sage science

BluePippin™

DNA Size Selection System

Operations Manual



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The BluePippin instrument and gel cassettes are covered by U.S. patents ***8,361,298*** *and* ***8,361,299****.*

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1 Safety and Regulatory Compliance

1.1 Safety Warnings and Icons Used

In this manual, the following icons will be used to provide the user with information pertinent to the use of the BluePippin.



Caution! Warns the user that injury or instrument damage may occur if the contents of the warning are not properly followed.



High Voltage! Warns of the risk of electrical shock if the contents of the warning are not properly followed.



Important! Provide important information about the proper use of the system that may influence the quality of the result.



Information. Provides additional information regarding the function of the system or applications for which is used.

1.2 Safe Use Guidelines

Environmental Conditions
The BluePippin system is designed to operate under the following environmental conditions:

- Pollution Degree 2
- Installation category 2
- Altitude 2000m
- Indoor use
- Ambient temperature 17-23^{o}C
- Humidity 10-80%, non-condensing

Laboratory Precautions
Standard laboratory precautions should be taken when handling BluePippin Gel cassettes and operating the BluePippin:

- Wear a lab coat, safety glasses, and gloves.
- Use in proximity of an eye wash station and/or running water.

Flammability
The BluePippin housing is made from polycarbonate/acrylonitrile-butadiene-styrene (PC/ABS) plastic. The plastic has a UL 94 V0 rating.

- **94 V0 rating**. Burning stops within 10 seconds on a vertical part allowing for drops of plastic that are not in flames.

Caution! The BluePippin should only be used as outlined in this manual and for the uses outlined herein. Improper usage can damage the instrument or could cause physical harm.

1.3 Regulatory Compliance

The BluePippin system has been tested and found to be in compliance with all applicable requirements of the following safety and electromagnetic standards:

CE / European Union harmonization legislation:

2014/30/EU Electromagnetic Compatibility Directive (EMC)
2014/35/EU EU Low Voltage Directive

CSA / Canada and US compliance:

CLASS 8721 85 – electrical equipment for laboratory use - Certified to US Standards
CLASS 8721 05 – Laboratory Equipment - Electrical

1.4 RoHS Compliance

The BluePippin system conforms to the Directive (EU) 2015/863 amendment of the RoHS DIRECTIVE 2011/65/EU, Article 4(1): and do not contain any of the specified hazardous substances above the specified limits.

1.5 Disposal

DO NOT throw this unit into a municipal trash bin when this unit has reached the end of its lifetime. To ensure the utmost protection of the global environment and minimize pollution, please recycle this unit.

The BluePippin system uses **disposable agarose gel cassettes**. The components of these products contain no substances which, at their given aqueous concentrations, are considered to be hazardous to health or ecosystems. Use standard laboratory disposal practices for disposal. Check with facility or local municipal regulations.

2 Introduction

Thank you for purchasing the BluePippin from Sage Science. The BluePippin is an automated preparative gel electrophoresis system.

We urge you to read this manual to familiarize yourself with the system's capabilities and precautions.

2.1 System Overview

There are three components to the BluePippin system:

- **Instrument –** The instrument includes epifluorescence detectors, an electrophoresis power supply, an electrode array, and a single-board computer. The system computer is accessed by an external LCD monitor, mouse, and keyboard. The electrode array is located in the top of the sliding instrument cover and is not user-accessible.

- **Software** – System software allows the user to enter parameters that define the DNA fragment size ranges to be collected and to run the system. The software also logs fluorescence and electrical current data which may be analyzed in a review screen.

- **Gel Cassettes** – Pre-cast, disposable agarose cassettes are manufactured by Sage Science. Cassettes are supplied in kits that contain 10 cassettes and sufficient reagents to complete all collections. Kits have been developed to address DNA size range collections at various size ranges. Please refer to **Section 23** for kit descriptions and ordering numbers.

Specifications for the BluePippin Instrument

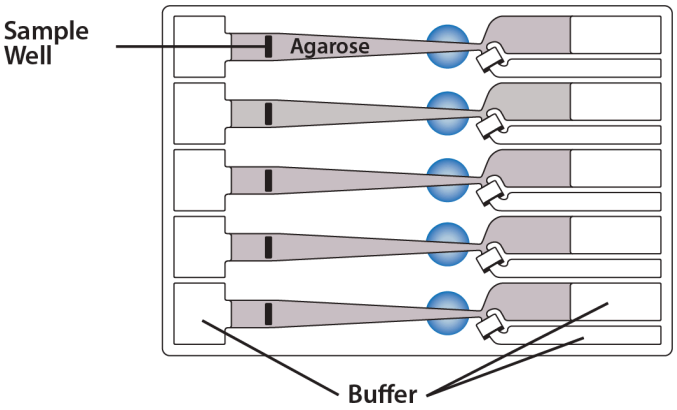
Electrophoresis Voltage	25V, 100V, 150V constant, 100 V pulsed
Optical detection	470 nm excitation, 525 nm emission
Power Requirements	100-240 VAC +/-10% VAC, 2.5 A, 50-60 Hz
Instrument Power (external power supply)	12VDC ⎓ 2.5A
Weight	15 lbs / 7 kg
Dimensions	7H X 11W X 21D (in.) / 18H X 28W X 53D (cm)
Regulatory Compliance	CE, CSA,
Country of Origin	United States



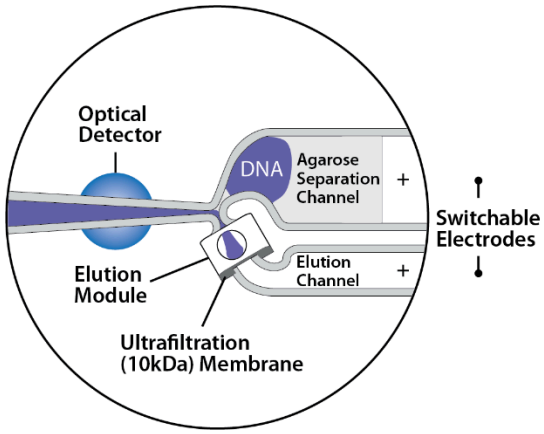
The BluePippin Instrument with cassette
(the monitor and keyboard are not shown)

2.2 How the System Works

The BluePippin systems use pre-cast and disposable agarose gel cassettes. DNA fractions are collected by electro-elution into a buffer-filled well using a branched channel configuration with switching electrodes. The timing of switching is determined by measuring the rate of DNA migration with optical detection of labelled markers. The position of the detectors beneath the gel cassette is shown in the schematic below.



Gel Cassette Schematic



Close-up of the collection well

2

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Note: *The agarose gel matrix does not contain intercalating dyes or gel stains, sample DNA is not viewable or monitored.*

Size Selections can be undertaken using one of the following strategies:

- ***Labeled internal standards*** *–Fluorescein labeled DNA internal standards are added to samples and run ahead of the input sample. The rates of migration of the internal standards are used to determine the collection timing within the sample lane. Up to 5 samples may be run per cassette.*

Note: *The DNA sequence of internal standards may be found at www.sagescience.com/support, on the BluePippin support page in the "Casssette Guides" section.*

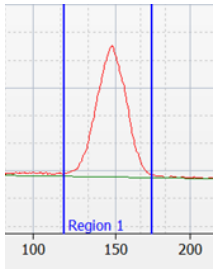
- ***Labeled external marker*** *– The fluorescein labeled DNA marker is loaded in a single dedicated lane during a run. The rates of migration of the markers are used to determine the collection timing for the remaining sample lanes.*

- ***Timed runs –*** *The beginning and end time of elution (size selection) is set by the user. Neither internal standards nor external markers are used.*

2.4 Collection Types: Tight, Range, High-Pass, Time, and Range+T

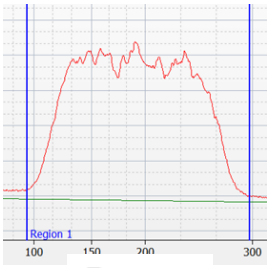
The BluePippin can be programmed to size select either targeted fractions or "High-Pass" collections. The fragment size distributions are described below:

1. ***Tight Size Collection ("Tight" setting).*** *Users enter a median value (BP Tight). The cassette lane will collect the narrowest distribution of fragments centered on the median value that is achievable given the resolution of the agarose gel concentration within the cassette. The distribution is Gaussian, and its range is typically +/- 16% (CV 8%) of the median fragment size.*



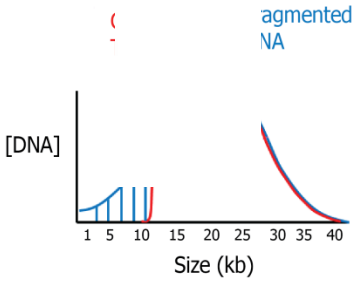
Tight

2. ***Range Size Collection ("Range" setting).*** *The cassette lane will collect a distribution of fragments between a starting base pair value (BP Start) and an ending base pair value (BP End). The actual start and end fragment sizes are typically within +/- 10% of the base pair values input into the software.*



Range

3. ***High-Pass Size Distribution (uses "Range*" setting).*** *Users enter a base pair threshold (BP Start) and the cassette lane will collect all fragments above that threshold. The method increases the average fragment length of the collection depending on threshold value and input DNA distribution profile. High-Pass protocols require an external marker to be run in one lane.*

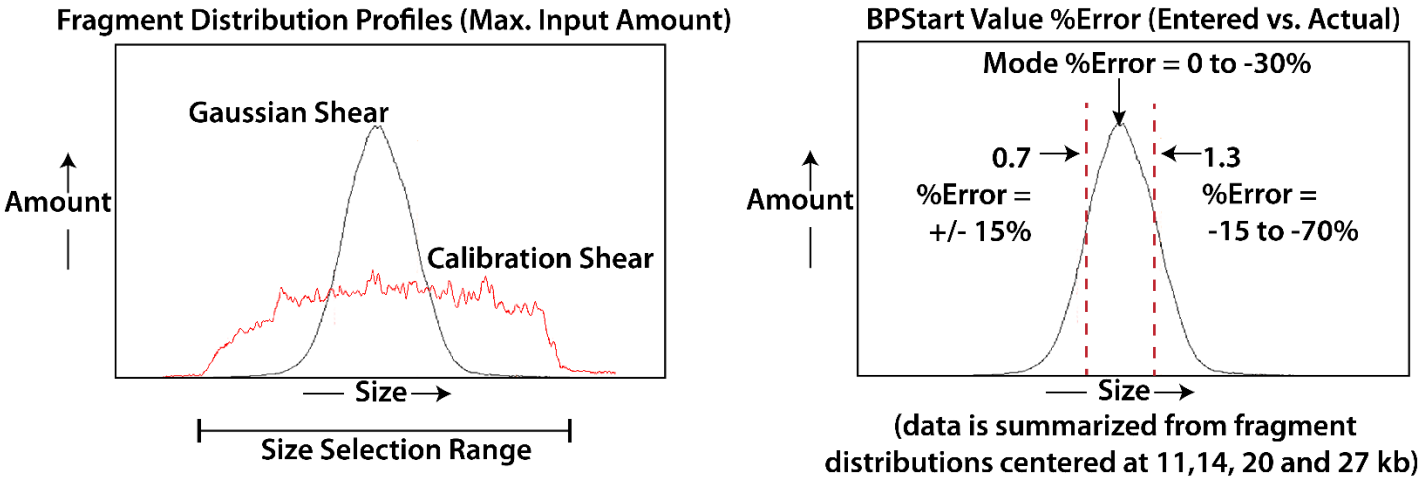


High Pass

* Although the cassette continues to collect until the end of the run, an end base pair value (50000) will auto-fill in the software protocol field. This value should be disregarded and should not be changed by users.

3. **Timed Programming (uses "Time" setting).** This allows users o start (TStart) of elution and the time to end elution (TEnd), and r :e selections. It also provides a setting to pause the elution in betw l end settings (TPause).

4. **Range + Time Programming (uses "Range + T" setting).** This allows users to enter a base pair threshold (BP Start) and set the total time of elution (TElution). This method can improve the overall accuracy of a collection, particularly with high molecular weight size selection. Gel cassette size selections are calibrated and QC'ed using sheared DNA with a fragment range that spans the specified size selection range of that cassette (e.g. 2-6 kb for 0.75% agarose cassette definitions). However, since many DNA shearing techniques produce narrow gaussian distributions, and since high concentrations of DNA migrate slower, fragments at the mode of the gaussian distribution migrate slower relative to the smaller fragments within the distribution. The practical consequence of this is that while the BP Start value (before the mode) will tend to be relatively accurate and reproduceable (the actual value vs. the software value), the BP END value (or BP Start value if it is after the mode) can be highly variable and inaccurate. To illustrate:



Range +T is a method to account for uncertainty in size selection BP End values.

To use Range + T:

1. It is best used on well characterized input samples with low variability.
2. The Range setting (Start) should be a value before or at the mode of the distribution.
3. The Time (TElution) setting will account for uncertainty in determining the "End" target of the fragment collection.
4. TElution value must be determined through trial and error.

3 Keys to Successful Collections

It is recommended that users closely follow the instructions outlined in this manual until they become thoroughly familiar with the system. The Quick Guide (included with cassettes) provides protocol reminders, but is not intended for beginning users.

Keys to successful collections are outlined in the following sections:

- ***Purity of the input sample*** *(Section 6)*
- ***Optical Calibration*** *(Section 9)*
- ***Preparation of the cassettes*** *(Section 10)*
- ***Sample loading*** *(Section 11)*

3.1 Common Misconceptions

Users should be aware of the following characteristics of the BluePippin System. These are part of normal operation of the system but may seem counterintuitive at first.

3.1.1 Narrowest is not always the best.

The BluePippin can produce very narrow size distributions from sheared genomic DNA. However, narrower size distributions will necessarily mean that a smaller fraction of the input DNA will be recovered. Users should broaden their collection ranges if the default tight settings do not produce enough DNA for their application.

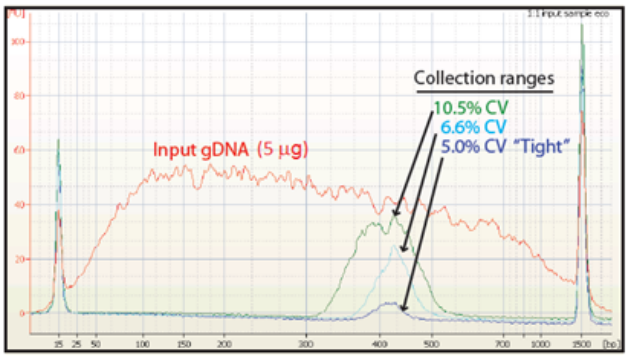


Figure 3.1. A comparison of the relative sample yield between tight and broader range size selections of DNA.

3.1.2 DNA undergoing elution is smaller than DNA at the detector. The branch point between the separation and elution channels is downstream from the detector position. **Figure 3.2** shows a cassette channel to illustrate. During normal operation, the leading edge of the DNA fraction scheduled for elution passes the detector ***before*** the start of elution (by up to several minutes). This offset can give rise to the impression that sample elution is late, even in runs that are functioning properly.

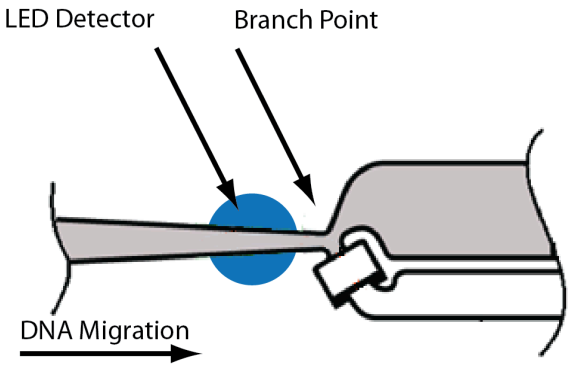


Figure 3.2. An illustration of the time and base pair difference between the detector and branch point.

3.1.3 The rate of electrophoresis is faster in the separation channel than in the elution channel. This may cause misconceptions with regard to the timing of broad size selections. For instance, if one sample is programmed to select from 400 – 600bp, and a second sample is programmed to select from 200 – 600 bp, the narrower range will finish eluting before the broader one, even though both elutions complete collection at 600 bp. The collections of large DNA fragments, especially with pulsed –field protocols, can also appear counter-intuitive with respect to timing.

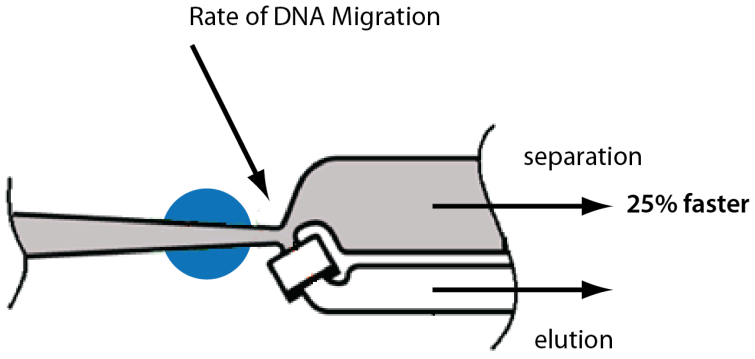


Figure 3.3. An illustration comparing the relative rate of electrophoresis in the separation and elution channels.

3.2 Improving Sample Yield

Users should expect an intrinsic yield of between 50-80% of the sample input. This is based on product validation studies in which restriction fragments have been run and collected, and the respective DNA amounts compared.

Yield can be affected by DNA becoming lightly bound to the low molecular weight cut-off membrane in the elution module. At the end of the run, the BluePippin will briefly reverse field (for 5 seconds). This improves sample yield by up to 20%.

Note: The reverse field feature may be disabled in the Systems Option screen.

3.3 Improving Sample Yield for HMW DNA

Recovering high molecular weight DNA (>2 kb, using all 0.75% agarose gel cassettes) tend to have lower yields than low molecular weight size selection. The following additional steps are highly recommended to improve product yield:

1. Wait **at least 45 min** after termination of the run before removing eluted DNA (it is OK to leave samples in instrument for hours, i.e. for an overnight run).

2. Rinse the elution module with Tween solution. Tween solution (0.1% Tween 20 in 0.5X electrophoresis buffer) is provided by Sage Science with cassette kits used with HMW collections.

   After removing the initial eluted volume:

   - Remove the eluted sample from elution module at the end of the run
   - Add 40µl of Tween solution (supplied with the gel cassettes) to the elution module
   - Wait 1 minute
   - Remove solution and pool with the original extracted sample, or process separately

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.

4 Unpacking and Installation

Unpacking the BluePippin

The BluePippin Instrument

The BluePippin instrumentation is shipped in two boxes: one will contain the BluePippin and Accessories and the second box will contain the computer monitor in the manufacturer's original packaging. With the boxes in the upright position, open and confirm that the following items are enclosed:

Monitor

- LCD computer monitor
- Video cable
- Power cord

BluePippin

- BluePippin Instrument
- Accessory box
  - Computer keyboard, USB
  - Computer mouse, USB
  - USB memory stick
  - Mousepad
  - Power supply
  - Power cord
  - Rinse cassette (for maintenance of electrodes)
  - Calibration fixture (for setting optical baseline before each run)
  - Operations Manual

Setting up the BluePippin

1. Remove the accessory box located on inside edge of box at the front end of the instrument. Remove the keyboard, mouse, and instrument power supply with cord from their packaging.

2. Firmly grip both sides of the instrument and lift it from the foam packaging insert. The BluePippin weighs approximately 15 lbs. Place the Pippin on a table or bench top.

3. Remove the computer monitor from the manufacturer's box and connect the monitor to BluePippin using supplied video cable.

4. Connect monitor to power using power supply supplied with monitor.

5. Insert USB connector from computer keyboard into port located in the back of the Instrument.

6. Connect mouse to BluePippin via USB or PS2 connector at back of instrument.

7. Connect monitor cable into the video port located in the back of the instrument.

8. Connect BluePippin instrument to power via supplied power supply and cable. Power connector is at rear of instrument.



Caution! Use of an unapproved replacement power supply or AC supply chord may damage the instrument. Contact Sage Science for an approved power supply replacement if needed.

9. Press the power switch located on the rear of the instrument and wait for the software to launch (approximately 30 seconds).

The BluePippin is ready for use. After the power switch is activated, the software will launch – allow ~30 seconds.

Figures 4.1 and 4.2 on the next page show the rear panel of the BluePippin.
Figure 4.3 on the following page shows the front panel.



Figure 4.1. Rear Panel Ports



Figure 4.2. Rear Panel Connections

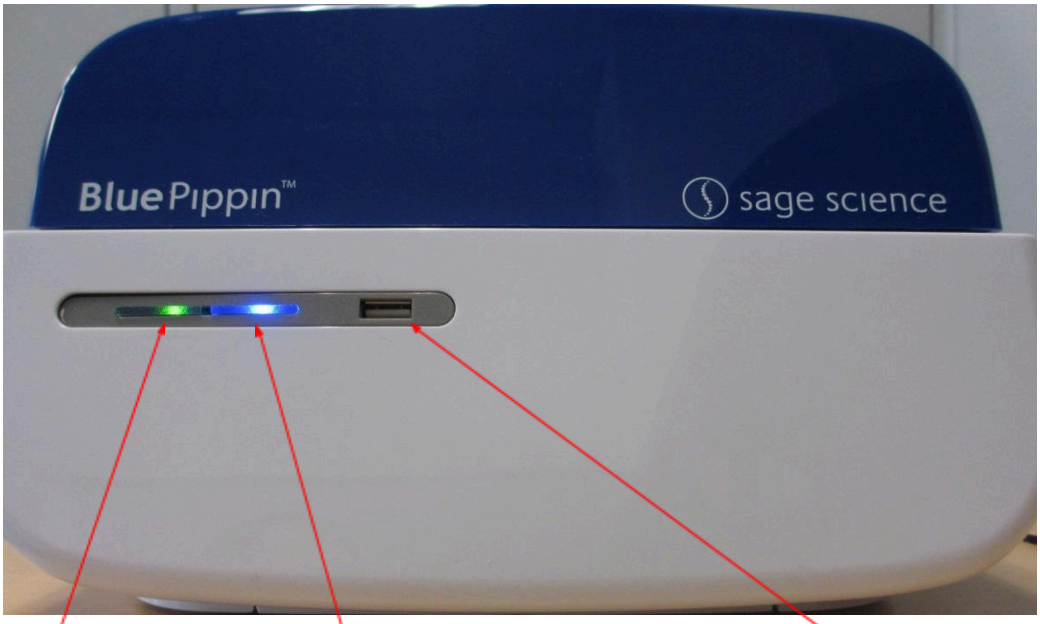


Figure 4.3. BluePippin front panel.

USB Port

Indicator Light:
Green when electrophoresis is underway (protocol is running).

Indicator Light:
Blue when instrument is ready for use (power is on, and software is active).

Unpacking Gel Cassettes

Gel cassettes are shipped in boxes in the following configurations. Ensure boxes are in the upright position and confirm that the following contents are present:

Dye-free gels with internal standards (50 samples, size selections 100 bp – 1.5 kb):

- 10 foil-sealed gel cassettes **(store at room temperature (R.T.))**
- 1 package of adhesive tape for sealing elution modules
- 1 reagent kit for 10 cassettes **(store at 4 °C)**
  - 1000 µl DNA internal standards/ loading solution mix (10 µl/sample)
  - 40 ml of spare running buffer

Dye-free gels with labeled external marker (40 samples, size selections >1 kb):

- 10 foil-sealed gel cassettes **(store at R.T.)**
- 1 package of adhesive tape for sealing elution modules
- 1 reagent kit for 10 cassettes **(store at 4 °C)**
  - 800 µl DNA size markers (1 x 40 µl load/ cassette)
  - 500 µl loading solution (10 µl/sample)
  - 2 X 800 µl 0.1% Tween 20 in running buffer*
  - 40 ml of spare running buffer

* Tween solution is used to improve DNA recovery of larger DNA fragments.



Important! *Fluorescein labels will degrade at room temperature – minimize time at RT for labeled standards and markers*

Storage Conditions for Cassettes and Reagents

The following storage requirements should be observed:

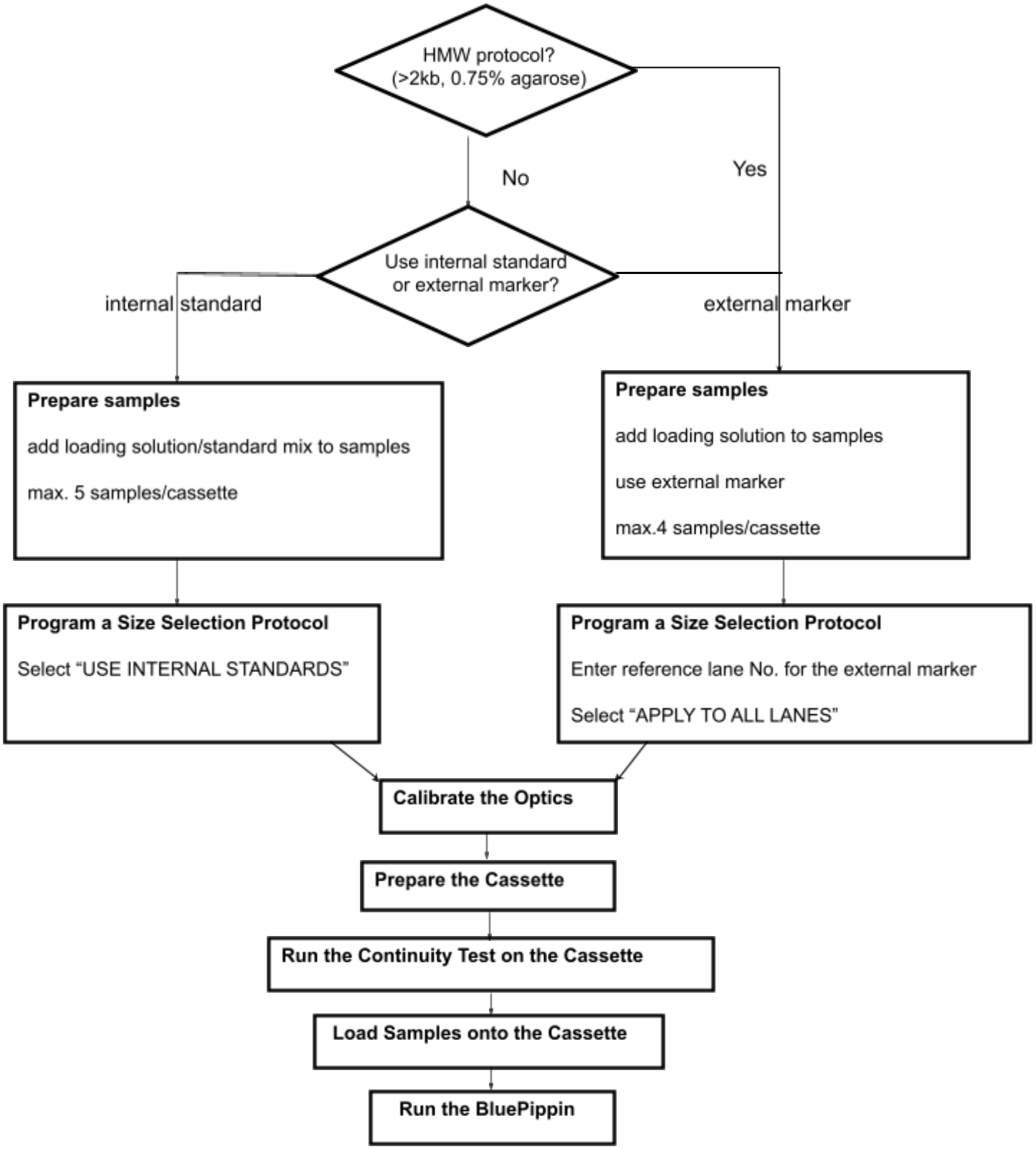
- **Gel Cassettes**- Store at room temperature (RT) in the foil bags until ready for use.
- **Internal standards or external markers** – store at 4ºC; minimize time at RT.
- **Electrophoresis buffer** – may be stored at 4 ºC or RT.
- **Loading solution** – store at 4 ºC or RT. Bring to RT before use if necessary.
- **Tween Solution** – store at 4 ºC or RT.

Important! Disposal: The components of gel cassettes contain no substances which, at their given aqueous concentrations, are considered to be hazardous to health or ecosystems. Use standard laboratory disposal practices for disposal. Check with facility or local municipal regulations.



Figure 4.4. Gel Cassette Kit

5 Workflow Summary



6 Sample Preparation

Input Sample Characteristics

When running the BluePippin, characteristics of input DNA can affect separation resolution and efficiency of product recovery. The following general guidelines should be followed:

- **Ionic strength:** The ionic strength of the sample should be lower than the ionic strength of the buffer (80mM monovalent ions). High salt concentrations can result in slower than expected DNA mobility.

- **Protein in the sample:** DNA-binding proteins such as ligases or polymerases can affect the mobility of fragments during separation. Proteins can also reduce DNA recovery from the elution module by increasing the binding of DNA to the ultrafiltration membrane at the back of the elution module. For best results, samples should be de-proteinized prior to loading whenever possible.

- **Input DNA size distribution:** A knowledge of the input size distribution is obviously important to program accurate size selection settings. BluePippin cassettes are calibrated using the Agilent Bioanalyzer to evaluate input and product sizes, and so, for best results, input size distributions should be evaluated using the Bioanalyzer. For low concentration samples, the Agilent HS chip is very useful.

Preparing DNA Samples for the BluePippin

1. Bring DNA sample up to 30µl with TE.
2. Bring internal standard mix (or loading solution if using an external marker) to room temperature.
3. For each sample, combine 30µl of DNA sample with 10µl of internal standard mix (or loading solution).
4. Mix samples thoroughly (vortex mixer). Briefly centrifuge to collect.

Recommended sample Load Guidelines

Minimum Sample Load:	15 ng sheared genomic DNA
Maximum Sample Load[1]:	2 ug sheared gDNA with 1.5% agarose 5 ug sheared gDNA all others 10ug sheared gDNA with High Pass Plus 4 ug restriction/PCR fragment

7 Protocol Editor Overview

The default screen on the BluePippin is a tabbed format. The Protocol Editor is the second tab.

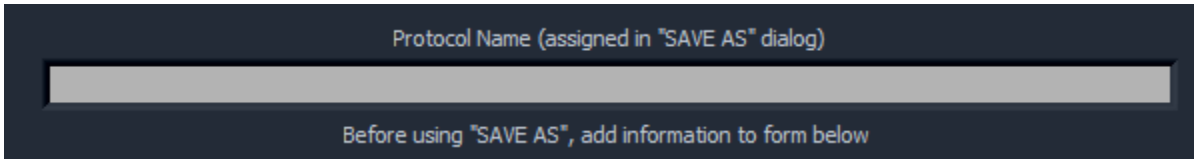
7.1 The Protocol Editor Tab

The protocol editor tab is the screen with which users create new protocols, or edit existing protocols, for DNA size selection. A protocol is created for a cassette type (within the applicable target range) and is saved as a named protocol file with a ".ppprot" file extension. When running a cassette, an appropriate protocol file is selected and applied to the run.



7.2 Protocol Name

Displays the name of the protocol. The user enters a name in a popup window when the protocol is saved.



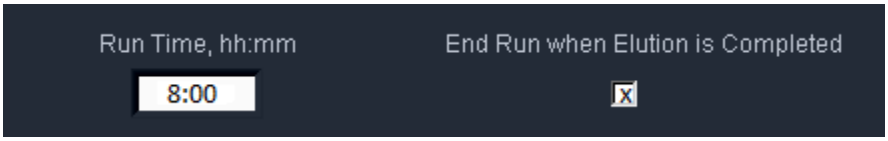
7.3 Cassette Type Selector

The user selects the cassette type/definition from a drop-down menu. This determines the cassette type that will be run, the electrophoresis waveform that will be used, and sets limits on the size selection parameters that may be entered. A quick guide is provided with every cassette kit that provides the cassette definition(s), directions for use, and expected run times.



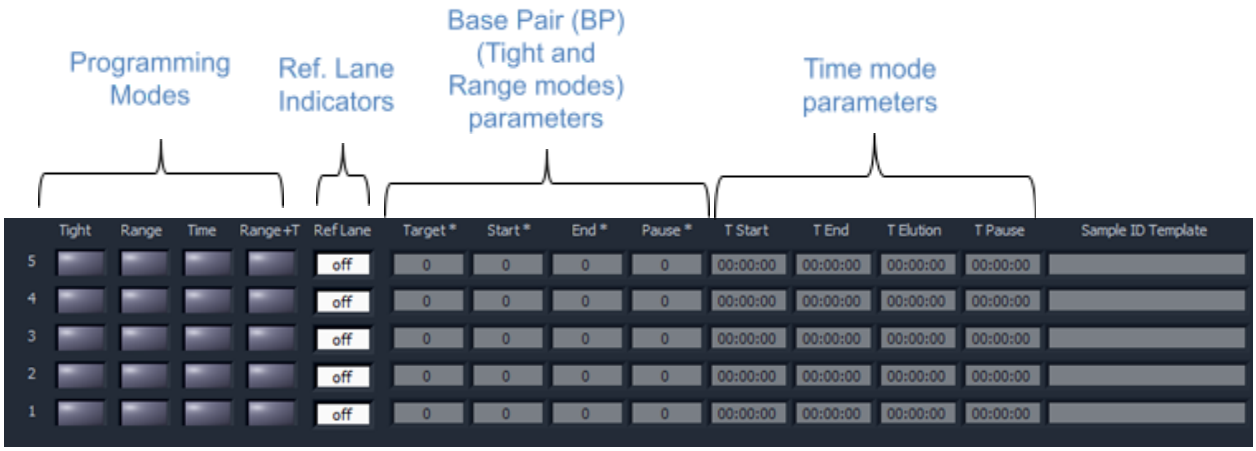
7.4 Run Time Selector

When checked, the run will end at the earliest possible time, after the final elution is complete. *This is checked by default, and run time is* based on the cassette definition selected. The "Run Time" value is a default value that ensures most runs will be completed if the "End Run when Elution Completed" box is left unchecked.

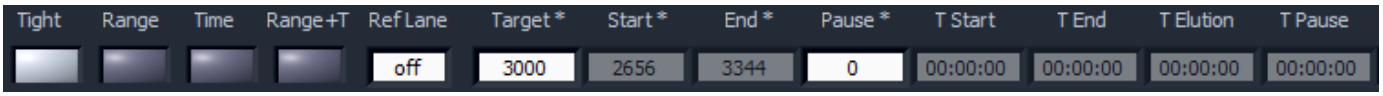


7.5 Protocol Parameters Detail

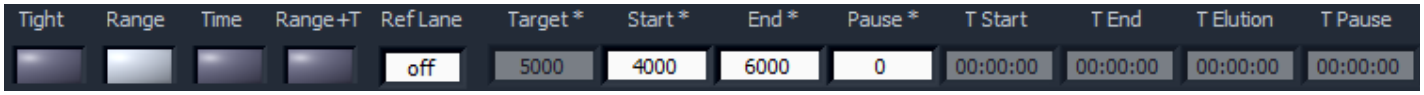
Programming Modes
The protocol parameters section is shown below. Users will select a programming mode for each sample and a lane to designate as a reference. Then, the size selection values are entered either as base pair (BP) values, or time values (hr:min:sec in "Time" mode). Individual lanes may have different parameters and different modes.



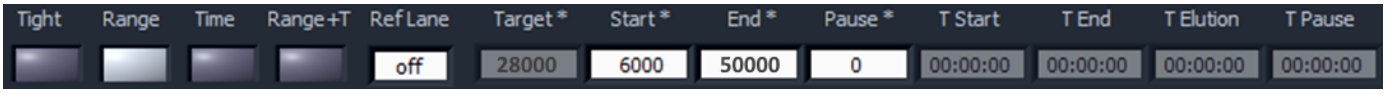
- **Tight** – collects minimum allowable distribution range of DNA fragments using the median target base pair value.



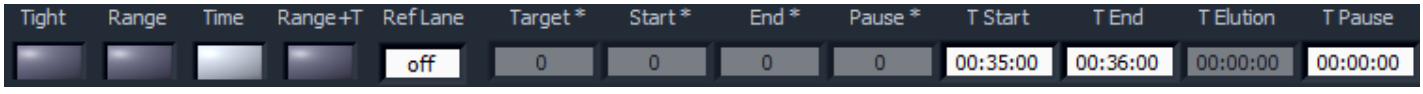
- **Range** – allows users to select the range to be collected using starting and ending base pair values.



- **High-Pass** (Range Mode) – allows users to select all fragments above a size threshold. A value of 50000 or 80000 is automatically populated in the End field.



- **Time** – users program size selection using the starting and ending elution time (hr:min:sec) values only (**a reference DNA marker is not used**)



- **Range + T** – collects a fragment range. The user enters a start base-pair value and an elution time in the size selection parameters.



- **Pause Fields**

The “Pause” feature allows sequential fraction to be collected for a single lane. Entering a value into the “Pause” field will stop the run during elution so that the user can remove the eluted fraction, replenish the buffer, and collect the reminder of the sample before elution ends. The value entered must be within the base pair range or the time range that has been entered.

Though two contiguous samples can be collected, there will be significant overlap of fragment sizes within the two samples. If users were to pause at the midpoint of progressively wider “Range” settings, the amount of overlap in the collections would become progressively smaller.

Two collections of a paused “Tight” setting will yield two nearly identical samples each with half the amount of a single Tight collection. This feature is not recommended for “Tight” mode.

- **Ref Lane indicators**

Ref Lane indicators display which lanes contain the reference marker to which that lane will calibrate, based on what is assigned by the user (see below). Protocols using internal standards will reference the same lane number. Protocols using an external marker will reference the lane into which the external marker will be run.

Ref Lane: Internal standards will be used.

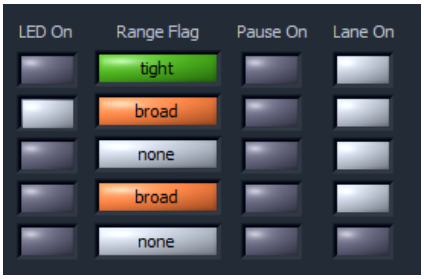




Ref Lane: External marker will be run in lane 2.

7.6 Status Indicators Detail

Displays the status summary of the programmed features.



- **LED On – user activated**

If clicked (light gray), the LED detector will remain ON for the entire run. In the default OFF state, the LEDs automatically turn off after marker detection during a run to avoid excess exposure of light to the DNA.

- **BP Range Flag**

If a “Tight” collection mode (minimum size range) has been selected for sample lane, or if the range in the “Range” mode fields contain the minimum size range, the “BP Range Flag” field for that sample will display “tight” and a green color.



When a “Range” collection mode (larger than the minimum) has been selected for a sample lane, the “BP Range Flag” field for that sample will display “broad” and an orange color.



- **Narrow Range Alert**

If the “Range” collection mode is selected, and the size range is narrower than the minimum, a yellow “narrow” flag will appear. Users should adjust the size selection parameters to “tight” or “broad” ranges before proceeding.



Note: *A collection range below the minimum will not collect the fragment range selected. Rather, the range will be reflected as the minimum allowable (as defined by the “Tight” setting) albeit with a lower sample yield that can be achieved by the “Tight” setting.*

- **Pause On**

If the pause feature has been entered for a sample lane, “Pause On” indicator will be displayed.



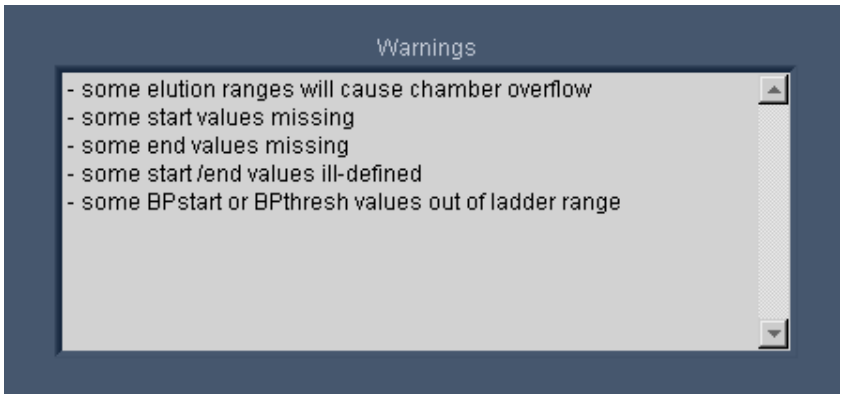
- **Lane On**

The “Lane On” indicates which lanes will be active during the run.



7.7 Warnings Text Box

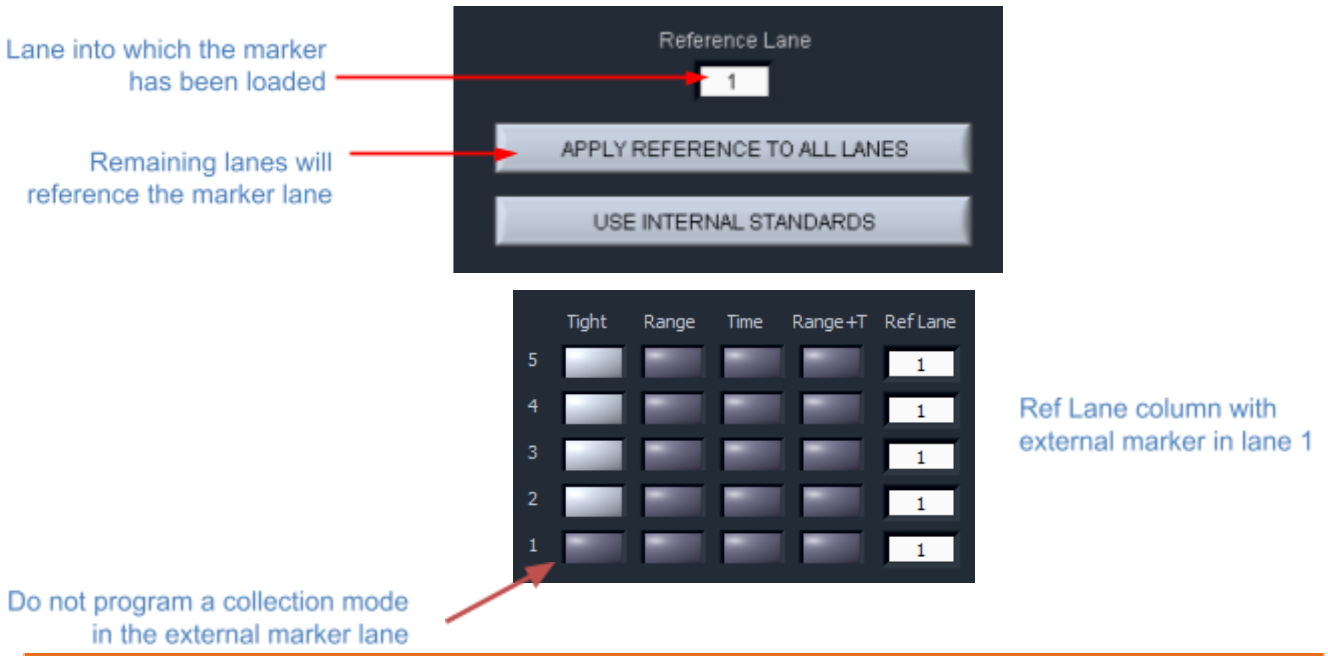
If there are errors or exceptions within a programmed protocol, or if a protocol has not been completed, a message will appear in the Warnings text box identifying the nature of the discrepancy or reminder to complete additional steps.



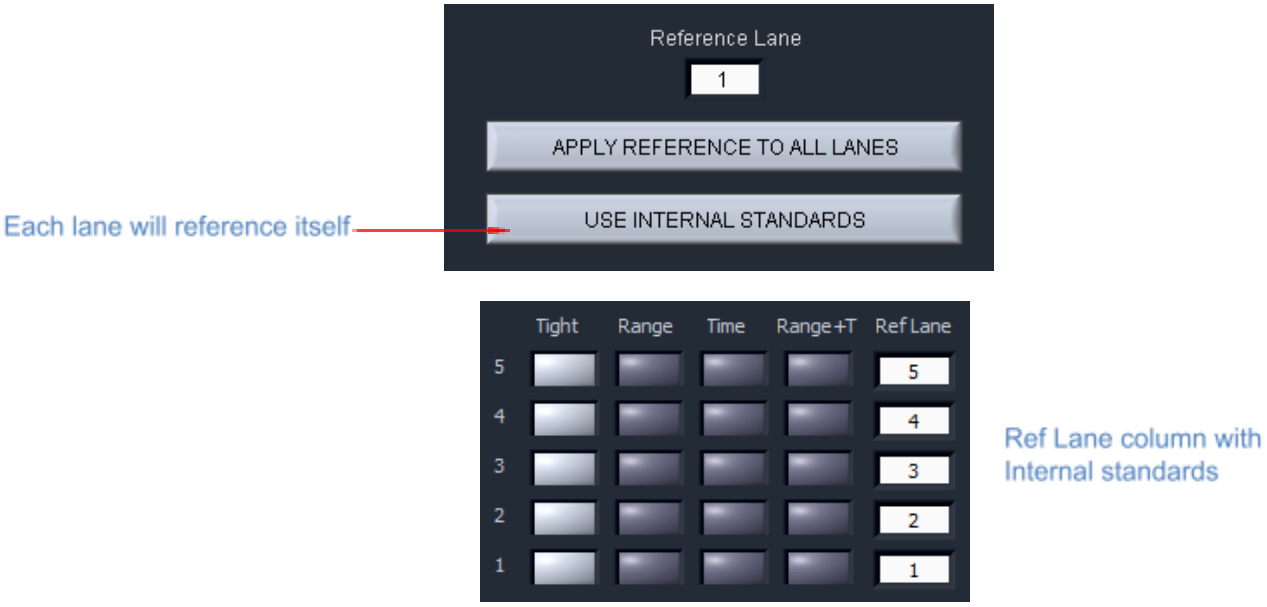
7.8 DNA Marker/Standards Lane Assignment

Users use these buttons and field to assign internal standards or external marker.

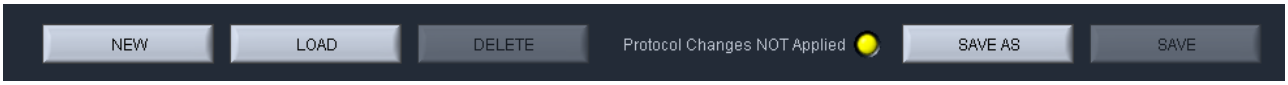
External Marker. For external markers, users press “APPLY REFERNCE TO ALL LANES”. The lane into which the marker will be loaded must be entered into the “Reference Lane” field by selecting from a drop-down menu. This lane number will populate each field in the “Ref Lane” column in the Protocol Parameters.



Internal Standard. For internal standards, users press “USE INTERNAL STANDARDS”. The “Reference Lane” field will display “off”. The numbers of each individual lane will populate the fields in the “Ref Lane” column in the Protocol Parameters.



7.9 File Commands - Details



New - Clears all fields and starts a new protocol

Load - Opens an existing protocol stored on the instrument directory.

Delete – Deletes an opened file from the instrument directory.

"Protocol Changes NOT Applied" - Flashes yellow when a new protocol has been programmed or an opened protocol has been edited. Indicates that the changes should be saved before use.

Save As – Saves an open protocol under a new name.

Save - Saves a new protocol with a pre-existing name.

8 Programming Size Selections

8.1 Tight Mode – programming minimum size range collections

The “Tight” programming mode is a method for selecting DNA size range collections by entering a target base pair value in software. The system will collect the minimum fragment distribution that is possible, centered on this base pair value.

For size reference, either internal DNA standard or external DNA markers (run in a separate lane) may be used, depending on cassette type and/or application. Cassette kits for smaller DNA size range collections (100-1500 bp) use internal standards and larger collections (>2 kb) require use of one cassette lane to run an external reference marker. Assignation of reference DNA is detailed in Section 7.8.

Note: Different programming modes maybe be run in multiple lanes on a cassette, with the exception of High-Pass protocols. High-Pass cassette definitions require High-Pass to be run in every lane, although multiple starting thresholds may be used.

In the protocol editor:

1. **Select an appropriate cassette definition from the cassette type selector**, if a new protocol is being created (Section 7.3)

2. **Assign the internal or external reference DNA** (Section 7.8)

    a. For cassettes using **internal standards**:

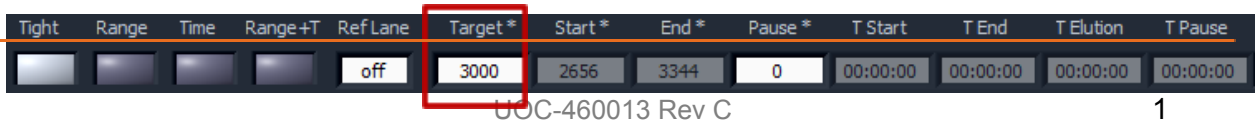
        i. press the “USE INTERNAL STANDARDS” button.

    b. For cassettes using an **external marker** lane:

        i. enter the lane into which the marker will be loaded, into the “Reference Lane” field (“1” is the default value)
        ii. press the “APPLY REFERENCE TO ALL LANES” button.
        iii. if using an external marker, make sure no collection modes are selected for that lane.

3. **Select the “Tight” programming mode**. Click the “Tight” button for every lane in which the “Tight” fragment range will be collected.

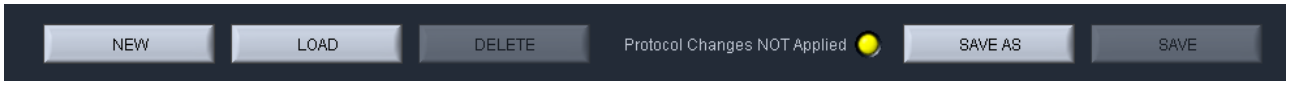
4. **Enter a value in the “BP Target” field**. This value should represent the median value for the desired size range to be collected. The actual range to be collected will auto-fill the “BP Start” and “BP End” fields. Make sure the size selection parameters are within the allowable range of for the cassette type as outlined in the cassette Quick Guide or Section 25 of this manual.



5. **(Optional) Enter a BP pause value**. This is not recommended for Tight mode. See Section 7.5 for a description of this feature.

6. **(Optional) Enter the Sample ID information.**

7. **Save.** After programming a new protocol, or editing an existing one, the protocol file must be saved prior to use. If the protocol is new, press "Save". If a protocol has been edited, a yellow alert will be displayed in the "Protocol Changes Not Applied" field. "Save" will save the file under the previously saved name, and "Save As" will allow a new name for the file to be applied.



All protocol files are saved in a file directory, /home/pippin/BluePippin/Protocols, and may be accessed in the File Manager tab.

8.2 Range Mode – programming broad size range collections

The "Range" programming mode is a method for selecting DNA size range collections by a starting and ending base pair value in software. The system will collect the fragment range between the start and end base pair values. This option should be used when collections that are broader than are provided by the "Tight" setting are needed.

For size reference, either internal DNA standard or external DNA markers (run in a separate lane) may be used, depending on cassette type and/or application. Cassette kits for smaller DNA size range collections (100-1500 bp) use internal standards and larger collections (>2 kb) require use of one cassette lane to run and external reference marker. Assignation of reference DNA is detailed in **Sections 7.5** and **7.6**.

Note: Different programming modes maybe be run in multiple lanes on a cassette, with the exception of High-Pass protocols. High-Pass cassette definitions require High-Pass to be run in every lane, although multiple starting thresholds may be used.

In the protocol editor:

1. **Select an appropriate cassette definition from the cassette type selector**, if a new protocol is being created (Section 7.3)

2. **Assign the internal or external reference DNA** (Section 7.8).

   a. For cassettes using **internal standards**:

      i. press the "USE INTERNAL STANDARDS" button.

   b. For cassettes using an **external marker** lane:

i. enter the lane into which the marker will be loaded, into the “Reference Lane” field (“1” is the default value)
ii. press the “APPLY REFERENCE TO ALL LANES” button.
iii. if using an external marker, make sure no collection modes are selected for that lane.

3. **Select the “Range” programming mode**. Click the “Range” button for the lane from which the cut will be extracted.

4. **Enter range values in the “BP Start” and “BP End” fields**. The median target value of the collection range will auto-fill the “BP Target” field. Make sure the size selection parameters are within the allowable range for the cassette type as outlined in the cassette Quick Guide or Section 25 of this manual.



5. **(Optional) Enter a BP pause value**. The instrument pauses the system to allow users to collect two serial collections. The value in the Pause field must be a value within the size range selected (i.e. if a 200-400 bp range is selected, the Pause value must be between 201 and 399). Each paused fraction must be wider than the “narrow” cut-off. This can be tested by entering the values in another range mode lane and making sure the “narrow” flag does not light (see Section 7-5 for more detail).

Important! *A pause will require the user to manually resume the protocol from the Main Screen controller. The instrument lid may be opened during the pause, and sample retrieved.*

6. **(Optional) Enter the Sample ID information**

7. **Save**. After programming a new protocol, or editing an existing one, the protocol file must be saved prior to use. If the protocol is new, press “Save”. If a protocol has been edited, a yellow alert will be displayed in the “Protocol Changes Not Applied” field. “Save” will save the file under the previously saved name, and “Save As” will allow a new name for the file to be applied.



All protocol files are saved in a file directory, /home/pippin/BluePippin/Protocols, and may be accessed in the File Manager tab.

A High-Pass size selection collects all fragments over a programmed size threshold.

For size reference, only external DNA markers (run in a separate lane) can be used. Assignation of reference DNA is detailed in Sections 7.8. A specific cassette definition must also be selected with which an electrophoresis waveform (typically pulsed-field) has been developed. "High-Pass" will be shown in the cassette definition name.

Note: High-Pass cassette definitions require High-Pass to be run in every lane of a cassette, Multiple starting thresholds may be used.

In the protocol editor:

1. **Select an appropriate cassette definition from the cassette type selector**, if a new protocol is being created (Section 7.3)

2. **Assign the external reference DNA** (Section 7.8)

   a. enter the lane into which the marker will be loaded, into the "Reference Lane" field ("1" is the default value)
   b. press the "APPLY REFERENCE TO ALL LANES" button.
   c. make sure no collection modes are selected for the external marker lane.

3. **Select the "Range" programming mode**. Click the "Range" button for the lane from which the cut will be extracted.

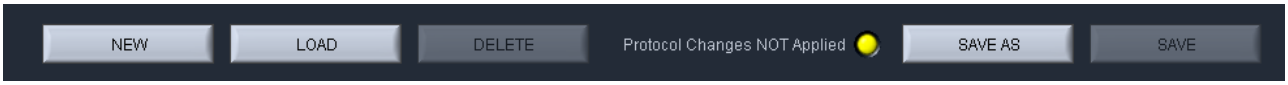
4. **Enter a threshold value in the BP "Start" field**. The cassette definition will auto-fill an end value in the BP "End" field. This is not editable and ensures all fragments above the size threshold will be collected. Make sure the size selection parameters are within the allowable range of for the cassette type as outlined in the cassette Quick Guide or Section 25 of this manual.



5. **(Optional) Enter a BP pause value**. This is not recommended for High Pass mode. See Section 7.5, for a description of this feature.

6. **(Optional) Enter the Sample ID information**

7. **Save**. After programming a new protocol, or editing an existing one, the protocol file must be saved prior to use. If the protocol is new, press "Save". If a protocol has been edited, a yellow alert will be displayed in the "Protocol Changes Not Applied" field. "Save" will save the file under the previously saved name, and "Save As" will allow a new name for the file to be applied.



All protocol files are saved in a file directory, /home/pippin/BluePippin/Protocols, and may be accessed in the File Manager tab.

8.4 Time Mode – programming timed collections

Important! *The time mode does not reference a standard. Cassette to cassette variablity and environmental running conditions will cause a wide variation in selection accuracy.*

The “Time” programming mode is a method for crude DNA collections where accuracy of the fragment size or range is not critical. The system will collect the fragment range between the elution start time and elution end time.

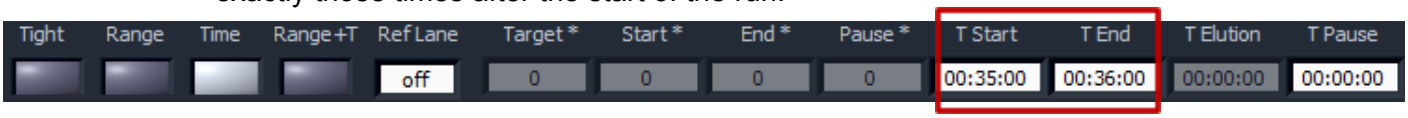
Note: Different programming modes maybe be run in multiple lanes on a cassette, with the exception of High-Pass protocols. High-Pass cassette definitions require High-Pass to be run in every lane, although multiple starting thresholds may be used.

In the protocol editor:

1. **Select a cassette definition** that encompasses the DNA size range from which the size selections should be collected.

2. **Select the “Time” programming mode**. Click the “Time” button for the lane from which the cut will be extracted. A DNA marker reference standard is not required.

3. **Enter time values in the “T Start” and “T End” fields**. Sample elutions will occur at exactly those times after the start of the run:



4. **(Optional) Enter a Time pause value**. If a value is entered into the pause field, the system will pause when that time has been reached. The pause will allow a user to remove the fraction and then resume the elution and collect a second fraction.

   The Pause value must be within the programmed elution range (between T Start and T End).

Important! *A pause will require the user to manually resume the protocol from the Main Screen controller. The instrument lid may be opened during the pause, and sample retrieved.*

5. **(Optional) Enter Sample ID information.**

6. **Save**. After programming a new protocol, or editing an existing one, the protocol file must be saved prior to use. If the protocol is new, press “Save”. If a protocol has been edited, a yellow alert will be displayed in the “Protocol Changes Not Applied” field. “Save” will save the file under the previously saved name, and “Save As” will allow a new name for the file to be applied.

   All protocol files are saved in a directory, /home/pippin/BluePippin/Protocols, and may be accessed in the File Manager tab.



8.5 Range+T Mode – programming Range+T collections

Important! *Range+T mode requires some method development to adjust for DNA input amount and fragment size distribution.*

The “Range+Time” programming mode is a method for highly accurate size selections based on input DNA characteristics (see Section 1-4) that may affect the accuracy of size selection, particularly on the “End” value.

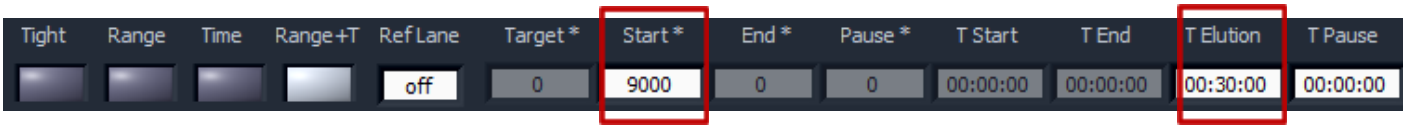
Note: Different programming modes maybe be run in multiple lanes on a cassette, with the exception of High-Pass protocols. High-Pass cassette definitions require High-Pass to be run in every lane, although multiple starting thresholds may be used.

In the protocol editor:

1. **Select an appropriate cassette definition from the cassette type selector**, if a new protocol is being created (Section 7.3)

2. **Select the “Range+T” programming mode**. Click the “Range + T” button for the lane from which the cut will be extracted. A DNA external marker is required for reference.

3. **Enter values in the BP “Start” and “T Elution” fields**. Sample elutions begin at the starting threshold value and continue for the programmed time. Users must analyze the size selections and then adjust the T Elution times and re-analyze, until the desired size selection range is collected. T Elution time recommendations are provided with the cassette quick guides and the cassette specification (page 25-16).



4. **(Optional) Enter a Time pause value**. If a value is entered into the pause field, the system will pause when that time has been reached. The pause will allow a user to remove the fraction, and then resume the elution and collect a second fraction.

   The Pause value must be within the programmed T Elution range. (see Section 7.5 for more information).

Important! *A pause will require the user to manually resume the protocol from the Main Screen controller. The instrument lid may be opened during the pause, and sample retrieved.*

5. **(Optional) Enter Sample ID information.**

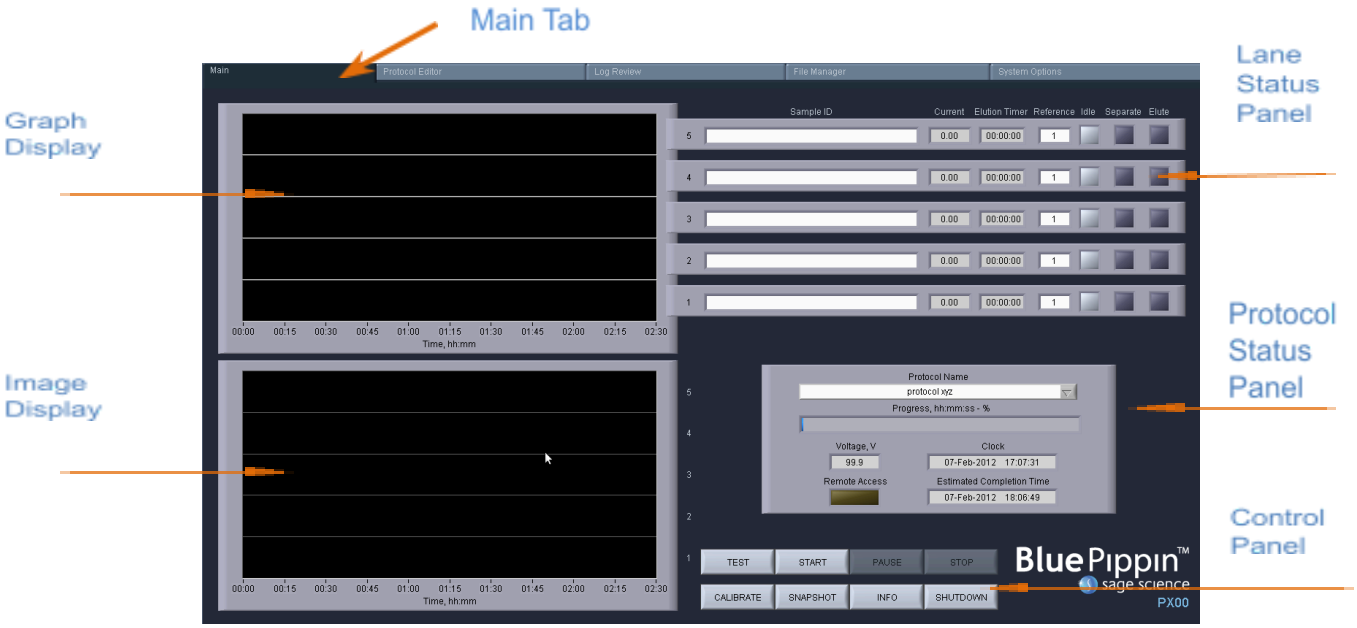
6. **Save**. After programming a new protocol, or editing an existing one, the protocol file must be saved prior to use. If the protocol is new, press “Save”. If a protocol has been edited, a yellow alert will be displayed in the “Protocol Changes Not Applied” field. “Save” will save the file under the previously saved name, and “Save As” will allow a new name for the file to be applied.



All protocol files are saved in a directory, /home/pippin/BluePippin/Protocols, and may be accessed in the File Manager tab.

9 Optical Calibration

The BluePippin optical system must be calibrated before every run. The instrument is supplied with a calibration fixture that fits over the LED detectors on the optical nest.



1. Press the Main Tab

2. Press “CALIBRATE” on the Control Panel



3. An “LED Calibration” window will launch. The “Calibration Status” field will contain the message “Ready for Calibration”.

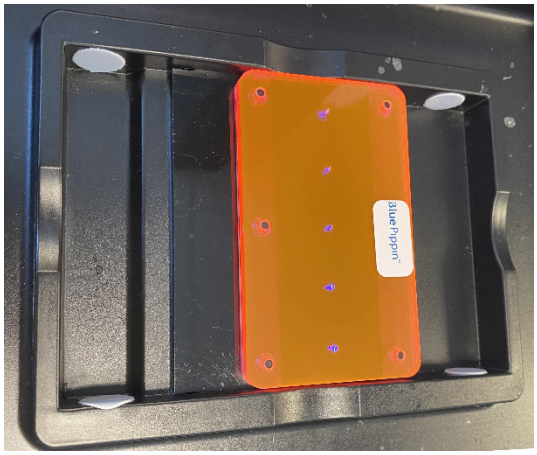
   Place the Calibration Fixture on the optical nest so that all five LED detectors are covered. The dark side of the fixture must be down (closest to the LEDs):



Top



Bottom



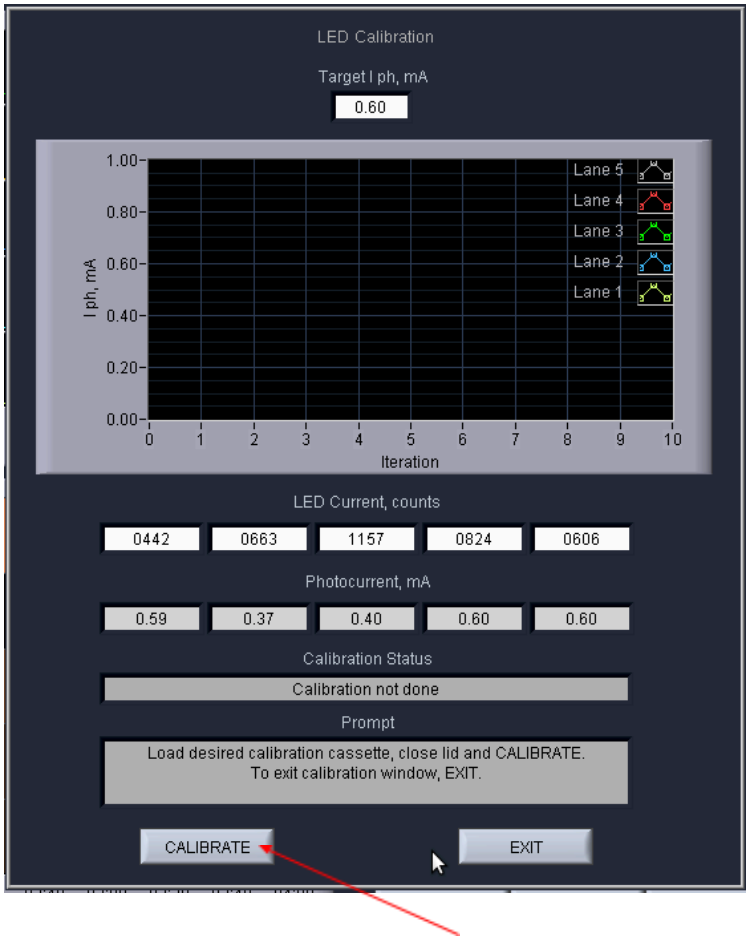
Correct Position
on Optical Nest



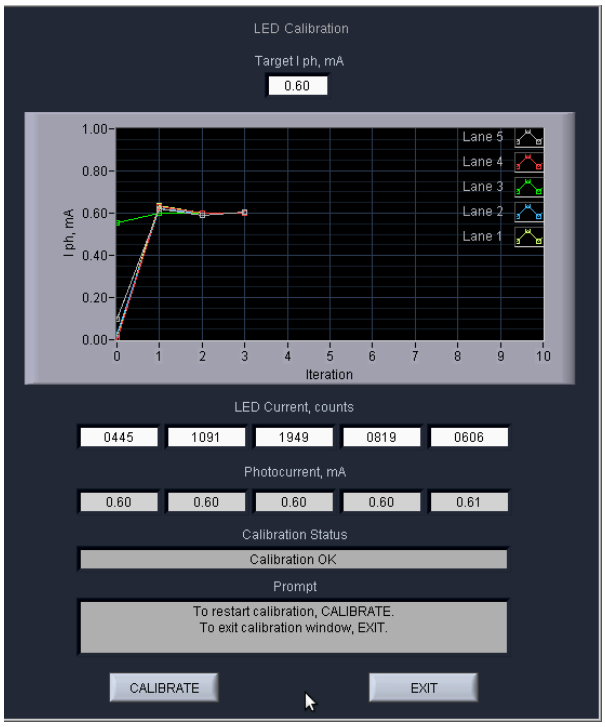
Protective Sock
for Storage

4. Close the lid.

5. Press “CALIBRATE”



5. The Calibration status will read "Calibration Underway" and the LED photocurrents will automatically adjust to a factory setting. Once calibration is successful the "Calibration Status" field will contain the message "Calibration OK".



6. Press “EXIT” to return to the Main Tab.

7. Open the lid and remove the fixture.

10 Preparing a Cassette

10.1 Visually Inspect the Cassette

1. **Remove the cassette from foil packaging.** Foil package is scored at one end to allow tearing.

2. **Inspect the levels of buffer in the buffer reservoirs.** Tip the cassette to consolidate any bubbles in the reservoirs, then hold the cassette in a horizontal position and look at the reservoirs from the top and bottom edges. Reservoirs should be nearly full of buffer and roughly equal in volume across the cassette. If the buffer level in any reservoir appears less than 50% full (compared with its neighbors), the low reservoir should be refilled with the supplied electrophoresis buffer prior to running. When refilling, a visual check of buffer levels is sufficient - a measured volume is not required.

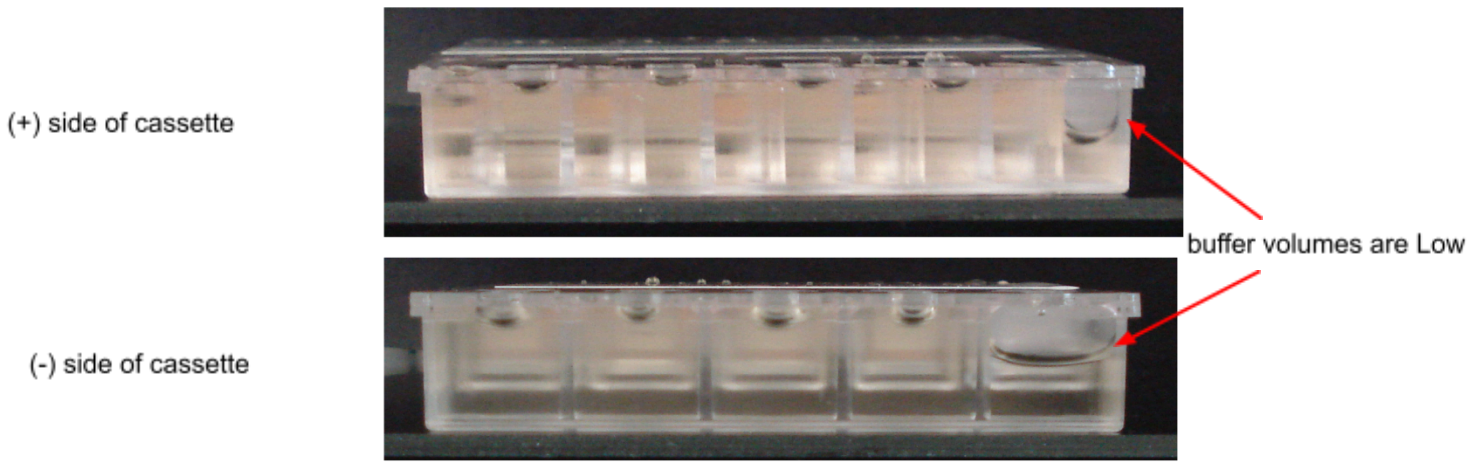


Figure 10.1. Low buffer levels in cassette buffer chambers.

3. **Inspect the gel columns.** Look for obvious breakage of the agarose column in each channel.

Important! *If there is obvious breakage, do not use the lane. Remaining lanes can be used.*

4. **Inspect bottom of cassette for bubbles in the detection region of the gel columns**. If a cassette has been jarred during shipping, the agarose column can delaminate from the plastic bottom of the separation channel, forming a thin flat bubble between the gel column and the bottom of the channel. If this happens in the region used by the optical detector (see illustration), DNA detection will be extremely unreliable. ***If a lane with such a bubble is used for the reference marker, the markers will not be properly identified and sample collection will occur at the wrong time or fail altogether.*** (Only the optical properties of the channel are affected - electrophoretic properties of the channel are unaffected, since the DNA travels through the center of the gel column, above the bubble.) To find such bubbles, turn the cassette upside-down, and view the bottom of the channels under a strong light, while tilting the cassette back and forth (see **Figure 10.2**, below). **Figure 10.3** shows the region of optical detection.

Important! *If a bubble is observed between the bottom surface of the gel column and the plastic bottom of the channel, do not use the lane to run the reference. It may be used for size selections using an external marker in a different reference lane.*

Important! *Flat bubbles between the top surface of the gel column and the plastic top of the channel will NOT affect run quality or optical detection. They may be used for markers or sample without any adverse effects.*

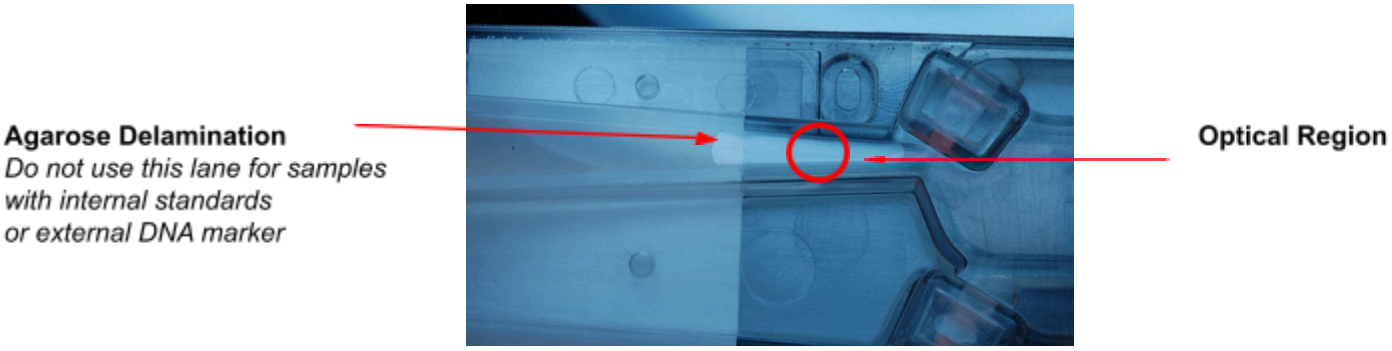


Figure 10.2. Bubble in optical path on bottom of lane. Do not use the lane for the DNA reference marker.

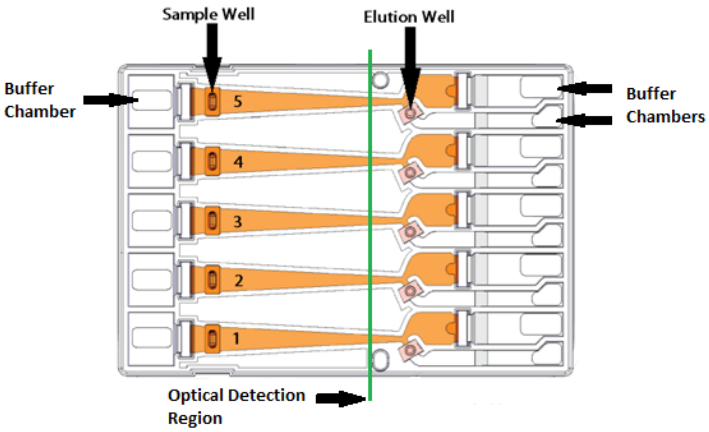


Figure 10.3. The region of optical detection

10.2 Prepare the Cassette for Loading

1. **Dislodge bubbles from behind the elution modules**. Return the cassette to the right-side up position. Tilt the cassette, sample well side down, to release any trapped bubbles behind the elution modules into the (+) buffer chambers. Gently tap the cassette if necessary. **Figure 10.4**, below, shows location to check for bubbles.

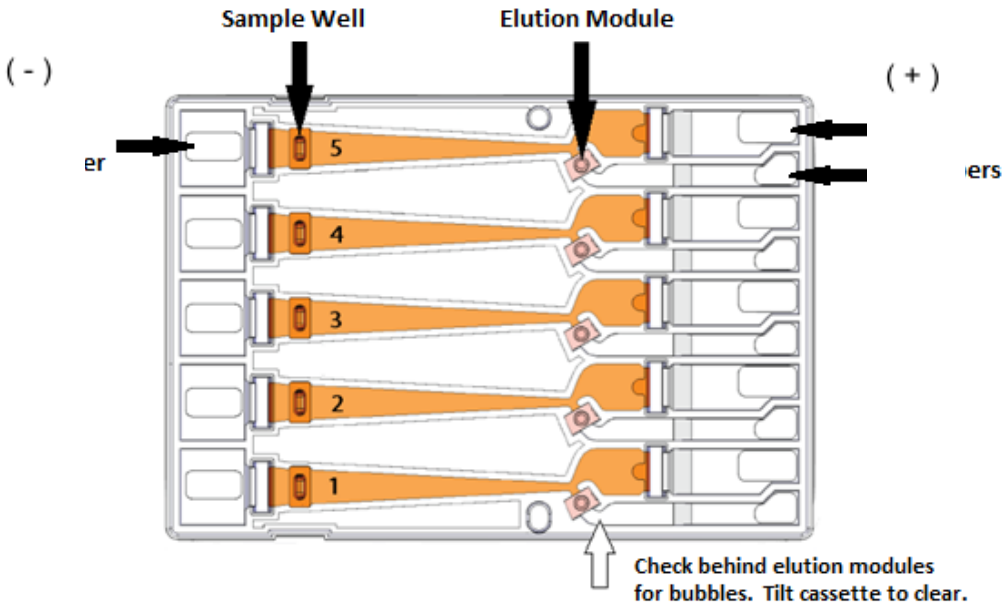


Figure 10.4. A schematic of a Gel Cassette.

2. **Place Cassette into the Pippin optical nest.** The cassette should be placed into the nest in the orientation shown in **Figure 10.4**, above, with the sample wells to the left side of the nest. When inserting the cassette into the nest, keep the (-) buffer chambers tilted down so that the bubbles in the elution reservoirs won't be trapped behind the elution modules.

⚠ **Important!** *Be sure the cassette is fully seated into the bottom of the nest. Detection of DNA within the cassette will fail if the bottom of the cassette is not properly seated against the optical region.*

3. **Remove adhesive strips from cassette.** Place one hand on the cassette and hold it firmly in the nest. Grab the white tabs of the tape and pull the strips firmly and slowly toward the front of the BluePippin until they are removed.

4. **Remove buffer from elution modules and replace with 40µl of fresh electrophoresis buffer**. Replenishing the buffer ensures proper electrophoretic continuity. When refilling empty modules, place tip all the way into the module and slowly fill from the bottom, withdrawing the pipette tip while dispensing, and taking care not to generate any bubbles that can occlude the current path. Use spare buffer that has been supplied with the cassette package, or pipette from the buffer chambers.

Important! *Make sure that the pipette tips used for step 4 extend all the way to the bottom of the elution modules without sealing the elution port opening. If the tips seal the port opening, it will be extremely difficult, or impossible, to empty and refill the elution module completely. Test tip fit using the empty rinse cassette supplied with the instrument.*

Note: *The total volume of the elution module when filled to the top is 65µl. The specified starting volume of 40µl only partially fills the module.*

5. **Seal the elution modules with the adhesive tape strips.** Tape for sealing the elution modules are supplied with cassette packaging. Long elutions will cause the elution modules to overflow if not sealed. Place tape over the elution modules and rub firmly to fix the tape in position. For best results, rub the tape at the boundary of the well with a smooth hard object such as the back end of a lab marker pen.

6. **Check the buffer levels in the sample wells.** Sample wells should be completely filled to the top with buffer. If any wells are under-filled, top them off with additional buffer.

Note: The total volume of the sample well is approximately 70*µl.*

10.3 Continuity Test

The continuity test measures the current in each separation and elution channel and determines whether they are within the expected values for a successful run.

Important! *Temperature affects electrical current readings. If cassettes have been refrigerated, they will fail the current test until the cassette is at least 17°C (62°F). Should this be the case, wait until the cassette temperature has equilibrated to room temperature and re-test.*

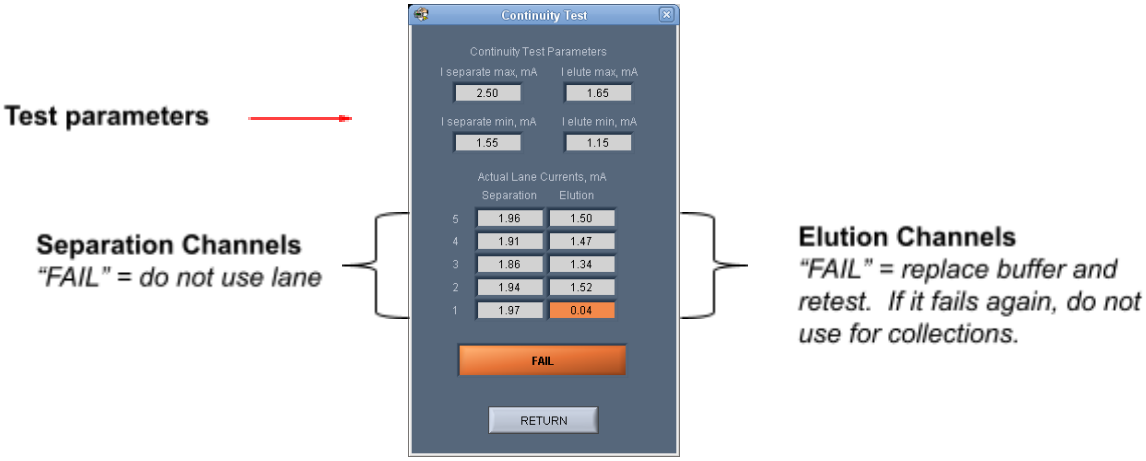
1. **Press "Test".** With the cassette in the optical nest, close the lid and press "TEST" on the Control Panel on the Main Tab.



2. **Continuity Test will Automatically Run.** The continuity test screen will launch. The separation and elution channel test parameters are displayed and the electrical current results will be listed after several seconds. If the test returns a "PASS" message, press "RETURN" and continue to baseline calibration.



3. A failed test is indicated by a "FAIL" message, and the failed channel is highlighted in orange. **Figure 10.5** shows a failed test screen (elution channel in lane 1). If an Elution channel has failed, it may be due to low buffer levels and may be rectified by refilling (see step 5 below).



:ontinuity Test screen. The elution cl

Important! Failure due to temperature.
The continuity reading can be affected by temperature. If this is the case, the continuity reading will only be **slightly different than the other lanes** (or they will all fail, but have similar values). This is likely due to the gel cassette having been stored in an environment that is outside the 17-23ºC operating range (e.g. refrigerated or exposed to sunlight). If this can be verified, allow the cassette to equilibrate to room temperature and test again.

4. **If a Separation lane has failed (left column) continuity.** Do not use that lane. Remaining passing lanes can be used if necessary.

5. **If an Elution channel (right column) has failed continuity.** Replace buffer in the elution module of the failed lane and refill with 40 ul of fresh electrophoresis buffer and retest the cassette. If the lane fails again, do not use the lane for collections.

11 Loading Samples

Proper sample loading is critical for best performance of BluePippin cassettes. For maximum reproducibility and accuracy, the sample should travel through the central section of the gel column, and should be bounded on all four sides by uniformly conductive media – either gel or electrophoresis buffer. The goal of the loading procedure is to produce this geometry in the sample loading well as illustrated in the bottom section of **Figure 11.1**. Properly prepared samples will be 40 ul in total volume consisting of 30 ul of DNA mixed with 10 ul of BluePippin internal standard mix or loading solution. The loading solution contains concentrated Ficoll as a densifying agent (see the Section 6 on Sample Preparation for details), and therefore the samples will sink and form a high density layer beneath the electrophoresis buffer when pipetted slowly into the sample wells. If there is insufficient conductive buffer over the sample, the electrophoretic forces lines will curve upward as the sample exits the well (see **Figure 11.1**, upper section), and the sample will be drawn to the top of the cassette where it can travel out of the gel into the gap between the gel column and the plastic top of the channel. Sample moving in this gap will travel at a different rate than the sample inside the gel column and will lead to elution of undesired size fractions in the eluted material. In such cases, the contaminating DNA will usually (but not always) be higher in molecular weight than the selected DNA.

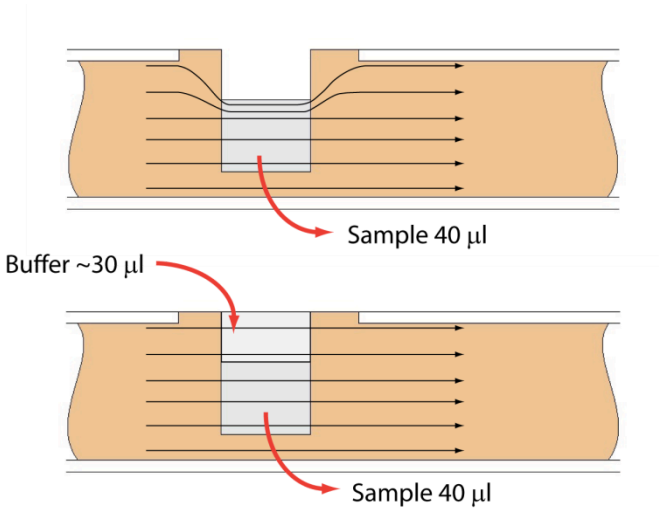


Figure 11.1. An illustration of the electrophoretic effect on a DNA sample when a sample well is not completely filled.

1. **Re-check the buffer level in the sample wells.** Make sure that sample wells are completely full to the top with electrophoresis buffer. Top off with additional buffer, if necessary. The total volume of the sample well is 70 ul.

2. **Remove 40µl of buffer from the first sample well, and load 40µl 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 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 (see **Figure 11.1**). 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 ~30ul of buffer left in the well. When adding sample, place tip of pipette just below the surface of the buffer, and follow the liquid level up with the tip as the well fills. 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 remaining wells.**

12 Running a Protocol

The default screen on the BluePippin is a tabbed format. The Main Tab is the first tab and the default screen.

12.1 Overview

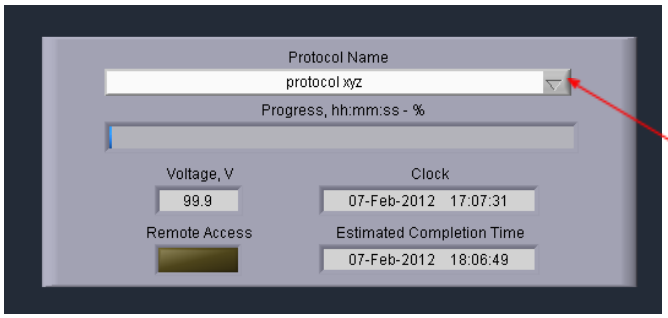
The Main Tab is the screen with which users load protocols, run the instrument, and monitor optical signal and electrophoresis.



12.2 Starting a Run

Important! The BluePippin should be optically calibrated prior to every run. If this has not been done, refer to **Chapter 9** for instructions.

1. If necessary, select the protocol to be run from the “Protocol Name” drop down menu in the Protocol Status Panel. The last protocol that has been programmed will automatically be loaded. **See Chapters 7 and 8** for instructions on programming.

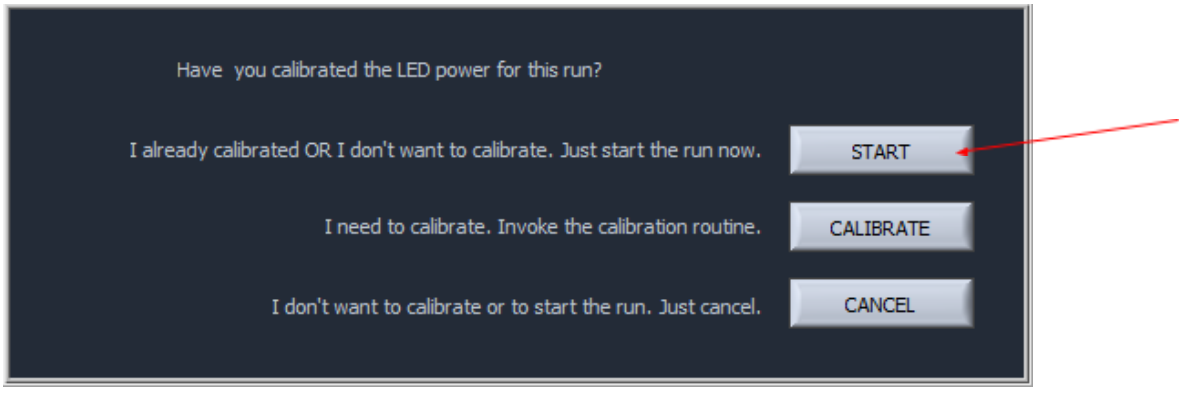


2. Load a properly prepared cassette (**Chapter 10)** into the optical nest. Typically, users will load samples while the cassette is on the nest (see **Chapter 11**). Ensure that adhesive tape has been placed over the elution modules.

3. Press “START” on the Control Panel.

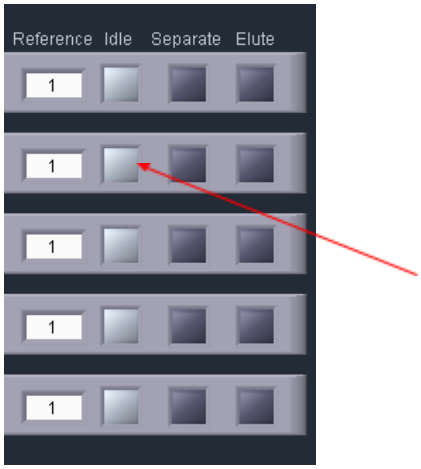


4. A pop-up window will appear to confirm that the LED calibration test was not inadvertently skipped. Press “START” to start the run.



12.3 Monitoring a Run

The Lane Status Pane has three indicator lights per lane. When the instrument is idle, all “Idle” indicator lights are light gray.



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



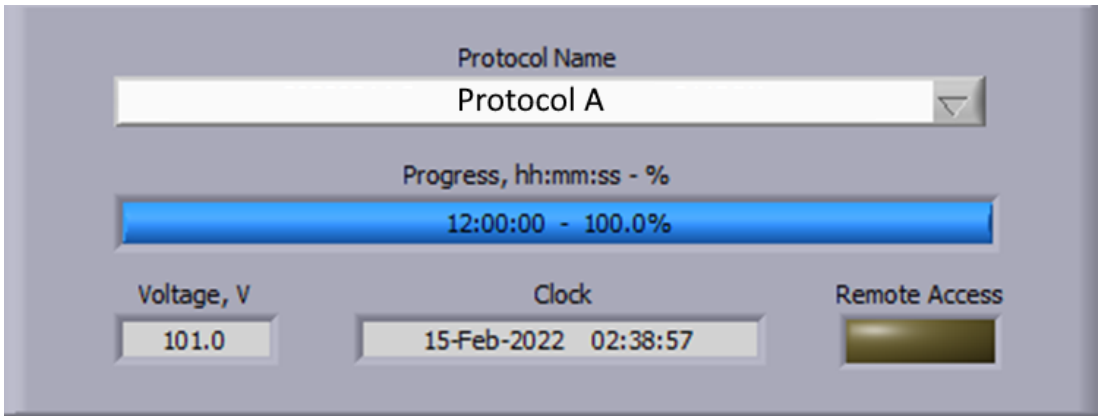
- **Green, Separation**. If a lane is separating fragments, the “Separate” indicator will be green.
- **Orange, Elution**. If a lane is eluting a size range, the “Elute” indicator will be orange, and the elution timer will be active.

Note: *If there is a time value in the “Elution Timer” field, and the orange “Elute” indicator is no longer lit, then the elution has been completed for that lane.*

The Elution Timer records the length of time of the elution. When elution is complete, the electrophoretic path will switch to either Idle state (if the "End Run When Elution is Completed" box is checked when the protocol was programmed (see **Figure 12.1**, below) or back to the "Separate" separation channel (if the box was not checked). The elution timer will show the value of the total elapsed elution time.



Run progress is monitored in the Protocol Status panel. The percent completion of the run time is indicated by a progress bar. The run time is determined by the "Run Time" field in the protocol editor (see **Figure 12.1**, below).



Important! If the "End Run when Elution is Completed" is checked in the protocol editor, the run may terminate before the total time is reached. The progress bar will jump to 100% at the end of the run.

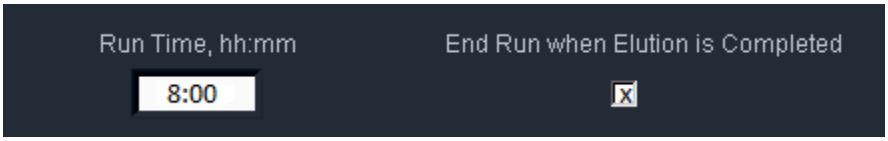


Figure 12.1 The Run Time field in the protocol editor. This time value is used to determine the progress of the run.

12.4 Log Files

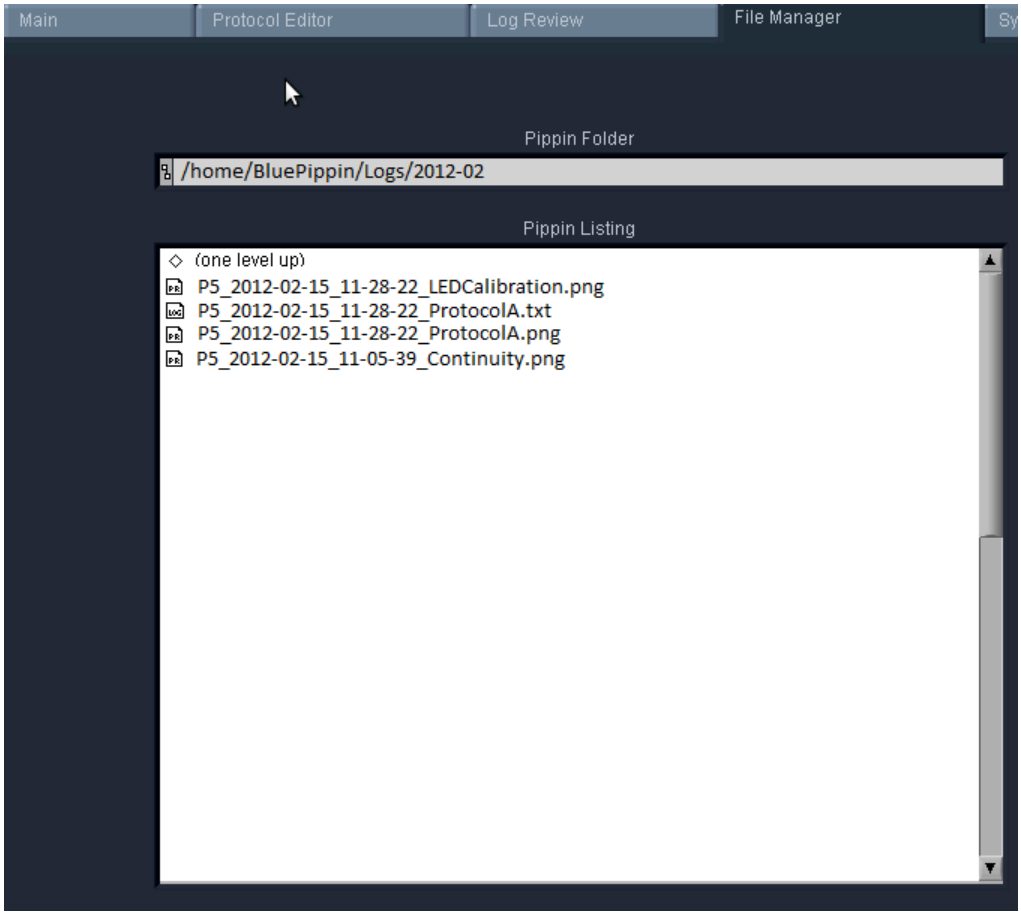
At the end of every run, the BluePippin will automatically save a log file (.txt) which can be viewed on the Log Review Tab (or the PC software version of Log Review). A screen image (.png) of the Main Tab is saved after the run has been finished. Screen images of all continuity tests and calibration tests are also automatically saved.

All files may be accessed from the File Manager Tab. The files are saved internally on the Pippin hard drive in the directory named **/home/pippin/BluePippin/Logs/** in a folder with a year-month (YYYY-MM) folder name.

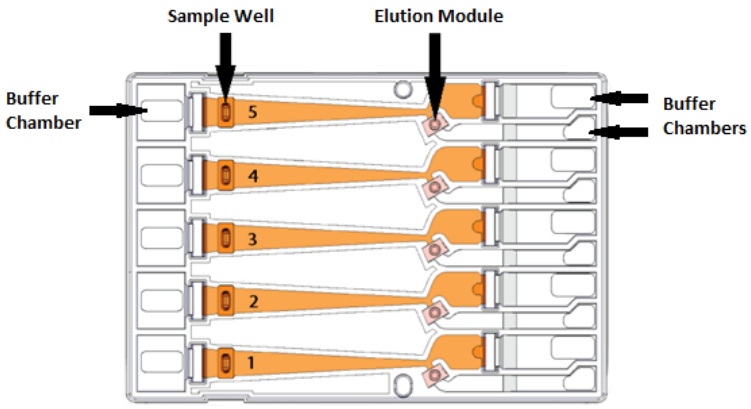
Log files names have the following convention:

[Instrument serial number/name] _[year]-[month]-[day]_[hour]-[minute]-[second]_[user input protocol name or test type].[file extention]

An example of the four types of log files, and file structure in the File Manager Tab, is shown below. The files will be saved every time these procedures are undertaken (see Section 19-2 for more information).



13 Sample Collection



1. Remove the sealing tape strip that covers the elution modules.
2. Samples can be removed from elution modules using a standard 100-200µl pipette.

Important! *Make sure that the pipette tips used for collection extend all the way to the bottom of the elution modules without sealing the elution port opening. If the tips seal the port opening, it will be extremely difficult, if not impossible, to completely recover the contents of the elution module. Test tip fit using the empty rinse cassette supplied with the instrument.*

3. Samples from standard cassettes with sealed elution modules will be recovered in approximately 40µl of electrophoresis buffer.
4. When all samples are collected, remove used cassette from instrument and dispose of it properly.

Important! *Used cassettes should never be allowed to sit in closed instruments for long periods of time (e.g., more than one day - overnight is acceptable). Under those conditions, humidity from the cassette reservoirs can accelerate corrosion of the electrode assembly, located in the sliding cover.*

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.*

Note. *Electrophoresis buffer is 50 mM Tris, 30 mM TAPS, 0.1 mM EDTA. During elution, EDTA does not rapidly equilibrate across the ultrafiltration membrane of the elution module, and so the final concentration of EDTA in eluted samples is elevated to 1-2 mM.*

Note: *Eluted samples can be used directly for ligation and amplification without buffer exchange.*

14 Sample Recovery and Yield

14.1 Overview

For most DNA collections, the intrinsic sample recovery, based on the input and recovery of restriction fragments, is between 50-80% (section 14.2). The amount of DNA recovered from a sheared/fragmented sample is dependent on the input DNA distribution profile, where the size selection occurred relative to that profile, and the range of fragments collected (section 14.3).

DNA may become entangled in the ultra-filtration membranes in the elution modules which can reduce yield. The BluePippin automatically adds a brief field reversal at the end of each elution to "push" DNA off of the membrane (Section 14.4). However for higher molecular weight DNA collections (>2kb), we recommend several user steps to help improve sample recovery (section 14.5).

14.2 Intrinsic Sample Recovery on the BluePippin

Intrinsic DNA recovery in BluePippin cassettes is determined by running known amounts of a plasmid restriction digest on the BluePippin, collecting a broad range of fragments from the digest, and comparing the input and product profile quantitatively using the Agilent Bioanlyzer 2100. The intrinsic recovery of DNA fragments for most Pippin cassette types is between 50-80%. **Figure 14.1** shows the recovery of a 5kb band from a 0.75% cassette.

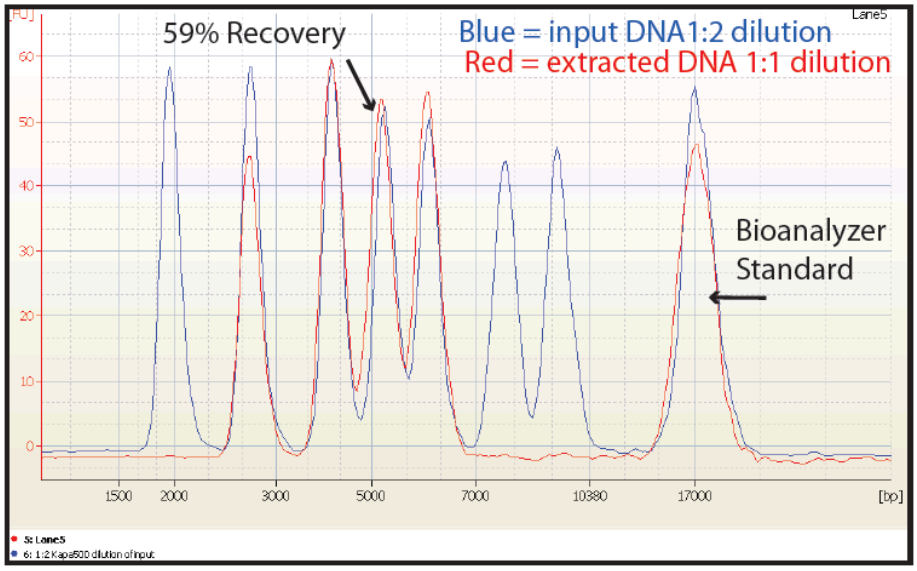
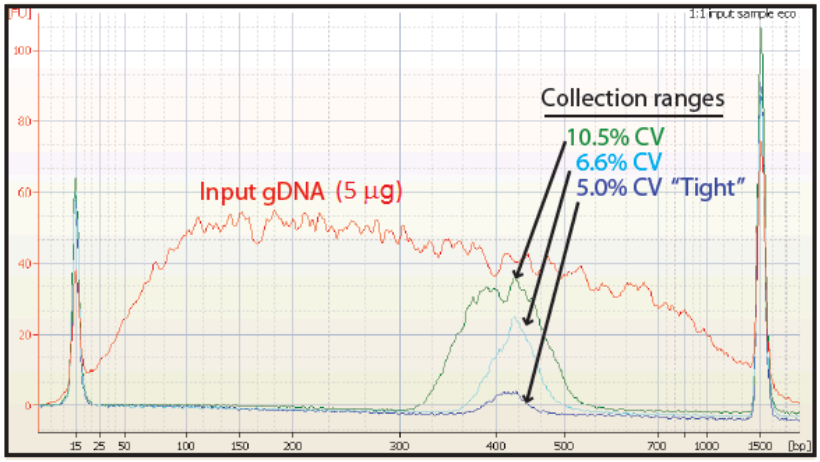


Figure 14.1. An example of the intrinsic recovery of 5 kb band on a 0.75% agarose cassette.

14.3 Improving Product Yield by Selecting a Wider Size Range

The BluePippin can produce very narrow size distributions from sheared genomic DNA. However, narrower size distributions will necessarily mean that a smaller fraction of the input DNA will be recovered. Users should broaden their collection ranges if the default tight settings do not produce enough DNA for their application.
Figure 14.2 shows an example of the effect of size range selection vs. yield.



Programmed Values - Pippin			Bioanalyzer Result			
Target	BP Start	BP End	Average Size (bp)	BP Start	BP End	Amt. (ng)
400	374	426	415	365	461	36
400	360	440	426	354	493	160
400	320	480	412	312	525	388

Figure 14.2. Increasing yield by widening the size selection range.

Note. *The percent recovery specification is determined by collecting known amounts of restriction fragments (Section 14.2). Product yield from a sheared DNA sample is influenced by the fragment distribution and input profile of the sample.*

14.4 Improving Product Recovery with the Field Reversal

The BluePippin provides a brief current reversal at the end of a sample elution can improve sample recovery by up to 20%. The field reversal is a default setting, located in the System Options Tab of the Pippin Software. The use of this option is recommended, but may be turned off by users. **Figure 14.3**, below, shows the improvement that can be achieved. **Figure 14.4** shows the setting option in software. When activated, the setting will be applied to all subsequent runs until the user again changes the settings in the System Options Tab.

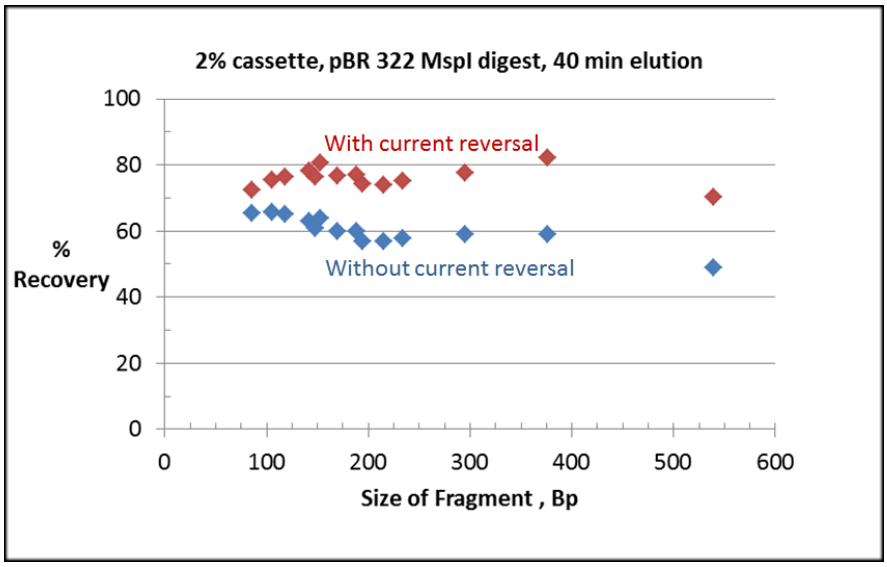


Figure 14.3. Improving recovery with a 5 sec. current reversal. A 40 minute elution of a plasmid restriction digest was run, and each data point represents an individual band.



Use this button to turn field reversal OFF/ON, and then press ("Accept"). The default setting is "ON".

14.5 HMW DNA: Recommendations to Improve Yield

Sage Science recommends the following procedures, in order of effectiveness, to maximize the yield for high molecular weight DNA (>2kb, and "high-pass" protocols).

1. Wait **at least 45 min** after termination of the run before removing eluted DNA (it is OK to leave samples in instrument for hours, i.e. for an overnight run).

2. Additional 10-30% yield can often be achieved by rinsing the elution module with Tween 20 (0.1% Tween 20 in 0.5X electrophoresis buffer). Tween solution is provided by Sage Science with cassette kits for HMW collections.

   After removing the initial eluted volume:

   - Remove eluted sample from elution module
   - Add 40µl Tween solution (supplied with the cassettes) to the elution module
   - Wait 1 minute
   - Remove solution and pool with the original extracted sample, or process separately



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.*

15 System Validation

Control DNA can be purchased from Sage Science to check the performance of the BluePippin system. The Control DNA is useful to test, refine, and troubleshoot BluePippin size fractionation protocols. For comparison to factory tested cassettes, an extraction protocol and typical Agilent Bioanalyzer results are provided with the kits. **Figure 15.1** illustrates QC size selection protocol for the 2% agarose control kit, and **Figure 15.2** shows a typical result from a 150 bp tight extraction from the kit guide.

BluePippin cassettes and instruments are functionally tested using restriction digests of genomic DNA from E. coli. For each cassette type, a different restriction digest is used, chosen so that size distribution of the digested DNA closely matches the useful fractionation range of the cassette, without any significant peaks or discontinuities. Following restriction digestion, the control DNA is purified by bead purification, and diluted into BluePippin electrophoresis buffer. Each kit contains sufficient control DNA to fully load four gel cassettes.

	Tight	Range	Time	Range+T	Ref Lane	Target *	Start *	End *	Pause *
5					1	500	450	550	0
4					1	400	360	440	0
3					1	275	248	302	0
2					1	150	135	165	0
1					off	0	0	0	0

Figure 15.1 Control DNA size selection protocol for a 2% agarose DNA control kit run (reference marker is assigned to lane 1).

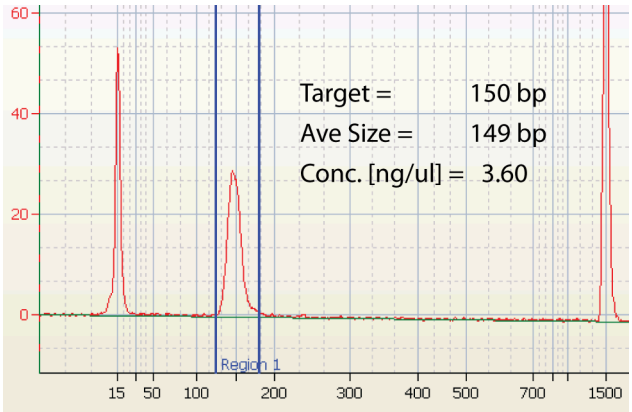


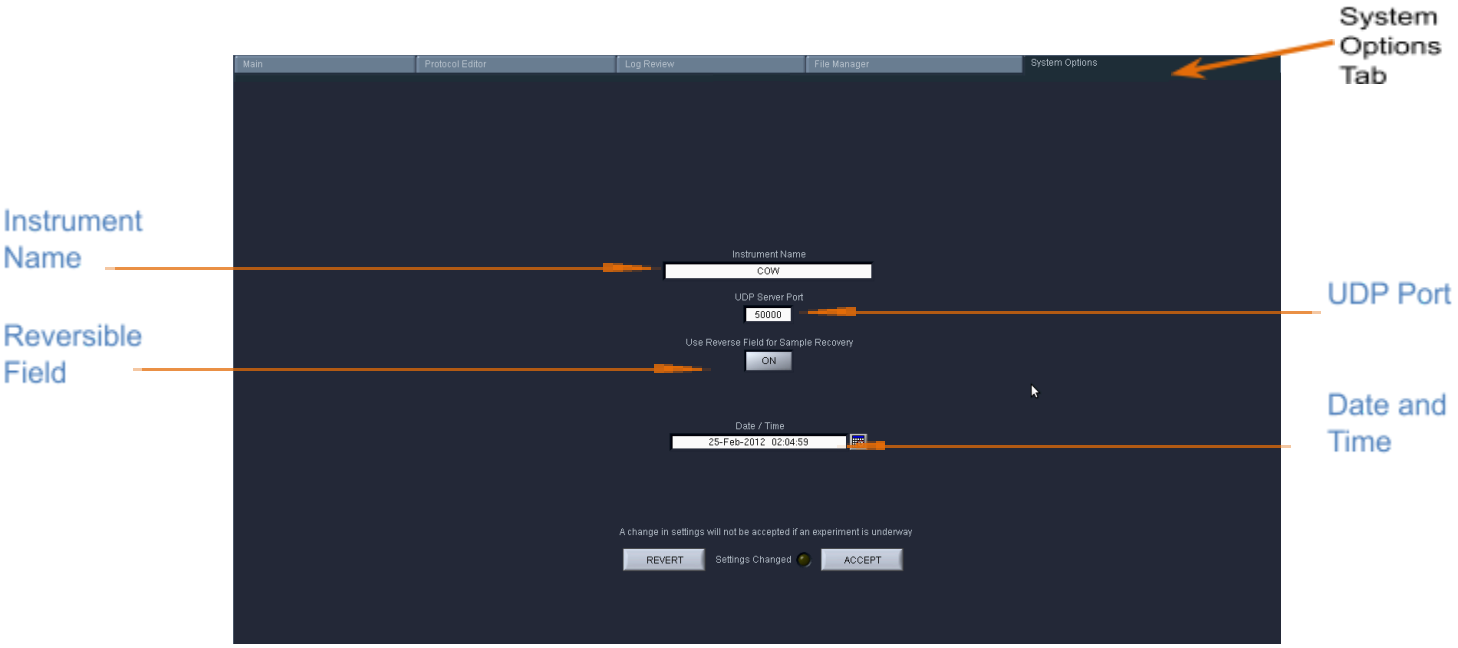
Figure 15.2 An example of a Bioanalyzer result for the 150 bp target of a Control DNA run on a 2% Agarose Gel Cassette

Important! Data are not intended to imply guaranteed results or performance. Control standards are intended to demonstrate that the BluePippin system is functioning as expected, and that proper operational technique is being used.

16 System Options Tab

The default screen on the BluePippin is a tabbed format. The System Options is the fifth tab.

Overview
The System Options Tab allows users to set or change global configurations for the software and BluePippin.



This screen allows users to set the following:

- **Instrument name**: A name can be given to (or changed for) the instrument that will be displayed in the Main Tab and in the log files. The default name is the instrument serial number.
- **UDP Server Port:** Provides an address for remote communications between the BluePippin and wireless control. Contact Sage Science for support.
- **Reversible Field for Sample Recovery:** When activated, all sample collections will terminate with a 5 second field reversal to improve recovery. When deactivated, all sample collections will not include the field reversal. The default setting is ON. When activated, the current will be reversed on all subsequent runs until the user changes the setting through the System Options tab.
- **Date/Time:** Displays the date and time settings for the instrument. The date and time settings are recorded in log files and log file names.

To Change the settings, press the “System Options” Tab.

Instrument name

1. Enter a text into the “Instrument Name” field.

2. The “Settings Changed” indicator light will activate. Press “Accept” to accept the new setting.

Reverse field option

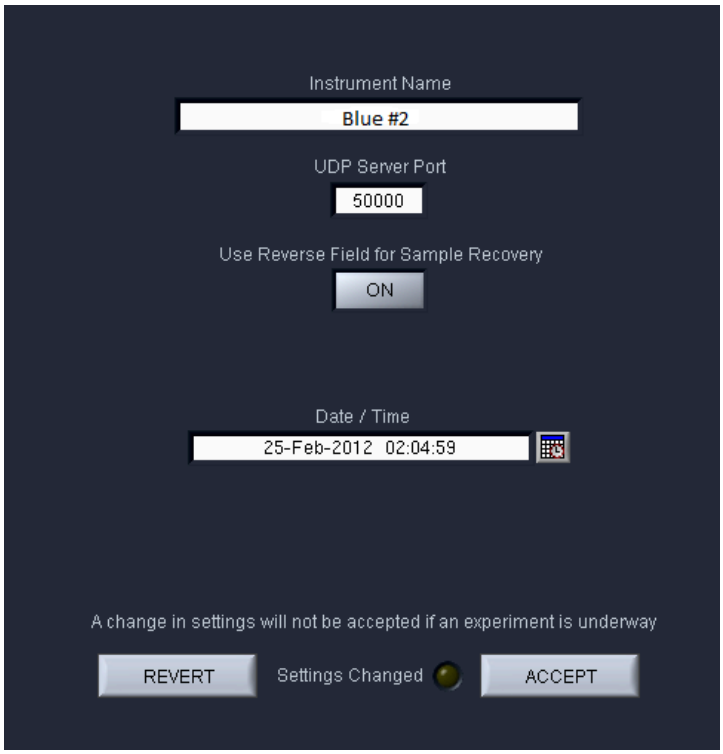
1. To turn on: press the “Use Reverse Field for Sample Recovery” button. The indicator display will change from dark gray to white, and the display will change from “OFF” to “ON”.

2. The “Settings Changed” indicator light will activate. Press “Accept” to accept the new setting.

Important! *The “reverse field” setting will apply to all runs until it is changed in this tab.*

Date and time

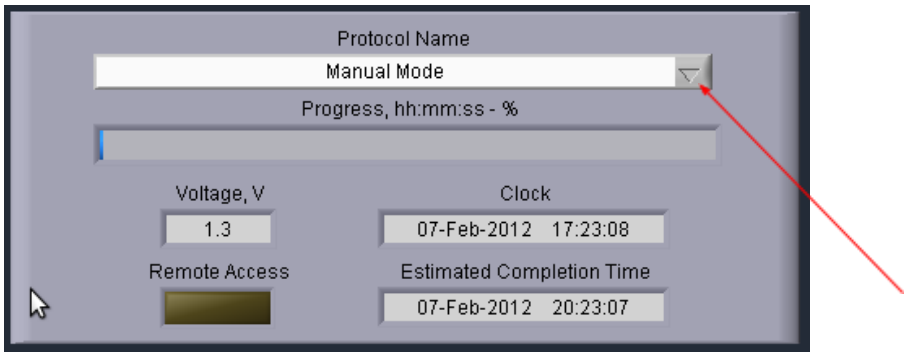
1. The time and date should be preset for a user’s time zone. For instructions on changing the time and date, please contact Sage Science support.



17 Running in Manual Mode

The BluePippin may be run without using a programmed protocol and with manual control.

1. Select the **Manual Mode** from the top of the “Protocol Name” drop down menu in the Run File Manager.

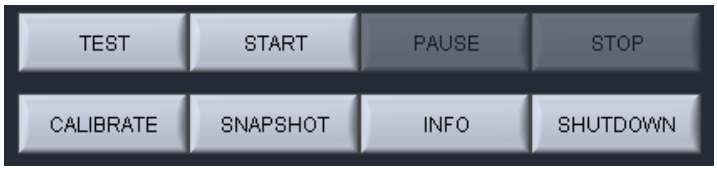


2. Press “Start” on the controller.

3. Sample lanes may be run individually by pressing the “Separate” button, and elutions may be carried out by pressing the “Elute” button. After elution, press the “Separate” button, to end the elution (and return to separation mode), or press the “Idle” button to stop the run in that lane.



4. The “Pause” / “Resume” and “Stop” buttons are also operative as in automated runs.



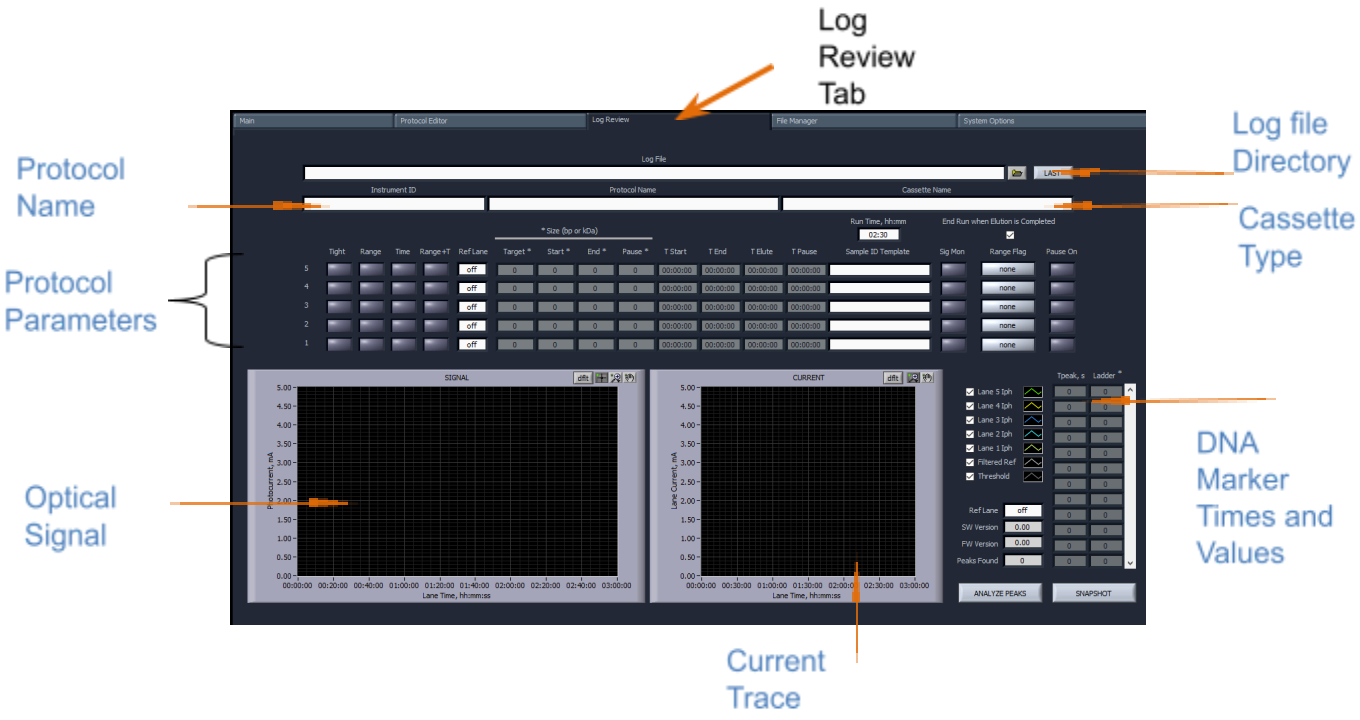
Note: *There is a delay between the time that a fragment is detected (and visible on the graph or image) and when it is in position to be eluted. See cassette specifications at the end of this manual to determine the best timing for manual runs.*

18 Analyzing Runs - Log Review Tab

The default screen on the BluePippin is a tabbed format. The Log Review is the third tab.

Overview

The Log Review screen is used to review data and programming parameters from previous size selection runs. All programming parameters are displayed as originally entered in the Protocol Editor. Optical and electrical current data from the run are displayed in graphical windows. Specific regions of either window can be selected and expanded as needed for detailed analyses. These data are useful for refining size selection performance and verifying instrument performance.

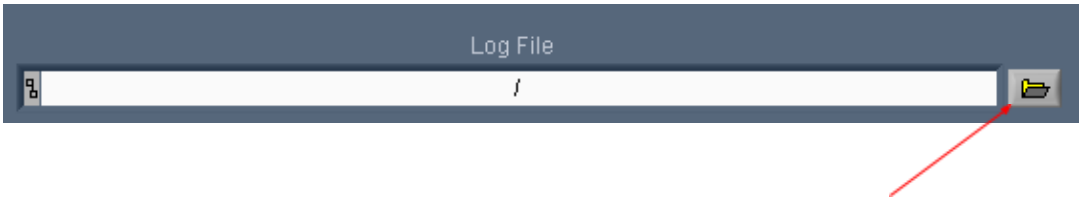


Opening a Log file in the Review Screen

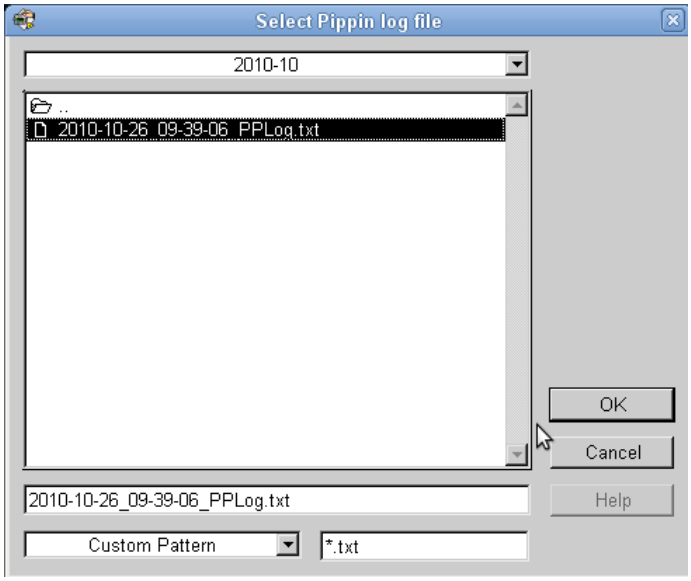
Note: *A stand-alone Review Log Program is available for PCs. Contact Sage Science support.*

1. Click the “Log Review” tab along the top of the screen.

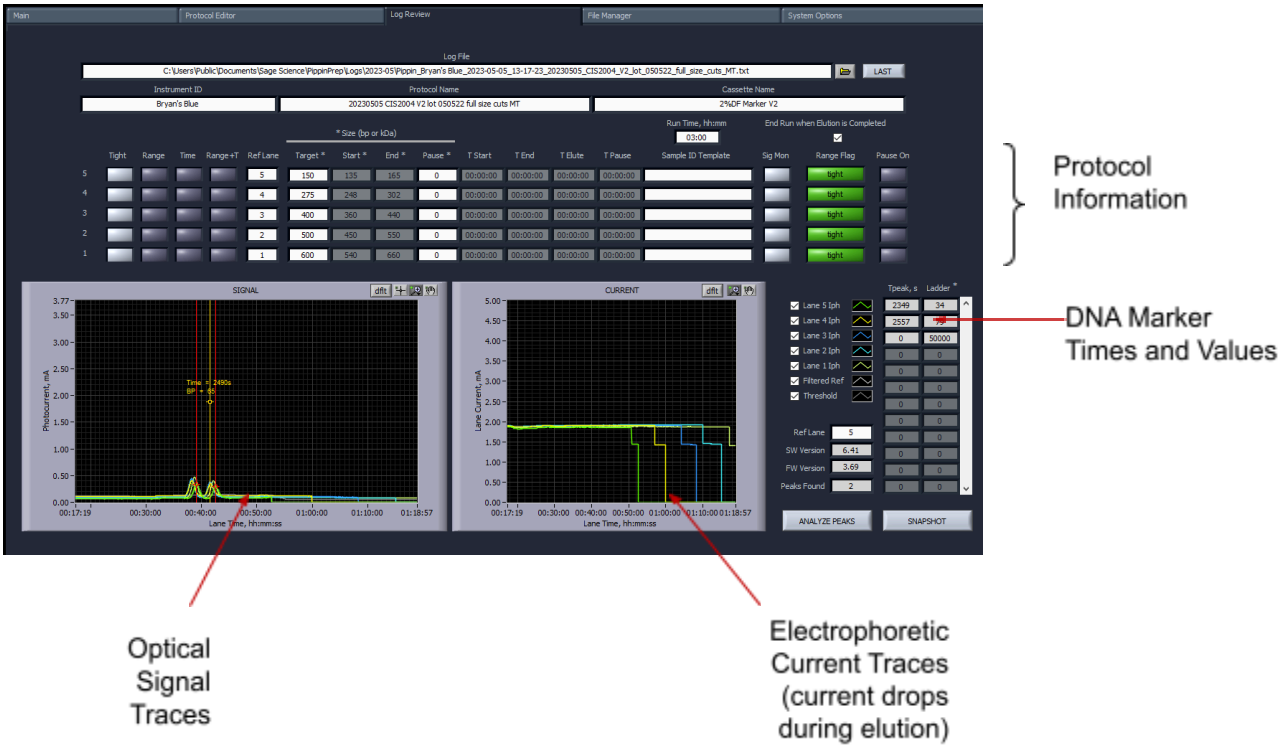
2. To select a log file, press the folder icon next to the “Log File” field.



3. Select a log (.txt) file.

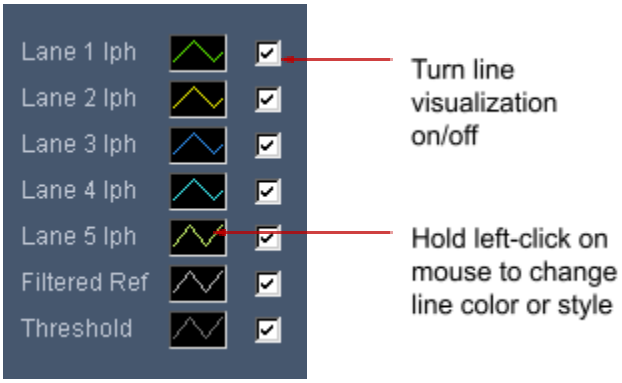


4. This will populate the Review Log Screen with data from the selected run log:



Visualizing Pippin Run Data

The current and optical signal traces are color-coded and a key is displayed to the right of the “Current” graph. A check mark next to each lane will toggle the visualization of that lane on and off. The type and color of the lines may also be adjusted by clicking inside the line field and holding the left-click on the mouse. The same styles are applied to both the signal and current traces.

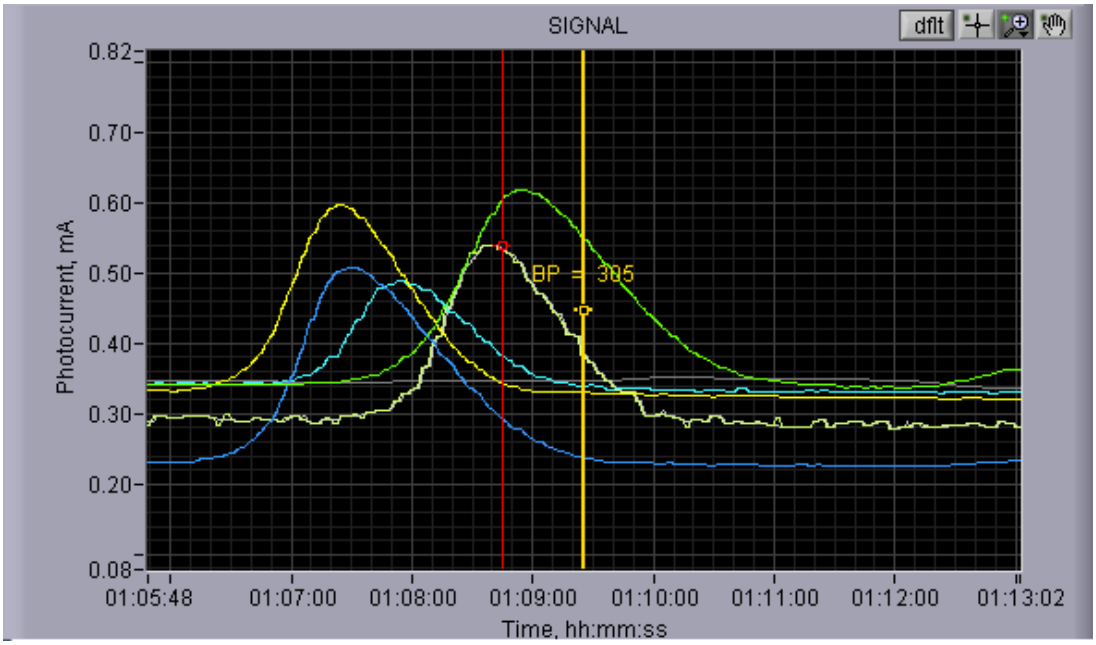


Signal Graph

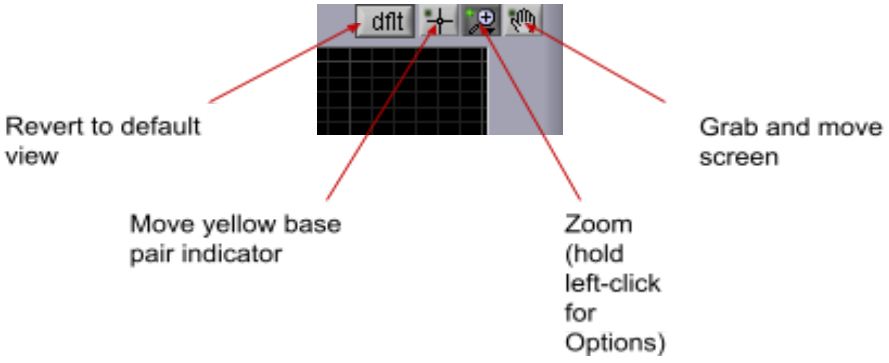
The “Signal” graph allows users to closely inspect the elution results of a Pippin run from a run log. The colored horizontal traces display the actual optical signal results in mA.

The vertical red lines indicate called reference DNA marker peaks that are listed in the DNA marker times and values list.

The yellow vertical line indicates the estimated base pair position at a given run time. The indicator may be moved (see below) along the x-axis showing the relative base pair position. If the perspective of the graph is changed (see below), the indicator will automatically default to the center of the x-axis.



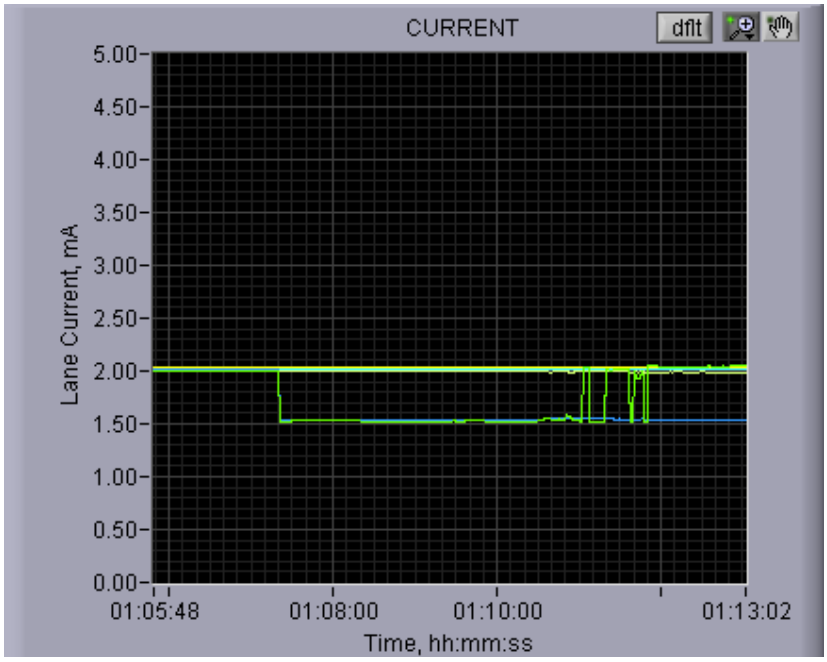
There are four icons in the upper right hand corner of the graph. The functions are listed below.



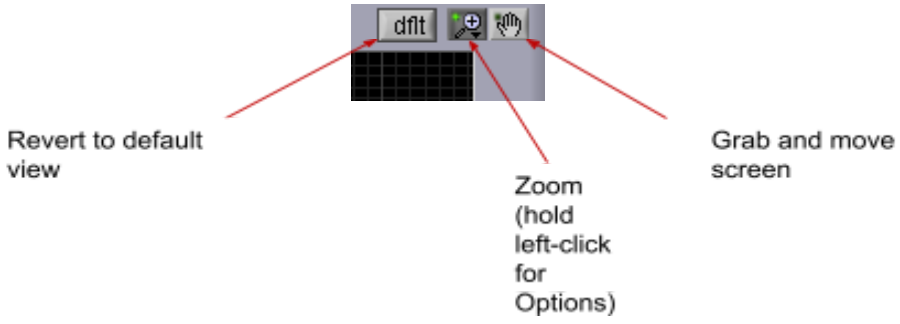
Current Graph

The “Current” graph allows users to closely inspect the electrophoretic current profile of a Pippin run from a run log. The colored horizontal traces display the actual current signal results in mA.

The timing of an elution (switch from separation to elution) is accompanied by a 0.5 mA drop in current.



There are three icons in the upper right hand corner of the graph. The functions are listed below.



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.



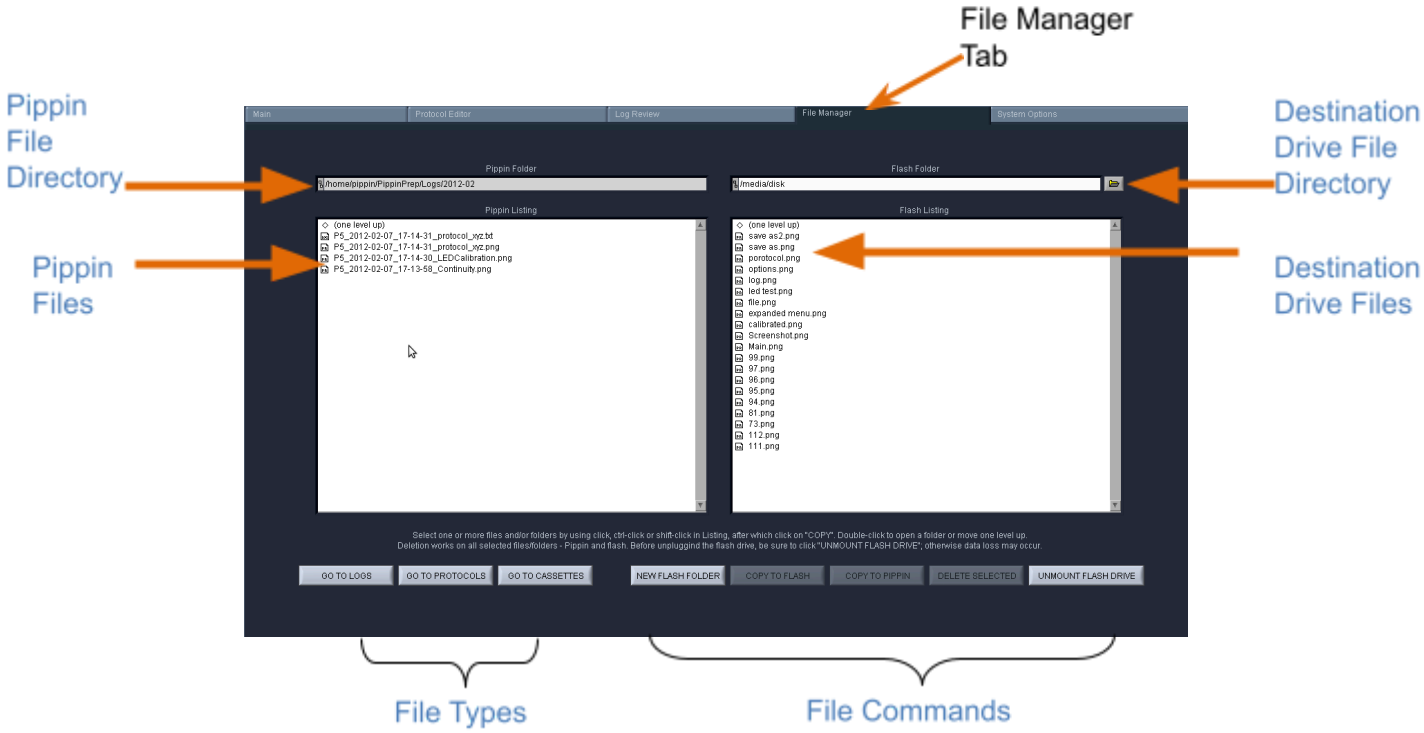
19 Managing Files -- File Manager Tab

The default screen on the BluePippin is a tabbed format. The File Manager Tab is the fourth tab.

19.1 Overview

The File Manager Tab allows users to access, copy, and delete BluePippin files. There are three types of files used by the system; log (text and image), protocol and cassette type.

The screen is divided into two sub-screens; the Pippin file directory on the left, and a target directory (portable “flash” media, i.e. USB key) on the right.



19.2 File Types

There are three types of files that are stored in different directories.



The file types are:

Log Files:

- **Optical Calibration** - at the end of every optical calibration routine, a screenshot of the result is saved as a .png file.
- **Continuity Test** – at the end of every continuity test, a screenshot of the result is saved as a .png file.
- **Main Screen** – at the end of every run, or if a run is stopped, a screenshot of the main screen is saved as a .png file.
- **Run Log** – at the end of every run, or if a run is stopped, a text (.txt) file is saved. This file contains optical data, electrical current data, and protocol and sample information from a run. Run Log data may be viewed in the Log Review screen (See Section 12).
- **Current Leak Test –** after every pulsed-field run (0.75% agarose cassettes) a current test is run between lanes. This is because salt build up can occur and create a current bridge between electrodes. If so, a warning pop-up will appear and the data will be stored in this log file that should be forwarded to Sage support for troubleshooting.

Protocol Files:

Size selection protocols that have been created, named, and saved by BluePippin users. These have a .ppprot file extension, and are only recognized by the BluePippin.

Cassette Files:

Cassette definition files. These are created by Sage Science and not editable by BluePippin users. These have a .ppcass file extension, and are added to the BluePippin during manufacture or through software upgrades.

Note. The BluePippin is equipped with 25 GB of storage. The combined files sizes for the log files saved for each run is about 150 kb, or a total storage capacity for 166,000 runs.

19.3 Transferring files

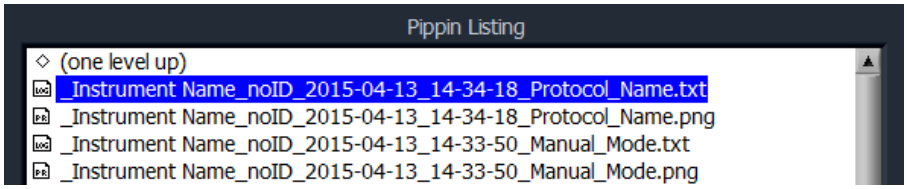
Files may be copied between the BluePippin and a USB flash drive. To transfer a file, place a USB drive into the USB port on the front panel of the instrument (the USB ports on the back panel may be used as well).

To transfer from the BluePippin to a USB Drive:

1. Select the directory from which the file will be copied.



2. Highlight the file to be copied from the BluePippin.



3. Press the “COPY TO FLASH” button.

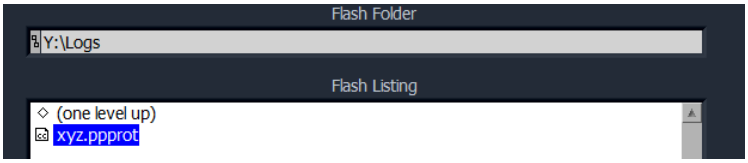


4. Press the “EJECT FLASH” button.

5. Remove the USB drive from the USB port.

To transfer from a USB Drive to the BluePippin:

1. Highlight the file to be copied.



2. Select the directory to which the file will be copied.



3. Press the “COPY TO PIPPIN” button.



4. Press the “EJECT FLASH” button.

5. Remove the USB drive from the USB port.

20 Upgrading Software

Pippin upgrade software is available for download at **www.sagescience.com/support/**. The files are provided in a zip file, and the contents must be extracted to the root directory of a USB flash drive.

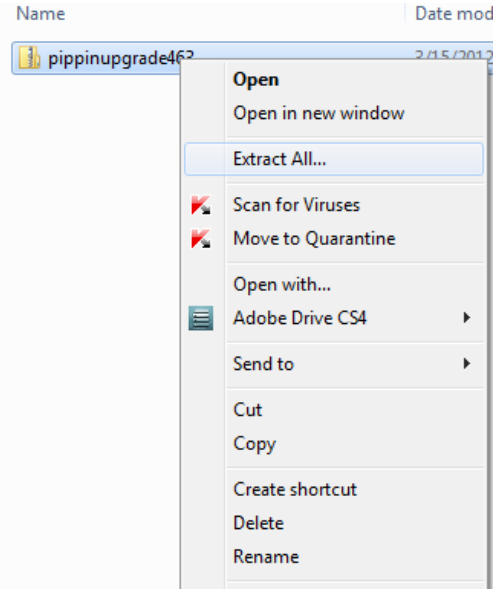
20.1 Extracting the Files to a USB flash drive

1. Download the Software Upgrade package by pressing the appropriate link on www.sagescience.com/support/ (in the Downloads section, under “Software”).

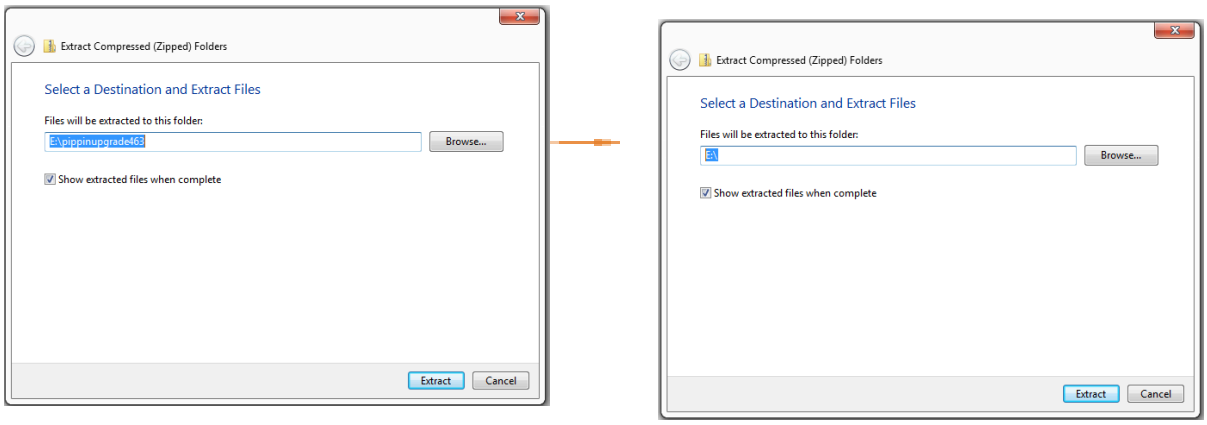
2. Press “Save File” when prompted. The zip file will likely save to a “Downloads” folder on your computer. The file will be named pippinupgrade[version number].zip.

3. Copy the zip file to a USB drive. Make sure the file is transferred to the root directory and not placed within a folder.

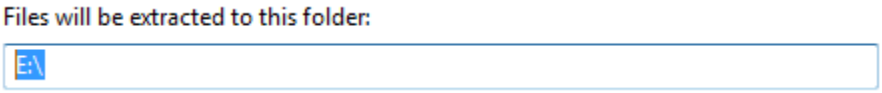
4. Right-click on the zip file, and select “Extract All....”:



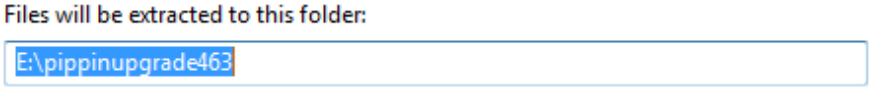
5. A window will launch to prompt you to select a destination where to extract files. The prompt will suggest creating folder based on the zip file name. Delete the destination folder name, so that only the root directory appears (E:\ in the example below).



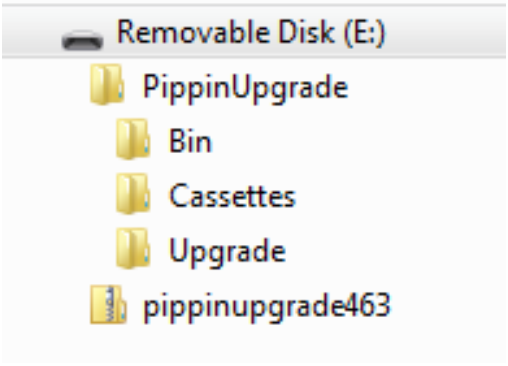
Correct:



Not Correct:



6. The final USB file structure should be as shown. The zip file may be deleted.



20.2 Upgrading the Pippin Instrument Software

1. Insert the USB drive with the upgrade folder into the USB port on the front panel of Pippin instrument.

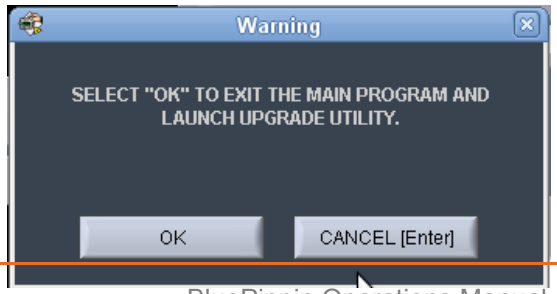
2. From the Main Tab, press “INFO”. This will launch the information window.

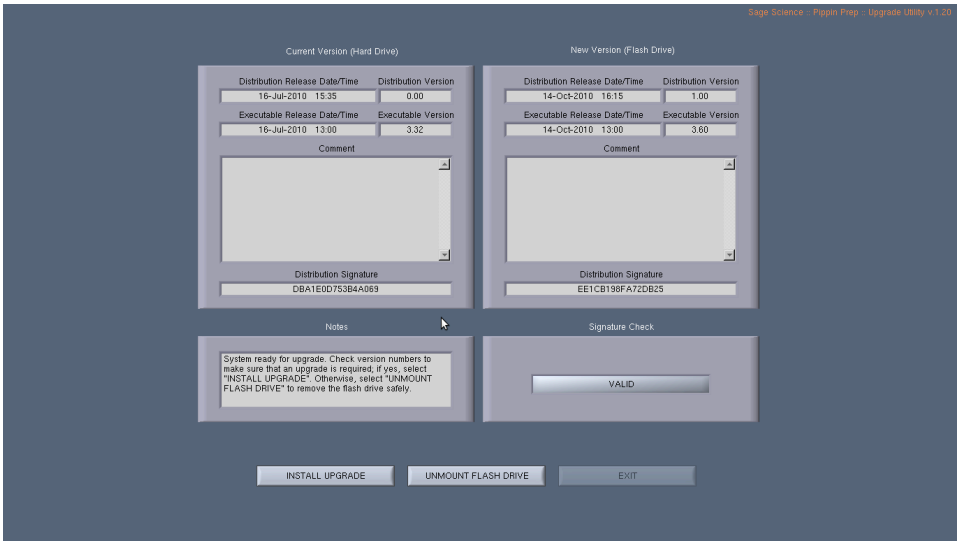


3. From the Information Window, press “SOFTWARE UPGRADE”



4. Press “OK” at the warning prompt, the Software Upgrade Window will launch.





5. Press “INSTALL UPGRADE”, this will take about 5 seconds



6. Press “UNMOUNT FLASH DRIVE”



Important! *Be sure to “Unmount Flash Drive” prior to removal. Failure to do so may cause data loss and problems with the upgrade procedure.*

7. Press “EXIT” to return to Main Menu. This will install the software. Installation may take up to 30 seconds.



8. To check that the software instrument has been updated, Press “INFO” again. In the Information window, the software version number is listed on the left side.



Note: *The system should be ready to use; re-boot is not necessary.*

21 *Preventative Maintenance – Rinsing Electrodes*

It is possible that salts will accumulate on the electrodes that are housed in the BluePippin lid assembly. This may alter the performance of the instrument over time. An empty cassette body, labelled "Rinse Cassette" is provided with the BluePippin. This should be used with the procedure below. No other preventative maintenance is required.



***Important!** Electrodes should be rinsed **after 5 runs or once per week**, whichever is more frequent.*

21.1 Electrode Rinse Procedure

1. Fill the rinse cassette with deionized water.
2. Load the rinse cassette onto the sample tray and close the BluePippin lid.
3. Allow the electrodes to rinse for at least 30 seconds.
4. Open the lid and remove the cassette.



Note: If a rinse cassette is lost or damaged contact Sage Science support for a replacement.

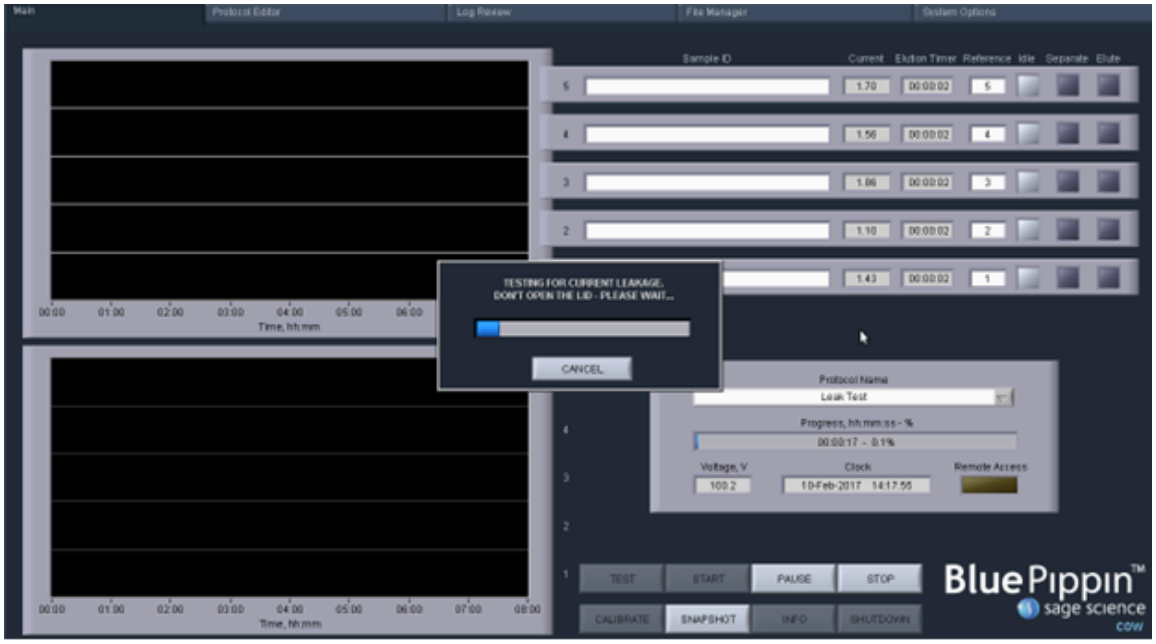
21.2 Current Leakage Test

After every pulsed-field run (0.75% agarose cassettes), a current leakage test is run between adjacent lanes. This is run because salt can accumulate on electrodes and create a current "bridge" between electrodes (an electrical current can jump from lane to lane). The relatively long duration of pulsed field runs, and having cassettes remain under a closed lid for long periods of time make this scenario more likely. Unchecked, a salt bridge can affect the performance of peak calling and size selection and ultimately cause the failure of the instrument.

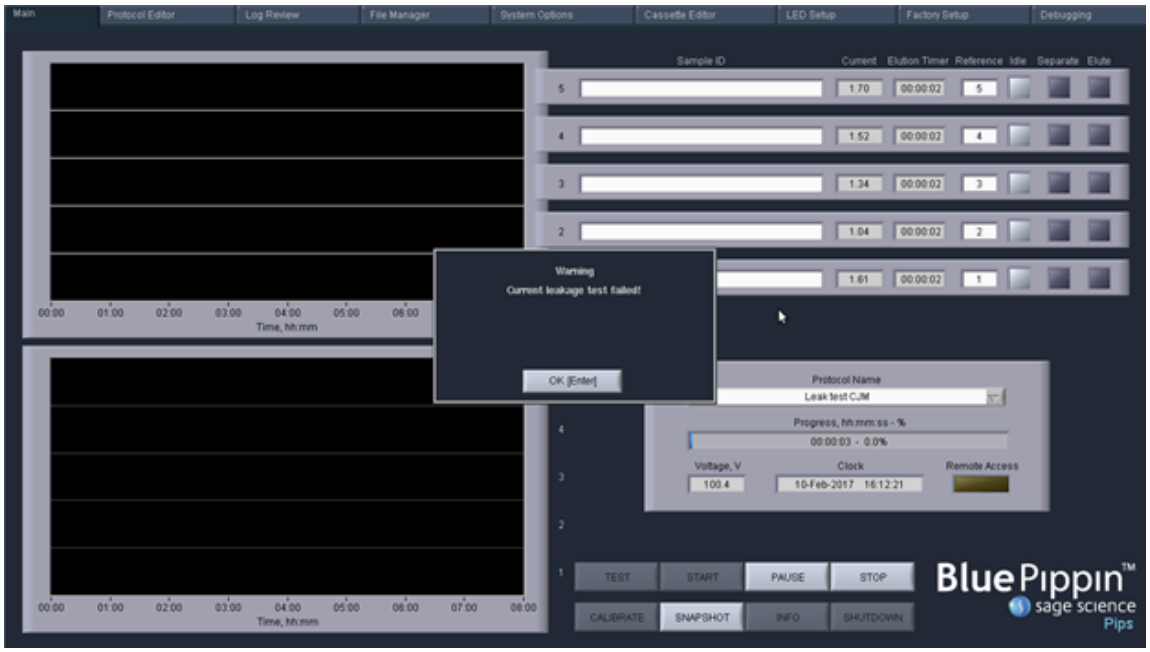


***Important!** Periodic rinsing of electrodes and minimizing time that cassettes are left in the instrument with a closed lid minimizes salt build up and extends the life of electrodes.*

At the end of every pulsed-field run, the BluePippin will automatically run a current leak test. The test lasts about 10 minutes and display a progress bar as it progresses:



If the test fails, a warning box will appear with the following message: “Warning! The Current Leakage Test Failed!”



Important! If the Current Leakage Test fails, contact Sage Science support! It is likely that the instrument will require servicing.

21.3 Cleaning the instrument surface

The surface of and nest of the BluePippin can cleaned with a moist lab tissue. 70% ethanol solution from a squeeze bottle can also be used. Cleaning of the detector area is not routinely necessary unless there is buffer spillage, in which case a moist lab tissue should be used to clean the area.



Warning! Do not use bleach or pour water or other liquids onto the instrument surface.

22 Warranty and Service

Warranty

Sage Science will offer a no charge repair or replacement of defective product if notified within the warranty period and product has not been misused, altered or damaged by disaster. This warranty is not transferable from the original purchaser to a subsequent purchaser.

Instruments – The BluePippin instrument and monitor have a standard 2-year parts and labour warranty. Instruments are subject to depot repair or replacement. On-site maintenance or repair is not implied. Do not attempt to repair or disassemble the BluePippin instrument. This will void the remaining warranty and possibly create a dangerous condition. Sage Science support staff may ask for a log file or image file to evaluate or troubleshoot instrument issues.



High Voltage! *The BluePippin contains high voltage apparatus. The instrument should be repaired by authorized personnel only. Do not disassemble under any circumstance.*

Cassettes – Cassettes have an 18-month warranty. If a cassette or sample lane is defective Sage Science will replace the cassette at no cost. The defective cassette should be disposed of as per regulations at the user's facility. Sage Science support staff may ask for a log file or image file to evaluate or troubleshoot cassette issues.

Cassette Disposal - The BluePippin system uses **disposable agarose gel cassettes**. The components of these products contain no substances which, at their given aqueous concentrations, considered to be hazardous to health or ecosystems. Use standard laboratory disposal practices for disposal. Check with facility or local municipal regulations.

Reagents and buffer should be disposed of according to internal lab safety policy.

Note: Dye-free cassettes use fluorescein dye bound to the reference external markers and internal standards.

23 Ordering Information

Products		
Instrument		**Catalogue No.**
BluePippin (Includes monitor, keypad, mouse and accessories)		**BLU0001**
Dye-Free Gel Cassettes – with Internal Standards (10/pk) **Size Selection Ranges (see section 25 for specifications)**	**Internal Standards**	**Catalogue No.**
3% agarose, dye-free, 100 – 250 bp	Q3	**BDQ3010**
2.5 % agarose, dye-free, 100 – 600 bp	V2	**BDF2510**
2% agarose, dye-free, 100 – 600 bp	V2	**BDF2010**
1.5% agarose, dye-free, 250 – 1500 bp	R2	**BDF1510**
1.5% agarose, dye-free, High-Pass >250 bp	R2	**BDF1510**
Dye-Free Gel Cassettes – with External Marker (10/pk) **Size Selection Ranges**	**External Marker**	**Catalogue No.**
2% agarose, dye-free, 100 – 600 bp	M1	**BEF2010**
0.75% agarose, low range, > 1 – 10 kb	S1	**BLF7510**
0.75% agarose, upper range, >10 – 18 kb*	U1	**BUF7510**
0.75% agarose, High-Pass > 4 – 10 kb*	S1	**BLF7510, PAC20KB**
0.75% agarose, High-Pass > 6 – 10 kb*	S1	**BLF7510, PAC20KB**
0.75% agarose, High-Pass >15 – 20 kb*	S1	**BLF7510, PAC20KB**
0.75% agarose, High-Pass >30 – 40 kb**	U1	**BUF7510, PAC30KB**
0.75% agarose, mid range, 18 – 27 kb	T1	**BMF7510**
0.75% agarose, high range, 50 kb	Z1	**BHZ7510**
High Pass Plus >10kb or >15 kb or >20 kb	U1	**BPLUS10**
Range + T 9-30kb	E2	**BRT7510**

* BLF7510 and PAC20KB (for PacBio users) consist of the same components, except for the user guide.
** BUF7510 and PAC30KB (for PacBio users) consist of the same components, except for the user guide.

Ordering Information Cont'd

Control DNA for Performance Validation	Catalogue No.
Control DNA for 3% Gel Cassette, (Int. Std) 100 – 250 bp, (5 µg/load), 20 loads	**CIS3004**
Control DNA, 2% Gel Cassette, (Int. Std) 100 – 600 bp, (5 µg/load), 20 loads	**CIS2004**
Control DNA for 1.5% Gel Cassette, (Int. Std) 250 – 1500 bp, (2 µg/load), 20 loads	**CIS1504**
Control DNA for 2% Gel Cassette, (Ext. Mkr.) 100 – 600 bp, (5 µg/load), 16 loads	**CON2004**
Control DNA for 0.75% Gel Cassette, (Ext. Mkr.), 1 -- 10 kb, (5 µg/load), 16 loads	**CON7504**
Control DNA for 0.75% Gel Cassette, (Ext. Mkr.),10 -- 27 kb, (5 µg/load), 16 loads	**CON7604**
Control DNA for 0.75% Gel Cassette, (Ext. Mkr.), High-Pass (5 µg/load), 16 loads	**CHP7510**
Reagent Replacement Kits – Dye Free with Internal Standards	**Catalogue No.**
BDF3010 reagent replacement kit for 10 cassettes, Marker Q3	**RBQ3010**
BDF2510, BDF2010 reagent replacement kit for 10 cassettes, Marker V2	**RBF2010**
BDF1510 reagent replacement kit for 10 cassettes, Marker R2	**RBF1510**
Reagent Replacement Kits – Dye Free with External Marker	**Catalogue No.**
BEF2010 reagent replacement kit for 10 cassettes, Marker M1	**RBE2010**
BLF7510 reagent replacement kit for 10 cassettes, Marker S1	**RBF7510**
BUF7510 reagent replacement kit for 10 cassettes, Marker U1	**RUK7510**
BMF7510 reagent replacement kit for 10 cassettes, Marker T1	**RMF7510**
BHZ7510 reagent replacement kit for 10 cassettes, Marker Z1	**RHZ7510**
BPLUS10 reagent replacement kit for 10 cassettes, Marker U1	**RUK7510**
BRT7510 reagent replacement kit for 10 cassettes, Marker E2	**RRT7510**

24 Instrument Specifications

Specifications for BluePippin Instrument

Electrophoresis Voltage	25V, 100V, 150V constant, 100 V pulsed
Optical detection	470 nm excitation, 525 nm emission
Power Requirements	100-240 VAC +/-10% VAC, 2.5 A, 50-60 Hz
Instrument Power (external power supply)	12VDC ⎓ 2.5A
Weight	15 lbs / 7 kg
Dimensions	7H X 11W X 21D (in.) / 18H X 28W X 53D (cm)
Regulatory Compliance	CE, CSA,
Country of Origin	United States

25 Gel Cassettes Specifications

Specifications – gel cassettes	
Minimum Sample Load	15 ng
Maximum Sample Load[1]	2 µg sheared gDNA with 1.5% agarose 5 µg sheared gDNA all others 10ug sheared gDNA High Pass Plus 4 µg restriction/PCR fragment
Accuracy[2]	specified on following pages
Reproducibility[3]	specified on following pages
Sample Recovery[4]	50-80%

1. Amounts DNA above the maximum may be loaded, however accuracy may be affected (DNA will migrate more slowly).
2. Deviation of actual target value from software input value divided by the actual value.
3. 2X standard deviation of replicate samples.
4. Recovery is measured using known amounts of plasmid marker ladder.

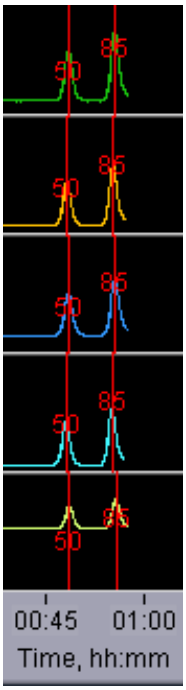
Important! *DNA input samples should be purified to eliminate DNA binding proteins such as ligases, polymerases, restriction enzymes. Bound proteins can alter mobility and reduce recovery.*

Specifications subject to change without notice.

25.1 3% Agarose, 100 bp-250 bp, Marker Q3 (internal standards)

Use Product Number: **BDQ3010**
Use Cassette Definition: **3% DF, Marker Q3**

DNA Marker Q3 -
Typical times to detector



Estimated Collection Times for
Minimum Size Range (Tight) Targets

Target (bp)	Time to Time to Collect* (min)
100	65
140	70
180	80
240	90

* *Run times are affected by temperature and broad range collections. Times should be used as an approximate guideline.*

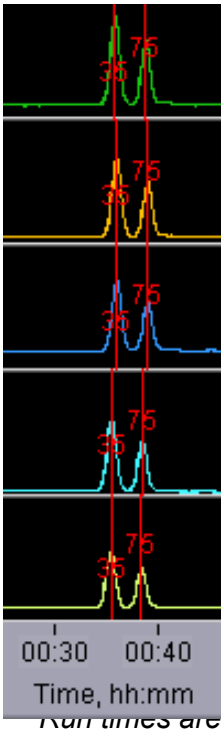
Performance Specifications*	***100-250 bp***
Minimum Size Distribution as CV	***<8%***
Sample Recovery	***50 – 80%***
Accuracy	***<5%***
Reproducibility	***< 5%***

Specifications subject to change without notice
**** see page 25-1 for specification definitions***

25.2 2.5% Agarose, 100 bp-600 bp, Marker V2 (internal standards)

Use Product Number: **BDF2510**
Use Cassette Definition: **2.5% DF, Marker V2**

DNA Marker V2 -
Typical times to detector



Estimated Collection Times for
Minimum Size Range (Tight) Targets

Target (bp)	Time to Time to Collect* (min)
150	60
260	75
400	90
600	115

Run times are affected by temperature and broad range collections.
Times should be used as an approximate guideline.

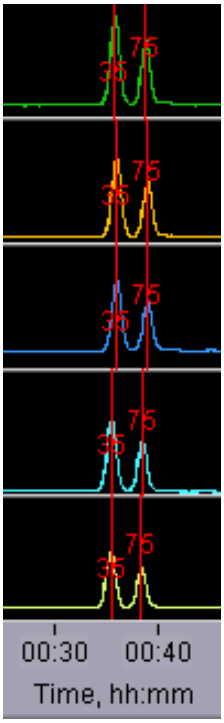
Performance Specifications*	***100-250 bp***	***275-500 bp***	***501-600 bp***
Minimum Size Distribution as CV	***<6.5%***	***<5%***	***<4%***
Sample Recovery	***50 – 80%***	***50 – 80%***	***50 – 80%***
Accuracy	***<8%***	***<6%***	***<2%***
Reproducibility	***< 3%***	***< 3%***	***< 1%***

Specifications subject to change without notice
**** see page 25-1 for specification definitions***

25.3 2% Agarose, 100 bp-600 bp, Marker V2 (internal standards)

Use Product Number: **BDF2010**
Use Cassette Definition: **2% DF, Marker V2**

DNA Marker V2 -
Typical times to detector



Estimated Collection Times for
Minimum Size Range (Tight) Targets

Target (bp)	Time to Time to Collect* (min)
100	49
200	57
300	63
400	71
500	79
600	86

* *Run times are affected by temperature and broad range collections. Times should be used as an approximate guideline.*

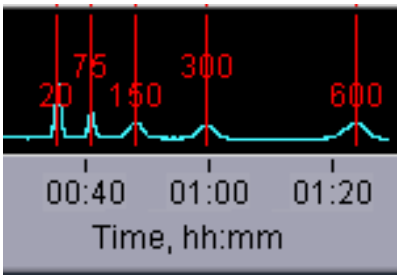
Performance Specifications*	***100-250 bp***	***275-500 bp***	***501-600 bp***
Minimum Size Distribution as CV	***<5%***	***<5%***	***<5%***
Sample Recovery	***50 – 80%***	***50 – 80%***	***50 – 80%***
Accuracy	***<15%***	***<10%***	***<7%***
Reproducibility	***< 6%***	***< 7%***	***< 5%***

Specifications subject to change without notice
**** see page 25-1 for specification definitions***

25.4 2% Agarose, 100 bp-600 bp, Marker M1 (external marker)

Use Product Number: **BEF2010**
Use Cassette Definition: **2% DF, Marker M1**

DNA Marker M1 - Typical times to detector



Estimated Collection Times for Minimum Size Range (Tight) Targets

Target (bp)	Time to Time to Collect* (min)
150	52
275	63
400	70
500	76
600	82

* *Run times are affected by temperature and broad range collections. Times should be used as an approximate guideline.*

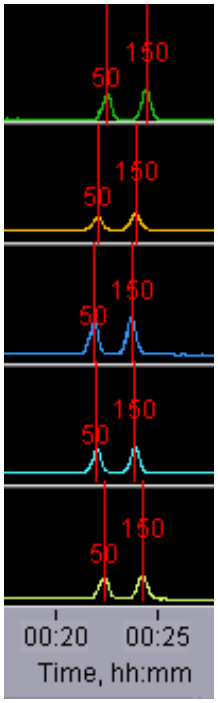
Performance Specifications*	***100-600 bp***
Minimum Size Distribution as CV	***<8%***
Sample Recovery	***50 – 80%***
Accuracy	***<5%***
Reproducibility	***< 5%***

Specifications subject to change without notice
**** see page 25-1 for specification definitions***

25.5 1. 5% Agarose, 250 bp-1.5 kb, Marker R2 (internal standards)

Use Product Number: **BDF1510**
Use Cassette Definition: **1.5% DF, Marker R2**

DNA Marker R2 -
Typical times to detector



Estimated Collection Times for
Minimum Size Range (Tight) Targets

Target (bp)	Time to Time to Collect* (min)
250	29
300	30
600	37
1200	49
1500	56

* *Run times are affected by temperature and broad range collections. Times should be used as an approximate guideline.*

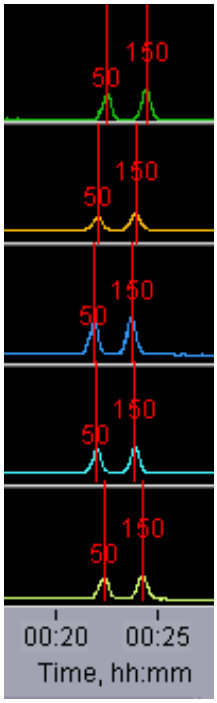
Performance Specifications*	***250-600 bp***	***800-1000 bp***	***1200-1500 bp***
Minimum Size Distribution as CV	***<8%***	***<8%***	***<15%***
Sample Recovery	***50 – 80%***	***50 – 80%***	***50 – 80%***
Accuracy	***<5%***	***<7%***	***<10%***
Reproducibility	***< 5%***	***< 8%***	***< 15%***

Specifications subject to change without notice
**** see page 25-1 for specification definitions***

25.6 1. 5% Agarose, High-Pass, Marker R2 (internal standards)

Use Product Number: **BDF1510**
Use Cassette Definition: **1.5% DF, Marker R2, 1000bp high-pass**

DNA Marker R2 -
Typical times to detector



Estimated Collection Times

Approximately 2 hours for all "BPStart" settings.

Collects fragments up to 8kb

Threshold settings may be programmed between 250bp-1.5kb

Performance Specifications*

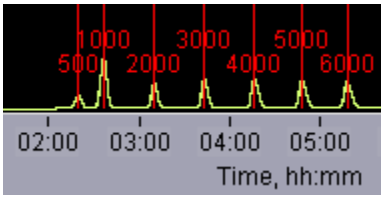
Accuracy, "BPStart"	***<10%***
Reproducibility, "BPStart"	***< 10%***

Specifications subject to change without notice
**** see page 25-1 for specification definitions***

25.7 0.75% Agarose, 1-6 kb, Marker S1, Low Voltage (external marker)

Use Product Number: **BLF7510**
Use Cassette Definition: 0.75% Agarose Dye Free / **0.75% DF Low Voltage 1 - 6kb Marker S1**

DNA Marker S1 - Typical times to detector



Estimated Collection Times for Minimum Size Range (Tight) Targets

Target (bp)	Time to Collect* (hr:min)
1,000	3:04
2,000	3:45
3,000	4:22
4,000	5:10
5,000	5:46
6,000	6:25

* *Run times are affected by temperature and broad range collections. Times should be used as an approximate guideline.*

Performance Specifications

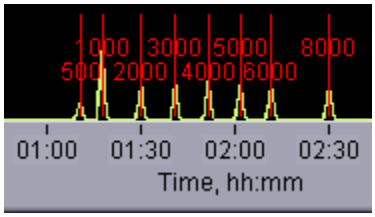
Minimum Size Distribution as CV	***10%***
Sample Recovery	***50 – 80%***
Reproducibility	***<10%***

Specifications subject to change without notice
**** see page 25-1 for specification definitions***

25.8 0.75% Agarose, 2-6 kb, Marker S1, Pulsed-Field (external marker)

Use Product Number: **BLF7510**
Use Cassette Definition: 0.75% Agarose Dye Free / **0.75% DF 2 - 6kb Marker S1**

DNA Marker S1 - Typical times to detector



Estimated Collection Times for Minimum Size Range (Tight) Targets

Target (bp)	Time to Collect* (hr:min)
2,000	1:51
3,000	2:01
4,000	2:09
5,000	2:20
6,000	2:29

* *Run times are affected by temperature and broad range collections. Times should be used as an approximate guideline.*

Performance Specifications

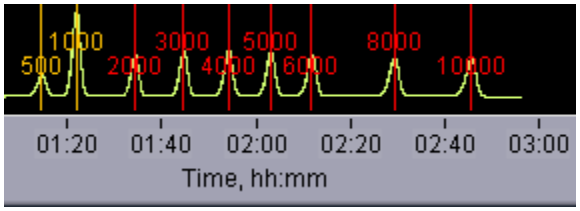
Minimum Size Distribution as CV	***20%***
Accuracy	***<10%***
Reproducibility	***<10%***

Specifications subject to change without notice
**** see page 25-1 for specification definitions***

25.9 0.75% Agarose, 3-10 kb, Marker S1, Pulsed-Field (external marker)

Use Product Number: **BLF7510**
Use Cassette Definitions: 0.75% Agarose Dye Free / **0.75% DF 3 - 10kb Marker S1**
0.75% Agarose Dye Free / **0.75% DF 3 - 10kb Marker S1 improved recovery**

DNA Marker S1 - Typical times to detector



Estimated Collection Times for Minimum Size Range (Tight) Targets

Target (bp)	Time to Time to Collect* (hr:min)
5,000	2:23
6,000	2:32
8,000	2:51
10,000	3:10

* *Run times are affected by temperature and broad range collections. Times should be used as an approximate guideline.*

Performance Specifications

Minimum Size Distribution as CV	***20%***
Accuracy	***<10%***
Reproducibility	***<10%***

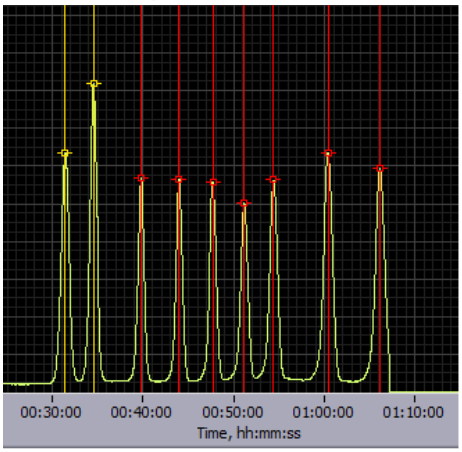
Specifications subject to change without notice
**** see page 25-1 for specification definitions***

25.10 0.75% Agarose, High-Pass Protocols (4-20kb thresholds)

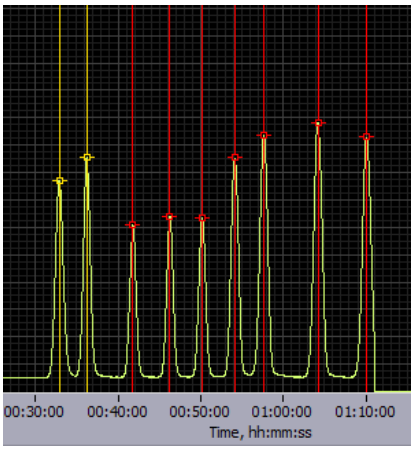
Use Product Number: **PAC20KB or BLF7510**
Use Cassette Definition: **Version 2**: **0.75% DF Marker S1 high-pass 4-10kb vs2**
Version 3: **0.75% DF Marker S1 high-pass 6-10kb vs3**
0.75%DF Marker S1 High Pass 15-20kb

DNA Marker S1 - Typical times to detector

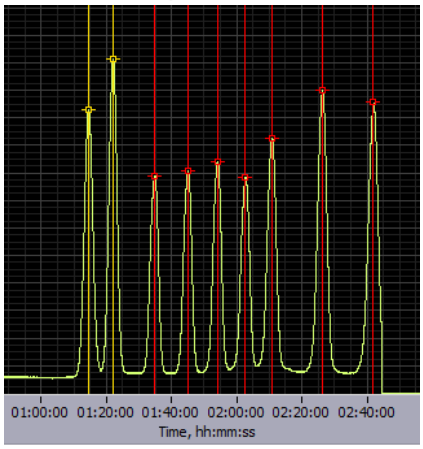
4-10kb high-pass



6-10kb high-pass



15-20kb high-pass



Estimated Collection Times

4-10kb high-pass	***~ 2 hours***
6-10kb high-pass	***~2 hours***
15-20kb high-pass	***5-6 hours***

Performance Specifications

	Version2	***Version3***
Accuracy, "BPStart"	***N/A****	***<10%***
Reproducibility, "BPStart"	***N/A****	***<10%***

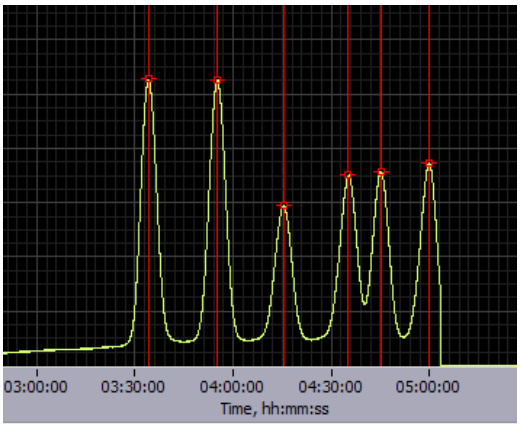
** Accuracy and reproducibility are affected by the sample load amount. Refer to the High-Pass user guide for recommendations.

Specifications subject to change without notice
**** see page 25-1 for specification definitions***

25.11 0.75% Agarose, High-Pass Protocols (30-40kb thresholds)

Use Product Number: **PAC30KB or BUF7510**
Use Cassette Definition: **Version 3**: **0.75% DF Marker U1 high-pass 30-40kb vs3**

DNA Marker U1 - Typical times to detector



Approximate Collection Time is 9.5-10 hours

Performance Specifications

Accuracy, "BPStart"	***<10%***
Reproducibility, "BPStart"	***<10%***

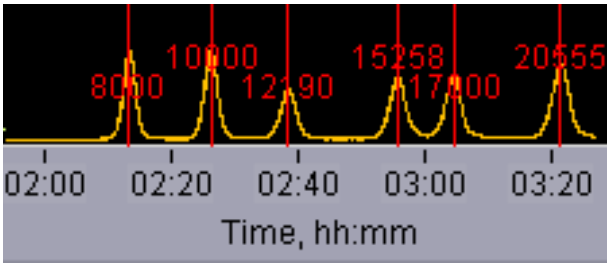
** Accuracy and reproducibility are affected by the sample load amount. Refer to the High-Pass user guide for recommendations.

Specifications subject to change without notice
**** see page 25-1 for specification definitions***

25.12 0.75% Agarose, 10-18 kb, Marker U1, Pulsed-Field (external marker)

Use Product Number: **BUF7510**
Use Cassette Definition: 0.75% Agarose Dye Free / **0.75% DF 10-18kb Marker U1**

DNA Marker U1 - Typical times to detector



Estimated Collection Times for Minimum Size Range (Tight) Targets

Target (bp)	Time to Time to Collect* (hr:min)
10,000	2:55
12,000	3:00
15,000	3:15
17,000	3:25
18,000	3:35

* *Run times are affected by temperature and broad range collections. Times should be used as an approximate guideline.*

Performance Specifications

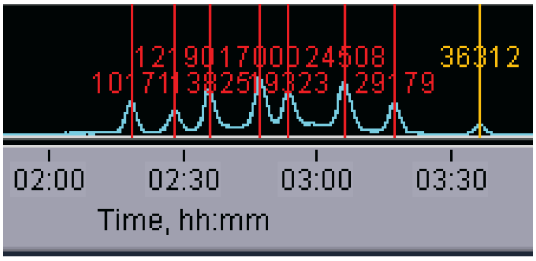
Minimum Size Distribution as CV	***20%***
Accuracy	***<10%***
Reproducibility	***<10%***

Specifications subject to change without notice
**** see page 25-1 for specification definitions***

25.13 0.75% Agarose, 18-27 kb, Marker T1, Pulsed-Field (external marker)

Use Product Number: **BMF7510**
Use Cassette Definition: 0.75% Agarose Dye Free / **0.75% DF 18 - 27kb Marker T1**

DNA Marker T1 - Typical times to detector



Estimated Collection Times for Minimum Size Range (Tight) Targets

Target (bp)	Time to Time to Collect* (hr:min)
18,000	3:45
23,000	4:00
27,000	4:15

* *Run times are affected by temperature and broad range collections. Times should be used as an approximate guideline.*

Performance Specifications

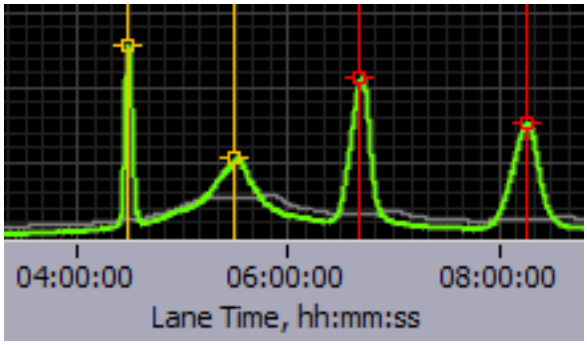
Minimum Size Distribution as CV	***20%***
Accuracy	***<10%***
Reproducibility	***<10%***

Specifications subject to change without notice
**** see page 25-1 for specification definitions***

25.14 0.75% Agarose, 50kb Target, Marker Z1, Pulsed- Field (external marker)

Use Product Number: **BHZ7510**
Use Cassette Definitions: 0.75% Agarose Dye Free / **0.75% DF 50 kb Marker Z1 - tight**
0.75% Agarose Dye Free / **0.75% DF 50 kb Marker Z1 - broad**

DNA Marker Z1 - Typical times to detector



Important!
"Range" mode programming is not allowed for this product. Only "Target" mode can be used. Two definitions are provided; "Tight" provides the narrowest distribution that is obtainable, and "Broad" provides a wider distribution around the 50 kb target for a higher product recovery.

Estimated Collection Times

Target	Run Time hr:min[1]	Additional Time/lane (min)[2]
50kb tight	7:50	15
50kb broad	8:50	30

[1]Time to elute one sample lane
[2]Add to time to each additional sample lane run

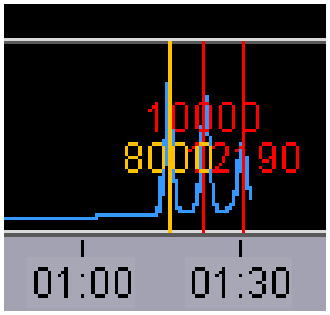
Target	50kb tight	50kb broad
Minimum Size Distribution as CV	***10%***	***14%***
Accuracy	***<10%***	***<10%***
Reproducibility	***<10%***	***<10%***

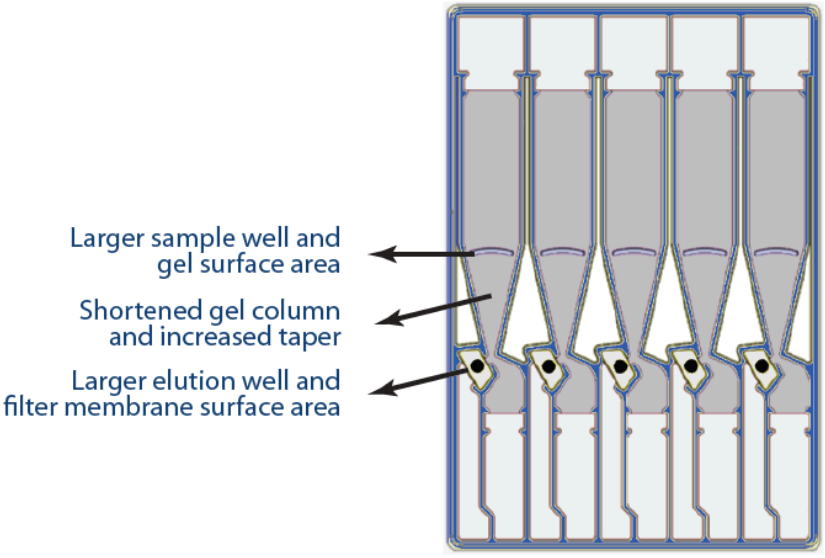
Specifications subject to change without notice
**** see page 25-1 for specification definitions***

25.15 High Pass Plus, Marker U1, (external marker)

Use Product Number: **BPLUS10**
Use Cassette Definitions: 0.75% Agarose Dye Free / **10kb High Pass Plus Marker U1**
0.75% Agarose Dye Free / **15kb High Pass Plus Marker U1**
0.75% Agarose Dye Free / **20kb High Pass Plus Marker U1**

DNA Marker U1 - Typical times to detector





High Pass Plus is an alternative gel cassette product that is designed to run High Pass protocols with a faster runtime than the standard cassette design. It will also accept a high DNA sample input (up to 10 ug) and produces a higher yield.

Estimated Collection Times

Target	hr:min
>10kb High Pass	2:00
>15kb High Pass	2:30

>20kb High Pass	2:50

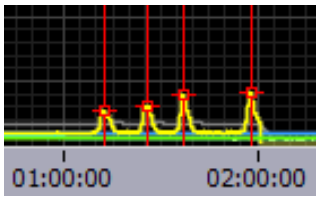
	At BP start
Accuracy	***<10%***
Reproducibility	***<10%***

Specifications subject to change without notice
**** see page 25-1 for specification definitions***

25.16 0.75% Agarose, Range + T 9-30 kb, Marker E2

Kit Numbers: **BRT7510**
Use Cassette Definition: **0.75% DF 9-30kb R+T Marker E2**

DNA Marker E2 - Typical times to detector (external marker)



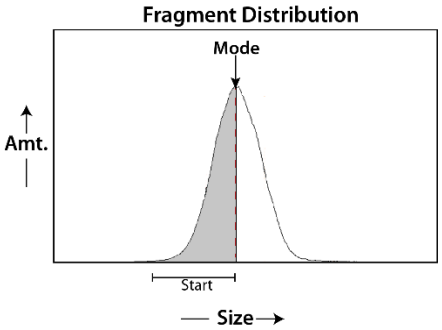
* Run times are affected by ambient temperature. Times should be used as an approximate guideline. Total Run Time is dependent on the TElution time.

Approx. Times to Start of Elution*

9-30kb Range +T Start (bp)	Time to start of elution (hh:mm)
9000	2:00
15000	3:00
16000	3:20
19000	3:40
22000	4:00
30000	5:00

Guidelines for Selection Range+ T values:

A. Range: Select a Range Start that is before the mode of the shear distribution:



B. T: add values, in minutes, between 5 and 30 minutes to begin to determine the optimal end point of the size selection.

e.g 5, 10, 15, 20 minutes

Do not exceed 30 minutes.

Performance Specifications* for Range Start, 9-30 kb

Minimum Size Distribution as CV	**N/A**
Sample Recovery	**50 – 80%**
Start Value Accuracy	**<15%**
Start Value Reproducibility	**< 15%**

Specifications subject to change without notice
*** see page 20-1 for specification definitions**

26 Revision Change Log

Last Rev	New Rev	Date	Page#	Notes
A	B	11/5/25	24-3	2% Agarose with External Standard (BEF2010) was listed twice and 2% Agarose with Internal Standard (BDF2010) was not listed. BDF2010 was added back in.
B	C	09/18/26	1-2	Removed Class I Laser warnings.



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