Preparing multiplexed amplicon libraries using SMRTbell® prep kit 3.0

Procedure & checklist

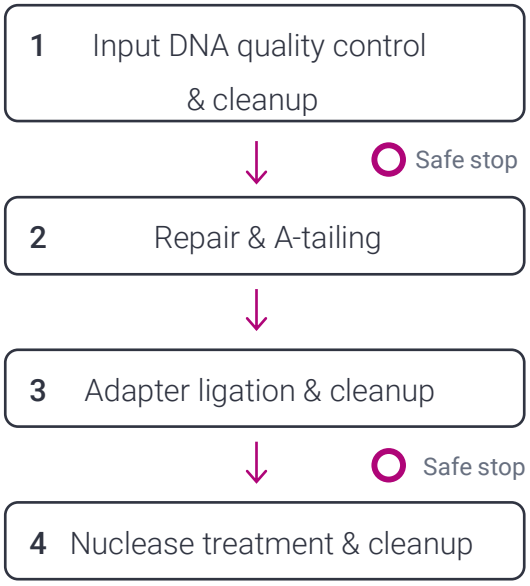
This procedure describes the workflow for constructing pre-indexed amplicon or target-enrichment libraries using the SMRTbell prep kit 3.0 for sequencing on PacBio® HiFi systems. For amplicon libraries requiring barcoding with indexed adapters, refer to the following protocol: Preparing multiplexed whole genome and amplicon libraries using the HiFi plex prep kit 96.

Overview	Number of pools per kit	Prep time
Pre-indexed pools per SMRTbell prep kit 3.0	24	3.5 hours

Minimum pooled DNA mass into library preparation for 1 SMRT® Cell			
Mean size	**Revio® + SPRQ™ chemistry**	**Revio (non-SPRQ) and Vega™ systems**	**Sequel® II/e systems**
1–3 kb	50 ng	200 ng	100 ng
3–5 kb	100 ng	400 ng	150 ng
5–10 kb	200 ng	800 ng	300 ng
>10 kb	300 ng	1000 ng	400 ng

102-359-000 REV05 APR2025

Workflow overview for pre-indexed samples



102-359-000 REV05 APR2025

Required materials and equipment

DNA sizing (one or more of the following)	
1% agarose gel, an electrophoresis unit, and imager	Any Major Lab Supplier (MLS)
2100 Bioanalyzer	Agilent technologies G2939BA
4200 TapeStation	Agilent technologies G2991BA
5300 or 5400 Fragment analyzer	Agilent technologies M5311AA or M5312AA
FEMTO Pulse system	Agilent Technologies M5330AA
DNA quantitation	
Qubit fluorometer	Thermo Fisher Scientific Q33238
Qubit 1X dsDNA HS assay kit	Thermo Fisher Scientific Q33230
SMRTbell library preparation	
SMRTbell prep kit 3.0	PacBio® 102-182-700
And only one of the following depending on which HiFi system and sequencing chemistry is being used:	
Revio® SPRQ™ polymerase kit ***or*** Vega™ polymerase kit ***or*** Revio® polymerase kit (non-SPRQ™)* ***or*** Sequel® II binding kit 3.2*	PacBio® 103-496-900 PacBio® 103-426-500 PacBio® 102-739-100 PacBio® 102-194-100
* Procedure for Revio polymerase kit (non-SPRQ) and Sequel II binding kit 3.2 can be found in SMRT® Link Sample Setup	
Other supplies	
200 Proof ethanol, molecular biology or ACS grade	Any MLS
Nuclease-free water, molecular biology grade	Any MLS
8-channel Pipettes, P20 and P200	Any MLS
0.2 mL 8-tube strips	USA Scientific TempAssure 1402-4708
Single-channel Pipettes (P10, P20, P100, P200, and P1000)	Any MLS
Microcentrifuge	Any MLS
Magnetic separation rack compatible with 0.2 mL 8-tube strips	Any MLS
Thermocycler compatible with 0.2 mL 8-tube strips	Any MLS
1.5 mL DNA LoBind® Tubes	Eppendorf 022431021

Before you begin

DNA input mass

The total amount of DNA input required for constructing the SMRTbell library is dependent on the mean size of the amplicons or DNA fragment (e.g., hybrid capture library).

Pre-pooled sample input mass

Pre-indexed samples can be pooled prior to library prep to satisfy the DNA input mass requirements (see Table 1). **The mass required *per sample* can be calculated by dividing the mass required from Table 1 by the number of samples per indexed pool.** Refer to the table below for the minimum required input DNA mass into library preparation per SMRT Cell for different insert sizes.

It is recommended to **pool amplicons by the bins specified in Table 1** to achieve optimal loading on PacBio sequencers. When amplicons are similar in size, it is acceptable to pool by mass for each sample. If the amplicons differ in size, pool by molarity to ensure even coverage. **A pooling calculator is available in SMRT Link** under Sample Setup → Add calculation → Loading calculator.

Table 1. Recommended DNA input mass, binned by size, into library preparation for different PacBio platforms.

Minimum pooled DNA mass into library preparation for 1 SMRT Cell			
Mean size	**Revio +SPRQ**	**Revio (non-SPRQ) and Vega systems**	**Sequel II/e systems**
1–3 kb	50 ng	200 ng	100 ng
3–5 kb	100 ng	400 ng	150 ng
5–10 kb	200 ng	800 ng	300ng
>10 kb	300 ng	1000 ng	400 ng

Hybrid capture samples

This procedure can be used to convert pre-indexed pools of hybridization probe capture libraries into SMRTbell libraries. Typically, genomic gDNA is sheared to 7–10 kb and a target enrichment library is prepared, which adds sample barcodes and enriches for regions of interest. Barcoded, enriched sequencing libraries are QC'ed and pooled before progressing to SMRTbell library construction, as described in this protocol.

Refer to Appendix for additional information on DNA shearing and multiplexing guidance.

Sample quality

Using gel-extracted amplicon products may results in lower sequencing performance due to the damage inherently caused by intercalating dyes such as ethidium bromide and exposure to UV radiation. If working with a gel-extracted product that has been stained with a dye, it is recommended to bring it through additional rounds of amplification to remove damage and/or dyes prior to library prep and sequencing.

Reagent handling

Room temperature is defined as any temperature in the range of 18–23°C for this protocol.

SMRTbell cleanup beads

- Bring SMRTbell cleanup beads to room temperature prior to use.
- Vortex immediately before any addition to sample. Failure to do this will result in low recovery.
- Pipette-mix all bead binding and elution steps until beads are distributed evenly in solution.

SMRTbell prep kit 3.0

- Thaw the Repair buffer, Nuclease buffer, SMRTbell adapter and Elution buffer at room temperature.
- Mix reagent buffers with a brief vortex prior to use. Enzyme mixes do not require vortexing.
- Quick-spin all reagents in microcentrifuge to collect liquid at bottom prior to use.
- Keep all temperature-sensitive reagents on ice.

Temperature-sensitive reagents		
Step Used	**Tube**	**Reagent**
Repair & A-tailing	Blue	End repair mix
	Green	DNA repair mix
Adapter ligation	Orange	SMRTbell adapter
	Yellow	Ligation mix
	Red	Ligation enhancer
Nuclease treatment	Light green	Nuclease mix

Bring Qubit 1X dsDNA HS reagents to room temperature prior to use.
Samples can be stored at 4°C at all safe stopping points listed in the protocol.

Annealing, binding, and cleanup

Thaw the following reagents at room temperature:

Component	Tube color
Annealing buffer	Light blue
Standard sequencing primer	Light green
Polymerase buffer	Yellow
Loading buffer	Green
Dilution buffer	Blue

Once thawed, store the Annealing and Polymerase buffers and sequencing primer on ice. The Loading buffer should be left at room temperature.

Note: The Loading buffer is light sensitive and should be protected from light when not in use.

Keep the following reagents on a cold block or ice:

- Sequencing polymerase
- Sequencing control

Bring the following reagents up to room temperature 30 minutes prior to use:

- Loading buffer
- SMRTbell cleanup beads

Safety precautions

Refer to the Safety Data Sheet (SDS) for information on reagent hazards and protocols for safe handling, use, storage, and disposal.

Thermocycler programs

Program thermocycler(s) prior to beginning the protocol for the first time.

Repair and A-tailing, adapter ligation, and nuclease treatment thermocycler steps can be combined into a single program and paused in between prep treatments if preferred.

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

1. Repair & A-tailing

Step	Time	Temperature
1	30 min	37°C
2	5 min	65°C
3	Hold	4°C

2. Adapter ligation

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

3. Nuclease treatment

Step	Time	Temperature
1	15 min	37°C
2	Hold	4°C

Procedure and checklist

1. Input DNA quality control & cleanup

Prior to library preparation, evaluate the quantity and size distribution of input DNA to determine whether it is suitable for the protocol.

✔	Step	Instructions
	1.1	Measure DNA concentration of each sample with a Qubit fluorometer using the **1X dsDNA HS kit** following manufacturer's instructions.
	1.2	**Recommended**: measure the DNA size distribution of the pool with the appropriate sizing technology following the manufacturer's instructions. • Amplicons ≤10 kb: Agilent 2100 Bioanalyzer, TapeStation, or Fragment Analyzer. • Amplicons ≥10 kb: Agilent FEMTO Pulse system.
	1.3	Proceed to the next step if sample concentration and quality is acceptable.
	1.4	Add the appropriate mass of each sample to a 0.2 mL PCR strip tube. If volume exceeds 100 µL, then use a 1.5 mL DNA LoBind tube instead.
		Cleanup with 1.3X SMRTbell cleanup beads
	1.5	Add **1.3X** volume per volume **(v/v)** of resuspended, room-temperature SMRTbell cleanup beads to each tube.
	1.6	Pipette-mix the beads until evenly distributed.
	1.7	Quick-spin the tube strip in a microcentrifuge to collect liquid.
	1.8	Incubate at **room temperature** for **10 minutes** to allow DNA to bind beads.
	1.9	Place tube strip in a magnetic separation rack until beads separate fully from the solution.
	1.10	Slowly remove the cleared supernatant without disturbing the beads. Discard the supernatant.
	1.11	Slowly dispense **200 µL**, or enough to cover the beads, of **freshly prepared 80% ethanol** into each tube. After **30 seconds**, remove the 80% ethanol and discard.
	1.12	Repeat the previous step.
	1.13	Remove residual 80% ethanol: • Remove the tube strip from the magnetic separation rack. • Quick-spin the tube strip in a microcentrifuge. • Place the tube strip back in a magnetic separation rack until beads separate fully from the solution. • Remove residual 80% ethanol and discard.
	1.14	Remove the tube strip from the magnetic rack. **Immediately** add **47 µL** of **low TE buffer** to each tube and resuspend the beads by pipetting 10 times or until evenly distributed.
	1.15	Quick-spin the tube strip in a microcentrifuge to collect liquid.
	1.16	Incubate at **room temperature** for **5 minutes** to elute DNA.
	1.17	Place the tube strip in a magnetic separation rack until beads separate fully from the solution.

	1.18	Slowly aspirate the cleared eluate without disturbing the beads. Transfer eluate to a **new 0.2 mL PCR tube strip**. Discard the old tube strip with beads.

SAFE STOPPING POINT – Store at 4°C

2. Repair & A-tailing

✔	Step	Instructions
	2.1	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 10% overage. For individual preps, add components directly to the sample from the previous step at the specified volumes and skip Repair master mix steps (Steps 2.2 to 2.4). **Repair master mix** ✔ / Tube / Component / Volume per pool Purple / Repair buffer / 8 µL Blue / End repair mix / 4 µL Green / DNA repair mix / 2 µL Total volume / 14 µL
	2.2	Pipette-mix **Repair master mix**.
	2.3	Quick-spin **Repair master mix** in a microcentrifuge to collect liquid.
	2.4	Add **14 µL** of the **Repair master mix** to each sample. The total reaction volume should be **60 µL**.
	2.5	Pipette-mix each sample.
	2.6	Quick-spin the strip tube in a microcentrifuge to collect liquid.
	2.7	Run the repair & A-tailing thermocycler program. Step / Time / Temperature 1 / 30 min / 37°C 2 / 5 min / 65°C 3 / Hold / 4°C
	2.8	Proceed to the next step of the protocol.

3. Adapter ligation & cleanup

<table>
<tr><th>✔</th><th>Step</th><th>Instructions</th></tr>
<tr><td></td><td>3.1</td><td>Add the following components in the order and volume listed below to a microfuge tube. Adjust component volumes for the number of samples being prepared, plus 10% overage. For individual preps, add components directly to each sample from the previous step in the order and volume listed below, then skip Ligation master mix steps (steps 3.3 to 3.5).
<table>
<tr><th colspan="4">Ligation master mix</th></tr>
<tr><th>✔</th><th>Tube</th><th>Component</th><th>Volume per pool</th></tr>
<tr><td></td><td>Orange</td><td>SMRTbell adapter</td><td>4 µL</td></tr>
<tr><td></td><td>Yellow</td><td>Ligation mix</td><td>30 µL</td></tr>
<tr><td></td><td>Red</td><td>Ligation enhancer</td><td>1 µL</td></tr>
<tr><td></td><td></td><td>Total volume</td><td>35 µL</td></tr>
</table></td></tr>
<tr><td></td><td>3.2</td><td>Pipette-mix Ligation master mix.</td></tr>
<tr><td></td><td>3.4</td><td>Quick-spin Ligation master mix in a microcentrifuge to collect liquid.</td></tr>
<tr><td></td><td>3.5</td><td>Add 35 µL of Ligation master mix to each sample from previous step. The total volume should be 95 µL.</td></tr>
<tr><td></td><td>3.6</td><td>Pipette-mix each sample.</td></tr>
<tr><td></td><td>3.7</td><td>Quick-spin the strip tube in a microcentrifuge to collect liquid.</td></tr>
<tr><td></td><td>3.8</td><td>Run the adapter ligation thermocycler program.
<table>
<tr><th>Step</th><th>Time</th><th>Temperature</th></tr>
<tr><td>1</td><td>30 min</td><td>20°C</td></tr>
<tr><td>2</td><td>Hold</td><td>4°C</td></tr>
</table></td></tr>
<tr><td colspan="3">Cleanup with 1.3X SMRTbell cleanup beads</td></tr>
<tr><td></td><td>3.9</td><td>Add 124 µL of resuspended, room-temperature SMRTbell cleanup beads to each sample.</td></tr>
<tr><td></td><td>3.10</td><td>Pipette-mix the beads until evenly distributed.</td></tr>
<tr><td></td><td>3.11</td><td>Quick-spin the tube strip in a microcentrifuge to collect all liquid from the sides of the tubes.</td></tr>
<tr><td></td><td>3.12</td><td>Incubate at room temperature for 10 minutes to allow DNA to bind beads.</td></tr>
<tr><td></td><td>3.13</td><td>Place the tube strip in a magnetic separation rack until beads separate fully from the solution.</td></tr>
<tr><td></td><td>3.14</td><td>Slowly remove the cleared supernatant without disturbing the beads. Discard the supernatant.</td></tr>
<tr><td></td><td>3.15</td><td>Slowly dispense 200 µL, or enough to cover the beads, of freshly prepared 80% ethanol into each tube. After 30 seconds, remove the 80% ethanol and discard.</td></tr>
<tr><td></td><td>3.16</td><td>Repeat the previous step.</td></tr>
</table>

	3.17	Remove residual 80% ethanol: • Remove the tube strip from the magnetic separation rack. • Quick spin the tube strip in a microcentrifuge. • Place the tube strip back in a magnetic separation rack until beads separate fully from the solution. • Remove residual 80% ethanol and discard.
	3.18	Remove the tube strip from the magnetic rack. **Immediately** add **40 µL** of **elution buffer** to each tube and resuspend the beads by pipetting 10 times or until evenly distributed.
	3.19	Quick-spin the tube strip in a microcentrifuge.
	3.20	Incubate at **room temperature** for **5 minutes** to elute DNA.
	3.21	Place the tube strip in a magnetic separation rack until beads separate fully from the solution.
	3.22	Slowly aspirate the cleared eluate without disturbing the beads. Transfer eluate to a **new tube strip**. Discard old tube strip with beads.
	SAFE STOPPING POINT – Store at 4°C	

4. Nuclease treatment & cleanup

✔	Step	Instructions
	4.1	Add the following components in the order and volume listed below to a microfuge tube. Adjust component volumes for the number of samples being prepared, plus 10% overage. For individual preps, add components directly to each sample from the previous step in the order and volume listed below, then skip **Nuclease master mix** steps (steps 4.2 to 4.4). **Nuclease master mix** ✔ / Tube / Component / Volume per pool Light purple / Nuclease buffer / 5 µL Light green / Nuclease mix / 5 µL Total volume / 10 µL
	4.2	Pipette-mix **Nuclease master mix**.
	4.3	Quick-spin **Nuclease master mix** in a microcentrifuge to collect liquid.
	4.4	Add **10 µL** of **Nuclease master mix** to each sample. Total volume should equal **50 µL.**
	4.5	Pipette-mix each sample.
	4.6	Quick-spin the strip tube in a microcentrifuge to collect liquid.
	4.7	Run the nuclease treatment thermocycler program. Step / Time / Temperature 1 / 15 min / 37°C

	2	Hold	4°C	

Cleanup with 1.3X SMRTbell cleanup beads

4.8	Add **65 µL** of resuspended, room-temperature SMRTbell cleanup beads to each sample.
4.9	Pipette-mix the beads until evenly distributed.
4.10	Quick-spin the tube strip in a microcentrifuge to collect all liquid from the sides of the tubes.
4.11	Incubate at **room temperature** for **10 minutes** to allow DNA to bind beads.
4.12	Place the tube strip in a magnetic separation rack until beads separate fully from the solution.
4.13	Slowly remove the cleared supernatant without disturbing the beads. Discard the supernatant.
4.14	Slowly dispense **200 µL**, or enough to cover the beads, of **freshly prepared 80% ethanol** into each tube. After **30 seconds**, remove the 80% ethanol and discard.
4.15	Repeat the previous step.
4.16	Remove residual 80% ethanol: • Remove the tube strip from the magnetic separation rack. • Quick-spin the tube strip in a microcentrifuge. • Place the tube strip back in a magnetic separation rack until beads separate fully from the solution. • Remove residual 80% ethanol and discard.
4.17	Remove the tube strip from the magnetic rack. **Immediately** add **26 µL** of **elution buffer** to each tube and resuspend the beads by pipetting 10 times or until evenly distributed.
4.18	Quick-spin the tube strip in a microcentrifuge.
4.19	Incubate at **room temperature** for **5 minutes** to elute DNA.
4.20	Place the tube strip in a magnetic separation rack until beads separate fully from the solution.
4.21	Slowly aspirate the cleared eluate without disturbing the beads. Transfer eluate to a **new tube strip**. Discard old tube strip with beads.
4.22	Take a **1 µL** aliquot from each tube and dilute with **9 µL** of **elution buffer or water**. Measure DNA concentration with a Qubit Fluorometer using the 1x dsDNA HS kit. Calculate the total mass.
4.23	Proceed to Section 5 to prepare 25 µL of library for sequencing on the Vega system or Revio (+SPRQ). If necessary, **dilute 25 µL of SMRTbell library to the concentrations indicated below**. Failure to normalize libraries or pools of libraries to the appropriate concentration prior to ABC may result in low sequencing yield.

SMRTbell library size	Concentration (ng/µL)
<3 kb	<10 ng/µL
3–10 kb	<20 ng/µL
>10 kb	<60 ng/µL

Or

Proceed to SMRT Link Sample Setup for sequencing on Revio (non-SPRQ) or Sequel II/e.

SAFE STOPPING POINT – Store at 4°C for up to 2 weeks or -20°C long-term

5. Annealing, binding, and cleanup (ABC)

This step is for preparing the libraries (25 µL) for sequencing on Revio or Vega systems. If samples are pooled prior to ABC or a custom volume is required, see Appendix section A1. **The Polymerase kit used will depend on which sequencer or chemistry is being used (see below).**

Kit	PN
Revio SPRQ polymerase kit	103-496-900
Revio polymerase kit	102-739-100
Vega polymerase kit	103-426-500

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

Annealing mix

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

	5.2	Pipette-mix the **Annealing mix** and quick-spin to collect liquid.
	5.3	Add **25 µL** of the **Annealing mix** to each library. Total volume should equal **50 µL.**
	5.4	Pipette-mix each sample and quick spin to collect liquid.
	5.5	Incubate at room temperature for **15 minutes.**
	5.6	During primer incubation, prepare the polymerase dilution (see below) and store on ice.
	5.7	To prepare the polymerase, add the following components to a new microcentrifuge tube on ice. Adjust component volumes for the number of samples being prepared, plus 10% overage.

Polymerase Dilution

✔	Tube	Component	Volume
	Yellow	Polymerase buffer	47 µL
	Purple	Sequencing polymerase	3 µL
		Total volume	**50 µL**

	5.8	Pipette mix the **polymerase dilution** and quick-spin to collect liquid.
	5.9	Add **50 µL of polymerase dilution** to primer annealed sample. Total volume should equal **100 µL**.
	5.10	Pipette-mix each sample and quick-spin to collect liquid.

5.11	Incubate at **room temperature for 15 minutes.**
5.12	Proceed immediately to the next step of the protocol to remove excess polymerase.

Post-binding cleanup with 1.3X SMRTbell cleanup beads

5.13	Add **130 µL** of resuspended, room-temperature SMRTbell cleanup beads to each sample
5.14	Pipette-mix the beads until evenly distributed and quick-spin if necessary to collect all liquid from the sides of the tube.
5.15	Incubate at **room temperature** for **10 minutes** to allow DNA to bind beads
5.16	Place sample on an appropriate magnet and allow beads to separate fully from the solution
5.17	Slowly remove the cleared supernatant without disturbing the beads. Discard the supernatant. **DO NOT USE EtOH.** Proceed immediately to the elution. It is important not to let the beads dry out.
5.18	Remove sample from the magnet and **immediately** add **Loading Buffer 96** to each tube and resuspend the beads by pipette mixing.

	Revio +SPRQ polymerase Kit	Vega/Revio (non-SPRQ) polymerase Kit
Loading buffer	25 µL	50 µL

5.19	Quick-spin the samples to collect any liquid from the sides of the tube.
5.20	Incubate at **room temperature** for **15 minutes** to elute DNA
5.21	Place sample on magnet and allow beads to separate fully from the solution.
5.22	Slowly remove the cleared eluate without disturbing the beads. Transfer eluate to a **new tube**. Discard the old tube with beads
5.23	Use **1 µL** of sample to measure DNA concentration with a Qubit fluorometer using the 1x dsDNA HS kit. **Important:** The **Qubit Flex** instrument is not compatible with measuring polymerase-bound library in Loading Buffer 96. Concentration readings will not be accurate.
5.24	Proceed to the **Loading Calculator** in SMRT Link v13.3 or higher to calculate the final dilution for adding the sample to the sequencing reagent plate. The recommended loading concentration is 200–300 pM.

PROTOCOL COMPLETE

Important: Polymerase-bound libraries can be stored at 4°C for up to 1 month, or at -20°C for up to 6 months prior to sequencing. Polymerase-bound libraries can withstand up to 4 freeze-thaw cycles. Note that the Loading buffer is light sensitive.

Appendix

A1. Annealing, binding, and cleanup (ABC) for custom volumes

This step is for preparing libraries for sequencing on PacBio sequencers. The sequencing polymerase is stable once bound to the HiFi library and can be stored at 4°C for 1 month or at -20°C for at least 6 months. Use the calculations below to determine reagent volumes based on input sample volume:

	SMRTbell library	Annealing buffer	Standard sequencing primer	Polymerase dilution
Volume (µL)	x	x/2	x/2	x*2
Example	100	50	50	200

A2. DNA quality requirement for hybrid capture libraries

We recommend DNA extraction methods that result in high molecular weight DNA with 70% of molecules larger than 10 kb and free of RNA. PacBio Nanobind® DNA extractions kits are a good option to ensure there is sufficient mass and quality of high molecular weight DNA for this protocol.

A3. DNA sharing for hybrid capture libraries

Target shear lengths refer to the mean or modal size of the distribution. The recommended size for shearing samples prior to target enrichment is 7–10 kb in order to maximize capture efficiency and HiFi read lengths. Use any desired mechanical shearing method (i.e., Megaruptor, Covaris g-TUBE, or MiniG). Please refer to the manufacturer's instructions for the Diagenode Megaruptor and Covaris g-TUBE for recommendations on fragmentation settings for preferred fragment sizes. See PacBio documentation for shearing parameters on 1600 MiniG from SPEX Sample Prep Technical note. Enzymatic shearing is also an option using the seqWell LongPlex™ Long Fragment Multiplex Kit.

A4. Multiplexing guidance for hybrid capture libraries

Capture pools consisting of 4–8 barcoded libraries should be QC'ed and combined in equal mass prior to beginning SMRTbell library prep using the SMRTbell prep kit 3.0. It is important to only combine samples from the same targeted panel into a SMRTbell library to ensure uniform coverage across samples. The table below recommends the number of samples and capture pools per SMRT Cell on Vega, Revio, and Sequel II systems. Based on the required input quantities per SMRT Cell for SMRTbell prep kit 3.0 and the number of capture pools per SMRT Cell, we also show the recommended quantities per capture pool.

The recommended sample multiplexing is intended to maximize the number of samples that can be run for each panel on a SMRT Cell. When pooling fewer samples than recommended, HiFi yield and P1 loading percentage may be suboptimal even though the recommended per-sample HiFi read depth may be achieved. In general, we recommend 50-fold or greater HiFi read depth for target regions.

Revio with SPRQ chemistry recommendation			
Panel target size	**Samples per SMRT Cell**	**Capture pools per SMRTbell prep kit 3.0 library***	**Quantity per capture**
20 Mb panel	16	2	150 ng
2 Mb panel	96	12	33 ng
100 kb panel	384	48	8 ng
Vega (Revio without -SPRQ chemistry) recommendation			
Panel target size	**Samples per SMRT Cell**	**Capture pools per SMRTbell prep kit 3.0 library**	**Quantity per capture**
20 Mb panel	12	2	200 ng
2 Mb panel	72	9	45 ng
100 kb panel	288	36	12 ng
Sequel II system recommendation			
Panel target size	**Samples per SMRT Cell**	**Capture pools per SMRTbell prep kit 3.0 library**	**Quantity per capture**
20 Mb panel	4	1	200 ng
2 Mb panel	24	3	67 ng
100 kb panel	96	12	17 ng

*This guidance assumes capture pools consist of 8 samples per pool except for the 20 Mb panel on Vega/Revio non-SPRQ with 6 samples per pool and on the Sequel II system where 4 samples are in the pool.

Revision history (description)	Version	Date
Initial release	01	Apr 2022
Clarify minimum DNA input requirements and best practices	02	Sep 2022
Updated to include recommendations for the Revio system	03	Dec 2023
Updated for SPRQ chemistry and the Vega system and to remove adapter-indexed workflow.	04	Dec 2024
Updated to add guidance for hybrid capture libraries.	05	Apr 2025