Technical note

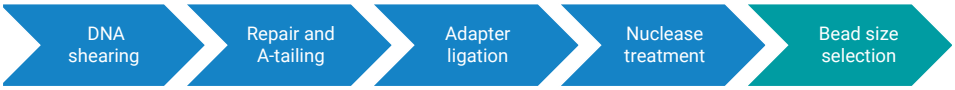
# GEL CASSETTE SIZE SELECTION METHODS FOR WGS HIFI LIBRARIES

## Introduction

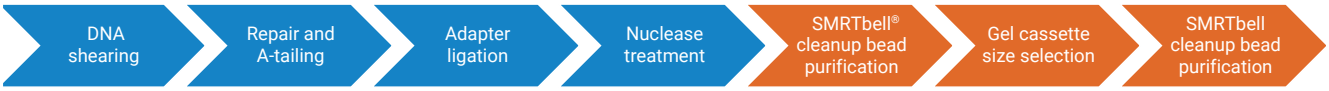
This technical note provides guidance for size selection of whole genome sequencing (WGS) HiFi libraries for sequencing on Sequel® II or Revio™ systems.

| | Size-selection beads (Standard) | Gel cassettes |
|---|---|---|
| **Size cutoff** | Fixed (progressive depletion up to 5 kb) | ✓ Adjustable |
| **Hands-on time** | ✓ Low | ✓ Low |
| **Run time** | ✓ Minutes | Hours |
| **Automation** | ✓ Standard liquid handler | Separate instrument |
| **Cost** | ✓ Low | High |
| **DNA recovery** | ✓ High | Low |
| **DNA input to library prep per SMRT® Cell** | 2 µg/Revio SMRT Cell<br>1 µg/Sequel II SMRT Cell | 3 µg/Revio SMRT Cell<br>1.5 µg/Sequel II SMRT Cell |

### Standard protocol with size-selection beads




### Gel cassette size selection




### 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 |
| **LightBench** | Yourgene Health | 12 | 3 hrs | High pass |

PacBio

## PippinHT system size-selection workflow

| | |
|---|---|
| **Min DNA input to library prep** | 1.5 µg/Sequel II SMRT Cell<br>3.0 µg/Revio 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/Sequel II SMRT Cell<br>3.0 µg/Revio 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/Sequel II SMRT Cell<br>3.0 µg/Revio 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.

## LightBench system size selection workflow

| | |
|---|---|
| **Min DNA input to library prep** | 1.5 µg/Sequel II SMRT Cell<br>3.0 µg/Revio SMRT Cell |
| **Samples per run** | 12 samples |
| **Run time** | 3 hrs |

## Required materials and equipment

| **Equipment** | **Part number** |
|---|---|
| *LightBench®* | CG-12500-03 |
| **Materials** | |
| *Cassette and Dual Dye Loading Buffer* kit | CG-14100-13-050-31-31 |
| **Includes** | |
| *Dual Dye Loading Buffer* 7 kb + 7 kb markers | CG-14000-31-31 |
| 0.5% *In-Channel Filter Size-selection* cassette | CG-10600-13-050 |

### Workflow steps

1. Prepare the *Ranger® Technology* software setup according to Yourgene *LightBench®* recommendations.
2. Prepare the samples by combining with *Dual Dye Loading Buffer* containing the 7 kb marker (CG-14000-31-31). Mix thoroughly to homogenize and spin down to remove any air bubbles.
3. Prepare the cassette and remove excess buffer from the reservoir.
4. To load samples into the cassettes, remove recommended volume of buffer from each loading well, and dispense the full volume of aspirated samples into loading wells.
5. Allow the electrophoresis process to proceed.
6. For sample extraction, remove the cassette from the instrument and rinse the extraction wells.
7. Refill extraction wells with 1×TBE buffer and insert the *In-Channel Filter* (ICF) array into the extraction well. Fill ICF collection chambers with 1×TBE buffer.
8. Return the cassette to the *LightBench®* drawer tray and allow the extraction process to proceed. Extract targeted material when prompted by the software.
9. Size selected fractions should be bead-cleaned and assessed for suitable concentration and fragment length distribution prior to sequencing.

# 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.



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