

Blue Pippin™ User Guide

High Pass DNA Size Selection

High Pass Plus™ Gel Cassettes

Part No.: BPLUS10

Using this Guide

This guide provides a comprehensive workflow for using the BluePippin for High-Pass DNA size-selection using High Pass Plus™ gel cassettes. High-Pass size selection is a process the BluePippin collects DNA fragments above a size threshold.

The instructions here provide special recommendations for software, hardware, and gel cassette usage for High-Pass DNA size selection. However, users should read the **BluePippin Operations Manual** to completely familiarize themselves with the system and features, and safety and warnings.

Cassette Kit Part No. BPLUS10	
High-Pass Threshold	Cassette Definition
>15 kb	15 Kb High Pass Plus Marker U1
>20 kb	20Kb High Pass Plus Marker U1

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A. Preparing DNA samples

1. Dilute up to 10 µg of a sheared DNA sample into a final volume of 30 µl TE .
2. Bring loading solution to room temperature and add 10 µl of loading solution to the 30 µl sample (40 µl total).
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 to room temperature.

B. Creating a Protocol

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 one of the cassette definitions shown below.

For software steps, a small representation of the screen is provided, with a yellow circle that indicates the area of the screen that is being referred to in the text.



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>15 kb	15 Kb High Pass Plus Marker U1
>20 kb	20Kb High Pass Plus Marker U1

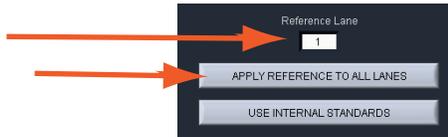
4. Select "Range" mode for the corresponding sample lanes to which the samples will be run. Do not select a collection mode for Lane 4 into which the DNA marker will be added.



Marker U1 must be loaded into Lane 4

Select Range Mode and de-select Lane 4 (for loading Marker U1)

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.



Cassette Definition	Enter in "BP Start" field
15 kb High Pass Plus Marker U1	15000
20 kb High Pass Plus Marker U1	20000



Note: The "BP End" values are automatically populated.

6. Enter Sample information into the "Sample ID" fields (optional).



7. Press "Save As" to name and save a new protocol.



Correct High Pass Plus protocol entry:

Cassette Type: BPLUS10
 Cassette Definition : 15kb High Pass Plus Marker U1
 High-Pass Threshold: 15,000 bp in lanes 1,2,3 and 5
 External Marker: MarkerU1 must be loaded into Lane 4

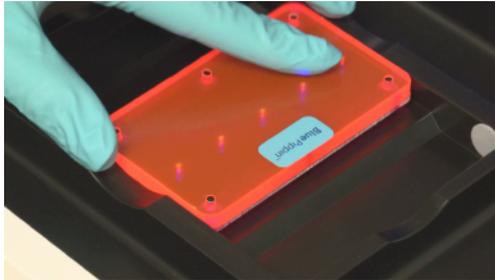
	Tight	Range	Time	Peak	Ref Lane	Target *	Start *	End *	Pause *
5					4	82500	15000	150000	0
4					4	0	0	0	0
3					4	82500	15000	150000	0
2					4	82500	15000	150000	0
1					4	82500	15000	150000	0

Cassette Type: BPLUS10
 Cassette Definition : 20kb High Pass Plus Marker U1
 High-Pass Threshold: 20000 bp in lanes 1,2,3 and 5
 External Marker: MarkerU1 must be loaded into Lane 4

	Tight	Range	Time	Peak	Ref Lane	Target *	Start *	End *	Pause *
5					4	85000	20000	150000	0
4					4	0	0	0	0
3					4	85000	20000	150000	0
2					4	85000	20000	150000	0
1					4	85000	20000	150000	0

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.



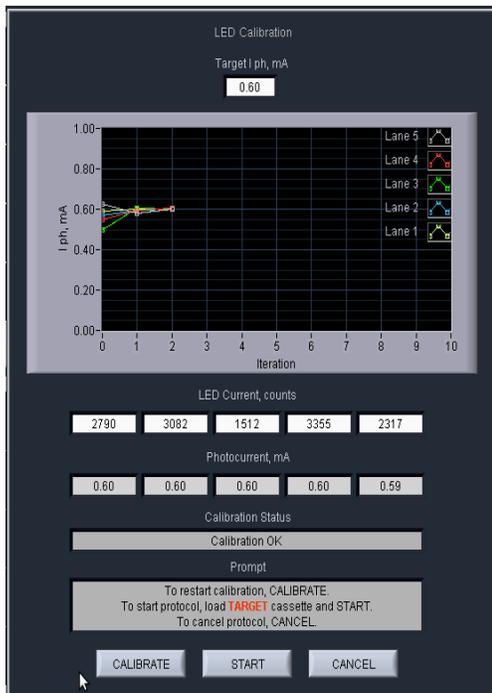
5. A calibration sub-window will launch.



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.



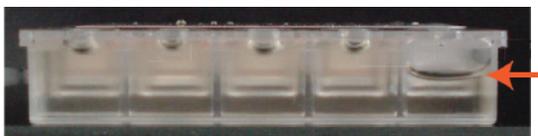
The Calibration sub-window after a successful calibration. If the status window reads “Calibration OK”, proceed to the next step.

Note: The optical calibration routine ensures that each LED light intensity is at the correct level (0.60 mA, in this case). The LEDs will adjust individual current (counts) values to achieve this intensity. If the count level is <200, the test will fail, likely due to light infiltration such as an open lid. The maximum count value is 4095, and if the LED cannot achieve the proper intensity at that level, the test will fail due to a faulty LED.

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).



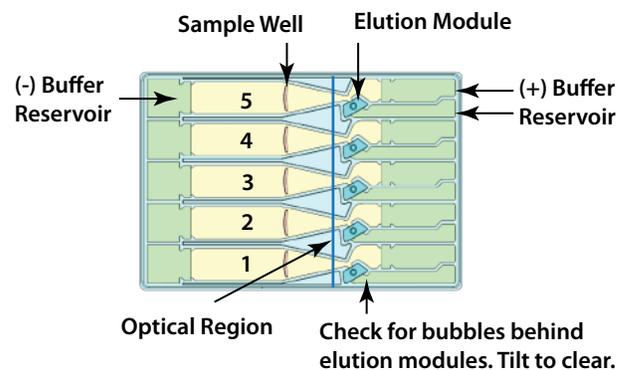
If a buffer reservoir is low, fill with spare buffer.

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 of sample DNA.



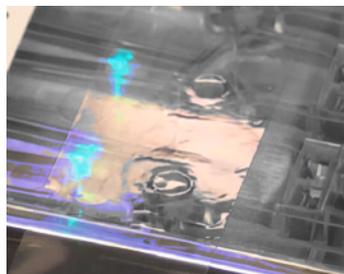
If the agarose has delaminated in the optical region, do not use that lane to run the reference marker.

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 80 μ l 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.

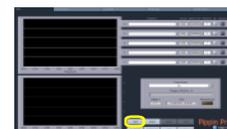
5. **Re-seal the elution wells with an adhesive tape strip (supplied with cassette packaging).** Place the tape over the elution wells and rub firmly to seal the elution ports. The port should be tightly sealed without any wrinkles around the edges of the port.



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.



Continuity Test Parameters

I separate max, mA	I elute max, mA
3.60	2.50
I separate min, mA	I elute min, mA
2.30	1.80

Actual Lane Currents, mA

	Separation	Elution
5	2.75	2.21
4	2.83	2.19
3	2.81	2.13
2	2.81	2.25
1	2.78	2.24

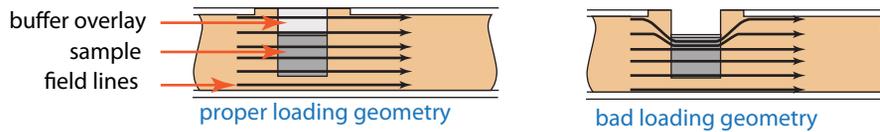
PASS

RETURN

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

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

F. Loading Samples

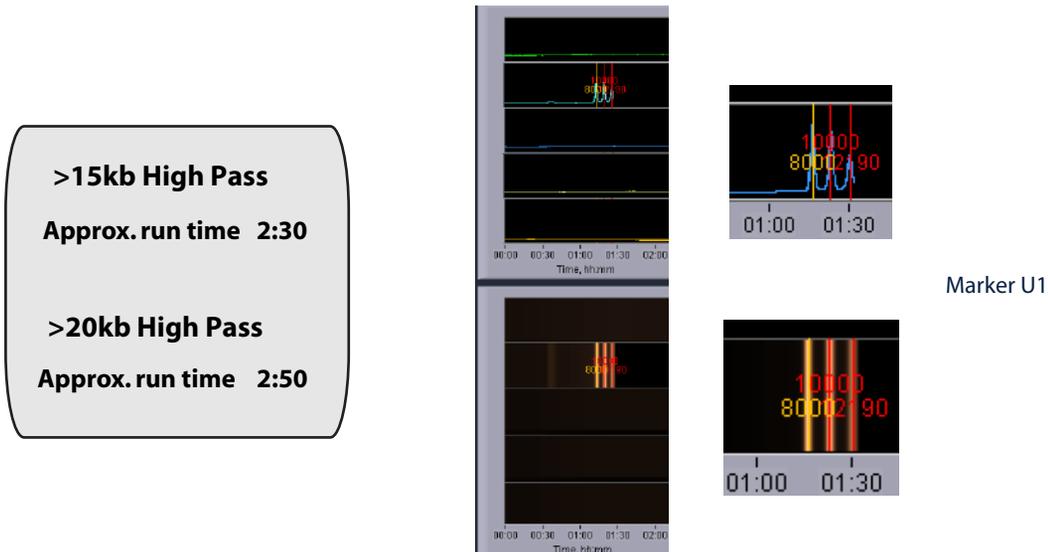


1. **Check the buffer levels in the sample wells.** Make sure that sample wells are completely full to the top with electrophoresis buffer. Fill with additional buffer if necessary.
2. **Remove 40 μ l of buffer from the first sample well, and load 40 μ l of sample or marker into that well.** Take care 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 ~70 μ l 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. The 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.

Note: Cassettes may be reused if 2-3 lanes are used (1-2 samples). Reseal the cassette with the adhesive tape provided and store at RT. However one remaining lane must again be loaded with the external marker for calibration.

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. Electropherogram images of the DNA markers in the Mains screen should appear as shown below. In either case the marker peaks should be detected in about 1.5 hours.



H. Collecting Fractions

1. For best recovery, wait at least 30-45 minutes after end of run before removing samples from the elution modules.
2. Remove samples using a standard 100-200µl pipette. Aspirate slowly to minimize breakage of large DNA fragments. Samples will be in a Tris-TAPS buffer at a volume of approximately 80µl.
3. **Optional: Additional 10-30% yield can often be achieved by rinsing the elution well with TE+0.1% Tween 20.** Tween solution is provided by Sage Science with cassette kits for HMW collections.
 - Add 80µl Tween solution (supplied with the cassettes) to the elution well
 - Wait 1 minute
 - Remove solution and pool with the original extracted sample, or process separately
4. Remove the cassette and dispose of properly. Do not keep used cassettes in the Pippin with the cover closed. Humidity from the cassette may cause damage to the electrodes.

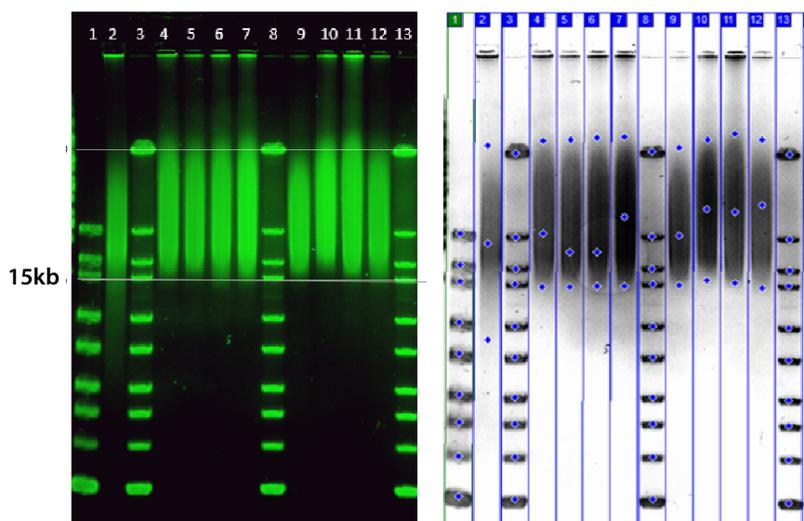
 **Important!** Qubit fluorometry is recommended for determining DNA concentrations of eluted samples. UV absorbance-based methods (eg. Nanodrop) give inaccurate results on post-Pippin products: an artifact from the cassette manufacturing process interferes with accurate quantification.

I. Gel Analysis Images of High-Pass Collections

The following shows analytical gel images of sheared samples after size selection with High Pass protocols. Gels were run using the Pippin Pulse pulsed-field electrophoresis power supply (PPI 0200).

>15kb High Pass Plus

Input: 2 µg DNA (lane 2)



>20kb High Pass Plus

Input: 2 μ g DNA (lane 2)

