sage science

User Manual

Pippin Pulse™

Electrophoresis Power Supply

Software Version 1.34

Pippin Pulse Power Supply Part #:	**PPI - 0200**
Sage Pulse Midi Gel Box Part #:	**PGB -1000**

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1.0 Introduction

Thank you for purchasing the Pippin Pulse electrophoresis power supply. The Pippin Pulse provides programmable pulsed-field power for running small to mid-sized (12 x 14 cm) agarose gels. A Windows PC running the Pippin Pulse software is required.

1.1 Pippin Pulse Specifications

Electrophoresis voltage:	25 – 175 V
Maximum current:	200 mA
Dimensions:	7H X 20W X 24D cm (3H X 8W X 10D in.)
Weight:	1.1 kg (2.5 lbs)

1.2 About the Sage Pulse Gel Box

The Sage Pulse Gel Box is a standard 12 x 14 cm gel box that is equipped with re-enforced large diameter platinum electrodes. Though a standard midi-gel box can be used with the Pippin Pulse, platinum electrodes can corrode and break over time under pulsing conditions.

It is highly recommended that user use the Sage Pulse Gel Box if heavy pulsed-field use is anticipated.

1.3 Warranties

The Pippin Pulse and Sage Pulse Gel box are subject to depot repair or replacement at the discretion of Sage Science. The warranty period is 1 year from the date of shipment for both devices.

2.0 About Pulsed-Field Electrophoresis

Pulsed-field gel electrophoresis is a strategy for resolving large fragments of DNA for analysis. When running a typical direct-current agarose gel, fragments above 10-15kb can migrate in a non-predictable manner that is not related to their size. Pulsed-field gels work by shuttling DNA back and forth in the gel, effectively slowing down large DNA fragments that might otherwise run at the same rate as smaller ones. By switching the direction of the electric field in a gel, DNA will change its direction of migration. Since smaller molecules can change direction faster than larger molecules, more differentiated separation can be achieved by rapidly switching, or pulsing, the electric field.

A single-pulse duration typically separates a relatively narrow range of DNA sizes. For best results, it is necessary to use a range of pulse times; this is accomplished by using a "ramp", progressively increasing the forward and/or reverse intervals from a lower limit to an upper limit.

The Pippin Pulse is programmed by entering a forward time step and reverse time step. A ramp is programmed by adding a time increment to either or both steps. An accelerated ramp can be achieved by adding additional time increment either or both increments. Lastly, the number of time steps is also entered, and the steps will continuously cycle for the duration of the run. Cycling allows the same protocol to be run for different lengths of time reproducibly.

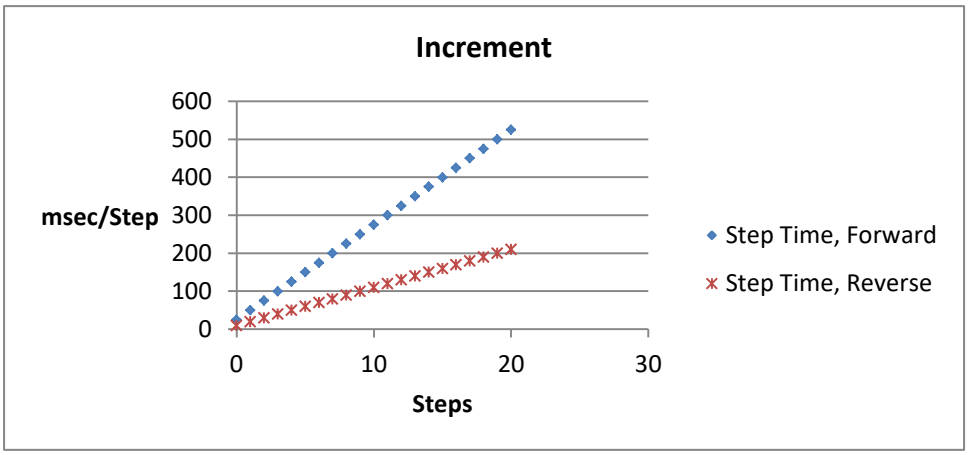


Fig 1. An example of time increments for the forward and reverse steps.
A 25 msec forward step, with a 25 msec increment, 10 msec reverse step with a 10 msec increment. 20 Steps/cycle.

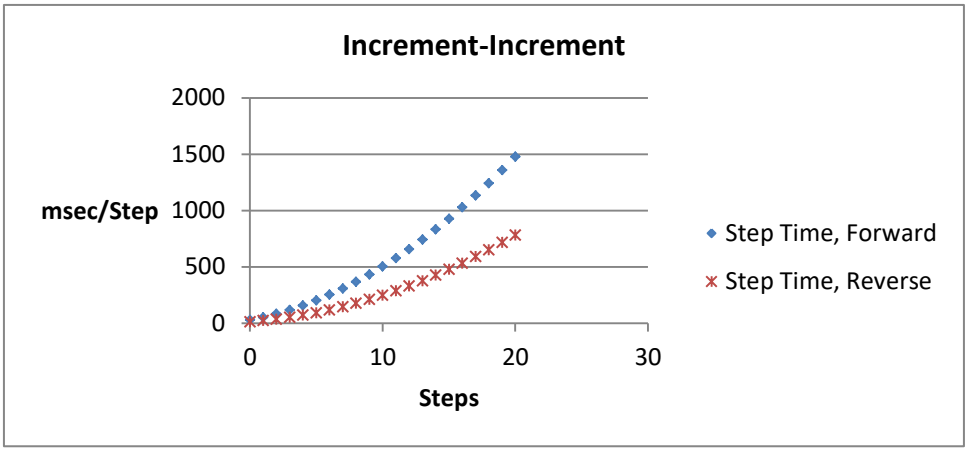


Fig 2. An example of second increments added.
5 msec was added to the forward increment and 3 msec increment, was added to the reverse increment.

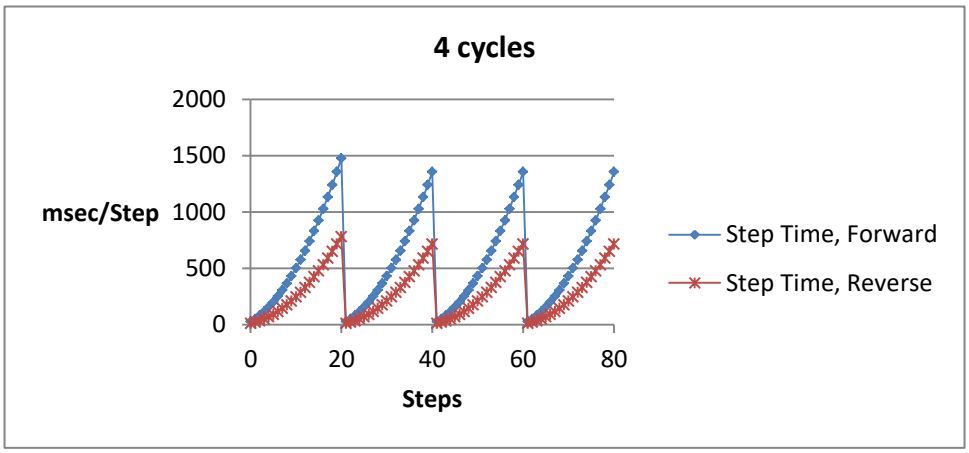


Fig 3. An Illustration of running four cycles of step of the previous protocol.
In this example, each cycle is about 6 seconds in length. Typical runs require 8-16 hours.

Note: If the reverse time is not long enough, large molecules may not completely change direction during the reversal, and may actually migrate faster than shorter molecules. This effect can be completely overcome by using a time ramp whose longest reverse time is long enough to separate all molecules of interest.

3.0 Using the Pippin Pulse

The Pippin Pulse software includes five preset protocols based on internal testing. These are a good starting point to analyze fragments from 0.5 – 430kb. Go to the *Pre-set Protocols* section of this manual (**Section 6**) for the buffer formulation and conditions for these runs. Go to *Programming the Pippin Pulse* (**Section 9**) for recommendations and guidelines for developing new protocols.

4.0 Unpacking

The Pippin Pulse (PPI0200) will arrive with the following components:

- Pippin Pulse electrophoresis power supply
- Power supply
- USB cable (to connect PC to a Pippin Pulse)
- USB storage device (contains the Pippin Pulse programming software)
- User manual (this document)

5.0 Set-up

- Remove the Pippin Pulse from its packaging.
- Connect the power supply to the unit, and plug the power cable into an electrical outlet.
- Insert the USB cable into the USB port found in the back of the unit.
- Insert the other end of the USB cable into a USB port of a PC.
- Install the Pippin Pulse software on the PC:
    - Insert the USB storage device into an available port on a PC
    - On the computer, go to the USB device (Computer/Removable Disk (E:))
    - Open the Pippin Pulse folder
    - Double click the “setup” executable file
    - Follow the installer instructions CDM drivers and Pippin Pulse software
    - After installation is complete, re-boot the computer

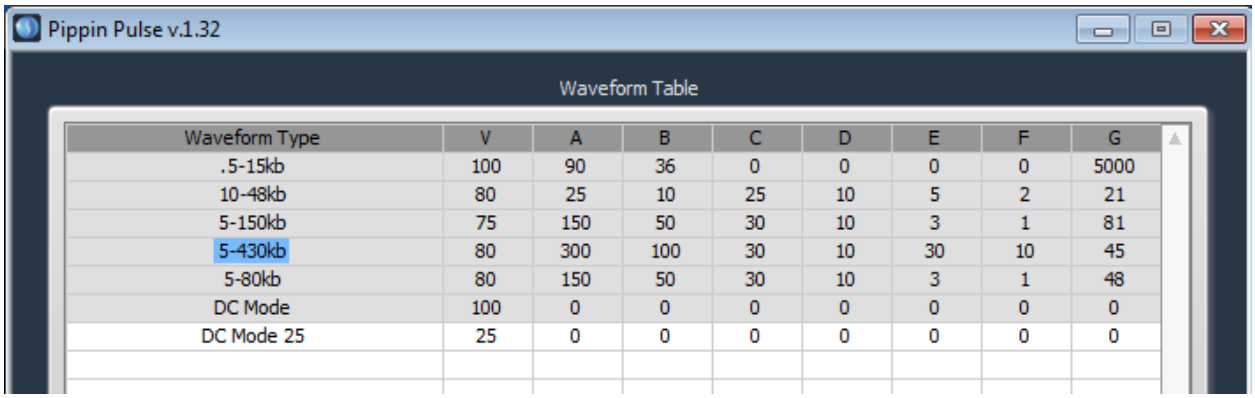
The following icon will appear on the computer desktop:

Pippin Pulse Desktop Icon

Double click the icon to launch the application.

6.0 Pre-set protocols

There are six preset fixed pulsed field protocols listed in the Pippin Pulse software. These are highlighted in gray in the software and cannot be modified or deleted, and indicate a fragment range over which DNA should be well-resolved (the "DC Mode 25" direct current protocol is not hightlighted in gray and can be quickly edited and re-saved). These protocols were developed by Sage Science specifically to work with higher size range workflows with the BluePippin DNA size selection system:



Waveform Type	V	A	B	C	D	E	F	G
.5-15kb	100	90	36	0	0	0	0	5000
10-48kb	80	25	10	25	10	5	2	21
5-150kb	75	150	50	30	10	3	1	81
5-430kb	80	300	100	30	10	30	10	45
5-80kb	80	150	50	30	10	3	1	48
DC Mode	100	0	0	0	0	0	0	0
DC Mode 25	25	0	0	0	0	0	0	0

6.1 Recommended gel conditions

The pre-set protocol gel images shown on the following pages were run using the following gel formulations in the Sage Pulse Gel Box. The Sage Pulse Gel Box is equipped with large diameter electrodes, specifically for pulsed-field use, Part No. **PGB1000.**

6.1.1 KBB Gel, pH 8.7

Note: 10X KBB buffer can be purchased from Sage Science, Part No. **KBB1001**.

	10X buffer g/liter	0.5X KBB running buffer (mM)
Tris (base)	124	51.18
TAPS (free acid)	140.16	28.806
EDTA (free acid)	0.48	0.082

0.5X KBB Buffer

Lonza SeaKem® GOLD	7.5 g	0.825 g
dH2O	950 ml	104.5 ml
10X KBB buffer	50 ml	5.5 ml
Total=	1 L	110 ml

0.75% Agarose

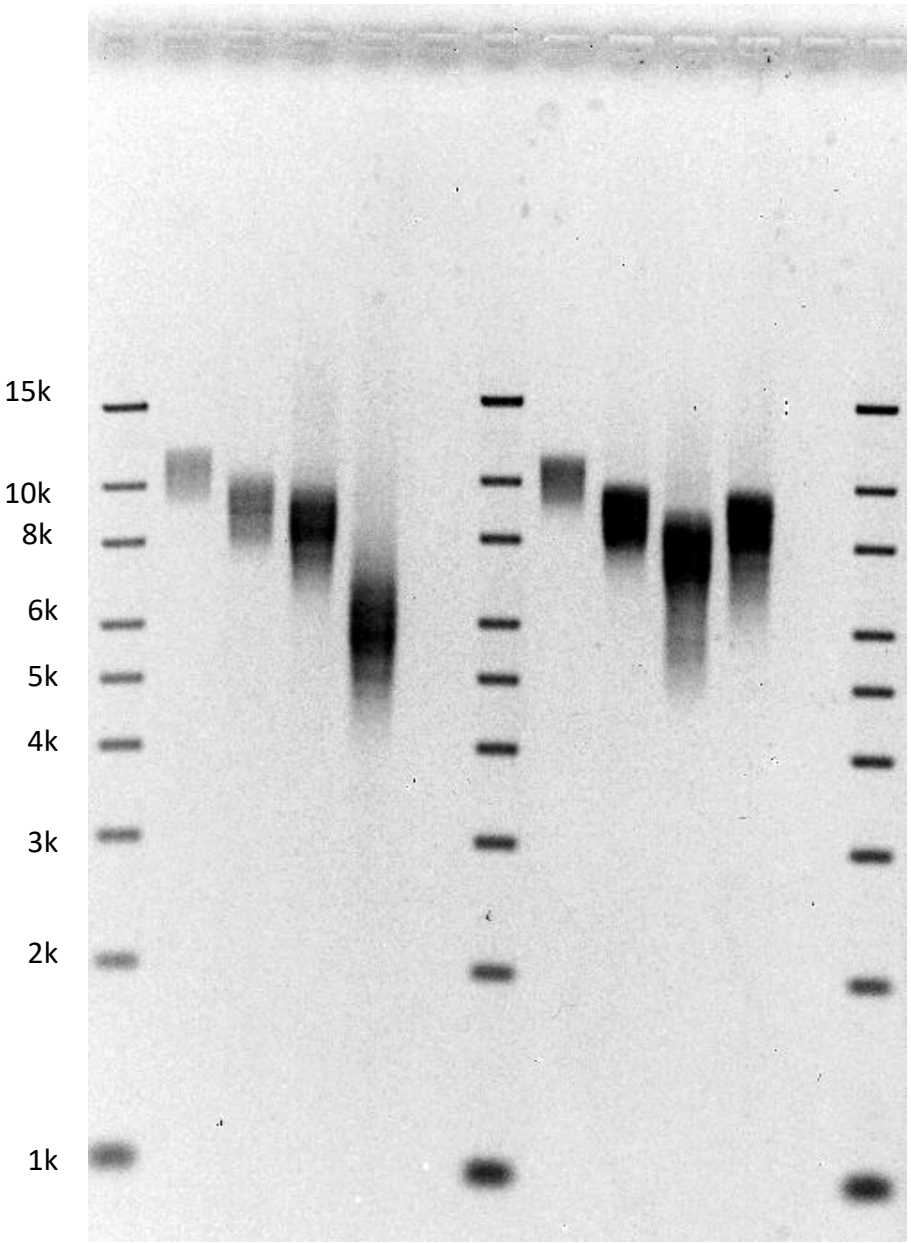
6.1.2 TBE Gel, pH 8.3

	10X buffer	0.5X TBE running buffer (mM)	
Tris (base)	108 g	44.5	
Boric Acid	55 g	44.5	
0.5M EDTA	40 ml	1	**0.5X TBE Buffer**
Total=	1 L		

Lonza SeaKem® GOLD	10 g	1.1 g	
dH2O	950 ml	104.5 ml	**1% Agarose**
10X TBE buffer	50 ml	5.5 ml	
Total=	1 L	110 ml	

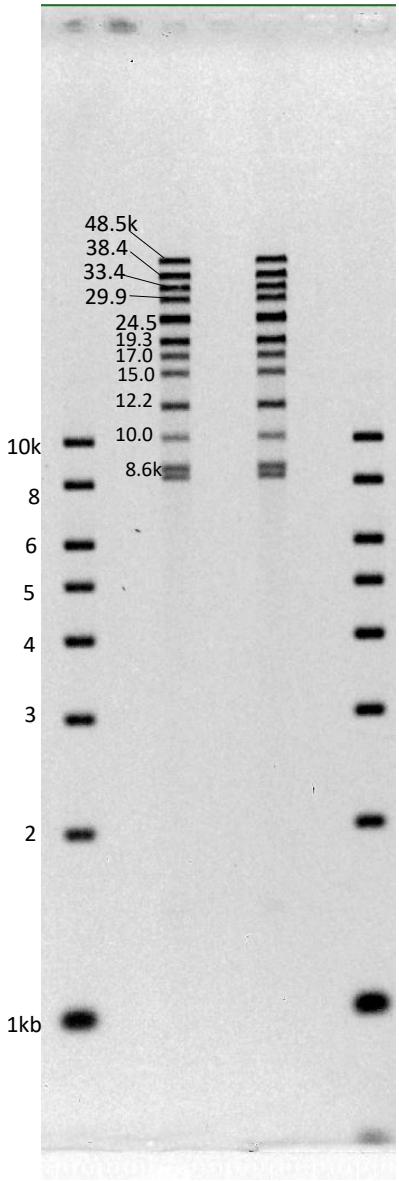
6.2 Pre-set protocol example 1, ".5-15kb"

Run time: 8 hours
Voltage: 100 V
Buffer: **0.5X KBB** (see page 7)
Agarose: **0.75%** Lonza SeaKem® GOLD, 110 ml



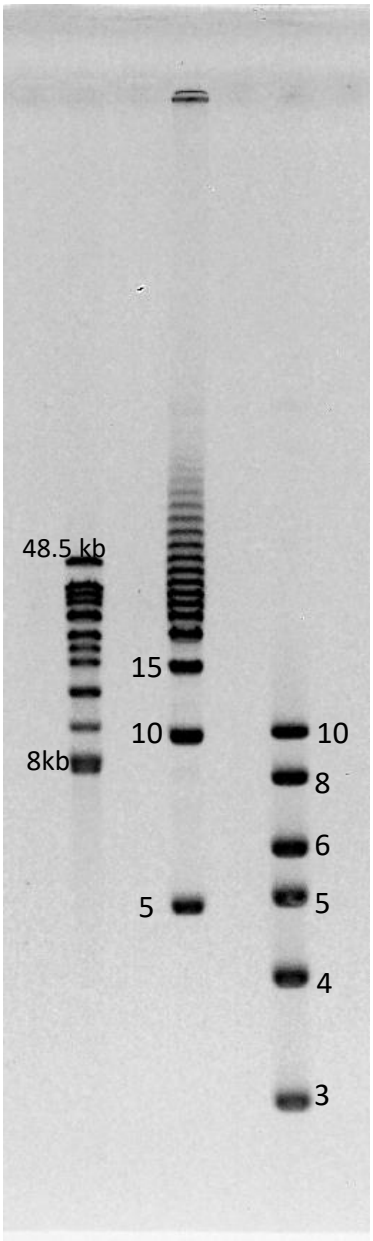
6.3 Pre-set protocol example 2, "10-48kb"

Run time: 9 hours
Voltage: 80 V
Buffer: **0.5X KBB** (see page 7)
Agarose: **0.75%** Lonza SeaKem® GOLD, 110 ml



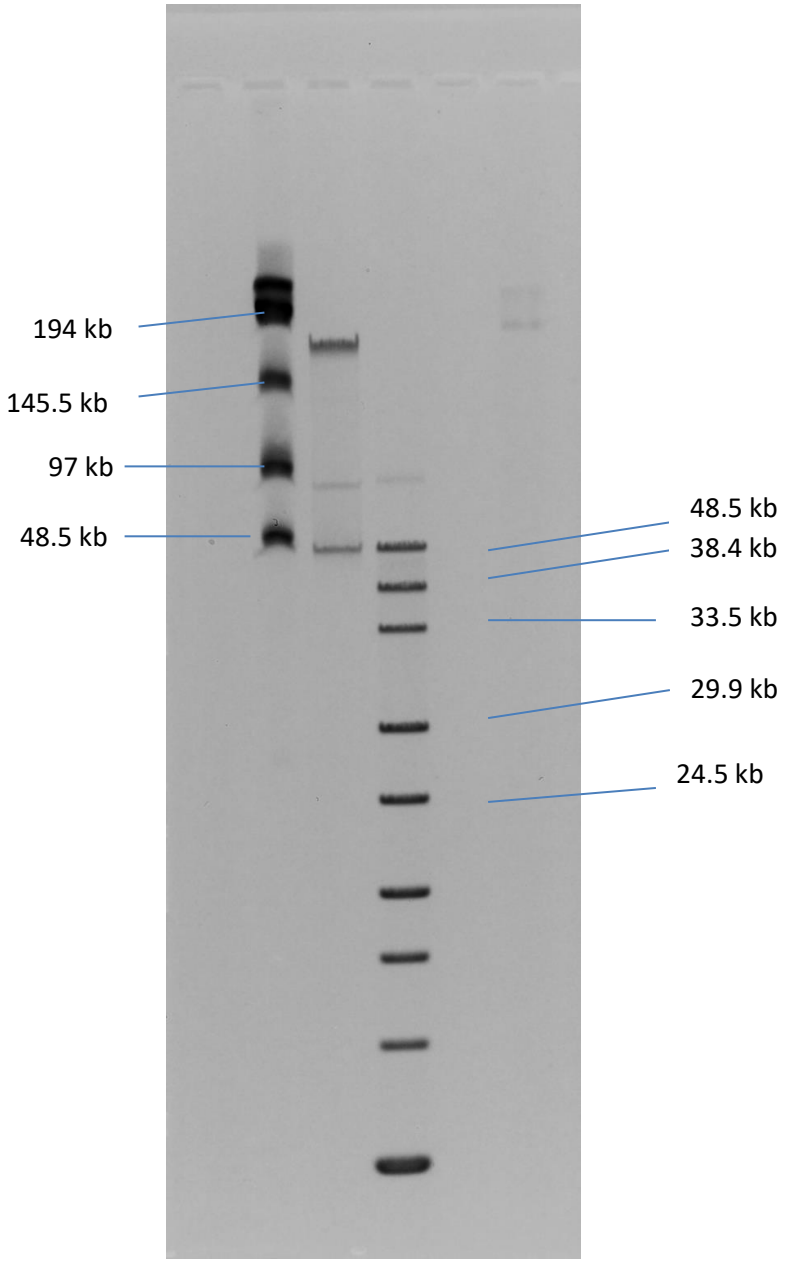
6.4 Pre-set protocol example 3, "5-80kb"

Run time: 14 hours
Voltage: 80 V
Buffer: **0.5X TBE** (see page 8)
Agarose: **1%** Lonza SeaKem® GOLD, 110 ml



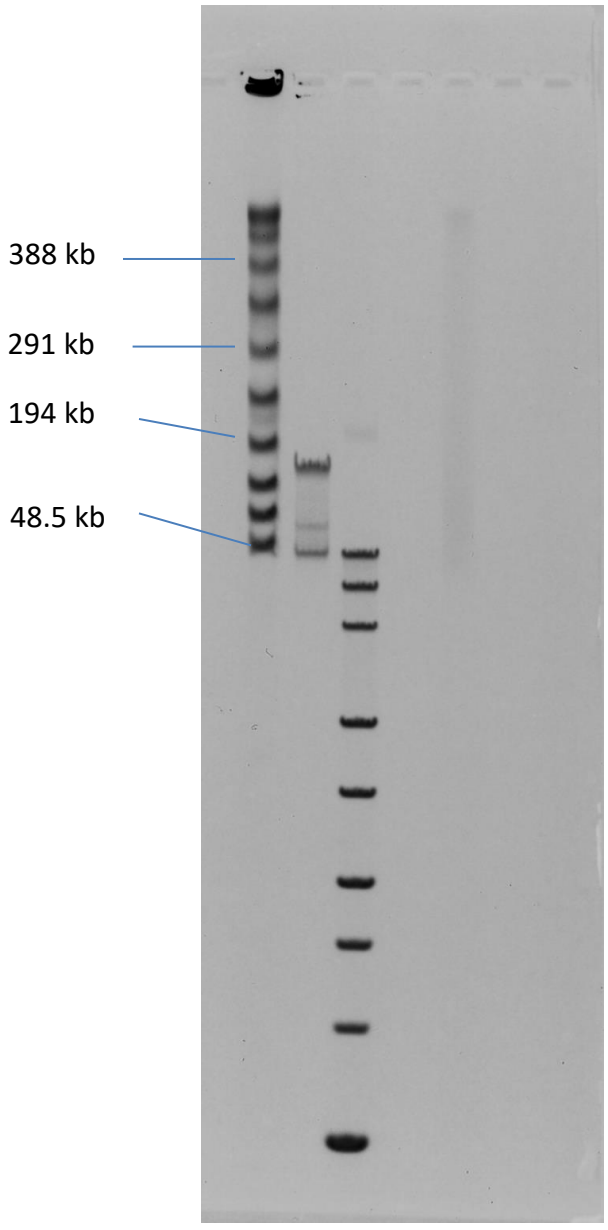
6.5 Pre-set protocol example 4, "5-150kb"

Run time: 16 hours
Voltage: 75 V
Buffer: **0.5X KBB** (see page 8)
Agarose: **0.75%** Lonza SeaKem® GOLD, 110 ml



6.6 Pre-set protocol example 5, "5-430kb"

Run time: 16 hours
Voltage: 75 V
Buffer: **0.5X KBB** (see page 8)
Agarose: **0.75%** Lonza SeaKem® GOLD, 110 ml

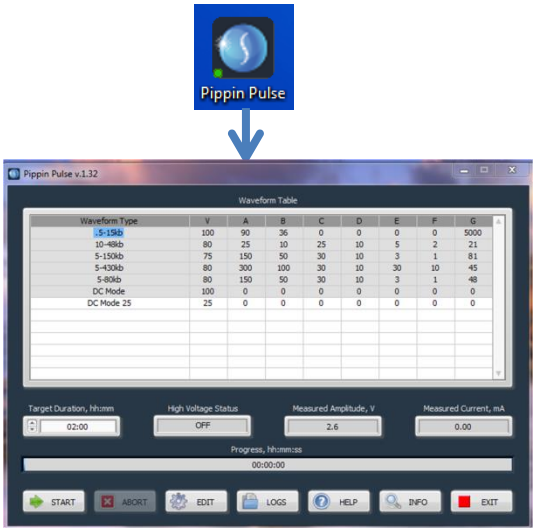


7.0 Running the Pippin Pulse

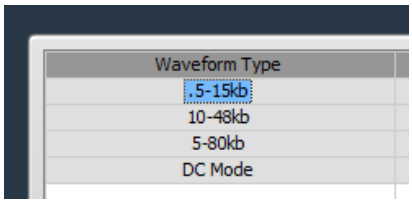
1. Set up the complete gel system, load the DNA samples, and connect the electrodes to the Pippin Pulse

2. Turn the Pippin Pulse on, by pressing the power button on the front panel.

3. Launch the Pippin Pulse application by double-clicking the Pippin Pulse Icon.

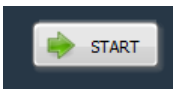


4. Using your mouse pointer, select the protocol, or "Waveform Type" name to be run. The selected protocol will be highlighted in blue.



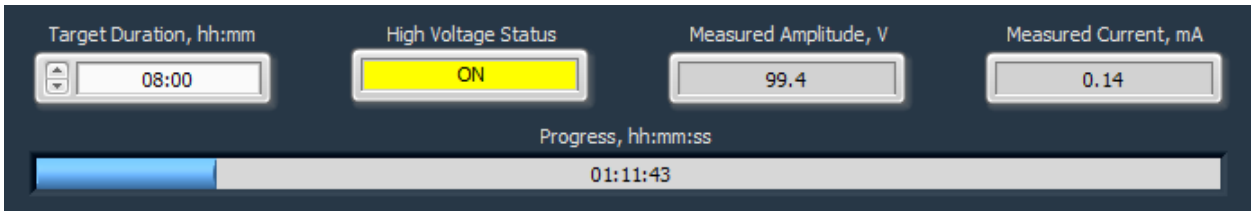
5. Enter a target duration for the run. This is very important; the default value is 2 hours.

6. Press "START"



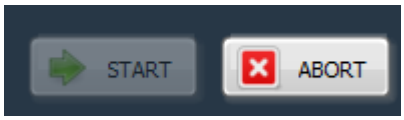
8.0 Monitoring a Pippin Pulse Run

1. When running, the Pippin Pulse High Voltage Status will indicate a yellow color.
2. The run voltage, current values, and run progress will be displayed.



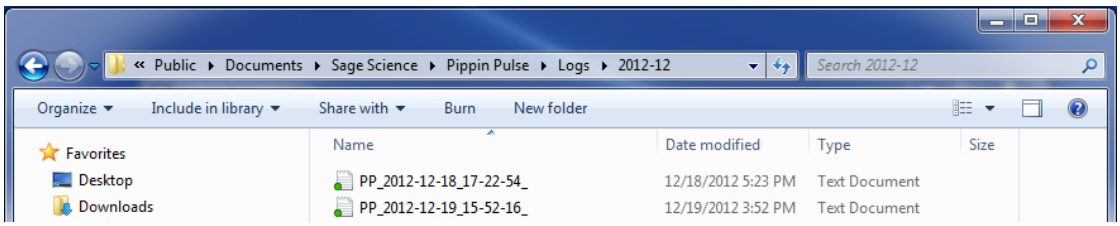
8.1 Ending a Run

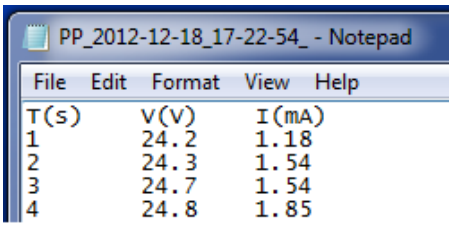
To abort a run before the target duration, press the "ABORT" button.



8.2 Log Files

A log file is saved after every run. The file name is saved as "PP_[year]-[month]-[day]_[hour]-[min]-[sec].txt". The files are saved in a sub-folder titled "Pippin Pulse" that is automatically placed in the computer's Documents folder during software installation. The file lists the measured voltage and current values at 1 second intervals for the entire run.





9.0 Programming the Pippin Pulse

9.1 What is a Pippin Pulse protocol?

A Pippin Pulse protocol is a series of parameters that control the run voltage, forward and reverse time steps, and number of steps per cycle. A more complete explanation of these parameters is described earlier in this manual (*About Pulsed Field Electrophoresis*, **Section 3**). The functions of the parameters are listed below, and appear on the Editing screen of the Pippin Pulse software application.

V. Voltage= Electrophoresis voltage, accept values between 25 -150 V.
A. Forward Time at start of run, 1 – 65,535 msec.
B. Reverse Time at start of run, 1 – 65,535 msec.
C. Increment added to A at each step, 0 – 255 msec.
D Increment added to B at each step, 0 – 255 msec.
E. Increment added to C at each step, 0 – 255 msec.
F. Increment added to D at each step, 0 – 255 msec.
G. Number of steps per cycle, 1 – 65,535.

9.2 Where to start

The table below may be used on a guideline to begin developing protocols for gel analysis of large DNA. These guidelines are based on 5-10 V/cm gels and 0.5X electrophoresis buffer (see Pre-set Protocols, **Section 6**), "Running the Pippin Pulse". The protocols that are highlighted in gray are pre-set in the Pippin Pulse software, the remaining protocols have not been tested, but are provided as a starting recommendation.

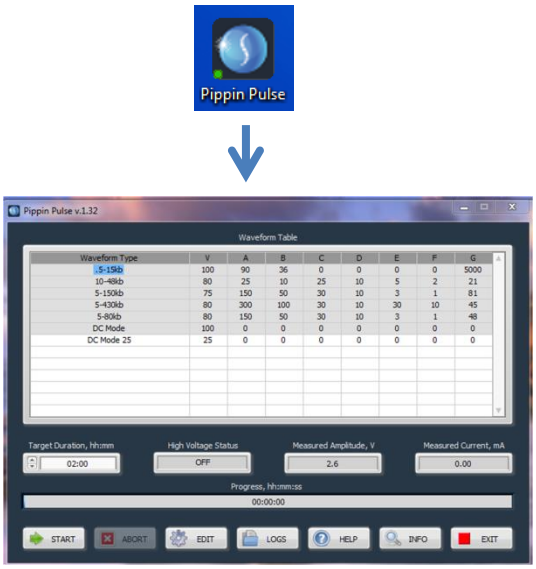
	Pippin Pulse Parameters							Approx. Resolved Size Range (kb)	Run Time
	A	**B**	**C**	**D**	**E**	**F**	**G**		
1	90	36	0	0	0	0	5000	.5 -15	8 hr
2	50	10	50	10	0	0	20	1-30	8 hr
3	25	10	25	10	10	4	9	1-30	16 hr
4	25	10	25	10	5	2	21	10-48	16 hr
5	150	50	30	10	3	1	48	5-80	16 hr
6	150	50	30	10	3	1	81	5-150	16 hr
7	300	100	30	10	30	10	45	5-430	16 hr
8	300	100	300	100	0	0	100	20-800	20 hr+
9	300	100	0	0	60	20	51	40-1000	20 hr+
10	300	100	300	100	0	0	250	40-2000	20 hr+

Fig 4. Pre-set protocols (highlighted in gray) have been validated by Sage Science.
Other protocols are suggested starting protocols for running gels between 5 – 10 V/cm.

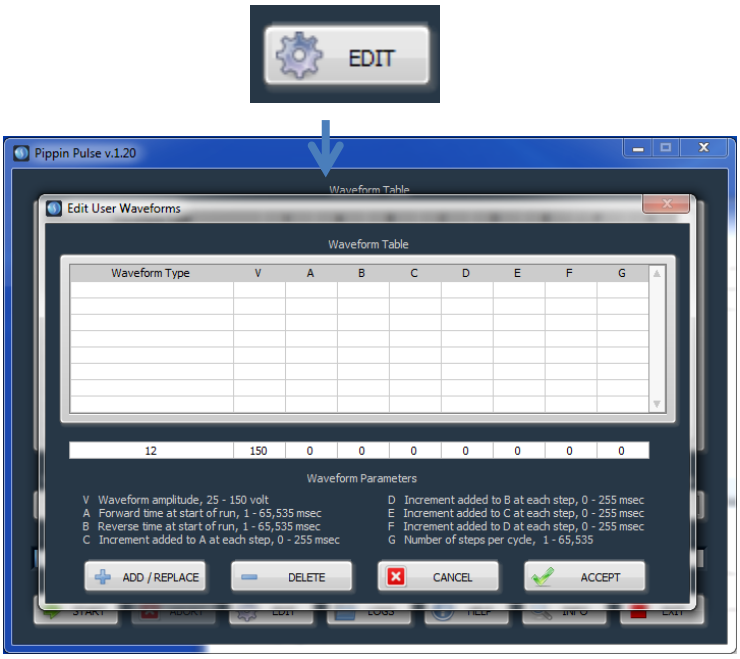
9.3 Programming the Pippin Pulse

1. Turn the Pippin Pulse on, by pressing the power button on the back panel.

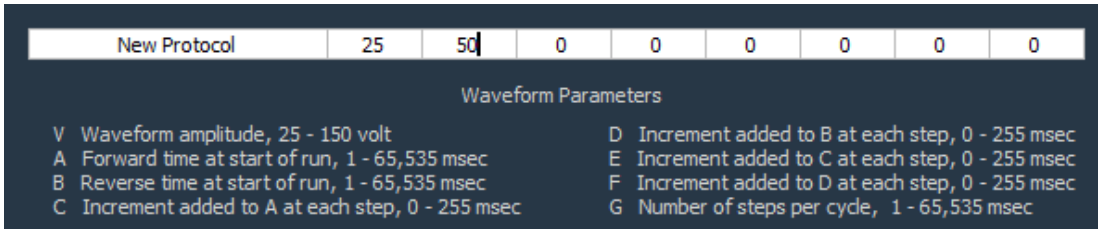
2. Launch the Pippin Pulse application by double-clicking the Pippin Pulse Icon.



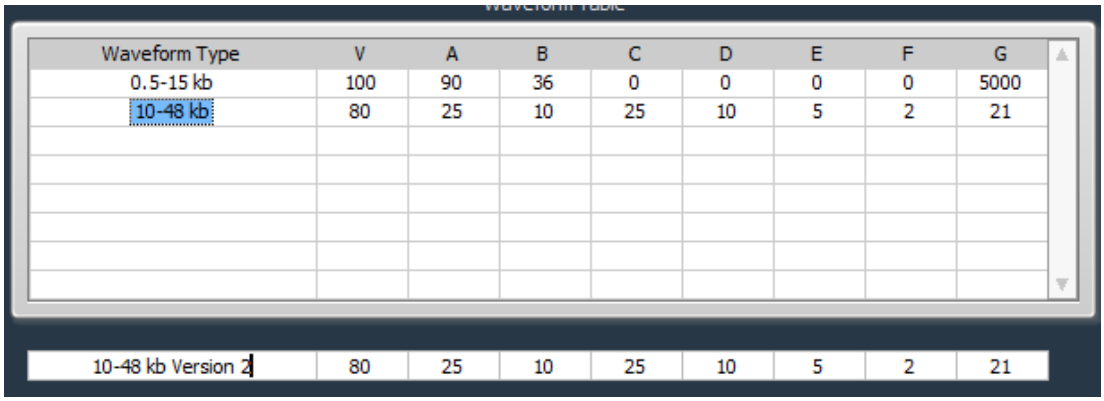
3. Press the “EDIT Button”. The editing screen will appear over the Main Screen.



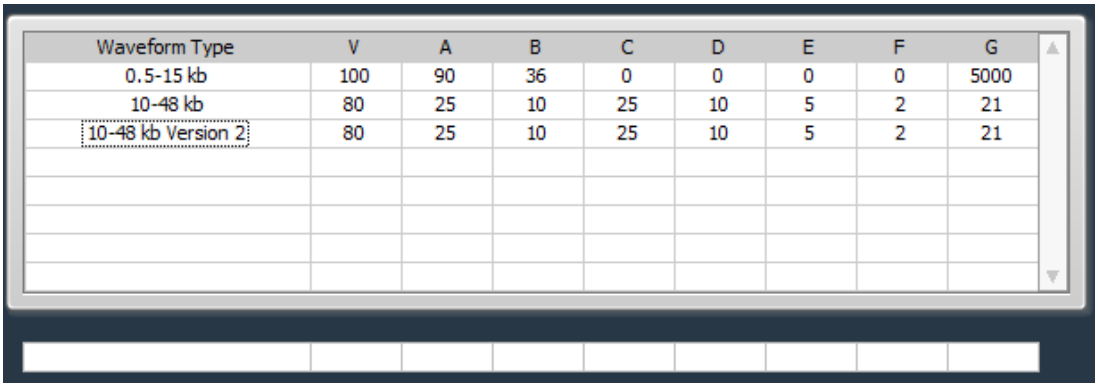
4. Place the mouse cursor in the Editing Field. Enter a name for the protocol (under "Waveform Type"), and add the values for each parameter.



5. If a previously written protocol is on the protocol list, clicking on the protocol will make it appear in the editing field and it may be modified.



6. Press "ADD/REPLACE" to add the new protocol to the list. If the same name appears in the "Waveform Type" field, the protocol will be replaced with the newer one.



7. Press "ACCEPT" to save the new list and return to the Main screen.

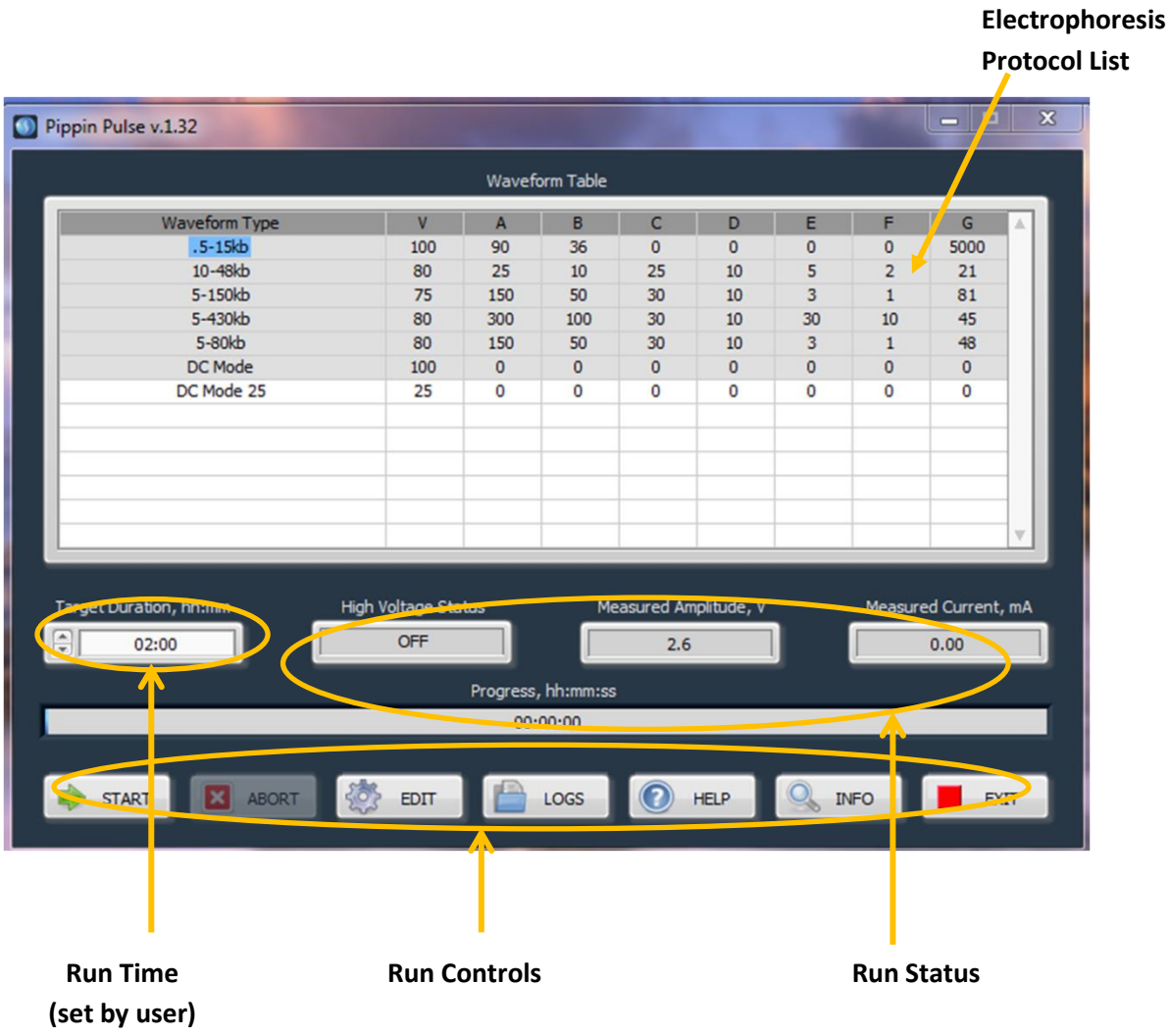
10.0 Software Overview

There are two screens in the Pippin Pulse software:

- The **Main Screen** lists the electrophoresis protocols, provides run controls, and displays the run status.
- The **Edit Screen** allows the programming, editing, and deleting of electrophoresis protocols

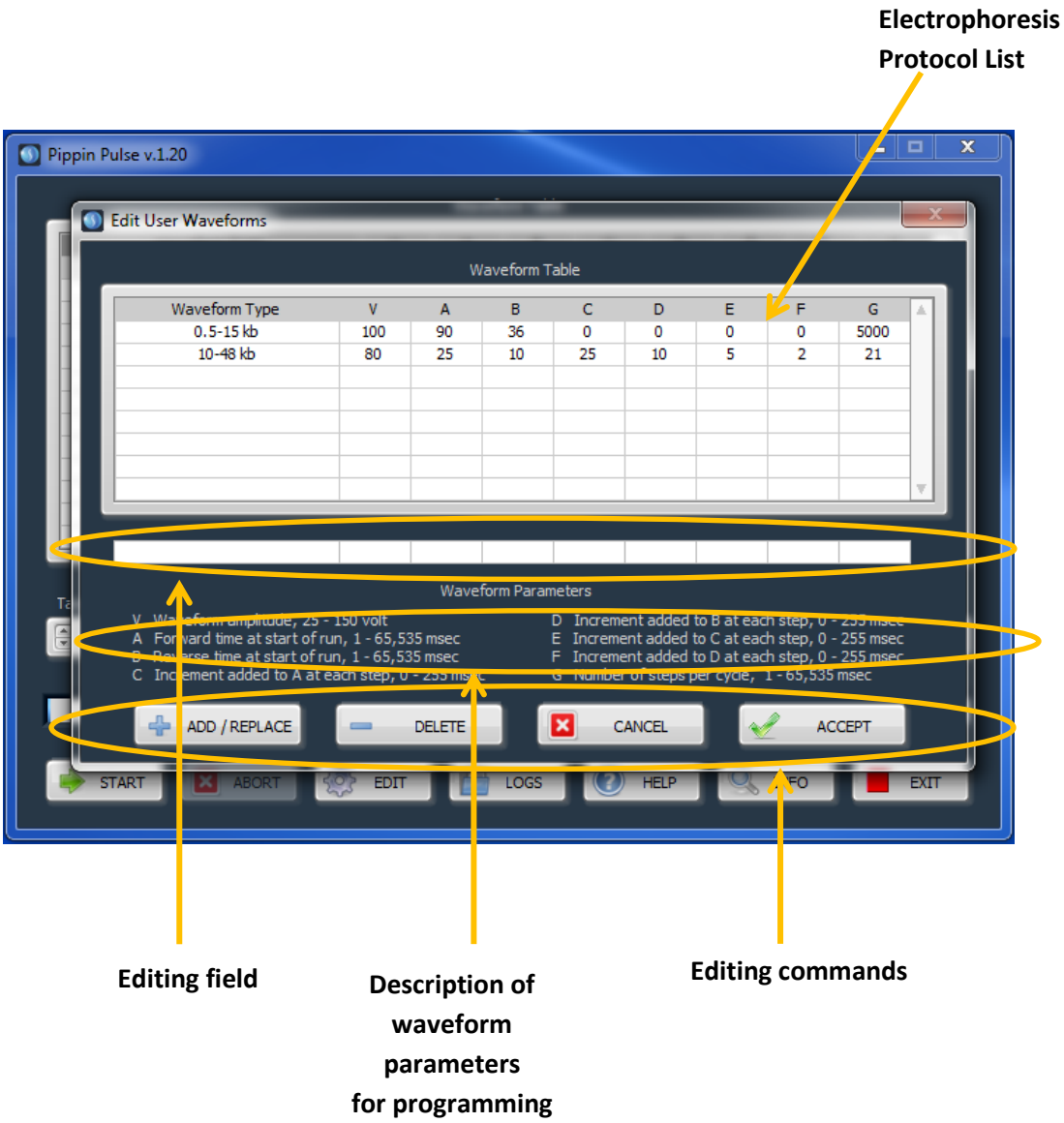
10.1 The Main Screen

When the Pippin Pulse application has been started, the following screen will launch:



10.2 The Edit Screen

The Edit Screen is accessed by pressing the “EDIT” button, and will appear over the Main Screen.



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