sage science

Operations Manual

MegaPulse™

Field-Inversion and Zero-Integrated-Field Electrophoresis

Electrophoresis Power Supply

MegaPulse Power Supply Part #: **MGP0200**

Sage Pulse Midi Gel Box Part #: **PGB1000**

Sage Science Inc.
Suite 2400
500 Cummings Center
Beverly, MA. 01915

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.

Table of Contents

1.0 Safety and Precautions

1.1 Safety Warnings and Icons Used

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

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

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

1.2 Safe Use Guidelines

Environmental Conditions

The MegaPulse system (MegaPulse power supply, agarose electrophoresis gel box, and PC controller) is designed to operate under the following environmental conditions:

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

Laboratory Precautions

Standard laboratory precautions should be taken when using the MegaPulse System:

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

Electrical Shock

The MegaPulse produces up to 150 V output at 100 mA. The Sage Science gel box is supplied with shielded banana plugs that cannot be removed from the gel box cover; use caution when using other gel box products. Do not connect or disconnect the banana plugs while the High Voltage (HV) indicator light is on. Do not operate the system in a cold room. Use only properly grounded outlets.

Caution!

Use the MegaPulse system only for its intended use and in accordance with this manual. Any use outside these instructions may result in equipment damage and/or personal harm.

1.3 Instrument Specifications

MegaPulse Power Input

- AC input to External Power Supply: 100-240 VAC, 2A, 50-60Hz
- External Power Supply DC output to MegaPulse: 24 VDC, 5A

MegaPulse Power Output

- Power is converted to +/-150 VDC at 100 mA. Useful working range is 20-150V DC
- Operates Sage Science one or two gel electrophoresis boxes (Sage PN# PGB1000) at a maximum of 50mA (per box)

MegaPulse Pulsed-Field Capabilities

- Voltage polarity is switchable for timed periods (pulses)
- Pulses may have durations between 10 milliseconds and 18,000 seconds.
- Pulse durations may increase time increments per pulse/step (i.e., 1 millisecond per pulse/step).
- Voltage amplitude may be altered coordinately with direction (i.e., has one voltage value in one pulse direction, and second voltage value in the reverse direction).
- The system includes pre-set validated electrophoresis protocols and new protocols may be programmed by users.

MegaPulse Physical Dimensions

- Size: 10H x 25W x 25D cm (4H x 10W x 10D in.)
- Weight: 2.45 kg (5.41 lbs)

Sage Science Gel Box Dimensions

- Size: 12 x 14 cm (4.7 x 5.5 in.)
- Weight: 0.883 kg (1.95 lbs)

1.4 Cleaning the Instrument

The exterior surface of the MegaPulse can be cleaned with a moist lab tissue. A 70% ethanol solution from a squeeze bottle can also be used. There is no user-accessible interior; cleaning should be limited to the external surface of the unit only.

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

2.0 About the MegaPulse

2.1 System Overview

Thank you for purchasing the MegaPulse electrophoresis power supply. A Windows PC running MegaPulse software is required to operate the system. The MegaPulse must be used with a Sage Science Midi Gel Box, which is equipped with reinforced, large-diameter platinum electrodes not found on other gel boxes and designed to withstand the demands of pulsed-field electrophoresis. The gel box is a standard midi size (12 × 14 cm) and includes both a 12-well and a 20-well comb. The wells are 1.5 mm thick with a maximum height of 12 mm. The 20-well comb has a well width of 4 mm, and the 12-well comb has a well width of 8 mm. The MegaPulse electrophoresis system is designed to resolve DNA fragments from 15 kb up to 1.6 Mb using standard agarose gel visualization methods. The MegaPulse can supply power to one or two gel boxes simultaneously, provided the same electrophoresis protocol is used.

2.2 Pulsed-Field Electrophoresis

Pulsed-field gel electrophoresis is a strategy for resolving large fragments of DNA for analysis. When running a typical direct-current (DC) agarose gel, fragments above approximately 30-50kb (depending on gel conditions) will migrate with the same mobility. Pulsed-field electrophoresis uses periodic changes in the electric field to enable resolution of larger DNA molecules. The MegaPulse supports two types of pulsed-field electrophoresis: field-inversion gel electrophoresis (FIGE) and zero-integrated-field electrophoresis (ZIFE).

In most FIGE programs, the duration of the forward field pulse is 2-3 times longer than the reverse pulse duration. During each reversal, the DNA fragments undergo brief periods of realignment with the new field direction, in which they have negligible movement. These realignment periods take longer for larger DNA molecules, resulting in lower overall mobility for larger fragments relative to smaller

fragments due to the increased realignment time.

Typical FIGE pulsed-field protocols use short pulses (milliseconds) and apply increasing/decreasing time increments in repeating cycles while maintaining a constant voltage. Using FIGE pulsed-field electrophoresis with the MegaPulse can resolve DNA molecules up to ~450 kb in size.

2.3 Zero-Integrated-Field Electrophoresis

In a typical FIGE pulsed-field workflow, DNA fragments over 450 kb cannot be separated, and instead run as an unresolved, low mobility "compression band". To resolve higher DNA sizes, the MegaPulse uses Zero-Integrated-Field Electrophoresis (ZIFE). ZIFE is an advanced application of pulsed-field electrophoresis that switches the magnitude, direction, and pulse time of the field simultaneously, such that the sum of the integrated applied field in each forward-reverse pulse cycle is equal to zero. In practice, our programs are not truly zero-integrated-field, but are slightly biased toward the forward direction. Under these near-zero-integrated-field conditions, larger molecules exhibit more complex realignment kinetics, allowing molecules 100s to 1000s of kb in size to be resolved.

Our near-ZIFE protocols typically use longer time increments (10's to 1000's of seconds) and usually require long runtimes, (~20-24 hours). The ZIFE protocols included in our preset programs work best at laboratory room temperatures between 17-23°C.

Our ZIFE protocols are based on those described by Jaan Noolandi and Chantal Turmel (Preparation, Manipulation, and Pulse Strategy for One-Dimensional Pulsed-Field Gel Electrophoresis (ODPFGE). *Methods in Molecular Biology*, Vol. 12: *Pulsed-Field Gel Electrophoresis*, 1992.

Additional details on waveform programming, waveform chains, and advanced protocol programming are provided in Section **7.0, Advanced Programming.**

3.0 Unpacking

3.1 The MegaPulse Electrophoresis Power Supply

The MegaPulse (MGP0200) will arrive with the following components:

- MegaPulse Unit
- External Power Supply for the MegaPulse
- USB-C cable (to connect PC to a MegaPulse)
- USB storage device (contains the MegaPulse programming software)
- User manual (this document)

3.2 Sage Pulse Midi Gel Box Assembly (Optional)

The Midi Gel Box Assembly (PGB1000) will arrive with the following components:

- Gel Box
- Gel Tray
- Gel Box cover with banana plug wires
- 12 well casting comb
- 20 well casting comb

3.3 Sage Supplied Mini PC (Optional)

- Mini PC tablet with touchscreen (MegaPulse software will be pre-loaded).
- Foldable 360 rotating tablet stand
- Power Supply

4.0 Installation

4.1 Installation on Sage supplied mini-PC tablet

1. Remove the MegaPulse from its packaging.
2. Connect the power supply to the unit, and plug the power cable into an electrical outlet.
3. Insert the USB-C cable into the USB-C port found in the back of the unit.
4. Insert the other end of the USB-C cable into a USB port of a PC.
5. Press the power switch located on the front of the MegaPulse
6. The following icon will appear on the mini PC:

7. Double click on the icon to launch the application

4.2 Installation on non-Sage purchased PC

1. Insert the USB storage device into an available port on a PC
2. On the computer, go to the USB device (Computer/Removable Disk (E:))
3. Open the MegaPulse folder
4. Double click the “setup” executable file
5. Follow the installer instructions
6. After installation is complete, re-boot the computer

4.3 MegaPulse Front and Rear Panels

Figure 4.4 on the next page shows the rear panel of the MegaPulse

Figure 4.5 on the next page shows the front panel of the MegaPulse

Figure 4.4 Rear Panel

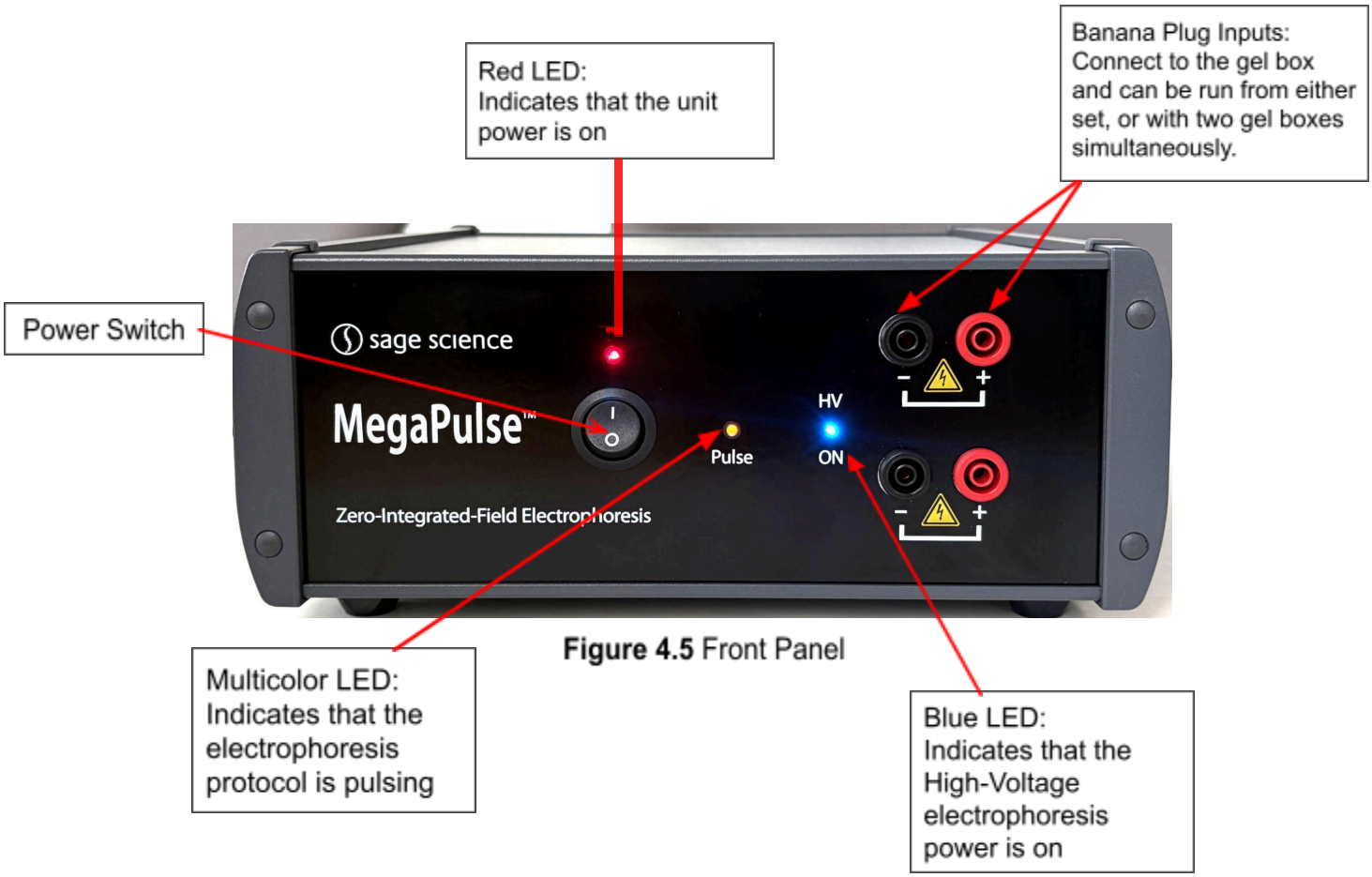


Figure 4.5 Front Panel

5.0 Using the MegaPulse

5.1 Before you Begin

To use a MegaPulse Assembly the following are required:

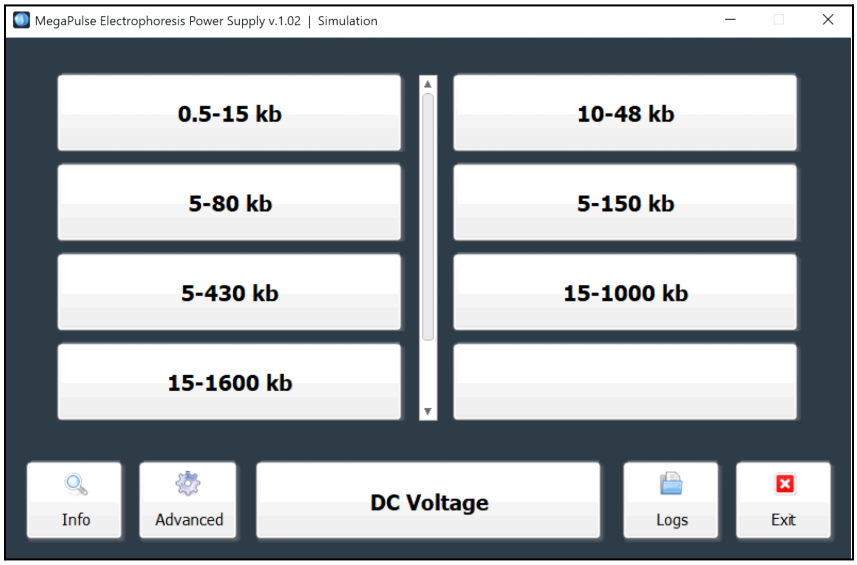
- MegaPulse Electrophoresis Power Supply
- Windows PC
- Sage Midi Gel Box (MegaPulse can run one or two Midi gel boxes using the same program for both)
- Gel cast using Lonza SeaKem GOLD agarose and Sage Science 0.5X KBB running Buffer (0.75% agarose gels recommended for DNA sizes up to 430kb, 1% agarose gels required for DNA sizes >430kb).
- Densifying agent for loading DNA and tracking dye (optional)
- Intercalating fluorescent dye for post-run gel staining/imaging

NOTE: Unless otherwise noted, all MegaPulse gels described in this manual were validated with the system as described. 10X KBB buffer can be purchased from Sage Science (PN KBB1001)

5.2 MegaPulse Software Overview

On the PC desktop, double-click on the MegaPulse icon to open the application. The MegaPulse software Main Screen opens and displays a list of seven pre-set protocols shown below.

MegaPulse Main Screen



Each protocol button is labeled with the target DNA fragment size range (kb) expected to be resolved at

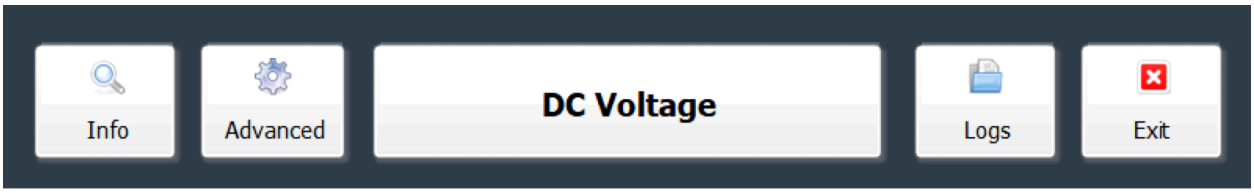
the end of the electrophoresis run for those protocols.

Summary of Pre-set Protocols

Separation Range (Kb)	Run Time (hh:mm)	Protocol Type
0.5-15	8:00	FIGE
10-48	9:00	FIGE
5-80	14:00	FIGE
5-150	16:00	FIGE
5-430	16:00	FIGE
15-1000	19:25	ZIFE
15-1600	24:27	ZIFE

NOTE: For the first five pre-set protocols (FIGE protocols), the run time may be adjusted by the user from the Main Screen. The 15–1000 kb and 15–1600 kb pre-set protocols (ZIFE protocols) are composed of multiple linked waveforms, and their run times are fixed and cannot be adjusted by the user.

In addition to the pre-set protocols, the following buttons are also found on the Main Screen:



Main Screen Buttons

Info: Opens an informational screen that displays the software revision number and other reference material (click within the window area to close the screen).

Advanced: Opens an interface where users can program custom waveforms and waveform chains (press “Main” button to return to the Main Screen).

DC Voltage: Opens the setup screen for Direct Current Electrophoresis runs (press “Main” button to return to the Main Screen).

Logs: Opens the PC folder where run log files are saved.

Exit: Closes the application.

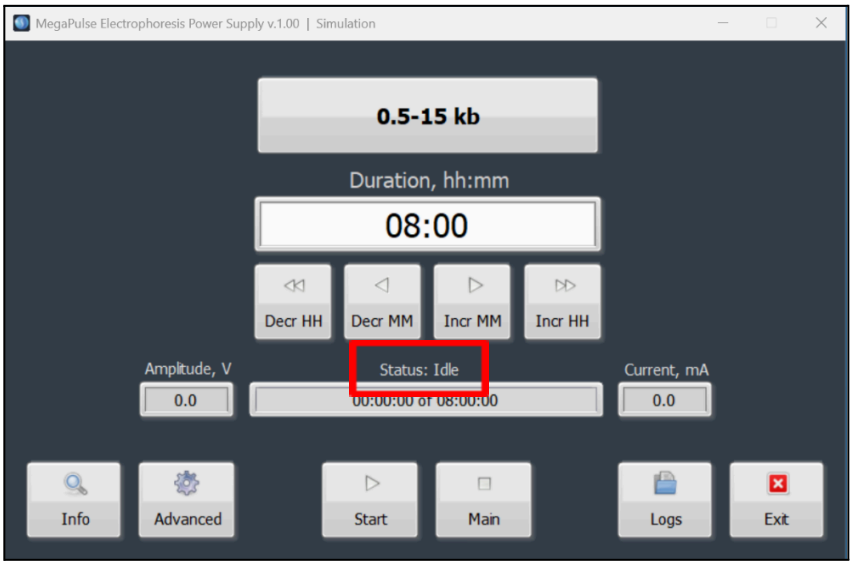
Instructions for running pre-set protocols and direct current electrophoresis protocols are provided in Sections 5.3 and 5.4.

5.3 Running a Pre-set Protocol

To run a pre-set protocol, follow the steps below from the Main Screen.

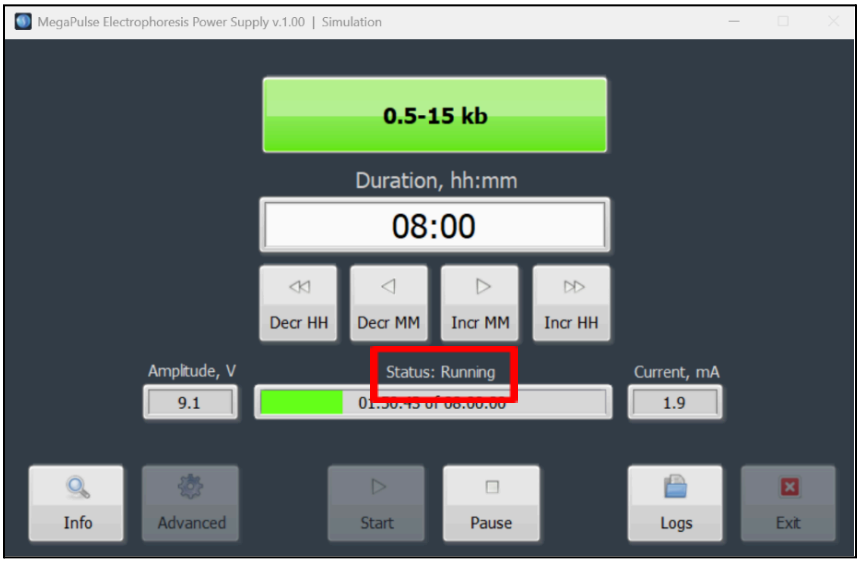
1. Prepare the agarose gel(s) and load samples into the wells.
2. Verify that the electrophoresis leads are connected to the MegaPulse with the correct polarity and orientation (red wire in the red input jack and black wire in the black input jack). One or two gels may run using the same protocol.
3. Select the protocol corresponding to the DNA fragment size range to be analyzed (0.5-15 kb for the instance shown below). This opens the STATUS: IDLE window. The required run time is displayed as hh:mm.

NOTE: For the first five pre-set protocols (FIGE protocols), the run time may be adjusted by the user from the Main Screen. The 15–1000 kb and 15–1600 kb pre-set protocols (ZIFE protocols) are composed of multiple linked waveforms, and their run times are fixed and cannot be adjusted by the user.



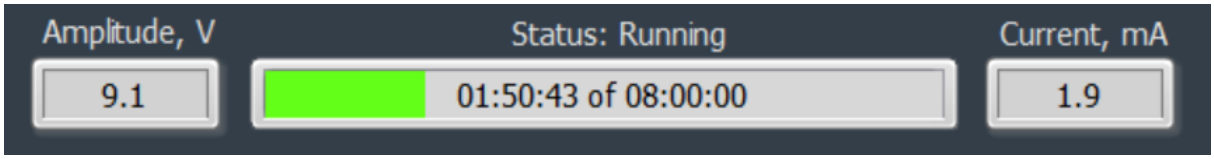
4. (Optional) The run time may be adjusted by the user by using Increment and decrement buttons. The run time can be adjusted +/- 1 minute or +/- 1 hour:

5. Press the “Start” button to initiate the run. The screen will change to STATUS: RUNNING. To indicate Running status, the protocol range label and elapsed time display will turn green.



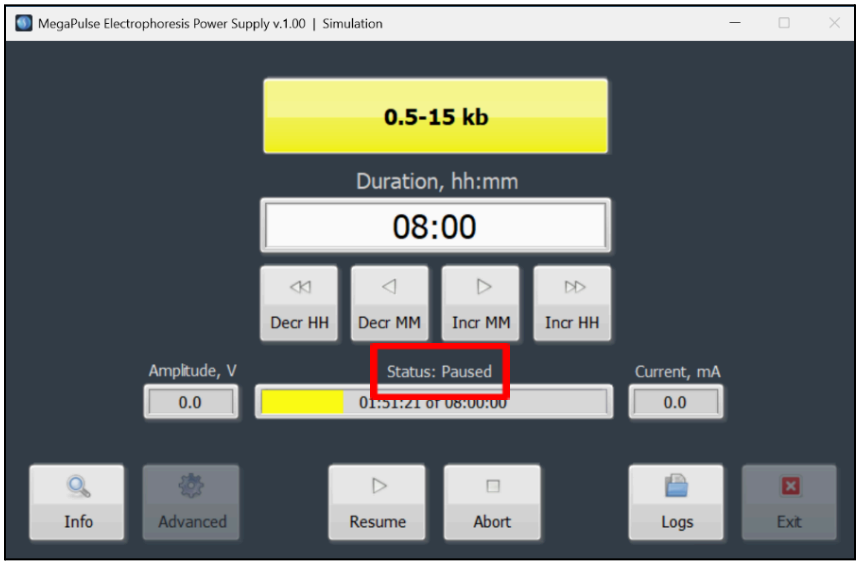
High Voltage! Starting a run will apply a voltage and potentially dangerous high voltage across the gel apparatus and across the electrode wires. Users should be aware, and use the product only as directed.

During the run, the display fields will indicate the Voltage (left) and Current (Right), and the elapsed time (hh:mm:ss). The green progress bar provides a visual indication of the run progress.

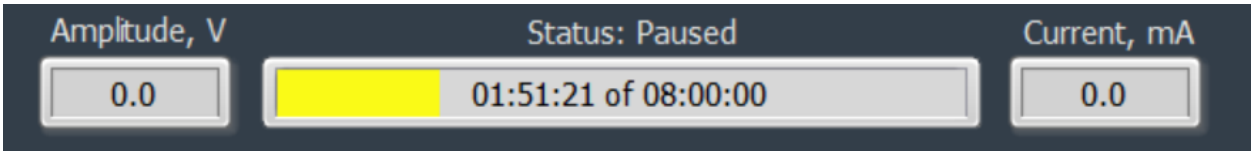


Note: The run may be paused from the Running screen. Pressing “Pause” pauses the run and disables the electrophoresis voltage. The run may be resumed or aborted from the Paused screen.

6. Press **"Pause"** to temporarily stop the run. The screen will change to **STATUS: PAUSED**. In the Paused screen, the protocol range label and elapsed time display turn yellow.



While paused, voltage and current are not applied and both display fields will read **0.0**. The yellow progress bar and elapsed time display indicate the point at which the run was paused relative to the total run time.

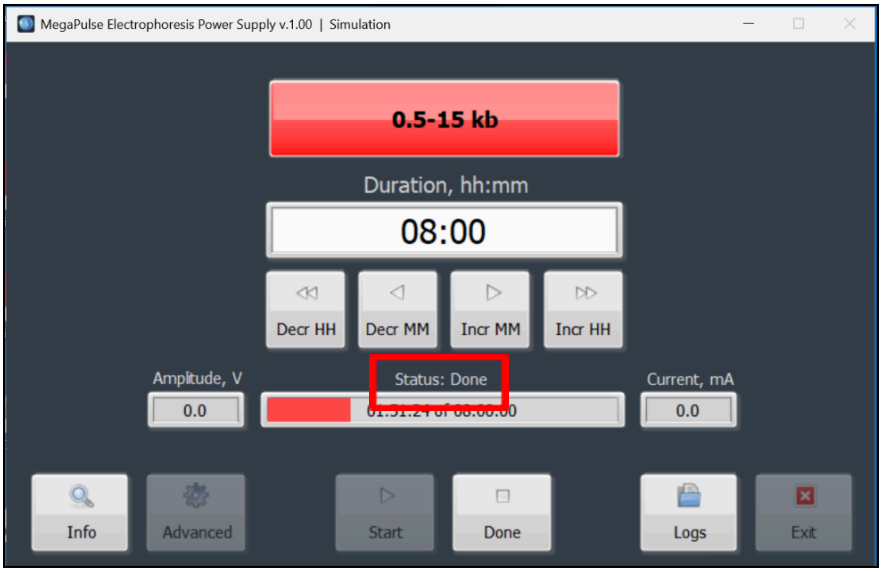


7. Press **"Resume"** to return to the Running screen. The electrophoresis run resumes from the point at which it was paused.

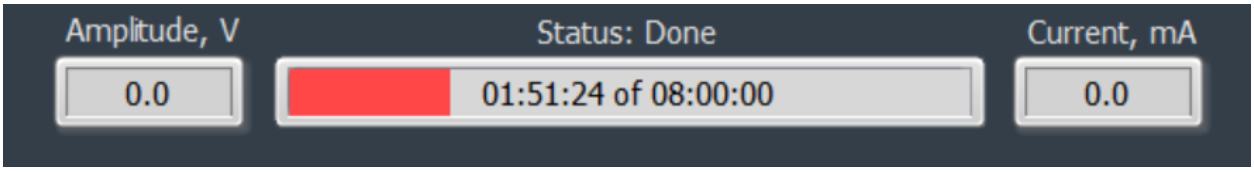
Note: Run time may be added or subtracted from the total run time from either the Running screen or the Paused screen using the increment and decrement buttons (MM and HH).

8. Press **"Abort"** to stop the run before completion. The screen will change to **STATUS: DONE**. In the Done screen, the protocol range label and elapsed time display will turn red.

Important! Aborting a run ends the run immediately and cannot be reversed.



After the run is aborted, voltage and current are no longer applied and both display fields will read **0.0**. The red progress bar and elapsed time display indicate the point at which the run was aborted relative to the total run time.



Note: The run also automatically enters STATUS: DONE when it reaches the pre-set run time.

9. Press **"Done"** to return to the **Idle** status screen. A new run with the same settings may be started from this screen.

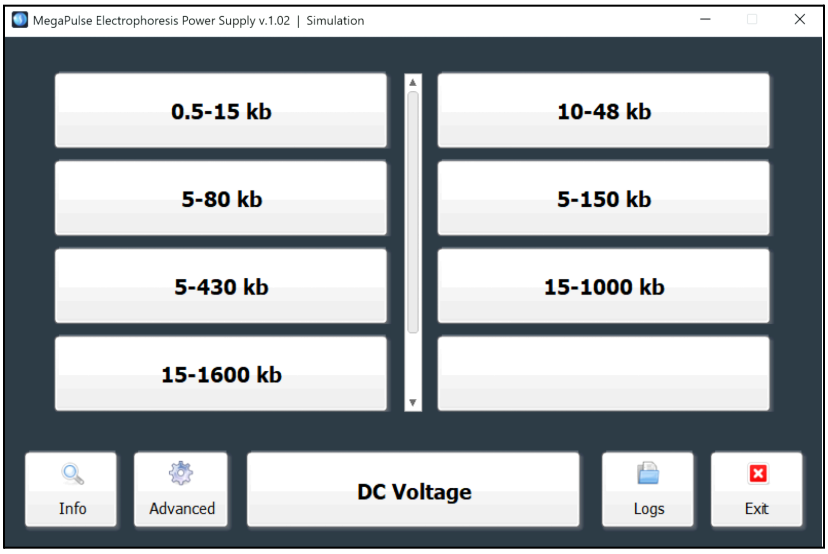
10. Press **"Main"** to return to the Main Screen.

5.4 Running a Direct Current Electrophoresis Protocol

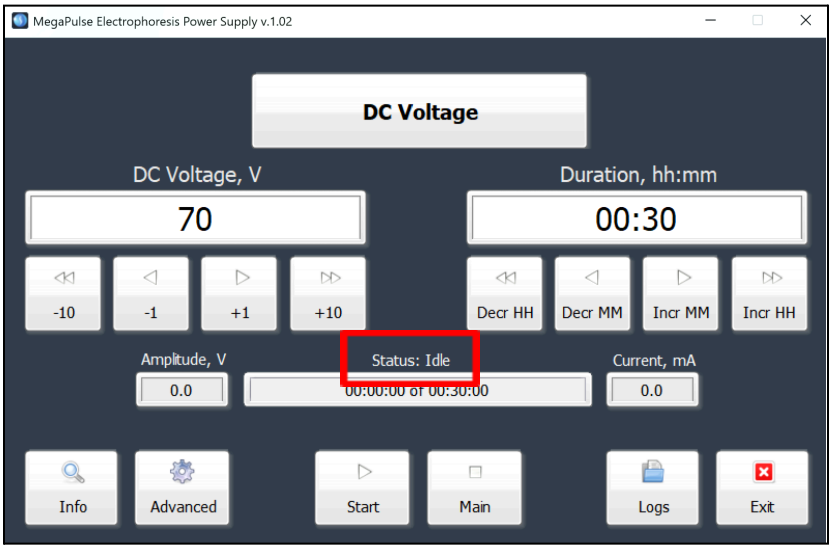
1. Prepare the agarose gel(s) and load samples into the wells.
2. Verify that the electrophoresis leads are connected to the MegaPulse with the correct polarity:

- **Red lead → red input jack**
- **Black lead → black input jack**

3. From the MAIN SCREEN, press the **"DC Voltage"** button.

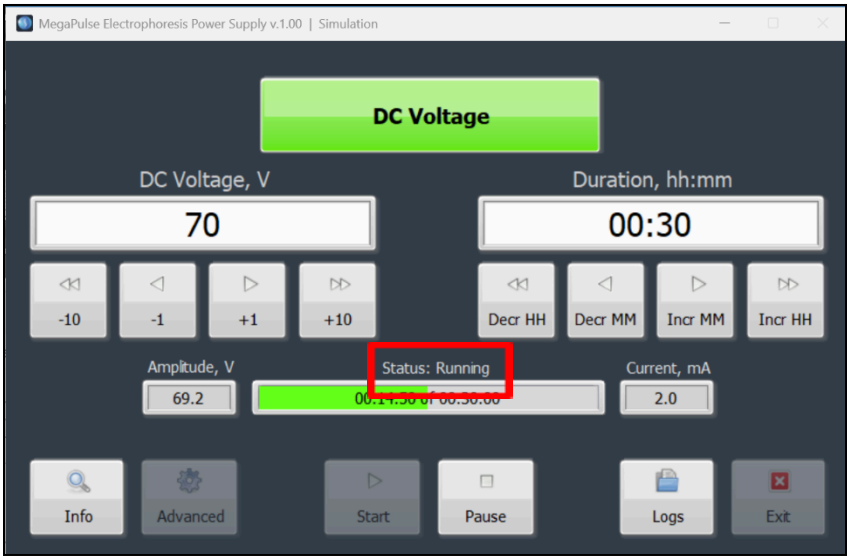


4. Set the desired **DC Voltage (V)** and **Duration (hh:mm)** using the increment and decrement buttons.



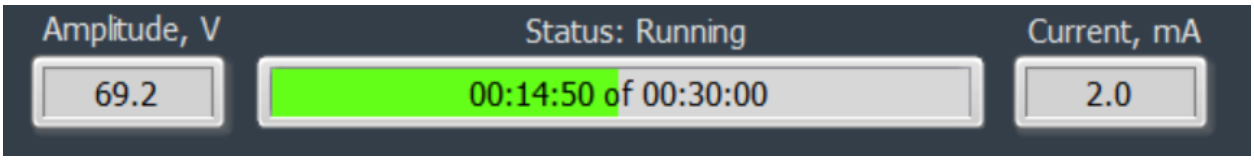
Important! The MegaPulse allows negative voltage values for non-standard applications. For DNA electrophoresis, the voltage should always be set to a positive value.

5. Press **"Start"** to initiate the run. The screen will change to **STATUS: RUNNING**. In the Running screen, the protocol label and elapsed time display turn green.



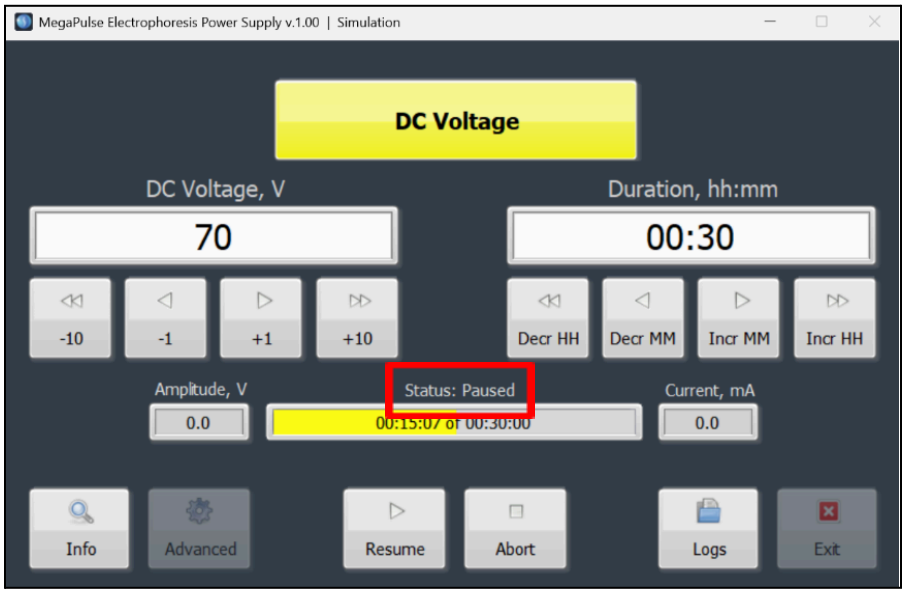
High Voltage! Starting a run applies voltage and potentially dangerous high voltage across the gel apparatus and electrode wires. Use caution, and do not disconnect the leads while the run is in progress.

During the run, the display fields show **Voltage** (left), **Current** (right), and **elapsed time** (hh:mm:ss). The green progress bar provides a visual indication of run progress.

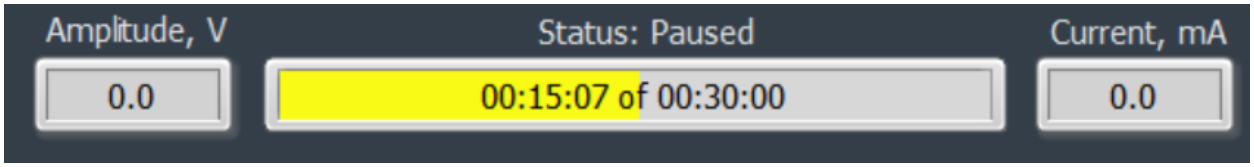


Note: The run cannot be stopped from the Running screen. Pressing "Pause" pauses the run and disables the electrophoresis voltage. From the Paused screen, the run may be resumed or aborted.

6. Press **"Pause"** to temporarily stop the run. The screen will change to **STATUS: PAUSED**. In the Paused screen, the protocol label and elapsed time display turn yellow.



While paused, voltage and current are not applied and both display fields will read **0.0**. The yellow progress bar and elapsed time display indicate the point at which the run was paused relative to the total run time.

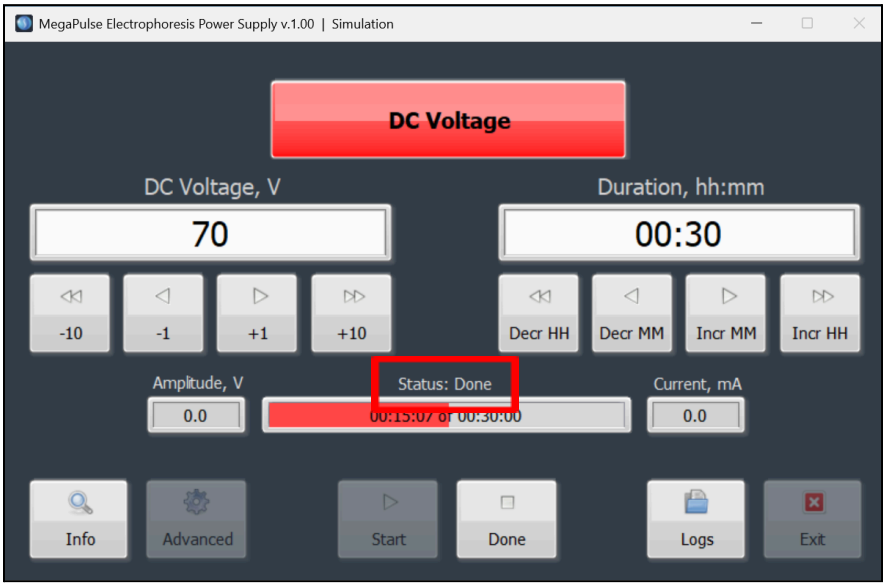


7. Press **"Resume"** to return to the Running screen. The electrophoresis run resumes from the point at which it was paused.

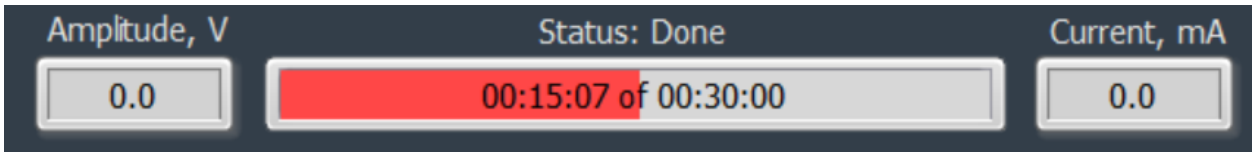
Note: Run time may be added or subtracted from the total run time from either the Running screen or the Paused screen using the increment and decrement buttons (MM and HH).

8. Press **"Abort"** to stop the run before completion. The screen will change to **STATUS: DONE**. In the Done screen, the protocol label and elapsed time display turn red.

Important! Aborting a run ends the run immediately and cannot be reversed.



After the run is aborted, voltage and current are no longer applied and both display fields will read **0.0**. The red progress bar and elapsed time display indicate the point at which the run was aborted relative to the total run time.



Note: The run also automatically enters STATUS: DONE when it reaches the pre-set run time.

9. Press **"Done"** to return to the **Idle** status screen. A new run with the same settings may be started from this screen.

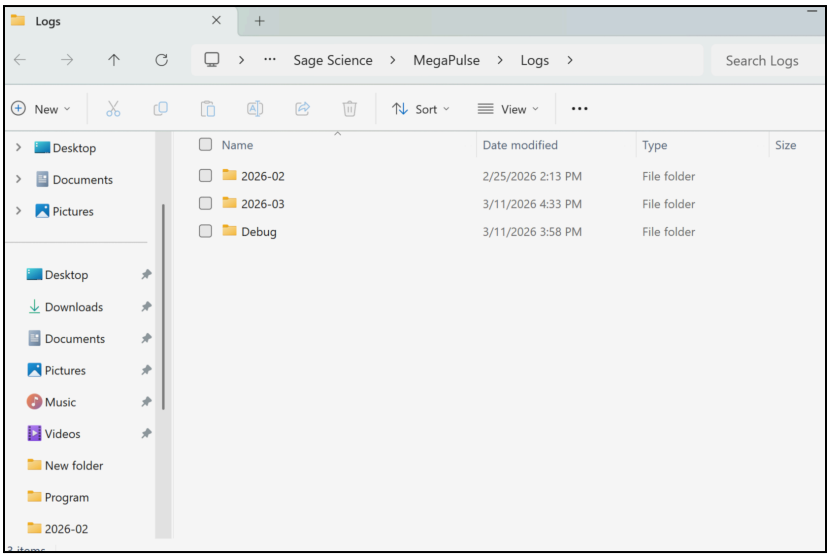
10. Press **"Main"** to return to the Main Screen.

6.0 Log Files and Run Records

Log files can be accessed by pressing the **"Logs"** button from any screen. Pressing **"Logs"** opens Windows Explorer to the MegaPulse log file directory:

(C:\Users\Public\Documents\Sage Science\MegaPulse\Logs).

The MegaPulse software and run status remain unaffected while the log file directory is open. Close the window to return to the MegaPulse software.



Log files are organized into monthly folders that are labeled YYYY-MM. Each MegaPulse run will generate a **.txt** log file using the following naming convention:
MPLog_[Protocol-name]_[yyyy-mm-dd]_[hh-mm-ss].txt

Example: MPLog_[10-48kb]_[2025-03-24]_[07-46-14].txt

Each log file records the run voltage and current values once per second. The log also includes the waveform and other programming parameters used during the run. When multiple waveforms are associated with a single protocol, those waveforms are referenced within the log file.

MegaPulse	1.02												
Firmware	9.39												
BuildDate	1/20/2026												
StartDate	3/25/2026												
StartTime	17:48:20												
ChainName	new 15-1600kb												
LinkNo	Name	Source	Duration	Interactiv	V+	V-	A	B	C	D	E	F	G
1	Detrap	factory	0:10:00	FALSE	75	75	00:01.0	00:00.1	00:00.0	00:00.0	00:00.0	00:00.0	1
2	DC75	factory	0:17:00	FALSE	75	0	00:00.0	00:00.0	00:00.0	00:00.0	00:00.0	00:00.0	0
3	Nool7	factory	12:00:00	FALSE	80	25	00:55.0	01:03.0	00:00.0	00:00.0	00:00.0	00:00.0	1
4	Nool7mod1	factory	12:00:00	FALSE	80	25	01:20.0	01:31.6	00:00.0	00:00.0	00:00.0	00:00.0	1
Time,s	LinkNo	Voltage,V	Current,mA										
1	1	75	30										
2	1	74.9	29.8										
3	1	74.9	29.7										
4	1	74.9	29.8										
5	1	74.9	29.8										

7.0 Advanced Programming

This section describes how to create, edit, save, and load custom electrophoresis programs in the MegaPulse software. Advanced Programming is intended for users who want to work with individual waveforms or combine multiple waveforms into waveform chains.

7.1 Pulsed-Field Waveforms

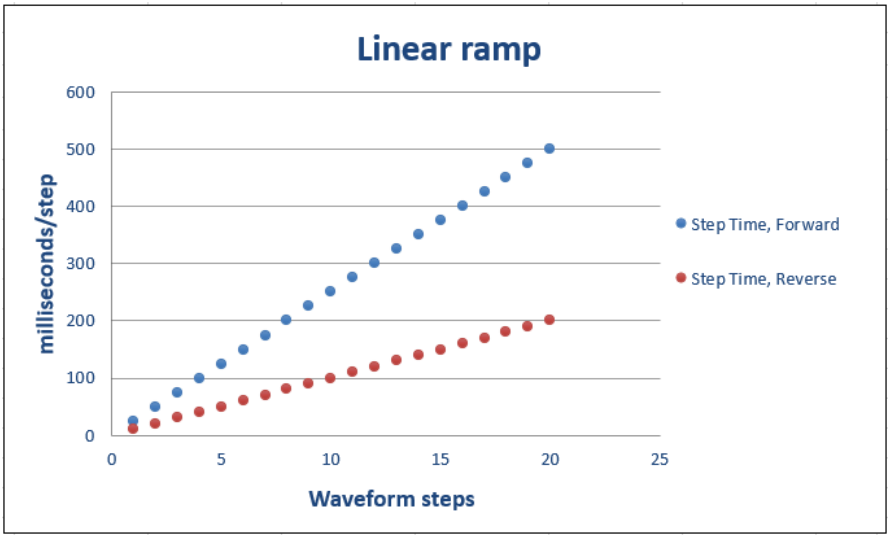
Each combination of forward and reverse pulse times typically separate a relatively narrow range of DNA sizes. To resolve a broader range of DNA sizes, a range of pulse times must be used. This is accomplished by using a "ramp," in which the forward and/or reverse pulse durations are progressively increased from a lower limit to an upper limit.

Pulsed-field waveforms are programmed by entering a forward time step and a reverse time step at the selected voltage magnitude. A ramp is programmed by adding a time increment to either or both steps. An accelerated ramp can be achieved by adding additional time increments to either or both increments. 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 reproducibly for different lengths of time.

Pulsed-field waveforms are defined by a set of parameters labeled V+, V-, and A through G. These include the starting forward and reverse pulse times (A and B), incremental time values that progressively increase the pulse durations to create a ramp (C–F), the number of steps per cycle (G), and the forward and reverse voltages (V+ and V−). Full parameter definitions and programming details are provided in Section 7.4.3.

Waveform input parameters

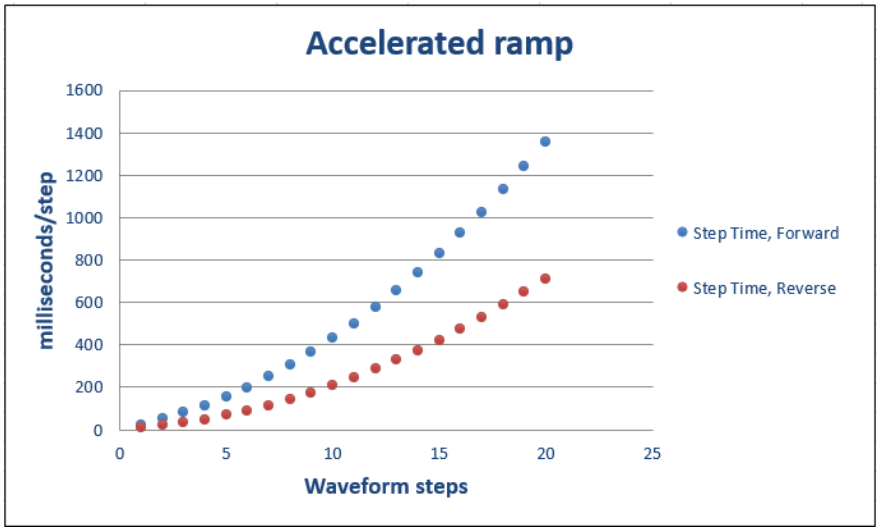
A	B	C	D	E	F	G
00:00:00:025	00:00:00:010	00:00:00:025	00:00:00:010	00:00:00:000	00:00:00:000	21



Example of time increments for the forward and reverse steps.
A 25 msec forward step (A) with a 25 msec increment (C) and a 10 msec reverse step (B) with a 10 msec increment (D) are shown. The waveform contains 21 steps per cycle (G).

Waveform input parameters

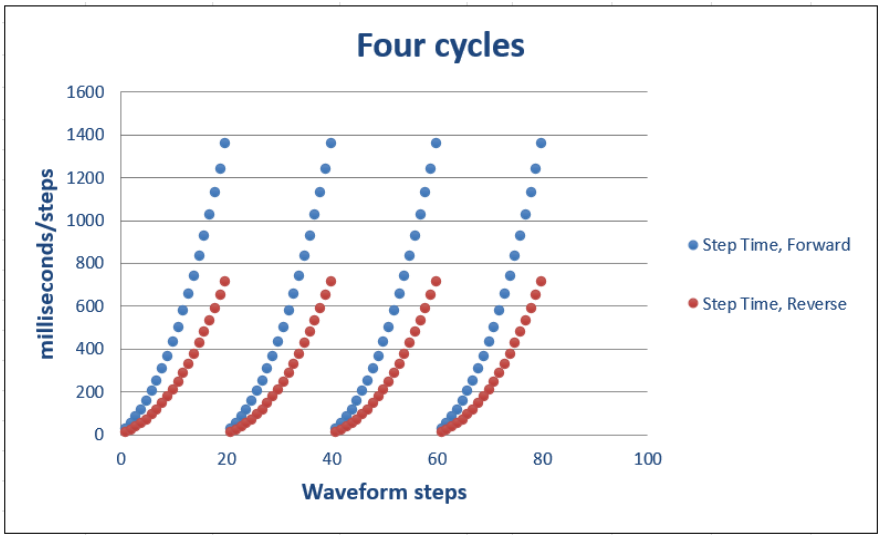
A	B	C	D	E	F	G
00:00:00:025	00:00:00:010	00:00:00:025	00:00:00:010	00:00:00:005	00:00:00:003	21



Example of second increments added to the forward and reverse increments.
A 25 msec forward step (A) with a 25 msec increment (C) and a 10 msec reverse step (B) with a 10 msec increment (D). 5 msec is added to the forward increment (E) and 3 msec is added to the reverse increment (F). The waveform contains 21 steps per cycle (G).

Waveform input parameters

A	B	C	D	E	F	G
00:00:00:025	00:00:00:010	00:00:00:025	00:00:00:010	00:00:00:005	00:00:00:003	21



Example of four cycles of the waveform shown in the previous graphs
In this example, each cycle is approximately 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.

7.2 ZIFE waveforms

Zero-Integrated-Field Electrophoresis (ZIFE) is an advanced pulsed-field mode in which both field direction and field magnitude are changed in concert. In ZIFE mode, the summed volt-time values integrated over the pulse cycle are zero, or nearly zero. Under these near-zero-integrated-field conditions, very large DNA fragments can be resolved on the basis of the time required for the DNA to reorient in response to changes in field direction. In practice, ZIFE electrophoresis is usually performed with a slight forward bias so that both large and small DNA molecules may be resolved.

7.3 Linking Waveforms and Chains

MegaPulse pulsed-field programs come in two flavors: FIGE and ZIFE. Both FIGE and ZIFE programs are composed of one or more waveforms. Each waveform is a program of electrophoresis field voltages, field directions, and pulse durations.

The pre-set programs 0.5–15 kb, 10–48 kb, 5–80 kb, 5–150 kb, and 5–430 kb each use a single waveform. These are FIGE protocols, and the size resolution of these programs is proportional to the total run time. Users may adjust the run time for these protocols as needed.

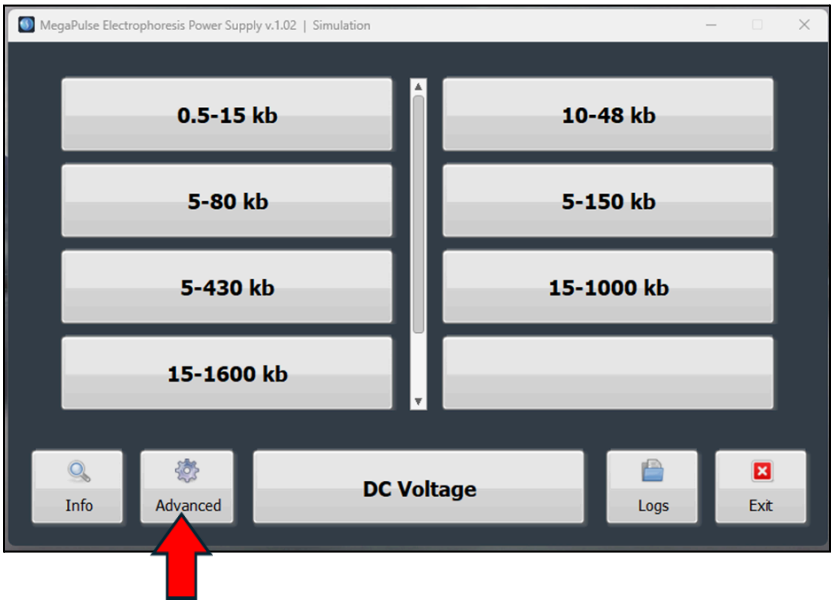
The pre-set programs 15–1000 kb and 15–1600 kb are ZIFE programs composed of four linked waveforms. In the Advanced Programming window, these linked combinations of waveforms are referred to as chains. For these programs, size resolution is a function of both the overall run time and the proportion of run time used by each waveform. For this reason, the factory pre-set chain runtimes have been optimized and cannot be changed.

Advanced users may use the Advanced Programming screen to create, save, and reload user-defined waveform chains.

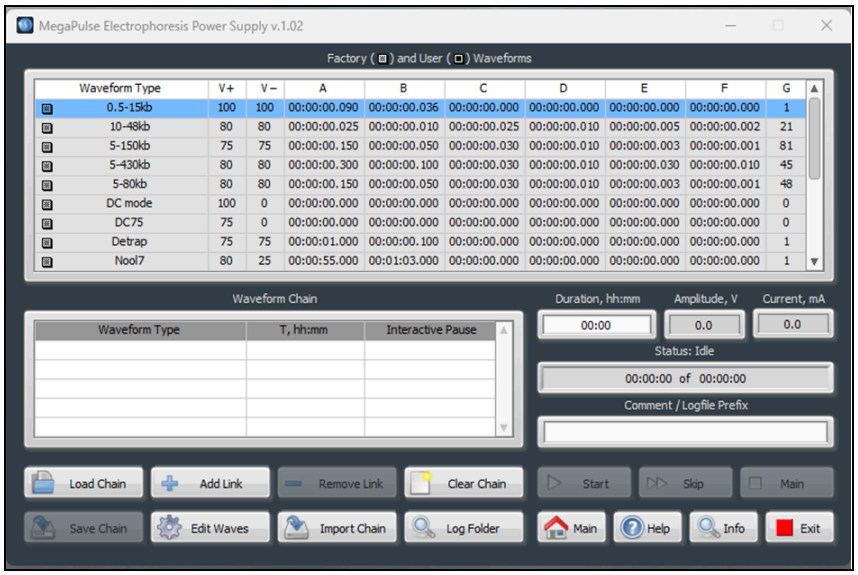
7.4 Using the Advanced Programming Interface

From the Main Screen, press **"Advanced"** to open the Advanced Programming screen. The Advanced Programming screen displays the available factory and user waveforms, the **Waveform Chain** window, and the controls used to build, save, load, and run waveform chains.

Main Screen



Advanced Programming Screen



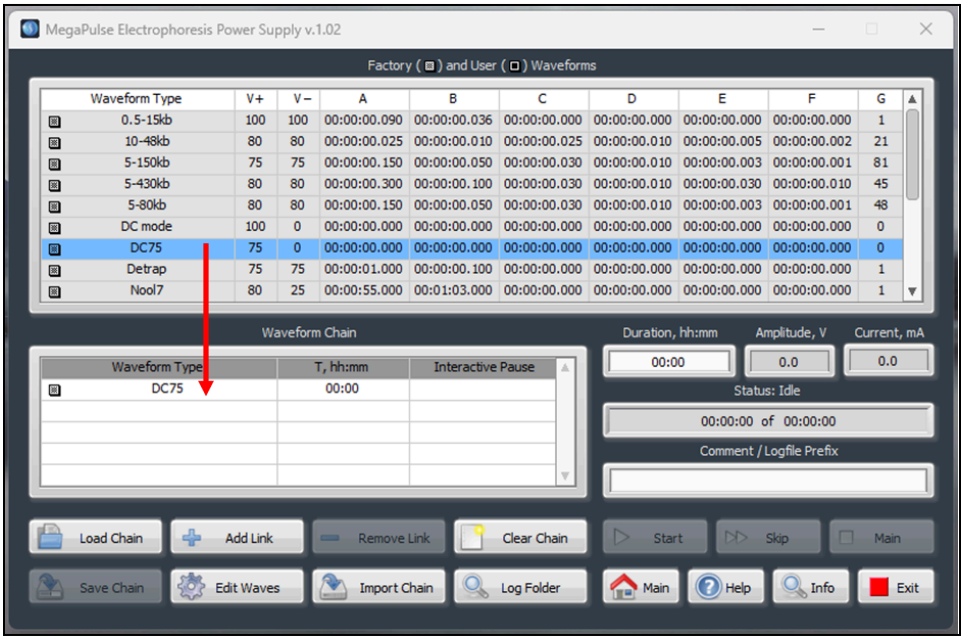
The waveform list at the top of the screen displays available factory and user waveforms. The **Waveform Chain** window below displays the waveforms selected for the current chain and their assigned durations. The controls at the bottom of the screen are used to load, save, edit, import, or clear waveform chains.

7.4.1 Creating and Saving a Waveform Chain

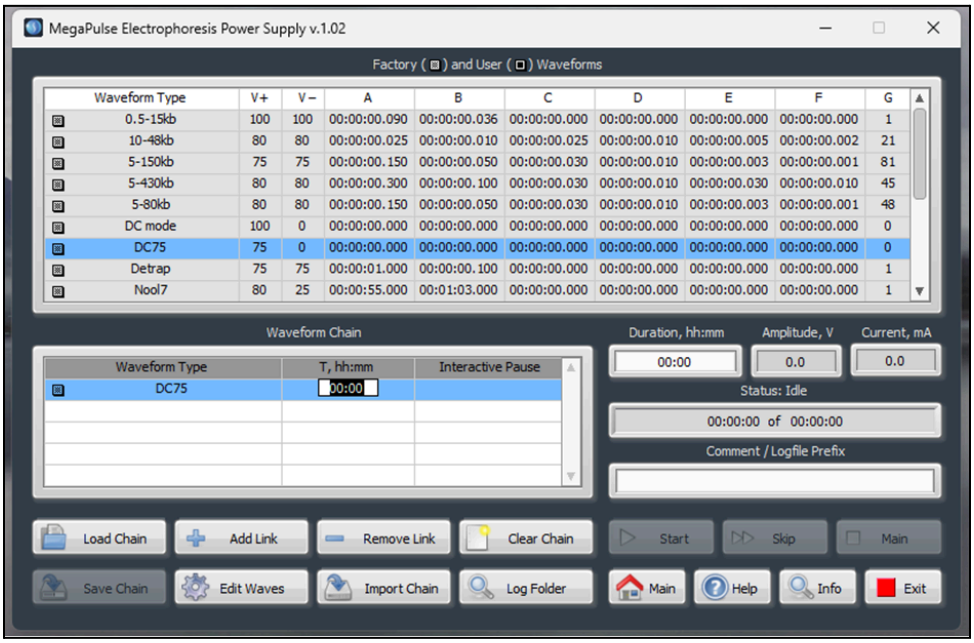
To create a waveform chain, begin from the Advanced Programming screen and follow the steps below.

1. In the Advanced Programming screen, select the desired waveform from the list of available factory or user waveforms.

2. Click and drag the waveform into the **Waveform Chain** window.

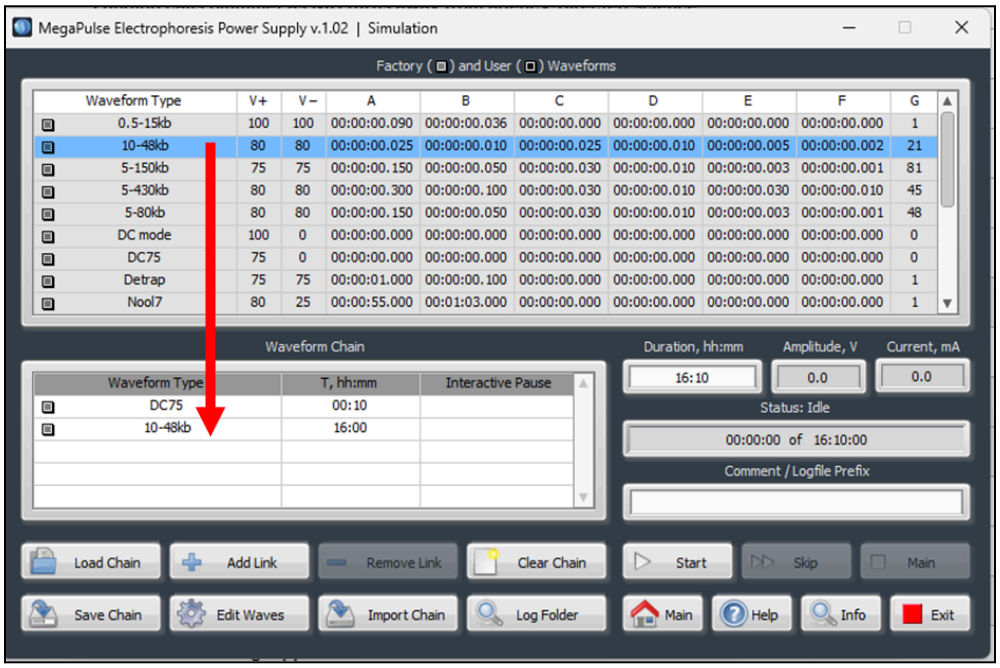


3. Double-click the **"T, hh:mm"** field and enter the desired waveform duration.



4. Repeat the drag-and-drop process as needed to add additional waveforms and create a waveform chain. Double-click on “T, hh:mm” and edit the duration of waveform time.

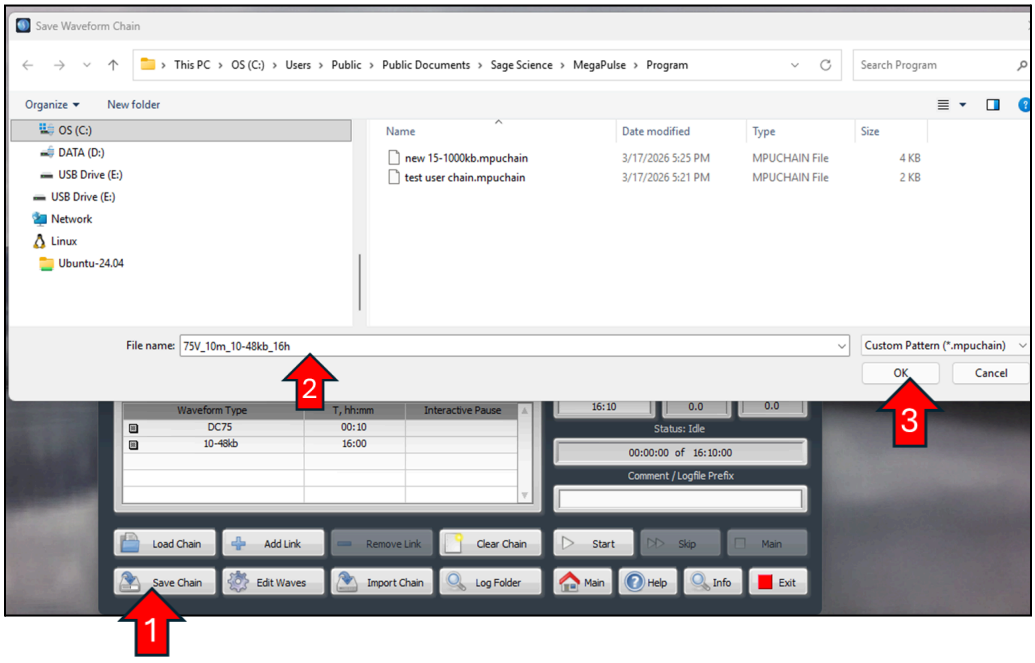
5. Review the total chain duration before starting or saving the chain.



6. Press “Save Chain.”

7. Enter the preferred chain name.

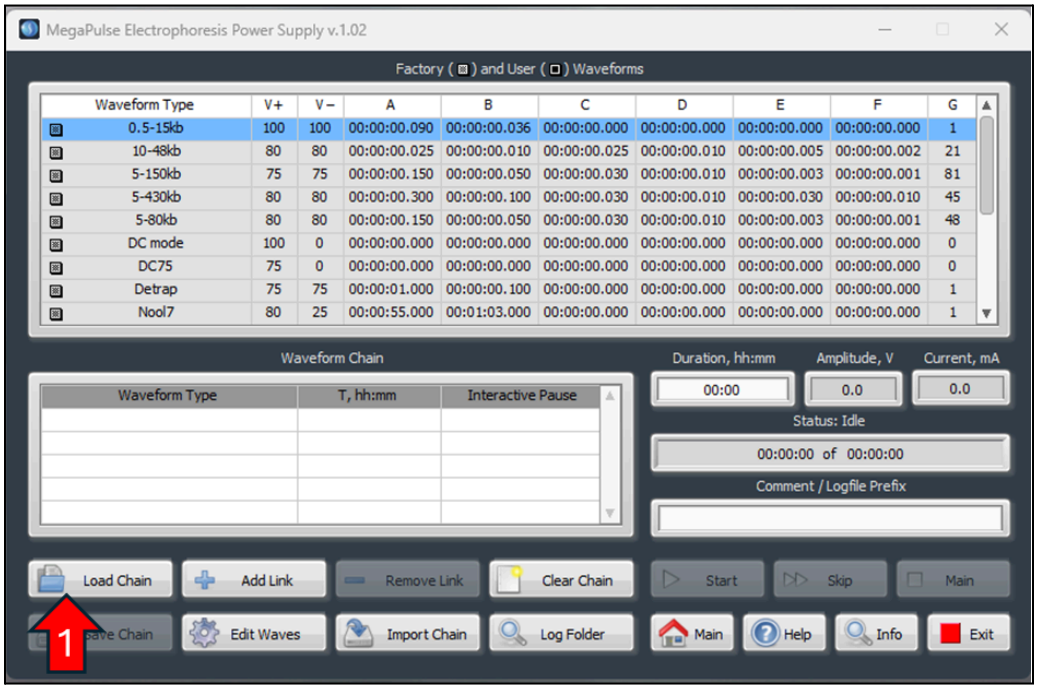
8. Press OK to save the chain.



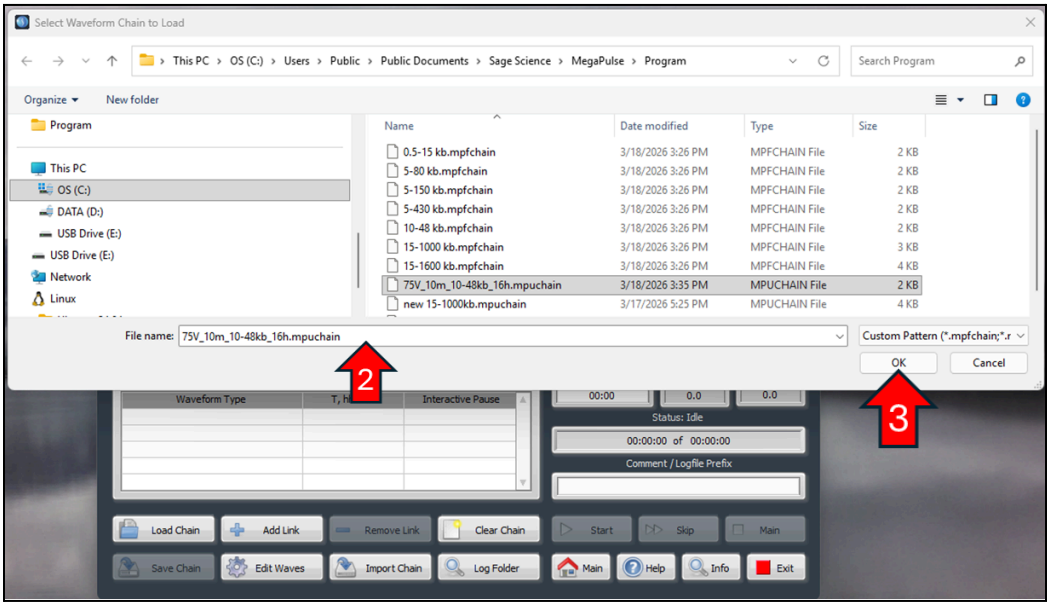
7.4.2 Loading a Saved Chain

To load and run a previously saved waveform chain, begin from the Advanced Programming screen and follow the steps below.

1. From the Advanced Programming screen, press **“Load Chain.”**

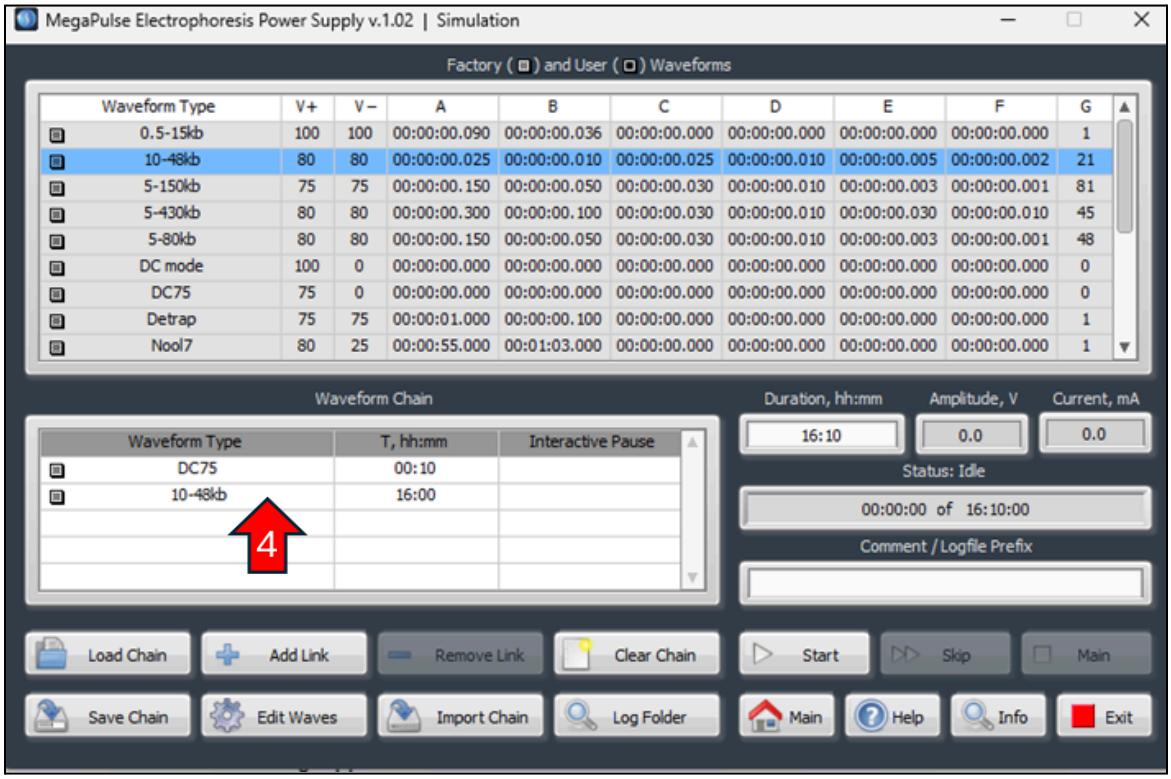


2. Select the desired chain from the file menu.



3. Press **“OK”** to load the selected chain.

4. The loaded chain will appear in the Waveform Chain window.
5. For user-generated chains, waveform durations may be edited as desired before starting the run.



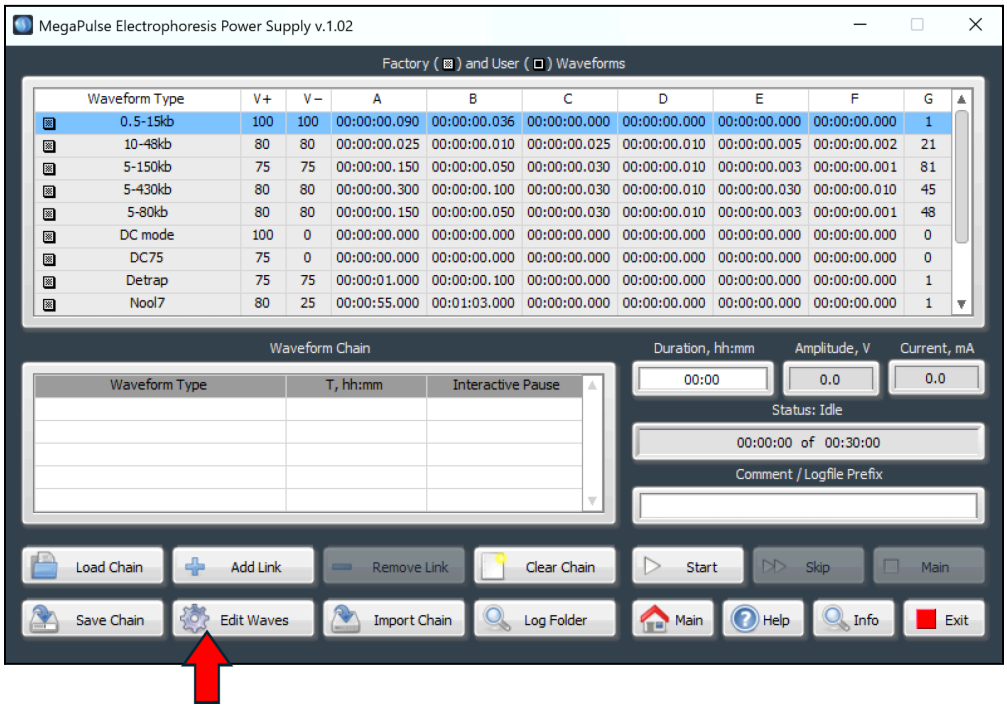
Note: In the **Chains** folder, user-generated chains have the suffix **".mpuchain"** and factory pre-set chains have the suffix **".mpfchain."** Factory chain files may be loaded, but they cannot be modified.

7.4.3 Programming and Editing Waveforms

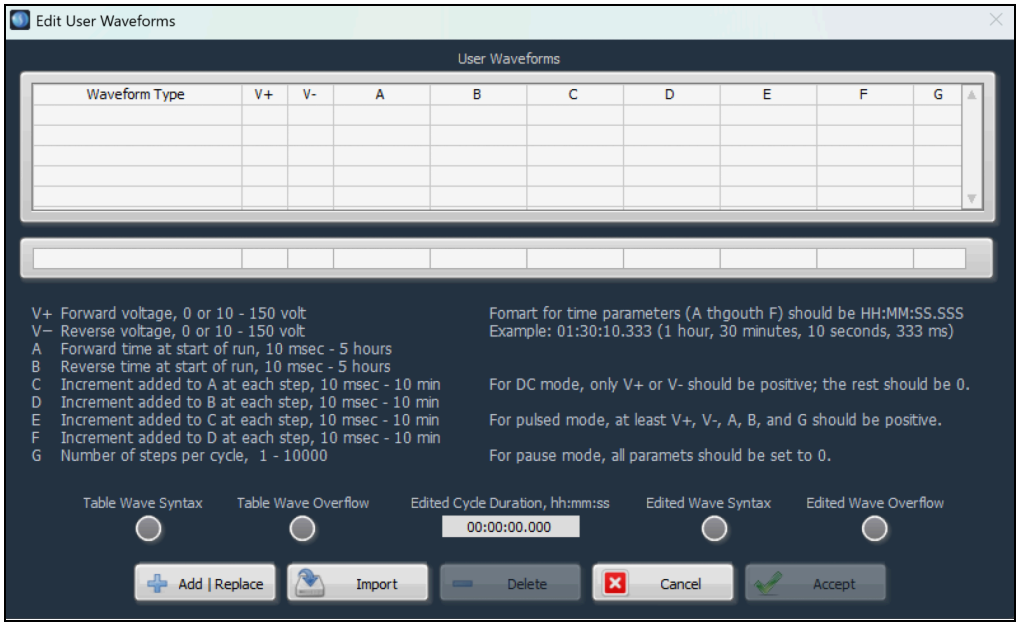
To create or edit an individual waveform, begin from the Main Screen and follow the steps below.

1. From the Main Screen, press **"Advanced."** The **Advanced Programming** screen will open and display the available factory and user waveforms.
2. Press **"Edit Waves."** The **Edit User Waveforms** screen will appear over the **Advanced Programming** screen.

Advanced Programming Screen



Edit User Waveforms Screen



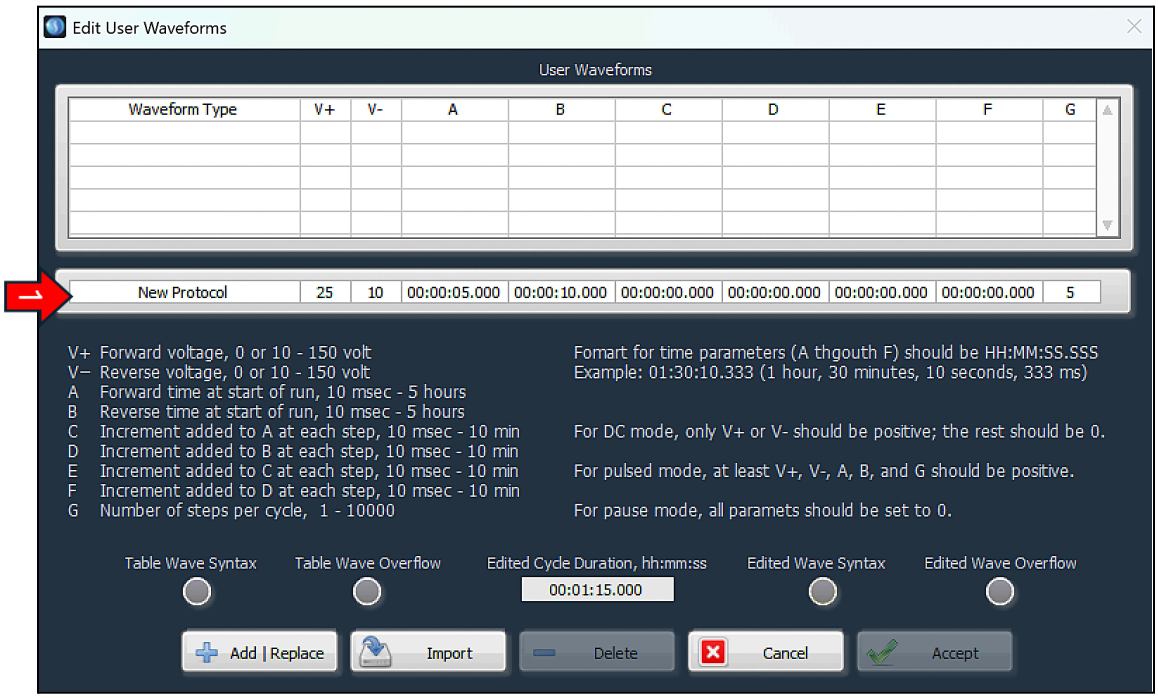
The waveform parameters used on the **Edit User Waveforms** screen are described below.

Waveform Parameters

V+ Forward voltage, 0 or 10 - 150 volt
V- Reverse voltage, 0 or 10 - 150 volt
A Forward time at start of run, 10 msec - 5 hours
B Reverse time at start of run, 10 msec - 5 hours
C Increment added to A at each step, 10 msec - 10 min
D Increment added to B at each step, 10 msec - 10 min
E Increment added to C at each step, 10 msec - 10 min
F Increment added to D at each step, 10 msec - 10 min
G Number of steps per cycle, 1 - 10000

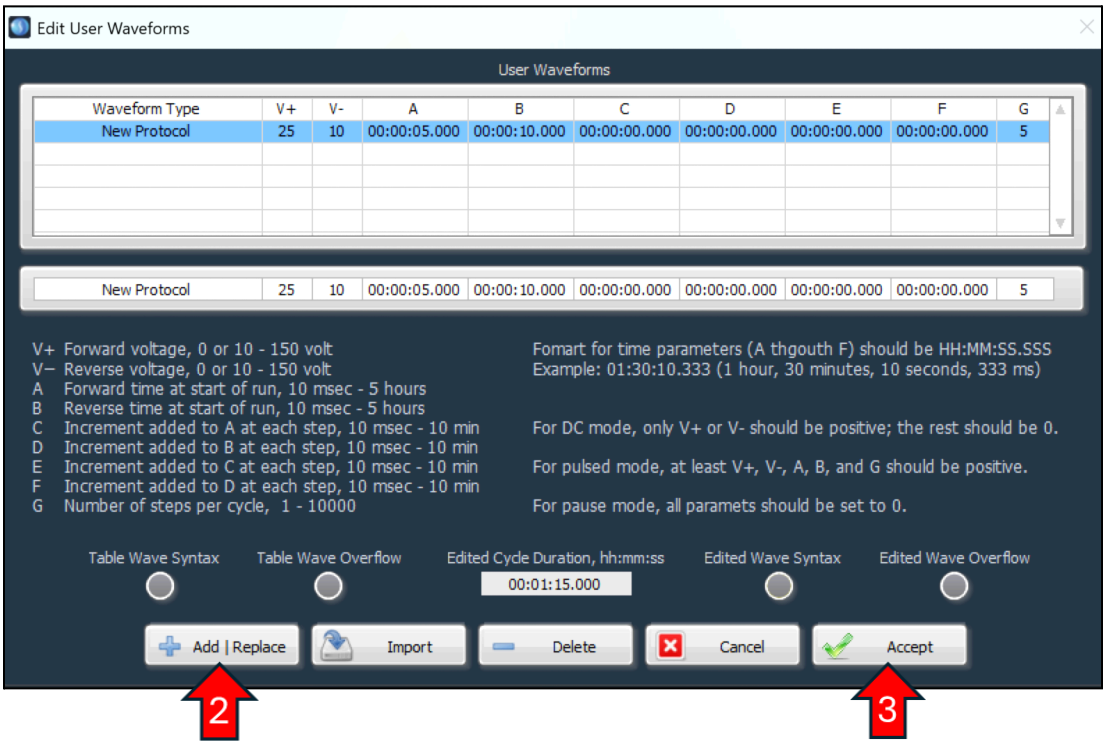
- Format for time parameters (A through F) should be HH:MM:SS.SSS
  Example: 01:30:10.333 (1 hour, 30 minutes, 10 seconds, 333 ms)
- For DC mode, only V+ or V- should be positive; the rest should be 0.
- For pulsed mode, at least V+, V-, A, B, and G should be positive.
- For pause mode, all parameters should be set to 0.

3. Place the mouse cursor in the editing field. Enter a name for the waveform under “Waveform Type,” and enter values for each parameter.



4. Press “Add/Replace” to add the new waveform to the waveform list. If a waveform with the same name already exists in the “Waveform Type” list, it will be overwritten.

5. Press **"Accept"** to save the new waveform and return to the **Advanced Programming** screen.



Note: If a previous user-defined waveform is shown in the waveform list, clicking on that waveform will cause it to appear in the editing field, where it may be modified and saved. The new waveform will replace the older user-defined waveform. Pre-set waveforms cannot be modified.

7.4.4 Pulsed Field Waveforms: Where to start

The tables below may be used as a starting point for developing protocols for gel analysis of large DNA. These guidelines are based on 5–10 V/cm gels and the electrophoresis conditions described in Section 5.3. The protocols highlighted in gray are included as pre-set protocols in the MegaPulse software.

7.4.4.1 FIGE pre-set protocol parameters

The first five pre-set pulsed-field programs are FIGE protocols composed of single waveforms.

Approx. Resolved Size Range (kb)	Run Time	Voltage		MegaPulse Parameters						
		V+	V−	A	B	C	D	E	F	G
0.5-15kb	8 hr	100	100	00:00:00.090	00:00:00.036	00:00:00.000	00:00:00.000	00:00:00.000	00:00:00.000	1
10-48kb	9 hr	80	80	00:00:00.025	00:00:00.010	00:00:00.025	00:00:00.010	00:00:00.005	00:00:00.002	21
5-150kb	16 hr	75	75	00:00:00.150	00:00:00.050	00:00:00.030	00:00:00.010	00:00:00.003	00:00:00.001	81
5-430kb	16 hr	80	80	00:00:00.300	00:00:00.100	00:00:00.030	00:00:00.010	00:00:00.030	00:00:00.010	45
5-80kb	14 hr	80	80	00:00:00.150	00:00:00.050	00:00:00.030	00:00:00.010	00:00:00.003	00:00:00.001	48

7.4.4.2 ZIFE pre-set chain waveform parameters

The 15–1000 kb and 15–1600 kb factory pre-set programs are ZIFE chains composed of multiple waveforms. The table below lists the component waveforms used in those chains.

Approx. Resolved Size Range (kb)	Waveform Type	Run Time (hh:mm)	Voltage		MegaPulse Parameters						
			V+	V−	A	B	C	D	E	F	G
15-1000kb	Detrap	00:10	75	75	00:00:01.000	00:00:00.100	00:00:00.000	00:00:00.000	00:00:00.000	00:00:00.000	1
	DC75	00:15	75	0	00:00:00.000	00:00:00.000	00:00:00.000	00:00:00.000	00:00:00.000	00:00:00.000	0
	Nool7	09:30	80	25	00:00:55.000	00:01:03.000	00:00:00.000	00:00:00.000	00:00:00.000	00:00:00.000	1
	Nool7mod1	09:30	80	25	00:01:20.000	00:01:31.600	00:00:00.000	00:00:00.000	00:00:00.000	00:00:00.000	1
15-1600kb	Detrap	00:10	75	75	00:00:01.000	00:00:00.100	00:00:00.000	00:00:00.000	00:00:00.000	00:00:00.000	1
	DC75	00:17	75	0	00:00:00.000	00:00:00.000	00:00:00.000	00:00:00.000	00:00:00.000	00:00:00.000	0
	Nool7	12:00	80	25	00:00:55.000	00:01:03.000	00:00:00.000	00:00:00.000	00:00:00.000	00:00:00.000	1
	Nool7mod1	12:00	80	25	00:01:20.000	00:01:31.600	00:00:00.000	00:00:00.000	00:00:00.000	00:00:00.000	1

8.0 Gel Images and Run Conditions

The pre-set protocol gel images shown in this section were generated using the gel formulations and conditions described below with the Sage Pulse Gel Box. The Sage Pulse Gel Box is equipped with large-diameter electrodes specified for pulsed-field use, Part No. **PGB-1000.**

8.1 Agarose Formulations: KBB Gel, pH 8.7

10X KBB Buffer and 0.5X KBB Running Buffer

Component	10X Buffer (g/L)	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

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

0.75% Agarose Gel

Component	1 L	110 mL
Lonza SeaKem GOLD	7.5 g	0.825 g
DH20	950 mL	104.5 mL
10X KBB Buffer	50 mL	5.5 mL

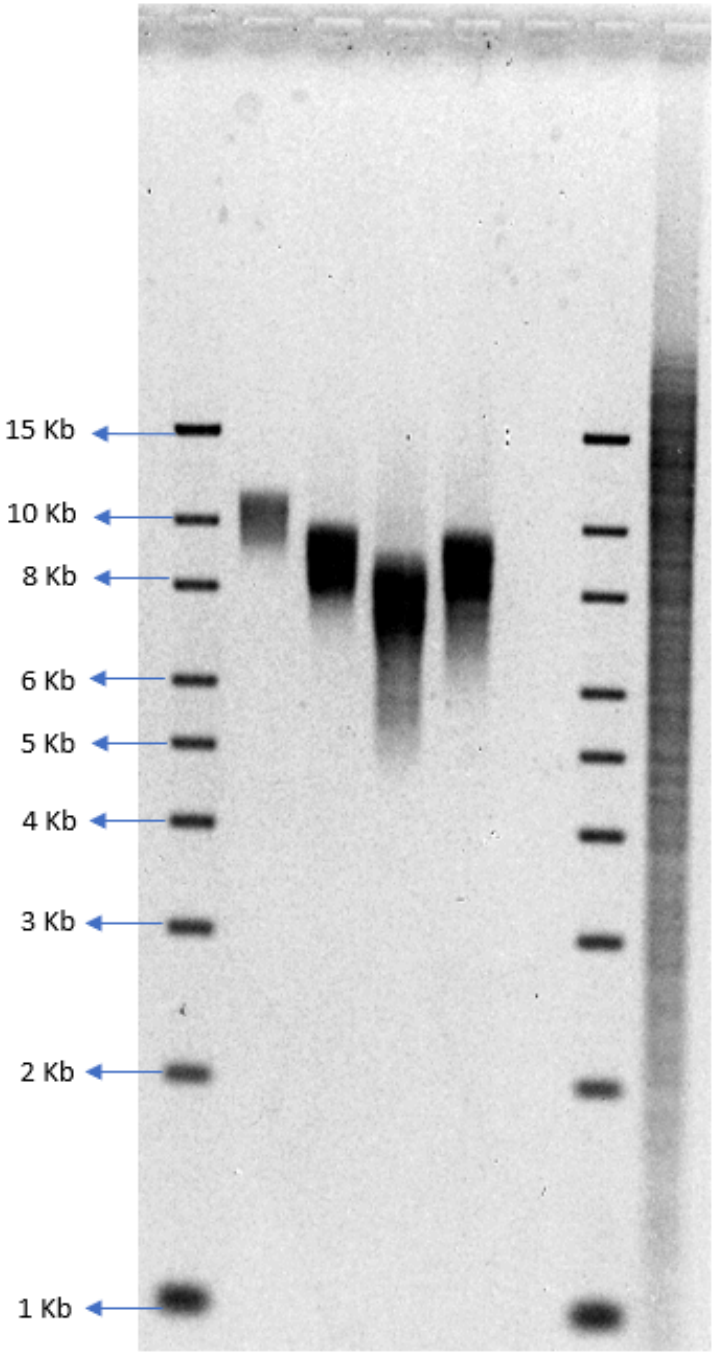
1.0% Agarose Gel

Component	1 L	110 mL
Lonza SeaKem GOLD	10 g	1.1 g
DH20	950 mL	104.5 mL
10X KBB Buffer	50 mL	5.5 mL

Note: For best image quality and reproducibility, run PF gels without intercalating dye and stain the gels after electrophoresis. Dyes bind tightly to DNA and affect mobility. Because the dyes are also mobile during electrophoresis, dye concentration at any one position in the gel will change over time, which can result in fuzzy bands.

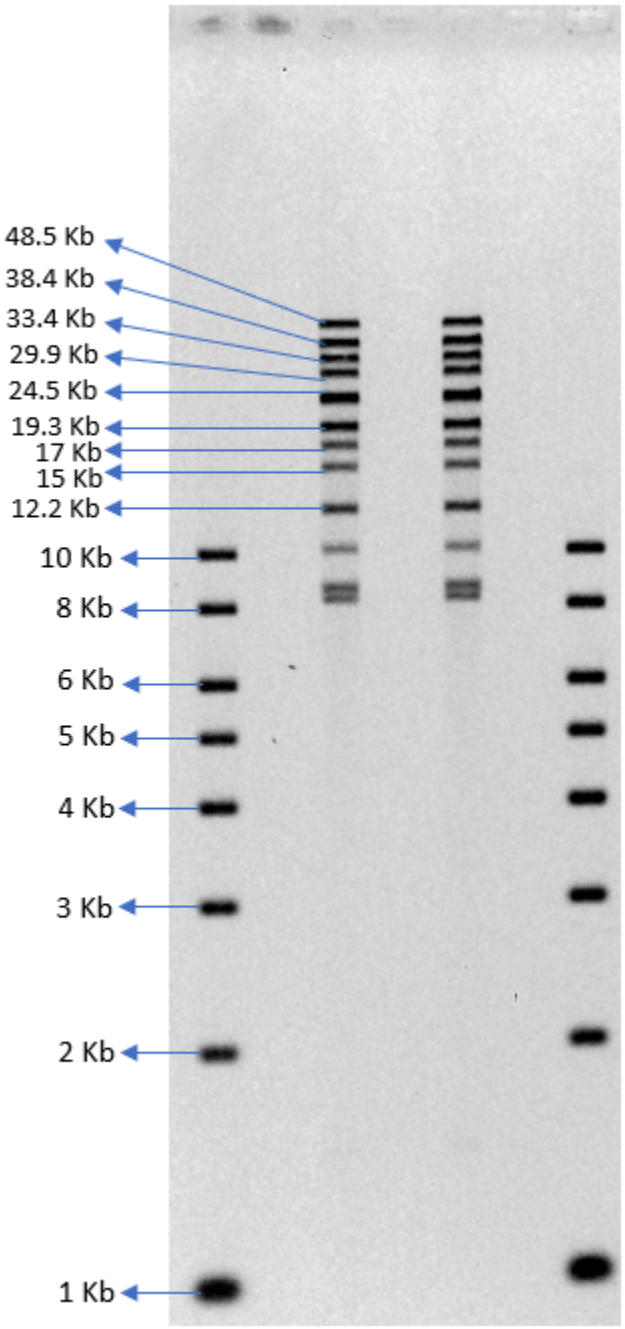
8.2 Pre-set protocol example 1: 0.5–15 kb

Run time: 8 hours
Voltage: 100 V
Buffer: 0.5X KBB (see section 8.1)
Agarose: 0.75% Lonza SeaKem GOLD, 110 mL
Ladder(s): Bio-Rad EZ Load 1 kb Molecular Ruler #170-8355



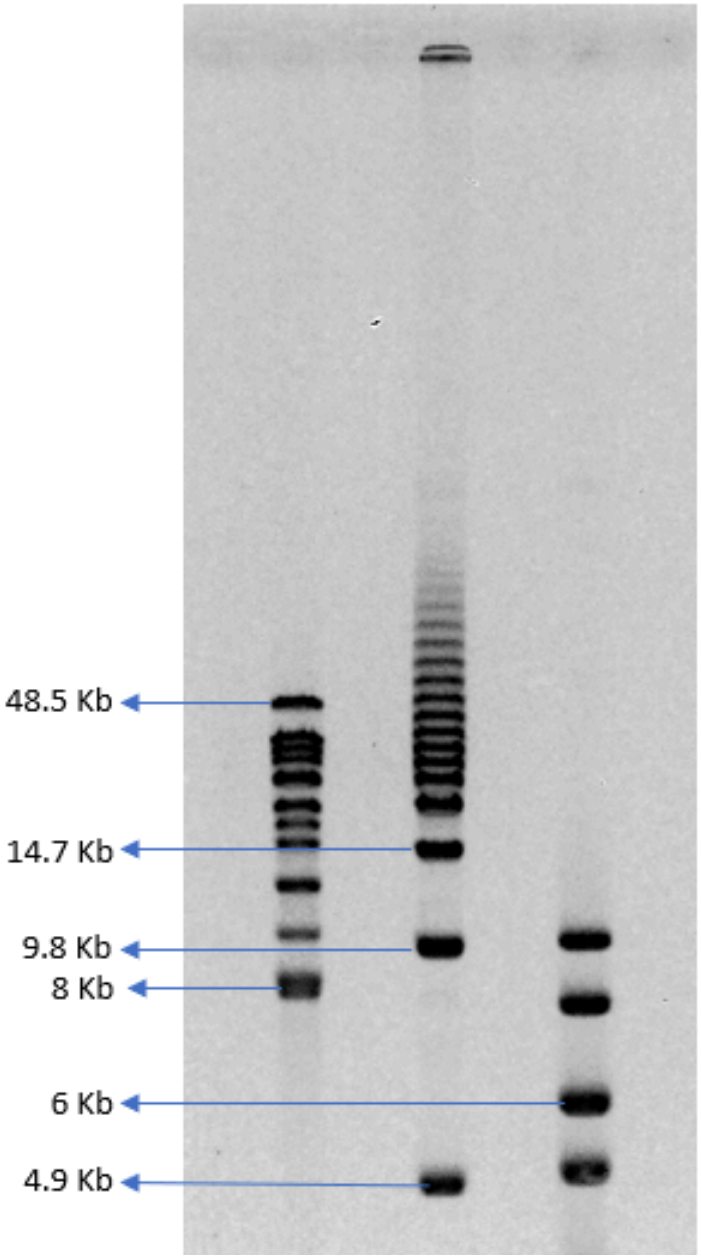
8.3 Pre-set protocol example 2: 10–48 kb

Run time: 9 hours
Voltage: 80 V
Buffer: 0.5X KBB (see section 8.1)
Agarose: 0.75% Lonza SeaKem GOLD, 110 mL
Ladder(s): NEB Quick-Load 1 kb Ladder #N0468S; Bio-Rad CHEF DNA Size Standards 8–48 kb #170-3707



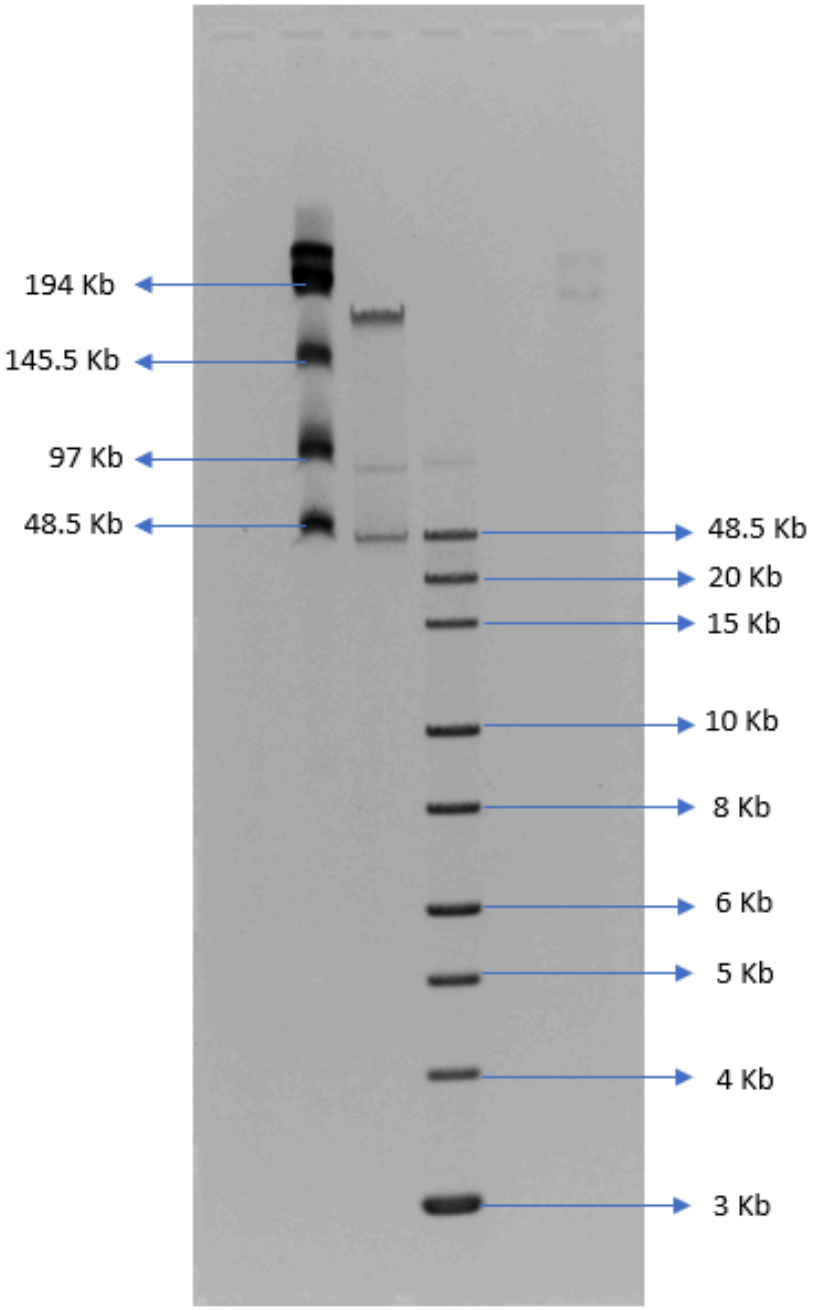
8.4 Pre-set protocol example 3: 5–80 kb

Run time: 14 hours
Voltage: 80 V
Buffer: 0.5X KBB (see section 8.1)
Agarose: 0.75% Lonza SeaKem GOLD, 110 mL
Ladder(s): Bio-Rad CHEF DNA Size Standards 8–48 kb #170-3707; Bio-Rad DNA Size Standard 5 kb Ladder #170-3624



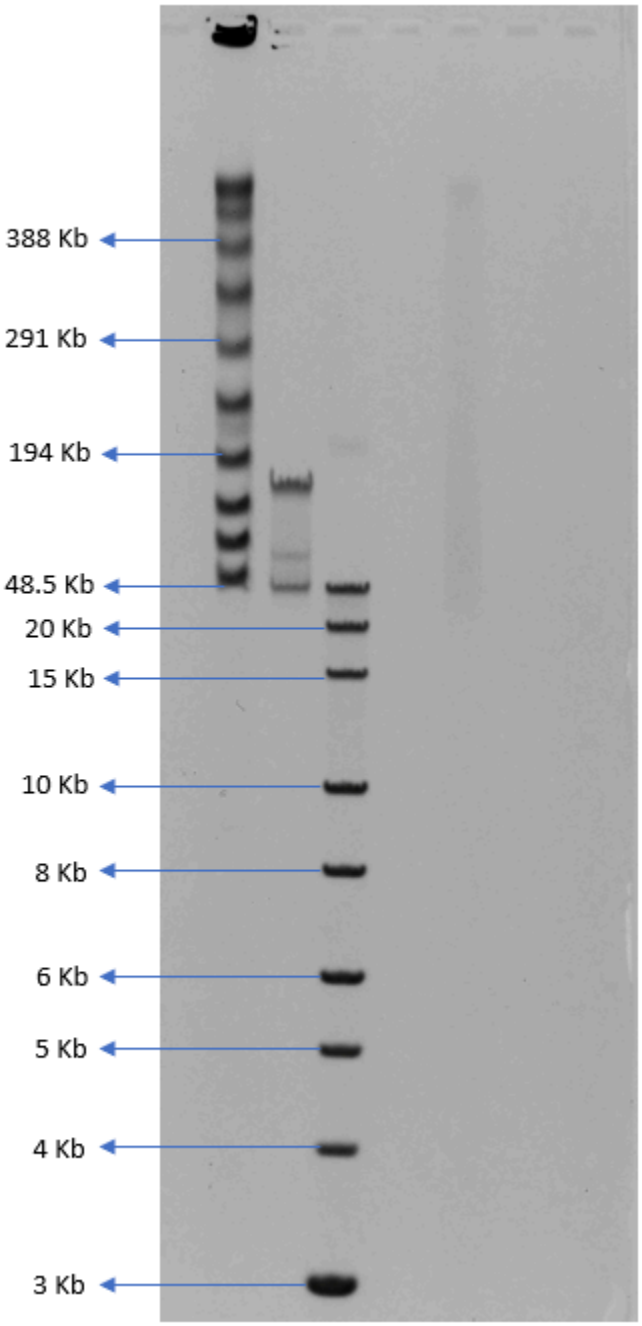
8.5 Pre-set protocol example 4: 5–150 kb

Run time: 16 hours
Voltage: 75 V
Buffer: 0.5X KBB (see section 8.1)
Agarose: 0.75% Lonza SeaKem GOLD, 110 mL
Ladder(s): NEB Lambda PFG Ladder #N0341S; NEB 1 kb Extend DNA Ladder #N3239



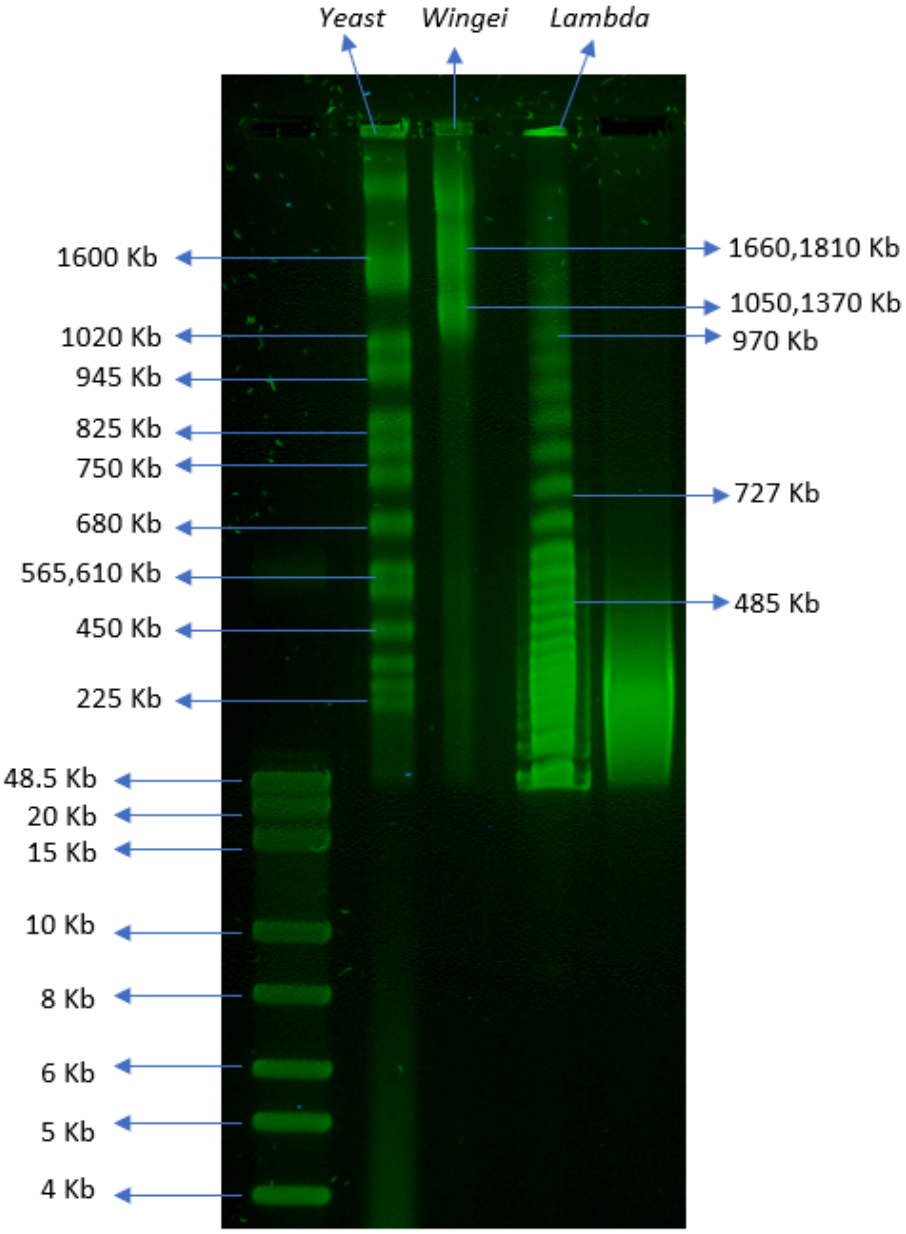
8.6 Pre-set protocol example 5: 5–430 kb

Run time: 16 hours
Voltage: 75 V
Buffer: 0.5X KBB (see section 8.1)
Agarose: 0.75% Lonza SeaKem GOLD, 110 mL
Ladder(s): NEB Lambda PFG Ladder #N0341S; Bio-Rad CHEF DNA Size Standards 8–48 kb #170-3707, NEB 1 kb Extend DNA Ladder #N3239



8.7 Pre-set protocol example 6: 15–1000 kb

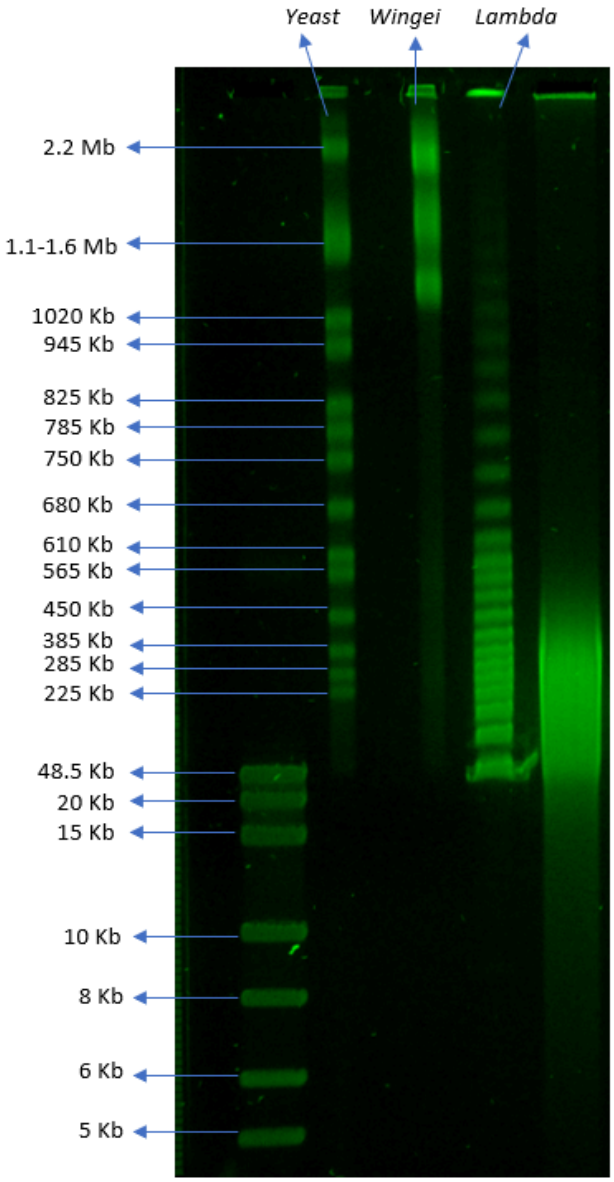
Run time: 19.25 hours
Protocol type: ZIFE waveform chain (see section 7.4.4.2)
Buffer: 0.5X KBB (see Section 8.1)
Agarose: 1.0% Lonza SeaKem GOLD, 250 mL
Ladder(s): Bio-Rad CHEF DNA Marker 0.2-2.2 Mb, S. cerevisiae #1703605, Bio-Rad CHEF DNA Marker 1-3.1 Mb, H. wingei #1703667, NEB Lambda PFG Ladder 48.5-1,018 kb #N0341S, NEB Quick-Load 1 kb Extend DNA ladder #N3239S



Note: Detailed marker-loading instructions for the 15–1000 kb and 15–1600 kb ZIFE protocols are provided in Appendix A.

8.8 Pre-set protocol example 7: 15–1600 kb

Run time: 24:27 hours
Protocol type: ZIFE waveform chain (see section 7.4.4.2)
Buffer: 0.5X KBB (see Section 8.1)
Agarose: 1.0% Lonza SeaKem GOLD, 250 mL
Ladder(s): Bio-Rad CHEF DNA Marker 0.2-2.2 Mb, S. cerevisiae #1703605, Bio-Rad CHEF DNA Marker 1-3.1 Mb, H. wingei #1703667, NEB Lambda PFG Ladder 48.5-1,018 kb #N0341S, NEB Quick-Load 1 kb Extend DNA ladder #N3239S



Note: Detailed marker-loading instructions for the 15–1000 kb and 15–1600 kb ZIFE protocols are provided in Appendix A.

Appendix A. Loading Markers for ZIFE Protocols

The procedure below applies to the **15–1000 kb** and **15–1600 kb** ZIFE protocols. Standard electrophoresis setup is assumed. These steps focus on the gel preparation and marker-loading practices that are most important for obtaining the best resolution in the **>500 kb** region.

Note: The Bio-Rad chromosome markers are supplied in a 10mM Tris + 100mM EDTA solution. For best results running the ZIFE protocols, transfer and store the *S. cerevisiae* and *H. wingei* chromosome marker slices in a **TE + 10 mM EDTA + 0.1% SDS** solution until ready to load.

Procedure

1. **Prepare 0.5X KBB running buffer**
   Dilute 10X KBB to 0.5X by adding 100 mL of 10X KBB to water and bringing the final volume to 2L. (see section 8.1)

2. **Prepare 1.0% agarose**
   Add 250 mL of 0.5X KBB to a bottle with a stir bar. Add 2.5 g of Lonza SeaKem GOLD agarose and stir to avoid clumping. Weigh bottle before heating.

Note: If agarose is stuck to the sides of the bottle, rinse it down with a small amount of water so that dry agarose does not burn during heating.

3. **Dissolve the agarose**
   Microwave carefully for approximately 2.5 minutes, stir, then microwave for an additional ~1 minute or until all particles are fully dissolved and the solution is clear. Reweigh the bottle and add water to restore the original weight. Stir briefly, then hold the solution at 65°C for 1 hour to ensure full dispersion.

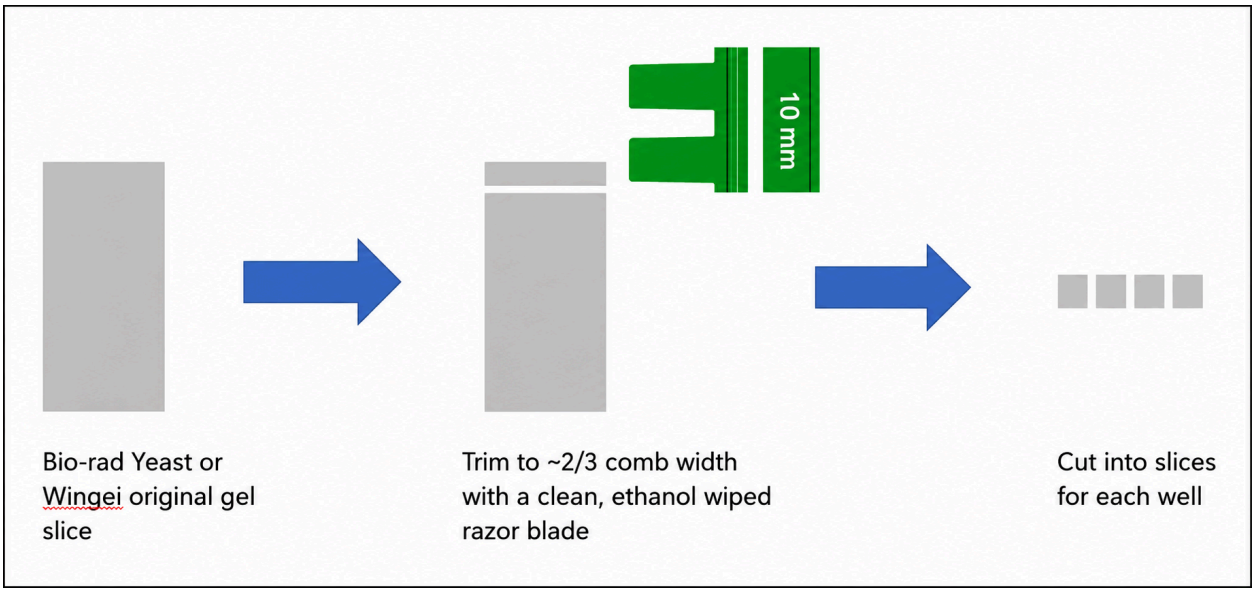
4. **Cast the gel**
   Place the gel box and tray on a balance and add 85 ± 3 g of the hot agarose solution. Insert a comb.

5. **Allow the gel to set**
   Leave the gel uncovered for ~1 hour before loading.

6. **Prepare the overlay agarose**
   Prepare a 0.6% overlay agarose by combining 4 mL of 0.5X KBB with 6 mL of the 1.0% agarose solution. Hold at 45°C until use.

Note: The 45°C hold helps keep the overlay fluid while minimizing the chance of softening the lambda slice during loading.

7. **Cut and load the chromosome marker slices**
   Cut the *S. cerevisiae* or *H. wingei* chromosome marker slices as shown in the gel slice cutting images (below). The slices should be trimmed to fit the well cleanly. Place the slices into the wells so that they stick flattened against the downstream face of the sample well. Proceed to add the 0.6% overlay agarose.



8. **Add the lambda ladder slice**
   Add the thinnest practical slice of the lambda ladder to the appropriate well.

9. **Add running buffer and ladder**
   Fill the gel box with 650-700 mL of 0.5X KBB buffer. Add 5 uL of the NEB 1kb Extend ladder (optional).

Note: The NEB 1 kb Extend ladder contains dye, which helps confirm that the gel is running properly and also provides a useful low-molecular-weight reference. If desired, the T4 DNA 164 kb may also be added.

10. **Run the selected ZIFE protocol. After electrophoresis, the gel may be stained and imaged as desired.**

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