

Pulsewave[®] 760 Switcher

Instruction Manual

Catalog Numbers 170-3600 through 170-3603

Version 3.0

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Section 1

Introduction

1.1 Preface

The technique of pulsed field electrophoresis resolves DNA fragments and chromosome size DNA from 200 to over 12,000 kilobase pairs. In this technique, directional switching of the electric field causes molecules to change direction in the gel. Separations are possible because the time each fragment takes to alter its shape to move in a new direction varies with its length.

The Pulsewave 760 switcher is a versatile and safe instrument for pulsed field DNA electrophoresis. The unit may be used with Orthogonal Field Alternating Gel Electrophoresis (OFAGE)¹ and its variants,^{2,3} Clamped Homogeneous Electric Fields (CHEF),⁴ and Field Inversion Gel Electrophoresis (FIGE).⁵ The Pulsewave 760 switcher interfaces with an external power supply and gel box, redirecting the output of the power supply to two pairs of electrodes (OFAGE). With the Programming Block accessory, the Pulsewave 760 switcher inverts the power supply output for the single pair of electrodes in a FIGE cell. The power supply plugs into the back of the Pulsewave 760 switcher. A maximum of 760 volts DC is allowed. When used with CHEF-DR® II system, the maximum is 300 V.

Pulsewave 760 switcher's integral microcomputer and 11 operating modes provide maximum control over the electrical wave pulses. Start and end times, and the forward/backward ratio, may be ramped to maximize resolution of DNA fragments in various size ranges. Parameters may be reset in the middle of an experiment, without operator intervention. A port for external computer control is provided for more complex pulsing requirements.

Features of the Pulsewave 760 switcher include:

- 0-760 volts, 0-500 milliamps, with safety circuitry
- Switch intervals from 0.1 to 65,000 seconds
- Adjustable forward/backward ratio from 0.1 to 99.9:1
- Off time insertion from 0.1 to 65,000 seconds
- Eleven modes for display, alarm, and specialized switching
- Programming Block available for two FIGE experiments
- Expander Block available for two OFAGE or two CHEF experiments

1.2 Safety

Warning: High voltages are present at both the input and output of the Pulsewave 760 switcher. Use extreme care in handling. Always turn off or turn down the power supply leading to the Pulsewave 760 switcher before plugging or unplugging wires.

- A. To prevent overheating, the sides and rear of the switcher require at least 6 cm of clearance. **DO NOT BLOCK THESE AREAS.**
- B. Do not operate the Pulsewave 760 switcher in extreme humidity ($\geq 95\%$), or where condensation can short the electrical elements.
- C. When operating the switcher in a coldroom, wrap it in a plastic bag and let it equilibrate to temperature for at least 2 hours. After this time, remove the bag and begin operation. Follow the same procedure for re-equilibration to room temperature.
- D. Do not store any item on top of the switcher.

Note: The Pulsewave 760 switcher will perform at temperatures between 0 and 35° C. It has also passed tests for operation with humidity between 0 and 95% under conditions where condensation is absent. Operating the switcher outside these conditions is not recommended, and will void the warranty.

The protection circuitry of the Pulsewave 760 switcher will protect the system against faults which may occur by supplying too much voltage at the inputs or overcurrenting at the outputs. The circuitry shorts the input device momentarily, which will usually cause the Pulsewave 760 fuse to blow. Replace the fuse (located next to the input jacks at the rear) with a 1A fast blow type. In extreme cases, when the protection circuitry is activated, the fuse in the power supply will blow. Replace with a fuse according to the power supply manufacturer's rating.

1.3 Specifications

Input voltage capacity	0-760 volts DC
Input current capacity	0-500 mA
Output terminals	Four jacks
Switch intervals	0.1 to 65,000 seconds
Forward/backward ratio	0.1 to 99.9:1, continuously adjustable, with 0.1 resolution
Overload and short protection	Fuse and electronic protection circuitry
Operating temperature	0-35° C
Operating humidity	0-95%, in the absence of condensation
Dimensions (W x L x H)	6.5 x 11.5 x 6.5 inches
Footprint	486 in ²
Weight	20 lb

1.4 Diagram of Front Panel Control Pads

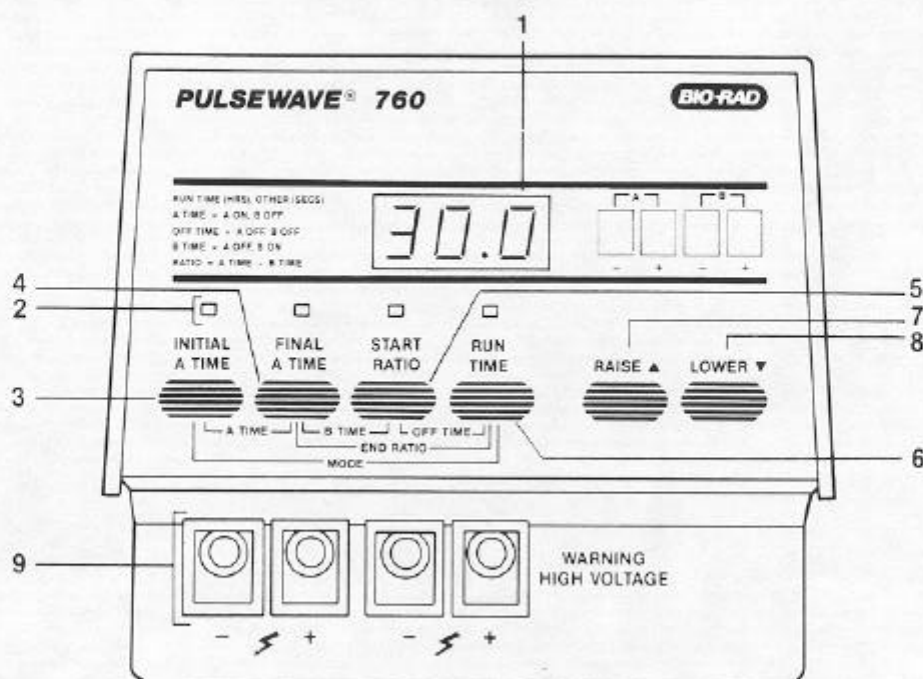


Fig. 1. Front Panel.

1.5 Description of Control Panel Functions

The area left of the LED display (1) shows the definitions of the output channels and the time units for run time and other pulse times. The area to the right diagrams the output of the A and B channels as used in orthogonal electrode electrophoresis chambers.

- | | |
|----------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1. LED display | Displays the initial and final A times, start ratio, off time, run time, and mode while they are being set. During a run, it can display all of the above, A and B time, ratio, and remaining run time. |
| 2. Display indicator | When a function button is pressed, the indicator light above it will illuminate, indicating the parameter which is being displayed. To change the display, use the raise and lower keys as described below. |
| 3. Initial A time | Press this button to set the A channel pulse time (seconds) at the beginning of a pulsed field run. For FIGE, this corresponds to the forward time. |
| 4. Final A time | Press this button to set the A channel pulse time for the end of a pulsed field run. If no ramp is required, the final A time is set equal to the initial A time. A linear ramp is generated if the final A time is different from the initial A time. |

5. Start ratio Press this button to set or view the ratio of forward to backward pulses. The backward time (or B channel) is automatically set given the ratio and forward time (A channel). For CHEF, PFG, and OFAGE, the ratio is set to 1 (the default condition).
6. Run time Press this button to set the run time (hours) or to view remaining run time. Only after 3, 4, 5, and 6 (above) are set will the switching begin.
7. Raise Press this button to raise the initial or final A time, ratio, end ratio, off time, or mode.
8. Lower Press this button to lower the initial or final A time, ratio, end ratio, off time, or mode.
9. Output terminals Connect the leads to the electrophoresis apparatus here. For FIGE, the Programming Block must be inserted in outlet jacks position. For two OFAGE or two CHEF experiments, the Extender Block must be inserted in the outlet jacks.

Other displays (refer to labels below left set of 4 keypads):

- A. A time Press initial A time and final A time together to view present A time. Display indicators light above the keypads.
- B. B time Press final A time and start ratio together to view present B time. Display indicators light above the keypads.
- C. Off time Press start ratio and run time together to display or insert an off time between A and B pulses, or forward and backward pulses. Display indicators light above the keypads.
- D. End ratio Press final A time and run time together to set or display the end ratio of forward to backward pulses. A linear transition from start to end ratio is generated. Two display indicators light.
- E. Mode Press initial A time and run time together to set or display the mode of operation. Two display indicators light, and a number from 1 to 11 appears on the LED.

Section 2

Operation

2.1 Operation for OFAGE and CHEF

Connecting Power Supply and Gel Chamber

The four output jacks on the front of the unit are connected by wires to a single OFAGE chamber. The two left-most jacks are the A output and the two rightmost jacks are the B output. This arrangement is delineated to the right of the LED display on the control panel. Next, hook up the power supply leads to the rear of the Pulsewave 760 switcher. Observe the proper polarity in input and output. The Pulsewave 760 switcher contains protection circuitry which operates if the improper polarity and/or input voltage is selected. Energizing the external power supply with a reversed hook up will cause the Pulsewave switcher's 1A FB fuse to blow.

To use the Pulsewave 760 switcher with the CHEF-DR II electrophoresis system, consult the CHEF-DR II instruction manual. To set up two CHEF-DR II basic units with one Pulsewave 760 switcher, order the Extender Block (catalog number 170-3604) and the Extender Cables (catalog number 170-3630).

Turn on the power to the Pulsewave 760 switcher with the switch at the rear, right. Pulsing does not commence until the initial and final A time, start ratio, and run time are set as described below. Finally, turn on the power supply.

Setting and Checking Parameters

Data is entered by using the keypads. The light above the key(s) will light. The display shows the present value of the function selected. Use raise and lower to change the value to that desired. The left-most key is set for the initial A channel time, in seconds. It can be as high as 65,000 (=18 hrs). At 1,000 seconds, the display format is a decimal number followed by a "lazy t" for thousands (also used in final A time and off time). Next, the final A time is set with the next key to the right. Then, the ratio is set, which automatically determines the B channel time. The ratio is the time of pulsing of the A time to B time. With OFAGE or CHEF, the ratio is set to 1.0. Last, the run time is set, in hours. The output of the Pulsewave 760 switcher will terminate after this time, with a continuous beep. The power supply should be shut off as soon as possible after this time. Pressing any Pulsewave 760 key stops the buzzer. Note that some power supplies lack adequate no-load sensors. In these instances, power should be shut off immediately after the run time has expired. With Bio-Rad's Model 200/2.0 power supply, the no-load sensor is tripped if the current exceeds 100 mA. Since this current may not always be obtained, it is good practice to set the timer on the power supply to be equal to or less than the total run time on the Pulsewave 760 switcher.

Once the four parameters are set as described above, the Pulsewave 760 switcher will produce electrical output. Turn up the voltage of the power supply at this time. The Pulsewave 760 switcher produces some minimal voltage drop with most loads, but this may be compensated for by increasing the power supply voltage. You may wish to check the voltage in your electrode chamber against the output of the supply with a volt meter.

Important! Turn off the power supply first before pausing or turning off the Pulsewave 760 switcher.

Additional functions are:

- (a) A time — press the two left-most keys. The display will show the A on time at the moment of display. The display will update as A changes throughout a run.
- (b) B time — press the second and third key from the left. The display will show the B on time at the moment of display. The display will update as B changes throughout a run.

(c) End ratio — press the second and fourth keys from the left. The display will show the value of the start ratio at the last time it was changed, assuming the end ratio was not changed later. End and start ratio are the same unless the operator specifically changes the end ratio to some other value. Therefore it is not necessary to be concerned with the end ratio if ramping of the ratio is not desired.

(d) Off time — press the third and fourth keys from the left. Use raise and lower to set. The off time inserts a pause between the A and B on. During the off time, both A and B are off.

(e) Mode — press the left-most and right-most keys. The default is 1. To pause during a run (e.g. to inspect a gel or change the voltage), set the mode to 0. After the pause, reset the mode to any other mode. Other modes are described in Section 2.3.

To check parameters during a run, press the appropriate key. (Voltage is changed via your power supply controls). The connecting lines below the key pads indicate which pairs of keys must be pressed in order to see a display. For example, pressing initial A time and initial B time will display the current A time in the run. It is good practice to check these after setting the ratio and before setting the run time, to be sure the values were entered.

An optional Extender Block (catalog number 170-3604) is available to provide jacks for two OFAGE or CHEF experiments. See Figure 2.

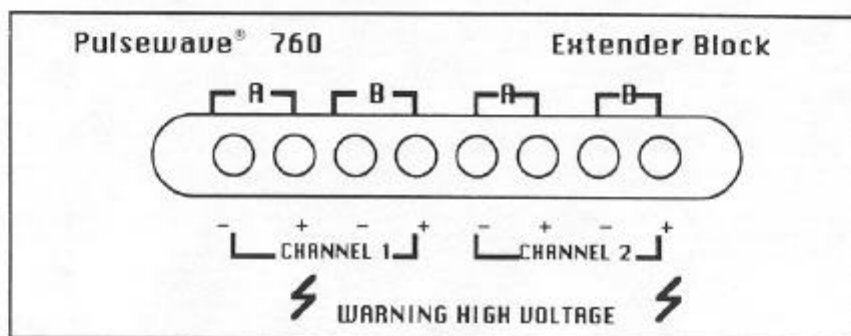


Fig. 2. Extender Block for two OFAGE or two CHEF experiments.

2.2 Operation for Field Inversion Electrophoresis

Connection to Power Supply and Gel Chamber

To use the Pulsewave 760 switcher for field inversion, first insert the Programming Block accessory into the jacks in front of the unit. The Programming Block provides jacks for two field inversion experiments (see Figure 3). Plug in the leads to the gel box, observing the correct polarity. The gel box should contain a single pair of electrodes, such as the Sub-Cell® submarine electrophoresis cell (catalog number 170-4300). Check that the power supply (Model 200/2.0 power supply, catalog number 165-4761) is turned off. Then, plug the power supply into the rear of the Pulsewave 760 switcher. Turn on the Pulsewave 760 switcher with the switch at the right rear. Finally, turn on the power supply.

Omit
Programming
Block for
single
gel box.

Important! Turn off the power supply first before pausing or turning off the Pulsewave 760 Switcher.

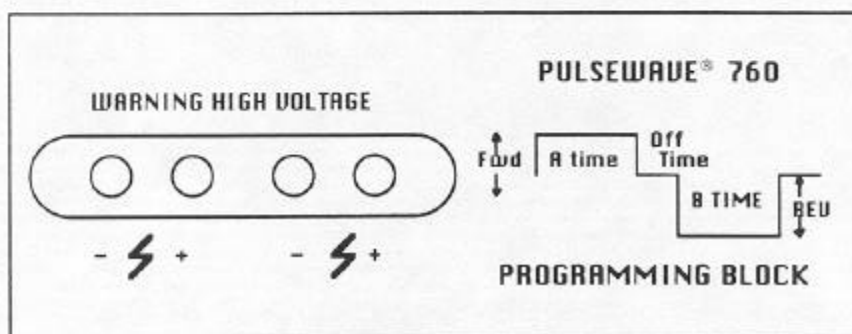


Fig. 3. Programming block for two field inversion experiments.

Setting and Checking Parameters

The A output is now the forward time, and the B output the backward time. Otherwise, parameters are set as in Section 2.1. The ratio is set to a number other than 1, e.g., 3.0. The backward time is automatically calculated and set after the forward time and ratio are set. Note, with a ratio of 3, the minimum initial A time is 0.3 seconds. During the run time selected, the Pulsewave 760 switcher will start with the selected A time and ramp to the final A time. The ratio of A/B may also change, if an end ratio was selected that differed from the start ratio. During the run, the run time display (if accessed) will count down, showing the time remaining. When run time reaches zero, the outputs turn off and the alarm will sound. To hear what the alarm sounds like, press both the raise and lower keys.

Parameters may be checked during a run as described in Section 2.1. To reset parameters on the Pulsewave 760 switcher, you may wait until the run time has expired, then select new values. Or, select mode 10 after the first set of conditions is established, and set parameters as described in Section 2.3. Next, select mode 11 to implement the mode 10 settings. Leave the unit in mode 11 during the run. The new parameters will become operational automatically after the first run-time expires. Shutting off the Pulsewave 760 switcher during a run will erase all parameters.

Section 2.3 Use of the Modes

Added flexibility in control of the pulses, as well as display, is provided with the mode function.

Mode Function

- 0 Pause. Suspends operations. Restart by selecting any other mode. It is not necessary to reset any other parameters.
- 1 Standard operating mode (default).
- 2 On-off electrophoresis. Uses the A output only. B is held in off condition for safety.
- 3 Computer interface. A special cable is connected from an external computer such as an IBM® PC, AT, Apple® II, or Macintosh™ computer to the round port at the rear of the Pulsewave 760 switcher, next to the power enter port. (Call Bio-Rad for details on the pinouts for the cable). The computer program controls the turn-on of the A and B outputs, and thus their ratio. A takes precedence, preventing A and B from being turned on simultaneously. For details, see the Appendix.
- 4 Link two Pulsewave 760 switchers. The output wires from the first unit are routed to the back of the second unit through a special cable. Information on this cable is available from Bio-Rad. For details, see the Appendix.
- 5 Beeper on. One beep at start of A cycle, two beeps at B start.
- 6 Countdown A and B times (when A or B displayed). No beep. After setting mode 6, press Initial A and Final A time keys simultaneously to display A and B times.
- 7 Alternates pulses between those defined by the initial A time and final A time.
- 8 Allows easy recovery from a power failure or interruption of a run. Set up all parameters as at the beginning of the run while in any other mode. Then select mode 8. Then select the approximate run time remaining at the time of the power failure. Other parameters will automatically be calculated.
- 9 Countdown with beep. Display countdown as in number 6 above.
- 10 Used with mode 11 to reset all parameters. Allows automatic change-over to new switch times, etc. during an experiment. Set the first set of parameters in mode 1. Then select mode 10 and set the second set of parameters. Finally, select mode 11. This causes the new values to go into effect when the first run time has counted down to 0. The Pulsewave 760 switcher must be left in mode 11 during the run.
- 11 Causes Pulsewave 760 switcher to run the parameters set with mode 10, after the first run time expires. In mode 11, the parameters displayed are the current values. The unit must be left in mode 11 during the run, although you can toggle to mode 10 to see new parameters at any time.

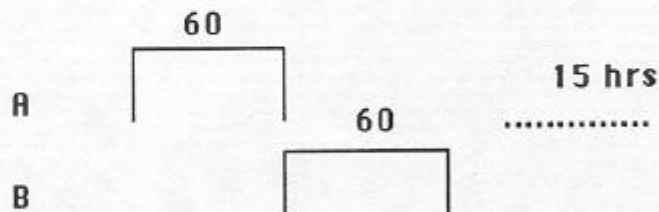
Section 3

Examples of Pulswave 760 Switcher Operation

The following examples illustrate operation of the Pulswave 760 switcher. The operation, keystrokes, and waveform are given for each. With the exception of examples 6 and 9, the Pulswave 760 switcher may also be left in modes 5, 6, or 8 during the run.

1. Pulsed field, typical conditions

Initial A time = 60 seconds
Final A time = 60 seconds
Start ratio = 1.0
Run time = 15 hours
Mode = 1



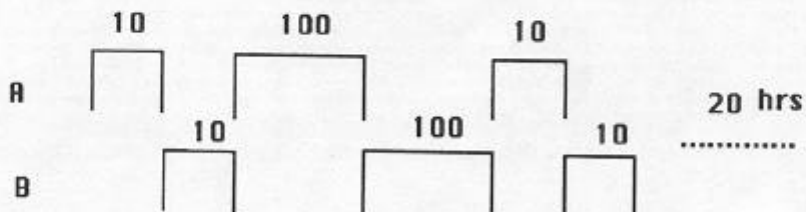
2. Pulsed field, time ramp

Initial A time = 20 seconds
Final A time = 80 seconds
Start ratio = 1.0
Run time = 20 hours
Mode = 1



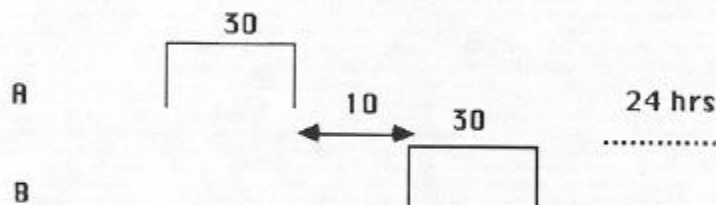
3. Pulsed field, alternating pulse times

Initial A time = 10 seconds
Final A time = 100 seconds
Start ratio = 1.0
Mode = 7
Run time = 20 hours
Mode = 1



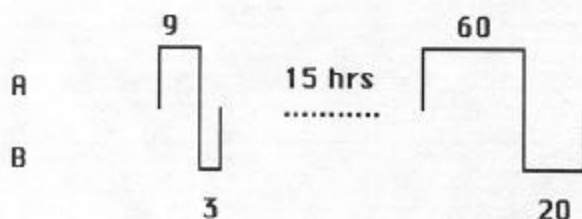
4. Pulsed field, with off-time

Initial A time = 30 seconds
 Final A time = 30 seconds
 Start ratio = 1.0
 Off time = 10 seconds
 Run time = 24 hours
 Mode = 1



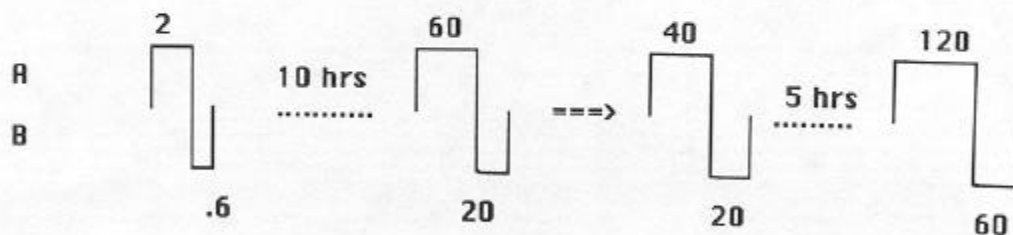
5. Field inversion, normal run

Initial A time = 9 seconds
 Final A time = 60 seconds
 Start ratio = 3.0
 Run time = 15 hours
 Mode = 1



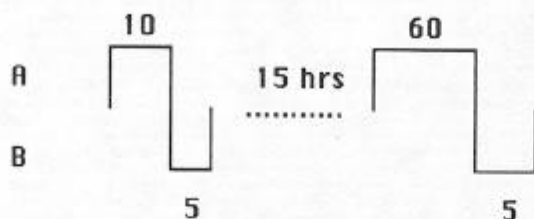
6. Field inversion, two steps

Mode = 1
 Initial A time = 2 seconds
 Final A time = 60 seconds
 Start ratio = 3.0
 Run time = 10 hours
 Mode = 10
 Initial A time = 40 seconds
 Final A time = 120 seconds
 Start ratio = 2.0
 Run time = 5 hours
 Mode = 11



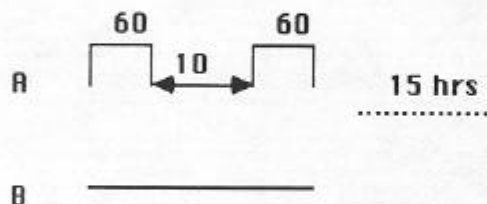
7. Field inversion, with ratio ramping (constant reverse times)

Initial A time = 10 seconds
Final A time = 60 seconds
Start ratio = 2.0
End ratio = 12.0
Run time = 15 hours
Mode = 1



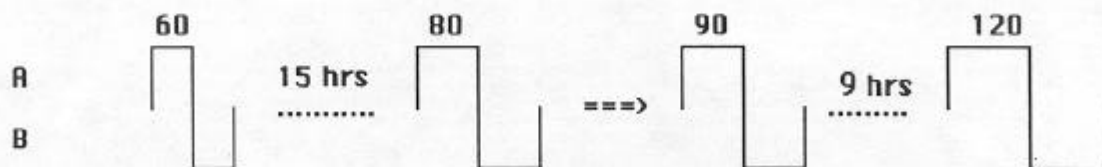
8. On-off electrophoresis

Initial A time = 60 seconds
Final A time = 60 seconds
Ratio = 6.0
Mode = 2
Off time = 0 seconds
Run time = 15 hours



9. CHEF electrophoresis, two steps, with ramps

Initial A time = 60 seconds
Final A time = 80 seconds
Ratio = 1
Run time = 15 hours
Mode = 10
Initial A time = 90 seconds
Final A time = 120 seconds
Ratio = 1
Run time = 9 hours
Mode = 11



Section 4

Applications

4.1 CHEF

CHEF is the most advanced pulsed field technique available, providing uniform fields and straight lanes. It is applicable to the separation of fragments ranging from 200 to 12 million bases.¹⁹ The equipment for CHEF is generally more complex and electrophoresis times are generally longer than those required for field inversion, described below. The following steps will set up the Pulsewave 760 switcher for resolution of 15-16 *Saccharomyces cerevisiae* (YNN 295) chromosomes:

1. Select mode 1
2. Set initial A time = 60 sec., final A time = 60 sec, start ratio = 1, and run time = 15 hr.
3. Select mode 10
4. Set initial A time = 90 sec., final A time = 90 sec, start ratio = 1, and run time = 9 hr.
5. Select mode 11 (Switching will begin)

The gel is 1% agarose in 0.5 x TBE. Electrophoresis is at 200 V, with buffer circulation and temperature maintained at 14° C.

Detailed protocols for CHEF sample preparation and electrophoresis are given in the CHEF-DR II instruction manual.

4.2 Field Inversion

Field inversion is the simplest and most economical of the pulsed field techniques. It is well suited for fragments under a million bases, especially in the 50,000 to 500,000 base range. It has the added advantages of short run times.

The Pulsewave system, which consists of Bio-Rad's Model 200/2.0 power supply (catalog number 165-4761), the Pulsewave 760 switcher, and the Sub-Cell® electrophoresis cell (catalog number 170-4300), can yield excellent results. In addition, you will need a small peristaltic pump with a flow rate of at least 0.5 gallons/min, such as GRI Model 14825-003. Tygon® tubing is connected to the pump and the ports of the Sub-Cell DNA cell, and to a water chiller (Bio-Rad's Model 4860 Refrigerated Water Recirculator) or to a passive cooling tank. The buffer should be equilibrated to between 9° and 14° C.

Caution: The circulating buffer is electrically conductive, and extreme care should be used in handling the pump, tubing, and other devices. Keep the tubing electrically insulated.
Bio-Rad is not liable for hazardous or improperly connected tubing.

Sample Preparation

DNA fragments may be loaded directly as described in reference 5. Chromosome-sized DNA generally must be prepared by the agarose block method, with digestion of protein *in situ* with Protease K². An excellent low melting agarose is Bio-Rad's Low Melt Preparative Grade Agarose (catalog number 162-0020).

Lambda ladders are excellent size standards up to approximately 600,000 bases. For larger DNAs, yeast chromosomal DNA prepared by the agarose block method is useful. The approximate sizes of yeast chromosomes have been described in *Microbiology Reviews*, Sept. 1985, 49:1-212.

Buffer and Gel

Pulsed field gels are typically 1-1.5% agarose (e.g. Bio-Rad's Molecular Biology Certified Agarose, catalog number 162-0133). The buffer is 0.5 X TBE. The buffer may need to be changed after 12 hours if the electrophoresis chamber is small.

Voltage and Current

FIGE gels are run at 10-15 volts/cm (200 V in the Sub-Cell electrophoresis cell). The current is typically 75 to 150 mA.

Pulsewave 760 Settings

Yeast chromosomes

Initial A time = 2 seconds, final A time = 60 seconds, ratio = 3:1, run time = 10 hours. After 10 hours, set mode = 0 and change the buffer. Set initial A time = 40 seconds and final A time = 120 seconds, and run time = 5 hours.

Fragments < 100 kb

To resolve fragments under 100,000 bases, very rapid switch times are required. A ramp of 0.3 to 1 second is suggested with a voltage gradient of 7.5 V/cm. Consult reference 24 for details.

The Importance of Ramping

A ramp is established when the switch interval at the start of the run is different from that at the end of the run. Ramping is important for two reasons. It allows you to selectively enhance resolution of fragments in a certain size class of DNAs, and it reduces or eliminates the tendency for very large DNAs to migrate faster than smaller DNAs (see reference 5).

The graph in Figure 4 shows the desired switch times (forward + reverse) needed for optimal resolution of DNAs in a given size class over 100,000 bases. The labels show the total switch time for the beginning and end of the run, with an arrow between them. The electrophoresis conditions were 1% agarose, 10 V/cm, 14° C, and 20 hours duration. A 3:1 ratio of forward to reverse interval was used. Conditions other than these will result in different curves.

Example

To resolve DNAs optimally between 200 and 300 kb, first find these sizes on the vertical axis. Then draw horizontal lines at 200 and 300 and note their intersections with the curves. The widest separation possible is achieved with the curve labeled 3 ->30 seconds. The 200 kb fragment will migrate 0.4 cm and the 300 kb fragment will migrate about 0.3 cm. Thus, to derive the switch times, divide each of the times on the graph (i.e. the 3 and the 30) so that the parts are in a 3:1 ratio. This is done by dividing the first time (3 sec) by 4. The result, 0.75, is the reverse time. The forward time is 3 times this number, or 2.25 seconds. (Total cycle is 2.25 + 0.75 = 3 sec.) Similar calculations are done with the 30 secs to get the final forward time. Thus, on the Pulsewave 760, the required ramp is Initial A time = 2.25 seconds forward, and Final A time = 22.5 seconds forward. The Pulsewave 760 switcher will automatically set up the reverse times (given the ratio) as 0.75 and 7.5 seconds, respectively.

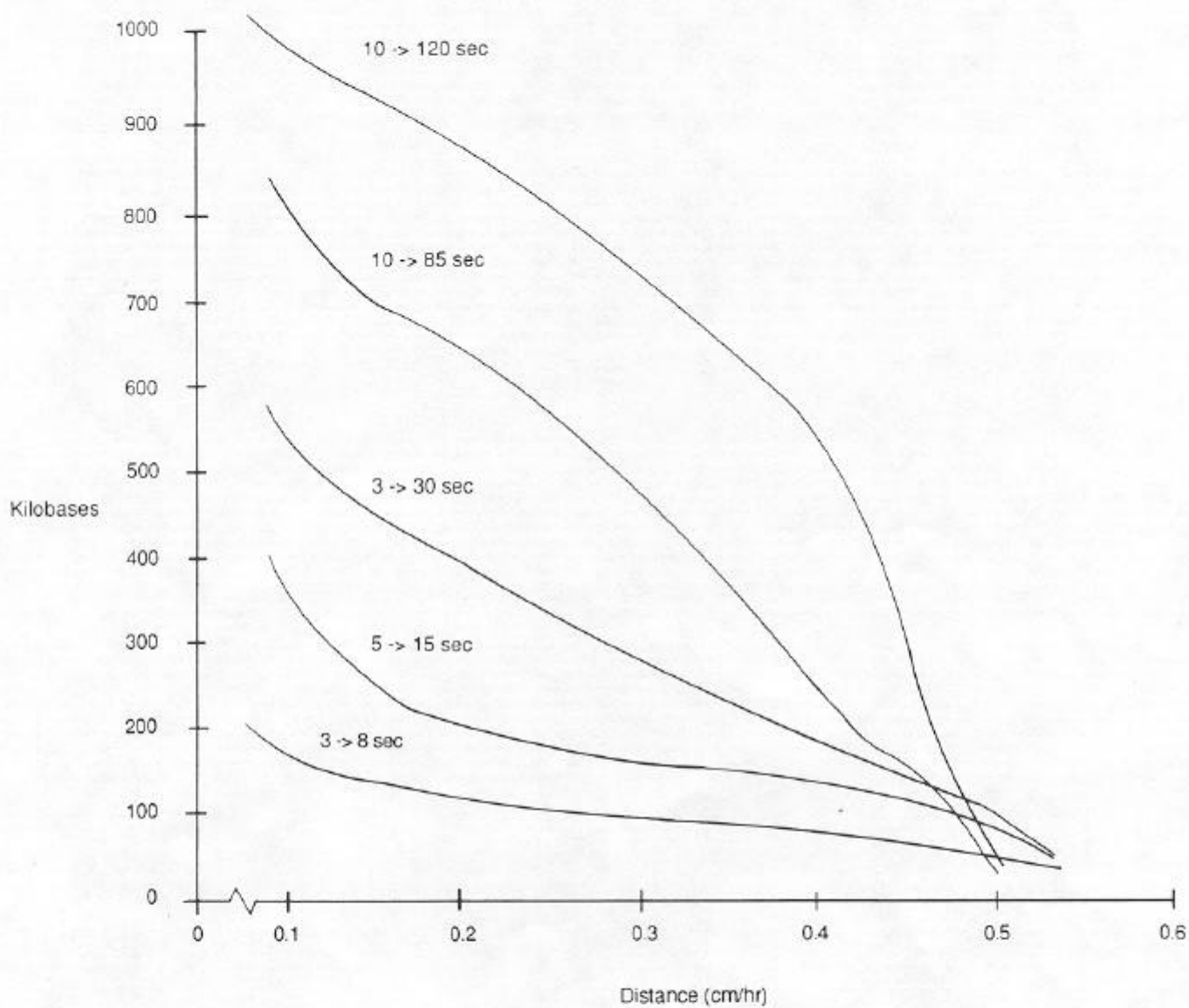


Fig. 4. Linear ramping conditions for field inversion.

4.3 Other Applications

1. Separation of open-circular DNA [FIGE].⁶
2. *Leishmania* chromosomal DNA, amplification of drug resistance genes [PFG*].⁷
3. Antigenetic variation in trypanosomes [PFG].⁸
4. Mammalian chromosomal DNA [PFG].⁹
5. *E. coli* chromosomal DNA [FIGE].¹⁰
6. Mammalian chromosome rearrangements [PFG].¹¹
7. Yeast chromosome mapping [OFAGE].¹²
8. Unidirectional pulsed field of single strand breaks in DNA.¹³
9. Mammalian chromosome mapping [FIGE].¹⁴
10. *C. albicans* chromosomal DNA [PFG].¹⁵
11. *Leishmania*, clinical diagnosis [PFG].¹⁶
12. Human gene linkage analysis [FIGE].¹⁷
13. Computer modeling [FIGE].¹⁸
14. Yeast chromosome analysis [CHEF].^{4,19}
15. DNA over 5 megabases [CHEF].^{20,21}
16. Plant DNAs [CHEF].²¹
17. Visualization of DNA migration during electrophoresis [FIGE, CHEF].²²
18. Sample preparation.²³
19. Optimized field inversion parameters [FIGE].²⁴
20. Review of pulsed field electrophoresis.^{25,26}

*Pulsed Field Gradient

Section 5 Troubleshooting

<u>Condition</u>	<u>Cause</u>	<u>Remedy</u>
Pulsewave 760 switcher		
1. LED fails to illuminate	1a. Power cord is not plugged in	1a. Check connections
	1b. Line surge or instrument failure	1b. Replace 1A SB fuse, contact Bio-Rad
2. Unit fails to switch	2a. One of four parameters was not set (initial & final A time, ratio, run time)	2a. Check parameters
	2b. Mode 0 or 10 set	2b. Change mode
	2c. 760 V or 1.25 A exceeded	2c. Replace 1 A FB fuse under round connector
3. Unit displays "ERR"	3a. Internal fault	3a. Contact Bio-Rad
Application		
4. Bands smeared in one section of lane	4a. Switch time or ramp incorrect	4a. Rerun with new parameters
5. Bands smeared, high background	5a. All protein not digested	5a. Increase protease concentration
	5b. DNA sheared, degraded	5b. Check sample preparation
	5c. Poor grade agarose	5c. Use higher grade
6. Broad bands	6a. Buffer breakdown	6a. Change after 10 hours or less
	6b. Too low agarose %	6b. Increase %
7. Lanes drift strongly to one side	7a. Ratio not equal to 1 (CHEF)	7a. Adjust ratio
	7b. Buffer flow not uniform	7b. Level the chamber
	7c. Internal fault	7c. Contact Bio-Rad

Section 6

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Section 7

Appendix

The following details the connection of special cables for modes 3 and 4.

- 1) Mate to the jack with a Switchcraft SL404M cord plug.
- 2) In Mode 3
 - a) Pin 1 controls "A" output; a low (0.4 V or less) turns on the "A" output. Pin 1 takes precedence over any control of the "B" output. Pin 1 must be high (3.5 V or higher) to allow actuation of the "B" output.
 - b) Pin 3 controls "B" output; a low (0.4 V or less) turns on the "B" output. Pin 1 must be high.
 - c) Pin 4 is ground reference.
 - d) The appropriately-wired interface cable (should be shielded if over 3 feet) must connect to two pins of a parallel port at the external computer. With Mode 3 selected on the Pulswave 760 switcher, control of Pins 1 and 3 will control "A" and "B" outputs as desired.
- 3) In Mode 4
 - a) The Pulswave 760 switcher which is operating in Mode 4 is denoted as "subsidiary." Pin 4 of the interface connector is ground, and Pin 1 is a synchronization line. Pin 1 must stay high the expected time of "A" time or longer, and must go low by the end of "B" time.
 - b) The "primary" Pulswave 760 switcher should be operated in any Mode except 3 and 4. The primary Pulswave 760 switcher will assert Pin 2 of the primary Pulswave 760 interface connector (will go high) at the start of primary time "A." Pin 2 of the primary Pulswave 760 switcher will go low at the start of primary time "B."
 - c) The interface cable should be wired so that Pin 2 of the primary Pulswave 760 switcher connects to Pin 1 of the subsidiary; Pin 4 of the primary (ground) should connect to Pin 4 of the subsidiary. Switchcraft SL40M cord plugs and shielded wire (if over 3 feet) should be used.

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