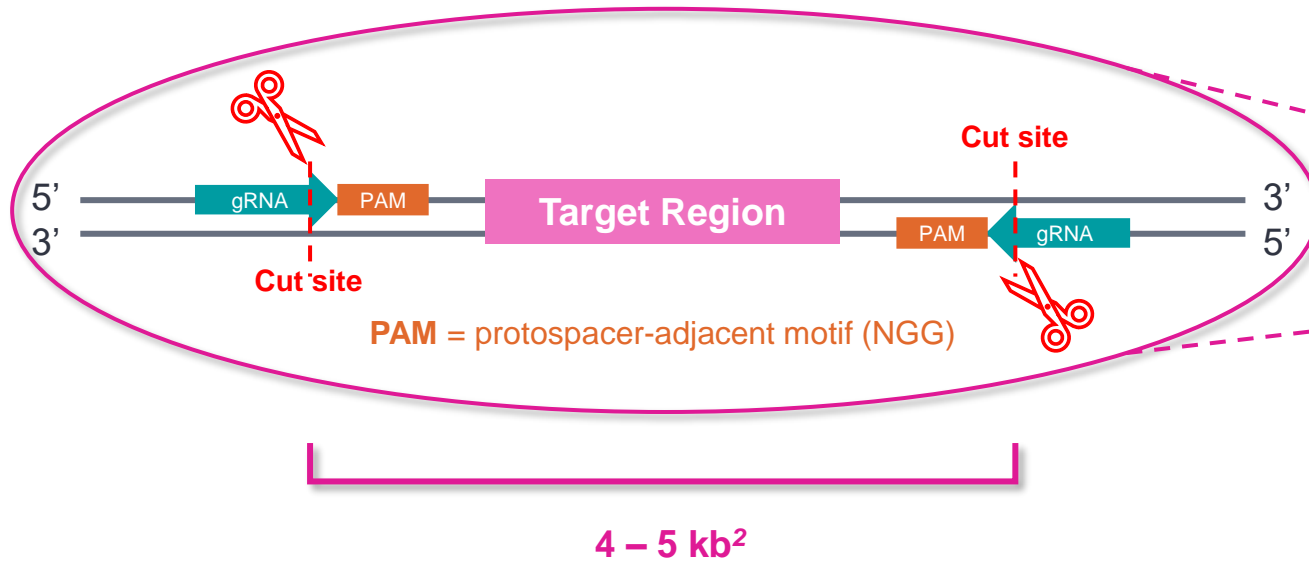
sgRNA is used in PureTarget library prep workflow

<sup>1</sup> Image modified from: <https://horizondiscovery.com/en/applications/gene-editing>

<sup>2</sup> 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

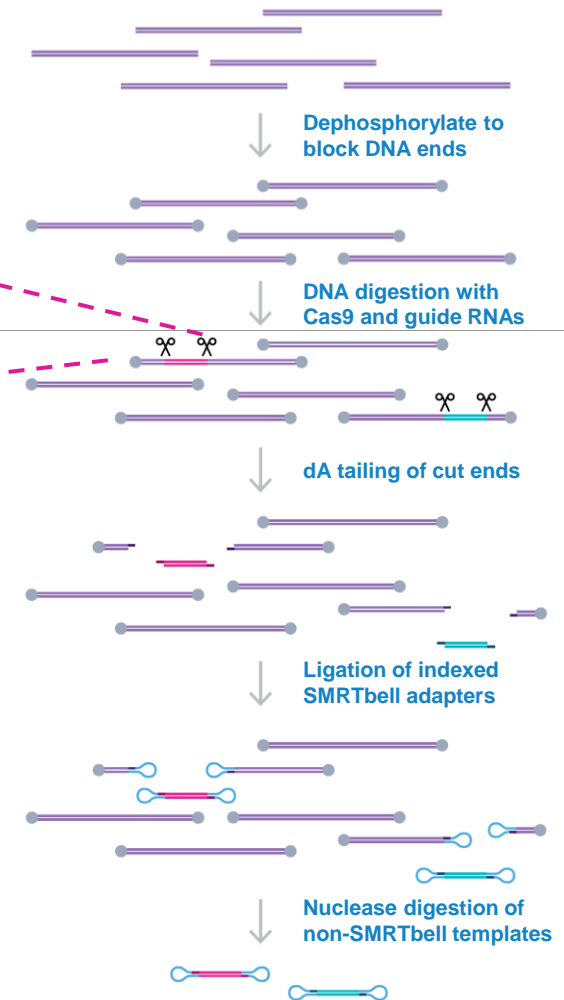
For PureTarget HiFi sequencing, CRISPR-Cas9 system is used to selectively enrich for regions of interest in a genomic sample prior to long-read sequencing of native DNA molecules<sup>1</sup>



## 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



<sup>1</sup> 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.).

<sup>2</sup> **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 repeat expansion panel library preparation procedure description

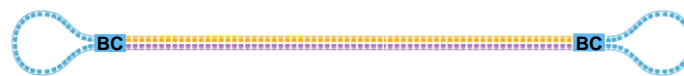
Procedure & checklist – Generating PureTarget repeat expansion panel libraries ([103-329-400](#)) describes the workflow for generating PureTarget repeat expansion libraries using the **PureTarget kit** for sequencing on PacBio Sequel II/Ile & Revio systems

Overview	
Samples	8–24 (processed in batches of 8)
Library prep time	8 hours +/- 2 hours for up to 24 samples
Annealing binding clean up time	1 hour +/- 10 minutes
DNA input	
Quantity	2 µg in Buffer EB, TE buffer (pH 8), or nuclease-free water
DNA size distribution	50% ≥30 kb
Sample multiplexing	
Sequel® II systems	Up to 24 samples
Revio™ system	Up to 48 samples



**PureTarget repeat expansion panel kit bundle**  
103-390-400 (24 rxn)

- Includes **20-gene panel** and targeting reagents
- Also includes **SMRTbell prep kit 3.0** reagents for library construction
- Supports **24** samples



**PureTarget library template (~4 – 5 kb)**  
Contains indexed SMRTbell adapters<sup>1</sup>

- PureTarget library preparation procedure supports up to **48-plex sample multiplexing** through use of 48 different SMRTbell indexed adapters<sup>1</sup>

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Generating PureTarget™ repeat expansion panel libraries

Procedure & checklist

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Before you begin

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

Overview	
Samples	8–24 (processed in batches of 8)
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**Note:** Procedure [102-329-400](#) includes instructions for PureTarget repeat expansion panel **SMRTbell library construction workflow** **and sequencing preparation (ABC<sup>2</sup>) workflow**

<sup>1</sup> To enable sample multiplexing, **SMRTbell adapter index plate 96A (102-009-200)** must be purchased separately from PureTarget repeat expansion panel kit bundle (103-390-400).

<sup>2</sup> For primer annealing, polymerase binding & complex cleanup (ABC) steps, follow sample setup instructions for PureTarget libraries in **Procedure & checklist – Generating PureTarget repeat expansion panel libraries (103-329-400)** (Step 11a for Revio systems or Step 11b for Sequel II/Ile systems) – Do not use SMRT Link Sample Setup.



# PureTarget repeat expansion panel kit bundle ([103-390-400](#))<sup>1,2</sup>

PureTarget panel kit bundle supports amplification-free targeted sequencing library prep workflow

## PureTarget repeat expansion targeting kit

- Includes reagents for PureTarget repeat expansion panel including Cas9 and guide RNAs for 20-target gene panel. Supports **24 samples**.








## PureTarget beads kit

- Includes reagents for performing SMRT boost bead washes in PureTarget library preparation workflows. Supports **3 bead washes** (batch size = 8).



## SMRTbell prep kit 3.0 (102-141-700)

- Includes reagents for SMRTbell library preparation. Supports **24 samples**.




### PureTarget targeting kit components

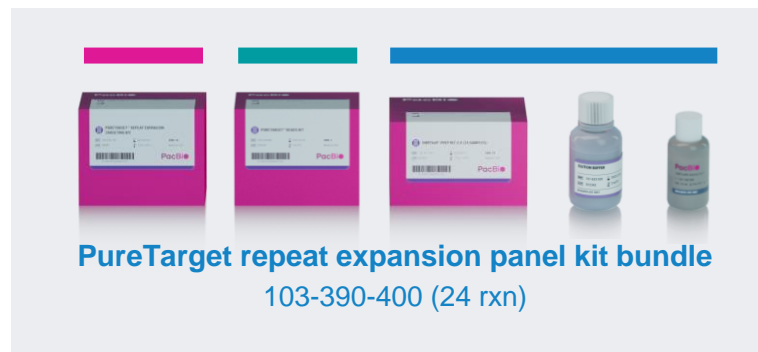
Component	Description
1 	<b>Cas9 buffer</b> • For Cas 9 digestion of gDNA
2 	<b>Phosphatase</b> • For blocking gDNA fragment ends
3 	<b>Cas9 nuclease</b> • For Cas 9 digestion of gDNA
4 	<b>gRNA mix</b> • For Cas 9 digestion of gDNA
5 	<b>dA tail buffer</b> • For A-tailing reaction
6 	<b>dATP</b> • For A-tailing reaction
7 	<b>Taq DNA polymerase</b> • For A-tailing reaction

### PureTarget beads kit components

Component	Description
1 	<b>SMRT boost beads</b> • For final cleanup of PureTarget repeat expansion panel libraries
2 	<b>SMRT boost buffer</b> • For preparing SMRT boost beads for bead wash steps prior to use

### SMRTbell prep kit 3.0 components

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



<sup>1</sup> PureTarget repeat expansion targeting kit (103-234-700) and PureTarget beads kit (103-234-800) can only be purchased as part of the PureTarget repeat expansion panel kit bundle product ([103-390-400](#)). SMRTbell prep kit 3.0 ([102-141-700](#)) may be purchased as a stand-alone product.

<sup>2</sup> PureTarget repeat expansion panel kit bundle (103-390-400) reagent volumes are **optimized for batches of 8** and reagent volumes may be insufficient to support batching of fewer than 8. Specifically, a total of 3 SMRT boost bead washes are supported by reagent volumes in the kit.

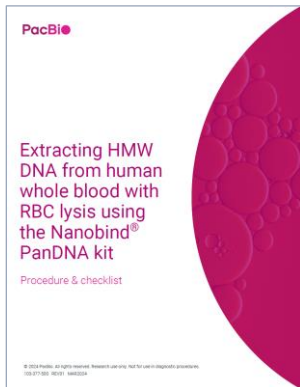
# Other recommended kits & consumables for PureTarget sample DNA extraction, sample multiplexing and SMRT sequencing

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

## HMW DNA extraction



**Nanobind PanDNA kit**  
(103-260-000)



**Procedure & checklist**  
(103-377-500)

- Supports **24 reactions** for HMW DNA extraction from cells, bacteria, blood, tissue, insect, and plant nuclei
- **Nanobind PanDNA kit** is recommended for DNA extraction from cultured cells and human whole blood with RBC lysis for PureTarget repeat expansion panel applications<sup>1</sup>

## Sample multiplexing



**SMRTbell adapter index plate 96A**  
(102-009-200)

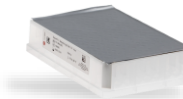
	1	2	3	4	5	6	7	8	9	10	11	12
A	bc2001	bc2009	bc2017	bc2025	bc2033	bc2041	bc2049	bc2057	bc2065	bc2073	bc2081	bc2089
B	bc2002	bc2010	bc2018	bc2026	bc2034	bc2042	bc2050	bc2058	bc2066	bc2074	bc2082	bc2090
C	bc2003	bc2011	bc2019	bc2027	bc2035	bc2043	bc2051	bc2059	bc2067	bc2075	bc2083	bc2091
D	bc2004	bc2012	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

- Contains **96 indexed SMRTbell adapters** in plate format (1 sample per index)
- Plate includes indexes bc2001–bc2096

## SMRT sequencing



**Revio polymerase kit<sup>2</sup>**  
(102-817-600)



**Revio sequencing plate**  
(4-rxn: 102-587-400)

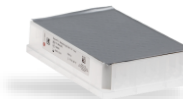


**Revio SMRT Cell tray**  
(4 cells: 102-202-200)

- Revio polymerase kit supports **12 Revio SMRT Cells** for PureTarget repeat expansion panel samples



**Sequel II binding kit 3.2 and cleanup beads**  
(102-333-300)



**Sequel II sequencing kit 2.0**  
(4-rxn: 101-820-200)



**Sequel II SMRT Cell 8M tray**  
(4 cells: 101-389-001)

- Sequel II binding kit supports **24 Sequel II SMRT Cells** for PureTarget repeat expansion panel samples

<sup>1</sup> 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) instead of extracting DNA directly from whole blood. For PureTarget repeat expansion panel applications, Nanobind CBB kit may also be used for HMW DNA extraction from cultured human cells.

<sup>2</sup> **Note:** PureTarget library preparation procedure (103-329-400) supports up to 48-plex sample multiplexing through use of 48 different SMRTbell indexed adapters.

# PureTarget repeat expansion panel kit product specifications for supported sample types

PureTarget enables comprehensive characterization of repeat expansions at scale<sup>1</sup>

Parameter	Specification	Notes
Target gene panel size	20	<ul style="list-style-type: none"> <li>See <i>Application note – Comprehensive genotyping with the PureTarget repeat expansion panel and HiFi sequencing</i> (<a href="#">102-326-614</a>) for list of target genes included in PureTarget repeat expansion panel kit (103-390-400)</li> </ul>
DNA input amount	2 µg DNA per sample	<ul style="list-style-type: none"> <li>1 – 4 µg DNA per sample supported</li> </ul>
DNA input quality	GQN30kb > 5	<ul style="list-style-type: none"> <li>50% of mass of DNA molecules longer than 30 kb as measured on Femto Pulse (Agilent)</li> </ul>
Target coverage	Mean target coverage: >200-fold	<ul style="list-style-type: none"> <li>Mean coverage for 2 µg of input DNA from supported sample types (Nanobind-extracted human blood and cell lines)<sup>2</sup> for unexpanded alleles</li> </ul>
	Minimum target coverage: 50-fold	<ul style="list-style-type: none"> <li>Minimum coverage for 2 µg of input DNA from supported sample types (Nanobind-extracted human blood and cell lines)<sup>2</sup> for unexpanded alleles</li> </ul>
Sample multiplexing	<b>Sequel II/IIe system:</b> Up to 24-plex	<ul style="list-style-type: none"> <li>Kit supports smaller batches in multiples of 8 samples</li> </ul>
	<b>Revio system:</b> Up to 48-plex	<ul style="list-style-type: none"> <li>Kit supports smaller batches in multiples of 8 samples</li> </ul>
Library insert size	4 – 5 kb	<ul style="list-style-type: none"> <li>Inserts with expanded alleles will be longer</li> </ul>
Methylation	5mC in CpG sites detected	<ul style="list-style-type: none"> <li>Methylation probabilities for CpG sites are encoded in BAM output file</li> </ul>
PureTarget library prep protocol	<b>Procedure &amp; checklist – Generating PureTarget repeat expansion panel libraries</b> ( <a href="#">103-329-400</a> )	<ul style="list-style-type: none"> <li>Requires PureTarget repeat expansion panel kit bundle (103-390-400)</li> </ul>
SMRT Link data analysis workflow	PureTarget repeat expansion analysis application	<ul style="list-style-type: none"> <li>SMRT Link v13.1+ required</li> </ul>

# PureTarget kit supported use cases and experimental design considerations

PureTarget kit offers a gene panel for 20 of the most important repeat expansions for human health

Disease	PureTarget gene panel targets
Spinocerebellar ataxia	<i>ATN1, ATXN1, ATXN2, ATXN3, ATXN7, ATXN8, ATXN10, CACNA1A, PPP2R2B, TBP</i>
CANVAS disease	<i>RFC1</i>
Fragile-X Disease	<i>FMR1</i>
Friedrich's ataxia	<i>FXN</i>
Huntington's Disease	<i>HTT</i>
Myotonic Dystrophy	<i>DMPK, CNBP</i>
Fuchs Endothelial Corneal Dystrophy	<i>TCF4</i>
ALS/FTD	<i>C9ORF72</i>
Spinal bulbar muscular atrophy	<i>AR</i>

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

- PureTarget kit includes a panel of **20 repeat expansion loci** with the panel capturing ~2 kb upstream and downstream of the repeat (total panel size is ~100 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**

## Note about using custom panel designs<sup>1</sup>

- **Custom PureTarget panels are not officially supported** by PacBio, but we can offer guidance to users interested in designing and optimizing their own panels
- In all cases, **we recommend first demonstrating success on the PureTarget repeat expansion panel using supported sample types** before adding new guide RNAs or testing a custom set of guides
- PureTarget repeat expansion panel is in total ~100 kb in length so **performance in panels that are much smaller or much larger is unknown** and may require optimization of wet lab protocol or reagents





# PureTarget library preparation workflow overview



# Procedure & checklist – Generating PureTarget repeat expansion panel libraries (103-329-400)

Procedure & checklist [103-329-400](#) describes the workflow for generating PureTarget repeat expansion libraries using the **PureTarget kit** for sequencing on PacBio Sequel II/IIe & Revio systems

## Procedure & checklist contents

1. DNA input QC recommendations and general best practices for reagent & sample handling.
2. Instructions for Cas9/gRNA complex formation.
3. Enzymatic workflow steps for performing targeted Cas9 digestion of input genomic DNA samples.
4. Enzymatic workflow steps for PureTarget SMRTbell library construction from Cas9-digested gDNA samples.
5. Workflow steps for final cleanup of PureTarget SMRTbell libraries using SMRT boost bead wash and SMRTbell cleanup beads
6. Workflow steps for sample setup ABC<sup>1</sup> to prepare samples for sequencing

**Note:** Procedure [102-329-400](#) includes instructions for PureTarget repeat expansion panel SMRTbell library construction workflow and sequencing preparation (ABC<sup>1</sup>) workflow

## Generating PureTarget™ repeat expansion panel libraries

Procedure & checklist

### Before you begin

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

Overview	
Samples	8–24 (processed in batches of 8)
Library prep time	8 hours +/- 2 hours for up to 24 samples
Annealing binding clean up time	1 hour +/- 10 minutes
DNA input	
Quantity	2 µg in Buffer EB, TE buffer (pH 8), or nuclease-free water
DNA size distribution	50% ≥30 kb
Sample multiplexing	
Sequel II systems	Up to 24 samples
Revio system	Up to 48 samples

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PacBio [Documentation](#) ([103-329-400](#))

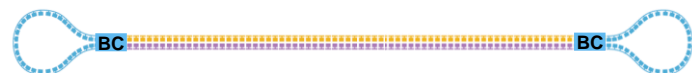
# PureTarget library construction workflow overview

Procedure & checklist – Generating PureTarget repeat expansion panel libraries ([103-329-400](tel:103-329-400))

	Cleanup	DNA QC	Walk-away time <sup>1</sup>
1 Input DNA quality control	N/A	Qubit DNA HS assay Femto Pulse gDNA 165 kb kit	N/A
2 Dephosphorylation	N/A	N/A	15 min
3 Cas9-gRNA complex formation	N/A	N/A	15 min
4 Cas-9 digestion & cleanup	1.0X SMRTbell cleanup beads	Qubit dsDNA HS assay	1.5 hrs
5 dA tail	N/A	N/A	30 min
6 Adapter ligation & cleanup	1.0X SMRTbell cleanup beads	Qubit dsDNA HS assay	1 hr
7 Nuclease Treatment	N/A	N/A	1 hr
8 Sample pooling & cleanup	1.0X SMRTbell cleanup beads	N/A	0.5 hrs
9 SMRT boost wash & cleanup	1.0X SMRTbell cleanup beads	N/A	45 min
10 Concentrate samples for ABC	1.0X SMRTbell cleanup beads	Qubit dsDNA HS assay	15 min

Including hands-on time, this library prep procedure takes **8 hrs (+/- 2 hrs)** to process up to 24 samples

<6 hrs<sup>1</sup>




<sup>1</sup> Excludes hands-on time for setting up enzymatic reaction steps and additional time required to perform DNA sizing QC and DNA concentration QC.

# General best practices recommendations for preparing PureTarget libraries

## Supported input sample types and DNA extraction methods

- It is recommended that users obtain high-quality genomic DNA with Nanobind extraction kits<sup>1</sup> from PacBio
- Officially supported sample types include:
  - Whole blood extracted using red blood cell (RBC) lysis method and the Nanobind PanDNA kit
  - Peripheral blood mononuclear cells (PBMCs) extracted with Nanobind CBB kit
  - Human cell lines extracted with Nanobind PanDNA or Nanobind CBB kit.
- When using sample types and extraction methods other than the above, we recommend users:<sup>3</sup>
  - 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
  - See table at right for more information about samples that are officially supported, have been tested, or are not supported

Guidance on sample types and extraction methods. Low plex means that fewer than 8 samples extracted with this method were pooled with other sample extraction types and run on a SMRT Cell at 8-plex or higher. High plex means 8 or more samples extracted with the method were pooled and run on a SMRT Cell.



**Nanobind PanDNA kit (103-260-000)**  
**Supports 24 reactions per kit and includes:**

- Nanobind PanDNA kit RT (103-260-300)
- Nanobind PanDNA kit 4C (103-260-400)

Human sample type	Extraction method	Category
Whole blood	Nanobind PanDNA kit (103-260-000); extracted using RBC-lysis method	Supported
Peripheral blood mononuclear cells (PBMC)	Nanobind CBB kit (102-301-900)	
Commercial lymphoblastoid cell lines	Nanobind CBB kit (102-301-900) Nanobind HT CBB kit – automated (102-762-700)	
Skeletal muscle Brain tissue Myoblasts	Nanobind PanDNA kit (103-260-000)	Tested in low plex
Whole blood	FlexiGene DNA Whole Blood Kit– automated (AutoGen AGKT-FG-640)	Tested in high plex
Whole blood	Genomic-Tip (Qiagen)	
Whole blood	QIAasymphony (Qiagen)	
Whole blood Corneal endothelial (CEC) cell culture	SP Blood and Cell Culture DNA Isolation Kit (Bionano 80042)	Tested
Whole blood	Monarch HMW DNA Extraction Kit for Cells & Blood New England Biolabs T3050S/T3050L	
Whole blood Fibroblasts	Genra Puregene Blood Kit (Qiagen 158467 / 158389) Genomic-Tip (Qiagen)	
Whole blood	chemagic DNA blood kit (Revity)	Not recommended

<sup>1</sup> Refer to PacBio [Documentation](#) site for a list of supported Nanobind HMW DNA extraction protocols.

<sup>3</sup> See [Brochure – Nanobind PanDNA kit \(102-326-604\)](#).

<sup>3</sup> See [Application note – Comprehensive genotyping with PureTarget repeat expansion panel and HiFi sequencing \(102-326-614\)](#)

# General best practices recommendations for preparing PureTarget libraries

## Reagent and sample handling

- Room temperature is defined as any temperature in the range of 18-23°C for this protocol
- Mix all reagents well prior to use
- Quick spin all reagents in microcentrifuge to collect liquid at bottom prior to use
- Keep all temperature-sensitive reagents on ice
- **Bring SMRTbell cleanup beads, SMRT boost beads, and Qubit reagents to room temperature** for 30 minutes prior to use
- Pipette-mix all reactions by pipetting up and down 10 times. Use **full-volume pipette mixing of all reactions** to ensure thorough mixing of all reaction components
- Samples can be stored at -20°C at all safe stopping points listed in the protocol

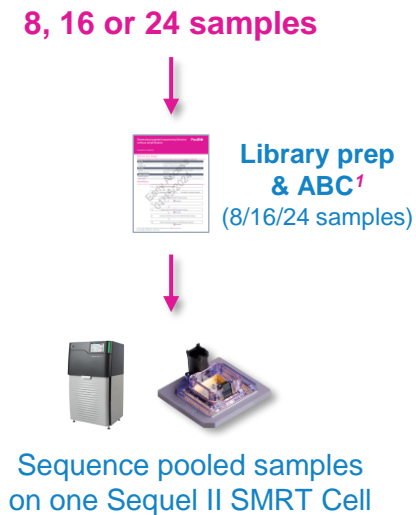
### Temperature-sensitive reagents

Protocol step	Reagent
Dephosphorylation	Phosphatase
Cas9 digestion	Cas9 Nuclease
Cas9 digestion	gRNA mix
dA Tailing	<i>Taq</i> DNA Polymerase
dA Tailing	dATP
Adapter ligation	SMRTbell adapter
Adapter ligation	Ligation mix
Adapter ligation	Ligation enhancer
Nuclease treatment	Nuclease mix
Reaction cleanups	SMRTbell cleanup beads

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

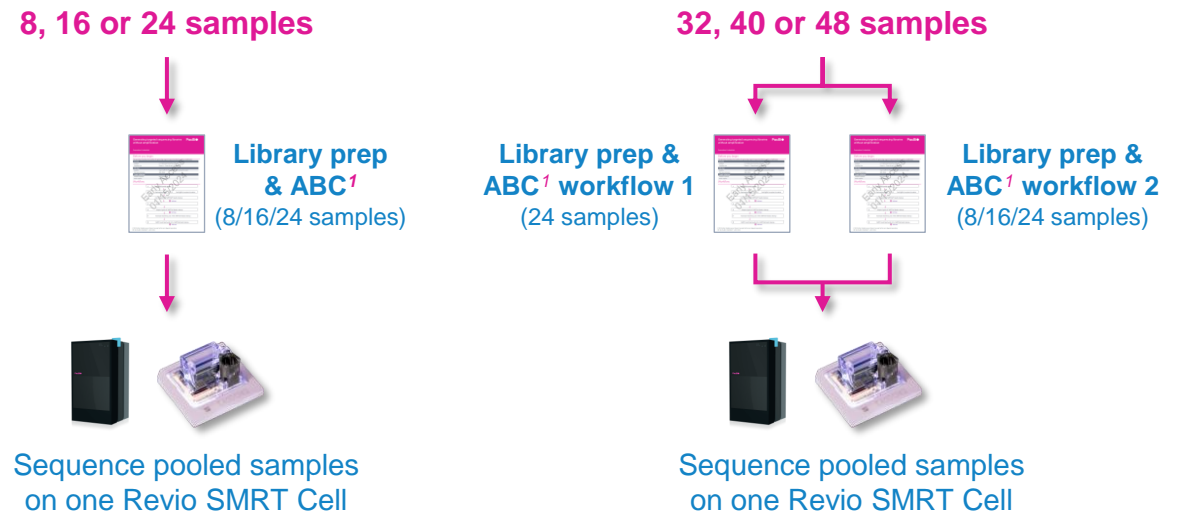
## Multiplexed sample processing

### Sequel II and Sequel IIe systems



- Up to **24** samples can be barcoded and sequenced per Sequel II SMRT Cell
- Multiplexed samples should be processed in **batches of 8/16/24 samples**
  - Follow protocol to process all samples in a **single** workflow

### Revio systems

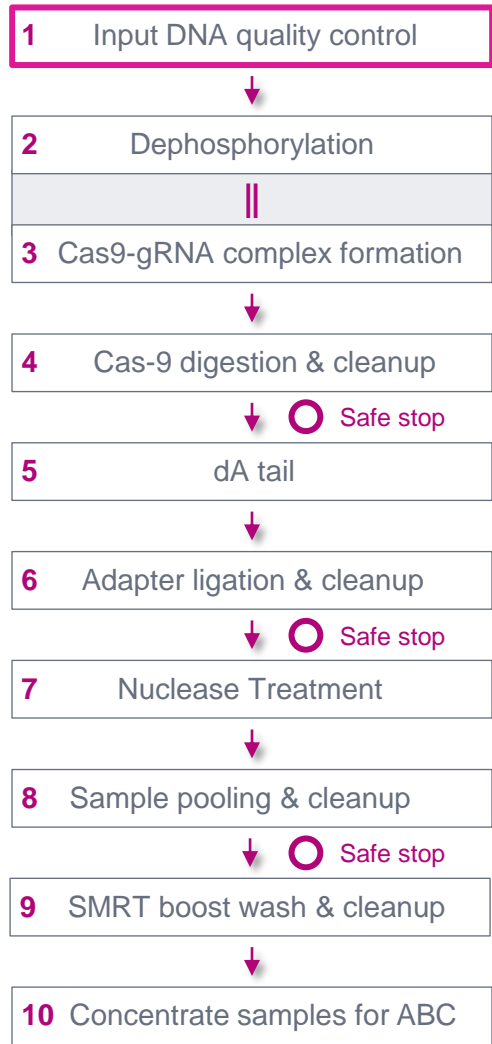


- Up to **48** samples can be barcoded and sequenced per Revio SMRT Cell
- Multiplexed samples should be processed in **batches of 8/16/24/32/40/48 samples**
- For Revio runs with 8/16/24 samples, follow protocol to **process all samples in a single workflow**
- For Revio runs with 32/40/48 samples, **process two workflows in parallel**:
  - **Workflow 1**: Process 24 samples following protocol; and
  - **Workflow 2**: Process an **additional** 8, 16 or 24 samples following protocol



# Input DNA quality control

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



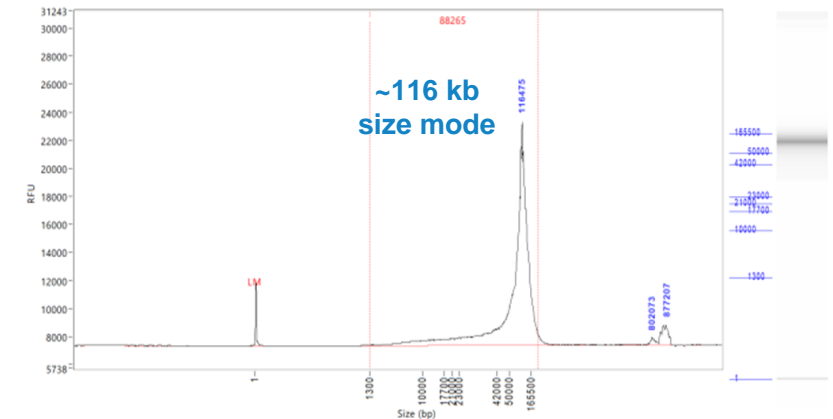
## 1. Input DNA quality control and dilution

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.  For viscous input DNA, it is important to homogenize the extracted DNA prior to start of the protocol. To homogenize the DNA, pulse-vortex 5 times and/or pipette-mix full sample volume 5 times, up and down 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 $\mu$ L aliquot from each sample and dilute with 9 $\mu$ L of elution buffer or water.
1.5	Measure DNA concentration with a Qubit fluorometer using the 1X dsDNA HS kit.
1.6	Dilute each aliquot to 250 $\mu$ g/ $\mu$ L in Femto Pulse dilution buffer based on the Qubit reading.
1.7	Measure DNA size distribution with a Femto Pulse system using the gDNA 165 kb analysis kit.
1.8	Aliquot or bring 1–4 $\mu$ g DNA to a final volume of 67 $\mu$ L per sample with nuclease-free water and transfer to an 8 tube PCR strip.

SAFE STOPPING POINT - Store at 4°C

- Protocol requires high-quality, high molecular weight (HMW) human gDNA with  $\geq 50\%$  of the mass of DNA in molecules of length  $\geq 30$  kb, or Genome quality number (GQN) at 30 kb of  $\geq 5$  based on Agilent Femto Pulse system.
- Recommended input DNA amount is 2  $\mu$ g per sample to ensure sufficient gene copies to load and maximize sequencing coverage. This protocol supports 1-4  $\mu$ g input DNA per sample.<sup>1</sup>

- For **viscous** input DNA, it is important to homogenize the extracted DNA prior to starting the protocol

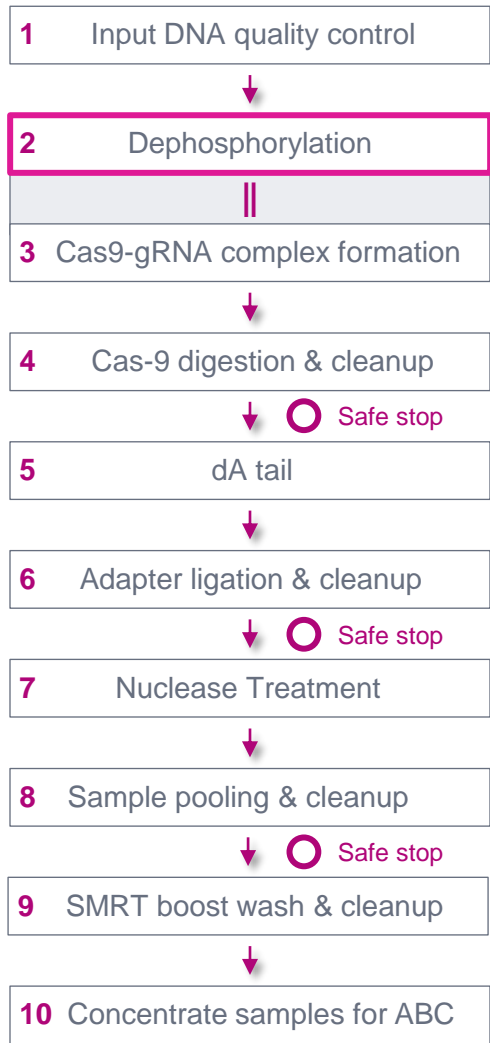


Example Femto Pulse genomic DNA sizing QC analysis results for high-molecular weight genomic DNA extracted from a human whole blood sample using Nanobind PanDNA kit.

<sup>1</sup> The standard DNA mass recommended for PureTarget libraries is 2  $\mu$ g. Users who wish to increase coverage of a sample may use up to 4  $\mu$ g of DNA in library prep. If higher coverage is needed, we recommend multiple preps of 4  $\mu$ g for the sample and combining the data during analysis.

# Dephosphorylation

This step enables dephosphorylation of genomic DNA 5' and 3' ends, which prevents subsequent adapter ligation to non-targeted genomic DNA ends



## 2. Dephosphorylation

✓ Step Instructions

Add the following components in the order and volume listed below to a new microcentrifuge tube. Adjust component volumes for the number of samples being prepared, plus 15% overage according to the table below.

Reaction Mix 1 (RM1)

2.1	Tube color	Component	Volume per sample	Volume per 8-plex With 15% overage	Volume per 16-plex With 15% overage	Volume per 24-plex With 15% overage
	Red	Cas9 buffer	8 µL	73.6 µL	147.2 µL	220.8 µL
	Blue	Phosphatase	5 µL	46 µL	92 µL	138 µL
		Total volume	13 µL	119.6 µL	239.2 µL	358.8 µL

2.2 Pipette-mix RM1 up and down 10 times (do not vortex). Pipette-mix full volume.

2.3 Quick-spin RM1 in a microcentrifuge to collect liquid.

2.4 Add 13 µL of the RM1 to each sample from step 1.8 for total reaction volume of 80 µL.

2.5 Pipette-mix each sample up and down 10 times. Pipette-mix full volume.

2.6 Quick-spin in a microcentrifuge to collect liquid.

2.7 Run the **dephosphorylation** thermocycler program. Set the lid temperature to 95°C. If the lid temperature is not programmable, it is acceptable to leave at 95–105°C.

Step	Time	Temperature
1	10 min	37°C
2	3 min	80°C
3	Hold	4°C

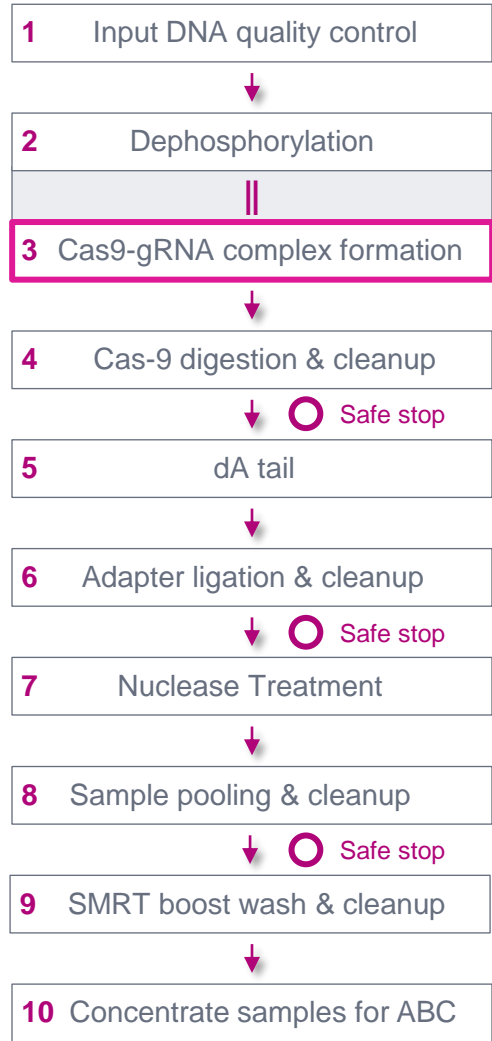
2.8 Proceed to the next step of the protocol.

- 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)

# Cas9-gRNA complex formation

This step enables Cas9-gRNA ribonucleoprotein complex formation required for subsequent targeting and digestion of genomic DNA



## 3. Cas9-gRNA complex formation

✓ Step Instructions

Add the following components in the order and volume listed below to a new PCR tube. Adjust component volumes for the number of samples being prepared, plus 25% overage according to the table below.

Reaction Mix 2 (RM2)						
✓	Reagent	Component	Volume per sample	Volume per 8-plex With 25% overage	Volume per 16-plex With 25% overage	Volume per 24-plex With 25% overage
		NF water	1.75 µL	17.5 µL	35 µL	52.5 µL
	Red	Cas9 buffer	0.5 µL	5 µL	10 µL	15 µL
	Green	Cas9 Nuclease	0.5 µL	5 µL	10 µL	15 µL
	Purple	gRNA mix (5 µM)	2 µL	20 µL	40 µL	60 µL
		<b>Total volume</b>	<b>4.75 µL</b>	<b>47.5 µL</b>	<b>95 µL</b>	<b>142.5 µL</b>

3.1

3.2 Pipette-mix RM2 up and down 10 times (do not vortex). Pipette-mix full volume.

3.3 Ensure RM2 is fully mixed and quick-spin in a microcentrifuge to collect liquid.

3.3 Proceed to incubating the RM2 Master Mix using thermocycler program below. The full master mix volume for 8, 16, or 24 samples should be incubated in one PCR tube.

Run the **Cas9 gRNA complex formation** thermocycler program.

3.4

Step	Time	Temperature
1	10 min	37°C
2	Hold	4°C

3.5 Proceed to the next step of the protocol.

Perform Cas9-gRNA complex formation during genomic DNA dephosphorylation reaction incubation

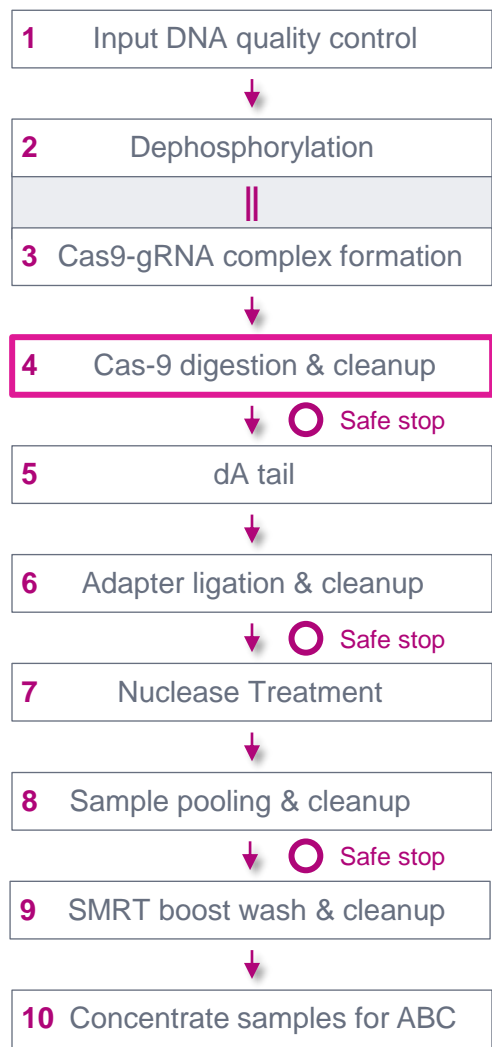
- Prepare a reaction master mix by adding the required components in the order and volume listed to a new microcentrifuge tube
- Adjust component volumes for the number of samples being prepared (8-plex, 16-plex or 24-plex), plus 25% overage according to table

- Run **Cas9 gRNA complex formation** thermocycler program
- Set the lid temperature to 75°C (if lid temp. is not programmable, leave at 95–105°C)



# Cas9 digestion & cleanup

This step enables digestion of double-stranded DNA at targeted regions using Master Mix RM2 prepared in Step 3



Safe stop

## 4.1 Cas9 digestion

✓ Step Instructions

Add the following components in the order and volume listed below to a new microcentrifuge tube. Adjust component volumes for the number of samples being prepared, plus 15% overage according to the table below. RM2 is Cas9 gRNA complex from step 3 after thermocycler program.

Reaction Mix 3 (RM3)						
4.1	✓ Reagent	Component	Volume per sample	Volume per 8-plex With 15% overage	Volume per 16-plex With 15% overage	Volume per 24-plex With 15% overage
		NF water	12.75 µL	117.3 µL	234.6 µL	351.9 µL
	Red	Cas9 buffer	1.5 µL	13.8 µL	27.6 µL	41.4 µL
		RM2 (Cas9 gRNA complex from Step 3)	4.75 µL	43.7 µL	87.4 µL	131.1 µL
		Total volume	19 µL	174.8 µL	349.6 µL	524.4 µL

- 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 the **Cas-9 digestion** thermocycler program.

Set the lid temperature to 75°C. If the lid temperature is not programmable, it is acceptable to leave at 95–105°C.

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

- Run **Cas-9 digestion** thermocycler program
- Set the lid temperature to 75°C (if lid temp. is not programmable, leave at 95–105°C)

4.8 Quick-spin in a microcentrifuge to collect liquid.

## 4.9 1X SMRTbell bead cleanup

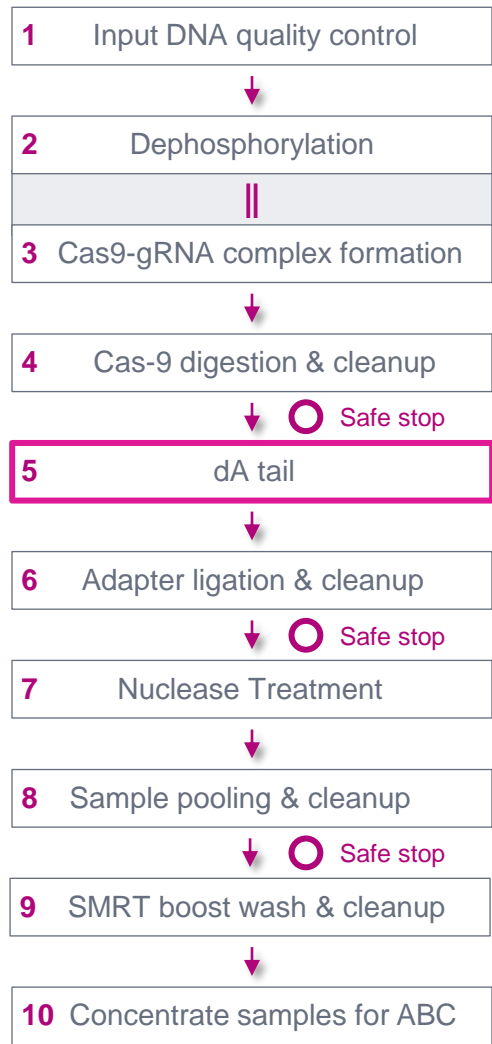
4.9 Add 100 µL of resuspended, room-temperature SMRTbell cleanup beads to each sample.

4.10 Pipette-mix the beads slowly 8–10x until evenly distributed. Bead clumping may occur and is not a concern, however, avoid over-pipetting as it may cause DNA/bead mixture to stick to the pipette tip.

- **Optional QC step after completing 1X SMRTbell bead cleanup:** Measure DNA concentration using Qubit 1X dsDNA HS kit
- Expected recovery is ~50-100% per sample relative to starting input DNA mass

# dA tail

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



Safe stop

## 4. dA tail

✓ Step Instructions

Add the following components in the order and volume listed below to a new microcentrifuge tube. Adjust component volumes for the number of samples being prepared, plus 15% overage according to the table below.

Reaction Mix 4 (RM4)					
✓ Reagent	Component	Volume per sample	Volume per 8-plex	Volume per 16-plex	Volume per 24-plex
5.1			With 15% overage	With 15% overage	With 15% overage
	NF water	2.5 µL	23 µL	46 µL	69 µL
	dA tail buffer	5 µL	46 µL	92 µL	138 µL
	dATP (10 mM)	1.25 µL	11.5 µL	23 µL	34.5 µL
	Taq DNA polymerase	1.25 µL	11.5 µL	23 µL	34.5 µL
	Total volume	10 µL	92 µL	184 µL	276 µL

5.2 Pipette-mix RM4 up and down 10 times (do not vortex). Pipette-mix full volume.

5.3 Quick-spin RM4 in a microcentrifuge to collect liquid.

5.4 Add 10 µL of the RM4 to 40 µL of each sample for a total reaction volume of 50 µL.

5.5 Pipette-mix each sample up and down 10 times. Pipette-mix full volume.

5.6 Quick-spin the tube strip in a microcentrifuge to collect liquid.

Run the **dA-tail** thermocycler program.

Set the lid temperature to 75°C. If the lid temperature is not programmable, it is acceptable to leave at 95–105°C.

Step	Time	Temperature
1	20 min	37°C
2	5 min	72°C
3	Hold	4°C

5.8 Proceed to the next step of the protocol.

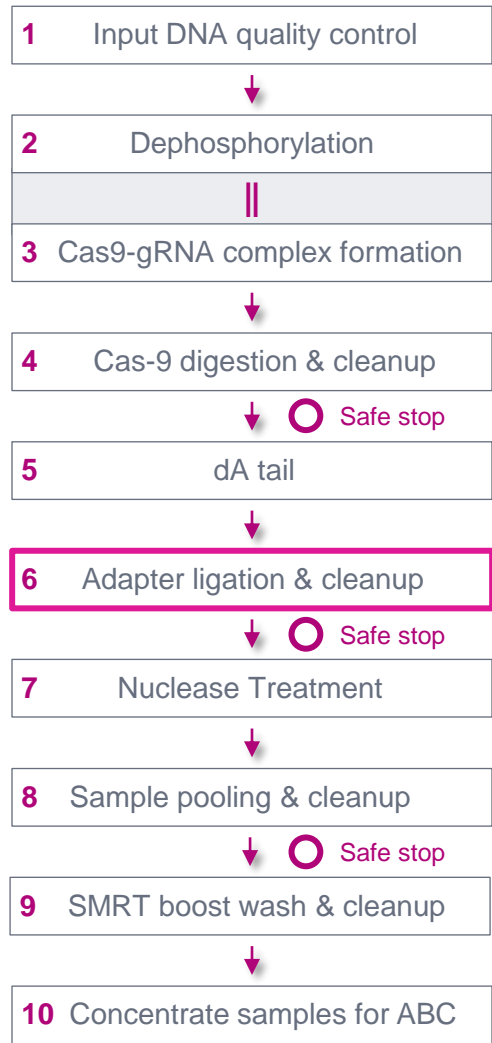
- Prepare a reaction master mix by adding the required components in the order and volume listed to a new microcentrifuge tube
- Adjust component volumes for the number of samples being prepared (8-plex, 16-plex or 24-plex), plus 15% overage according to table

- Run **dA-tail** thermocycler program
- Set the lid temperature to 75°C (if lid temp. is not programmable, leave at 95–105°C)



# Adapter ligation & cleanup

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



## 6. Adapter ligation

6.1 To a PCR strip with 50  $\mu\text{L}$  DNA from Step 5, add 4  $\mu\text{L}$  SMRTbell barcoded adapter 3.0 to each sample. Tap-mix or pipette up and down 10 times (do not vortex).

Add the following components in the order and volume listed below to a new microcentrifuge tube. Adjust component volumes for the number of samples being prepared, plus 15% overage according to the table below.

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

Reaction Mix 5 (RM5)					
Reagent	Component	Volume per sample	Volume Per 8-plex With 15% overage	Volume Per 16-plex With 15% overage	Volume Per 24-plex With 15% overage
	NF water	2 $\mu\text{L}$	18.4 $\mu\text{L}$	36.8 $\mu\text{L}$	55.2 $\mu\text{L}$
Purple	Repair buffer	8 $\mu\text{L}$	73.6 $\mu\text{L}$	147.2 $\mu\text{L}$	220.8 $\mu\text{L}$
Yellow	Ligation mix	30 $\mu\text{L}$	276 $\mu\text{L}$	552 $\mu\text{L}$	828 $\mu\text{L}$
Red	Ligation enhancer	1 $\mu\text{L}$	9.2 $\mu\text{L}$	18.4 $\mu\text{L}$	27.6 $\mu\text{L}$
Total volume		41 $\mu\text{L}$	377.2 $\mu\text{L}$	754.4 $\mu\text{L}$	1131.6 $\mu\text{L}$

- Add 4  $\mu\text{L}$  **SMRTbell adapter index plate 96A** to each sample and then tap-mix or pipette up and down 10 times (do not vortex)

- 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

6.8 Run the **adapter ligation** thermocycler program.

Set the lid temperature to 75°C. If the lid temperature is not programmable, it is acceptable to leave at 95–105°C.

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

- Run **adapter ligation** thermocycler program
- Set the lid temperature to 75°C (if lid temp. is not programmable, leave at 95–105°C)

## 1X SMRTbell bead cleanup

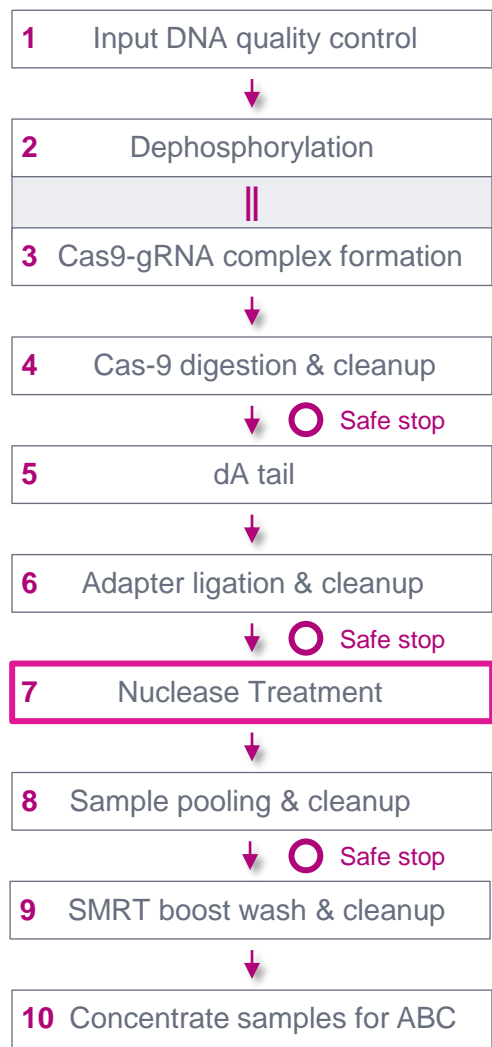
6.9 Add 95  $\mu\text{L}$  of resuspended, room-temperature SMRTbell cleanup beads to each sample.

6.10 Pipette-mix the beads 10 times until evenly distributed.

- **Optional QC step after completing 1X SMRTbell bead cleanup:** Measure DNA concentration using Qubit 1X dsDNA HS kit
- Expected recovery is ~50-100% per sample relative to starting input DNA mass

# Nuclease treatment

Nuclease treatment step removes unligated DNA fragments<sup>1</sup> and also removes leftover SMRTbell adapters from the sample



## 7. Nuclease treatment

Step	Instructions				
7.1	Add the following components in the order and volume listed below to a new microcentrifuge tube. Adjust component volumes for the number of samples being prepared, plus 15% overage according to the table below.				
<b>Reaction Mix 6 (RM6)</b>					
✓ Reagent	Component	Volume per sample	Volume per 8-plex With 15% overage	Volume per 16-plex With 15% overage	Volume per 24-plex With 15% overage
Light Purple	Nuclease buffer	5 µL	46 µL	92 µL	138 µL
Light Green	Nuclease mix	5 µL	46 µL	92 µL	138 µL
Total volume		10 µL	92 µL	184 µL	276 µL

- 7.2 Pipette-mix RM6 up and down 10 times (do not vortex). Pipette-mix full volume.
- 7.3 Quick-spin RM6 in a microcentrifuge to collect liquid.
- 7.4 Add 10 µL of RM6 to 40 µL of each sample. The total volume should equal 50 µL.
- 7.5 Pipette-mix each sample up and down 10 times. Pipette-mix full volume.
- 7.6 Quick-spin the tube strip in a microcentrifuge to collect liquid.

Run the **nuclease treatment** thermocycler program.

Set the lid temperature to 75°C. If the lid temperature is not programmable, it is acceptable to leave at 95–105°C.

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

- 7.8 Proceed to the next step of the protocol (pooling and cleanup with 1x SMRTbell cleanup beads).

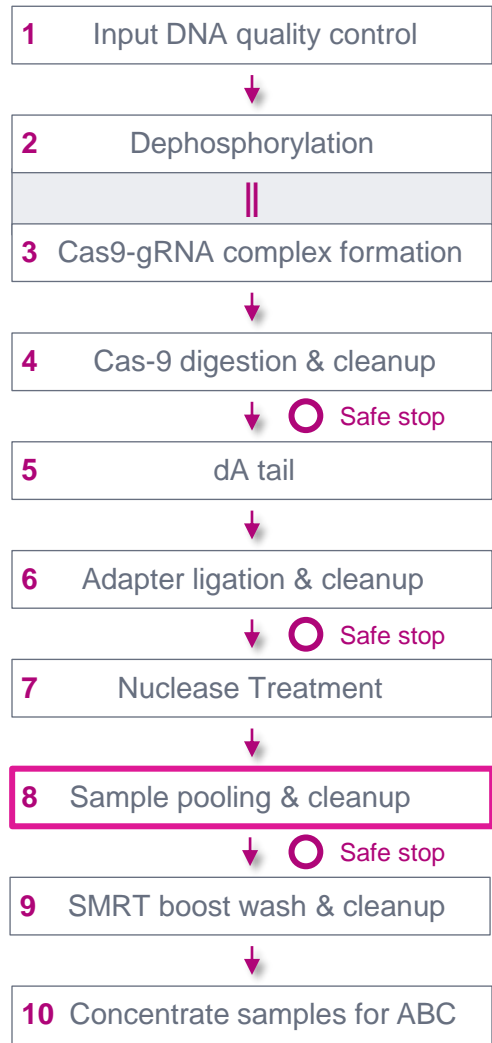
- Prepare a reaction master mix by adding the required components in the order and volume listed to a new microcentrifuge tube
- Adjust component volumes for the number of samples being prepared (8-plex, 16-plex or 24-plex), plus 15% overage according to table

- Run **nuclease treatment** thermocycler program
- Set the lid temperature to 75°C (if lid temp. is not programmable, leave at 95–105°C)

<sup>1</sup> Unligated DNA fragments comprise the majority of DNA fragments present prior to this step.

# Sample pooling & cleanup

After nuclease treatment of individual samples, pool SMRTbell templates in units of 8 samples and perform cleanup with 1x SMRTbell cleanup beads



## 8. Pooling samples for multiplex and 1X SMRTbell bead cleanup

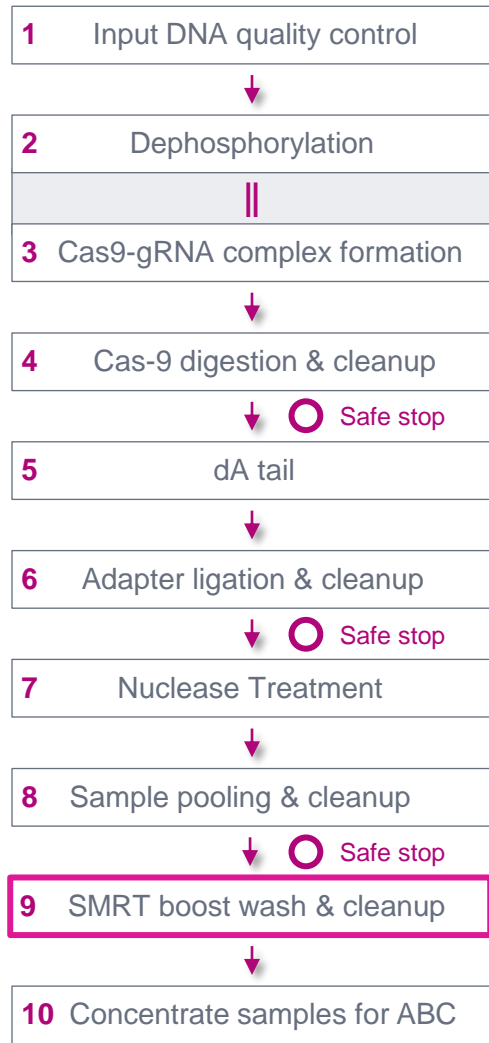
✓ Step	Instructions for pooling, bead binding, washing, and sample elution
8.1	In a 1.5 mL DNA LoBind tube combine nuclease treated libraries from step 7.7 in groups of 8 (8 x 50 µL) for a final sample volume of 400 µL.
8.2	Add 400 µL of SMRTbell cleanup beads to each pooled nuclease treated sample.
8.3	Pipette-mix up and down 10 times until the beads are evenly distributed. Pipette-mix full volume.
8.4	Quick-spin the tube strip in a microcentrifuge to collect all liquid from the sides of the tubes.
8.5	Leave at room temperature for 10 minutes to allow DNA to bind beads.
8.6	Place the tube strip in a magnetic separation rack until beads separate fully from the solution.
8.7	Slowly pipette off the cleared supernatant without disturbing the beads. Discard the supernatant.
8.8	Slowly dispense 1 mL of freshly prepared 80% ethanol into each tube. After 30 seconds, pipette off the 80% ethanol and discard.
8.9	Repeat the previous step.
	Remove residual 80% ethanol:
	<ul style="list-style-type: none"><li>Remove the tube strip from the magnetic separation rack.</li><li>Quick-spin the tube strip in a microcentrifuge.</li><li>Place the tube strip back in a magnetic separation rack until beads separate fully from the solution.</li><li>Pipette off residual 80% ethanol and discard.</li></ul>
8.10	
8.11	Remove the tube strip from the magnetic rack. Immediately add 100 µL of elution buffer to each tube and resuspend the beads by pipetting up and down 10 times.
8.13	Leave at room temperature for 5 minutes to elute DNA.
8.14	Place the tube strip in a magnetic separation rack until beads separate fully from the solution.
8.15	Slowly pipette off the cleared supernatant without disturbing the beads. Transfer supernatant to a 1.5 mL DNA LoBind tube. Discard old tube with beads.
	Proceed to the next step of the protocol.

- Combine nuclease treated libraries from previous step in groups of 8 (8 x 50 µL = 400 µL total pooled sample volume)

- Transfer supernatant to a 1.5 mL DNA LoBind tube
- DNA concentration QC measurement is not performed after completing this 1X SMRTbell bead cleanup step

# SMRT boost bead wash of SMRTbell templates & cleanup

Perform SMRT boost bead wash followed by cleanup with 1X SMRTbell cleanup beads



## 9.1 SMRT boost bead wash of SMRTbell templates

Step	Instructions for bead washing and sample collection			
9.1	Prepare the beads by washing in SMRT boost buffer <ul style="list-style-type: none"> <li>To a 1.5 mL DNA LoBind tube, first add 1 mL of room-temperature SMRT boost buffer and then 100 <math>\mu</math>L of resuspended, room-temperature SMRT boost beads.</li> <li>Pulse-vortex 5 times to mix.</li> <li>Quick-spin the tube and magnetically separate for 2 minutes until the supernatant is clear.</li> <li>Remove the supernatant and wash once more by adding 1 mL of SMRT boost buffer.</li> <li>Pulse-vortex 5 times to mix.</li> <li>Quick-spin the tube and magnetically separate for 2 minutes until the supernatant is clear. Remove the supernatant.</li> <li>Resuspend the beads in 200 <math>\mu</math>L SMRT boost buffer by pulse-vortexing 5 times to mix.</li> <li>Quick-spin to collect the beads.</li> </ul>			
9.2	Prepare the SMRTbell templates for SMRT boost bead washing by mixing together one or more 8-plex pools (each at 100 $\mu$ L) from Step 8. Bring the total volume to 300 $\mu$ L with Elution Buffer if combining less than 3 pools of 8, as shown in the table below.			
	# of Samples in Batch	8	16	24
	8-plex pool 1	100 $\mu$ L	100 $\mu$ L	100 $\mu$ L
	8-plex pool 2	0 $\mu$ L	100 $\mu$ L	100 $\mu$ L
	8-plex pool 3	0 $\mu$ L	0 $\mu$ L	100 $\mu$ L
	Elution Buffer	200 $\mu$ L	100 $\mu$ L	0 $\mu$ L
	Total sample volume	300 $\mu$ L	300 $\mu$ L	300 $\mu$ L
9.3	Add 300 $\mu$ L of SMRTbell templates to 200 $\mu$ L SMRT boost beads in wash buffer from step 9.1. Pulse-vortex 5 times to mix. Spin down to collect.			
9.4	Gently rotate-mix for 30 minutes at RT using a rotator at low speed (~10 rpm).			
9.5	Spin down to collect and magnetically separate for 2 minutes until the supernatant is clear.			
9.6	Aliquot 500 $\mu$ L of the <b>SMRTbell-containing supernatant</b> into a fresh 1.5 mL LoBind tube.			

## 9.7 1X SMRTbell bead cleanup

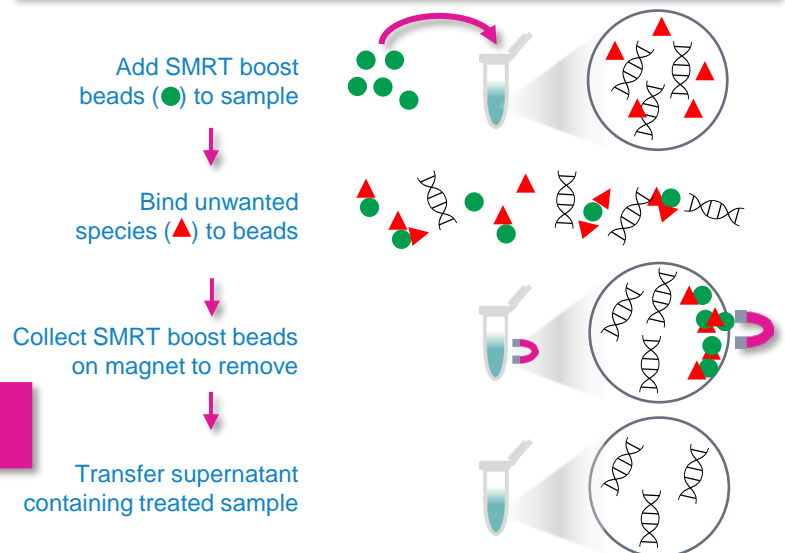
Step	Instructions
9.7	Add 500 $\mu$ L of resuspended, room-temperature SMRTbell cleanup beads to 500 $\mu$ L <b>SMRTbell-containing supernatant</b> from step 9.6.
9.8	Pipette-mix the beads 8–10 times until evenly distributed.

- Note:** SMRT boost beads are only for use with the PureTarget repeat expansion panel SMRTbell library prep workflow – use of SMRT boost beads with other types of PacBio library construction workflows is **not supported**
- Perform one SMRT boost bead cleanup reaction per final pool of up to 24 PureTarget samples
- For >24 PureTarget samples → perform two SMRT boost bead cleanups with  $\leq 24$  samples each

Prior to use, bring SMRT boost buffer and SMRT boost beads to room temperature, vortex and then spin down

Prepare templates for SMRT boost bead washing by mixing together one or more 8-plex pools (each 100  $\mu$ L)

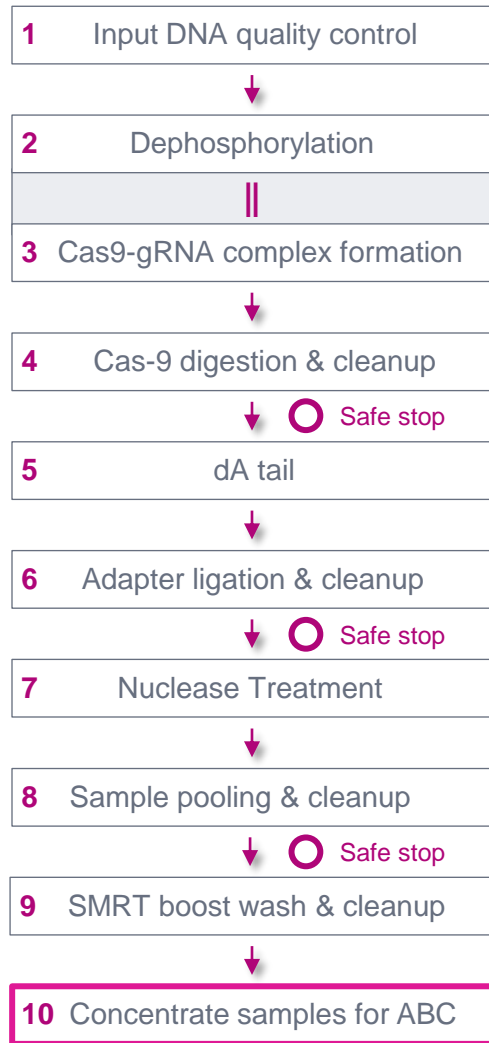
Bring total batch volume to 300  $\mu$ L with Elution Buffer if combining less than 3 pools of 8 (see table)



Serves to clean up & concentrate sample down from 500  $\mu$ L to 100  $\mu$ L

# Concentrate samples for ABC

Perform concentration step with 1X SMRTbell cleanup beads to reduce final sample volume prior to ABC (primer annealing / polymerase binding / complex cleanup)



## 10. Concentrating samples for ABC

Step	Instructions for bead binding, washing, and sample elution
10.1	Add 100 $\mu$ L SMRTbell cleanup beads to a 1.5mL DNA LoBind tube with 100 $\mu$ L of SMRTbell templates.
10.2	Pipette-mix slowly up and down 10 times until the beads are evenly distributed.
10.3	Quick-spin the tube in a microcentrifuge to collect all liquid from the sides of the tubes.
10.4	Leave at room temperature for 10 minutes to allow DNA to bind beads.
10.5	Place the tube in a magnetic separation rack until the beads separate fully from the solution.
10.6	Slowly pipette off the cleared supernatant without disturbing the beads. Discard the supernatant.
10.10	Remove the tube from the magnetic rack. Immediately add 16 $\mu$ L of elution buffer to each tube and resuspend the beads.
10.11	Leave at room temperature for 5 minutes to elute DNA.
10.12	Place the tube in a magnetic separation rack until beads separate fully from the solution.
10.13	Slowly pipette off the cleared eluate (supernatant) without disturbing the beads. Transfer supernatant to a 1.5 mL DNA LoBind tube. Discard old tube with beads.
10.14	<p>QC step: Take 1 <math>\mu</math>L of eluted DNA for quantification and measure DNA concentration with a Qubit fluorometer using the 1X dsDNA HS kit. Do NOT use Qubit dsDNA BR assay kit as the concentration may be too low to measure. Do NOT dilute sample 1:10 as concentration may be too low to measure.</p> <p>Expect recovery of 0.5% or less (range 0.02% - 0.5%) relative to input starting mass. For example, starting with 2 <math>\mu</math>g input per sample (or 16 <math>\mu</math>g per pool of 8 samples), the final mass recovered at this step is expected to be less than 80 ng total (range 3 ng–80 ng). Note, however, it is possible to observe recoveries outside of this range and still get good sequencing yield.</p>

Proceed to the next step of the protocol.  
SAFE STOPPING POINT - Store at -20°C

- Add **100  $\mu$ L** of SMRTbell cleanup beads to **100  $\mu$ L** of sample volume containing a pooled mixture of up to 24 SMRTbell templates

Elute concentrated sample (containing up to 24 SMRTbell templates) into **16  $\mu$ L** of EB

- **Take 1  $\mu$ L** to perform DNA concentration QC using Qubit dsDNA HS kit
- Use remaining **15  $\mu$ L** to proceed with sample setup (ABC<sup>1</sup>)

- **QC step:** Measure DNA concentration using Qubit 1X dsDNA HS kit
- Expected recovery is **~0.02 – 0.5% (e.g., ~3 ng – 80 ng per 8-plex)** relative to starting input DNA mass
- **Note:** Significantly higher recoveries could indicate an issue with the nuclease treatment step

<sup>1</sup> For primer annealing, polymerase binding & complex cleanup (ABC) steps, follow sample setup instructions for PureTarget libraries in [Procedure & checklist – Generating PureTarget repeat expansion panel libraries \(103-329-400\)](#) (Step 11a for Revo systems or Step 11b for Sequel II/IIe systems) – Do not use SMRT Link Sample Setup

Proceed to **Step 11** of this Procedure & Checklist (103-329-400) to perform **ABC<sup>1</sup>** sample setup (**do not use SMRT Link Sample Setup**)

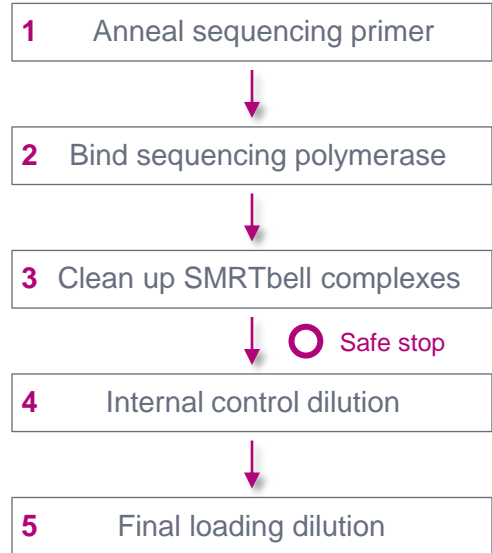




# PureTarget sequencing preparation workflow overview

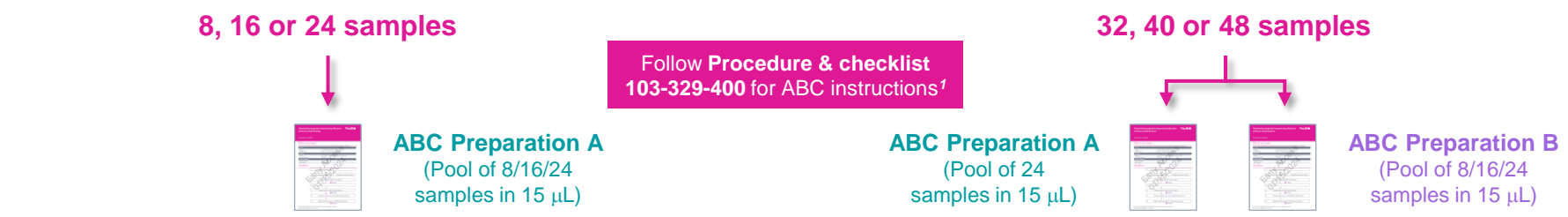
# Sample setup workflow overview for PureTarget libraries – Revio system

Follow sample setup instructions for PureTarget libraries in **Procedure & checklist – Generating targeted sequencing libraries without amplification** (103-329-400) – Do not use SMRT Link Sample Setup



- Use **entire volume (15 µL)** of pooled SMRTbell templates per ABC reaction
- **Note:** Always use the fixed reagent volumes shown at each ABC step for each pooled batch

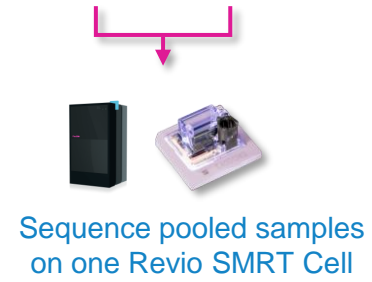
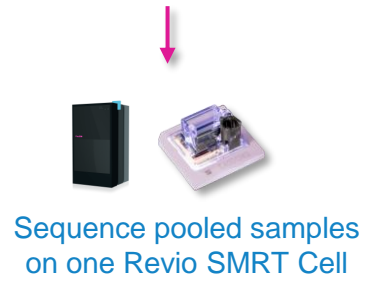
## Revio system ABC workflow for PureTarget repeat expansion panel samples



Step	Instructions																		
11a.1	<p><b>Note:</b> Always use these values for each pooled preparation. Do not adjust based on measured concentration, value, or plex level.</p> <p>Annealing Sequencing primer Combine the following components in a new low-binding tube and pipette to mix.</p> <table border="1"> <thead> <tr> <th></th> <th>Preparation A</th> <th>Preparation B</th> </tr> </thead> <tbody> <tr> <td># of Samples in Preparation</td> <td>8 or 16 or 24</td> <td>8 or 16 or 24</td> </tr> <tr> <td>Sample (SMRTbell templates)</td> <td>15 µL</td> <td>15 µL</td> </tr> <tr> <td>Annealing buffer</td> <td>7.5 µL</td> <td>7.5 µL</td> </tr> <tr> <td>Sequencing primer</td> <td>7.5 µL</td> <td>7.5 µL</td> </tr> <tr> <td>Total Volume</td> <td>30 µL</td> <td>30 µL</td> </tr> </tbody> </table> <p>Incubate at room temperature for 15 minutes then proceed to the next step.</p>		Preparation A	Preparation B	# of Samples in Preparation	8 or 16 or 24	8 or 16 or 24	Sample (SMRTbell templates)	15 µL	15 µL	Annealing buffer	7.5 µL	7.5 µL	Sequencing primer	7.5 µL	7.5 µL	Total Volume	30 µL	30 µL
	Preparation A	Preparation B																	
# of Samples in Preparation	8 or 16 or 24	8 or 16 or 24																	
Sample (SMRTbell templates)	15 µL	15 µL																	
Annealing buffer	7.5 µL	7.5 µL																	
Sequencing primer	7.5 µL	7.5 µL																	
Total Volume	30 µL	30 µL																	

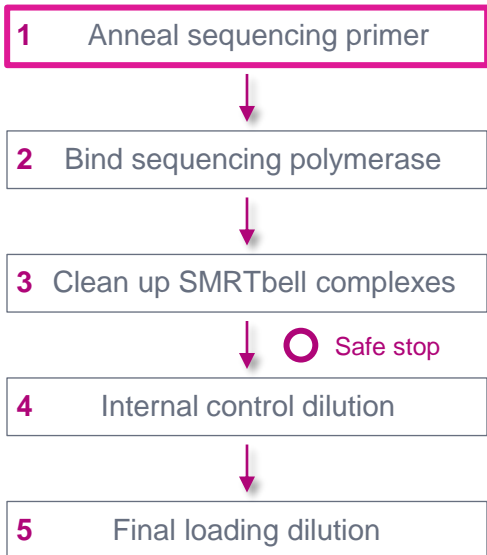
Step	Instructions																		
11a.1	<p><b>Note:</b> Always use these values for each pooled preparation. Do not adjust based on measured concentration, value, or plex level.</p> <p>Annealing Sequencing primer Combine the following components in a new low-binding tube and pipette to mix.</p> <table border="1"> <thead> <tr> <th></th> <th>Preparation A</th> <th>Preparation B</th> </tr> </thead> <tbody> <tr> <td># of Samples in Preparation</td> <td>8 or 16 or 24</td> <td>8 or 16 or 24</td> </tr> <tr> <td>Sample (SMRTbell templates)</td> <td>15 µL</td> <td>15 µL</td> </tr> <tr> <td>Annealing buffer</td> <td>7.5 µL</td> <td>7.5 µL</td> </tr> <tr> <td>Sequencing primer</td> <td>7.5 µL</td> <td>7.5 µL</td> </tr> <tr> <td>Total Volume</td> <td>30 µL</td> <td>30 µL</td> </tr> </tbody> </table> <p>Incubate at room temperature for 15 minutes then proceed to the next step.</p>		Preparation A	Preparation B	# of Samples in Preparation	8 or 16 or 24	8 or 16 or 24	Sample (SMRTbell templates)	15 µL	15 µL	Annealing buffer	7.5 µL	7.5 µL	Sequencing primer	7.5 µL	7.5 µL	Total Volume	30 µL	30 µL
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# of Samples in Preparation	8 or 16 or 24	8 or 16 or 24																	
Sample (SMRTbell templates)	15 µL	15 µL																	
Annealing buffer	7.5 µL	7.5 µL																	
Sequencing primer	7.5 µL	7.5 µL																	
Total Volume	30 µL	30 µL																	

**NOTE:**  
ALWAYS USE THESE FIXED VOLUMES FOR EACH POOLED BATCH  
→ Do not adjust reagent volumes based on measured sample concentration value or plex level of batch



# Sample setup procedure for PureTarget libraries – Revio system

## Anneal sequencing primer (ABC)



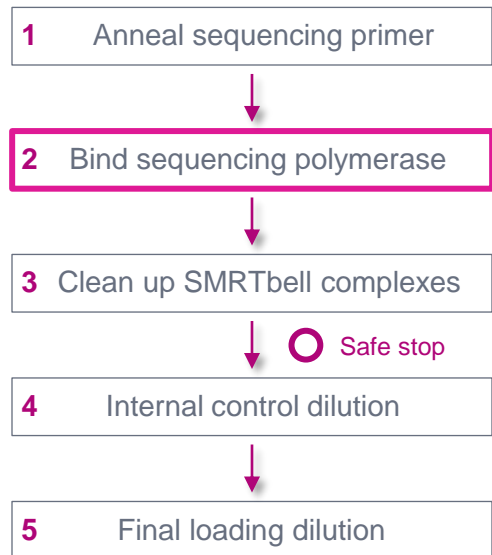
### Anneal sequencing primer

Step	Instructions																		
✓	<b>Note:</b> Always use these values for each pooled preparation. Do not adjust based on measured concentration, value, or plex level.																		
	<b>Annealing Sequencing primer</b> Combine the following components in a new low-binding tube and pipette to mix.																		
11a.1	<table border="1"><thead><tr><th></th><th>Preparation A</th><th>Preparation B</th></tr></thead><tbody><tr><td># of Samples in Preparation</td><td>8 or 16 or 24</td><td>8 or 16 or 24</td></tr><tr><td>Sample (SMRTbell templates)</td><td>15 µL</td><td>15 µL</td></tr><tr><td>Annealing buffer</td><td>7.5 µL</td><td>7.5 µL</td></tr><tr><td>Sequencing primer</td><td>7.5 µL</td><td>7.5 µL</td></tr><tr><td>Total Volume</td><td>30 µL</td><td>30 µL</td></tr></tbody></table>		Preparation A	Preparation B	# of Samples in Preparation	8 or 16 or 24	8 or 16 or 24	Sample (SMRTbell templates)	15 µL	15 µL	Annealing buffer	7.5 µL	7.5 µL	Sequencing primer	7.5 µL	7.5 µL	Total Volume	30 µL	30 µL
	Preparation A	Preparation B																	
# of Samples in Preparation	8 or 16 or 24	8 or 16 or 24																	
Sample (SMRTbell templates)	15 µL	15 µL																	
Annealing buffer	7.5 µL	7.5 µL																	
Sequencing primer	7.5 µL	7.5 µL																	
Total Volume	30 µL	30 µL																	
	Incubate at room temperature for 15 minutes then proceed to the next step.																		

**NOTE:**  
**ALWAYS USE THESE FIXED VOLUMES FOR EACH POOLED BATCH**  
→ Do not adjust reagent volumes based on measured sample concentration value or plex level of batch

# Sample setup procedure for PureTarget libraries – Revio system

## Bind sequencing polymerase (ABC)



### Dilute sequencing polymerase

✓ Step Instructions

Combine the following components in a single low-bind tube and pipette to mix. The prepared volume of diluted polymerase is sufficient to process all specified samples in a batch.

11a.2

	Preparation A	Preparation B
# of Samples in preparation	8 or 16 or 24	8 or 16 or 24
Polymerase stock	3.5 µL	3.5 µL
Polymerase buffer	26.5 µL	26.5 µL
Total Volume	30 µL	30 µL

Diluted polymerase must be used immediately.

• **Note:** For PureTarget repeat expansion samples, polymerase working solution concentration after performing polymerase dilution step is **higher** than for Kinnex & WGS samples

### Bind sequencing polymerase

✓ Step Instructions

Add annealed sample to diluted polymerase and finger tap or pipette to mix.

11a.3

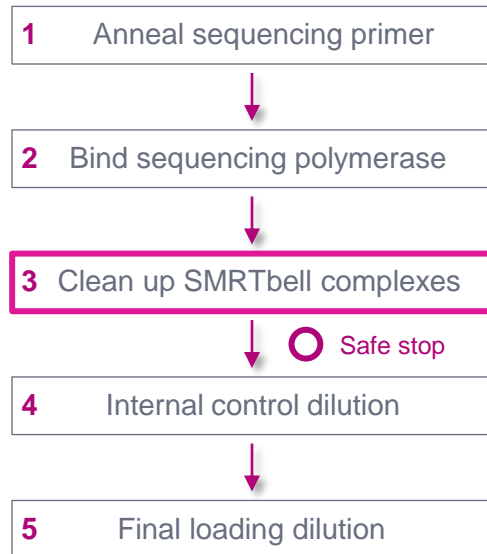
	Preparation A	Preparation B
# of Samples in preparation	8 or 16 or 24	8 or 16 or 24
Annealed sample	30 µL	30 µL
Diluted Polymerase	30 µL	30 µL
Total Volume	60 µL	60 µL

Incubate at room temperature for 15 minutes. The bound complex can be stored at 4°C for 4 weeks.

**NOTE:**  
**ALWAYS USE THESE FIXED VOLUMES FOR EACH POOLED BATCH**  
→ **Do not adjust reagent volumes** based on measured sample concentration value or plex level of batch

# Sample setup procedure for PureTarget libraries – Revio system

## Clean up SMRTbell complexes (ABC)



### Purification of polymerase-bound SMRTbell complexes

✓ Step	Instructions															
1.	Equilibrate the SMRTbell cleanup beads and the loading buffer to room temperature at least 30 min before use.															
2.	Add the following buffer volumes to each sample in each batch, as indicated: <table border="1" data-bbox="901 486 1498 648"> <thead> <tr> <th></th> <th>Preparation A</th> <th>Preparation B</th> </tr> </thead> <tbody> <tr> <td># of Samples in preparation</td> <td>8 or 16 or 24</td> <td>8 or 16 or 24</td> </tr> <tr> <td>Binding Reaction</td> <td>60 µL</td> <td>60 µL</td> </tr> <tr> <td>Dilution Buffer</td> <td>40 µL</td> <td>40 µL</td> </tr> <tr> <td>Total Volume</td> <td>100 µL</td> <td>100 µL</td> </tr> </tbody> </table>		Preparation A	Preparation B	# of Samples in preparation	8 or 16 or 24	8 or 16 or 24	Binding Reaction	60 µL	60 µL	Dilution Buffer	40 µL	40 µL	Total Volume	100 µL	100 µL
	Preparation A	Preparation B														
# of Samples in preparation	8 or 16 or 24	8 or 16 or 24														
Binding Reaction	60 µL	60 µL														
Dilution Buffer	40 µL	40 µL														
Total Volume	100 µL	100 µL														
3.	Add the indicated volume of SMRTbell cleanup beads to each sample in each preparation and gently pipette-mix. Incubate on the benchtop for 10 minutes. <table border="1" data-bbox="901 711 1498 872"> <thead> <tr> <th></th> <th>Preparation A</th> <th>Preparation B</th> </tr> </thead> <tbody> <tr> <td># of Samples in preparation</td> <td>8 or 16 or 24</td> <td>8 or 16 or 24</td> </tr> <tr> <td>Binding Reaction</td> <td>100 µL</td> <td>100 µL</td> </tr> <tr> <td>SMRTbell cleanup Beads</td> <td>120 µL</td> <td>120 µL</td> </tr> <tr> <td>Total Volume</td> <td>220 µL</td> <td>220 µL</td> </tr> </tbody> </table>		Preparation A	Preparation B	# of Samples in preparation	8 or 16 or 24	8 or 16 or 24	Binding Reaction	100 µL	100 µL	SMRTbell cleanup Beads	120 µL	120 µL	Total Volume	220 µL	220 µL
	Preparation A	Preparation B														
# of Samples in preparation	8 or 16 or 24	8 or 16 or 24														
Binding Reaction	100 µL	100 µL														
SMRTbell cleanup Beads	120 µL	120 µL														
Total Volume	220 µL	220 µL														
4.	Place each tube in a magnetic bead rack until the beads collect to the side of the tube and the solution appears clear. Discard the supernatant. DO NOT wash the collected bead pellet with ethanol.															
5.	Immediately resuspend the beads in the indicated volumes of room temperature Loading buffer and pipette-mix: <table border="1" data-bbox="901 1029 1498 1129"> <thead> <tr> <th></th> <th>Preparation A</th> <th>Preparation B</th> </tr> </thead> <tbody> <tr> <td># of Samples in preparation</td> <td>8 or 16 or 24</td> <td>8 or 16 or 24</td> </tr> <tr> <td>Loading Buffer</td> <td>49 µL</td> <td>49 µL</td> </tr> </tbody> </table>		Preparation A	Preparation B	# of Samples in preparation	8 or 16 or 24	8 or 16 or 24	Loading Buffer	49 µL	49 µL						
	Preparation A	Preparation B														
# of Samples in preparation	8 or 16 or 24	8 or 16 or 24														
Loading Buffer	49 µL	49 µL														
6.	To elute the polymerase-bound complexes, incubate the samples on the benchtop for at least 10 minutes at room temperature.															
7.	Place each tube in a magnetic bead rack until the beads collect to the side of the tube and the solution appears clear.															
8.	Transfer eluates to new low-binding tube. Place on ice and protect from light.															

11a.4

• Equilibrate SMRTbell cleanup beads and loading buffer to room temperature at least 30 min before use

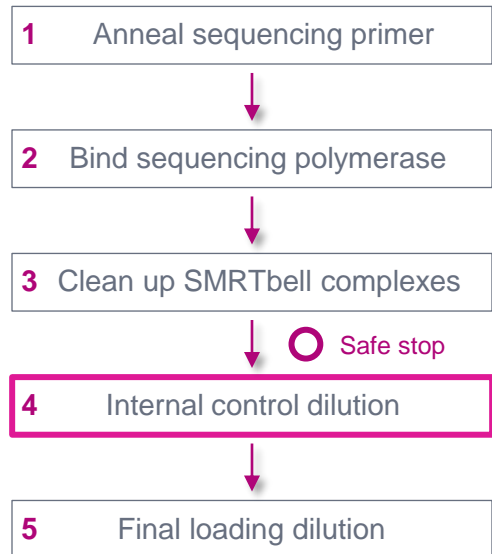
**NOTE:**  
**ALWAYS USE THESE FIXED VOLUMES FOR EACH POOLED BATCH**  
 → Do not adjust reagent volumes based on measured sample concentration value or plex level of batch

• Do not wash bead pellet with ethanol



# Sample setup procedure for PureTarget libraries – Revio system

## Internal control dilution



### Internal control dilution – First dilution

Step	Instructions								
11a.5	Prepare only one control dilution, regardless of number of samples (up to 48 samples). 1st Dilution. Mix well by flicking the tube by hand and pulse-spin to collect contents and keep on ice.								
	<table border="1"><thead><tr><th>Reagent</th><th>Internal Control</th></tr></thead><tbody><tr><td>Dilution buffer</td><td>19 <math>\mu</math>L</td></tr><tr><td>Sequencing control</td><td>1.0 <math>\mu</math>L</td></tr><tr><td>Total volume</td><td>20 <math>\mu</math>L</td></tr></tbody></table>	Reagent	Internal Control	Dilution buffer	19 $\mu$ L	Sequencing control	1.0 $\mu$ L	Total volume	20 $\mu$ L
Reagent	Internal Control								
Dilution buffer	19 $\mu$ L								
Sequencing control	1.0 $\mu$ L								
Total volume	20 $\mu$ L								

- Prepare only one control dilution reaction, regardless of number of samples (up to 48 samples per SMRT Cell for Revio system)

### Internal control dilution – Second dilution

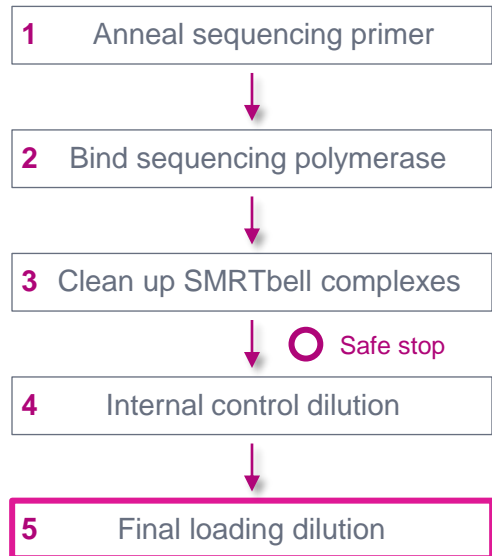
Step	Instructions								
11a.6	2 <sup>nd</sup> Dilution. Mix well by flicking the tube by hand and pulse-spin to collect contents and keep on ice.								
	<table border="1"><thead><tr><th>Reagent</th><th>Internal Control</th></tr></thead><tbody><tr><td>Dilution buffer</td><td>19 <math>\mu</math>L</td></tr><tr><td>Sequencing control</td><td>1.0 <math>\mu</math>L</td></tr><tr><td>Total volume</td><td>20 <math>\mu</math>L</td></tr></tbody></table>	Reagent	Internal Control	Dilution buffer	19 $\mu$ L	Sequencing control	1.0 $\mu$ L	Total volume	20 $\mu$ L
Reagent	Internal Control								
Dilution buffer	19 $\mu$ L								
Sequencing control	1.0 $\mu$ L								
Total volume	20 $\mu$ L								

### Internal control dilution – Third dilution

Step	Instructions								
11a.7	3 <sup>rd</sup> Dilution. Mix well by flicking the tube by hand and pulse-spin to collect contents and keep on ice.								
	<table border="1"><thead><tr><th>Reagent</th><th>Internal Control</th></tr></thead><tbody><tr><td>Dilution buffer</td><td>19 <math>\mu</math>L</td></tr><tr><td>Sequencing control</td><td>1.0 <math>\mu</math>L</td></tr><tr><td>Total Volume</td><td>20 <math>\mu</math>L</td></tr></tbody></table>	Reagent	Internal Control	Dilution buffer	19 $\mu$ L	Sequencing control	1.0 $\mu$ L	Total Volume	20 $\mu$ L
Reagent	Internal Control								
Dilution buffer	19 $\mu$ L								
Sequencing control	1.0 $\mu$ L								
Total Volume	20 $\mu$ L								

# Sample setup procedure for PureTarget libraries – Revio system

## Final loading dilution



## Final loading dilution

Step	Instructions												
✓	Combine the following and protect from light:												
11a.8	<b>If loading ≤24 samples</b>												
	<table border="1"><thead><tr><th># of Samples in preparation</th><th>≤24-plex</th></tr></thead><tbody><tr><td>Prepared Sample (Preparation A)</td><td>48.5 μL</td></tr><tr><td>Loading buffer</td><td>48.5 μL</td></tr><tr><td>Diluted internal control (Dilution 3)</td><td>3 μL</td></tr><tr><td><b>Total volume</b></td><td><b>100 μL</b></td></tr></tbody></table>	# of Samples in preparation	≤24-plex	Prepared Sample (Preparation A)	48.5 μL	Loading buffer	48.5 μL	Diluted internal control (Dilution 3)	3 μL	<b>Total volume</b>	<b>100 μL</b>		
# of Samples in preparation	≤24-plex												
Prepared Sample (Preparation A)	48.5 μL												
Loading buffer	48.5 μL												
Diluted internal control (Dilution 3)	3 μL												
<b>Total volume</b>	<b>100 μL</b>												
	Load 100 μL of sample per well and/or store at 4°C for up to 24 hours before use.												
	<b>If loading &gt;24 samples</b>												
	<table border="1"><thead><tr><th># of Samples in preparation</th><th>&gt;24-plex</th></tr></thead><tbody><tr><td>Prepared sample (Preparation A)</td><td>48.5 μL</td></tr><tr><td>Prepared sample (Preparation B)</td><td>48.5 μL</td></tr><tr><td>Loading buffer</td><td>0 μL</td></tr><tr><td>Diluted internal control (Dilution 3)</td><td>3 μL</td></tr><tr><td><b>Total volume</b></td><td><b>100 μL</b></td></tr></tbody></table>	# of Samples in preparation	>24-plex	Prepared sample (Preparation A)	48.5 μL	Prepared sample (Preparation B)	48.5 μL	Loading buffer	0 μL	Diluted internal control (Dilution 3)	3 μL	<b>Total volume</b>	<b>100 μL</b>
# of Samples in preparation	>24-plex												
Prepared sample (Preparation A)	48.5 μL												
Prepared sample (Preparation B)	48.5 μL												
Loading buffer	0 μL												
Diluted internal control (Dilution 3)	3 μL												
<b>Total volume</b>	<b>100 μL</b>												
	Load 100 μL of sample per well and/or store at 4°C for up to 24 hours before use.												

- If loading ≤24 samples onto Revio SMRT Cell, add 48.5 μL Preparation A + 48.5 μL Loading buffer + 3 μL diluted internal control  
→ Transfer entire mixture (100 μL) to appropriate sample well in Revio sequencing plate

- If loading >24 samples onto Revio SMRT Cell, add 48.5 μL Preparation A + 48.5 μL Preparation B + 3 μL diluted internal control  
→ Transfer entire mixture (100 μL) to appropriate sample well in Revio sequencing plate

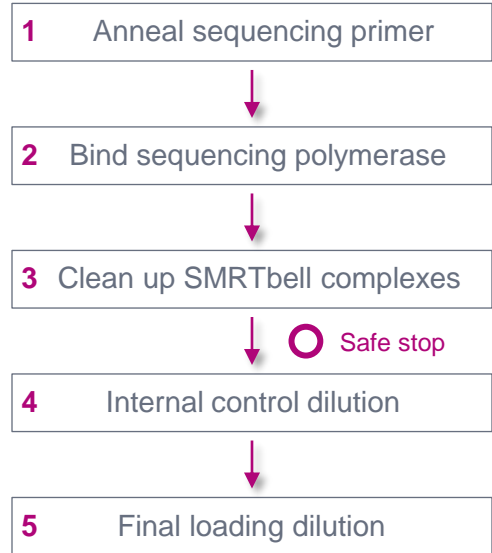
### NOTE:

**ALWAYS USE THESE FIXED VOLUMES FOR EACH POOLED BATCH**

- Do not adjust reagent volumes based on measured sample concentration value or plex level of batch

# Sample setup workflow overview for PureTarget libraries – Sequel II/Ile system

Follow sample setup instructions for PureTarget libraries in **Procedure & checklist – Generating PureTarget repeat expansion panel libraries** (103-329-400) – Do not use SMRT Link Sample Setup



- Use **entire volume (15 µL)** of pooled SMRTbell templates per ABC reaction
- **Note:** Always use the fixed reagent volumes shown at each ABC step for each pooled batch

## Sequel II/Ile system ABC workflow for PureTarget repeat expansion panel samples

Follow **Procedure & checklist 103-329-400** for ABC instructions

8, 16 or 24 samples



**ABC<sup>1</sup>**  
(Pool of 8/16/24 samples in 15 µL)

Step	Instructions										
11b.1	<p><b>Note:</b> Always use these values for each pooled batch. Do not adjust based on measured concentration, value, or plex level of batch.</p> <p>Annealing Sequencing primer</p> <p>Combine the following components in a new low-binding tube and pipette to mix.</p> <table border="1"><tr><td># of Samples in Batch</td><td>8 or 16 or 24</td></tr><tr><td>Sample (SMRTbell templates)</td><td>15 µL</td></tr><tr><td>Annealing Buffer</td><td>7.5 µL</td></tr><tr><td>Sequel II Primer 3.2</td><td>7.5 µL</td></tr><tr><td>Total volume</td><td>30 µL</td></tr></table> <p>Incubate at room temperature for 15 minutes then proceed to the next step.</p>	# of Samples in Batch	8 or 16 or 24	Sample (SMRTbell templates)	15 µL	Annealing Buffer	7.5 µL	Sequel II Primer 3.2	7.5 µL	Total volume	30 µL
# of Samples in Batch	8 or 16 or 24										
Sample (SMRTbell templates)	15 µL										
Annealing Buffer	7.5 µL										
Sequel II Primer 3.2	7.5 µL										
Total volume	30 µL										

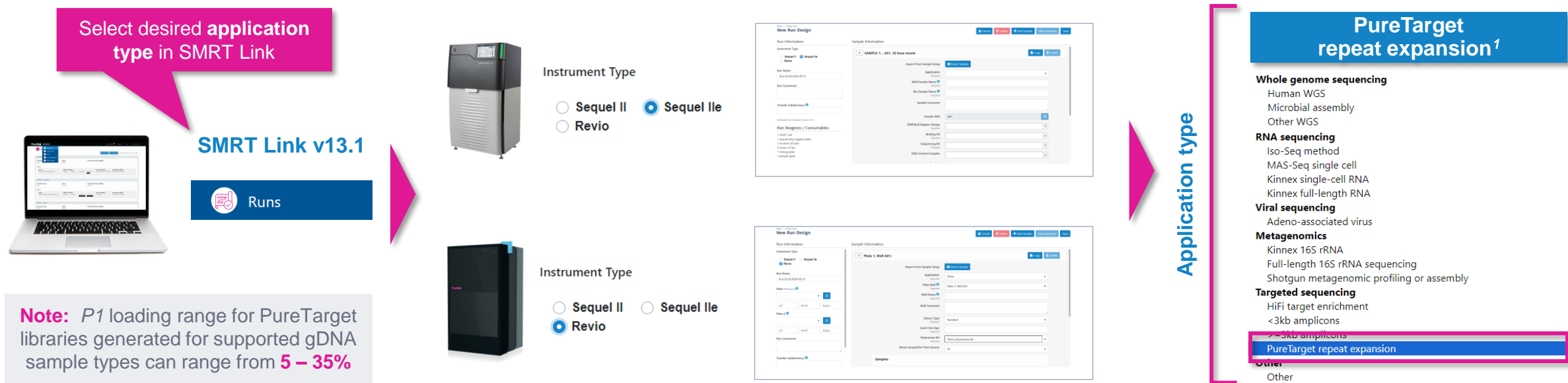
**NOTE:**  
**ALWAYS USE THESE FIXED VOLUMES FOR EACH POOLED BATCH**  
→ Do not adjust reagent volumes based on measured sample concentration value or plex level of batch



Sequence pooled samples on one Sequel II SMRT Cell

# SMRT Link Run Design workflow overview for PureTarget libraries

## SMRT Link run design recommendations for PureTarget repeat expansion panel samples



SMRT Link module	Key setup parameters for PureTarget libraries	Sequel II/IIe system recommended settings for PureTarget libraries	Revo system recommended settings for PureTarget libraries
	Adapter / Library type	SMRTbell Adapter Design = Overhang – SMRTbell Prep Kit 3.0	Library type = Standard
	Movie collection time	30 hrs	24 hrs
Runs → Run design	Use adaptive loading		NO
	On-instrument CCS	CCS Analysis Output - Include Low Quality Reads = YES CCS Analysis Output - Include Kinetics Information = YES	Consensus Mode = MOLECULE Include Base Kinetics = NO

# SMRT Link Run Design procedure for PureTarget libraries – Revio system

## Run information and sample information

Run Information

Run Name  
PureTarget\_Run\_Design\_Demo

Plate 1 Required ⓘ  
Revio sequencing plate  
012345 12345 20241231

Plate 2 ⓘ  
Lot Serial Expiry

Run Comments

Transfer Subdirectory ⓘ

Use Adaptive Loading  
 YES  NO

Sample Information

▼ Plate 1, Well A01: PureTarget\_library\_demo

Import from Sample Setup

Application Required PureTarget repeat expansion

Plate Well Required Plate 1, Well A01

Well Name Required PureTarget\_library\_demo

Well Comment

Library Type Required Standard

Insert Size (bp) Required 5000

Polymerase Kit Required Revio polymerase kit

Movie Acquisition Time (hours) 24

Samples

Select **PureTarget repeat expansion** for application type to autofill Library Type, Polymerase Kit & Movie Acquisition Time recommended settings

Specify **Standard** library type (instead of Kinnex or AAV)<sup>1</sup>

Specify **Insert Size**  
→ For PureTarget repeat expansion panel samples, specify 5000 bp<sup>2</sup>

Specify **Revio polymerase**

Recommend **24 hrs** movie collection for PureTarget samples

**IMPORTANT!** For Revio system PureTarget repeat expansion panel samples, specify **Use Adaptive Loading = NO**<sup>3</sup>

- **Note:** **Use Adaptive Loading field** is a run-level setting and the default value for new Revio run designs is YES – however, **PureTarget repeat expansion samples require Use Adaptive Loading = NO**
- 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**

<sup>1</sup> **Library Type** field determines which adapter finding algorithm is used during post-primary analysis.

<sup>2</sup> **Note:** This '5000 bp' value only serves as a **placeholder** since the **actual average insert size distribution of PureTarget repeat expansion samples may vary**.

<sup>3</sup> **Note:** In SMRT Link v13.1, Adaptive Loading is ON by default for all Revio system run designs. For PureTarget repeat expansion panel samples, specify **Adaptive Loading = NO** to enable correct sample immobilization conditions to be used on the Revio system. PureTarget repeat expansion panel samples should not be included in the same run design as other sample types that require Adaptive Loading to be enabled. After starting a run with PureTarget repeat expansion panel samples, users should wait until the Revio system door is unlocked to pre-load samples that require adaptive loading



# SMRT Link Run Design procedure for PureTarget libraries – Revio system (cont.)

## Sample indexing (barcoding) information

**Samples**

Sample is indexed  YES  NO

Indexes Required: SMRTbell adapter indexes

Same Barcodes on Both Ends of Sequence  YES  NO

Biosample names Required: Interactively | From a File

Default = YES for Sample is indexed

Specify Indexes FASTA = SMRTbell adapter indexes

Specify YES for Same barcodes on both ends of sequences

### Example PureTarget library molecule containing SMRTbell indexed adapters<sup>1</sup> at both ends



Both forward & reverse terminal SMRTbell adapters contain the same barcode sequence

### Example interactive biosample name specification for a multiplexed PureTarget library sample

Barcode Selector and Sample Name Editor

Available Barcodes		Included Barcodes	
Barcode ID		Barcode ID	Bio Sample ID
<input type="checkbox"/>	bc2001--bc2001		
<input type="checkbox"/>	bc2002--bc2002		
<input type="checkbox"/>	bc2003--bc2003		
<input type="checkbox"/>	bc2004--bc2004		

Barcode Selector and Sample Name Editor

Available Barcodes		Included Barcodes	
Barcode ID		Barcode ID	Bio Sample ID
<input type="checkbox"/>	bc2003--bc2003		
<input type="checkbox"/>	bc2004--bc2004		
<input type="checkbox"/>	bc2005--bc2005		
<input checked="" type="checkbox"/>	bc2001--bc2001		PureTarget_Sample_1
<input checked="" type="checkbox"/>	bc2002--bc2002		PureTarget_Sample_2

SMRT Link



Data Management

#### SMRTbell adapter indexes

```
>bc2001
ATCGTGCGACGAGTAT
>bc2002
TGCATGTCATGAGTAT
>bc2003
ACGAGTGCTCGAGTAT
>bc2004
TGCAGTGCTCGAGTAT
```

# SMRT Link Run Design procedure for PureTarget libraries – Revio system (cont.)

## Run options and data options

### Run Options

Library Concentration (pM)   
Required

#### Specify Library Concentration

- For PureTarget repeat expansion panel samples, specify on-plate loading concentration (OPLC) = **200 pM**
- **Note:** This '200 pM' value only serves as a **placeholder** since the **actual loading concentration of PureTarget repeat expansion samples may vary**

### Data Options

Include Base Kinetics  YES  NO

Specify Include Base Kinetics = NO

Consensus Mode  MOLECULE  STRAND

Specify Consensus Mode = MOLECULE<sup>1</sup>

Assign Data To Project

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

# SMRT Link Run Design procedure for PureTarget libraries – Sequel Ii system

## Sample information and run information

- Select **PureTarget repeat expansion** from **Application** field drop-down menu
- Following fields are **auto-populated** with default recommended values and high-lighted in **green**:
  - SMRTbell Adapter Design**
    - Overhang – SMRTbell Prep Kit 3.0
  - Binding Kit**
    - Sequel II Binding Kit 3.2
  - Sequencing Kit**
    - Sequel II Sequencing Plate 2.0 (4 rxn or 1 rxn)
  - DNA Control Complex**
    - Sequel II DNA Internal Control Complex 3.2
  - Movie Time per SMRT Cell**
    - 30 hrs
- For PureTarget samples, specify **Use Pre-extension = NO** (default)

Example sample information entered into a Sequel Ii system run design worksheet for a PureTarget repeat expansion panel library sample.

<sup>1</sup> **SMRTbell Adapter Design** field determines which adapter finding algorithm is used during post-primary analysis.

<sup>2</sup> **Note:** This '5000 bp' value only serves as a **placeholder** since the **actual average insert size distribution of PureTarget repeat expansion samples may vary.**

<sup>3</sup> **Note:** This '85 pM' value only serves as a placeholder since the actual loading concentration of PureTarget repeat expansion samples may vary.

<sup>4</sup> Specify **Use Pre-extension = No** to enable optimal sequencing performance for PureTarget repeat expansional panel samples.

# SMRT Link Run Design procedure for PureTarget libraries – Sequel IIe system

## Advanced options

- For PureTarget library samples, leave the following **Advanced Options** fields at their **default settings**
  - Use Adaptive Loading**  
→ NO
  - Maximum Loading Time**  
→ 4 hours
  - CCS Analysis Output - Include Low Quality Reads**  
→ YES
  - CCS Analysis Output - Include Kinetics Information**  
→ YES
- If desired, specify to use an alternative project folder for the **Add Data to Project** field

Advanced Options

Use Adaptive Loading  YES  NO

Immobilization Time (hrs) 4

CCS Analysis Output - Include Low Quality Reads  YES  NO

CCS Analysis Output - Include Kinetics Information  YES  NO

Add Data to Project

**IMPORTANT!** For Sequel II/Ile system PureTarget samples, specify **Use Adaptive Loading = NO<sup>1</sup>**

**IMPORTANT!** For Sequel II/Ile system PureTarget samples, specify **Include Low Quality Reads = YES<sup>2</sup>**

**IMPORTANT!** For Sequel II/Ile system PureTarget samples, specify **Include Kinetics Info = YES<sup>3</sup>**

Example default Advanced Options settings entered into a Sequel IIe system run design worksheet for a PureTarget repeat expansion panel library sample.

<sup>1</sup> Post-Cas9 digestion & cleanup yields typically range from ~50% to ~100% (1 – 2 µg) per sample when using supported genomic DNA types for PureTarget library construction.

<sup>2</sup> For PureTarget repeat expansion panel samples, low quality reads are saved by default to enable improved sensitivity for genotyping expanded tandem repeat alleles.

<sup>3</sup> Since low quality reads are saved by default for PureTarget libraries, 5mC detection cannot be performed on-instrument for Sequel IIe systems and, therefore, kinetics information must be saved in the CCS analysis output file to enable base modification detection analysis to be performed in SMRT Link.

# SMRT Link Run Design procedure for PureTarget libraries – Sequel IiE system

## Barcoded sample options

- For PureTarget library samples, can leave most **Barcoded Sample Options** fields at their **default settings**

Specify Bio Sample Names, either interactively or by downloading a CSV file (**Interactively** or **From a file**)

Optionally specify to perform barcode demultiplexing in **SMRT Link<sup>1</sup>** (Default)

Barcoded Sample Options

Sample Is Barcoded  YES  NO

Barcode Set Required SMRTbell adapter indexes

Same Barcodes on Both Ends of Sequence ?  YES  NO

Assign Bio Sample Names to Barcodes ? Required Interactively From a File

Demultiplex Barcodes  ON INSTRUMENT  IN SMRT LINK  DO NOT GENERATE

Example default Barcoded Sample Options settings entered into a Sequel IiE system run design worksheet for a PureTarget repeat expansion panel library sample.



# SMRT Link Run Design analysis options for **Revio system** and **Sequel II/Ie systems**

**Analysis Options**

Add Analysis  YES  NO

Analysis Name Required: PureTarget\_demo\_analysis\_job\_name

Select Analysis Workflow Required: PureTarget repeat expansion

Reference Genome Required: Human Genome hg38, with Gencode v39 annotations

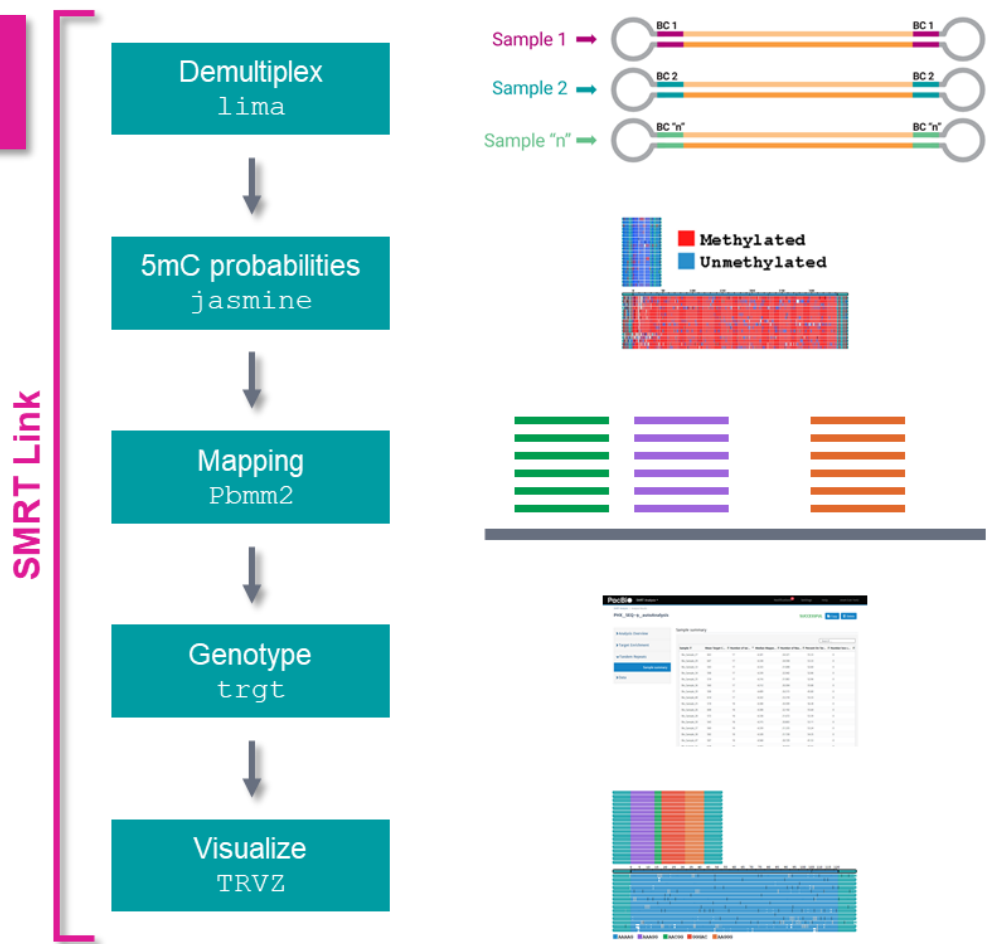
Target and repeat definitions: PureTarget repeat expansion panel

[Advanced Parameters](#)

Default = YES for Add Analysis

Analysis Workflow is automatically filled in (Default = PureTarget repeat expansion)

## PureTarget repeat expansion analysis application



- Analyze multiplexed PureTarget panel samples using [PureTarget repeat expansion analysis application](#)<sup>1</sup>
- The analysis produces **target enrichment summary statistics** and uses the tandem repeat genotyping tool (**TRGT**) for variant calling and visualization

<sup>1</sup> Users have two options for analysis when setting up sequencing runs in SMRT Link. For the fastest turnaround time and seamless analysis, users can include the PureTarget repeat expansion 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 do automatic demultiplexing only. Demultiplexed BAM files may then be transferred for command line analysis starting at the mapping step..

<sup>2</sup> See *SMRT Link User Guide* ([Documentation](#)) for detailed descriptions of parameter settings for PureTarget repeat expansion analysis application.

# SMRT Link Run Design analysis options for **Revio system** and **Sequel II/Ie systems**

**Analysis Options**

Add Analysis  YES  NO

Analysis Name  
Required PureTarget\_demo\_analysis\_job\_name

Select Analysis Workflow  
Required PureTarget repeat expansion

Reference Genome Required Human Genome hg38, with Gencode v39 annotations

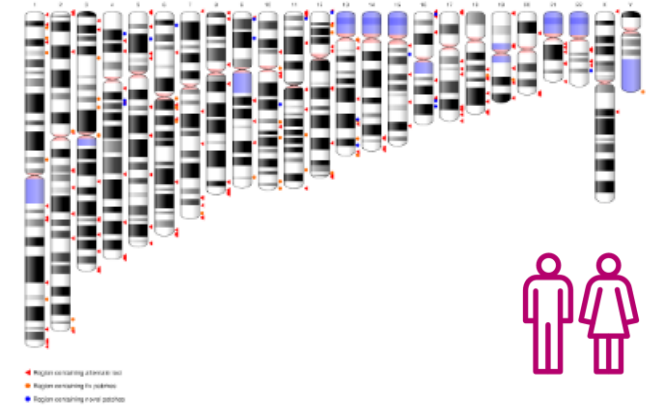
Target and repeat definitions  
PureTarget repeat expansion panel

Advanced Parameters

**Default Reference Genome = Human Genome hg38**

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

## Reference genome specification



## pbmm2

Sequence alignment program for aligning PacBio sequencing data against a reference database

# SMRT Link Run Design analysis options for **Revio system** and **Sequel II/Ie systems**

▼ Analysis Options

Add Analysis  YES  NO

Analysis Name Required

Select Analysis Workflow Required

Reference Genome Required

**Target and repeat definitions**

**Specify Target and repeat definitions BED dataset**

## Target and repeat definitions specification



\*.PureTarget\_repeat\_expansion\_panel.bedset.xml

### Example tandem repeat definition

```
chr4 3074876 3074966 ID=HTT,MOTIFS=CAG,CCG;STRUC=(CAG)nCAACAG(CCG)n
```

- Repeat region has coordinates chr4:3074876-3074966
- Identifier is HTT
- Region contains two tandem repeats with motifs CAG and CCG and these tandem repeats are expected to be separated by a short interrupting sequence CAACAG



**TRGT**

Tandem repeat genotyping tool  
for PacBio sequencing data

- Specify a target and repeat definition (browser extensible data) BED dataset
  - The default set is [PureTarget repeat expansion panel](#)
- Only reads that map within the target regions in the BED file are included in the analysis
  - To “in-silico” mask data from targets included in the 20 gene Repeat Expansion panel, create a new BED dataset **without** that target
- To analyze data from a custom panel, create a new BED dataset with those additional targets
  - Note that this analysis workflow will **only** work for tandem repeat regions
  - See [TRGT documentation](#) in [GitHub](#) for more information on the required BED file format for tandem repeats

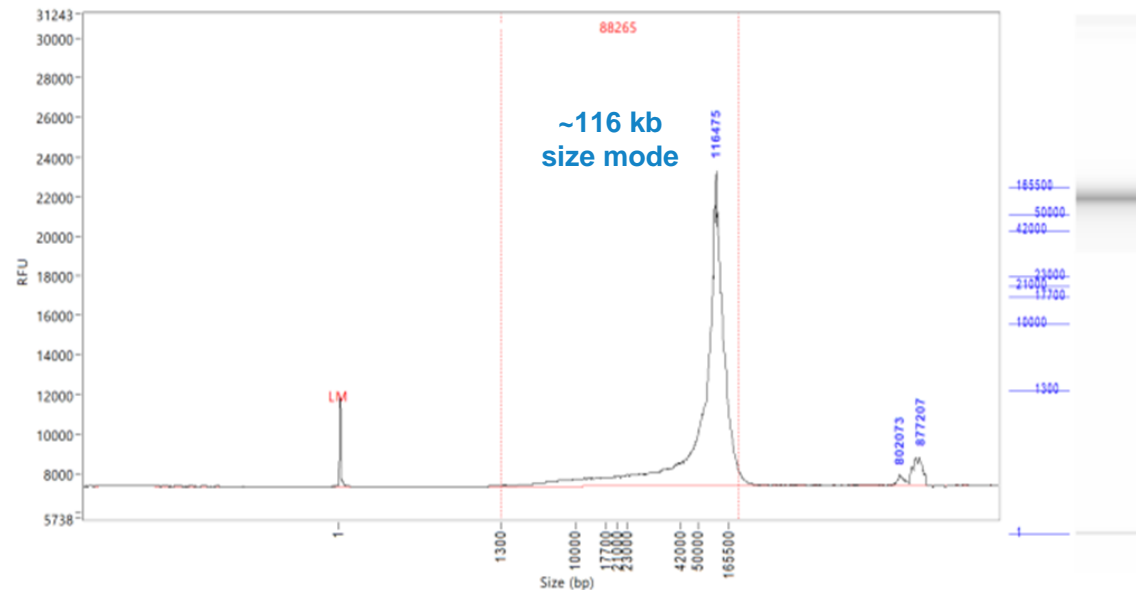


# PureTarget example sequencing performance data

# Example PureTarget repeat expansion panel library preparation QC results

Multiplexed PureTarget library prepared with genomic DNA isolated from human blood 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	Yield
Starting input genomic DNA	2,000 ng per sample
Post-Cas9 digestion & cleanup yield <sup>1</sup>	1,340 ng (67%) per sample
Post-Adapter ligation & cleanup yield <sup>2</sup>	960 ng (48%) per sample
Post-nuclease treatment & final library cleanup yield <sup>3</sup>	10 ng (0.063%) per 8-plex

Example library preparation step yield results for PureTarget repeat expansion library prepared from a human whole blood sample.

Final PureTarget library yield is typically sufficient to load **1 SMRT Cell**

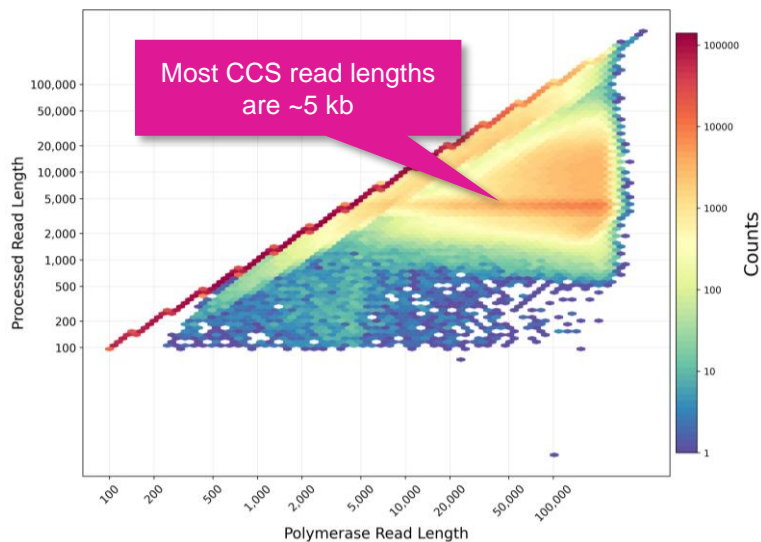
<sup>1</sup> Post-Cas9 digestion & cleanup yields typically range from ~50% to ~100% (1 – 2 µg) per sample when using supported genomic DNA types for PureTarget library construction.  
<sup>2</sup> Post-Adapter ligation & cleanup yields typically range from ~50% to ~100% (1 – 2 µg) per sample when using supported genomic DNA types for PureTarget library construction.  
<sup>3</sup> Post-nuclease treatment & final cleanup yields typically range from ~0.02% to ~0.5% (3 – 80 ng) per 8-plex when using supported genomic DNA types for PureTarget library construction.



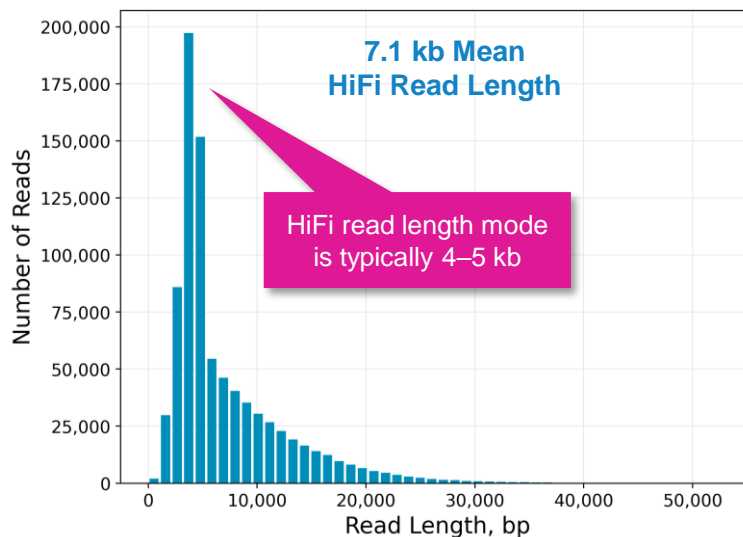
# Example sequencing performance for 48-plex PureTarget repeat expansion panel library prepared from human blood samples (Revio system)

48-plex PureTarget repeat expansion panel Revio system example data<sup>1</sup>

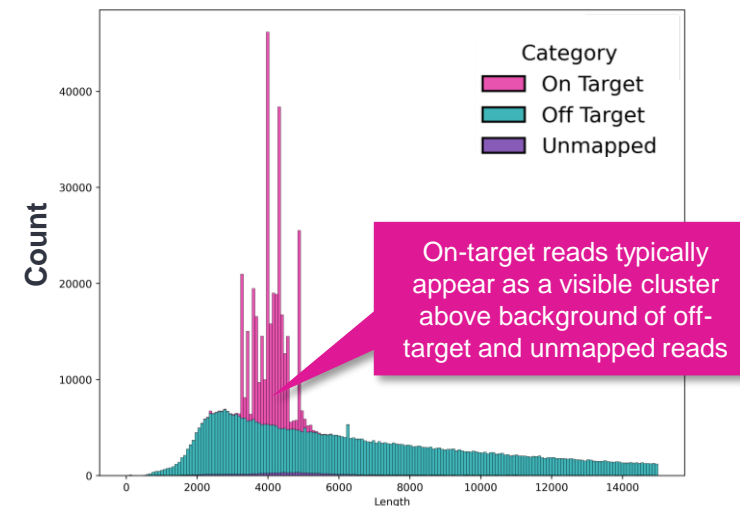
## Raw Data Report



## HiFi Read Length



## Target Enrichment Summary Metrics



Raw Base Yield	161 Gb
Mean Polymerase Read Length	18.0 kb
P0	64%
P1	36%
P2	1%

Example metrics for a 48-plex PureTarget repeat expansion panel library run on a Revio system with Revio polymerase kit using a 24-hrs movie time. Revio system *P1* range for 48-plex PureTarget repeat expansion panel libraries was typically ~25%–38%.

HiFi Reads	838.2 K
HiFi Base Yield	6.0 Gb
Mean HiFi Read Length	7.1 kb
Median HiFi Read Quality	Q38
HiFi Read Mean # of Passes	21

For 48-plex PureTarget repeat expansion panel libraries, per-Revio SMRT Cell HiFi read counts were typically ~0.8 M–1.0 M depending on the final library insert size and *P1* loading performance.

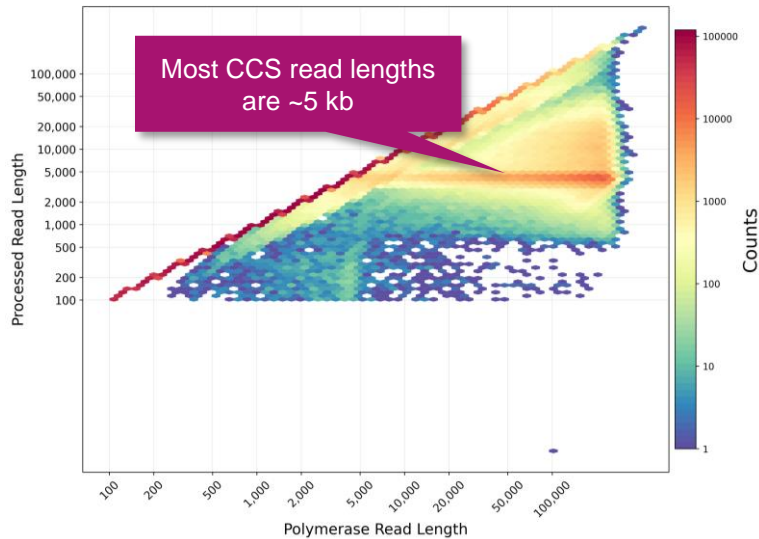
Total Bases*	6,547,987,725
Total Reads*	886,509
Median Read Length*	4,855 bp
Median Read Quality*	Q36
Sample Count	48
Target Regions	20

\* Includes HiFi + non-HiFi data. For 48-plex PureTarget repeat expansion panel libraries, per-Revio SMRT Cell total read counts were typically ~0.8 M–1.0 M.

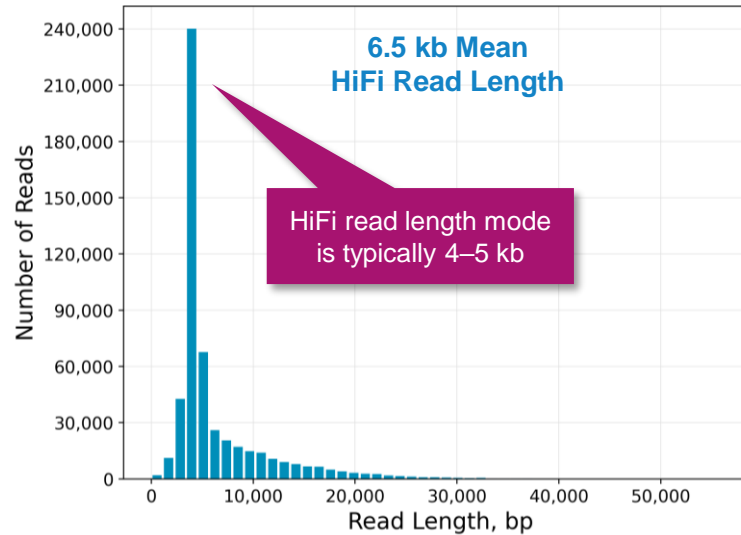
# Example sequencing performance for 24-plex PureTarget repeat expansion panel library prepared from human blood samples (Revio system)

24-plex PureTarget repeat expansion panel Revio system example data<sup>1</sup>

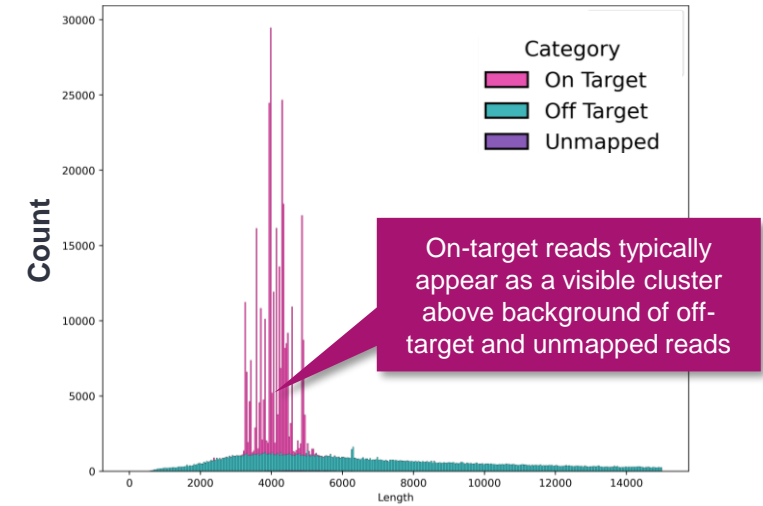
## Raw Data Report



## HiFi Read Length



## Target Enrichment Summary Metrics



Raw Base Yield	98 Gb
Mean Polymerase Read Length	16.1 kb
P0	75%
P1	24%
P2	0%

Example metrics for a 24-plex PureTarget repeat expansion panel library run on a Revio system with Revio polymerase kit using a 24-hrs movie time. Revio system *P1* range for 24-plex PureTarget repeat expansion panel libraries was typically ~15%–30%.

HiFi Reads	522.6 K
HiFi Base Yield	3.37 Gb
Mean HiFi Read Length	6.5 kb
Median HiFi Read Quality	Q43
HiFi Read Mean # of Passes	25

For 24-plex PureTarget repeat expansion panel libraries, per-Revio SMRT Cell HiFi read counts were typically ~0.3 M–0.5 M depending on the final library insert size and *P1* loading performance.

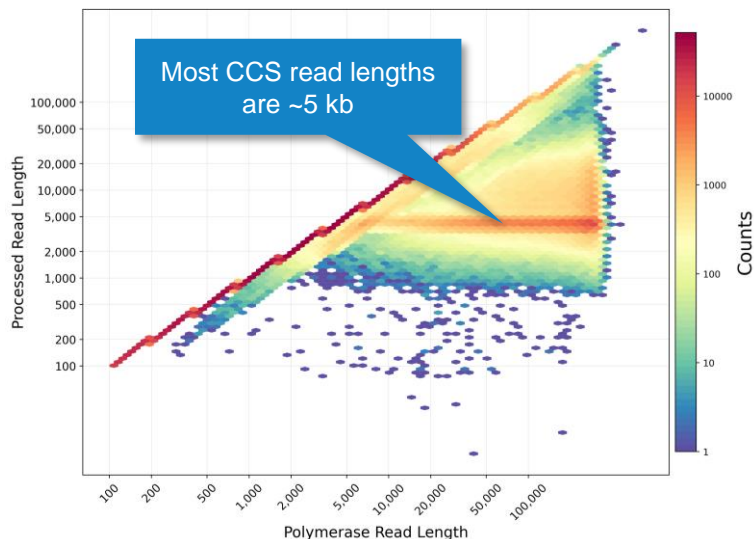
Total Bases*	3,648,915,399
Total Reads*	553,106
Median Read Length*	4,342 bp
Median Read Quality*	Q41
Sample Count	24
Target Regions	20

\* Includes HiFi + non-HiFi data. For 24-plex PureTarget repeat expansion panel libraries, per-Revio SMRT Cell total read counts were typically ~0.3 M–0.5 M.

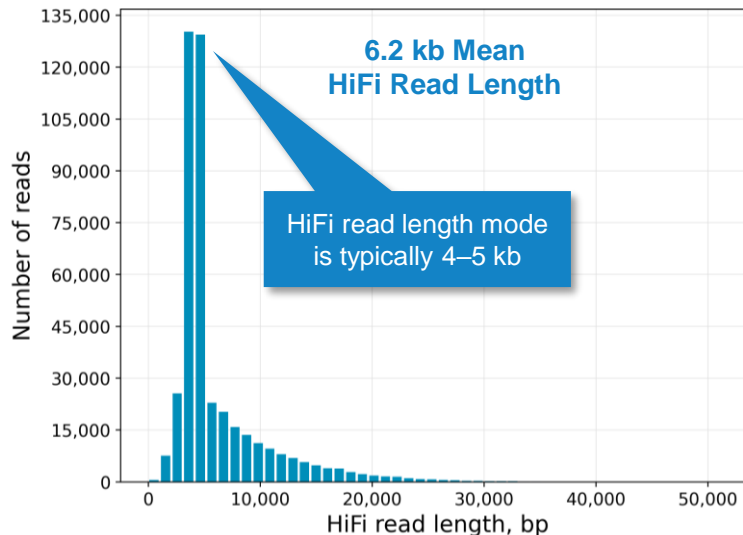
# Example sequencing performance for 24-plex PureTarget repeat expansion panel library prepared from human blood samples (Sequel IIe system)

24-plex PureTarget repeat expansion panel Sequel IIe system example data<sup>1</sup>

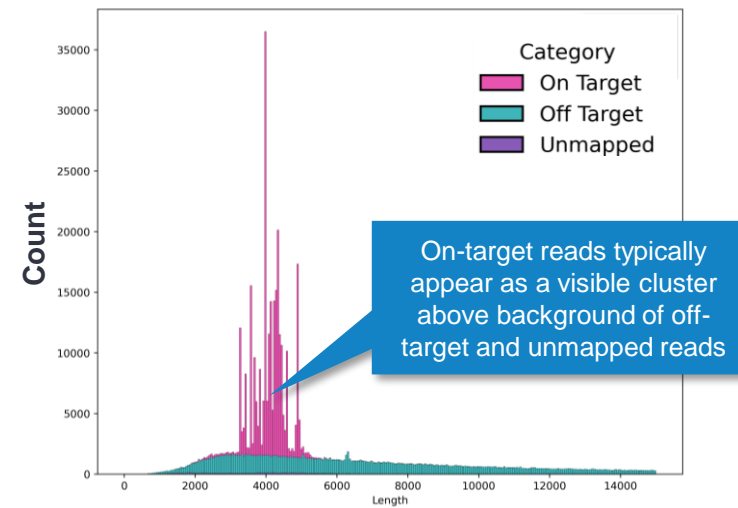
## Raw Data Report



## HiFi Read Length



## Target Enrichment Summary Metrics



Raw Base Yield	96 Gb
Mean Polymerase Read Length	29.0 kb
P0	56%
P1	41%
P2	2%

Example metrics for a 24-plex PureTarget repeat expansion panel library run on a Sequel IIe system with Sequel II binding kit 3.2 using a 30-hrs movie time. Sequel IIe system *P1* range for 24-plex PureTarget repeat expansion panel libraries was typically ~5%-50%.

HiFi Reads	435.8 K
HiFi Base Yield	2.7 Gb
Mean HiFi Read Length	6.2 kb
Median HiFi Read Quality	Q40
HiFi Read Mean # of Passes	18

For 24-plex PureTarget repeat expansion panel libraries, per-Sequel II SMRT Cell HiFi read counts were typically ~0.4 M–0.5 M depending on the final library insert size and *P1* loading performance.

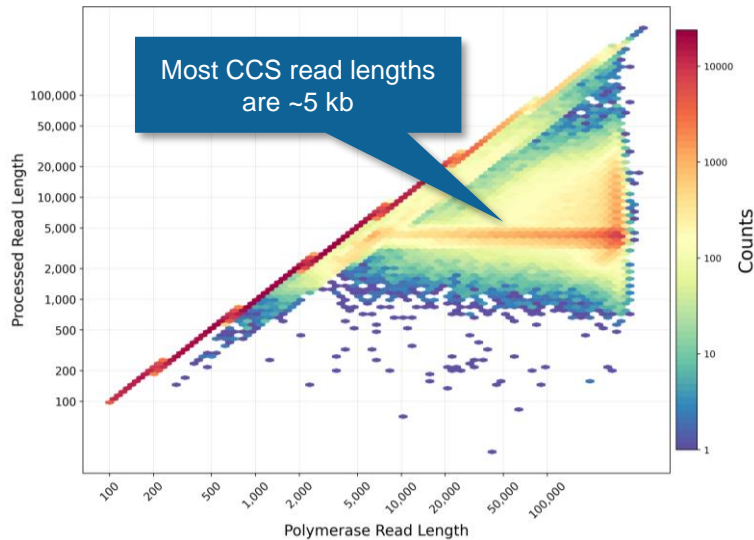
Total Bases*	3,679,524,534
Total Reads*	522,722
Median Read Length*	4,374 bp
Median Read Quality*	Q37
Sample Count	24
Target Regions	20

\* Includes HiFi + non-HiFi data. For 24-plex PureTarget repeat expansion panel libraries, per-Sequel II SMRT Cell total read counts were typically ~0.4 M–0.5 M.

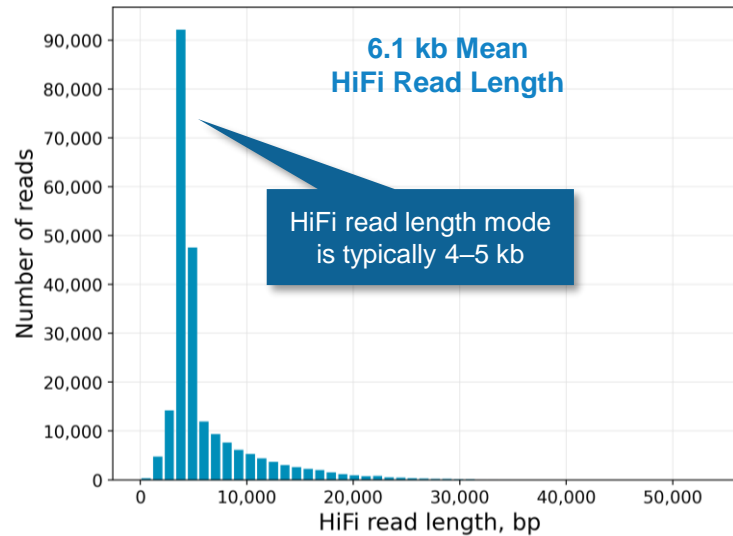
# Example sequencing performance for 8-plex PureTarget repeat expansion panel library prepared from human blood samples (Sequel IIe system)

8-plex PureTarget repeat expansion panel Sequel IIe system example data<sup>1</sup>

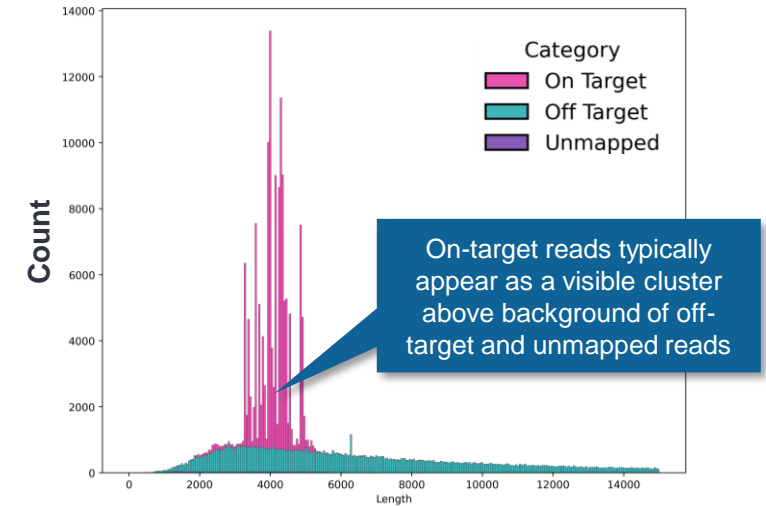
## Raw Data Report



## HiFi Read Length



## Target Enrichment Summary Metrics



Raw Base Yield	53 Gb
Mean Polymerase Read Length	31.3 kb
P0	78%
P1	21%
P2	1%

Example metrics for a 8-plex PureTarget repeat expansion panel library run on a Sequel IIe system with Sequel II binding kit 3.2 using a 30-hrs movie time. Sequel IIe system *P1* range for 8-plex PureTarget repeat expansion panel libraries was typically ~5%-35%.

HiFi Reads	225.4 K
HiFi Base Yield	1.4 Gb
Mean HiFi Read Length	6.1 kb
Median HiFi Read Quality	Q43
HiFi Read Mean # of Passes	20

For 24-plex PureTarget repeat expansion panel libraries, per-Sequel II SMRT Cell HiFi read counts were typically ~0.1 M–0.2 M depending on the final library insert size and *P1* loading performance.

Total Bases*	1,671,935,304
Total Reads*	255,625
Median Read Length*	4,325 bp
Median Read Quality*	Q41
Sample Count	8
Target Regions	20

\* Includes HiFi + non-HiFi data. For 24-plex PureTarget repeat expansion panel libraries, per-Sequel II SMRT Cell total read counts were typically ~0.1 M–0.2 M.

# Example coverage performance for PureTarget repeat expansional panel library (Revio system)

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

## DNA sample preparation

- 2 µg DNA per sample extracted from 24 human whole blood samples
- *Procedure & checklist – Extracting HMW DNA from human whole blood with RBC lysis using the Nanobind PanDNA kit* ([103-377-500](#))

## PureTarget library preparation

- 20-gene target panel library constructed using PureTarget repeat expansional panel kit (103-390-400)
- Multiplexed PureTarget library containing 24 human DNA samples

## Sequencing run design

- Revio system
- 24 hrs movie time
- No adaptive loading

## Coverage (per gene per sample)

- Mean = 387
- Max = 678
- Min = 74

Gene	Motif	Motif repeat number and PureTarget sequencing coverage			
		Allele 1	Coverage	Allele 2	Coverage
<i>ATN1</i>	CAG	19	207	17	236
<i>ATXN2</i>	GCT	22	240	23	234
<i>ATXN8</i>	CTA	9	271	11	254
<i>ATXN3</i>	GCT	16	204	20	232
<i>CACNA1A</i>	CTG	13	185	13	184
<i>ATXN10</i>	ATTCT	13	251	13	242
<i>ATXN7</i>	GCA	10	194	12	199
<i>PPP2R2B</i>	GCT	10	217	12	238
<i>ATXN1</i>	TGC	31	174	35	170
<i>TBP</i>	GCA	34	199	37	206
<i>FXN</i>	GAA	8	123	9	137
<i>DMPK</i>	CAG	13	163	19	203
<i>C9ORF72</i>	GGCCCC	5	184	5	165
<i>TCF4</i>	CAG	17	241	25	232
<i>PABPN1</i>	GCG	6	205	6	222
<i>AR</i>	GCA	21	421		
<i>RFC1</i>	AAAAG	10	94	10	94
<i>CNBP</i>	CAGG	15	258	15	257
<i>FMR1</i>	CGG	29	304	31	278
<i>HTT</i>	CAG	16	155	21	138

# Example coverage performance for PureTarget repeat expansional panel library (Revio system) (cont.)

On-target coverage results for selected repeat expansions in different human cell line samples

Gene		Motif	Motif repeat number and PureTarget sequencing coverage			
			Allele 1	X Coverage	Allele 2	X Coverage
<i>FXN</i>	NA14519	GAA	9	324	1048	243
<i>FMR1</i>	NA07537	CGG	29	462	336	475
<i>FMR1</i>	NA06968	CGG	33	157	113	56
<i>FXN</i>	NA16212	GAA	8	54	509	35
<i>PABPN1</i>	NA23629	GCG	6	363	9	397
<i>AR</i>	NA23709	GCA	48	194		
<i>C9orf72</i>	ND06751	GGCCCC	8	166	731	69

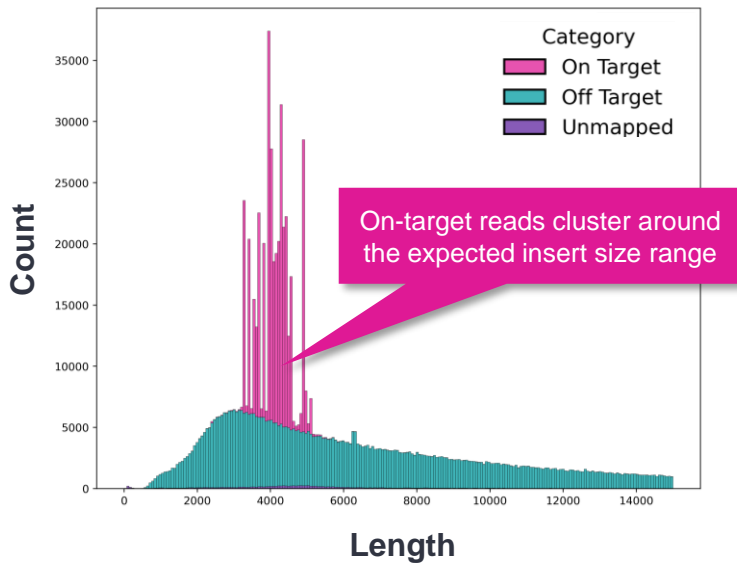
PureTarget repeat expansional panel kit can accurately detect expanded alleles containing >100 repeat expansions



# Recommended guidance for evaluating PureTarget repeat expansion panel sequencing run performance

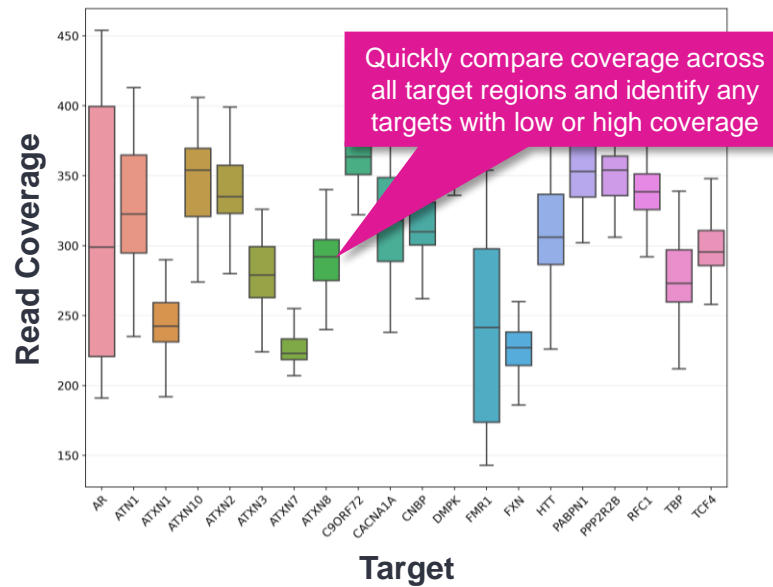
When evaluating PureTarget runs, it is generally more useful to examine the **secondary analysis results** (e.g., on-target coverage) since primary sequencing metrics like Productivity ( $P0$ ,  $P1$ ,  $P2$ ) are mostly dominated by 'background' non-targeted reads

### Read Categories<sup>1</sup>



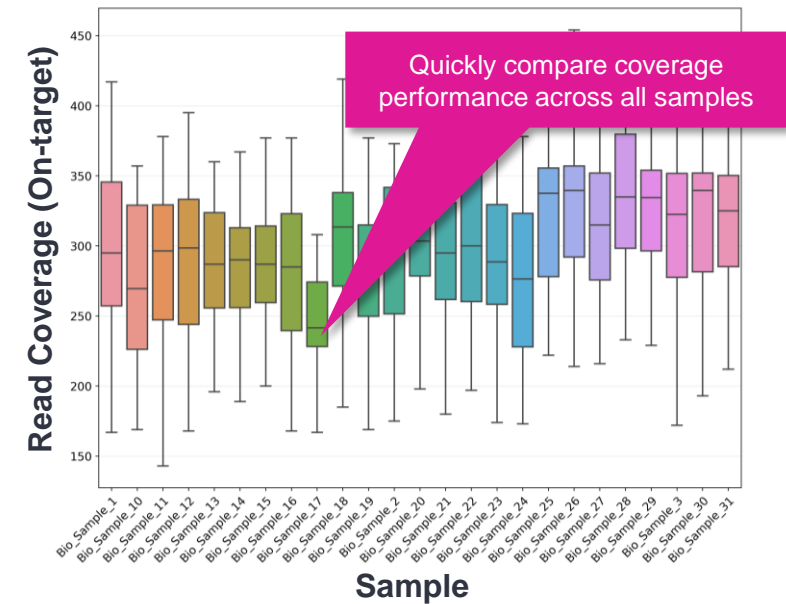
- Histogram mapping the length of On target, Off target, and Unmapped reads in the sample

### Target Coverage



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

### Sample Coverage



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

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

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

The screenshot shows the PacBio Run Details interface. The 'Overview' section displays run metadata such as 'Run Created', 'Run Start', 'Run Complete', 'Created By', 'Instrument Name', 'Completed Cells', 'Failed Cells', 'Time remaining for PostProcessing', 'Transfer Status', 'Run ID', 'Instrument SN', 'Instrument Control SW Version', 'Instrument Chemistry Build', and 'Primary SW Version'. Below this is a table of consumables. The 'Barcode Counts' section is highlighted with a pink box and shows a table with columns for Barcode ID, HiFi reads, and HiFi read length (mean, bp).

This section provides a detailed view of the Barcode Counts table. It includes filters for 'Well name' (PHX\_SEQ-12) and 'Time point' (23 hr). The table has three columns: 'Barcode ID', 'HiFi reads', and 'HiFi read length (mean, bp)'. A search bar is located at the top right. Three callouts provide definitions for the metrics:

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

Barcode ID	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
bc2006--bc2006	2.4%	6627
bc2007--bc2007	2.4%	6063
bc2008--bc2008	1.2%	8257
bc2009--bc2009	1.2%	6463
bc2010--bc2010	4.7%	10685
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

# 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**

PHX\_SEQ-12-Cell1 (all samples)

Copy Analyze Export Delete

Dataset Overview

Run Preview

Run Preview

CCS Analysis Report

5mC CpG Report

Adapter Report

Loading Report

Raw Data Report

Control Report

Barcodes

Summary Metrics

**Barcode Data**

Barcoded Read Statistics

Barcoded Read Binned Histograms

Analyses

Data

Sample Name	Barcode	Barcode Qual.	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 3	bc2003--bc20...	97.7	25,236	6,484	Q38	163,630,897	120,873
Bio Sample 4	bc2004--bc20...	96.0	23,954	6,630	Q38	158,825,582	121,598
Bio Sample 5	bc2005--bc20...	97.0	24,809	6,534	Q38	162,760,887	121,678
Bio Sample 6	bc2006--bc20...	97.7	23,360	6,734	Q38	152,786,356	121,771
Bio Sample 7	bc2007--bc20...	97.7	23,360	6,605	Q38	141,095,083	120,761
Bio Sample 8	bc2008--bc20...	97.5	22,467	6,662	Q38	158,100,389	122,254
Bio Sample 9	bc2009--bc20...	97.4	27,277	6,828	Q38	186,250,674	121,427
Bio Sample 10	bc2010--bc20...	97.3	22,084	6,641	Q38	146,668,330	120,977
Bio Sample 11	bc2011--bc20...	97.1	22,351	6,539	Q38	146,158,985	120,805
Bio Sample 12	bc2012--bc20...	97.6	21,407	6,594	Q39	141,171,468	121,334
Bio Sample 13	bc2013--bc20...	97.4	22,347	6,675	Q39	147,845,367	121,793
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 25	bc2025--bc20...	97.5	14,985	6,874	Q39	100,264,900	120,846
Bio Sample 26	bc2026--bc20...	97.4	15,678	7,042	Q39	110,418,581	121,313
Bio Sample 27	bc2027--bc20...	96.3	14,540	7,060	Q39	102,663,839	122,298
Bio Sample 28	bc2028--bc20...	97.9	14,674	6,879	Q39	100,869,814	121,259
Bio Sample 29	bc2029--bc20...	96.8	16,168	7,189	Q39	116,341,083	121,688
Bio Sample 30	bc2030--bc20...	97.5	15,112	7,117	Q39	107,556,363	121,329
Bio Sample 31	bc2031--bc20...	97.4	15,283	7,312	Q39	111,762,389	122,442
Bio Sample 32	bc2032--bc20...	97.3	14,740	7,377	Q39	105,492,469	121,769
Bio Sample 33	bc2033--bc20...	97.3	15,320	6,873	Q39	106,839,105	121,794
Bio Sample 34	bc2034--bc20...	97.4	15,868	7,034	Q39	111,621,343	121,645
Bio Sample 35	bc2035--bc20...	97.3	15,809	7,117	Q39	112,514,643	122,392
Bio Sample 36	bc2036--bc20...	97.5	14,012	7,077	Q39	99,766,762	121,131
Bio Sample 37	bc2037--bc20...	97.4	15,376	7,244	Q39	111,695,520	122,290
Bio Sample 38	bc2038--bc20...	97.6	14,470	7,444	Q39	103,382,534	122,633
Bio Sample 39	bc2039--bc20...	97.5	15,277	7,286	Q39	111,316,012	121,982
Bio Sample 40	bc2040--bc20...	96.7	15,195	7,323	Q39	111,281,396	120,718
Bio Sample 41	bc2041--bc20...	97.2	13,970	6,889	Q39	93,224,058	120,817
Bio Sample 42	bc2042--bc20...	97.5	12,714	6,726	Q39	85,516,208	120,429
Bio Sample 43	bc2043--bc20...	97.5	14,753	7,045	Q39	103,935,396	121,444
Bio Sample 44	bc2044--bc20...	97.6	15,508	7,169	Q39	111,183,795	121,566
Bio Sample 45	bc2045--bc20...	97.6	13,848	7,076	Q39	98,001,581	120,904
Bio Sample 46	bc2046--bc20...	97.3	14,362	7,107	Q39	102,074,985	121,586
Bio Sample 47	bc2047--bc20...	97.3	15,416	7,148	Q39	110,509,529	121,808
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

**PacBio Data Management**

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

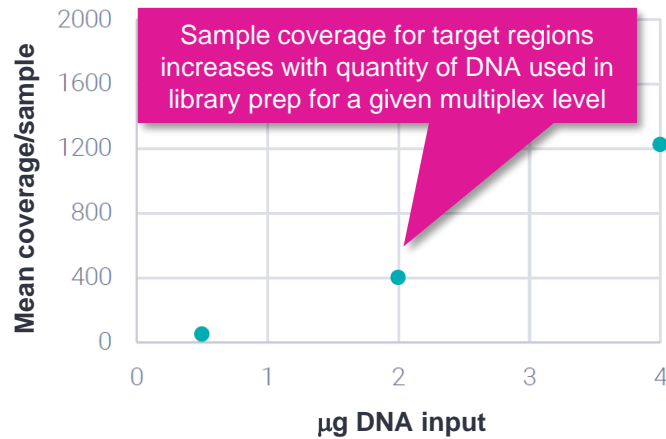
Data

Sample Name	Barcode	Barcode Qua...	HiFi Reads	HiFi Read Le...	HiFi Read Q...	HiFi Yield (bp)	Polymerase ...
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Bio Sample 11	bc2011--bc20...	97.1	22,351	6,539	Q38	146,158,985	120,805
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Bio Sample 13	bc2013--bc20...	97.4	22,347	6,675	Q39	147,845,367	121,793
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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<sup>1</sup>

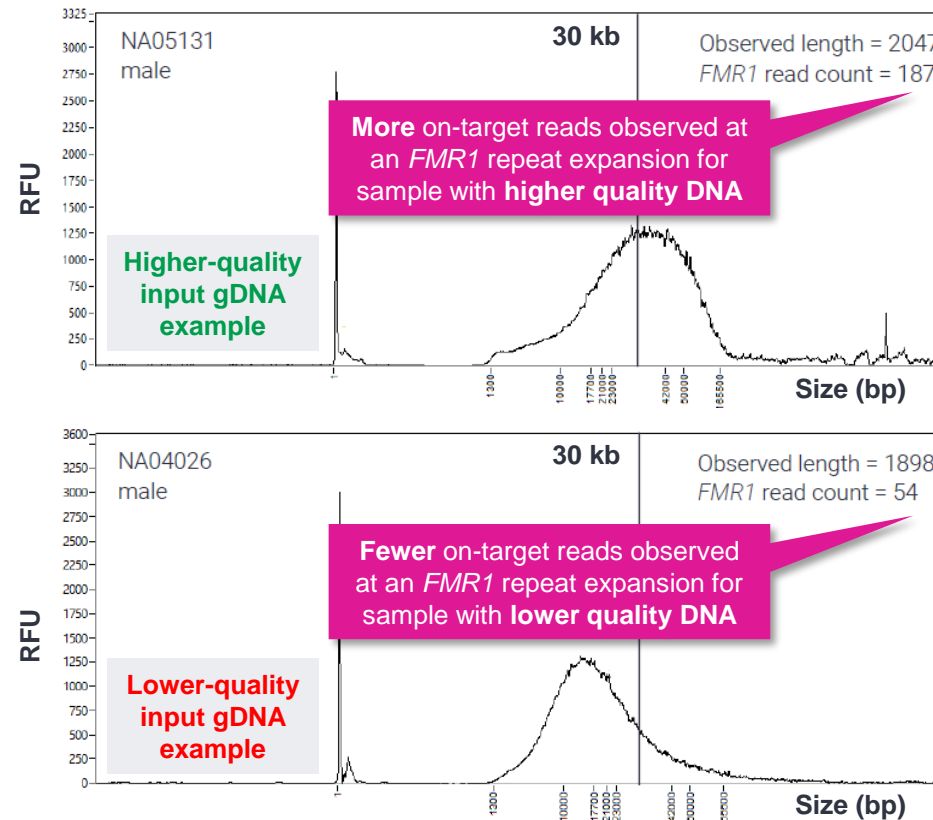
## DNA Input Quantity vs. Coverage



**DNA input quantity versus coverage.** DNA was extracted from whole blood using the Nanobind PanDNA kit and run in an 8-plex.

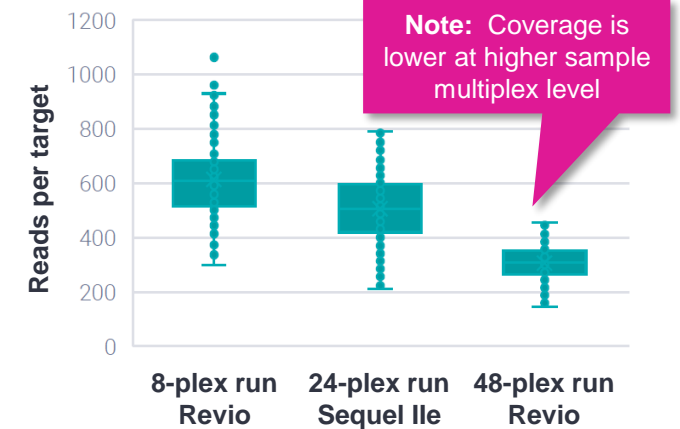
- PureTarget libraries do not use amplification to enrich targets but rather retain targets of interest and deplete off-target molecules
- As such, the library quantity loaded on the SMRT Cell is lower than other library types like WGS and the **sequencing yield for a sample can be influenced by how much starting DNA is used in the library prep**

## Impact of Input Genomic DNA Quality on Coverage



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

## Sample Multiplex Level vs. Coverage



**Sample multiplex level versus coverage.** Samples were prepared from 2.0–2.5 µg of high molecular weight (HMW) DNA extracted with Nanobind PanDNA from whole blood.

- Deep coverage is achieved across 20 panel targets for 8-plex through 48-plex experimental designs



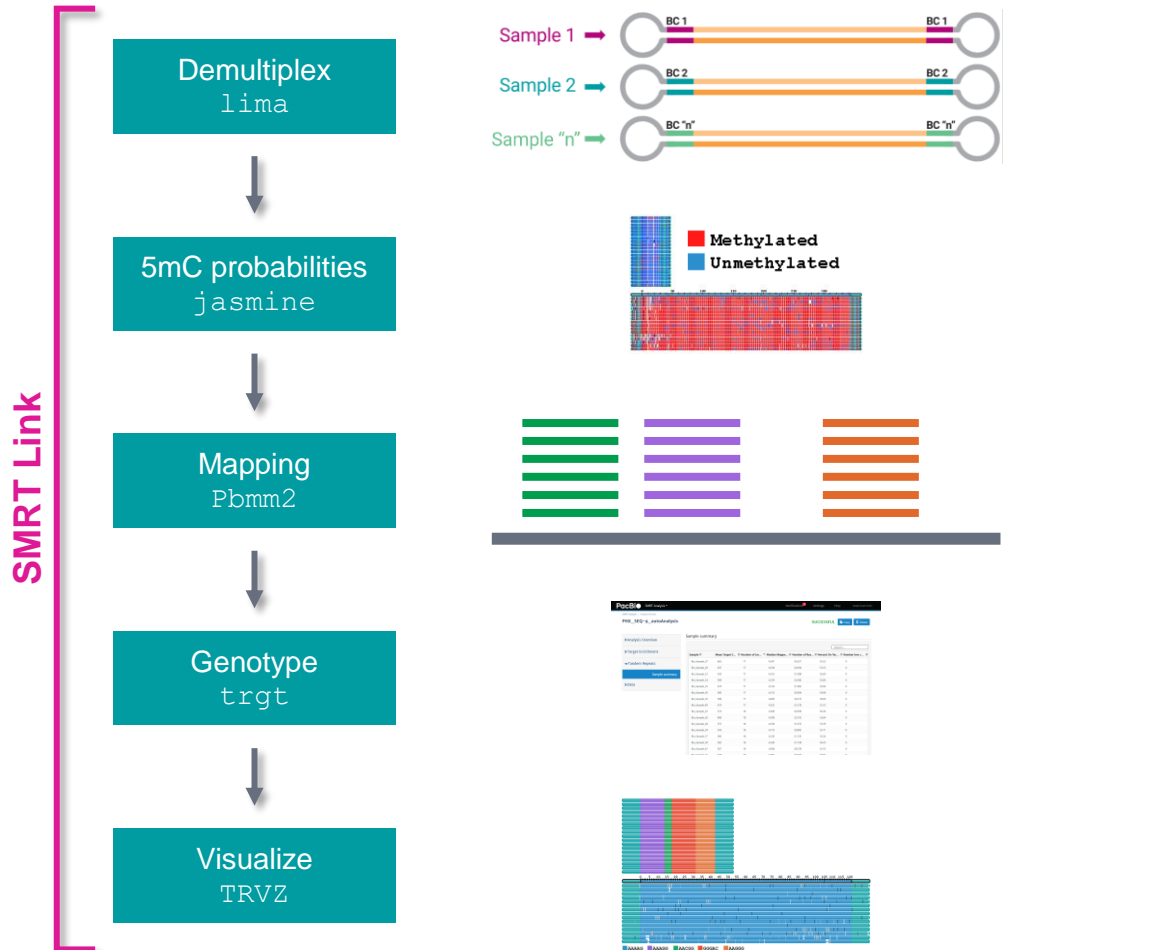
# PureTarget data analysis workflow overview



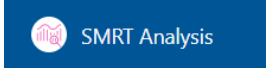
# PureTarget data analysis workflow overview

Analysis of PureTarget repeat expansion libraries can be performed in SMRT Link using the PureTarget repeat expansion analysis workflow or at the command line<sup>1</sup>

## SMRT Link PureTarget bioinformatics workflow<sup>2</sup>



SMRT Link v13.1+



### Demultiplex

- Samples are demultiplexed using `lima`

### 5mC probabilities

- 5mC methylation probabilities for CpG sites are called with `jasmine`

### Mapping

- Reads are mapped to the hg38 reference genome with `pbmm2`

### Genotype

- Repeat genotypes are called with `TRGT`

### Visualize

- Visualizations are produced with `TRVZ`.



# PureTarget data analysis workflow overview (cont.)

## File Downloads tab

Edit Output File Name Prefix **Example:**analysis-[multiple]-2928

File ↑	Size ↓↑	Type
Analysis Log		log
Input - Target BED file used in run	2 KB	unknown
QC - Target Genotype Table	68 KB	csv
SMRT Link Log	28 KB	log
TRGT - BAM (input for TRVZ)	14 MB	zip
TRGT - VCF files	194 KB	zip
TRVZ - all target plots (meth; allele)	39 MB	zip
TRVZ - all target plots (meth; waterfall)	38 MB	zip
TRVZ - all target plots (motifs; allele)	22 MB	zip
TRVZ - all target plots (motifs; waterfall)	26 MB	zip
Tandem Repeats	8 KB	JsonReport

- **QC – Target Genotype Table**
  - CSV file containing repeat unit sequence, allele count, min/max/consensus repeat array length, motif count, and motif span information

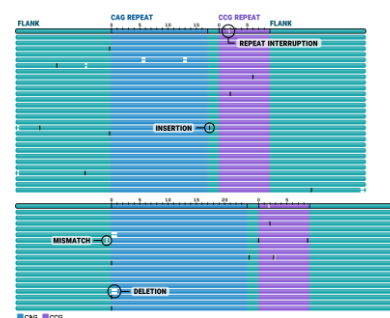
Sample	Bio_Sample_1
CNBP repeat unit	CAGG:CAGA:CA
CNBP read count allele 0	146
CNBP consensus size allele 0	139
CNBP min size allele 0	129
CNBP max size allele 0	317
CNBP motif counts allele 0	15_8_23
CNBP motif spans allele 0	0(0-61)_1(61-93)_2(93-139)

- **TRVZ - all target plots (meth, allele):**
  - Depicts consensus repeat alleles and reads aligning to them. Bases in repeats are colored by methylation levels.
- **TRVZ - all target 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.
- **TRVZ - all target plots (motifs, allele):**
  - Depicts consensus repeat alleles and reads aligning to them. Bases in repeats are colored by repeat motif.
- **TRVZ - all target plots (motifs, 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.

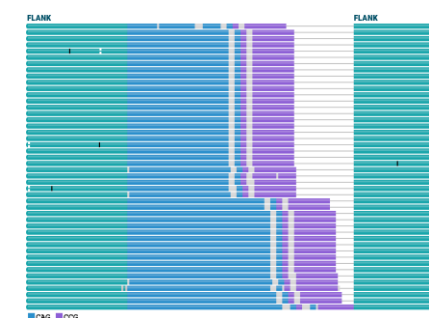
### TRVZ is a companion visualization program for TRGT

- 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.
- **Color code repeat structure** on allele and waterfall plots
- **Display 5mCpG methylation levels** on allele and waterfall plots

#### Allele plot



#### Waterfall plot





# 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](#))
- Brochure – Comprehensive genotyping with PureTarget repeat expansion panel ([102-326-609](#))
- Procedure & checklist – Generating PureTarget repeat expansion panel libraries ([103-329-400](#))
- Technical overview – PureTarget repeat expansion panel library preparation using PureTarget kit ([103-418-100](#))

## Data analysis resources

- Application note – Analysis guide for PureTarget repeat expansion panel ([102-326-616](#))
- SMRT Link software installation guide [ [Link](#) ]
- SMRT Link user guide [ [Link](#) ]
- SMRT Tools reference guide [ [Link](#) ]

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

## Publications and posters

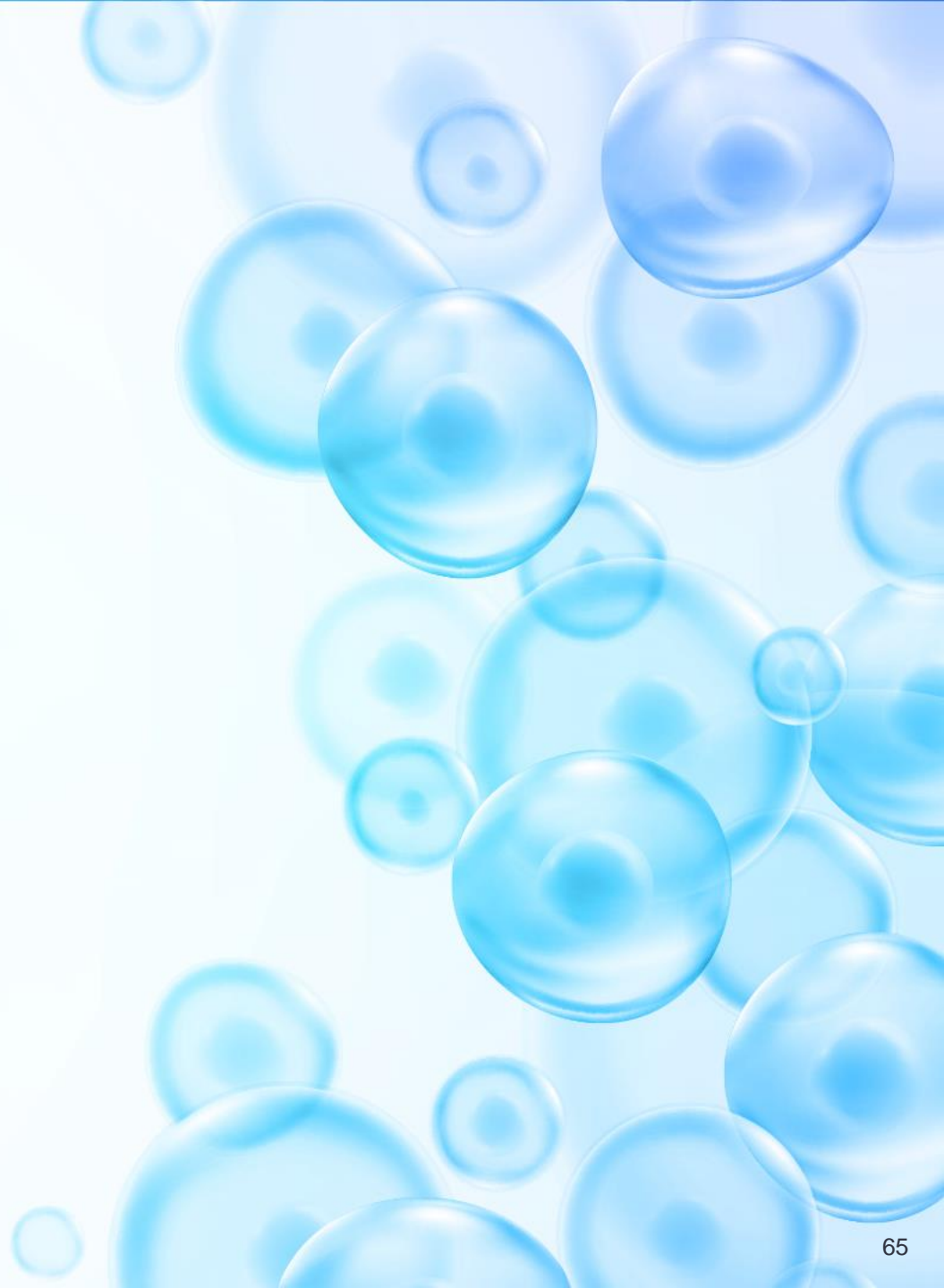
- ACMG 2024 Poster abstract - Fuligni, F. et al. (2024) Multiplex detection and quantification of neurological disease-associated repeat expansions using the PacBio Sequel IIe Platform. Genetics in Medicine Open. [ [DOI](#) ]

## Example PacBio data sets

Application	Dataset	Data type	PacBio system
PureTarget repeat expansion panel	Repeat expansion panel Coriell samples [ <a href="#">Link</a> ]	HiFi long read	Sequel IIe system
	Repeat expansion panel HG001 and HG002 48-plex [ <a href="#">Link</a> ]	HiFi long read	Revio system
	Repeat expansion panel HG001 and HG002 8-plex [ <a href="#">Link</a> ]	HiFi long read	Sequel IIe system

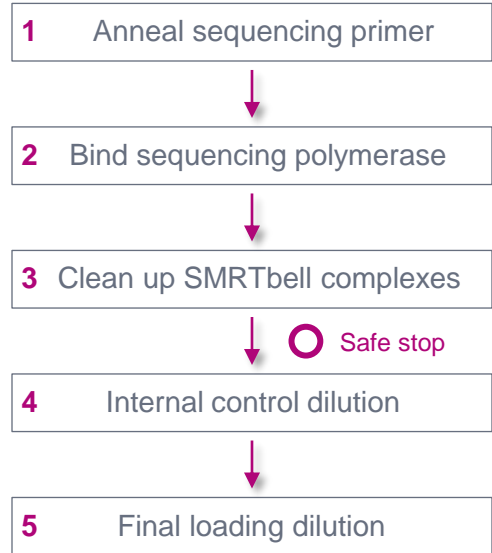


# Appendix



# Sample setup workflow overview for PureTarget libraries – Sequel II/Ile system

Follow sample setup instructions for PureTarget libraries in **Procedure & checklist – Generating PureTarget repeat expansion panel libraries** (103-329-400) – Do not use SMRT Link Sample Setup



- Use **entire volume (15 µL)** of pooled SMRTbell templates per ABC reaction
- **Note:** Always use the fixed reagent volumes shown at each ABC step for each pooled batch

## Sequel II/Ile system ABC workflow for PureTarget repeat expansion panel samples

Follow **Procedure & checklist 103-329-400** for ABC instructions

8, 16 or 24 samples



**ABC<sup>1</sup>**  
(Pool of 8/16/24 samples in 15 µL)

Step	Instructions										
11b.1	<p><b>Note:</b> Always use these values for each pooled batch. Do not adjust based on measured concentration, value, or plex level of batch.</p> <p>Annealing Sequencing primer</p> <p>Combine the following components in a new low-binding tube and pipette to mix.</p> <table border="1"><tr><td># of Samples in Batch</td><td>8 or 16 or 24</td></tr><tr><td>Sample (SMRTbell templates)</td><td>15 µL</td></tr><tr><td>Annealing Buffer</td><td>7.5 µL</td></tr><tr><td>Sequel II Primer 3.2</td><td>7.5 µL</td></tr><tr><td>Total volume</td><td>30 µL</td></tr></table> <p>Incubate at room temperature for 15 minutes then proceed to the next step.</p>	# of Samples in Batch	8 or 16 or 24	Sample (SMRTbell templates)	15 µL	Annealing Buffer	7.5 µL	Sequel II Primer 3.2	7.5 µL	Total volume	30 µL
# of Samples in Batch	8 or 16 or 24										
Sample (SMRTbell templates)	15 µL										
Annealing Buffer	7.5 µL										
Sequel II Primer 3.2	7.5 µL										
Total volume	30 µL										

**NOTE:**  
**ALWAYS USE THESE FIXED VOLUMES FOR EACH POOLED BATCH**  
→ **Do not adjust reagent volumes** based on measured sample concentration value or plex level of batch

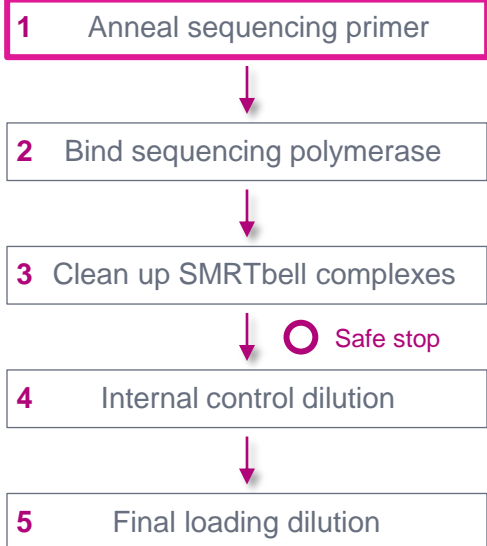


Sequence pooled samples on one Sequel II SMRT Cell



# Sample setup procedure for PureTarget libraries – Sequel II/Ile system

## Anneal sequencing primer



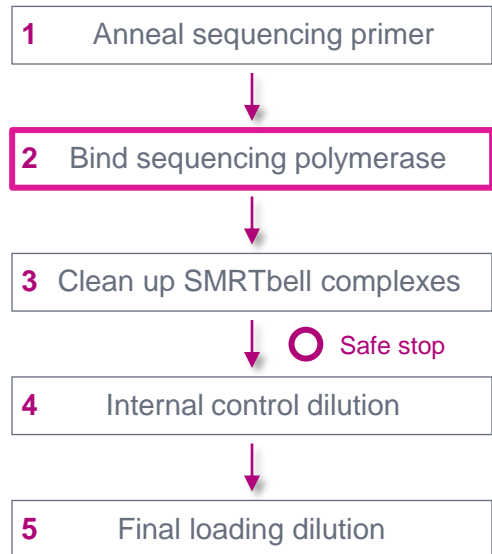
## Anneal sequencing primer

✓ Step	Instructions										
	<b>Note:</b> Always use these values for each pooled batch. Do not adjust based on measured concentration, value, or plex level of batch.										
	<b>Annealing Sequencing primer</b> Combine the following components in a new low-binding tube and pipette to mix.										
11b.1	<table border="1"><tbody><tr><td># of Samples in Batch</td><td>8 or 16 or 24</td></tr><tr><td>Sample (SMRTbell templates)</td><td>15 <math>\mu</math>L</td></tr><tr><td>Annealing Buffer</td><td>7.5 <math>\mu</math>L</td></tr><tr><td>Sequel II Primer 3.2</td><td>7.5 <math>\mu</math>L</td></tr><tr><td>Total volume</td><td>30 <math>\mu</math>L</td></tr></tbody></table>	# of Samples in Batch	8 or 16 or 24	Sample (SMRTbell templates)	15 $\mu$ L	Annealing Buffer	7.5 $\mu$ L	Sequel II Primer 3.2	7.5 $\mu$ L	Total volume	30 $\mu$ L
# of Samples in Batch	8 or 16 or 24										
Sample (SMRTbell templates)	15 $\mu$ L										
Annealing Buffer	7.5 $\mu$ L										
Sequel II Primer 3.2	7.5 $\mu$ L										
Total volume	30 $\mu$ L										
	Incubate at room temperature for 15 minutes then proceed to the next step.										

**NOTE:**  
**ALWAYS USE THESE FIXED VOLUMES FOR EACH POOLED BATCH**  
→ Do not adjust reagent volumes based on measured sample concentration value or plex level of batch

# Sample setup procedure for PureTarget libraries – Sequel II/Ile system (cont.)

## Bind sequencing polymerase



### Dilute sequencing polymerase

Step	Instructions								
11b.2	Combine the following components in a single low-bind tube and pipette to mix. The prepared volume of diluted polymerase is sufficient to process all specified samples in a batch.								
	<table border="1"><thead><tr><th># of Samples in Batch</th><th>8 or 16 or 24</th></tr></thead><tbody><tr><td>Polymerase Stock</td><td>3.5 <math>\mu</math>L</td></tr><tr><td>Sequel II polymerase dilution buffer</td><td>26.5 <math>\mu</math>L</td></tr><tr><td>Total Volume</td><td>30 <math>\mu</math>L</td></tr></tbody></table>	# of Samples in Batch	8 or 16 or 24	Polymerase Stock	3.5 $\mu$ L	Sequel II polymerase dilution buffer	26.5 $\mu$ L	Total Volume	30 $\mu$ L
# of Samples in Batch	8 or 16 or 24								
Polymerase Stock	3.5 $\mu$ L								
Sequel II polymerase dilution buffer	26.5 $\mu$ L								
Total Volume	30 $\mu$ L								
	Diluted polymerase must be used immediately.								

• **Note:** For PureTarget repeat expansion samples, polymerase working solution concentration after performing polymerase dilution step is **higher** than for Kinnex & WGS samples

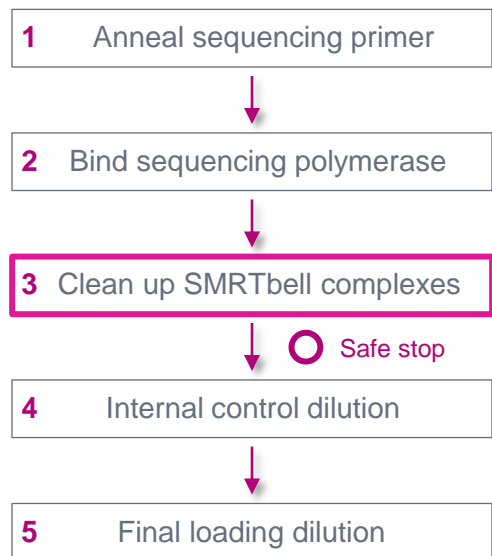
### Bind sequencing polymerase

Step	Instructions								
11b.3	Add annealed sample to diluted polymerase and finger tap or pipette to mix.								
	<table border="1"><thead><tr><th># of Samples in Batch</th><th>8 or 16 or 24</th></tr></thead><tbody><tr><td>Annealed sample</td><td>30 <math>\mu</math>L</td></tr><tr><td>Diluted polymerase</td><td>30 <math>\mu</math>L</td></tr><tr><td>Total volume</td><td>60 <math>\mu</math>L</td></tr></tbody></table>	# of Samples in Batch	8 or 16 or 24	Annealed sample	30 $\mu$ L	Diluted polymerase	30 $\mu$ L	Total volume	60 $\mu$ L
# of Samples in Batch	8 or 16 or 24								
Annealed sample	30 $\mu$ L								
Diluted polymerase	30 $\mu$ L								
Total volume	60 $\mu$ L								
	Incubate at room temperature for 15 minutes. Bound complex can be stored at 4°C for 4 weeks.								

**NOTE:**  
**ALWAYS USE THESE FIXED VOLUMES FOR EACH POOLED BATCH**  
→ **Do not adjust reagent volumes** based on measured sample concentration value or plex level of batch

# Sample setup procedure for PureTarget libraries – Sequel II/Ile system (cont.)

## Clean up SMRTbell complexes



## Purification of Polymerase Bound SMRTbell Complexes

✓ Step	Instructions								
1	1. Equilibrate the cleanup beads and Sequel II loading buffer 3.2 to room temperature at least 30 min before use.								
2	2. Add the following buffer volumes to each sample in each batch, as indicated: <table border="1"><thead><tr><th># of Samples in Batch</th><th>8 or 16 or 24</th></tr></thead><tbody><tr><td>Binding reaction</td><td>60 µL</td></tr><tr><td>ABC buffer</td><td>40 µL</td></tr><tr><td>Total Volume</td><td>100 µL</td></tr></tbody></table>	# of Samples in Batch	8 or 16 or 24	Binding reaction	60 µL	ABC buffer	40 µL	Total Volume	100 µL
# of Samples in Batch	8 or 16 or 24								
Binding reaction	60 µL								
ABC buffer	40 µL								
Total Volume	100 µL								
3	3. Add the indicated volume of cleanup beads to each sample in each batch and gently pipette-mix. Incubate on the benchtop for 10 minutes. <table border="1"><thead><tr><th># of Samples in Batch</th><th>8 or 16 or 24</th></tr></thead><tbody><tr><td>Binding reaction</td><td>100 µL</td></tr><tr><td>Cleanup beads</td><td>120 µL</td></tr><tr><td>Total Volume</td><td>220 µL</td></tr></tbody></table>	# of Samples in Batch	8 or 16 or 24	Binding reaction	100 µL	Cleanup beads	120 µL	Total Volume	220 µL
# of Samples in Batch	8 or 16 or 24								
Binding reaction	100 µL								
Cleanup beads	120 µL								
Total Volume	220 µL								
4	4. Place each tube in a magnetic bead rack until the beads collect to the side of the tube and the solution appears clear. Discard the supernatant. DO NOT wash the collected bead pellet with ethanol.								
5	5. Immediately resuspend the beads in the indicated volumes of room temperature Sequel II loading buffer 3.2 and pipette-mix: <table border="1"><thead><tr><th># of Samples in Batch</th><th>8 or 16 or 24</th></tr></thead><tbody><tr><td>Sequel II loading buffer 3.2</td><td>50 µL</td></tr></tbody></table>	# of Samples in Batch	8 or 16 or 24	Sequel II loading buffer 3.2	50 µL				
# of Samples in Batch	8 or 16 or 24								
Sequel II loading buffer 3.2	50 µL								
6	6. To elute the polymerase-bound complexes, incubate the samples on the benchtop for at least 10 minutes at room temperature.								
7	7. Place each tube in a magnetic bead rack until the beads collect to the side of the tube and the solution appears clear.								
8	8. Transfer eluates to new low-binding tube. Place on ice and protect from light.								

11b.4

- Equilibrate SMRTbell cleanup beads and loading buffer to room temperature at least 30 min before use

### NOTE:

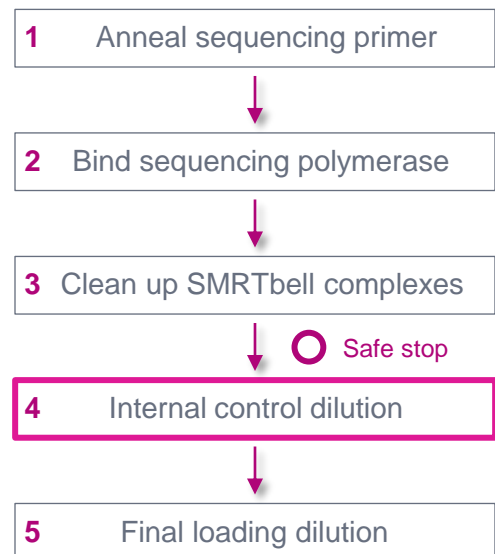
**ALWAYS USE THESE FIXED VOLUMES FOR EACH POOLED BATCH**

- Do not adjust reagent volumes based on measured sample concentration value or plex level of batch

- Do not wash bead pellet with ethanol

# Sample setup procedure for PureTarget libraries – Sequel II/Ile system (cont.)

## Internal control dilution



### Internal control dilution – First dilution

Step	Instructions								
11b.5	1st Dilution. Mix well by flicking the tube by hand and pulse-spin to collect contents and keep on ice.								
	<table border="1"><thead><tr><th>Reagent</th><th>Internal Control</th></tr></thead><tbody><tr><td>ABC buffer</td><td>19 <math>\mu</math>L</td></tr><tr><td>Sequel II DNA internal control complex 3.2</td><td>1.0 <math>\mu</math>L</td></tr><tr><td>Total volume</td><td>20 <math>\mu</math>L</td></tr></tbody></table>	Reagent	Internal Control	ABC buffer	19 $\mu$ L	Sequel II DNA internal control complex 3.2	1.0 $\mu$ L	Total volume	20 $\mu$ L
Reagent	Internal Control								
ABC buffer	19 $\mu$ L								
Sequel II DNA internal control complex 3.2	1.0 $\mu$ L								
Total volume	20 $\mu$ L								

- Prepare only one control dilution reaction, regardless of number of samples (up to 24 samples per SMRT Cell for Sequel II/Ile system)

### Internal control dilution – Second dilution

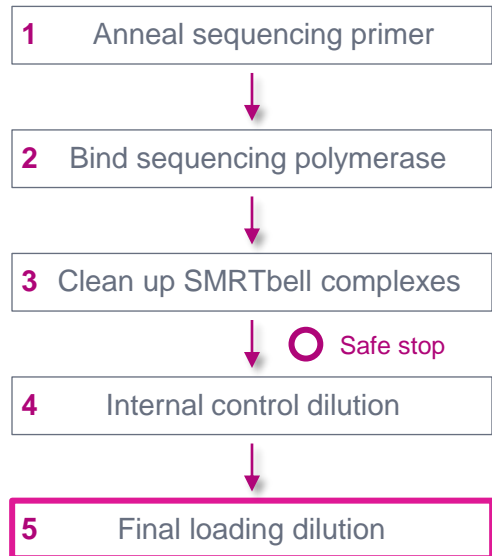
Step	Instructions								
11b.6	2nd Dilution. Mix well by flicking the tube by hand and pulse-spin to collect contents and keep on ice.								
	<table border="1"><thead><tr><th>Reagent</th><th>Internal Control</th></tr></thead><tbody><tr><td>ABC buffer</td><td>19 <math>\mu</math>L</td></tr><tr><td>Sequel II DNA internal control complex 3.2</td><td>1.0 <math>\mu</math>L</td></tr><tr><td>Total volume</td><td>20 <math>\mu</math>L</td></tr></tbody></table>	Reagent	Internal Control	ABC buffer	19 $\mu$ L	Sequel II DNA internal control complex 3.2	1.0 $\mu$ L	Total volume	20 $\mu$ L
Reagent	Internal Control								
ABC buffer	19 $\mu$ L								
Sequel II DNA internal control complex 3.2	1.0 $\mu$ L								
Total volume	20 $\mu$ L								

### Internal control dilution – Third dilution

Step	Instructions								
11b.7	3rd Dilution. Mix well by flicking the tube by hand and pulse-spin to collect contents and keep on ice.								
	<table border="1"><thead><tr><th>Reagent</th><th>Internal Control</th></tr></thead><tbody><tr><td>ABC buffer</td><td>19 <math>\mu</math>L</td></tr><tr><td>Sequel II DNA internal control complex 3.2</td><td>1.0 <math>\mu</math>L</td></tr><tr><td>Total volume</td><td>20 <math>\mu</math>L</td></tr></tbody></table>	Reagent	Internal Control	ABC buffer	19 $\mu$ L	Sequel II DNA internal control complex 3.2	1.0 $\mu$ L	Total volume	20 $\mu$ L
Reagent	Internal Control								
ABC buffer	19 $\mu$ L								
Sequel II DNA internal control complex 3.2	1.0 $\mu$ L								
Total volume	20 $\mu$ L								

# Sample setup procedure for PureTarget libraries – Sequel II/Ile system (cont.)

## Final loading dilution



## Final loading dilution

✓ Step	Instructions										
	Combine the following and protect from light:										
11b.8	<table border="1"><thead><tr><th># of Samples in batch</th><th>Sample 1 (24-plex or less)</th></tr></thead><tbody><tr><td>Prepared sample</td><td>50 <math>\mu</math>L</td></tr><tr><td>Sequel II loading buffer 3.2</td><td>67 <math>\mu</math>L</td></tr><tr><td>Diluted internal control (dilution 3)</td><td>3 <math>\mu</math>L</td></tr><tr><td>Total volume</td><td>120 <math>\mu</math>L</td></tr></tbody></table>	# of Samples in batch	Sample 1 (24-plex or less)	Prepared sample	50 $\mu$ L	Sequel II loading buffer 3.2	67 $\mu$ L	Diluted internal control (dilution 3)	3 $\mu$ L	Total volume	120 $\mu$ L
# of Samples in batch	Sample 1 (24-plex or less)										
Prepared sample	50 $\mu$ L										
Sequel II loading buffer 3.2	67 $\mu$ L										
Diluted internal control (dilution 3)	3 $\mu$ L										
Total volume	120 $\mu$ L										
	Load 115 $\mu$ L of sample per well and/or store at 4°C for up to 24 hours before use.										
PROTOCOL COMPLETE											

- Add 50  $\mu$ L Prepared sample + 67 Sequel II loading buffer 3.2 + 3  $\mu$ L diluted internal control  
→ Transfer entire mixture (100  $\mu$ L) to appropriate sample well in Revo sequencing plate

**NOTE:**  
**ALWAYS USE THESE FIXED VOLUMES FOR EACH POOLED BATCH**  
→ Do not adjust reagent volumes based on measured sample concentration value or plex level of batch



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