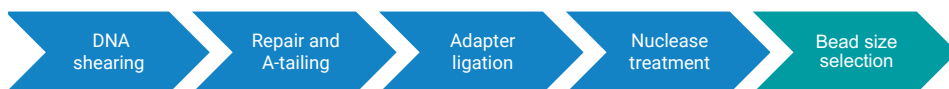# ALTERNATIVE SIZE SELECTION METHODS FOR SMRTBELL® PREP KIT 3.0

Introduction

In *Procedure & checklist — Preparing whole genome and metagenome sequencing libraries using SMRTbell prep kit 3.0*, size-selection beads are used to remove short DNA fragments and enrich for the long fragments that are most valuable for downstream analysis. Although size-selection beads have many advantages, alternative methods offer more flexibility in defining a size cutoff. This technical note provides guidance for size selection of WGS libraries prepared with the SMRTbell prep kit 3.0.

	Size-selection beads (Standard)	Gel cassettes	Short read eliminator
Size cutoff	Fixed (>5 kb)	✓ Adjustable	Fixed (> 10 kb)
Hands-on time	✓ Low	✓ Low	✓ Low
Run time	✓ Minutes	Hours	✓ Minutes
Automation	✓ Standard liquid handler	Separate instrument	n/a
Cost	✓ Low	High	✓ Low
DNA recovery	✓ High	Low	Low
DNA input to library prep per SMRT® Cell	✓ 1 µg	1.5 µg	1.5 µg

Standard protocol with size-selection beads

DNA shearing → Repair and A-tailing → Adapter ligation → Nuclease treatment → Bead size selection

Gel cassette size selection

DNA shearing → Repair and A-tailing → Adapter ligation → Nuclease treatment → SMRTbell cleanup bead purification → Gel cassette size selection → SMRTbell cleanup bead purification

Short read eliminator

Short read eliminator → DNA shearing → Repair and A-tailing → Adapter ligation → Nuclease treatment → Bead size selection

PacBio

Gel cassette size-selection options

Equipment	Vendor	Samples per run	Run time	Size selection
PippinHT system	Sage Science	≤20	2 hrs	High pass
BluePippin system	Sage Science	≤4	4.5 hrs	High pass
SageELF system	Sage Science	≤2	4.5 hrs	Fractionation

PippinHT system size-selection workflow

Min DNA input to library prep	1.5 µg / SMRT Cell
Samples per run	≤ 20
Run time	2 hrs

Required materials

Material	Part number
PippinHT system	Sage Science HTP0001
0.75% Agarose gel cassettes, marker 75E	Sage Science HPE7510
SMRTbell cleanup beads	PacBio 102-158-300
DNA Lo-Bind microfuge tubes	Eppendorf 022431021
Magnetic separation rack compatible with 1.5 mL tubes	Any major lab supplier
Qubit fluorometer	ThermoFisher Scientific Q33238
Qubit 1x dsDNA HS assay kit	ThermoFisher Scientific Q33230
Elution buffer	PacBio 101-633-500

Verify that your PippinHT system software is up to date and follow the procedure below to remove fragments <10 kb. For the latest PippinHT system Operations Manual and guidance on size-selection protocols, contact **Sage Science**.

1. Starting after the nuclease treatment step described in *Procedure & checklist — Preparing whole genome sequencing and metagenome libraries using SMRTbell prep kit 3.0* (102-166-600), perform a SMRTbell cleanup bead purification by following the steps outlined in Appendix A located at the end of this document, eluting in a **21 µL** elution volume. Dilute **1 µL** of eluted sample with **9 µL** elution buffer and measure DNA concentration with a Qubit system to calculate total mass. After completing, proceed to step 2 below.

2. Prepare **~1.5 µg** of SMRTbell library from step 1 in a final volume of **20.0 µL** of elution buffer for each PippinHT lane.

3. Bring the loading solution to room temperature, and then add **5.0 µL** of the loading solution to the **20.0 µL** DNA sample and mix well. For loading multiple lanes with the same sample, scale up the volumes proportionally. The loading solution is viscous, so pipette slowly to ensure complete transfer into the DNA sample.

   a. Spin briefly to collect the contents at the bottom of the 1.5 mL tube.

4. Follow the manufacturer's recommendations to set up a run protocol.

   a. Select the "**6−10kb High Pass Marker 75E**" Cassette Definition File.

   b. Using the "Range" selection mode, enter a desired "BPstart" value of 10000 and a "BPend" value of 50000.

   c. Be sure to assign a marker lane.

5. Load the 75E marker and samples into the gel cassette and start the run. Run time is approximately **1 hr 15 mins**.

6. To maximize recovery of eluted DNA, wait at least **45 minutes** after the run terminates before removing the sample from the elution chamber.

   a. Collect the eluate (**~30 µL**) into a new 1.5 mL tube.

   b. Wash the elution well with **30 µL** PippinHT 0.1% Tween20 (supplied with the cassettes). Wait **1 minute** and add the recovered wash liquid to the eluted sample.

   c. Wash the elution well with **30 µL** of elution buffer and add the recovered wash liquid to the eluted sample.

   d. The total volume after pooling the recovered washes with the original eluted sample is **~90 µL**.

7. Repeat SMRTbell cleanup bead purification by following the instructions in Appendix A (at the end of this document), eluting in **10 µL** elution buffer.

8. Dilute **1 µL** of eluted sample with **9 µL** elution buffer and measure DNA concentration with a Qubit system to calculate concentration. Using the same dilution, obtain library size distribution with an appropriate DNA sizing system.

9. Proceed to Sample Setup in SMRT® Link to prepare the SMRTbell library for sequencing.

BluePippin system size-selection workflow

Min DNA input to library prep	1.5 µg / SMRT Cell
Samples per run	≤ 4
Run time	4.5 hrs

Required materials

Material	Part Number
BluePippin system	Sage Science BLU0001
0.75% Agarose gel cassettes, marker S1	Sage Science BLF7510
SMRTbell cleanup beads	PacBio 102-158-300
DNA Lo-Bind microfuge tubes	Eppendorf 022431021
Magnetic separation rack compatible with 1.5 mL tubes	Any major lab supplier
Qubit fluorometer	ThermoFisher Scientific Q33238
Qubit 1x dsDNA HS assay kit	ThermoFisher Scientific Q33230
Elution buffer	PacBio 101-633-500

Verify that your BluePippin system software is up to date and follow the procedure below to remove fragments <10 kb using the BluePippin system. For the latest BluePippin system Operations Manual and guidance on size-selection protocols, contact **Sage Science**.

1. Starting after the nuclease treatment step (section 5) described in *Procedure & checklist — Preparing whole genome sequencing and metagenome libraries using SMRTbell prep kit 3.0* (102-166-600), perform a SMRTbell cleanup bead purification by following the steps outlined in Appendix A (at the end of this document), eluting in **31 µL** elution buffer. Dilute **1 µL** of eluted sample with **9 µL** elution buffer and measure DNA concentration with a Qubit system to calculate total mass. After completing, proceed to step 2 below.

2. Prepare **~1.5 µg** of SMRTbell library from step 1 in a final volume of **30.0 µL** of elution buffer for each BluePippin lane.

3. Bring the loading solution to room temperature, and then add **10.0 µL** of the loading solution to the **30.0 µL** DNA sample and mix well. For loading multiple lanes with the same sample, scale up the volumes proportionally. The loading solution is viscous, so pipette slowly to ensure complete transfer into the DNA sample.

   a. Spin briefly to collect the contents at the bottom of the 1.5 mL tube.

4. Follow the manufacturer's recommendations to set up a run protocol.
   a. Select the "**0.75%DF Marker S1 High-Pass 6–10kb vs3**" Cassette Definition File.
   b. Using the "Range" selection mode, enter a desired "BPstart" value of 10000 and a "BPend" value of 50000.
   c. Be sure to assign a marker lane.
5. Load the S1 marker and samples into the gel cassette and start the run. Run time is approximately **4 hrs 30 mins**.
6. To maximize recovery of eluted DNA, wait at least **45 minutes** after the run terminates before removing the sample from the elution chamber.
   a. Collect the eluate (**~40 μL**) into a new 1.5 mL tube.
   b. Wash the elution well with **40 μL** PippinHT 0.1% Tween20 (supplied with the cassettes). Wait **1 minute** and add the recovered wash liquid to the eluted sample.
   c. Wash the elution well with **40 μL** of elution buffer and add the recovered wash liquid to the eluted sample.
   d. The total volume after pooling the recovered washes with the original eluted sample is **~120 μL**.
7. Repeat SMRTbell cleanup bead purification by following the instructions in Appendix A, eluting in **10 μL** elution buffer.
8. Dilute **1 μL** of eluted sample with **9 μL** elution buffer and measure DNA concentration with a Qubit system to calculate concentration. Using the same dilution, obtain library size distribution with an appropriate DNA sizing system.
9. Proceed to Sample Setup in SMRT Link to prepare the SMRTbell library for sequencing.

SageELF system size-selection workflow

Min DNA input to library prep	1.5 μg / SMRT Cell
Samples per run	≤ 2 (1 sample per cassette)
Run time	4.5 hrs

Required materials

Material	Part Number
SageELF system	Sage Science ELF0001
0.75% Agarose gel cassettes, marker 75	Sage Science ELD7510
SMRTbell cleanup beads	PacBio 102-158-300
DNA Lo-Bind microfuge tubes	Eppendorf 022431021
Magnetic separation rack compatible with 1.5 mL tubes	Any major lab supplier
Qubit fluorometer	ThermoFisher Scientific Q33238
Qubit 1x dsDNA HS assay kit	ThermoFisher Scientific Q33230
Elution buffer	PacBio 101-633-500
Femto Pulse system	Agilent Technologies, Inc. P-0003-0817

Verify that your SageELF system software is up to date and follow the size selection procedure below. For the latest SageELF user manual and guidance on size-selection protocols, contact **Sage Science**.

1. Starting after the nuclease treatment step (section 5) described in *Procedure & checklist — Preparing whole genome sequencing and metagenome libraries using SMRTbell prep kit 3.0* (102-166-600), perform a SMRTbell cleanup bead purification by following the steps outlined in Appendix A, eluting in **31 μL** elution buffer. Dilute **1 μL** of eluted sample with **9 μL** elution buffer and measure DNA concentration with a Qubit system to calculate total mass. After completing, proceed to step 2 below.

2. Follow the SageELF manufacturer's instructions to calibrate the instrument. A new calibration is recommended before each run.

3. Inspect the gel cassette:

    a. Ensure that the buffer wells are full.

    b. Ensure that there is no separation of the gel from the cassette.

4. Prepare the gel cassette by following the manufactures instructions.

5. Prepare **>1 μg** of SMRTbell library from step 1 in a final volume of **30.0 μL** of elution buffer for each sample.

6. Add **10.0 μL** of Marker 75 to the **30.0 μL** DNA sample and mix well.

    a. Spin briefly to collect the contents at the bottom of the 1.5 mL tube.

7. Follow the manufacturer's recommendations to set up a run protocol.

    a. In the "Protocol Editor" tab, click on the "New Protocol" button.

    b. Select the "0.75% 1–18kb v2" in the cassette definition menu.

    c. Select "size-based" for separation mode.

    d. Enter **3450** in the "Target Value" field and move the bar slider to select well #12.

    e. Save as a new protocol.

    f. On the Main screen, clear the previous run data, select cassette description, cassette definition and protocol, enter sample ID(s).

    g. Select in the Nest Selector the cartridge that will be run.

8. Load the sample/marker mixture into the gel cassette and start the run. Run time is approximately **4 hrs 30 mins**.

9. Once the run is complete collect **30 μL** of the respective fractions from the elution wells. Fractions of interest are typically ~11 kb, ~13 kb, ~15 kb and ~17 kb.

10. Check the sizes of all fractions collected on a Femto Pulse system.

11. Pool together fractions that have an average library size between 10–20 kb.

12. Repeat SMRTbell cleanup bead purification by following the instructions in Appendix A (at the end of this document), eluting in **10 μL** elution buffer.

13. Dilute **1 μL** of eluted sample with **9 μL** elution buffer and measure DNA concentration with a Qubit system to calculate concentration. Using the same dilution, obtain library size distribution with an appropriate DNA sizing system.

14. Proceed to Sample Setup in SMRT Link to prepare SMRTbell library for sequencing.

Short read eliminator

Required materials

Material	Part Number
Short read eliminator kit	PacBio SS-100-101-01
Centrifuge	Eppendorf 5425 or similar
Heat block (or water bath)	Any capable of 50°C
200 μL wide bore pipette tips	USA Scientific 1011-8410
200 proof ethanol, molecular biology or ACS grade	Any major lab supplier
Qubit fluorometer	ThermoFisher Scientific Q33238
Qubit 1x dsDNA HS assay kit	ThermoFisher Scientific Q33230
Elution buffer	PacBio 101-633-500

The Short read eliminator kit can be used on samples containing many 5–10 kb fragments prior to shearing to improve the sample genome quality number (GQN). The eluted gDNA sample is then processed with the standard workflow (including a final bead size selection) found in *Procedure & checklist — Preparing whole genome sequencing and metagenome libraries using SMRTbell prep kit 3.0*
(102-166-600).

Follow the manufacturer's protocol found in the **Short read eliminator handbook**, including volume and concentration sample requirements. Elute in 101 μL, allowing 1 μL for DNA quantification with the Qubit system.

Appendix A

SMRTbell cleanup bead purification

1. Add **1.0X** v/v of resuspended, room-temperature SMRTbell cleanup beads to each sample to be purified.

2. Mix by pipetting 10 times.

3. Spin briefly to collect the contents at the bottom of the 1.5 mL tube/s.

4. Leave at **room temperature** for **10 minutes** to allow DNA to bind to the beads.

5. Place the tube/s in a magnetic separation rack allowing beads to separate fully from the solution.

6. Slowly pipette off the cleared supernatant without disturbing the beads. Discard the supernatant.

7. Slowly dispense enough **freshly prepared 80% ethanol** to completely cover the beads on the side of the tube/s. After **30 seconds**, pipette off the 80% ethanol and discard.

8. Repeat previous step.

9. Remove residual 80% ethanol:

    a. Remove tube/s from the magnetic separation rack.

    b. Quick spin the tube/s.

    c. Place tube/s back in the magnetic separation rack until beads separate to the side of the tube/s.

    d. Pipette off residual 80% ethanol and discard.

10. Remove tube/s from magnetic rack. **Immediately** add volume of elution buffer specified in respective sections above and mix by pipetting 10 times to resuspend beads.

11. Spin briefly to collect the contents at the bottom of the tube/s.

12. Leave at **room temperature** for **5 minutes** to elute DNA.

13. Place tube/s in a magnetic separation rack until beads separate fully from the solution.

14. Slowly pipette off the cleared supernatant without disturbing the beads. Transfer supernatant to a **new tube**. Discard old tube with beads.

15. Proceed to next step in respective sections above.

PacBio