

# Blue Pippin™ User Guide

## High-Pass™ DNA Size Selection of ~20kb SMRTbell™ Templates

for PacBio® System Sequencing

Agarose Gel Cassette Kit

**P/N BLF7510**

Cassette Definition

**0.75%DF Marker S1 high-pass 4-10kb vs2**

software v.5.91

Thank you for purchasing BluePippin High-Pass gel cassettes for size-selection of SMRTbell™ templates. The system is designed to collect DNA above a user-set threshold. Size-selection of ~20kb SMRTbell templates prior to PacBio® System Sequencing results in significantly longer subread lengths.

### Using this Guide

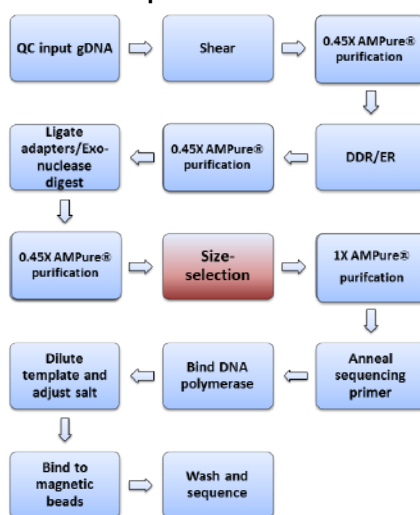
This guide is meant to provide a comprehensive workflow for using the BluePippin to size-select ~20kb SMRTbell templates (prepared according to PacBio's SampleNet protocol "Guidelines for Preparing Size-Selected ~20kb SMRTbell™ Templates" available for download on Pacific Biosciences' SMRT® Community website, [www.smrtcommunity.com](http://www.smrtcommunity.com), under *Sample Net / Shared Protocols*).

The instructions in this document include software, hardware, and gel cassette usage. However, users should read the **BluePippin Operations Manual** to completely familiarize themselves with the system and features, and safety and warnings.

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### Size Selection step within the SMRTbell workflow



Users should direct their attention to the area of the screen indicated by the yellow circle.

### Conventions

In this guide, procedural steps are numbered and additional comments are provided in blue. For software steps, a small representation of a screen is provided to the right of the step with a yellow circle that indicates the area of the screen that is being referenced.

### Installing pre-set protocols

To load the pre-set protocol definitions, go to [www.sagescience.com/support](http://www.sagescience.com/support), and:

1. Make sure your BluePippin software is the current version.
2. Download the PacBio cassette definition set (0.75%DF Marker S1 high-pass 4-10kb vs2) to a USB drive.
3. Follow the software update instructions used for software version updates.

### Downloads

#### Software, BluePippin

- BluePippin v5.91 cassette definition set 10
- PacBio SMRTbell cassette definition set
- software update instructions

## A. Preparing DNA samples

1. Dilute up to 5µg SMRTbell template into a final volume of 30 µl EB (PacBio Elution Buffer).

**Note:** Run 500 ng to 5 µg SMRTbell template per lane. We do not recommend running less than 500 ng per lane.

2. Bring loading solution to room temperature and add 10 µl of loading solution to the 30 µl sample.

**Note:** Loading solution is viscous; pipet slowly to ensure complete transfer of the loading solution into the DNA sample.

3. Mix thoroughly by gentle pipetting. Do not vortex. Spin briefly to collect the contents at the bottom of the tube.
4. Bring the DNA marker (Marker S1) to room temperature.

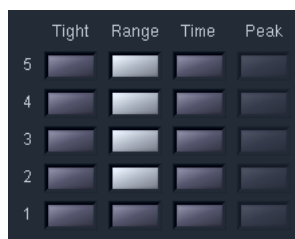
## B. Creating a Protocol

The following instructions show how to program the size selection protocol for SMRTbell template preparation. Optional pre-programmed protocols may also be loaded onto a BluePippin, and are shown in the gray box on the next page.

1. In the BluePippin Software, click the Protocol Editor Tab.
2. Press the “NEW” button, to program a new protocol.

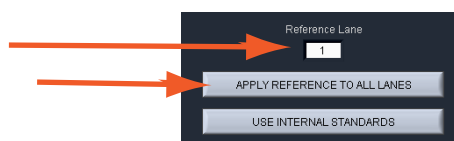
3. Click “Cassette Type” folder icon, and select the “0.75% DF Marker S1 high-pass 4-10kb vs2” cassette definition from the expanded menu selection.

4. Select “Range” mode for the corresponding sample lanes to which the samples will be run. Do not select a collection mode for the sample lane to which the DNA marker will be added.



In this example, DNA reference marker will be loaded into lane 1, and the DNA samples will be loaded into lanes 2-5.

5. Enter the lane number to which the DNA marker will be loaded into the “Reference Lane” field, and press the “APPLY REFERENCE TO ALL LANES” button.



- 6. Enter base pair threshold (BP Start ) for DNA size selection. For input DNA amounts between 2-5 µg enter 10000, for amounts between 0.5-2 µg enter 7000.
- 7. Enter 50000 into the (BP End ) field. Examples of correctly programmed protocols are shown below.



### B.1 Loading optional pre-set protocols for SMRTbell™ ~20kb templates

The protocols may be downloaded from [www.sagescience.com/support](http://www.sagescience.com/support). Use the typical software update instructions. See page 2 for more information.

- 1. Press the “LOAD” button.



- 2. Select the appropriate SMRTbell protocol from the protocol list.



- 3. The protocol names and settings are shown below.



#### Protocol: SMRTbell 20kb, 10000bp High-Pass

	Tight	Range	Time	Peak	Ref Lane	BP Target	BP Start	BP End	BP Pause
5					1	30000	10000	50000	0
4					1	30000	10000	50000	0
3					1	30000	10000	50000	0
2					1	30000	10000	50000	0
1					1	0	0	0	0

#### Protocol: SMRTbell 20kb, 7000bp High-Pass

	Tight	Range	Time	Peak	Ref Lane	BP Target	BP Start	BP End	BP Pause
5					1	28500	7000	50000	0
4					1	28500	7000	50000	0
3					1	28500	7000	50000	0
2					1	28500	7000	50000	0
1					1	0	0	0	0

8. Enter Sample information into the “Sample ID” fields (optional).

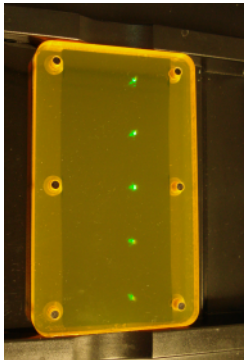


9. Press “Save As” to name and save a new protocol.



### C. Calibrating the Optics with the Calibration Fixture

- 1. In the BluePippin Software, click the Main Tab.
- 2. Place the calibration fixture onto the optical nest. The dark side of the fixture must be down, and completely cover all LED detectors.



Correct placement of the calibration fixture.

- 3. Close the Lid.
- 4. Press the “CALIBRATE” button.



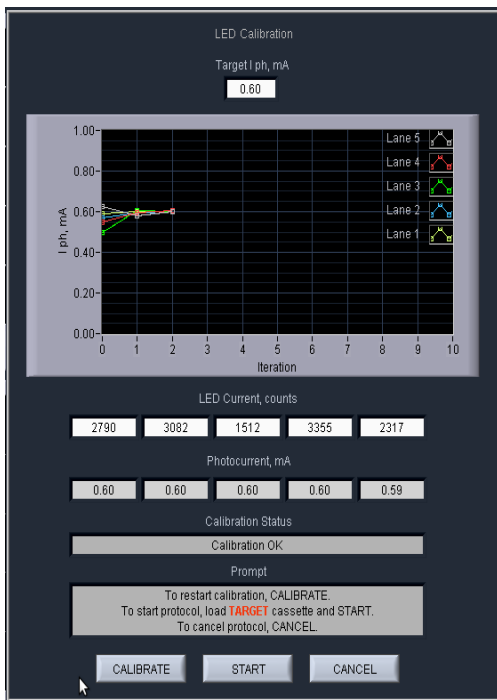
5. A calibration sub-window will launch.



6. Check that the calibration target box (“Target I ph, mA”) at the top of the window reads “0.60”. If not, manually change the target value to “0.60”.



7. Press “CALIBRATE” button within the calibration window.

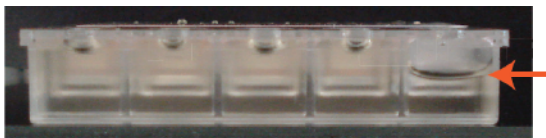


The Calibration sub-window after a successful calibration. If the status window reads “Calibration OK”, proceed to the next step.

8. Press “EXIT” to return to the Main Screen.

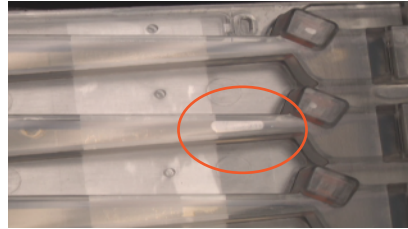
### D. Inspecting the Gel Cassette

1. Remove the gel cassette from the foil packaging.
2. Inspect the levels of buffer in all buffer reservoirs. Reservoirs should be nearly full. If the buffer level in any reservoir appears lower, or less than 50% full, fill with spare electrophoresis buffer (provided with the BluePippin reagent kit).



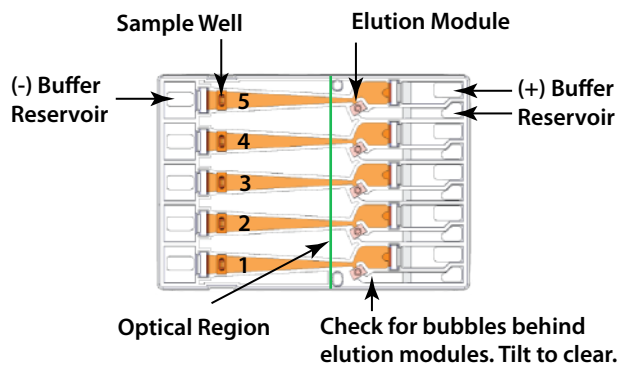
If a buffer reservoir is low, fill with spare buffer.

3. **Inspect for bubbles due to delamination of agarose from the bottom of the cassette in the region used for optical detection of DNA.** To inspect, turn the cassette upside down and gently tilt the cassette under a light source. If a bubble is detected, do not use the affected lane to run the DNA reference marker. Affected lanes can be used for size selection.



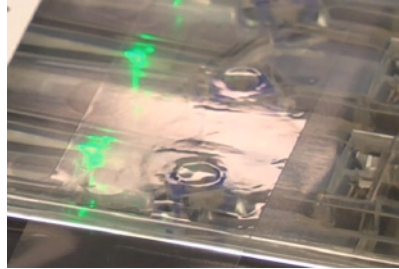
If the agarose has delaminated in the optical region, do not use that lane to run the reference marker.

## E. Preparing the Cassette for Loading



1. **Dislodge air bubbles from behind the elution wells.** Tilt the cassette sample well side down, and tap gently to release the bubbles.
2. **Place the cassette into the optical nest.** Keep the cassette slightly tilted down so that the air bubbles do not return to the area behind the elution modules. Make sure the cassette is fully seated into the bottom of the nest to ensure proper optical alignment.
3. **Remove the white tabbed adhesive strips from the cassette.** Place one hand on the cassette, and hold it firmly in the nest. Grab the white tabs and pull the strips firmly and slowly toward the front of the instrument until they are removed.
4. **Completely remove all buffer from each elution module, and replace with 40 µl of fresh electrophoresis buffer** (provided with the BluePippin reagent kit). When refilling, dispense the fresh buffer slowly, raising the pipette tip slowly from the bottom of the elution module, to avoid trapping air bubbles in the module.

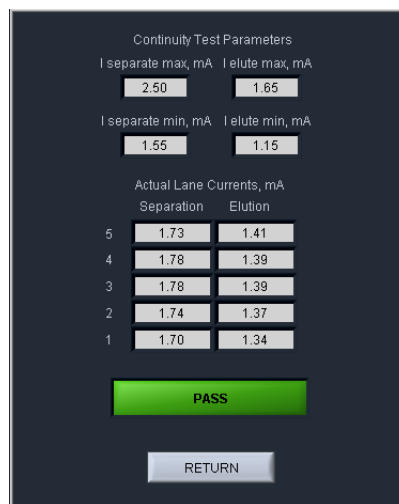
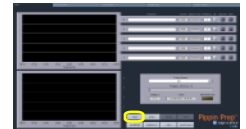
5. **Re-seal the elution wells with an adhesive tape strip (supplied with cassette packaging).** Place the tape over the elution wells and rub firmly to seal the elution ports. The port should be tightly sealed without any wrinkles around the edges of the port.



Rub firmly to ensure a tightly sealed elution port.

6. **Close the lid.**

7. **Perform the Continuity Test.** Press the “Test” button. The test sub-window will open, and the test routine will automatically measure the current in each separation and elution channel. A successful continuity test will return a “PASS” indication. The cassette temperature must be above 17°C (62°F). If a lane fails due to low elution current (the affected lane will be highlighted with an orange color), refilling the elution module (see step 4 above) will usually resolve the problem.

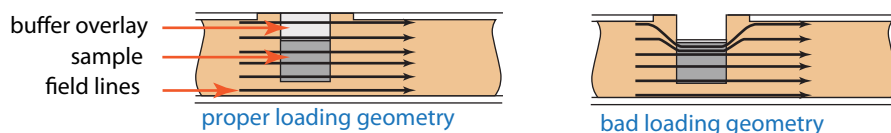


A successful continuity test will return a “PASS” indication.

8. **Press “Return” to close the Continuity Test sub-window.**



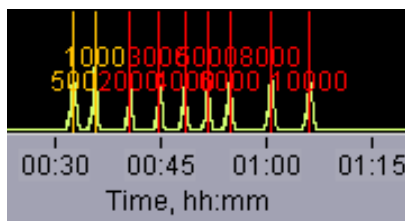
## F. Loading Samples



1. **Check the buffer levels in the sample wells.** Make sure that sample wells are completely full to the top with electrophoresis buffer. Fill with additional buffer if necessary.
2. **Remove 40µl of buffer from the first sample well, and load 40µl of sample into that well.** Take care not to pierce the agarose with the pipette tip. There is gel on all sides and at the bottom of the sample well. In addition, there is an agarose “chimney” surrounding the top of the sample well that protrudes up through the cassette cover. When removing buffer, some users find it useful to immerse the pipette tip just below the surface of the buffer and follow the liquid level down with the tip as the buffer is removed. When buffer removal is completed, there will be ~30µl of buffer left in the well. When adding sample, place the tip of the pipette just below the surface of the buffer, and slowly eject the sample. Don't be concerned if the sample well slightly overfills. Then density of the sample will allow it to sink before it can flow out of the well.
3. Repeat step 2 for the remaining four wells.

## G. Running

1. Close the lid, go to the Main Tab, and make sure the proper protocol is loaded in the “Protocol Name” field.
2. Press “START”. The run will automatically stop when every collection is complete.



Run time: 2 hours

DNA Marker S1 should appear as shown at the end of the run.

## H. Collecting Fractions

1. **Remove samples using a standard 100-200µl pipette.** Samples will be in a Tris-TAPS buffer at a volume of approximately 40µl. Do not let samples remain the cassette overnight.
2. **Remove the cassette and dispose of properly.** Do not keep used cassettes in the BluePippin with the cover closed. Humidity from the cassette may cause damage to the electrodes.