Generating PureTarget™ repeat expansion panel libraries

Procedure & checklist

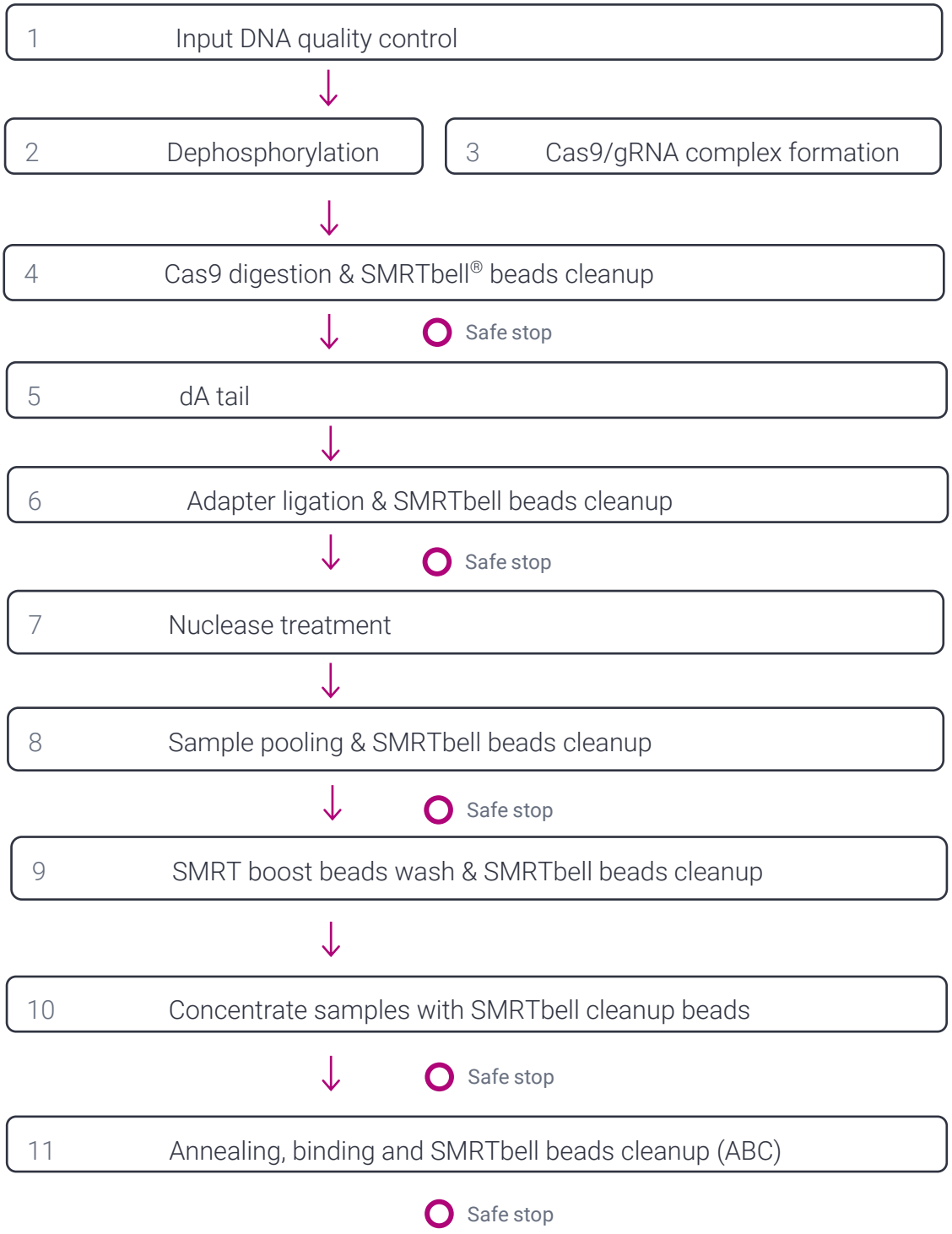
Before you begin

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, 0.1mM EDTA), or nuclease-free water
DNA size distribution	50% ≥30 kb
Sample multiplexing	
Sequel® II systems	Up to 24 samples
Vega™ system	Up to 48 samples
Revio® system	Up to 48 samples

103-329-400 REV04 APR2025

Workflow overview



103-329-400 REV04 APR2025

Required materials and equipment

DNA sizing	
Femto Pulse system	Agilent Technologies, Inc. M5330AA
Femto Pulse gDNA 165kb analysis kit	Agilent Technologies, Inc. FP-1002-0275
DNA quantitation	
Qubit fluorometer	ThermoFisher Scientific Q33238
Qubit 1X dsDNA HS assay kit	ThermoFisher Scientific Q33230
Target enrichment and library preparation	
PureTarget™ repeat expansion panel	PacBio® 103-390-400
PureTarget™ beads kit (store at 4°C upon arrival)	PacBio® 103-234-800*
PureTarget™ repeat expansion targeting kit	PacBio® 103-234-700*
SMRTbell® prep kit 3.0	PacBio® 102-141-700*
SMRTbell® Cleanup Beads	PacBio® 102-158-300*
Low TE buffer (pH 8.0)	PacBio® 102-178-400*
SMRTbell® adapter index plates (for barcoding)	PacBio® 102-009-200 PacBio® 102-547-800 PacBio® 102-547-900 PacBio® 102-548-000
Revio® SPRQ™ polymerase kit *or* Vega™ polymerase kit *or* Revio® polymerase kit *or* Sequel® II binding kit 3.2	PacBio® 103-496-900 PacBio® 103-426-500 PacBio® 102-793-100 PacBio® 102-194-100
Other supplies	
200 Proof ethanol, molecular biology or ACS grade	Any major lab supplier (MLS)
Mini-tube rotator	Any MLS (e.g., Fisher Scientific 05-450-127)
8-channel pipettes	Any MLS
0.2 mL 8-tube strips	USA Scientific TempAssure 1402-4708
Microcentrifuge	Any MLS
Magnetic separation rack compatible with 0.2 mL 8-tube strips	Any MLS (e.g., V&P Scientific VP 772F4-1 8-strip or Permagen MSR812 24-strip)

Magnetic separation rack compatible with 1.5 mL tubes	Any MLS (e.g., Invitrogen DynaMag™-2 Magnet 12321D)
Thermocycler compatible with 0.2 mL tube strips	Any MLS
Nuclease-free (NF) water	Any MLS, molecular biology grade
1.5 mL DNA LoBind tubes	Eppendorf 022431021

*Sold together as part of the PureTarget™ repeat expansion panel kit (103-390-400)

Before you begin

DNA input

For optimal performance, this protocol requires high-quality, high molecular weight (HMW) human gDNA with at least 50% of the mass of DNA in molecules at ≥30 kb in length, or genome quality number (GQN) of ≥5 at 30 kb based on the Agilent Femto Pulse system.

The supported sample type is high-quality, HMW genomic DNA extracted with the Nanobind PanDNA kit (PacBio 103-260-000). For human whole blood we recommend the RBC lysis extraction method. For human cell lines Nanobind PanDNA or Nanobind® CBB kit (PacBio 102-301-900) is supported.

The recommended mass of gDNA is **2 µg per sample** to ensure there are sufficient gene copies to load and maximize sequencing coverage. This protocol is suitable for 1–4 µg per sample. We recommend a minimum total DNA of 16 µg on the Sequel, Vega and Revio systems to yield a measurable library mass, and a maximum total DNA of 75 µg on the Sequel system, 100 µg on Vega and Revio (non-SPRQ), and 50 µg on Revio +SPRQ, across all multiplexed samples.

System	Min gDNA input	Max gDNA input
Sequel II/e	16 µg	75 µg
Vega	16 µg	100 µg
Revio (non-SPRQ)	16 µg	100 µg
Revio (+SPRQ)	16 µg	50 µg

Multiplexing samples

Sequel II/ Sequel IIe systems

Up to 24 samples can be barcoded and sequenced per SMRT® Cell on the Sequel II and Sequel IIe systems. These samples should be processed in batches of 8, 16, or 24 samples.

Vega system

Up to 48 samples can be barcoded and sequenced per Vega SMRT Cell. These samples should be processed in batches of 8, 16, 24, 32, 40, or 48 samples.

For Vega runs with 8, 16, or 24 samples, follow this protocol as is.

For Vega runs with 32, 40, or 48 samples, process two workflows in parallel: 1) 24 samples following this protocol and 2) an additional 8, 16, or 24 samples following this protocol.

Revio system

Up to 48 samples can be barcoded and sequenced per SMRT Cell on the Revio system. These samples should be processed in batches of 8, 16, 24, 32, 40, or 48 samples.

For Revio runs with 8, 16, or 24 samples, follow this protocol as is.

For Revio runs with 32, 40, or 48 samples, process two workflows in parallel: 1) 24 samples following this protocol and 2) an additional 8, 16, or 24 samples following this protocol.

Each PureTarget repeat expansion panel kit supports the preparation of 24 samples. Therefore, two library prep kits are required to generate a Revio run of 32, 40, or 48 samples.

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. Vortex-mix all buffers prior to use. Do not vortex enzymes.
- Quick-spin all reagents in a microcentrifuge to collect liquid at the bottom prior to use.
- Thaw all temperature-sensitive reagents on ice and keep on ice prior to use. Keep master mixes involving temperature-sensitive reagents on ice until use.

Temperature-sensitive reagents		
Step used	**Tube**	**Reagent**
Dephosphorylation	Blue	Phosphatase
Cas9 digestion	Green	Cas9 Nuclease
Cas9 digestion	Purple	gRNA mix
dA Tailing	Light Blue	*Taq* DNA Polymerase
dA Tailing	Yellow	dATP
Adapter ligation		SMRTbell adapter index plate
Adapter ligation	Yellow	Ligation mix
Adapter ligation	Red	Ligation enhancer
Nuclease treatment	Light green	Nuclease mix

- Bring SMRTbell cleanup beads, SMRT boost beads, SMRT boost buffer, 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 4°C overnight or -20°C long term for all safe stopping points listed in the protocol.

Anneal, bind, 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, place reaction buffers and sequencing primer on-ice prior to making master mix. 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 conditions

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

1.Dephosphorylation program

Set the lid temperature to 95°C.

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

2. Cas9 gRNA complex formation program

For this and the rest of thermocycler programs, set the lid temperature to 75°C.

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

3. Cas9 digestion program

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

4. dA-tail program

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

5. Adapter ligation program

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

6. Nuclease treatment program

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

Workflow steps

1. Input DNA quality control and dilution

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 (we recommend HMW DNA with GQN of ≥5 at 30 kb).

✔	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 µL** aliquot from each sample and dilute with 9 **µL** of **elution buffer or water.**
	1.5	Measure DNA concentration with a Qubit fluorometer using the **1X dsDNA HS kit.**
	1.6	Dilute each aliquot to **250 pg/µL** 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 µg DNA to a final volume of 67 µL **per sample** with nuclease-free water and transfer to an 8 tube PCR strip.

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

2. Dephosphorylation

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

✔	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 15% overage according to the table below.
	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.
	2.8	Once the dephosphorylation program is complete, take out the sample and keep on ice until step 4. Meanwhile, proceed to the next step of the protocol, Step 3 below.

Reaction Mix 1 (RM1)

✔	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**

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

3. Cas9 gRNA complex formation

This step enables Cas9-gRNA ribonucleoprotein complex formation required for subsequent targeting and digestion of genomic DNA in Step 4.

✔	Step	Instructions
	3.1	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
		Total volume	**4.75 µL**	**47.5 µL**	**95 µL**	**142.5 µL**

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. **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.**
3.4	Run the **Cas9 gRNA complex formation** thermocycler program. Set the lid temperature to 75°C.

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

3.5	Proceed to the next step of the protocol.

4. Cas9 digestion and SMRTbell cleanup

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

✔	Step	Instructions
	4.1	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. **RM2 is Cas9 gRNA complex from step 3 after thermocycler program.**

Reaction Mix 3 (RM3)

✔	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**

4.2	Pipette-mix **RM3** up and down 10 times (do not vortex). Pipette-mix full volume.
4.3	Quick-spin in a microcentrifuge to collect liquid.
4.4	Add **19 µL** of the RM3 to each sample from Step 2 for total reaction volume of **99 µL.**
4.5	Pipette-mix each sample up and down 10 times. Pipette-mix full volume.
4.6	Spin down the tube strip for 15–30 seconds in a microcentrifuge to collect liquid and remove bubbles.
4.7	Run the **Cas-9 digestion** thermocycler program. Set the lid temperature to 75°C.

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

4.8	Quick-spin in a microcentrifuge to collect liquid.
	Cleanup with 1X SMRTbell cleanup beads
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. Avoid over-pipetting as it may cause DNA/bead mixture to stick to the pipette tip.**
4.11	Quick-spin the tube strip in a microcentrifuge to collect all liquid from the sides of the tubes.
4.12	Incubate at **room temperature** for **10 minutes** to allow DNA to bind beads.
4.13	Place the tube strip in a magnetic separation rack for 3–5 minutes until beads separate fully from the solution.

4.14	Slowly remove the cleared supernatant without disturbing the beads. Discard the supernatant.
4.15	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.
4.16	Repeat the previous step.
4.17	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.
4.18	Remove the tube strip from the magnetic rack. **Immediately** add **41 µL** of **low-TE buffer** to each tube and resuspend the beads.
4.17	Quick-spin the tube strip in a microcentrifuge.
4.18	Incubate at **room temperature** for **5 minutes** to elute DNA.
4.19	Place the tube strip in a magnetic separation rack for 3–5 minutes until beads separate fully from the solution.
4.20	Slowly aspirate the cleared eluate without disturbing the beads. Transfer eluate to a **new tube strip**. Discard old tube strip with beads.
4.21	Optional QC step: Take a 1 µL aliquot from each sample and dilute with 9 µL of **elution buffer or water**. Measure DNA concentration with a Qubit fluorometer using the **1X dsDNA HS kit.** **Expect recovery of 50-100% per samples relative to starting mass.**
4.22	Proceed to the next step of the protocol.

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

5. dA-tail

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

✔	Step	Instructions
	5.1	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.

Reaction Mix 4 (RM4)

✔	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	2.5 µL	23 µL	46 µL	69 µL
	Orange	dA tail buffer	5 µL	46 µL	92 µL	138 µL
	Yellow	dATP (10 mM)	1.25 µL	11.5 µL	23 µL	34.5 µL
	Light Blue	*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.
	5.7	Run the **dA-tail** thermocycler program. Set the lid temperature to 75°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.

6. Adapter ligation and SMRTbell cleanup

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

<table>
<tr><th>✔</th><th>Step</th><th>Instructions</th></tr>
<tr><td></td><td>6.1</td><td>To a PCR strip with 50 µL DNA from Step 5, add 4 µL SMRTbell adapter index to each sample. Tap-mix or pipette up and down 10 times (do not vortex).</td></tr>
<tr><td></td><td>6.2</td><td>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.
<table>
<tr><th colspan="6">Reaction Mix 5 (RM5)</th></tr>
<tr><th>✔</th><th>Reagent</th><th>Component</th><th>Volume per sample</th><th>Volume Per 8-plex
With 15% overage</th><th>Volume Per 16-plex
With 15% overage</th><th>Volume Per 24-plex
With 15% overage</th></tr>
<tr><td></td><td></td><td>NF water</td><td>2 µL</td><td>18.4 µL</td><td>36.8 µL</td><td>55.2 µL</td></tr>
<tr><td></td><td>Purple</td><td>Repair buffer</td><td>8 µL</td><td>73.6 µL</td><td>147.2 µL</td><td>220.8 µL*</td></tr>
<tr><td></td><td>Yellow</td><td>Ligation mix</td><td>30 µL</td><td>276 µL</td><td>552 µL</td><td>828 µL</td></tr>
<tr><td></td><td>Red</td><td>Ligation enhancer</td><td>1 µL</td><td>9.2 µL</td><td>18.4 µL</td><td>27.6 µL</td></tr>
<tr><td></td><td></td><td>Total volume</td><td>41 µL</td><td>377.2 µL</td><td>754.4 µL</td><td>1131.6 µL</td></tr>
</table>
*The actual fill volume in the Repair buffer tube is >225 µL</td></tr>
<tr><td></td><td>6.3</td><td>Pipette-mix RM5 up and down 10 times (do not vortex). Pipette-mix full volume.
Ensure slow mixing and dispensing of RM5 to prevent liquid adhering to inside wall of tip and volume loss.</td></tr>
<tr><td></td><td>6.4</td><td>Quick-spin RM5 in a microcentrifuge to collect liquid.</td></tr>
<tr><td></td><td>6.5</td><td>Add 41 µL of RM5 to each sample from previous step for a total volume of 95 µL.</td></tr>
<tr><td></td><td>6.6</td><td>Pipette-mix each sample up and down 10 times. Pipette-mix full volume.</td></tr>
<tr><td></td><td>6.7</td><td>Quick-spin the tube strip in a microcentrifuge to collect liquid.</td></tr>
<tr><td></td><td>6.8</td><td>Run the adapter ligation thermocycler program. Set the lid temperature to 75°C.
<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 1X SMRTbell cleanup beads</td></tr>
<tr><td></td><td>6.9</td><td>Add 95 µL of resuspended, room-temperature SMRTbell cleanup beads to each sample.</td></tr>
<tr><td></td><td>6.10</td><td>Pipette-mix the beads 10 times until evenly distributed.</td></tr>
</table>

6.11	Quick-spin the tube strip in a microcentrifuge to collect all liquid from the sides of the tubes.
6.12	Incubate at **room temperature** for **10 minutes** to allow DNA to bind beads.
6.13	Place the tube strip in a magnetic separation rack for 3–5 minutes until beads separate fully from the solution.
6.14	Slowly remove the cleared supernatant without disturbing the beads. Discard the supernatant.
6.15	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.
6.16	Repeat the previous step.
6.17	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.
6.18	Remove the tube strip from the magnetic rack. **Immediately** add **41 µL** of **elution buffer** to each tube and resuspend the beads.
6.19	Quick-spin the tube strip in a microcentrifuge.
6.20	Incubate at **room temperature** for **5 minutes** to elute DNA.
6.21	Place the tube strip in a magnetic separation rack until beads separate fully from the solution.
6.22	Slowly aspirate the cleared eluate without disturbing the beads. Transfer eluate to a **new tube strip**. Discard the old tube strip with beads.
6.23	Optional QC step: Take a **1 µL** aliquot from each sample and dilute with **9 µL** of **elution buffer or water**. Measure DNA concentration with a Qubit fluorometer using the **1X dsDNA HS kit.** **Expect recovery of 50-100% per sample relative to starting mass.**
6.23	Proceed to the next step of the protocol.

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

7. Nuclease treatment

This step removes DNA fragments that have not formed SMRTbell templates. This step removes the vast majority of starting DNA fragments.

✔	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.

Reaction Mix 6 (RM6)

✔	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.
7.7	Run the **nuclease treatment** thermocycler program. Set the lid temperature to 75°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).

8. Sample pooling

Pool SMRTbell templates **in units of 8** samples after nuclease treatment of individual samples

✔	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	Incubate 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 remove 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**, remove the 80% ethanol and discard.
	8.9	Repeat the previous step.
	8.10	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.
	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	Incubate 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 remove the cleared supernatant without disturbing the beads. Transfer supernatant to a **1.5 mL DNA LoBind tube**. Discard old tube with beads.
		SAFE STOPPING POINT – Store at 4°C overnight or -20°C long term

9. SMRT boost beads wash of SMRTbell templates

The SMRT boost beads wash will prepare the library for sequencing.

Wash pooled SMRTbell templates with SMRT boost beads.

Perform one SMRT boost beads wash per final pool of up to 24 samples. For >24 samples you need to perform two SMRT boost beads wash with up to 24 samples each.

Bring SMRT boost buffer and SMRT boost beads to room-temperature prior to use.

Vortex room-temperature SMRT boost buffer prior to use. Vortex room-temperature SMRT boost beads prior to use and spin down to collect.

✔	Step	Instructions
	9.1	Prepare the beads by washing in SMRT boost buffer • To a 1.5 mL DNA LoBind tube, first add 1 mL of **room-temperature SMRT boost buffer** and then 100 µL of resuspended, **room-temperature SMRT boost beads**. • Pulse-vortex 5 times to mix. • Quick-spin the tube and magnetically separate until supernatant is clear (about 2-3 minutes). • Remove the supernatant and wash once more by adding **1 mL of SMRT boost buffer**. • Pulse-vortex 5 times to mix. • Quick-spin the tube and magnetically separate until supernatant is clear (about 2-3 minutes). Remove the supernatant. • Resuspend the beads in **200 µL SMRT boost buffer** by pulse-vortexing 5 times to mix. • Quick-spin to collect the beads.
	9.2	Prepare the SMRTbell templates for SMRT boost bead washing by mixing together one or more 8-plex pools (each at 100 µL) from Step 8. Bring the total volume to **300 µL with Elution Buffer** if combining fewer than 3 pools of 8, as shown in the table below.
	9.3	Add **300 µL of SMRTbell templates** to **200 µL SMRT boost beads** in SMRT boost 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). If you don't have a rotator, keep beads in suspension by manually inverting every 5 min.
	9.5	Spin down to collect and magnetically separate for 2 minutes until the supernatant is clear.
	9.6	Aliquot **500 µL** of the SMRTbell-containing supernatant into a fresh 1.5 mL LoBind tube.

# of Samples in Batch	8	16	24
8-plex pool 1	100 µL	100 µL	100 µL
8-plex pool 2	0 µL	100 µL	100 µL
8-plex pool 3	0 µL	0 µL	100 µL
Elution Buffer	200 µL	100 µL	0 µL
Total sample volume	300 µL	300 µL	300 µL

	Cleanup with 1X SMRTbell cleanup beads
9.7	Add **500 µL** of resuspended, room-temperature SMRTbell cleanup beads to **500 µL** SMRTbell-containing supernatant from step 9.6.
9.8	Pipette-mix the beads 8–10 times until evenly distributed.
9.9	Quick–spin the tube in a microcentrifuge to collect all liquid from the sides of the tubes.
9.10	Incubate at **room temperature** for **10 minutes** to allow DNA to bind beads.
9.11	Place the tube in a magnetic separation rack until beads separate fully from the solution.
9.12	Slowly remove the cleared supernatant without disturbing the beads. Discard the supernatant.
9.13	Slowly dispense **1 mL** of **freshly prepared 80% ethanol** into each tube. After **30 seconds**, remove the 80% ethanol and discard.
9.14	Repeat the previous step.
9.15	Remove residual 80% ethanol: • Remove the tube from the magnetic separation rack. • Quick-spin the tube in a microcentrifuge. • Place the tube back in a magnetic separation rack until beads separate fully from the solution. • Remove residual 80% ethanol and discard.
9.16	Remove the tube from the magnetic rack. **Immediately** add **100 µL** of **elution buffer** to each tube and resuspend the beads.
9.17	Incubate at **room temperature** for **5 minutes** to elute DNA.
9.18	Place the tube in a magnetic separation rack until beads separate fully from the solution.
9.19	Slowly aspirate the cleared eluate without disturbing the beads. Transfer eluate to a **1.5 mL DNA LoBind tube**. Discard old tube with beads.

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

10. Concentrate samples with SMRTbell cleanup beads for ABC

Concentrate up to 24 SMRTbell templates into 15 µL volume for ABC.

✔	Step	Instructions
	10.1	Add **100 µL** SMRTbell cleanup beads to a 1.5mL DNA LoBind tube with **100 µL 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	Incubate 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 remove the cleared supernatant without disturbing the beads. Discard the supernatant.
	10.7	Slowly dispense **250 µL**, or enough to cover the beads, of **freshly prepared 80% ethanol** into each tube. After **30 seconds**, remove the 80% ethanol and discard.
	10.8	Repeat the previous step.
	10.9	Remove residual 80% ethanol: • Remove the tube from the magnetic separation rack. • Quick-spin the tube in a microcentrifuge. • Place the tube back in a magnetic separation rack until beads separate fully from the solution. • Remove residual 80% ethanol and discard.
	10.10	Remove the tube from the magnetic rack. **Immediately** add **16 µL** of **elution buffer** to each tube and resuspend the beads.
	10.11	Incubate 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 aspirate the cleared eluate without disturbing the beads. Transfer eluate to a **1.5 mL DNA LoBind tube**. Discard old tube with beads.
	10.14	QC step: Take **1 µL** 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.** **Expect recovery of 0.5% or less (range 0.02% - 0.5%) relative to input starting mass.** For example, starting with 2 µg input per sample (or 16 µg 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.
		SAFE STOPPING POINT – Store at 4°C overnight or -20°C long term

11a. Annealing, binding & SMRTbell cleanup (ABC) for Revio +SPRQ

Use the entire volume of 15 µL pooled SMRTbell templates per ABC preparation and loading.

Ensure each ABC preparation is per 24 samples or fewer. Thus, if processing 24 SMRTbell templates or fewer (15 µL total) go into Preparation A; If processing more than 24 SMRTbell templates, the rest of the SMRTbell templates (also pooled in 15 µL total) go into Preparation B.

✔	Step	Instructions

11a.1

Note: Always use these values for each pooled preparation. Do not adjust based on measured concentration, value, or plex level.

Annealing sequencing primer

Combine the following components in a new low-binding tube and pipette to mix.

✔	Tube color		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
	Light blue	Annealing buffer	7.5 µL	7.5 µL
	Light green	Standard 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.

11a.2

Binding sequencing polymerase

Dilute sequencing polymerase

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.

✔	Tube color		Preparation A	Preparation B
		# of samples in preparation	8 or 16 or 24	8 or 16 or 24
	Purple	Sequencing polymerase	3.5 µL	3.5 µL
	Yellow	Polymerase buffer	26.5 µL	26.5 µL
		Total Volume	**30 µL**	**30 µL**

Diluted polymerase must be used immediately.

11a.3

Bind sequencing polymerase

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

✔	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.

Purification of polymerase bound SMRTbell complexes

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:

Tube color		Preparation A	Preparation B
	# of samples in preparation	8 or 16 or 24	8 or 16 or 24
	Binding Reaction	60 µL	60 µL
Blue	Dilution Buffer	40 µL	40 µL
	Total Volume	**100 µL**	**100 µL**

3. Combine Preparation A and Preparation B into a single 1.5 mL new Eppendorf tube if preparing more than 24 samples. Add the indicated volume (1.2X) of SMRTbell cleanup beads to each sample in each preparation and gently pipette-mix. Incubate on the benchtop for 10 minutes.

11a.4

	Preparation A	Preparation A+B
# of samples in preparation	8 or 16 or 24	> 24
Binding Reaction	100 µL	200 µL
SMRTbell cleanup Beads	120 µL	240 µL
Total Volume	**220 µL**	**440 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:

Tube color		Preparation
	# of samples in preparation	8 or 16 or 24 or more
Green	Loading Buffer	25 µL

6. To elute the polymerase-bound complexes, incubate the samples on the benchtop for at least 15 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**. The bound complex can be stored at 4°C for 4 weeks.

Internal control dilution

Prepare only one control dilution, regardless of number of samples (up to 48 samples).

11a.5

1st Dilution. Mix well by flicking the tube by hand and pulse-spin to collect contents and keep on ice.

Tube color	Reagent	Internal control
Blue	Dilution buffer	19 µL
Red	Sequencing control	1.0 µL

Total volume	20 µL

11a.6 2nd Dilution. Mix well by flicking the tube by hand and pulse-spin to collect contents and keep on ice.

✔	Tube color	Reagent	Internal control
	Blue	Dilution buffer	19 µL
		Sequencing control (dilution 1)	1.0 µL
		Total volume	20 µL

11a.7 3rd Dilution. Mix well by flicking the tube by hand and pulse-spin to collect contents and keep on ice.

✔	Tube color	Reagent	Internal control
	Blue	Dilution buffer	19 µL
		Sequencing control (dilution 2)	1.0 µL
		Total Volume	20 µL

11a.8 **Final loading dilution**

Combine the following and **protect from light**:

# of samples in preparation	
Eluate of polymerase bound sample	25 µL
Diluted internal control (dilution 3)	1 µL
Total volume	26 µL

Load exactly 23 µL of sample (11a.8) per well and/or store at 4°C for up to 24 hours before use. Before loading, inspect the sample wells for bubbles. If present, pop bubbles in the sample well using a single forceful tap of the sealed sequencing plate on a benchtop and spin the Revio-SPRQ sequencing plate down for 30 sec at 1200 rpm.

11b. Annealing, binding & cleanup (ABC) for Vega and Revio (non-SPRQ)

Use the entire volume of 15 µL pooled SMRTbell templates per ABC preparation and loading. **The Polymerase kit used will depend on which sequencer or chemistry is being used (see below).**

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

Ensure each ABC preparation is per 24 samples or fewer. Thus, if processing 24 SMRTbell templates or fewer (15 µL total) go into Preparation A; If processing more than 24 SMRTbell templates, the rest of the SMRTbell templates (also pooled in 15 µL total) go into Preparation B.

✔	Step	Instructions
	11b.1	**Note:** Always use these values for each pooled preparation. Do not adjust based on measured concentration, value, or plex level. **Annealing sequencing primer** Combine the following components in a new low-binding tube and pipette to mix.

✔	Tube color		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
	Light blue	Annealing buffer	7.5 µL	7.5 µL
	Light green	Standard 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.

11b.2

Binding sequencing polymerase

Dilute sequencing polymerase

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.

✔	Tube color		Preparation A	Preparation B
		# of samples in preparation	8 or 16 or 24	8 or 16 or 24
	Purple	Sequencing polymerase	3.5 µL	3.5 µL
	Yellow	Polymerase buffer	26.5 µL	26.5 µL
		Total Volume	**30 µL**	**30 µL**

Diluted polymerase must be used immediately.

11b.3

Bind sequencing polymerase

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

✔		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.

Purification of polymerase bound SMRTbell complexes

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:

Tube color		Preparation A	Preparation B
	# of samples in preparation	8 or 16 or 24	8 or 16 or 24
	Binding Reaction	60 µL	60 µL
Blue	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.

11b.4

	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:

Tube color		Preparation A	Preparation B
	# of samples in preparation	8 or 16 or 24	8 or 16 or 24
Green	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**. The bound complex can be stored at 4°C for 4 weeks.

Internal control dilution

Prepare only one control dilution, regardless of number of samples (up to 48 samples).

11b.5

1st Dilution. Mix well by flicking the tube by hand and pulse-spin to collect contents and keep on ice.

Tube color	Reagent	Internal control
Blue	Dilution buffer	19 µL
Red	Sequencing control	1.0 µL

Total volume	20 µL	

11b.6 2nd Dilution. Mix well by flicking the tube by hand and pulse-spin to collect contents and keep on ice.

✔	Tube color	Reagent	Internal control
	Blue	Dilution buffer	19 µL
		Sequencing control (dilution 1)	1.0 µL
		Total volume	20 µL

11b.7 3rd Dilution. Mix well by flicking the tube by hand and pulse-spin to collect contents and keep on ice.

✔	Tube color	Reagent	Internal control
	Blue	Dilution buffer	19 µL
		Sequencing control (dilution 2)	1.0 µL
		Total Volume	20 µL

11b.8 **Final loading dilution**

Combine the following and **protect from light**:

If loading ≤24 samples

✔	Tube color	# of samples in preparation	≤24-plex
		Prepared sample (preparation A)	48.5 µL
	Green	Loading buffer	48.5 µL
		Diluted internal control (dilution 3)	3 µL
		Total volume	100 µL

Load 100 µL of sample per well and/or store at 4°C for up to 24 hours before use.

If loading >24 samples

✔	Tube color	# of samples in preparation	>24-plex
		Prepared sample (preparation A)	48.5 µL
		Prepared sample (preparation B)	48.5 µL
	Green	Loading buffer	0 µL
		Diluted internal control (dilution 3)	3 µL
		Total volume	100 µL

Load 100 µL of sample per well and/or store at 4°C for up to 24 hours before use.

11c. Annealing, binding, & SMRTbell cleanup (ABC) for Sequel II systems

Use entire volume of 15 µL pooled SMRTbell templates per ABC batch and loading.

✔	Step	Instructions
	11c.1	**Note:** Always use these values for each pooled batch. Do not adjust based on measured concentration, value, or plex level of batch. **Annealing sequencing primer** Combine the following components in a new low-binding tube and pipette to mix. ✔ / Tube color / # of samples in batch / 8 or 16 or 24 Sample (SMRTbell templates) — 15 µL Light blue — Annealing Buffer — 7.5 µL Light green — Sequel II Primer 3.2 — 7.5 µL Total volume — 30 µL Incubate at room temperature for 15 minutes then proceed to the next step.
	11c.2	**Binding sequencing polymerase** **Dilute sequencing polymerase** 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. ✔ / Tube color / # of samples in batch / 8 or 16 or 24 Purple — Sequel II DNA Polymerase 2.2 — 3.5 µL Yellow — Sequel II polymerase dilution buffer — 26.5 µL Total Volume — 30 µL Diluted polymerase must be used immediately.
	11c.3	**Bind sequencing polymerase** Add annealed sample to diluted polymerase and finger tap or pipette to mix. ✔ / # of samples in Batch / 8 or 16 or 24 Annealed sample — 30 µL Diluted polymerase — 30 µL Total volume — 60 µL Incubate at room temperature for 15 minutes.

Purification of polymerase bound SMRTbell complexes

1. Equilibrate the cleanup beads and Sequel II loading buffer 3.2 to room temperature at least 30 min before use.
2. Add the following buffer volumes to each sample in each batch, as indicated:

✔	Tube color	# of samples in batch	8 or 16 or 24
		Binding reaction	60 µL
	Blue	ABC buffer	40 µL
		Total volume	**100 µL**

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.

11c.4

✔	# of samples in batch	8 or 16 or 24
	Binding reaction	100 µL
	Cleanup beads	120 µL
	Total volume	**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 Sequel II loading buffer 3.2 and pipette-mix:

✔	Tube color	# of samples in batch	8 or 16 or 24
	Green	Sequel II loading buffer 3.2	50 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**. The bound complex can be stored at 4°C for 4 weeks.

Internal control dilution

1st Dilution. Mix well by flicking the tube by hand and pulse-spin to collect contents and keep on ice.

11c.5

✔	Tube color	Reagent	Internal control
	Blue	ABC buffer	19 µL
	Red	Sequel II DNA internal control complex 3.2	1.0 µL
		Total volume	**20 µL**

2nd Dilution. Mix well by flicking the tube by hand and pulse-spin to collect contents and keep on ice.

11c.6

✔	Tube color	Reagent	Internal control
	Blue	ABC buffer	19 µL
		Sequel II DNA internal control complex 3.2 (dilution 1)	1.0 µL
		Total volume	20 µL

11c.7

3rd Dilution. Mix well by flicking the tube by hand and pulse-spin to collect contents and keep on ice.

✔	Tube color	Reagent	Internal control
	Blue	ABC buffer	19 µL
		Sequel II DNA internal control complex 3.2 (dilution 2)	1.0 µL
		Total volume	20 µL

11c.8

Final loading dilution

Combine the following and **protect from light**:

✔	Tube color	# of samples in batch	Sample 1 (24-plex or less)
		Prepared sample	50 µL
	Green	Sequel II loading buffer 3.2	67 µL
		Diluted internal control (dilution 3)	3 µL
		Total volume	120 µL

Load 115 µL of sample per well and/or store at 4°C for up to 24 hours before use.

PROTOCOL COMPLETE

Quick start for run design and analysis

PureTarget libraries must be sequenced with the **PureTarget repeat expansion run design** and analyzed with the **PureTarget repeat expansion analysis** in **SMRT® Link v13.1** and later.

The default run design settings should be used:

Option	Selection
Application	PureTarget repeat expansion
Library type	Standard
Insert size	5000
Library concentration	<Calculated by user>
Use adaptive loading	OFF

Note: Run conditions are not impacted by insert size or library concentration. If you calculate your library concentration, enter it, but if not, enter "0".

It is recommended that the **PureTarget repeat expansion analysis workflow** is run (even if genotype information is not desired such as for service providers) because it provides useful performance metrics. The following metrics are key for assessing performance:

- **Target coverage** and **Sample coverage** plots in the **Target Enrichment** section show coverage across panel targets and across samples, respectively. The later plot is useful for reviewing sample performance across the run.
- **Mean target coverage** in the **Tandem Repeats Sample Summary** is expected to be > 200-fold for Nanobind-extracted human blood or cell line DNA samples with GQN_{30kb} > 5 for non-expanded alleles. Coverage may be lower for expanded alleles, lower quality DNA samples, or different tissue types.

Please see the latest SMRT Link User guide for more information.

Revision history (description)	Version	Date
Initial release	01	March 2024
Minor updates for clarity	02	April 2024
Updated with SPRQ chemistry and the Vega system, adapter index plates, color guide for ABC steps, Quick Start Guide for run design and analysis, and minor changes for clarity.	03	December 2024
Moved ABC safe-stop point from from post-polymerase binding to post-cleanup and added ABC reagents to the Reagent handling section	04	April 2025

103-329-400 REV04 APR2025