Operation of the Blue Pippin for Size Selection

I. Purpose

To operate the Blue Pippin in order to size select DNA from miRNA libraries.

II. Scope

All procedures are applicable to the BCGSC (Library Core) Group.

III. Policy

This procedure will be controlled under the policies of the Genome Sciences Centre, as outlined in the Genome Sciences Centre High Throughput Production Quality Manual (QM.0001). Do not copy or alter this document. To obtain a copy see a QA associate.

IV. Responsibility

It is the responsibility of all personnel performing this procedure to follow the current protocol. It is the responsibility of the (Library Core) Group Leader to ensure personnel are trained in all aspects of this protocol. It is the responsibility of Quality Systems Team to audit this procedure for compliance and maintain control of this procedure.

V. References

Document Title	Document Number
N/A	N/A

VI. Related Documents

Document Title	Document Number
Automated MicroRNA Library Construction using randomized adapters for Illumina Sequencing	LIBPR.0136

VII. Safety

All Laboratory Safety procedures will be complied with during this procedure. The required personal protective equipment includes a laboratory coat and gloves. See the safety data sheet (SDS) for additional information.

VIII. Materials and Equipment

Name	Supplier	Number	Model or Catalogue #	
Gilson P10 pipetman	Mandel	GF-44802		✓
Gilson P200 pipetman	Mandel	GF-23601		✓
Diamond Filter Tips 10 µL	Mandel	GF-F171203		✓
Diamond Filter Tips 200 µL	Mandel	GF-F171503		✓
Blue Pippin	D-Mark	SAG-BLU0001		✓
3% Agarose, dye-free. w/internal standards, Blue Pippin, 100-250bp, Marker Q3	D-Mark	SAG-BDQ3010		✓
Safe-Lock Tubes, 1.5 mL	Eppendorf	022363604		✓

IX. Procedure

1. Blue Pippin Start up

1.1. **Powering up the Blue Pippin**. The Blue Pippin instrument has to be rebooted each time it is used in order to connect to the server. Once it is turned off it loses the connection. Either reboot or if the device is turned off prior to use, locate the black power button at the rear of Blue Pippin. Push the button and the Blue Pippin will start up.

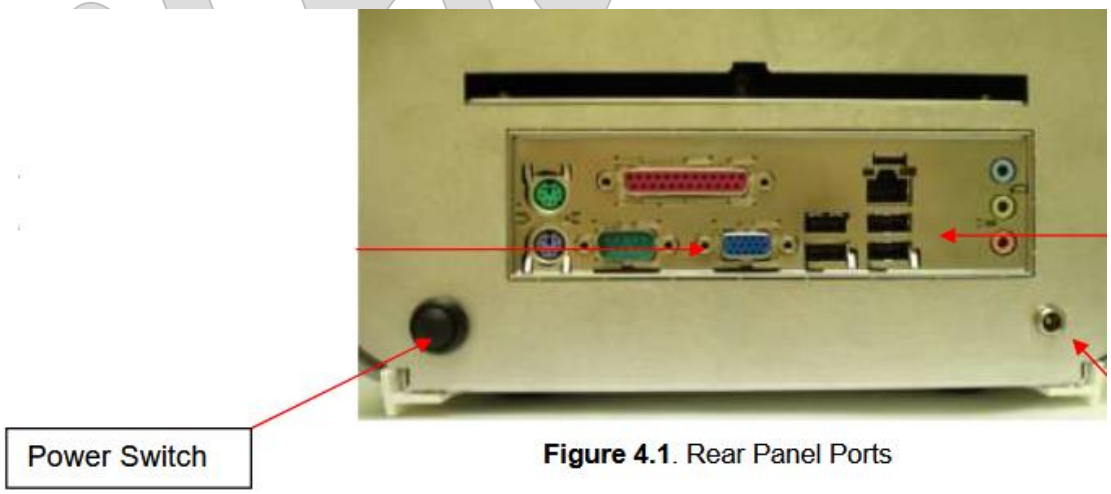


Figure 4.1. Rear Panel Ports

1.2. A powered up Blue Pippin is indicated by a blue light on the front of the Blue Pippin. When samples are running a green light also appears (See Figure 4.3).

Figure 4.3. BluePippin front panel.

2. Sample Preparation for loading

2.1. Discuss with the APC which samples you will be loading and how many lanes you will need. A maximum of 5 lanes can be loaded per cassette and a max sample volume of 30 µL (+ 10 µL of loading solution) can be loaded per lane of the cassette.

2.2. With your pre-pooled sample, consider the volume of the sample. If the sample volume is less than 30 µL, bring up the volume of the sample to 30 µL with TE buffer (pH 8.0).

2.3. After sample is brought up to 30 µL, add 10 µL of loading solution (contains internal standards) to bring the final volume to 40 µL. The loading solution is stored at 4°C. Mix samples thoroughly (vortex mixer). Briefly centrifuge to collect.

2.4. Sample or samples are now ready to load onto the Blue Pippin.

3. Selecting software parameters for size selection.

3.1. The number of lanes being run will vary depending on the instructions from the APC, thus a need to setup program software may vary from run to run. The basic layout of the software can be seen below:

3.2. If running less than the full 5 lanes of the cassette, follow the instructions here. If running all 5 lanes, proceed to next step. For the lanes not being run, go to the “Type of cut” column and deselect the unused lanes (lanes will go dark grey). Next go to “Ref lane” column and turn “off” the unused lanes. Next go to the “LED On” column and deselect the unused lanes (lanes will go dark grey). Proceed to next step in order to set parameters for the lanes being run.

3.3. Select the "Protocol Editor" tab, then select "New" from the File Commands, then click on "Cassette Type Selector". Depending of the samples being run, select a cassette type (i.e. for miRNA5, select 3% agarose, dye-free).

3.4. Make sure the "Run Time End Selector" has the "End Run when Elution is Completed" box checked off.

3.5. Next go to the "Protocol Name" box and enter the run name, Project/library type/date/initials (i.e. SU2C_miRNA5_190416SSC).

3.6. Next, from the "Protocol Parameters" select the lanes that will be run (1-5). For the purposes of miRNA5 we will be selecting the range parameter. Once the range is

selected, you will need to input a BP range for the run. For miRNA5 input 140bp for "BP start" and 150bp for the "BP end" (140-150bp range). A bp average will automatically be calculated once a range is input. Make sure to input a range for each lane selected.

3.7. Proceed to the DNA Marker/Standards Lane Assignment section. For miRNA5 samples, select "Use Internal Standards". This will auto fill the "Ref Lane" fields with reference marker internal to the sample. The software will determine the timing of elution based on the migration of the DNA marker within its own lane.

3.8. Examine the "warnings text box" for any warnings or errors. The range selected for the miRNA5 will produce a warning indicating the range is smaller than the minimum. This will be ok and run will proceed. The range flag will be highlighted "yellow".

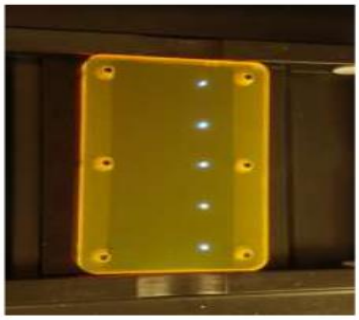
3.9. Select "Save" from the file commands menu.

4. Optical Calibration

4.1. An optical calibration must be run on the Blue Pippin before each run. Complete this step now. Remove the Calibration Plate from its sleeve and place in the optical nest. Make sure the top of the plate faces up and the LED's are covered by the grey section of the optical plate (See image below). **Close the lid of the Blue Pippin.**

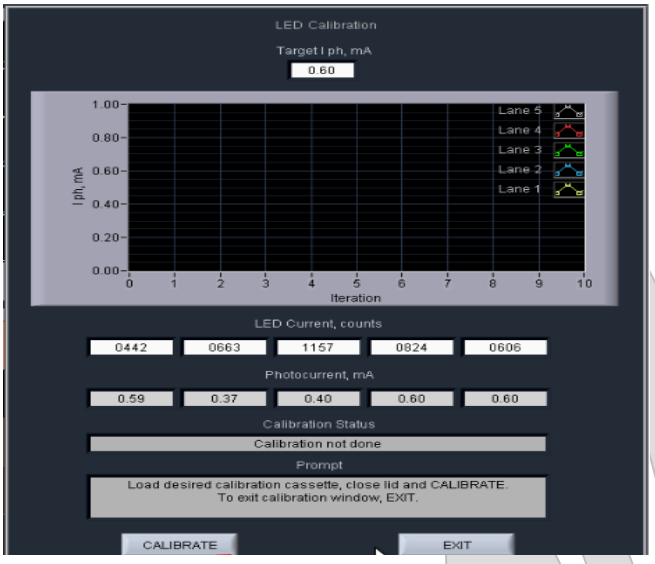
Top

Bottom



Correct Position
on Optical Nest

4.2. Once the calibration plate is accurately placed within the optical nest and the lid is shut, select the "Main tab" from the software. Near the bottom of the screen look for the "Calibration" tab. Click the "Calibration" tab. A new window will open containing LED Calibration. Press "Calibrate". The Calibration will be done within 30 seconds and will produce a calibration status of "Calibration OK" or " Calibration Failed". If "Calibration OK" appears, open lid and remove the optical plate and return it to the sleeve. Proceed with the next step.



5. Prepping Cassettes

5.1. Before loading the samples into the cassettes some prep work will need to be done. Open a new or previously run (with unused lanes) 3% Agarose, dye-free 100-250 bp cassette located at room temperature next to the Blue Pippin. Examine the cassette, if there are any bubbles try concentrate them to one location by tilting and moving the cassette. Once again examine the ends of the cassette to check the level of the buffer in the wells. Inspect each lane for gel quality, if there are any broken/cracked gels avoid using that lane, remaining lanes can be used if the gel is ok in those lanes.

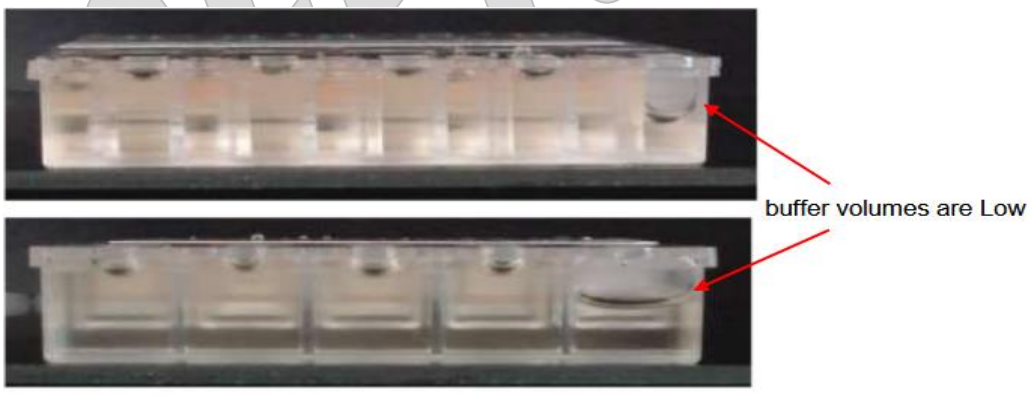
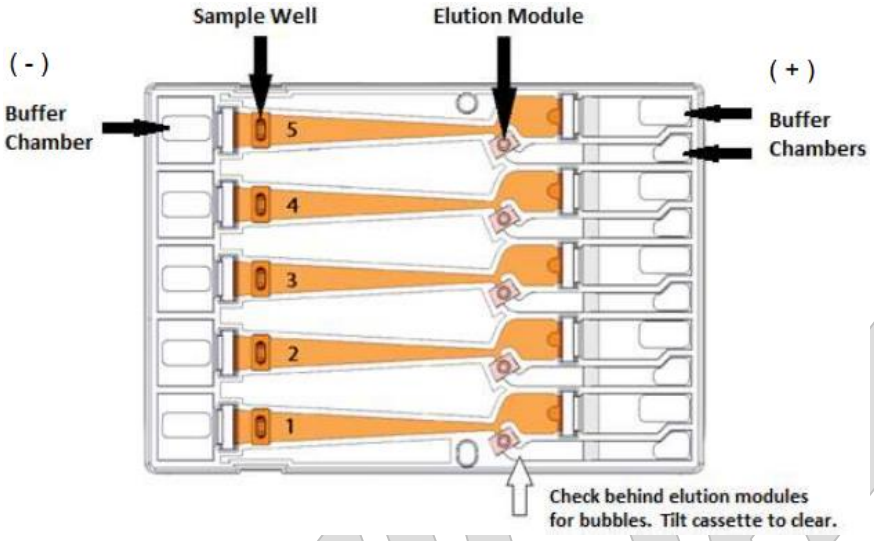


Figure 10.1. Low buffer levels in cassette buffer chambers.

5.2. In reference to the image below, look at the cassette and examine the area behind the elution module. If there are any bubbles present behind the elution module tilt the cassette in order to move the bubbles away from the elution module.



5.3. If there are no bubbles behind the elution module, place the cassette into the optical nest, while tilting the negative (-) down in order to minimize bubbles returning to the elution module.

5.4. While holding the cassette in the nest with one hand, remove the adhesive strip with the other hand. There are two strips, one covering the positive and one covering the negative end.

5.5. Using a P200 tip remove the existing buffer from the elution module (~40 µL) for each lane being run, replace with 40 µL of fresh Electrophoresis Buffer (supplied with the cassette kit). When refilling the elution module make sure the tip completely enters the module and be careful not to disperse any bubbles into the module.

5.6. Seal the elution wells with the supplied adhesive tape, using a sealing tool or sharpie ensure that the adhesive tape is firmly attached to the edges of the lanes.

5.7. Check the volume of the sample well and if needed top off with electrophoresis buffer. Max volume of the sample wells should be 70 µL.

5.8. **CONTINUTITY TEST:** In order to test the current in each separation and elution module a continuity test needs to be done. With the cassette in the optical nest, close the lid. In the "Main" tab of the software look for the "Test" tab, press the button. The

continuity test will proceed automatically. If the test returns a "PASS" message, press "RETURN" and continue to baseline calibration.
A failed test is indicated by a "FAIL" message, and the failed channel is highlighted in orange. If a separation lane has failed continuity. Do not use that lane. Remaining passing lanes can be used if necessary. If an Elution channel has failed continuity. Replace buffer in the elution module of the failed lane and refill with 40 µL of fresh electrophoresis buffer and retest the cassette. If the lane fails again, do not use the lane for collections.

6. Loading Cassettes

6.1. The total volume of the sample well is 70 µL. Using a 200 µL tip remove 40 µL from each sample well being run, leaving behind a residual volume of 30 µL (Reference section 5.2 for position of sample well). Be careful not to pierce the gel located on all four sides of the sample well. Start by pipetting from the top of the well and following the liquid down. The removed 40 µL of electrophoresis will be replaced by the sample + loading solution prepared in Section 2.3 (combined volume of 40 µL).

6.2. Using a 200 µL tip add the prepared sample + loading solution (40 µL) to the sample well. Add by pipetting just below the top of the liquid level in the well and follow the liquid up the well. If the volume slightly overfills that's ok, the sample will sink down into the bottom of the well.

6.3. Repeat Section 6.2 for remaining lanes.

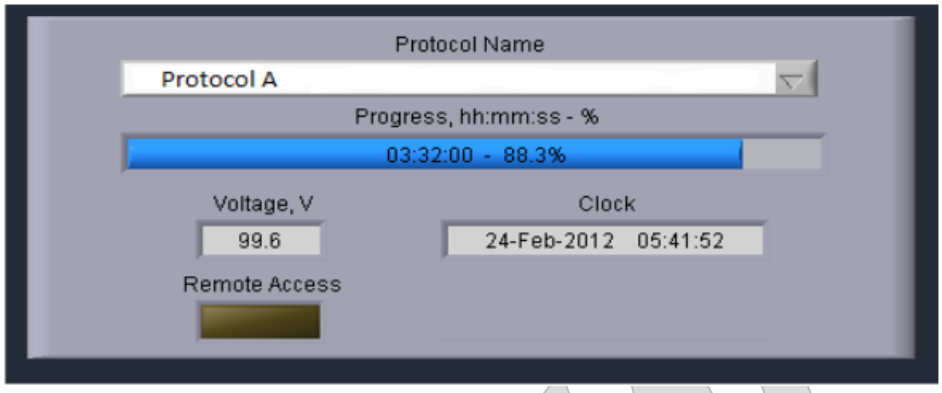
6.4. Close the lid of the Blue Pippin. Samples are now ready to run.

7. Starting the Blue Pippin run

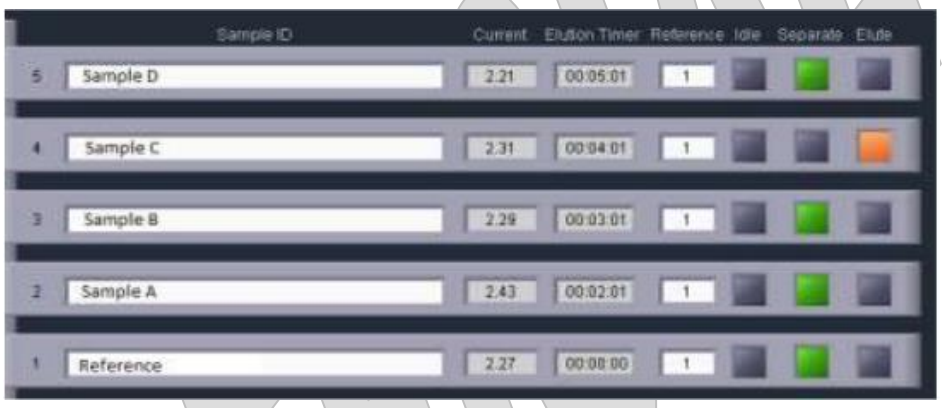
7.1. Click on "Main" tab on the software and scroll down to "Start" tab. Press "Start" tab.

7.2. A pop-up window will appear to remind users that the LED calibration and continuity tests were not inadvertently skipped. Press "OK" to start the run.

7.3. The run time for a miRNA5 run should be approximately 75 mins. A progress bar, as seen below, will track the progress of the run. A run may finish before the end of the progression bar is complete. If the display in Section 7.4 has reverted to idle in all the lanes being run, the run has finished.



7.4. When a protocol is running, the following indicator panel will be displayed:



-Green, Separation. If a lane is separating fragments, the "Separate" indicator will be green.
-Orange, Elution. If a lane is eluting a size range, the "Elute" indicator will be orange, and the elution timer will be active.

8. Sample collection

8.1. When the run is complete, open the lid of the Blue Pippin. Remove the adhesive tape from the elution modules.

8.2. Using a 200 µL pipette, pipette out 40 µL of sample from the elution module (Refer to Section 5.2). Transfer to a pre-labeled 1.5 mL safe-lock tube.

8.3. Sample can now be used for downstream applications.

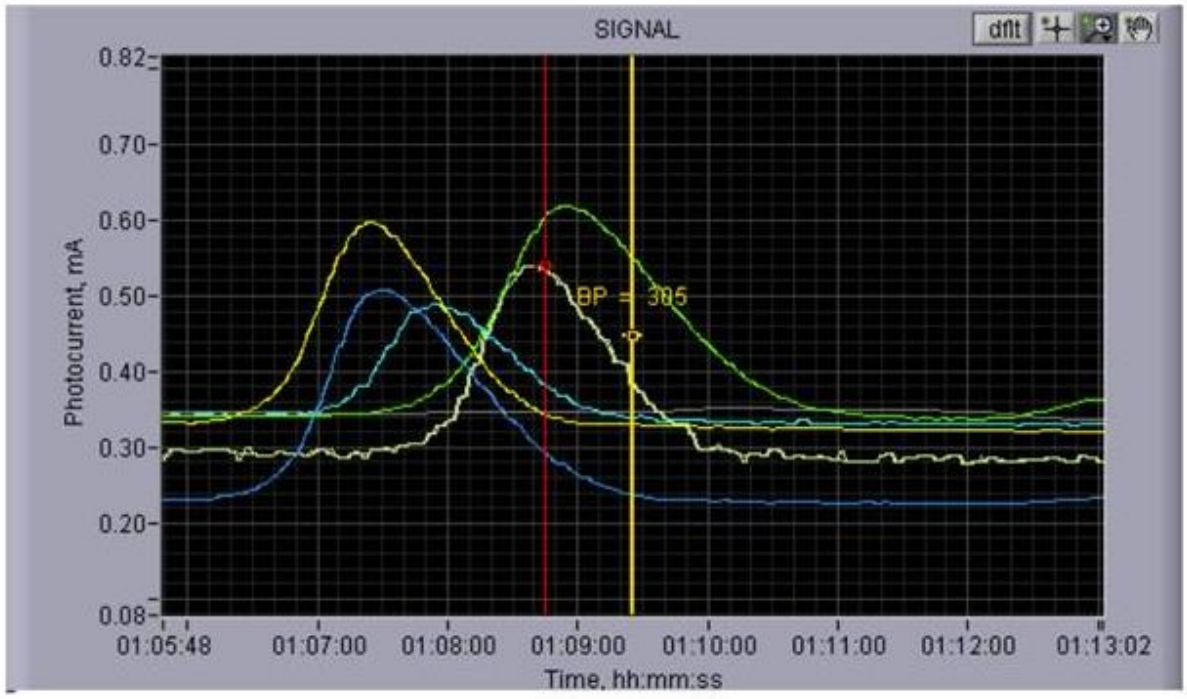
8.4. Reseal any lanes that have not been used and mark and date the lanes that have been used. Store cassette back into dark, dry pouch to be used at a later date. The cassette will remain useful until its original expiry date.

9. Data Analysis

Saving a Review Image

An image from the review screen may be captured and automatically saved by pressing the "Snapshot" button. The .png file will date and time stamped and saved in the Pippin log file directory.

The indicator may be moved (see below) along the x-axis showing the relative base pai position. If the perspective of the graph is changed (see below), the indicator will automatically default to the center of the x-axis.



10. Cleaning Blue Pippin

10.1. **IMPORTANT:** Cleaning will need to be done after every run. Retrieve the rinse cassette next to the Blue Pippin. Fill up the cassette to 3/4 full with de-ionized H_20. Open the lid of the Blue Pippin. Insert the cassette. Shut the lid for 30 seconds. Open the lid and remove the cassette, shake out the water from the cassette into a sink. Cleaning is now complete.