



Pippin PrepTM

DNA Size Selection System

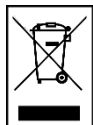
Operations Manual

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All other brands and name mentioned herein are property of their owners.

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



Indicates disposal instruction.

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

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1 Introduction

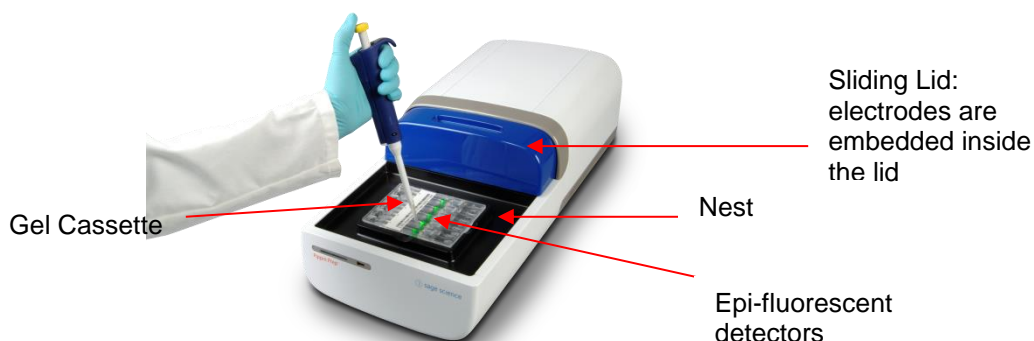
Thank you for purchasing the Pippin Prep from Sage Science. The Pippin Prep is an automated preparative gel electrophoresis system.

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

1.1 System Overview

There are three components to the Pippin Prep system:

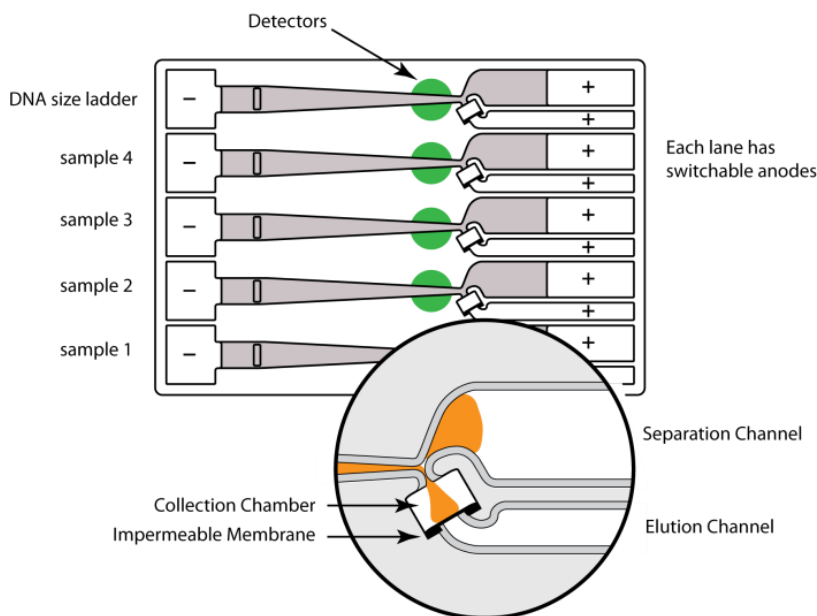
- **Instrument** – The instrument contains an epifluorescence detector, an electrophoresis power supply, an electrode array, and a Linux-based single-board computer. The system computer is accessed by an external LCD monitor, mouse, and keyboard. The electrode array is located in the top of the sliding instrument cover and is not user-accessible.
- **Software** – System software allows the user to enter parameters that define the DNA fragment size ranges or DNA bands that are to be collected. 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 includes sufficient reagents to complete all collections. Kits have been developed to address DNA size range collections at various size ranges. Standards and sample loading solution are supplied with each cassette kit. There are two categories of gel cassette:
 - **Dye-free Cassettes** – The DNA size marker has been labeled with a fluorescent dye (TAMRA). The sample DNA is not detected by the system. These cassettes are used for size-selecting sheared DNA. Two types of strategies are used: 1) External marker – a DNA marker is run in a dedicated lane to determine elution timing. Capacity is 4 samples/cassette. 2) Internal standards – DNA markers are added to each sample to determine elution timing. Capacity is 5 samples/cassette.
 - **Cassettes with intercalating dye (ethidium bromide)** – The agarose gel and running buffer contains ethidium bromide. Ethidium bromide is an intercalating dye which allows the system to detect DNA above a certain threshold. These use external markers only.



The PippinPrep Instrument
(the monitor and keyboard are not shown)

1.2 How the System Works

The Pippin Prep system uses 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 with close-up of a collection well

1.3 Collection Strategies

Collection timing (start, duration, and end) is based on the factory calibration settings provided with a cassette definition file, and the user-input size range settings in software.

DNA size selections can be undertaken using one of the following strategies:

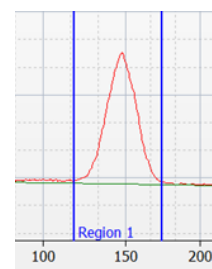
- **Dye-free gel with labeled internal standards** – TAMRA-labeled DNA internal standards are added to samples, and run ahead of the input sample. The rates of migration of the internal standards are used to determine the collection timing* within the sample lane. Up to 5 samples may be run per cassette. The internal standards are viewable with software.
- **Dye-free gel with labeled external marker** – An ethidium homodimer-labeled DNA marker is loaded in a single dedicated sample lane during a run. The rates of migration of the markers are used to determine the collection timing the remaining sample lanes. One DNA marker and 4 samples must be run per cassette. The marker is viewable with software.
- **Ethidium bromide stained gel with external marker** – A native DNA marker is loaded into a single dedicated sample lane during a run. The rates of migration of the markers are used to determine the collection timing the remaining sample lanes. One DNA marker and 4 samples must be run per cassette. The sample DNA and marker are viewable with software.
- **Timed runs** – The beginning and end time of elution (size selection) is set by the user. Neither internal standards or external markers are used.



Note: The DNA sequence of internal standards may be found at www.sagescience.com/support section, on the Pippin Prep resources page.

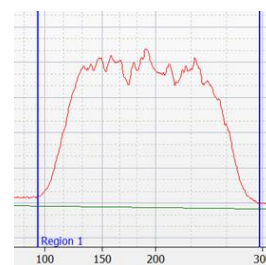
1.4 Collection Types

1. **Minimum Size Distribution (“Tight” setting).** The cassette lane will collect the narrowest distribution of fragments 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 Distribution (“Range” setting).** The cassette lane will collect a distribution of fragments between a starting base pair value and an ending base pair value. The actual start and end fragment sizes are typically within +/- 10% of the base pair values input into the software.



Range

2 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:

The keys to successful operation are:

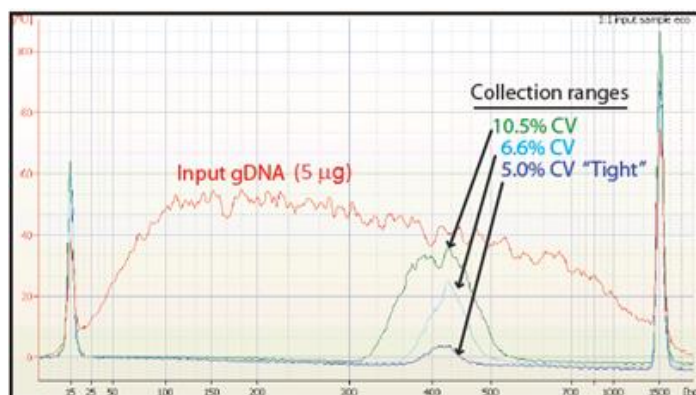
- **Purity of the input sample** (See Section 6)
- **Optical Calibration** (Section 9)
- **Preparation of the cassettes** (Section 10)
- **Sample loading** (See Section 11)

2.1 Common Misconceptions

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

2.1.1 Narrowest is not always the best.

The Pippin Prep 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. See **Section 14.3** for details.



2.1.2 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 2.2** 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.

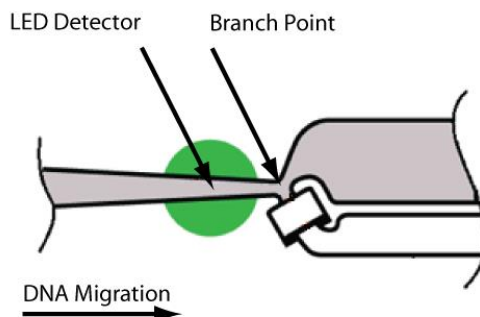


Figure 2.2. An illustration of the time and base pair difference between the detector and branch point.

2.1.3 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.

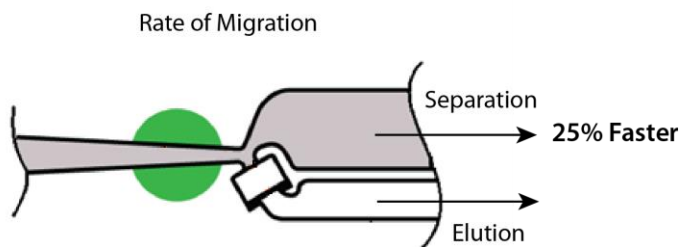


Figure 2.3. An illustration comparing the relative rate of electrophoresis in the separation and elution channels

2.1.4 Elution creates a jump in the optical background of the sample lanes.

Since ethidium bromide is positively charged, it will migrate toward the (-) electrode during electrophoresis – moving in the opposite direction from the DNA. During the initial phase of a Pippin Prep run, the separation channel will become partially depleted of ethidium bromide. However, when elution begins, a front of fresh ethidium bromide from the unused elution channel will migrate up through the separation, past the detector, and create a jump in the ethidium bromide background signal, which can be misinterpreted as a DNA signal. This is illustrated in **Figure 2.4** and **Figure 2.5**. The ethidium bromide front passes the detector approximately 4-5 minutes after start of elution. The ethidium bromide front may not be noticeable when DNA input is high.

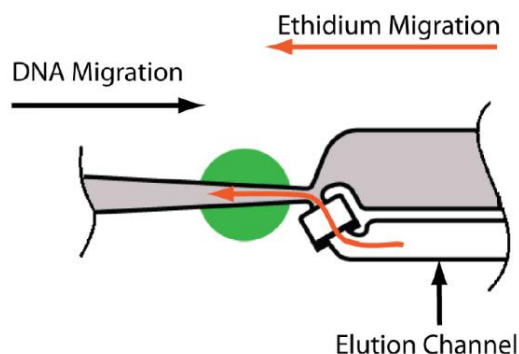


Figure 2.4. An illustration showing the direction of ethidium bromide migration during electrophoresis.

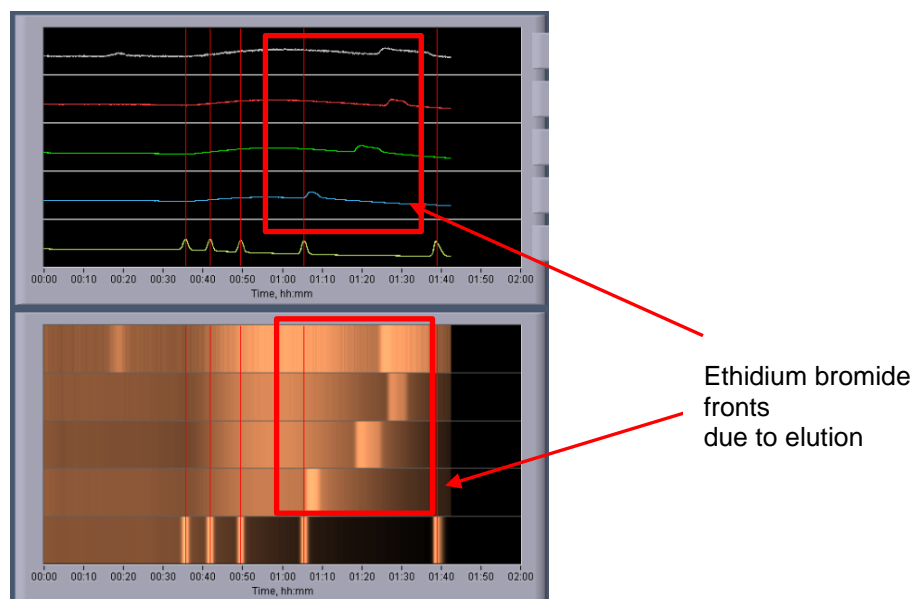


Figure 2.5. Relatively low input sheared DNA sample with broad distributions (300-500 bp) in lanes 2-5. Sample loading is the same in all lanes but different DNA ranges were eluted. Brighter bands are ethidium bromide fronts due to elution.

2.2 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 well. At the end of the run, the Pippin Prep will briefly reverse field (for 5 seconds) at the end of the sample elution. This improves sample yield by up to 20%.



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

3 Safety and Precautions

Icons Used

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



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.

Safe Use Guidelines

The Pippin Prep system is designed to operate under the following environmental conditions:

- Pollution Degree 2
- Installation category 2
- Altitude 2000m
- Indoor use
- Ambient temperature 17-32°C
- Humidity 10-80%, non-condensing

Standard laboratory precautions should be taken when handling Pippin Prep Gel cassettes and operating the Pippin Prep:

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

Disposal



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



Important! *For ethidium bromide-containing gel cassettes, running buffer and spare electrophoresis buffer contain **5 ug/ml of Ethidium bromide**. Cassettes and buffer should be disposed of as laboratory waste according to institutional lab policy.*

4 Unpacking the Pippin Prep

The Pippin Prep Instrument

The Pippin Prep instrumentation is shipped in two boxes: one will contain the Pippin Prep 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

Pippin Prep

- Pippin Prep Instrument
- Accessory box
 - Computer keyboard, USB
 - Computer mouse, USB
 - Power supply
 - Power cord
 - Rinse cassette (for maintenance of electrodes)
 - Optical calibration fixture (for setting optical baseline before each run)

Setting up the Pippin Prep

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 from their packaging.
2. Firmly grip both sides of the instrument and lift it from the foam packaging insert. The Pippin Prep weighs approximately 14 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 Pippin Prep using supplied video cable. Connect monitor to power using power supply supplied with monitor.
4. Insert USB connector from computer key board into port located in the back of the Instrument.
5. Connect mouse to Pippin Prep via USB connector in the back of the instrument.
6. Connect monitor cable into the video port located in the back of the instrument.
7. Connect monitor to power using power supply and cords supplied with monitor.
8. Connect Pippin Prep instrument to power via supplied power supply and cable. Power connector is at rear of instrument.
9. Press power switch located on the rear of the instrument, and wait for software to launch (approximately 30 seconds).

The Pippin Prep is ready for use. The software should automatically launch – allow 30 seconds.

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

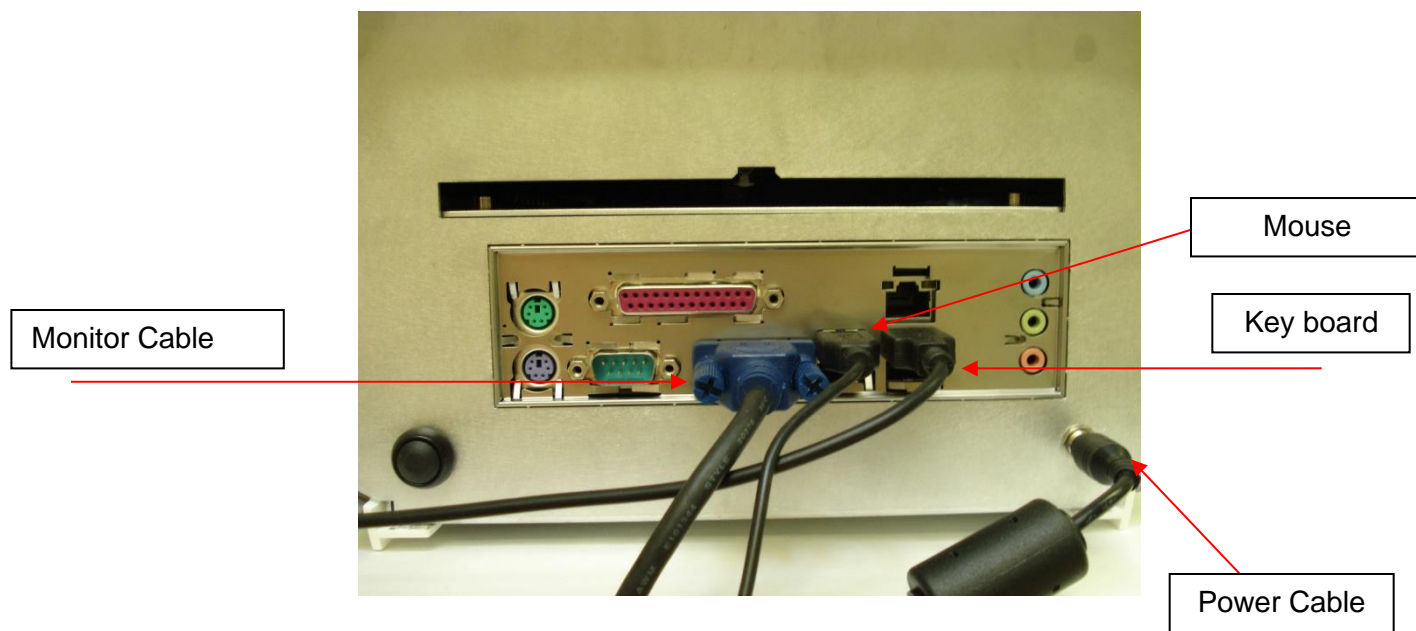
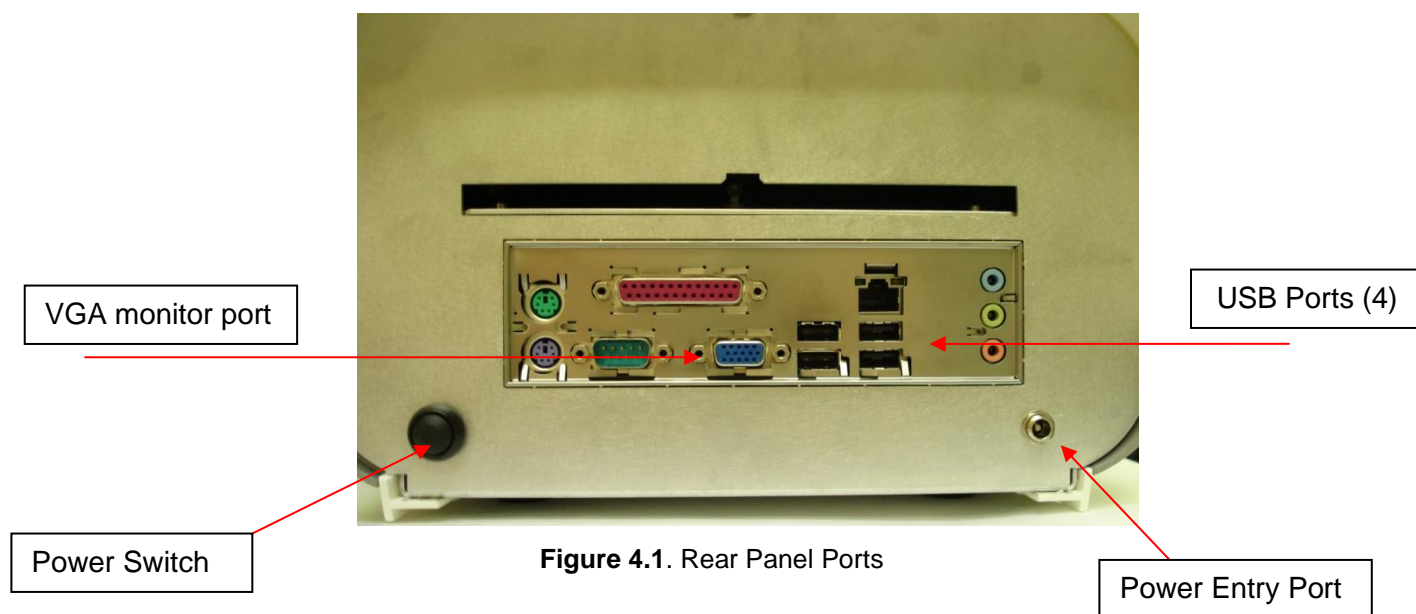




Figure 4.3. Pippin Prep front panel.

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).

USB Port

Unpacking Gel Cassettes

Gel cassettes are shipped in boxes in the following configurations. Ensure boxes are in the upright position and confirm that following contents are present. DNA size markers and loading solution should be stored at 4°C. (Storage of electrophoresis buffer at 4°C is optional.) The cassettes should be stored at room temperature:

- 10 cassettes with reagents to run 40-50 samples
 - 10 foil-sealed gel cassettes (**store at R.T.**)
 - 1 reagent kit for 10 cassettes (**store at 4 °C**)
 - 40 ml of running buffer
 - 800 µl DNA size markers (external marker)
 - 500 µl loading solution
 - or-
 - 1000 µl marker/loading solution mix (internal standard)
 - 1 package of adhesive tape for sealing elution wells



Important! *DNA size markers and loading solution should be stored at 4°C. Loading solution is viscous should be equilibrated at room temperature prior to use.*



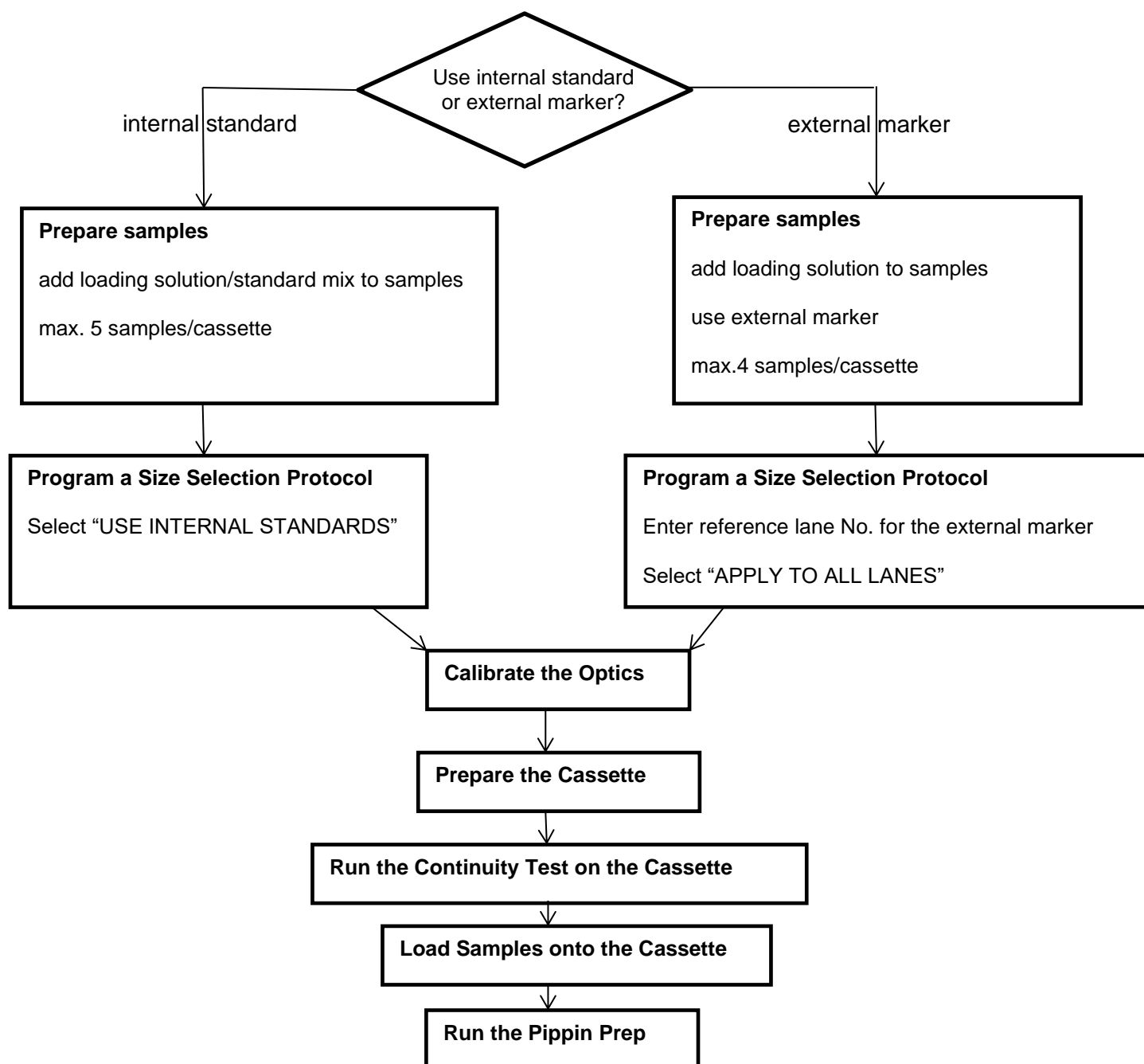
Important! *Cassette should be stored at room temperature in the sealed foil packaging. They are light sensitive and should not be removed until prior to use. Shelf life is 18 months.*

Storage Conditions for Cassettes and Reagents

The following storage requirements should be observed:

- **Gel Cassettes-** Store at room temperature in the foil bags until ready for use.
- **DNA Markers (unlabeled)** – store at 4°C.
- **DNA Internal standards and markers (labeled)** – store at 4°C minimize time at room temperature.
- **Electrophoresis buffer** – may be stored at room temperature or 4 °C
- **Loading solution** – store at 4 °C. Bring to room temperature before use.

5 Workflow Summary



6 Sample Preparation

Input Sample Characteristics

When running the Pippin Prep, 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. Pippin Prep 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 Pippin Prep

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

Recommended sample Load Guidelines

Maximum Load: 5 µg sheared genomic DNA*

Minimum Load: 15 ng sheared genomic DNA*

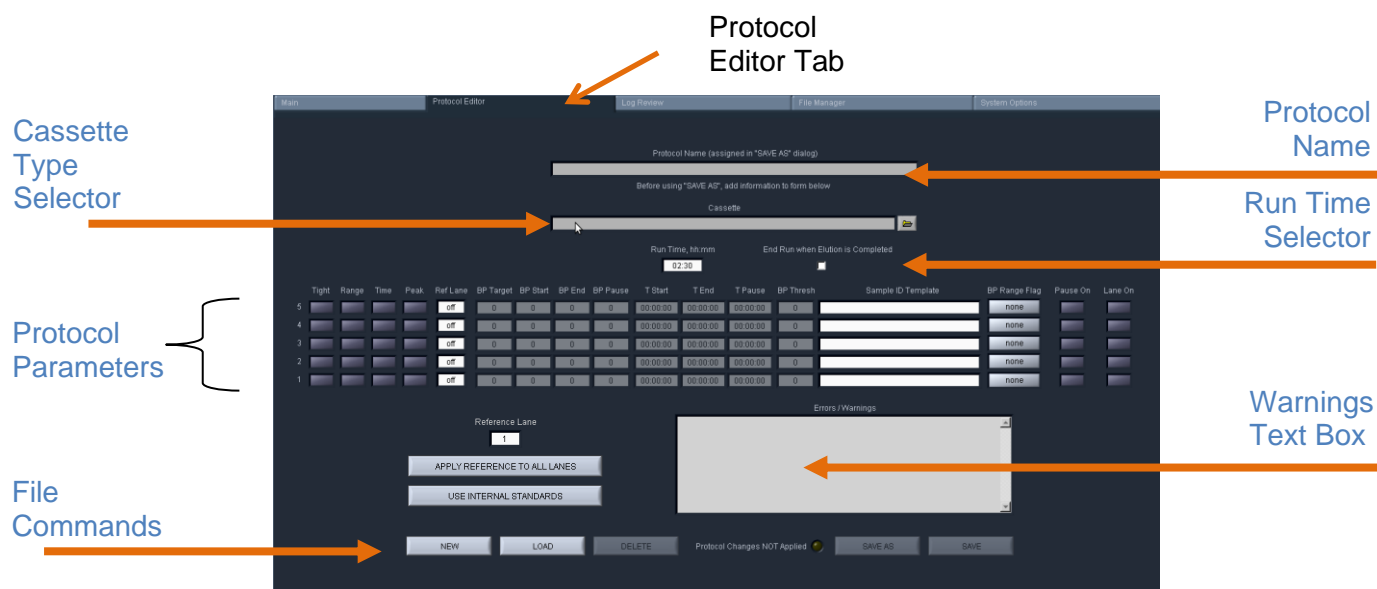
Optical Sensitivity
(ethidium bromide cassettes): approx. 200ng sheared genomic DNA

7 Writing and Editing a Protocol

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

7.1 Overview

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. A protocol file may be applied to any run, provided the cassette type is correct.



Protocol Parameters Detail

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.

Diagram illustrating the Protocol Parameters section layout:

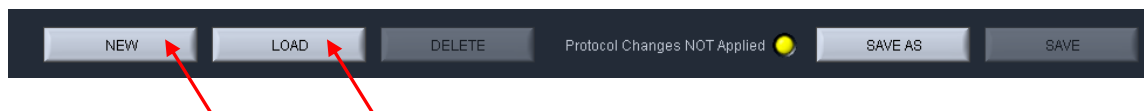
- Programming Modes** (Tight, Range, Time, Peak)
- Ref. Lane Indicators** (Ref Lane)
- Base Pair (BP) values** (BP Target, BP Start, BP End, BP Pause)
- Time values** (T Start, T End, T Pause, BP Thresh)

	Tight	Range	Time	Peak	Ref Lane	BP Target	BP Start	BP End	BP Pause	T Start	T End	T Pause	BP Thresh	Sample ID Template
5					off	0	0	0	0	00:00:00	00:00:00	00:00:00	0	
4					off	0	0	0	0	00:00:00	00:00:00	00:00:00	0	
3					off	0	0	0	0	00:00:00	00:00:00	00:00:00	0	
2					off	0	0	0	0	00:00:00	00:00:00	00:00:00	0	
1					off	0	0	0	0	00:00:00	00:00:00	00:00:00	0	

7.2 Programming a New Protocol – Quick Guide

The following is a summary of the steps required to program a protocol. Detailed instructions are provided in the subsequent sections.

1. Click the “Protocol Editor” tab along top of the screen.
2. Select “NEW”, to create a new protocol. To edit an existing protocol, press the “LOAD” button to select the protocol to be edited.



3. Select a Cassette Type from “Cassette” menu, by clicking the folder icon. Protocol fields cannot be edited unless a cassette type has been selected.



4. Adjust the run time value, if necessary.

Run Time, hh:mm	End Run when Elution is Completed
<input type="text" value="8:00"/>	<input checked="" type="checkbox"/>

5. Program the size selection protocol, and assign the DNA reference marker lane. There are four modes of programming on the Pippin Prep:

- **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.
- **Time** – allows users to program extractions using the starting and ending elution time (hr:min:sec) values only (**a reference DNA marker is not used**)
- **Peak** – collects the next peak (restriction fragment or PCR band) after the set threshold base pair value has been reached.

	Tight	Range	Time	Peak	Ref Lane
5	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text" value="off"/>
4	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text" value="off"/>
3	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text" value="off"/>
2	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text" value="off"/>
1	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text" value="off"/>



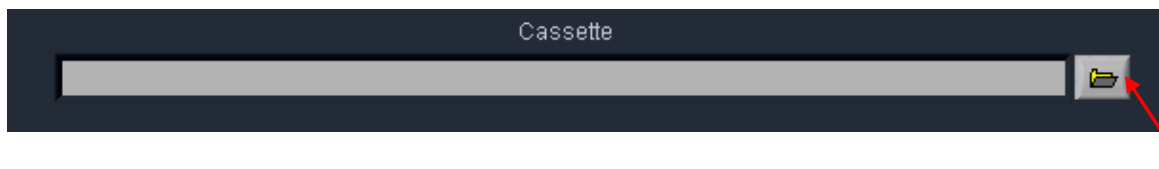
Note: Programming modes may be independently applied to individual sample lanes

6. Enter Sample ID or description (optional).

7. Press “Save As” button, enter a name for the protocol.

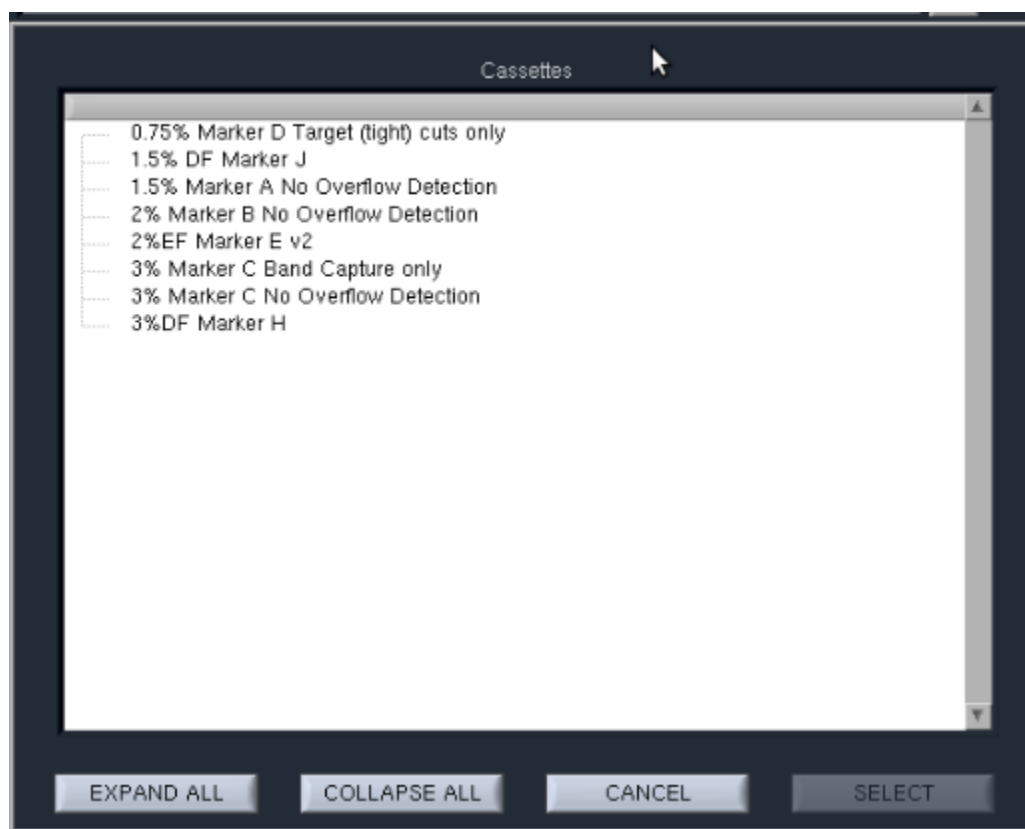
7.3 Selecting a Cassette Definition

Cassettes types are selected from the “Cassette” drop down menu.



Cassette files are presented in a list. The appropriate cassette type to select is based on the cassette product that has been purchased, and in some instances the application to be used (i.e. different electrophoresis protocols are used with the same cassette product).

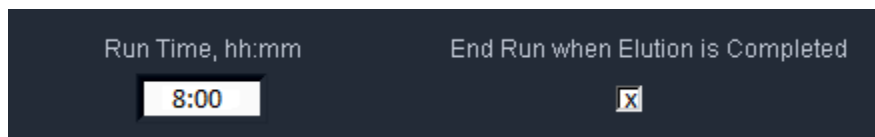
Highlight the appropriate cassette definition file and press “SELECT”. See Appendix A for summary of marker types and calibration



Note: “No Overflow Detection” is a legacy designation. This refers to cassettes that are run with open elution modules – large collections can cause modules fill and possibly overflow if not sealed with tape. Software limits previously prevented mis-programming, and the “No Overflow” definitions were provided for user who used tape to seal the modules. Module sealing tape is now provided with each cassette package and is recommended for all protocols. “No Overflow Detection” is effectively included with each existing cassette type (and future cassette type) but the term will no longer be used.

7.4 Selecting a Run Time

Protocols can be run with widely variable run times – up to 7 hours for low voltage runs. The Run Time Selector consists of a field and a check box, with the following default settings.

The image shows a dark-themed control panel. On the left, the text "Run Time, hh:mm" is displayed above a white rectangular input field containing the text "8:00". To the right of this field, the text "End Run when Elution is Completed" is displayed above a checked checkbox, which contains a small 'x' icon.

The 8 hour default value in the Run Time field ensures that a run is not unintentionally ended early. The checked box in the “End Run when Elution is Completed” field will automatically end a run when every lane that has a programmed elution has completed its collection.

Users may adjust the desired run time by referring to **Section 21**, where estimated run times are listed for each cassette definition. Users should be aware of the following when adjusting the run time parameters:

- If a run time is entered based on run time estimates from **Section 21**, make sure adequate time is added to the estimate as a safety
- The “Run Time” field will take precedence—When the run time is achieved, the run will end regardless of whether the elutions are complete.

To end a run manually, go to the Main Tab and press “STOP”. Completed elutions may be verified in the Lane Status Panel if there is a value in the “Elution Timer” field and the “Separate” button is lit:



7.5 Assigning a Reference Marker – external standard

1. Determine the lane into which the reference marker will be loaded.
2. In the protocol editor, enter the reference lane number into the “Reference Lane” field. Click “APPLY REFERENCE TO ALL LANES”. **Figure 7.1** shows the proper settings for running a DNA marker in lane 1, with programmed “Tight” extractions in lanes 2-5.

Lane to which marker has been loaded.

Remaining lanes will reference off of marker lane

Reference Lane

1

APPLY REFERENCE TO ALL LANES

USE INTERNAL STANDARDS

	Tight	Range	Time	Peak	Ref Lane
5					1
4					1
3					1
2					1
1					1

Figure 7.1. The correct configuration for a protocol with an external marker loaded in lane 1.



Note: When using external markers, make sure the collection mode is selected for that lane.

7.6 Assigning Reference Markers – internal standards

1. Markers must be loaded into every lane with the sample (30 ul of sample with 10 ul of loading solution/marker mix).
2. In the protocol editor, press “USE INTERNAL STANDARDS”. This will auto-fill the “Ref Lane” fields with reference marker internal to the sample. The software will determine the timing of elution based on the migration of the DNA marker within its own lane.
3. **Figure 7.2** shows the correct assignment of marker lanes for internal standard runs.

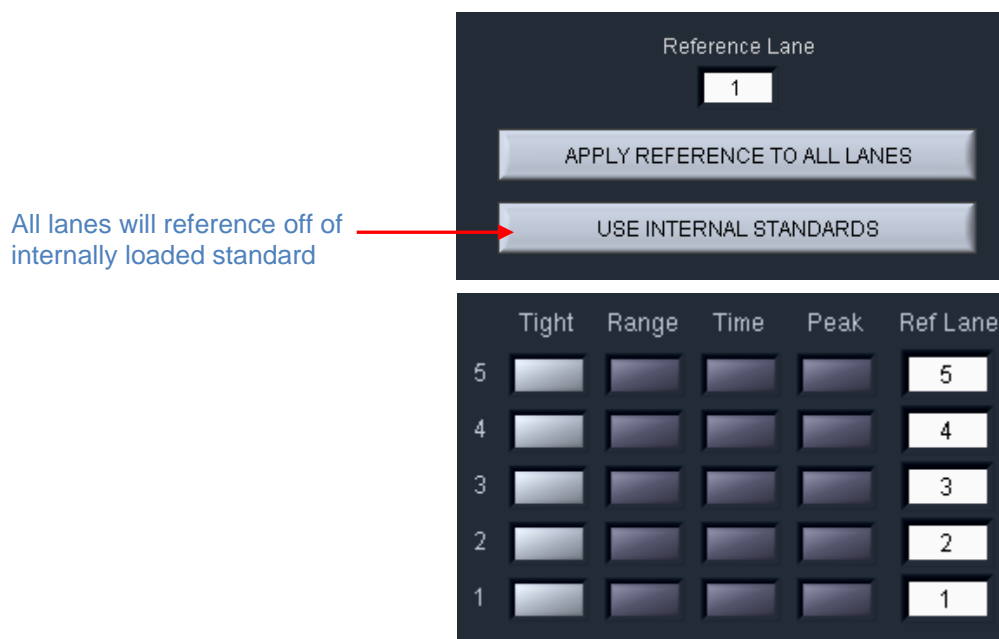


Figure 7.2. The correct configuration for a protocol using internal standards.



Important! When using internal standards all samples must be prepared with the Pippin Prep loading solution/marker mix which is formulated for the cassette type and size range to be used.

7.7 Warnings and Indicators

BP Range Flag

When a “**Tight**” collection mode (minimum size range) has been selected for sample lane, 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 Flag

If the “Range” collection mode is selected, and the size range is narrower than the minimum, a yellow “narrow” flag will appear.



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.

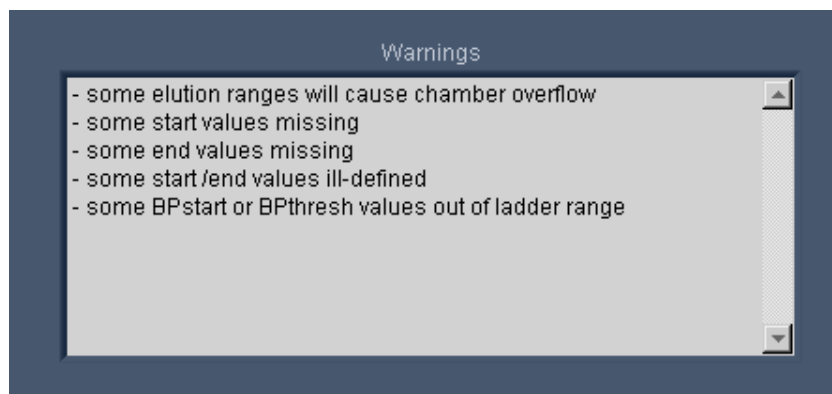
Pause Enabled Indicator

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



Warnings Text Box

If there are errors or exceptions within a programmed protocol, a message will appear in the Warnings text box identifying the nature of the discrepancy.



8 Programming Size Selections – Tight, Range, and Timed Modes

8.1 Programming a “Tight” Collection

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. Assignment of reference DNA is detailed in **Sections 7.5** and **7.6**.

In the protocol editor:

1. **Select an appropriate cassette type for size selection**, if a new protocol is being created.
2. **Assign the internal or external reference DNA.**
 - 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.
3. **Select the “Tight” programming mode.** Click the “Tight” button for the lane in which the tight cut will be extracted. If using an external marker, make sure no collection modes are selected for that lane.
4. **Enter a value in for the “BP Target”.** 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.

5. **(Optional) Enter a BP pause value.** This is not recommended for Tight mode. See Section 8.2, below, for a description of this feature.
6. **Enter Sample ID information.**
8. **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/Pippin Prep/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. Assignment of reference DNA is detailed in **Sections 7.5 and 7.6**.

In the protocol editor:

1. **Select an appropriate cassette type for size selection**, if a new protocol is being created.
2. **Assign the internal or external reference DNA.**
 - 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.

7. **Select the “Range” programming mode.** Click the “Range” button for the lane from which the cut will be extracted. If using an external marker, make sure no collection modes are selected for that lane.
8. **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.
9. **(Optional) Enter a BP pause value.** If a value is entered into the pause field, the system will pause when that (estimated) base-pair value has been reached. The pause will allow a user to remove the fraction, and then resume the elution and collect a second fraction.

The BP Pause value must be within the programmed elution range (between BP Start and BP End). The pause function temporarily turns off power to the electrodes and suspends run timers, allowing users to remove the sample, rinse the elution well (if desired), and then resume the instrument run to collect additional sample.

This will result in the collection of two contiguously sized fractions, with overlapping fragment sizes. Two collections of a paused “Tight” setting (not recommended) will yield two nearly identical samples each with half the amount of a single Tight collection. If users were to pause at the midpoint of progressively wider “Range” settings, the amount of overlap in the collections would become progressively smaller.



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.

10. Enter the Sample ID information

11. **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/ Pippin Prep/Protocols, and may be accessed in the File Manager tab.

8.3 Time Mode – programming timed collections



Important! *The time mode does not reference a standard. Cassette to cassette variability 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.

In the protocol editor:

1. **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.
2. **Enter a time values in the “T Start” and “T End” fields.** Sample elutions will occur at exactly those times after the start of the run.
3. **(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). The pause function temporarily turns off power to the electrodes and suspends run timers, allowing users to remove the sample, rinse the elution well (if desired), and then resume the instrument run to collect additional sample.

This will result in the collection of two contiguously sized fractions, with overlapping fragment sizes. Two collections of a paused “Tight” setting (using comparable timing, this not recommended) will yield two nearly identical samples each with half the amount of a single Tight collection. If users were to pause at the midpoint of progressively wider “Range” settings, the amount of overlap in the collections would become progressively smaller.



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.*

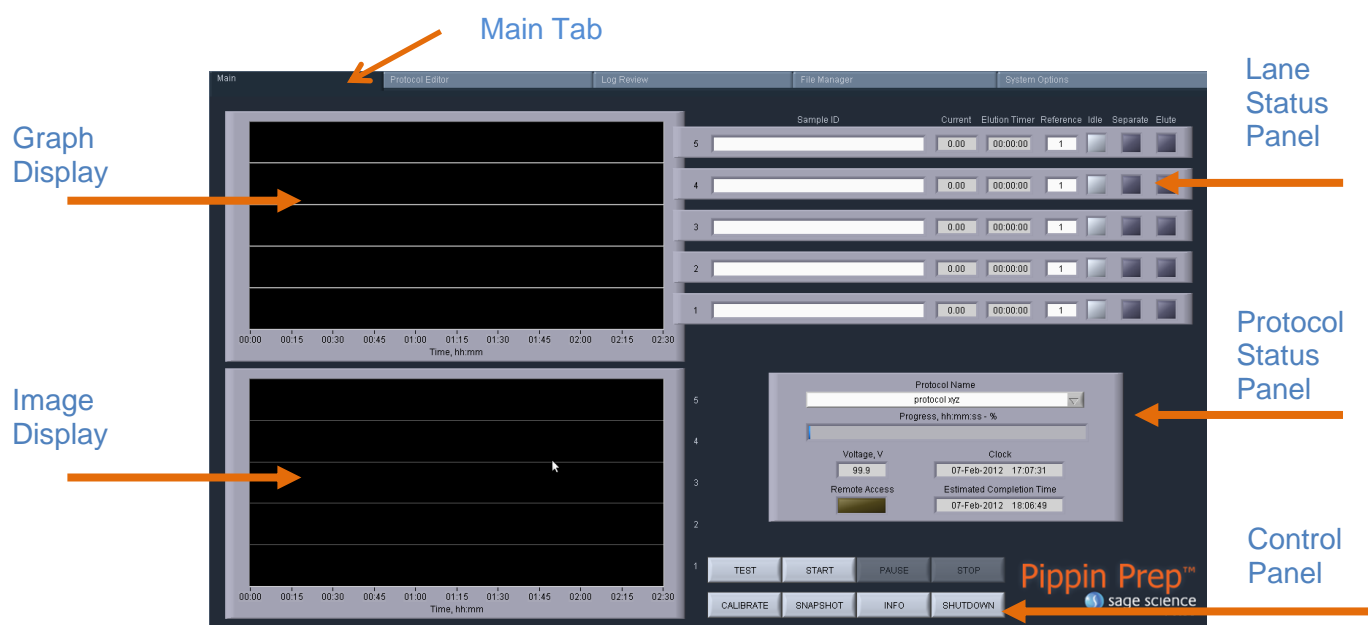
4. **Enter Sample ID information.**
5. **Save.** After programming a new protocol, or editing an existing one, the protocol file must be saved prior to use. If the protocol is new, press “Save”. If a protocol has been edited, a yellow alert will be displayed in the “Protocol Changes Not Applied” field. “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/ Pippin Prep/Protocols, and may be accessed in the File Manager tab.

9 Optical Calibration

The Pippin Prep optical system should be calibrated prior to every run. The instrument is supplied with a calibration fixture that fits into the optical nest.



1. Press "CALIBRATE" on the Control Panel



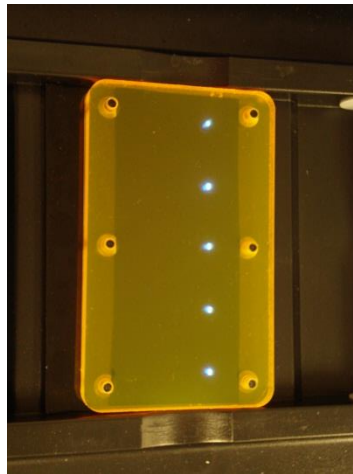
2. An “LED Calibration” window will launch. The “Calibration Status” field will contain the message “Calibration not done”.
3. Place the Calibration fixture onto the optical nest, so that all five LED detectors are covered. The dark side of the fixture must be down (closest to the LEDs):



Top



Bottom

Correct Position
on Optical Nest

4. Close the lid.
5. Press “CALIBRATE”



6. The LED photocurrents will automatically adjust to a factory setting, and the “Calibration Status” field will contain the message “Calibration OK”.



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

10 Preparing a Cassette

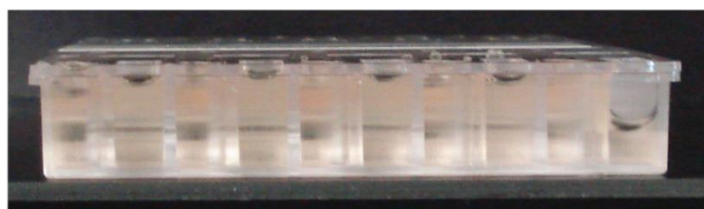
10.1 Visually Inspect the Cassette



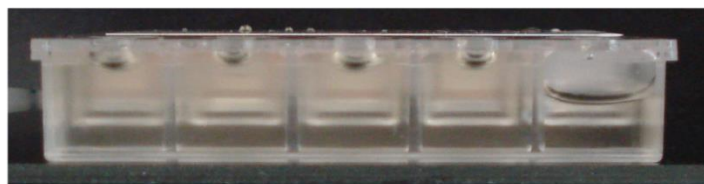
Caution! Some cassettes contain the DNA-binding dye ethidium bromide. Use appropriate protective equipment (disposable gloves, lab coat, and lab glasses) when handling cassettes.

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 prior to running. When refilling, a visual check of buffer levels is sufficient -- a measured volume is not required.

(+) side of cassette



(-) side of cassette



buffer volumes are Low

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.



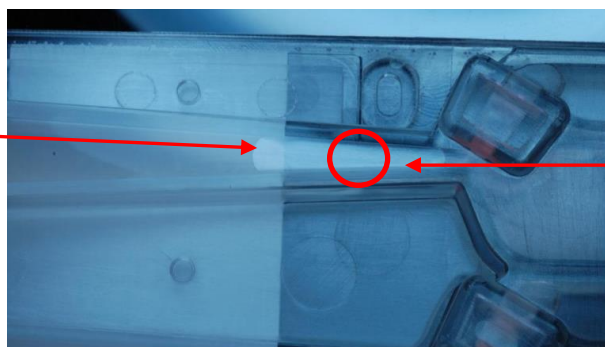
Important! *If a bubble is observed, do not use the lane to run the reference marker or for peak capture mode applications. It may be used for size-based fractionations (tight or bp range modes).*



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.*

Agarose Delamination

Do not use this lane for DNA marker



Optical Region

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

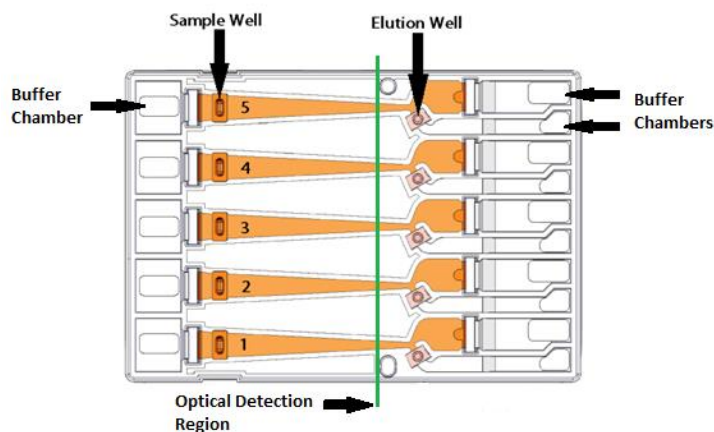


Figure 10.3. The region of optical detection

10.2 Prepare the Cassette for Loading

1. **Dislodge bubbles from behind the elution wells.** 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.

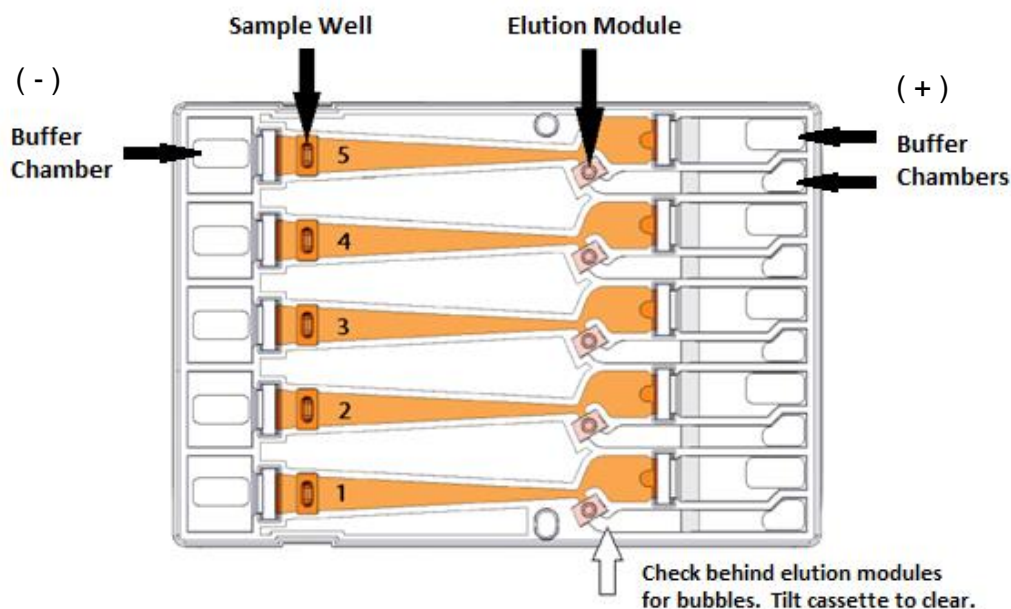


Figure 10.4. A schematic of a Gel Cassette.

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 Pippin Prep 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 65 μ l. The specified starting volume of 40 μ l only partially fills the module.

5. **Seal the elution wells with the adhesive tape strips.** Tape for sealing the elution wells are supplied with cassette packaging. Long elutions will cause the elution wells to overflow if not sealed. Place tape over the elution wells 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 μ l.

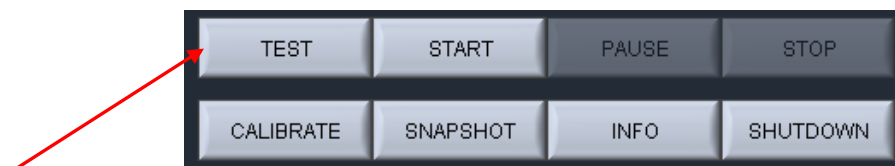
10.3 Continuity Test

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.

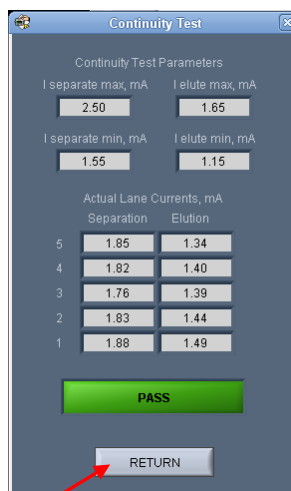


Important! Temperature affects electrical current readings. If cassettes have been refrigerated, they will fail the current test until the cassette is at least 17°C (62°F). Should this be the case, wait until the cassette temperature has equilibrated to room temperature and re-test.

1. **Press “Test”.** With the cassette in the optical nest, close the lid and press “TEST” on the controller 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.



Failed Continuity Tests

1. A failed test is indicated by a “FAIL” message, and the failed channel is highlighted in orange. **Figure 6.5** shows a failed test screen (elution channel in lane 1).
2. **If a Separation lane has failed (left column) continuity.** Do not use that lane. Remaining passing lanes can be used
3. **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 samples. However, the failed lane may be used to run an external reference marker.

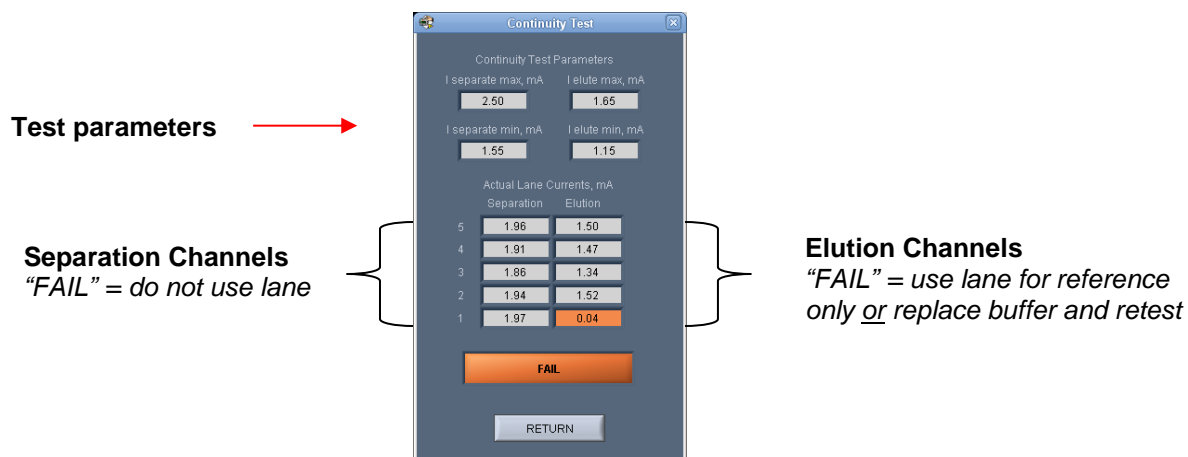


Figure 6.5. The Continuity Test screen. The elution channel in lane 1 has failed

11 Loading Samples

Proper sample loading is critical for best performance of Pippin Prep 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 μ l in total volume consisting of 30 μ l of DNA mixed with 10 μ l of Pippin Prep loading solution. The loading solution contains concentrated Ficoll as a densifying agent (see following chapter 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.

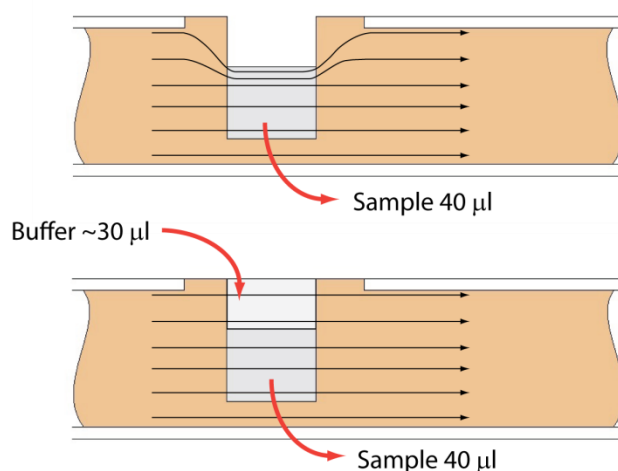


Figure 11.1. An illustration of the electrophoretic effect on a DNA sample when a sample well is not completely filled.

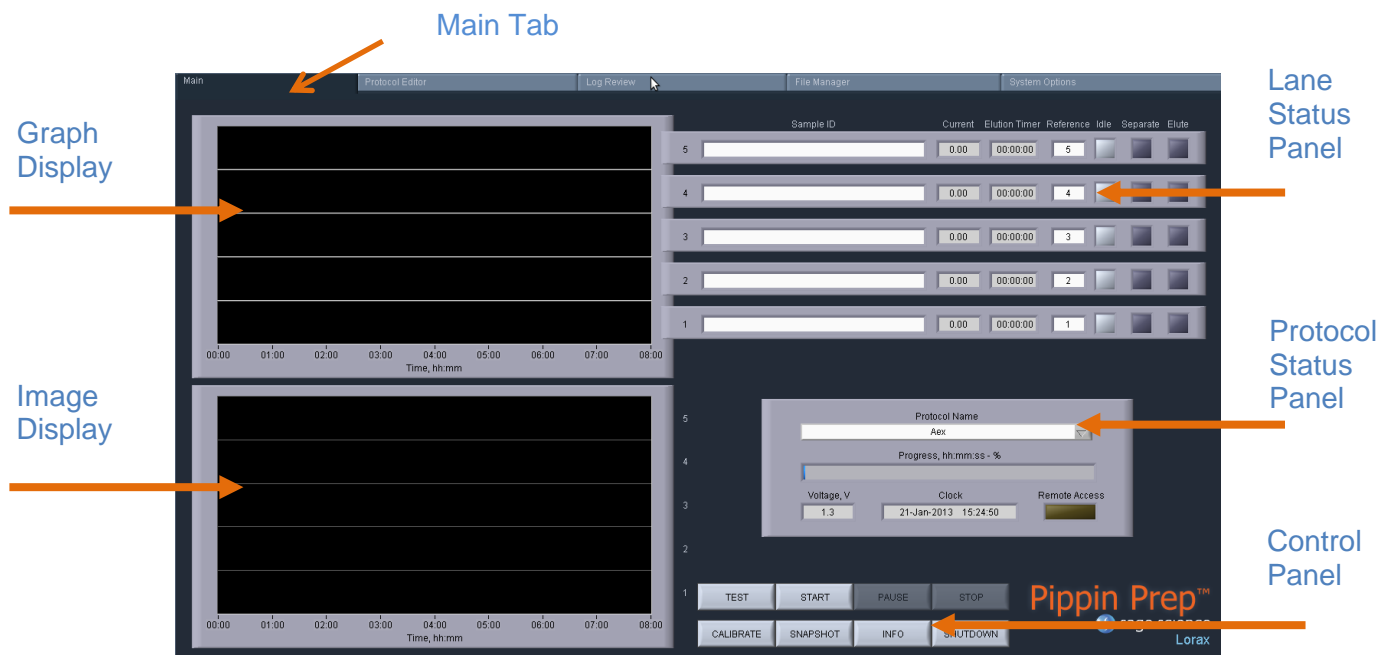
1. **Re-check the buffer level in the sample wells.** Make sure that sample wells are completely full to the top with electrophoresis buffer. Top off with additional buffer, if necessary. The total volume of the sample well is 70 μ l.
2. **Remove 40 μ l of buffer from the first sample well, and load 40 μ l of sample (or marker) into that well.** Take care not to pierce the agarose with the pipette tip. There is gel on all sides and 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**, previous page). 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 ~30 μ l 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 Pippin Prep 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.

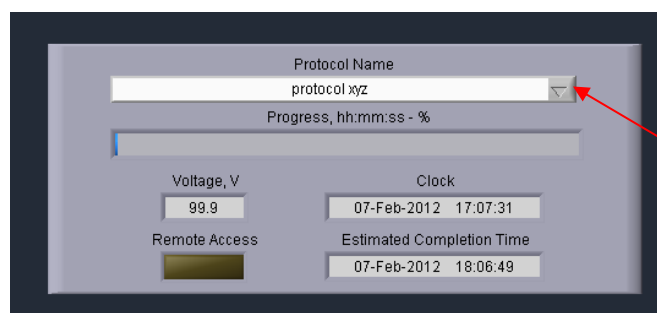


12.2 Starting a Run

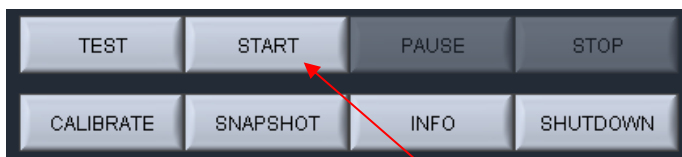


Important! The Pippin Prep should be optically calibrated prior to every run. If this has not been done, refer to **Chapter 9** for instructions.

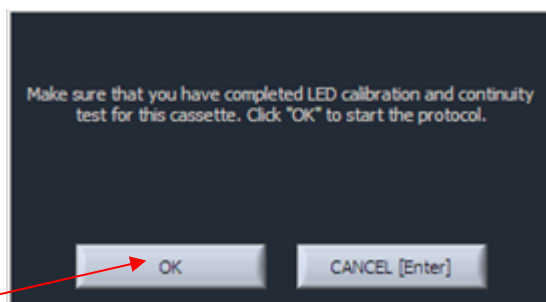
1. If necessary, select the protocol to be run from the “Protocol Name” drop down menu in the Protocol Status Panel. The last protocol that has been programmed will automatically be loaded. **See Chapters 7 and 8** for instructions on programming.



2. A properly prepared cassette (**Chapter 10**) with loaded samples should be in 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 wells.
3. Press “START” on the Control Panel.

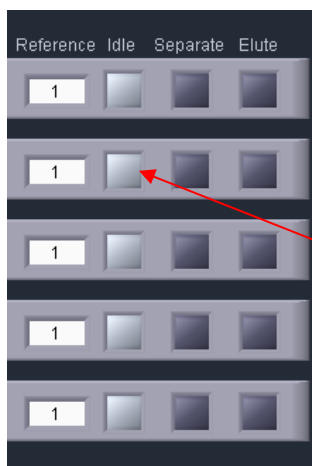


4. A pop-up window will appear to check that the LED calibration and continuity tests were not inadvertently skipped. Press “OK” to start the run.



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:

	Sample ID	Current	Elution Timer	Reference	Idle	Separate	Elute
5	Sample D	2.21	00:05:01	1			
4	Sample C	2.31	00:04:01	1			
3	Sample B	2.29	00:03:01	1			
2	Sample A	2.43	00:02:01	1			
1	Reference	2.27	00:00:00	1			

- **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.

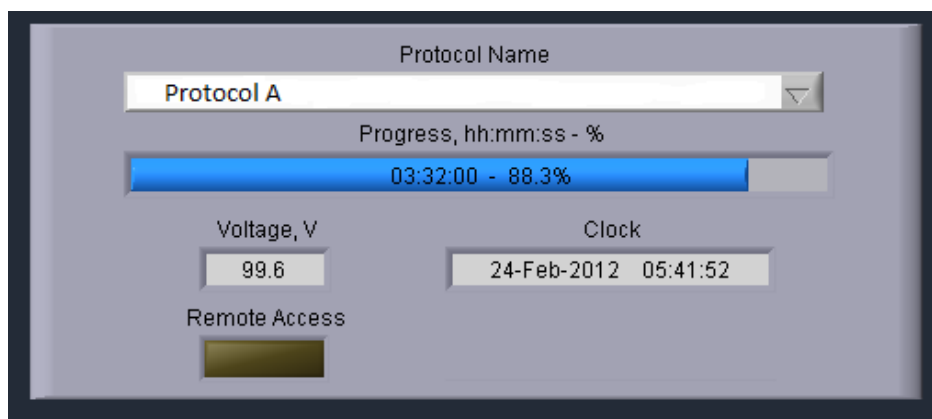


Note: If there is a time value in the “Elution Timer” field, and the orange “Elute” indicator is no longer lit, then the elution has been completed for that lane.

The Elution Timer records the length of time of the elution. When elution is complete, the electrophoretic path will switch to either the 1) Idle state (if the “End Run When Elution is Completed” box is checked when the protocol was programmed. See **Figure 12.1**, below), or 2) 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). However, the run may end at a sooner time if all elutions have been completed.



Important! If the “End Run when Elution is Completed” is checked in the protocol editor, the run may terminate before the progress bar reaches 100%

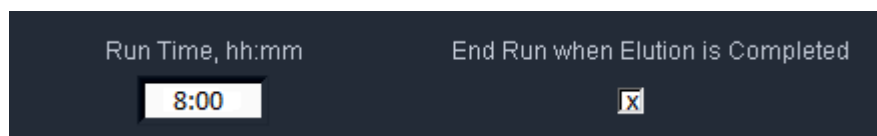


Figure 12.1 The Run Time field in the protocol editor. This time value is used to determine the progress of the run.

12.4 Log Files

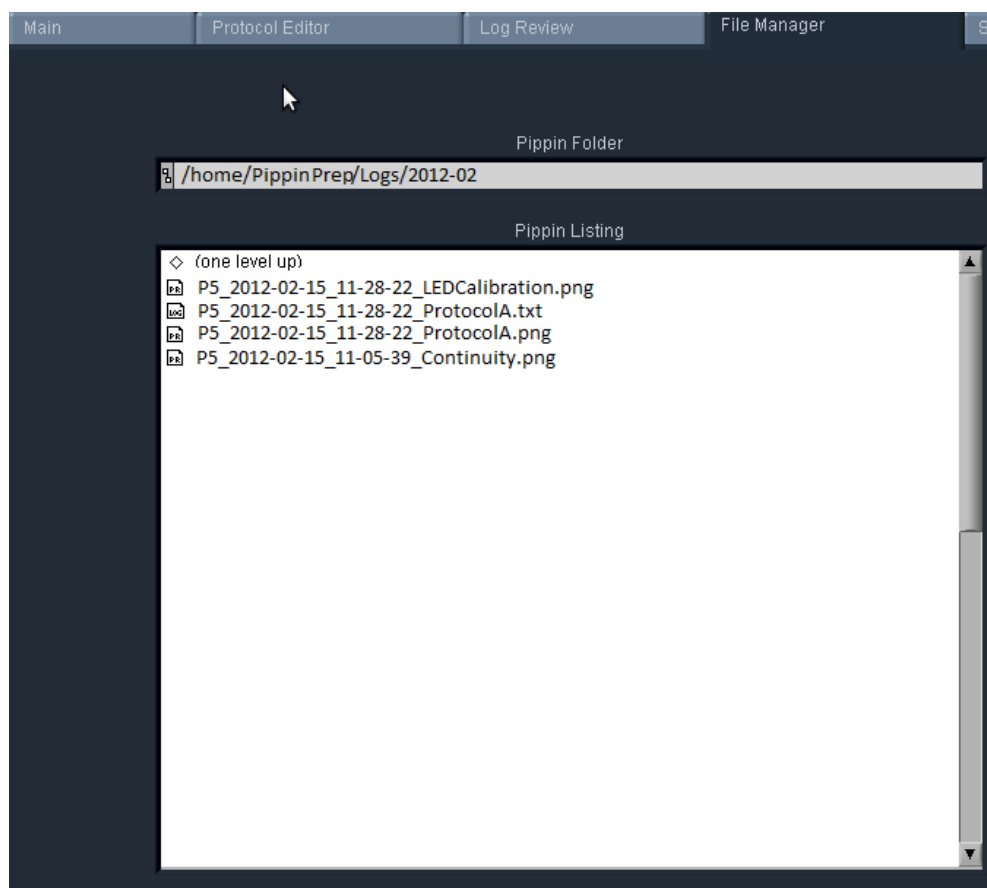
At the end of every run, the Pippin Prep will automatically save a log file (.txt) which can be viewed on the Log Review Tab (or the PC software version of Log Review). A screen image (.png) of the Main Tab is saved after the run has been finished. Screen images of all continuity tests and calibration 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/Pippin Prep/Logs/** in a folder with a year-month (YYYY-MM) folder name.

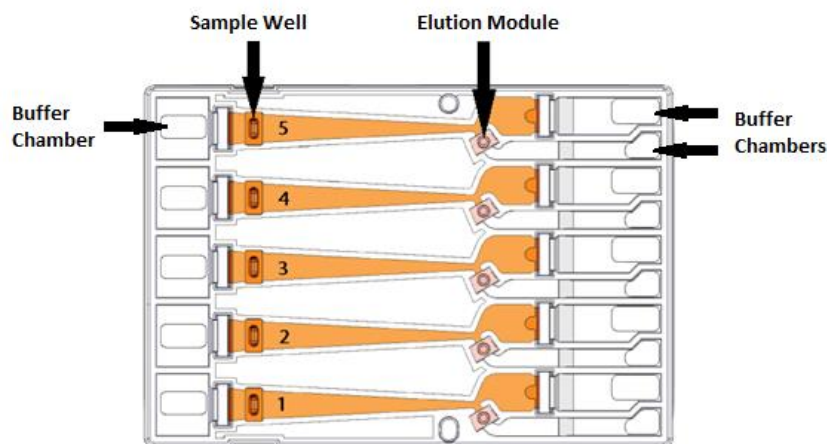
Log files names have the following convention:

[software version]_[year]-[month]-[day]_[hour]-[minute]-[second]_[user input protocol name or test type].[file extension]

An example of the four types of log files, and file structure in the File Manager Tab, is shown below.



13 Sample Collection



13.1 Overview

1. Remove the sealing tape strip that covers the elution wells.
2. Samples can be removed from elution modules using a standard 100-200 μ l 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 40 μ l 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.



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 is 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 Pippin Prep automatically adds a brief field reversal at the end of each elution to “push” DNA off of the membrane (Section 14.4).

14.2 Intrinsic Sample Recovery on the Pippin Prep

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

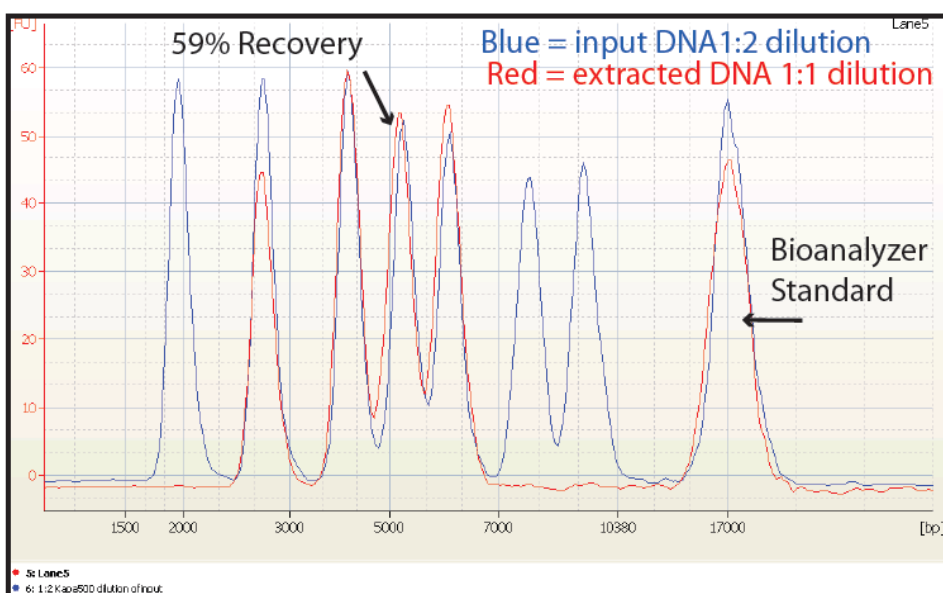


Figure 14.1. An example of the intrinsic recovery of 5 kb band on a 0.75% agarose cassette.

14.3 Improving Product Yield by Selecting a Wider Size Range

The Pippin Prep 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.

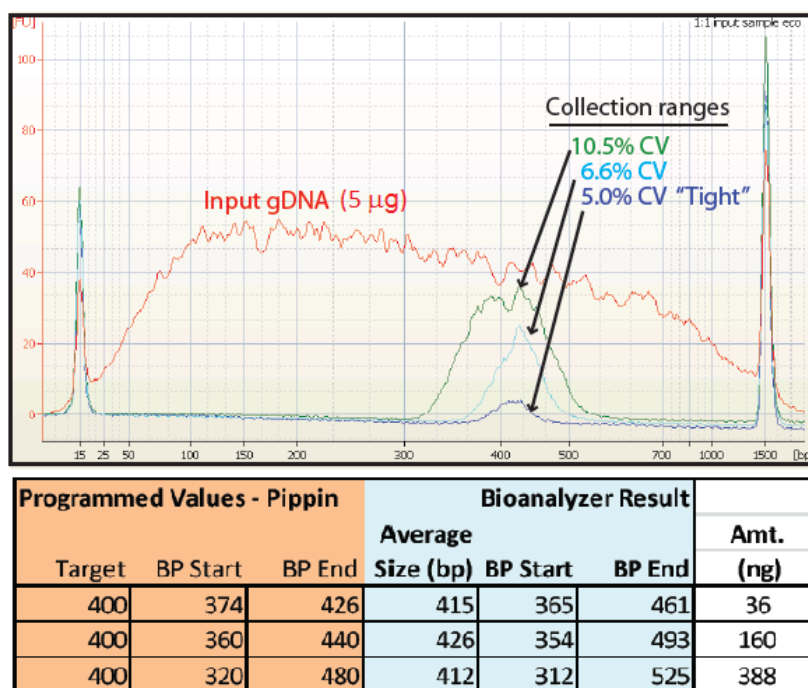


Figure 14.2. Increasing yield by widening the size selection range.



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 Pippin Prep 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.

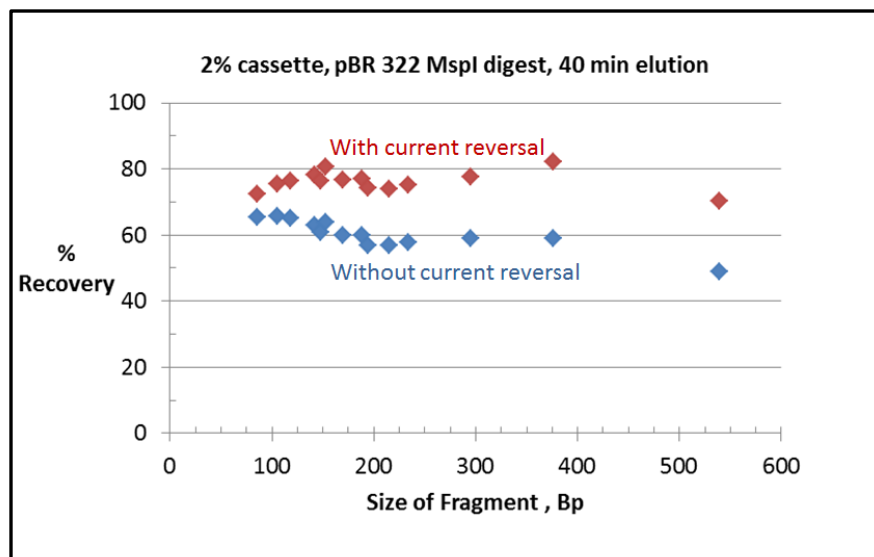


Figure 14.3. Improving recovery with a 5 sec.current reversal. A 40 minute elution of a plasmid restriction digest was run, and each data point represents an individual band.



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

Figure 14.4. System Options Tab with expanded view of Reverse Field Option.

15 System Validation

Control DNA can be purchased from Sage Science to check the performance of the Pippin Prep system. The Control DNA is useful to test, refine, and troubleshoot Pippin Prep size fractionation protocols. For comparison to factory tested cassettes, an extraction protocol with typical Agilent Bioanalyzer results is provided with the kits.

Figure 15.1 shows the DNA profile and extraction protocol for the 2% agarose control kit, and **Figure 15.2** shows a typical result from a 150 bp tight extraction from the DNA control Kit.

Pippin Prep 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 phenol:chloroform extraction, dialyzed, and diluted into Pippin Prep electrophoresis buffer (without Ethidium bromide).

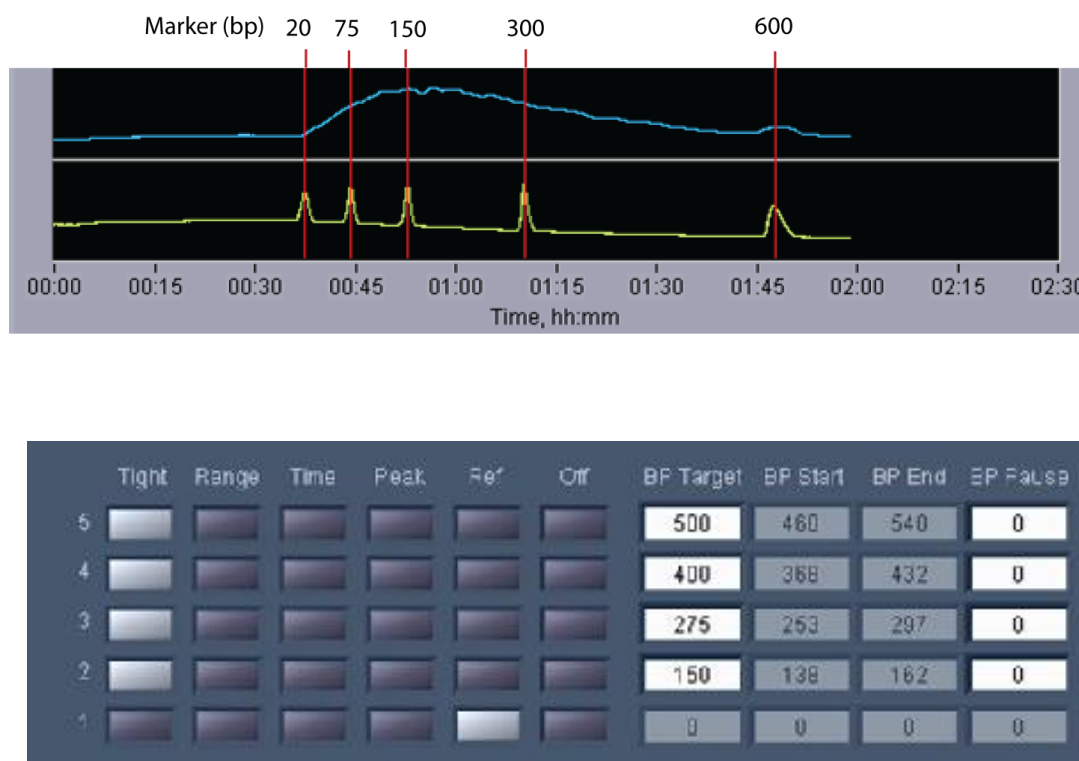


Figure 15.1 Control DNA profile (with reference marker) and DNA extraction protocol for a 2% agarose DNA control kit run.

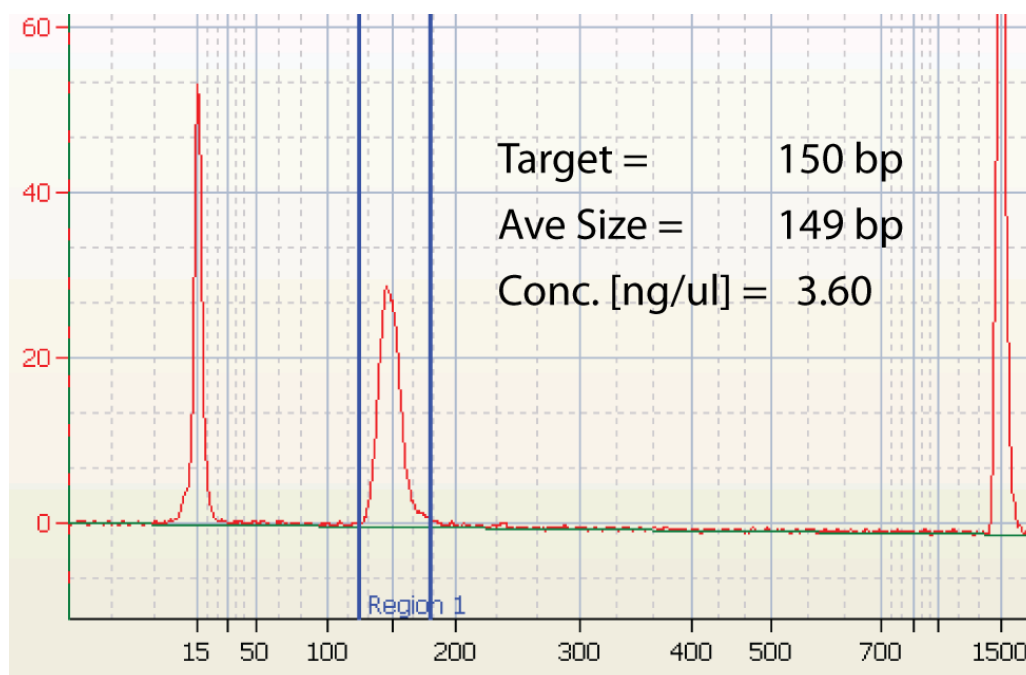


Figure 15.2 Example of a Bioanalyzer result for the 150 bp target of a Control DNA run on a 2.0% Agarose Cassette



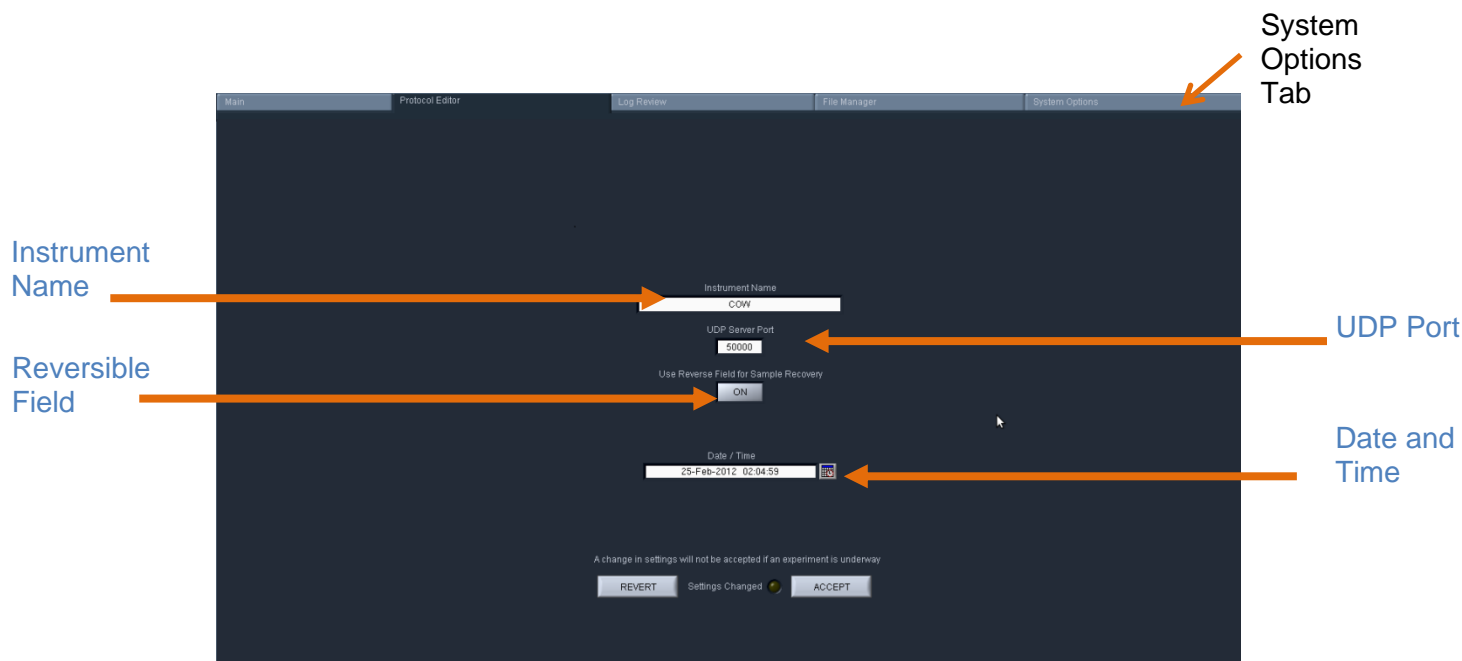
Important! Data are not intended to imply guaranteed results or performance. Control standards are intended to demonstrate that the Pippin Prep system is functioning as expected, and that proper operational technique is being used.

16 System Options Tab

The default screen on the Pippin Prep is a tabbed format. The System Options Tab is the fifth tab.

Overview

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



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.
- **UDP Server Port:** Provides an address for remote communications between the Pippin Prep 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:** This allows users to set or change 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. Press the “Use Reverse Field for Sample Recovery” button. The indicator display will change from dark gray to white, and display 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

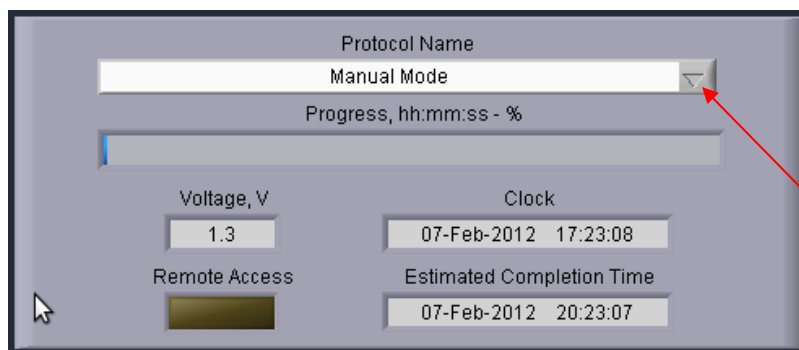
1. Edit the date or time display directly in the “Time/Display” field, or select the calendar icon next to the field and edit the time or select a date.
2. The “Settings Changed” indicator light will activate. Press “Accept” to accept the new setting

The screenshot shows a dark-themed interface for system settings. At the top, there's a label "Instrument Name" above a text input field containing "Blue #2". Below that is a label "UDP Server Port" above a text input field containing "50000". Next is a label "Use Reverse Field for Sample Recovery" above a button labeled "ON". Below that is a label "Date / Time" above a text input field containing "25-Feb-2012 02:04:59" and a small calendar icon to its right. At the bottom, there's a message: "A change in settings will not be accepted if an experiment is underway". Below this message are three buttons: "REVERT", "Settings Changed" (which has a small green indicator light next to it), and "ACCEPT".

17 Running in Manual Mode

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

1. Select the **Manual Mode** from 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 are carried out by pressing the “Elute” button. After elution, press the “Separate” button, to end the elution (and return to separate mode), or press the “Idle” button to stop the run on that lane.



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



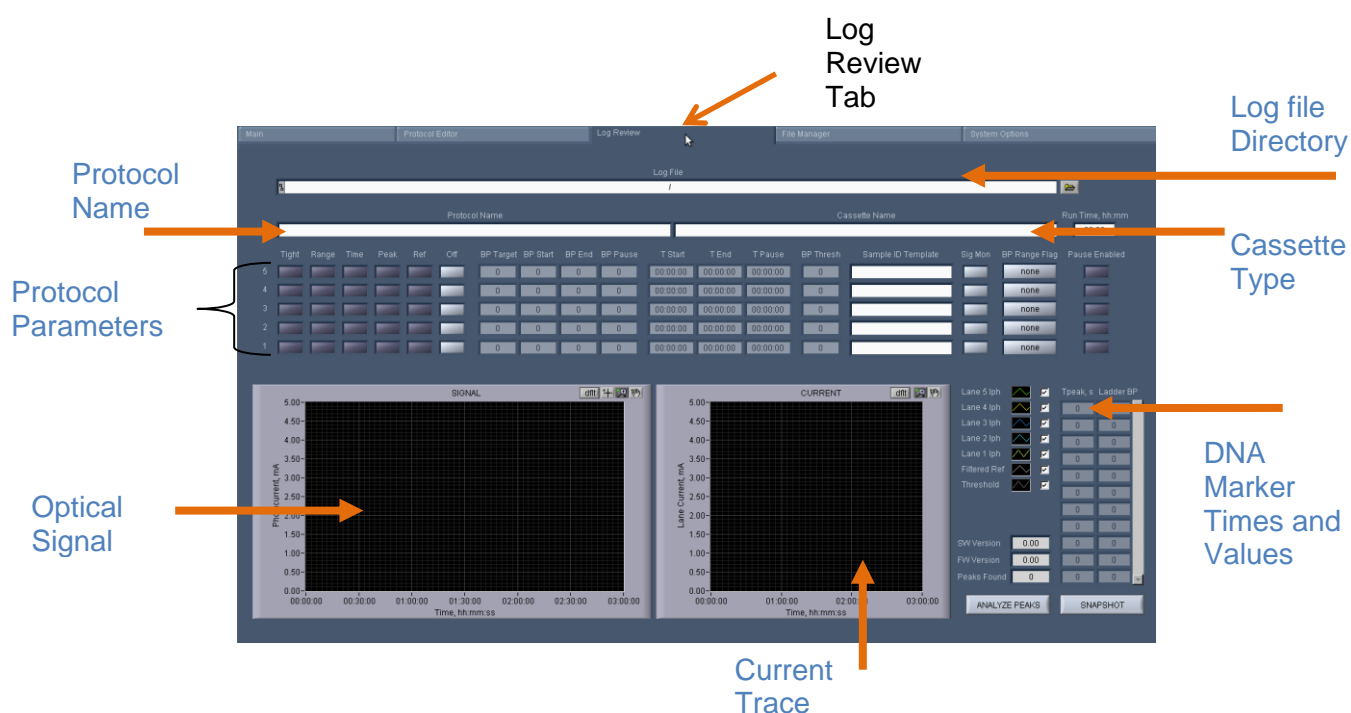
Note: *There is a delay between the time that a fragment is detected (and visible on the graph or image) and when it is in position to be eluted. See cassette specifications at the end of this manual to determine the best timing for manual runs.*

18 Analyzing Runs - Log Review Tab

The default screen on the Pippin Prep 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

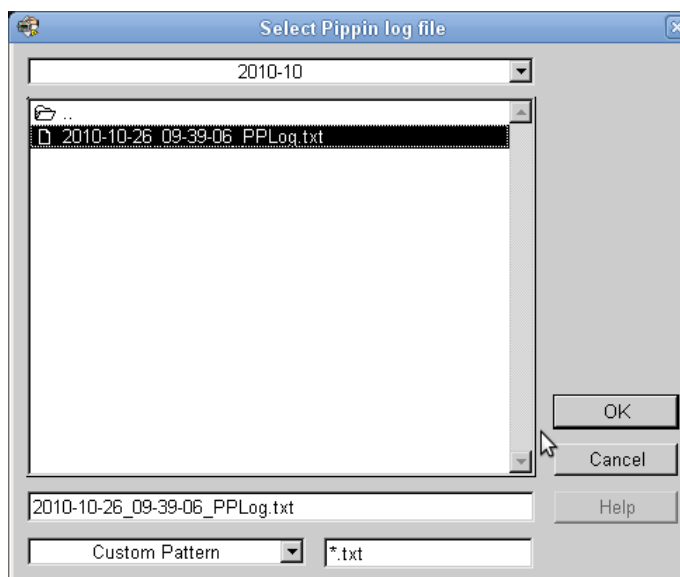


Note: A stand-alone Review Log Program is available for PCs. Contact sage science support.

1. Click the “Log Review” tab along top of the screen.
2. To select a log file, press the folder icon in 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:



Protocol
Information

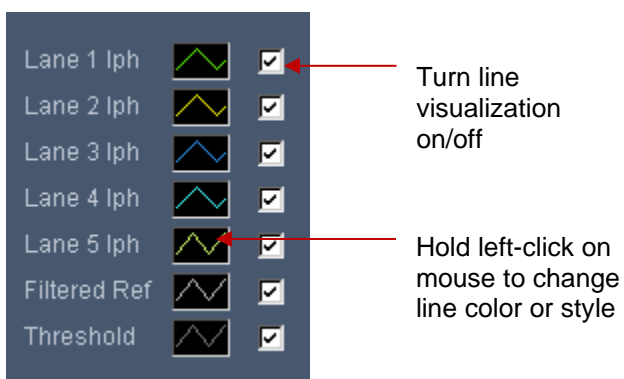
DNA Marker
Times and
Values

Optical
Signal
Traces

Electrophoretic
Current Traces
(current drops
during elution)

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.



Turn line
visualization
on/off

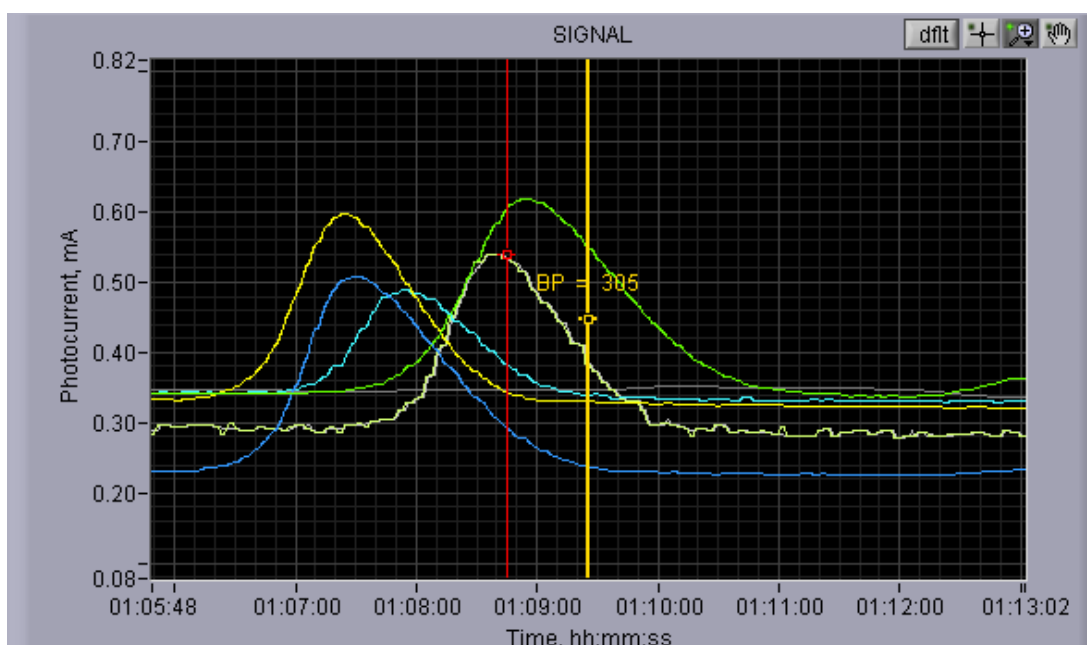
Hold left-click on
mouse to change
line color or style

Signal Graph

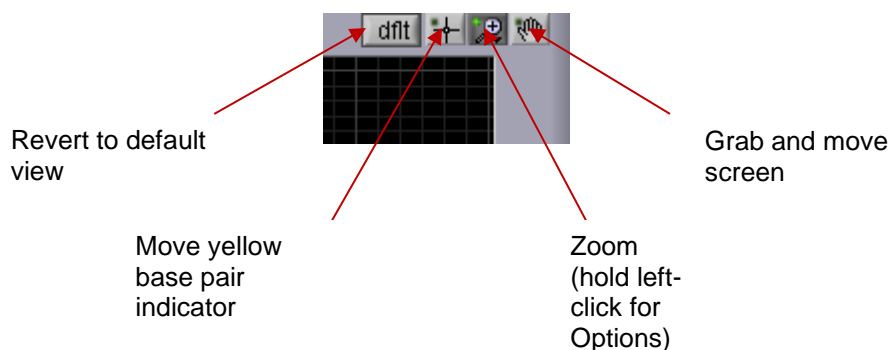
The “Signal” graph allows users to closely inspect the elution results of a Pippin run from a run log. The colored horizontal traces display the actual optical signal results in mA.

The vertical red lines indicate called reference DNA marker peaks that are listed in the DNA marker times and values list. There is an “Analyze Peaks” button to recall the markers.

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.



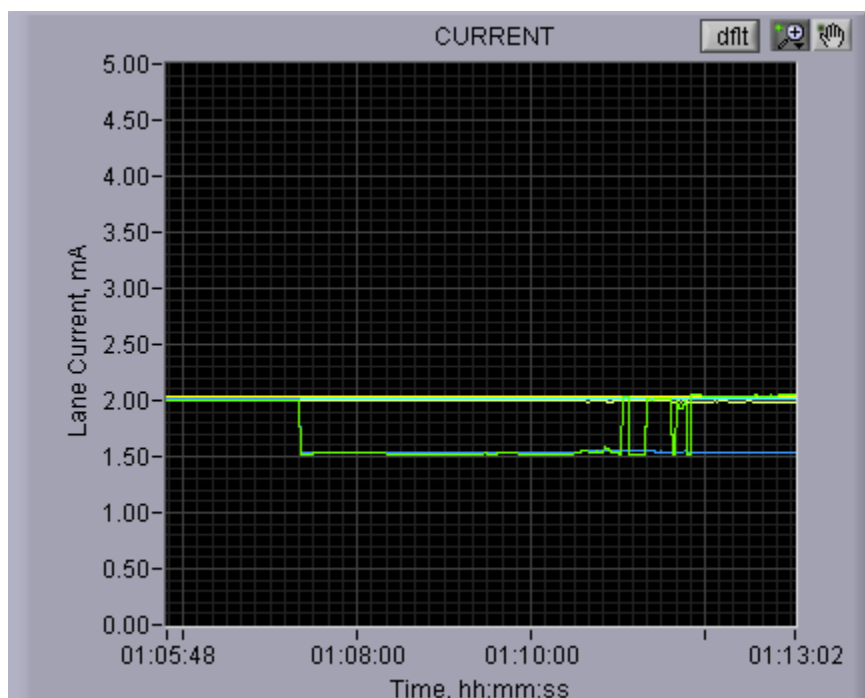
There are four icons in the upper right hand corner of the graph. The functions are listed below.



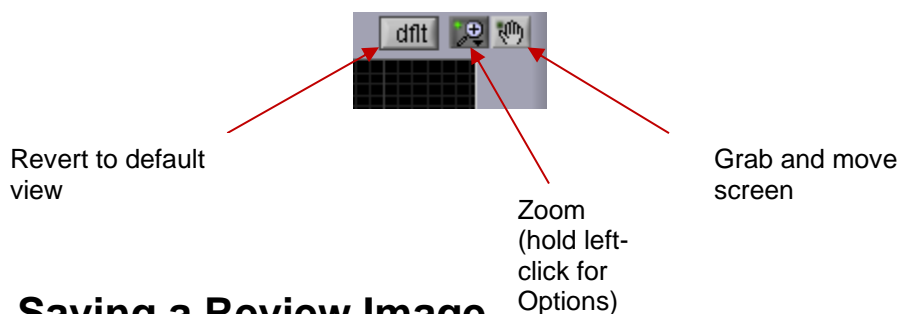
Current Graph

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.



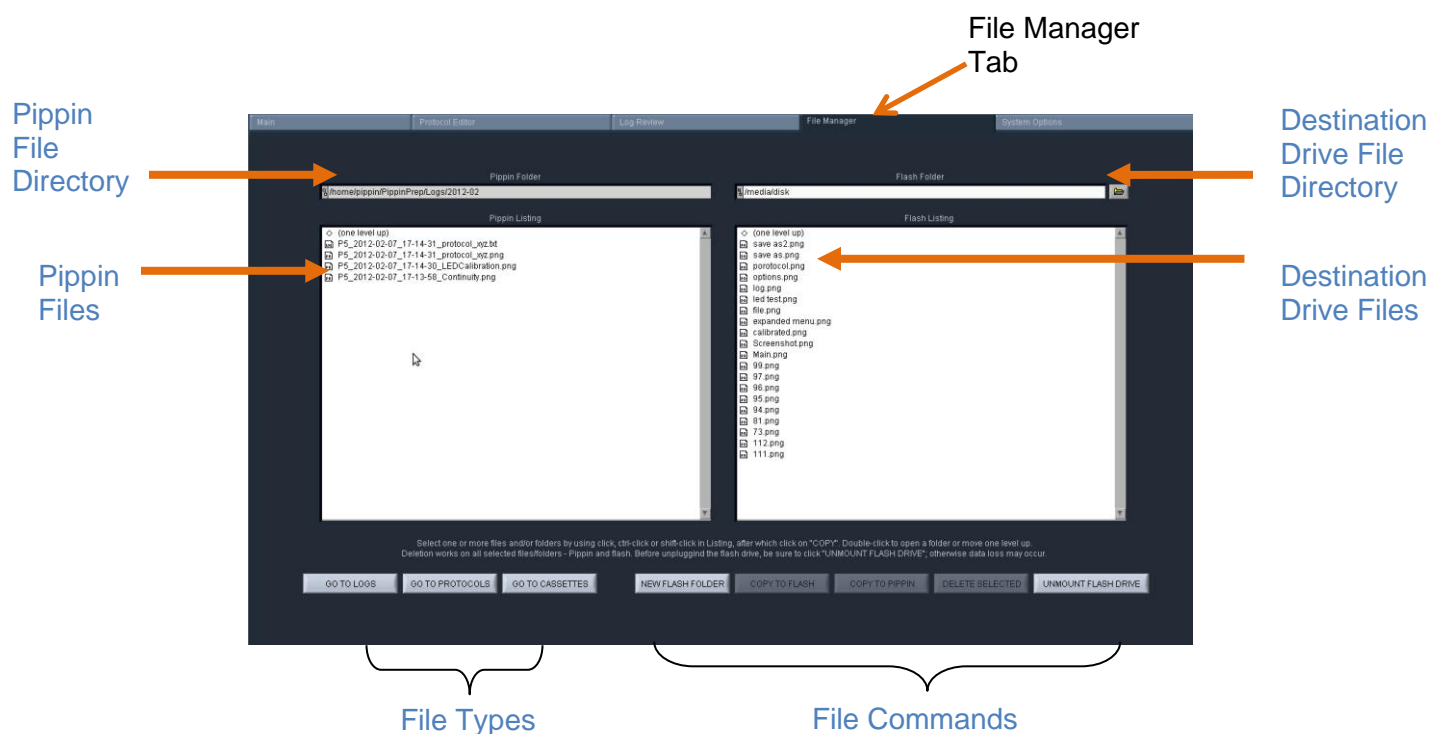
19 Managing Files -- File Manager Tab

The default screen on the Pippin Prep 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 Pippin Prep 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).

Protocol Files:

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

Cassette Files:

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



Note. The Pippin Prep 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

In addition, a screenshot may be manually saved at any time during a run (as a .png file) by pressing “Snapshot” in the Main Tab on the controller. The same file convention is used, but with the word “Screenshot” in the file name.



19.3 Transferring files

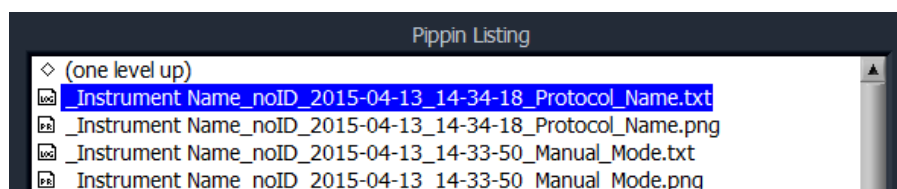
Files may be copied between the Pippin Prep and a USB flash drive. To transfer a file, place a USB drive into the USB port on the front panel of the instrument (the USB ports on the back panel may be used as well).

To transfer from the Pippin Prep to a USB Drive:

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



2. Highlight the file to be copied from the Pippin Prep.



3. Press the “COPY TO FLASH” button.



4. Press the “EJECT FLASH” button.

5. Remove the USB drive from the USB port.



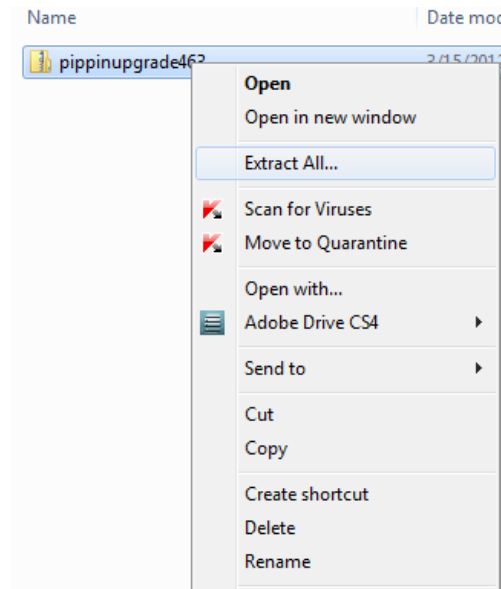
Warning! Be sure to “Unmount Flash Drive” prior to removal. Failure to do so may cause data loss. Failure to do so may cause data loss and problems with future upgrade procedures.

20 Upgrading Software

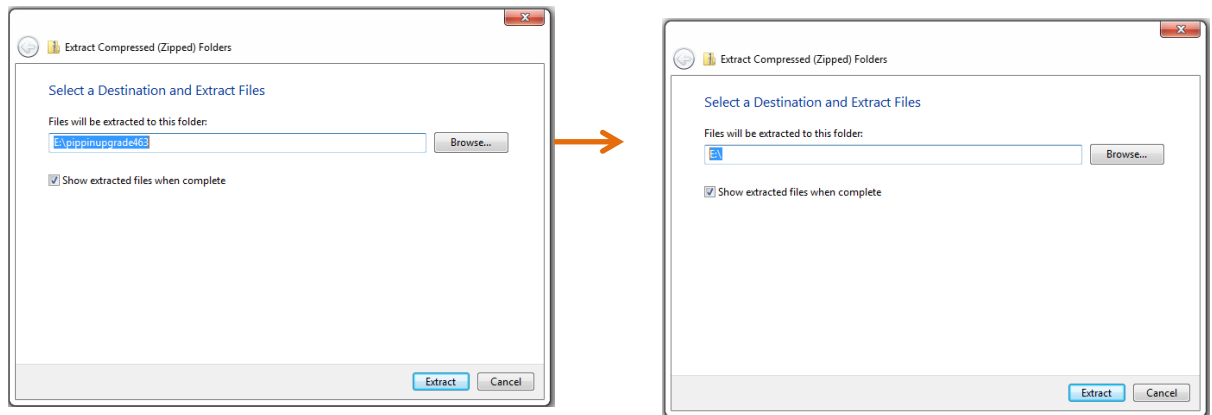
Pippin upgrade software is available for download at **www.sagescience.com/support/**. The files are provided in a zip file, and the contents must be extracted to the root directory of a USB flash drive.

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:**

Files will be extracted to this folder:

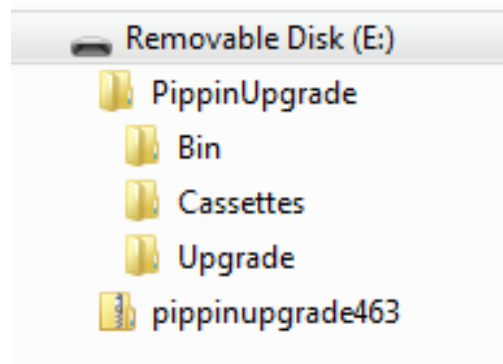
E:\

Not Correct:

Files will be extracted to this folder:

E:\pippinupgrade463

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



20.2 Upgrading the Pippin Instrument Software

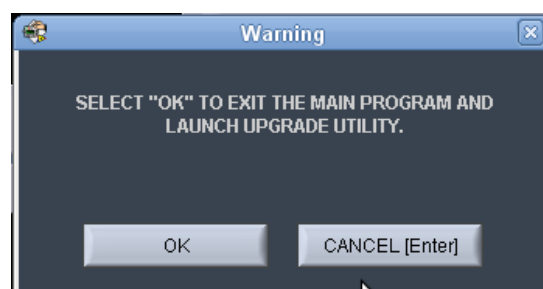
1. Insert the USB drive with the upgrade folder into the USB port on the front panel of Pippin instrument.
2. From the Main Tab, press “INFO”. This will launch the information window.



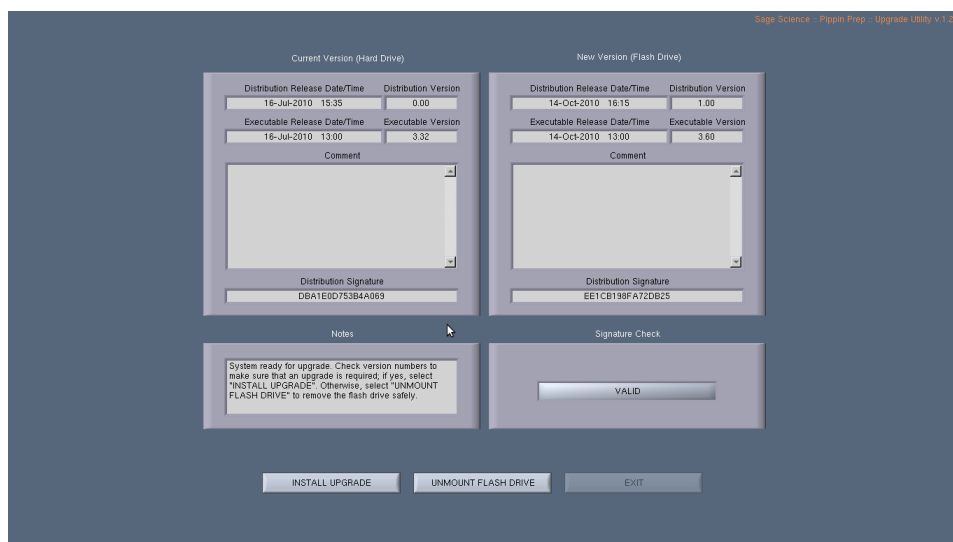
3. From the Information Window, press “SOFTWARE UPGRADE”



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



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



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



7. Press “UNMOUNT FLASH DRIVE”



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

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



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

```
Instrument Name: Pip1
SW Version: 5.30
IP Address: 127.0.0.1
```



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 Pippin Prep lid assembly. This may alter the performance of the instrument over time. An empty cassette body, labelled “Rinse Cassette” is provided with the Pippin Prep. This should be used with the procedure below. No other preventative maintenance is required.

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



Important! Electrodes should be rinsed **after 5 runs or once per week**, whichever is more frequent. If switching from a ethidium bromide cassette to a dye-free cassette, rinsing the electrodes is required.

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 Pippin Prep lid.
3. Allow the electrodes to rinse for approx. 30 secs.
4. Open the lid and remove the cassette.



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

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 Pippin Prep instrument and monitor have a standard 2-year parts and labor 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 Pippin Prep 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 Pippin Prep 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

Running buffer should be disposed of according to internal lab safety policy. Running buffer contains up to **5 ug/ml of Ethidium bromide**. Cassettes should be disposed of as laboratory waste.



Caution! *Ethidium bromide is a suspected mutagen. Many labs have require disposal of ethidium bromide as a hazardous waste.*



Note: Dye-free cassettes contain TAMRA dye bound to the reference marker.

23 Ordering Information

Products		
Instrument		Catalogue No.
Pippin Prep (Includes monitor, keypad, mouse and accessories)		PIP0001
Gel Cassettes with Ethidium Bromide– with External Marker (10/pk)	External Marker	Catalogue No.
3% agarose, with EtBr, 90 – 250 bp	C	CSD3010
2% agarose, with EtBr, 100 – 600 bp	B	CSD2010
1.5% agarose, with EtBr, 250 – 1500 bp	A	CSD1510
Dye-Free Gel Cassettes – with Internal Standards (10/pk)	Internal Standards	Catalogue No.
3% agarose, dye-free, 100 – 250 bp	P	CDP3010
2% agarose, dye-free, 100 – 600 bp	L	CDF2010
1.5% agarose, 250 bp – 1.5 kb	E	CDF1510
Dye-Free Gel Cassettes – with External Marker (10/pk)	External Marker	Catalogue No.
2% agarose, 100 – 600 bp	K	CEF2010

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 for 3% Gel Cassette, (Ext. Mrk.) 90 – 250 bp, (5 µg/load), 16 loads	CON3004
Control DNA, 2% Gel Cassette, (Int. Std). 100 – 600 bp, (5 µg/load), 20 loads	CIS2004
Control DNA for 2% Gel Cassette, (Ext. Mkr.) 100 – 600 bp, (5 µg/load), 16 loads	CON2004
Control DNA for 1.5% Gel Cassette, (Int. Std) 250 – 1500 bp, (2 µg/load), 20 loads	CIS1504
Control DNA for 1.5% Gel Cassette, (Ext. Mkr.) 250 – 1500 bp, (2 µg/load), 16 loads	CON1504
Reagent Replacement Kits – Ethidium Bromide with External Marker	Catalogue No.
CSD3010 reagent replacement kit for 10 cassettes, Marker C	RPK3010
CSD2010 reagent replacement kit for 10 cassettes, Marker B	RPK2010
CSD1510 reagent replacement kit for 10 cassettes, Marker A	RPK1510
Reagent Replacement Kits – Dye Free with Internal Standards	Catalogue No.
CDP3010 reagent replacement kit for 10 cassettes, Marker P	RIP3010
CDF2010 reagent replacement kit for 10 cassettes, Marker L	RIK2010
CDF1510 reagent replacement kit for 10 cassettes, Marker K	RIK1510
Reagent Replacement Kits – Dye Free with External Marker	Catalogue No.
CEF2010 reagent replacement kit for 10 cassettes, Marker E	REK2010

24 Instrument Specifications

Specifications for Pippin Prep Instrument

Electrophoresis Voltage	100 V or 150V, constant
Typical current per sample lane	2.0 mA
Optical detection	535 nm excitation, 640 nm emission
Power Requirements	100-240 VAC +/-10% VAC, 2.5 A, 50-60 Hz
Weight	15 lbs / 7 kg
Dimensions	7H X 11W X 21D (in.) / 18H X 28W X 53D (cm)
Approvals	CE, CSA
Country of Origin	United States

25 Specifications for Gel Cassettes

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
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.

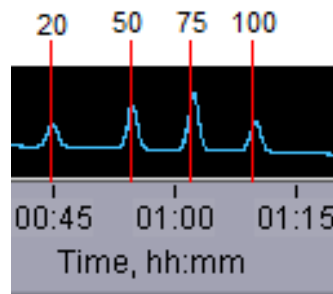
Specifications subject to change without notice.

25.1 3% Agarose, ethidium bromide, 90 – 250 bp, Marker C

Product Number: **CSD3010**

Use Cassette Definition: **3% Agarose, Marker C**

DNA Marker C - Typical times to detector



Estimated Collection Times for Minimum Size Range (Tight) Targets

Target (bp)	Time to Time to Collect* (min)
100	67
150	82
250	118

* 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	5%
Accuracy	<5%
Reproducibility	<5%

Specifications subject to change without notice

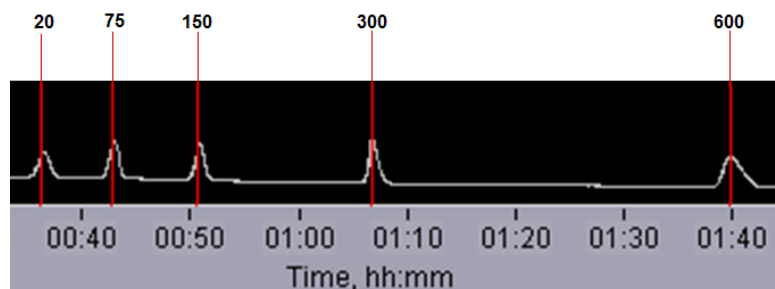
*** see page 21-1 for specification definitions**

25.2 2% Agarose, ethidium bromide, 100 – 600 bp, Marker B

Product Number: **CSD2010**

Use Cassette Definition: **2% Agarose, Marker B**

DNA Marker B - Typical times to detector



Estimated Collection Times for Minimum Size Range (Tight) Targets

Target (bp)	Time to Time to Collect* (min)
150	57
300	73
600	111

* 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	8%
Accuracy	<5%
Reproducibility	<5%

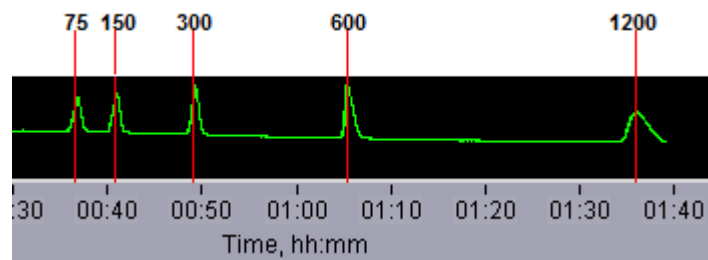
Specifications subject to change without notice

*** see page 21-1 for specification definitions**

25.3 1.5% Agarose, ethidium bromide, 250 bp – 1.5kb, Marker A

Product Number: **CSD1510**
 Use Cassette Definition: **1.5% Agarose, Marker A**

DNA Marker A - Typical times to detector



Estimated Collection Times for Minimum Size Range (Tight) Targets

Target (bp)	Time to Time to Collect* (min)
300	53
600	64
1,200	105
1,500	130

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

Performance Specifications

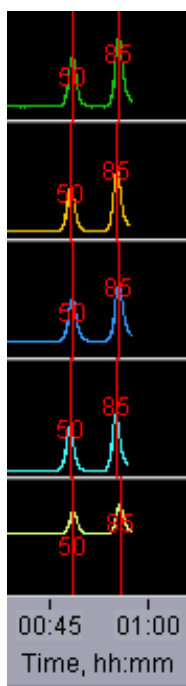
Minimum Size Distribution as CV	8%
Accuracy	<5%
Reproducibility	<5%

Specifications subject to change without notice
 * see page 21-1 for specification definitions

25.4 3% Agarose, Dye-Free, 100 bp-250 bp, Marker P (internal standards)

Use Product Number: **CDP3010**
 Use Cassette Definition: **3% DF, Marker P**

DNA Marker P - Typical times to detector



*Estimated Collection Times for
Minimum Size Range (Tight) Targets*

Target (bp)	Time to Time to Collect* (min)
100	63
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

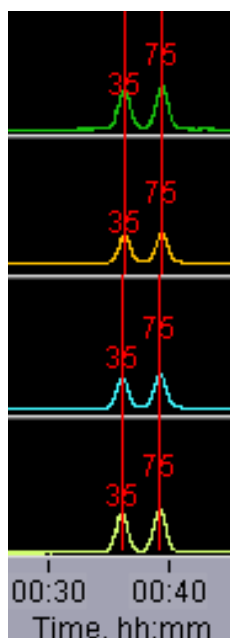
Minimum Size Distribution as CV	<5%
Sample Recovery	50 – 80%
Accuracy	<5%
Reproducibility	< 5%

Specifications subject to change without notice
 * see page 21-1 for specification definitions

25.5 2% Agarose, Dye-Free, 100 bp-600 bp, Marker L (internal standards)

Use Product Number: **CDF2010**
 Use Cassette Definition: **2% DF, Marker L**

DNA Marker L - *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

Minimum Size Distribution as CV	<8%
Sample Recovery	50 – 80%
Accuracy	<5%
Reproducibility	< 5%

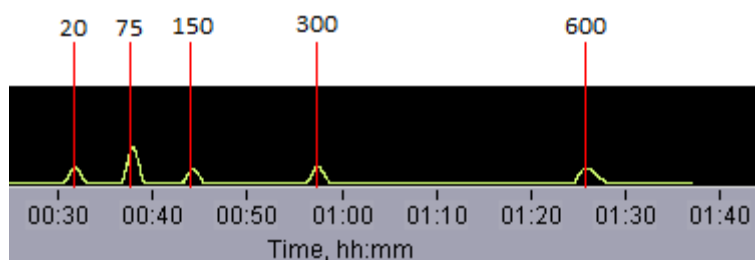
Specifications subject to change without notice

*** see page 21-1 for specification definitions**

25.6 2% Agarose, Dye-Free, 100 bp-600 bp, Marker E (external marker)

Use Product Number: **CEF2010**
 Use Cassette Definition: **2% EF, Marker E**

DNA Marker E - Typical times to detector



Estimated Collection Times for Minimum Size Range (Tight) Targets

Target (bp)	Time to Time to Collect* (min)
150	51
300	67
600	100

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

Performance Specifications*

100-600 bp

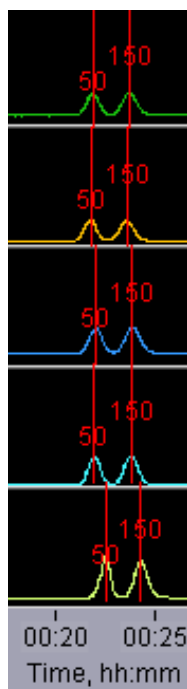
Minimum Size Distribution as CV	<8%
Sample Recovery	50 – 80%
Accuracy	<10%
Reproducibility	< 10%

Specifications subject to change without notice
 * see page 21-1 for specification definitions

25.7 1. 5% Agarose, Dye-Free, 250 bp-1.5 kb, Marker K (internal standards)

Use Product Number: **CDF1510**
 Use Cassette Definition: **1.5% DF, Marker K**

DNA Marker K - Typical times to detector



Estimated Collection Times for Minimum Size Range (Tight) Targets

Target (bp)	Time to Time to Collect* (min)
250	29
300	31
600	36
1200	47
1500	50

* 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%	<10%
Sample Recovery	50 – 80%	50 – 80%	50 – 80%
Accuracy	<5%	<7%	<10%
Reproducibility	< 5%	< 8%	< 15%

Specifications subject to change without notice

* see page 21-1 for specification definition



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