

1. Pulse Controller Function and Description

1.A. PC 500 Pulse Controller

The PC 500 SwitchBack Pulse Controller is designed for performing field inversion gel electrophoresis (FIGE) of double-stranded DNA molecules. The SwitchBack is used in conjunction with a power supply to run either a horizontal or vertical electrophoresis unit. When used in the reverse field mode for FIGE, the SwitchBack periodically reverses the polarity of the electrodes. As a result, molecules in the gel migrate in a forward direction and then, when the polarity is reversed, reorient themselves and travel backward. Because the forward pulse is always longer than the reverse, this results in a net forward migration. By selecting the appropriate pulse times, FIGE enables you to maximize resolution of DNA fragments in desired regions, from the kilobase to megabase size range. The SwitchBack also provides an alternating (cross-field) mode, for OFAGE and TAFE.

The SwitchBack can be programmed for single- or multi-phase runs. A single-phase run is controlled by one program. A multi-phase run is controlled by several single programs concatenated together. A single-phase run can have a constant pulse time throughout, or a ramped pulse time that increases linearly during the run. For a ramped separation, all you need to enter is the total run time and the initial and final pulse times; the SwitchBack automatically computes and executes the intermediate pulse times. A multi-phase run is a concatenation of up to five single-phase runs. As many as 30 single-phase and 30 multi-phase programs can be stored in the SwitchBack memory.

1.B. Applications and Theory

Applications of Pulsed Field Gel Electrophoresis (PFGE) are numerous and diverse. These include preparing large DNA fragments for cloning in yeast artificial chromosomes (YAC's) and P1 vectors, identifying restriction fragment length polymorphisms

(RFLP's) and construction of physical maps, detecting in vivo chromosome breakage and degradation, and determining the number and size of chromosomes (electrophoretic karyotype) from yeasts, fungi, and parasites such as Leishmania, Plasmodium, and Trypanosoma.

Although the theory of pulsed field electrophoresis is still a matter of research, some qualitative statements can be made about the movement of DNA in agarose gels during PFGE. Briefly, during continuous field electrophoresis, DNA above 30-50 kb migrates with a mobility independent of size. This is seen on a gel as a single large diffuse band. If, however, the DNA is forced to change directions during electrophoresis, different sized fragments within this diffuse band begin to separate from each other. With each reorientation of the electric field relative to the gel, smaller sized DNA will begin moving in the new direction more quickly than the larger DNA. Thus, the larger DNA lags behind, providing a separation from the smaller DNA.

Many types of PFGE instrumentation are available. The simplest equipment is designed for FIGE, which periodically inverts the polarity of the electrodes during electrophoresis, subjecting DNA to a 180° reorientation. Only an electrical field switching module is needed and any standard vertical or horizontal gel box with temperature control can be used. Although extremely useful for separating relatively small DNA (4-1000 kb), FIGE's reorientation angle of 180° results in a separation range limited to under 2000 kb. Like other PFGE techniques, FIGE may exhibit mobility inversions during electrophoresis in which larger DNA can move ahead of smaller DNA. Pulse length ramping, in which the orientation pulse length is constantly increased during separation, will minimize inversions, and this capability is included in the SwitchBack.

Ideally, the DNA should migrate in straight lanes to simplify lane-to-lane comparisons. The original pulsed field systems used inhomogeneous electric fields that did not produce straight lanes, making interpretation of gels difficult. The simplest way to achieve straight lanes is to periodically reverse the electric field with a Pulse Controller. A standard gel box with parallel electrodes produces a homogeneous electric field.

1.C. Specifications

| | |
|-------------------------------|--|
| Power Requirements | 115 V~, 60 Hz, 5 W 230 V~, 50 Hz, 5 W |
| Fuse values | 115 V~ input: T 0.25A, 250V, 5x20 mm output: F 0.5A, 250V, 5x20 mm 230 V~ input: (2) F 0.16A, 250V, 5x20 mm output: F 0.5A, 250V, 5x20 mm |
| Programmable power parameters | Switched Voltage: 0–500 V== Switched Current: 500 mA max. |
| Timer parameters | Minimum: 1 min Maximum: 299 hrs, 59 min Run-in Time: 0 - 99 min Pulse Time Range: 0.01 s - 99.99 hrs Pulse Increments: 0.01 s, 0.01 min or 0.01 hr Forward and Reverse Pulse Ratio: 2.0:1 to 4.0:1 in increments of 0.1 |
| Memory storage | 30 single and 30 multi-phase programs (An internal battery maintains memory when power is shut off or interrupted) |
| Operating environment | Indoor use, 15–40 °C ambient |
| Relative humidity | ≤80% for 15–31 °C, decreasing linearly to 50% for 31–40 °C |
| Altitude | ≤2000 m |
| Installation category | II |
| Pollution Degree | 2 |
| Dimensions (wxdxh) | 22x23x13 cm (8.6x9x5 in.) |
| Weight | 1.6 kg (3.54 lbs) |
| Product Certifications | UL3101–1, CSA C22.2 1010.1, CE |

This declaration of conformity is only valid for the instrument when it is:

- ⇒ used in laboratory locations,
- ⇒ used as delivered from Amersham Biosciences except for alterations described in the User Manual, and
- ⇒ connected to other CE labeled instruments or products recommended or approved by Amersham Biosciences.

2. Important Information

Electrical Safety

The following items should be checked prior to initiating any experiment in electrophoresis.

Important: The instrument must always be used with the earth lead of the power cord correctly grounded to earth at the mains outlet.

Work area. The bench and floor should be dry.

High-voltage connections. All high-voltage leads used should be intact and not frayed. All plugs should have a fixed protective plastic sleeves that shields the plug as it is inserted into the power supply or Pulse Controller. Exposed plugs are a shock hazard and should be replaced with shielded plugs. Stackable leads that connect more than one gel unit to a single outlet are not recommended and should be replaced with shielded-style plugs.

Electrophoresis chambers. These should be covered when in use, with no openings large enough to allow fingers or other objects to make contact with the electrified buffer or electrodes.

If this equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

Only accessories and parts approved or supplied by Amersham Biosciences may be used for operating, maintaining, and servicing this product.



Informations importantes

Securité Electrique

Les points suivant doivent être vérifiés avant de commencer toute experience électrophorétique.

Important: l'instrument doit toujours être utilisé proprement connecté à la prise de terre.

Place de travail: la paillasse et le sol doivent être secs.

Connexions de haut voltage: Toutes les prises de courant à haut voltage, utilisé doivent être intactes et non effilochées. Toutes les prises ont une manche protectrice fixes en plastique qui les protègent quand elles sont branchées au générateur de courant ou le Pulse Controller. Des prises exposées (non protégées) sont un danger d'électrocution et doivent être remplacées par des prises avec protection. L'utilisation de multi-prises qui permettent de brancher plus d'un gel sur une sortie électrique du générateur ne sont pas recommandées et devraient être remplacées par des prises protégées.

Cuves électrophorétiques: elles devraient être couvertes durant leur utilisation. Assurez-vous qu'il n'y aie aucune ouverture suffisamment grande pour permettre à des doigts ou autre objets d'occasionner des contacts avec le tampon ou les électrodes.



Si l'instrument n'est pas utilisé en conformité avec les recommandations du fabriquant, les protections de sécurité qui équipent cet appareil peuvent être rendues inefficaces.

Seulement les accessoires et pièces détachées approuvés ou fournis par Amersham Biosciences sont recommandés pour l'utilisation, l'entretien et réparation de cet appareil.

3. User Interface

The user interface includes two sets of keys: the eight function keys are below the liquid crystal display (LCD); and the twelve alpha-numeric keys are at the right side of the control panel. (See Figure 1.)

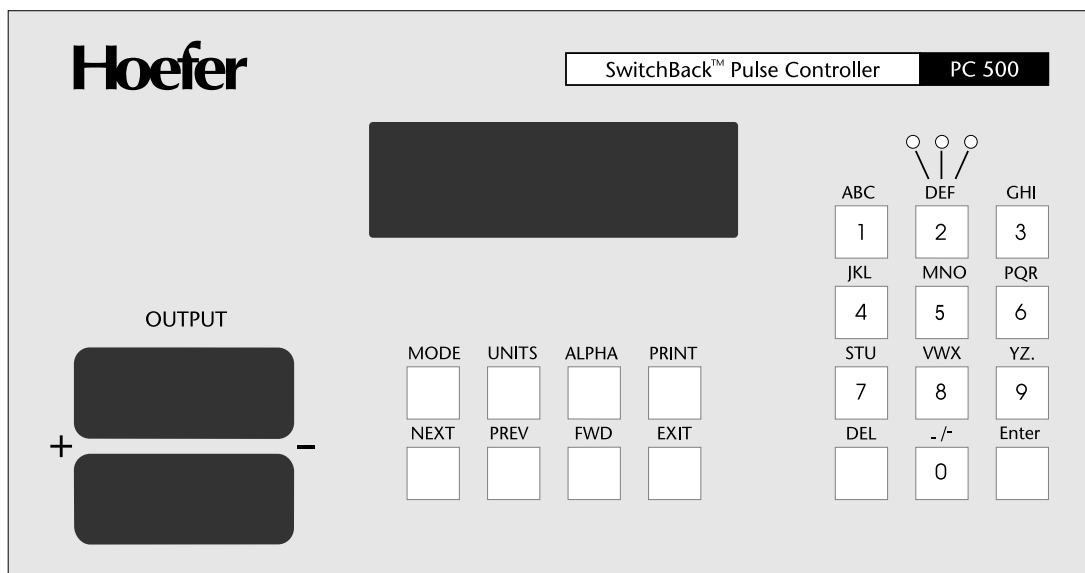


Figure 1. Switchback front panel

Function keys

| | |
|-------|--|
| MODE | When editing a single-phase program, the MODE key changes the operation from reverse field (REV) to alternating (ALT). The default mode for FIGE is reverse field. Pressing the MODE key toggles between the Single Main Menu and the Multi Main Menu. |
| UNITS | When editing a single-phase program, pressing the UNITS key cycles the Pulse Time (PT) setting through seconds, minutes, and hours. |
| ALPHA | Determines the selection of either the number or one of the three characters above the alpha-numeric keys (indicated by a lit lamp above the 2 key). The ALPHA key only operates while naming a file (see Step 5 in Section 5.B: Saving a Single-phase Program). |
| PRINT | Prints set up screens and directories. |
| NEXT | Scrolls down in the file directory. |
| PREV | Scrolls up in the file directory. |

| | |
|------|--|
| FWD | Moves the cursor to the next item. |
| EXIT | Exits the current screen. When a run is in progress, pressing EXIT pauses the run. Pressing EXIT again cancels the run. |

Alpha-numeric keys

The twelve keys to the right of the control keys are similar to the keys on a touch-tone telephone in that each key represents either the number on the key or one of the three letters or characters above the key. When naming files, pressing the ALPHA key sequentially lights the lamps above the 2 (also D, E, F) key. The position of the lit lamp indicates which of the three characters is activated (press the ALPHA key until the correct position is selected). When all three lamps are off, the numeric keys are active.

The DEL key deletes an entry. The Enter key completes a program file entry.

4. Operating Instructions

General Operating Tips

- Use longer pulse times to separate larger DNA fragments.
- A longer ramp (the difference between the initial and the final pulse time) will separate a wider range of DNA sizes.
- Band sharpness can be improved by using a higher concentration and/or a higher quality agarose.
- If the sample contains DNA fragments larger than 50 kb, the sample must be in agarose plugs for sample loading.
- If the sample contains DNA fragments larger than 200 kb, the gel should be cooled to 10–14 °C.

Operating Procedure

1. Cast the gels and assemble your horizontal or vertical electrophoresis unit. If the unit has cooling capability, we recommend cooling to 10 °C during FIGE.
2. Connect the power cord to the MAINS IN socket on the back panel of the Pulse Controller and plug into a properly grounded wall outlet. (See Figure 3.)
3. Connect the leads from your electrophoresis unit to one of the two sets of OUTPUT receptacles on the front of the Pulse Controller.
4. Connect the INPUT leads from the back of the SwitchBack to an approved power supply. Plug the power supply into a grounded wall outlet. (Figure 2 illustrates instrument hook up.) Do not turn the power supply on yet.
5. **Optional:** If you intend to print screens or programs, connect the printer cable to the parallel printer port at the back of the Pulse Controller.
6. For ease in viewing the screen, a retractable metal stand is attached to the underside near the front feet of the unit. To use, pull the stand forward and allow it to snap securely into place.
7. Turn the Pulse Controller on by setting the mains power switch to the on (–) position.
8. To program the Pulse Controller, see either Section 5: Programmng and Performing a Single-phase run, or Section 6: Programming and Performing a Multi-phase run.
9. When programming is complete, prepare and load your samples.

10. Turn on the power supply. We recommend using constant voltage. Maximum voltage is 500 V $\overline{=}$; maximum current is 500 mA. See Section 13: Running Parameter Guidelines.
11. Perform the run according to the instructions in Section 5.C.: Performing a Single-phase run, or Section 6.C.: Performing a Multi-phase run.

Note: In the event of a power interruption, the SwitchBack automatically continues the programmed run once power is restored.

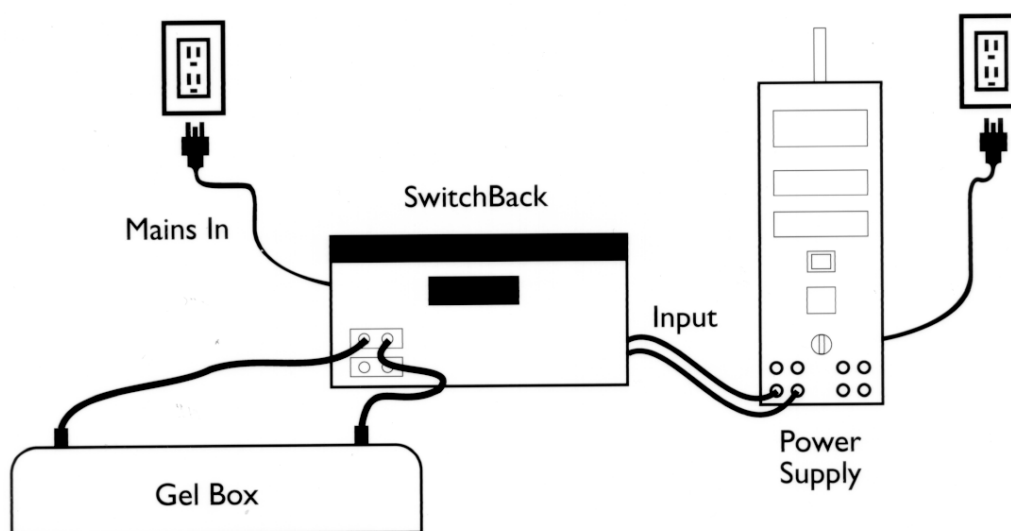


Figure 2. Instrument hook up

5. Single-phase runs

5.A. Programming a Single-phase run

1. When the mains power switch is set to on (–), three long beeps sound and the screen displays:

```
SwitchBack  
Pulse Controller  
Rev X.XX  
Scientific
```

2. Press any key. The screen will now display:

```
= SINGLE MAIN MENU =  
<1> Edit   <2> Run  
<3> Filer Menu  
<4> Print File
```

3. Press 1 (EDIT) to begin programming a single-phase run. The screen will now display:

```
Run-in 0 m blank:S00  
Run 0:00 (hr:min)  
PT 00:00 to 00:00 Sec  
Mode REV  F/R 3. 0:1
```

(blank:S 00 is the default name and location of an as-yet unspecified file.)

4. The default mode of operation is reverse field (REV) for FIGE. To switch to alternating mode for OFAGE and TAFE, press the MODE key.

The edit screen for alternating mode will appear:

```
blank:S 00  
Run 0:00 (hr:min)  
PT 00:00 to 00:00 Sec  
MODE ALT
```

The programming for the alternating mode is similar to that for the reverse field mode except there is no run-in time or forward/reverse pulse time ratio to be set. Saving, loading, creating multi-phase runs and all other functions are identical to those for the reverse field mode.

5. Enter the values for each parameter:
 - a. Key in the desired value. The cursor itself does not move, but the numbers entered scroll from right to left. Press the DEL key to delete the right-most character.
 - b. Press the FWD key.
 - c. Enter in the next value and press the FWD key.

Run-in: Run-in time is an optional setting that designates a conventional electrophoresis period before the pulse program begins. Run-in time is only programmable in the reverse field mode (REV). Run-in time can be set from 0 to 99 min. **Note:** It is **not** included as part of the run time.

Run: Run time can be set from 1 to 299 hrs, 59 min.

Pulse Time: When entering the pulse time (PT), the initial and final pulse times must have the same time units, *i.e.* s, min, or hr. To switch between time units, press the UNITS key. Initial and final pulse times can be set from 0.01 to 99.99 s, 0.01 to 99.99 min, or 0.01 to 99.99 hr. The intermediate pulses used during the run are automatically determined by the program.

Mode: The default mode is reverse field (REV) for FIGE. Alternating mode (ALT) for OFAGE or TAFE can be selected by pressing the MODE key.

Forward/Reverse Ratio (F/R): When you select reverse field (REV), F/R indicates the forward/reverse ratio of each pulse. It can be set from 2.0:1 to 4.0:1. The default setting is 3.0:1.

- d. To change a value, press the DEL key. The value will be deleted from right to left. Enter the correct value.
 - e. If you enter a value that is outside the programmable range, the closest allowable value will automatically display. For example, if you enter an F/R ratio of 1.0:1, the lowest allowable value of 2.0:1 would be set; and if you enter an F/R ratio of 5.0:1, the highest allowable value of 4.0:1 would be set. Press the FWD key to see the programmed value.
6. To print this screen, press the PRINT key.
7. After entering the last value (for the F/R pulse time ratio) and printing the screen (optional), press the Enter key. The Single Main Menu screen will display.
8. To save the program, press 3 (FILER MENU) and proceed to Section 5.B.: Saving a Single-phase Program. We recommend that you always save the program. Otherwise, the program will be lost when the SwitchBack is turned off at the end of a run or when the program is aborted.
9. To start the run, proceed to Section 5.C.: Performing a Single-phase run.

5.B. Saving a Single-phase Program

1. After you have finished entering or editing a program and pressed the Enter key, the Single Main Menu screen will display. To save the program, press 3 (FILER MENU). The Single Filer Menu screen will appear:

```
= Single Filer Menu =
<1> Save <2> Del
<3> Load <4> Format
<5> Dir <6> Prt Dir
```

2. The Directory can store up to 30 programs. To save a program, press 1 (SAVE) to access the directory. Each file in the directory is identified by a number and can be named. When you are setting up your first file, the screen will look like this:

```
SØ1:empty SØ2:empty
SØ3:empty SØ4:empty
SØ5:empty SØ6:empty
Store program at SØ1
```

The program defaults to the next empty file for storage. If you wish to store the program in a different file, use the delete key to delete the default number and then use the numeric keys to enter the desired file number.

3. Press the Enter key. The screen will now show:

```
File will be saved
as _ _ _ _ .
```

(Note: File name is optional)

4. **Optional**—naming a file. If you do not wish to name it, press the Enter key to return to the Single Main Menu. The file will be saved as the number selected in Step 2 above. To begin the run, proceed to Section 5.C: Performing a Single-phase run. To name the file, proceed to Step 5 below.
5. A file name may contain up to five characters. The characters may be alphabetical or numeric or a combination. To name a file, use the ALPHA key in combination with the 12 keys on the right side of the unit:

a. Note the three small red lamps above the DEF key. When all of the lamps are off, the key pad is in numeric mode. Any lit lamp indicates that: the key pad is in the alphabetical mode, and the active letter position is the same as the active lamp position for positions 0–9.

b. If you wish to type alphabetical characters and no lamps are lit, press the ALPHA key. The lamp above the D (farthest left) will light. . In this position, the letter or symbol to the left will be entered when a key (0-9) is pressed.

If you press ALPHA again, the center lamp (above the E) will light. In this position, the letter or symbol in the center will be entered when a key (0-9) is pressed.

If you press ALPHA a third time, the lamp on the right (above the F) will light. In this position, the letter or symbol at the right will be entered when a key (0-9) is pressed.

If you press ALPHA a fourth time, no lamps will be lit, indicating the keyboard is in numeric mode.

c. To change a character, press the DEL (delete) key and enter a new character.

6. After entering the file name, press the Enter key to save the program. The screen will momentarily display before returning to the Single Filer Menu screen:

-Filer Message-
File is saved.

7. Press the EXIT key to return to the Single Main Menu. At this point you may print the file (see Steps 8 and 9 below) or run the program (see Section 5.C. below).
8. To print a file after it has been entered and saved, press 4 (PRINT FILE). The screen will display the first six files and the message PRINT PROGRAM AT S_1. If you wish to print a different file, use the DEL key to delete the default number. Use the numerical keys to enter the desired file number. Press the Enter key. The screen will display:

File now printing
Press any key
to abort...

9. After the printing is complete, the Single Main Menu screen will appear. To run the program, proceed to Section 5.C. below.

5.C. Performing a Single-phase run

1. Make sure the INPUT leads from the SwitchBack Pulse Controller are securely connected to an approved power supply. Make sure the leads from your electrophoresis unit are securely connected to the receptacles marked OUTPUT on the front of the Pulse Controller. Load the samples, turn on the power supply, and set the voltage. We recommend using constant voltage. Maximum voltage is 500 V $\overline{=}$; maximum current, 500 mA. For suggested running conditions, See Section 13.
2. To start the run, go to the Single Main Menu and press 2 (RUN). If you have programmed a run-in time, the Run-in screen will appear:

```

=== Run-in ===
File TEST1:S01
Elapsed Time 01:15
Program Time 02:00
  
```

3. After the run-in is complete, the run will begin and the Run Screen will appear:

```

TEST1:S01 Run Time
                0:00:10E
F/R 3.0:1    4:10:00S
Mode:REV 01.00 Sec PT
  
```

In this example, the single-phase run file name is TEST1. The single-phase run file number is S01. The elapsed time (E) is 10 seconds. The forward/reverse ratio (F/R) is 3:1; the set time (S) is 4 hrs, 10 min. The unit is running in the reverse field (REV) mode and the current pulse time (PT) is 1 second.

4. To pause a run, press the EXIT key. When the run is paused, the screen will read:

```

Run is paused...

To continue, 'ENTER'
to end run 'EXIT'
  
```

To continue the run, press the Enter key. To cancel the run, press the EXIT key a second time.

Note: Always use the EXIT-EXIT sequence to cancel a run. Turning off the unit does **NOT** end the run; it only interrupts it. This feature causes the run to resume once power is restored (this is also the case when power is restored after a power failure.)

5. Once the run is complete, the Pulse Controller will sound several beeps and the screen will show the program parameters in effect at the end of the run.

Note: Due to rounding error, the elapsed time at the end of a run may be slightly less than the programmed run time. This discrepancy can be particularly noticeable with very short pulse times.

6. Press the EXIT key to return to the Single Main Menu.
7. Turn off the Pulse Controller and turn off the power supply. Disconnect the power supply leads. Disconnect the leads to the electrophoresis unit.

6. Multi-phase runs

As many as five single-phase programs may be concatenated to form a multi-phase program. After programming and saving single-phase programs according to the instructions in Section 5.A. and 5.B. above, follow the instructions below to create a multi-phase program.

6.A. Programming a Multi-phase run

1. From the Single Main Menu, press the MODE key to switch to the Multi Main Menu. The screen will show:

```
= Multi Main Menu =  
<1> Edit    <2> Run  
<3> Filer Menu  
<4> Print File
```

2. To program a multi-phase run from the Multi Main Menu, press 1 (EDIT). The screen will show the file numbers and names of the first four single-phase run files as well as a line of SØØ characters which you will use to select the single file numbers to be concatenated into your multiple (multi) run.

```
SØ1:TEST1    SØ2:TEST2  
SØ3:TEST3    SØ4:empty  
Multi-Run:blank:MØØ  
SØØ,SØØ,SØØ,SØØ,SØØ
```

To display other single-phase programs on the screen, press the NEXT or PREV keys. Two files are displayed at a time. The entire directory of 30 files can be scrolled through by pressing these keys.

Note: For a printed directory of the single files, refer to Section 12: Printing the Directory.

3. Key in up to five single-phase file numbers in the order you wish them to run. Press the FWD key to advance the cursor from one file number to the next. SØØ is considered the end marker for multi-phase runs if there are fewer than five single-phase programs.

Note: When a single program is used to create a multiple-program, the run-in time is still executed. If this is not desired, edit and save a version of the single-program without a run-in time. (See Section 9: Editing and Saving a Program.)

4. After the last file number is entered, press the Enter key to return to the Multiple Main Menu screen.
5. To save the program, press 3 (FILER MENU) and proceed to Section 6.B.: Saving a Multi-phase program. **We recommend always saving the multi-phase program** because it will be lost when the Pulse Controller is turned off at the end of a run or when the program is cancelled.
6. If you are ready to start the run, proceed to Section 6.C.: Performing a Multi-phase run.

6.B. Saving a Multi-phase Program

1. After you have entered the single file numbers you wish to concatenate into a multi-phase file and pressed Enter, the Multi Main Menu Screen will appear.
2. From the Multi Main Menu, Press 3 (FILER MENU). The Multi Filer Menu screen will appear.

```
= Multi Filer Menu =
<1> Save <2> Del
<3> Load <4> Format
<5> Dir <6> Prt Dir
```

3. Press 1 (SAVE). The screen will change to the Multi-phase Directory.

```
MØ1:empty MØ2:empty
MØ3:empty MØ4:empty
MØ5:empty MØ6:empty
Store program at MØ1
```

The program will automatically point to the first empty file for storage. If you wish to store the program in a different file, use the DEL key to delete the file number and then enter the desired file number using the numeric keys.

4. Press the ENTER key. The screen will display:

```
File will be saved
as_ _ _ _ _
```

(Note: File name is optional.)

5. **Optional:** Naming a Multi-phase file. The procedure for naming a multi-phase program is the same as for a single-phase program. For instructions, see Step 5 in Section 5.B.: Saving a Single-phase Program.

If you do not wish to name the file, press the Enter key. The file will be saved as the number you selected in Step 3 above.

6. After entering the file name, press the Enter key. The screen will momentarily display:

```
-Filer Message-
File is saved.
```

Then the Multi Filer Menu screen will appear.

7. To go back to the Multi Main Menu, press the EXIT key. If you are ready to start the multi-phase run, proceed to Section 6.C. below.

6.C. Performing a Multi-phase run

1. Make sure the leads from an approved power supply are securely connected into the receptacle marked INPUT at the back of the Pulse Controller. Make sure the leads from your electrophoresis unit are securely connected to the receptacles marked OUTPUT on the front of the Pulse Controller. Load your samples, turn on the power supply, and set the voltage. We recommend using the constant voltage setting. Maximum voltage is 500 V $\overline{---}$; maximum current, 500 mA. For suggested running conditions, See Section 13.
2. To start the run, go to the Multi Main Menu and press 2 (RUN). The screen will display:

```
Starting a Multi
File Run. . .
```

Then:

```
Starting a Single
File run from
a Multi File. . .
```

3. The run screen will then appear. If a run-in time was specified, the screen will be similar to the following example in which a ten minute run-in time was specified:

```
=== Run-In ===
File TEST1:S Ø1 1/5
Elapsed time ØØ:ØØ
Program time 1Ø:ØØ
```

In this example, there are five single-phase programs concatenated into a multi-phase program. The name of the multiple file is TEST1 and the single file number currently running is SØ1. "1/5" (indicated at the end of the second line) reports that the first of five files is currently running.

4. After the run-in time has elapsed the run screen will appear:

```
TEST1:SØ1 Run Time
Multi 1/5 Ø:ØØ:1ØE
F/R 3:Ø:1 4:ØØ:ØØ S
Mode:REV Ø1.ØØ Sec PT
```

In this example, the multi-phase program name is TEST1. The single-phase run in progress is SØ1. Multi 1/5 indicates that the run in progress is the first of five concatenated runs. The elapsed time (E) is 10 s. The forward/reverse ratio (F/R) is 3:1; the set time (S) is 4 hr. The unit is running in the reverse field (REV) mode and the pulse time (PT) is 1 s.

5. To stop a run once it has started, press the EXIT keyThe display will show:

```
Run is paused . . .
To continue 'ENTER'

To end run 'EXIT'
```

- When the multi-phase run is complete, the Pulse Controller will sound several beeps and the final screen will show:

```
Multiple run is now
completed.

Press any key. . .
```

- Press the EXIT key to return to the Multi Main Menu.
- Turn off the Pulse Controller and turn off the power supply. Disconnect the power leads. Disconnect the leads to the electrophoresis unit.

7. Loading a Program

- To load a single-phase program, go to the Single Filer Menu Screen. To load a multi-phase run, go to the Multi File Menu screen. Press 3 (LOAD). The screen will now show either the directory of single files or multiple files. If you had two single-phase programs saved and named, the Single File Directory would look like this:

```
SØ1NAME1  SØ2NAME2
SØ3empty  SØ4empty
SØ5empty  SØ6empty
LOAD PROGRAM AT SØ1
```

If you are loading a multi-phase program, the screen will look the same as above except that the file numbers will begin with “M”.

- The default setting is SØ1 for single-phase run files; MØ1 for multi-phase run files. To load a different program, use the DEL key to delete the 1 and then enter the desired file number, *e.g.*, Ø2, Ø3. Press the Enter key. The screen will display:

```
- - Filer Message - -

File is loaded.
```

- The screen will return to the Single or Multiple Filer Menu. Press the EXIT key. The Single or Multi Main Menu screen will appear.
- To run the program, press 2 (RUN). The screen will momentarily display:

```
Starting a Single (or
Multi)

File Run . . .
```

- For a complete description of run progress, see either Section 5.C.: Performing a Single-phase run or Section 6.C.: Performing a Multi-phase run.

8. Editing and Saving a Program

1. To edit a single or multi-phase program, first load it into memory: See Section 7: Loading a Program.
2. Once the program is loaded, press the EXIT key to return to the Single or Multi Filer Menu.
3. Press 1 (Edit).
4. Use the FWD key to step the cursor to the desired position. Use typeover, or the DEL key, to change values.

To cancel the edit, press the EXIT key. This will return to the Main Menu without altering either the program copy loaded into memory, or the original program stored in the directory.

5. To load the changes into memory, press the Enter key. This will return you to the Main Menu. If desired, you may test the edited program by pressing 2 (Run).
6. To save the edited program to the Directory, press 3 (Filer Menu) from the Main Menu. Then press 1 (Save). You can either replace the original program (see “a” below) or save it as a new program (see “b” below).
 - a. To accept these defaults, press the Enter key. This replaces the original program in the Directory with the edited version.
 - b. To change the default name and location, use typeover, or the DEL key, to specify a new name and location. Then press the Enter key. This saves the edited version to the Directory, without replacing the original program.

9. Deleting a Program

1. To delete a single-phase file, go to the Single Filer Menu and press 2 (DEL).
2. To delete a multi-phase run file, go to the Multi Filer Menu and press 2 (DEL).
3. The screen will display the first six files in the directory along with the message Delete file at SØ1 (for the Single File Directory) or MØ1 (for the Multiple File Directory). To see other files in the directory, press the NEXT key. To review previous files, press the PREV key.

Note: The default setting is SØ1 or MØ1. To delete a different file, press the delete key (DEL) to delete the number 1 and then enter the correct file number.

4. Press the Enter key. The screen will momentarily display the message that the file has been deleted, and the Single or Multi Filer Menu will appear.

10. Formatting the Directory

To reformat and clear the entire directory of single-phase and multi-phase files, bring up the Single or Multi Filer Menu and press 4 (FORMAT). The screen will display the message “Are you sure?”. Press Ø for no, or 1 for yes. If you want to completely clear all stored files, press 1. The screen will momentarily display “Format complete” and then return to the Filer Menu.

11. Viewing the Directory

To view the directory, go to the Single or Multiple Filer Menu and press 5 (DIR). The first eight files will appear. To view more files, press the NEXT key. To review previous files, press the PREV key. In this way you can page through the entire directory. To return to the Filer Menu press the EXIT key.

12. Printing the Directory

For a printout of the file names and numbers in a particular directory, go to either the Single Main Menu or the Multi Main Menu and press 3 (FILER MENU). On the Filer Menu Screen, press 6 (PRT DIR). The screen will display:

```
Printing directory
Press any key
to abort...
```

When the printout is complete, the Filer Menu screen will display. Press the EXIT key to return to the Single or Multi Main Menu.

13. Running Parameter Guidelines

For each set of conditions, these factors were the same: 1% GTG agarose gels were run on the HE 100 SuperSub Horizontal Electrophoresis Unit with buffer circulation. The 0.5X TBE buffer was cooled to 10 °C. The power supply was operated in constant voltage mode.

Single-phase program for DNA in the size range of 0.5–25 Kb

Voltage: 230 V_{rms} (7.9 V/cm)
 Run-in Time: 10 min
 Run Time: 16 hrs
 Pulse Time: 50 msec (no ramp)
 Mode: Reverse (REV)
 F/R Ratio: 2.5:1

Single-phase program for DNA in the size range of 8.3 to 48 kb

| | | | |
|--------------|---------------------------------|------|---------------------------------|
| Voltage: | 150 V _{rms} (5.2 V/cm) | —OR— | 150 V _{rms} (5.2 V/cm) |
| Run-in Time: | 10 min | | 10 min |
| Run Time: | 32 hrs | | 28 hrs |
| Pulse Time: | 0.7 to 2.3 s | | 0.6 to 2.0 s |
| Mode: | Reverse (REV) | | Reverse (REV) |
| F/R Ratio: | 2.0:1 | | 3.0:1 |

Multi-phase program for DNA in the size range of 5 to 250 kb

| | RUN 1 | RUN 2 |
|--------------|---------------------------------|---------------------------------|
| Voltage: | 200 V _{rms} (6.9 V/cm) | 200 V _{rms} (6.9 V/cm) |
| Run-in Time: | 10 min | 0 |
| Run Time: | 8 hrs | 8 hrs |
| Pulse Time: | 1 to 20 s | 0.8 to 1.5 s |
| Mode: | Reverse (REV) | Reverse (REV) |
| F/R Ratio: | 3.0:1 | 3.0:1 |

Note: If the sample DNA is larger than 50 kb, it must be in agarose plugs.

Single-phase program for DNA from 50 to 1000 kb, including lambda ladders and *S. cerevisiae* chromosomes

Voltage: 200 V_{rms} (6.9 V/cm)
 Run-in Time: 0
 Run Time: 28 hrs
 Pulse Time: 1 to 50 s
 Mode: Reverse (REV)
 F/R Ratio: 3.0:1

Note: The sample must be in agarose plugs.

14. Care and Maintenance

Cleaning

Do not immerse any part in water. Wipe the unit components with a soft damp cloth after each run. Do not use any strong detergents or acids or bases.

Replacing fuses

Important! Fuses protect equipment by disconnecting loads too large for the circuit design, so it is imperative that replacement fuses conform to the fuse rating specified on the label. Figure 3 shows the back panel and Figure 4 illustrates how to replace input fuses in the mains power module.

- 1 Caution! Turn the mains power supply switch off and detach the power cord before replacing input fuse(s)!
- 2 Open the fuse compartment by inserting a small flat-blade screwdriver into the slot at the end of the power module opposite the power cord receptacle. Twist the screwdriver 1/8-turn, pulling the hinged compartment cover open.

Note: The 115 V model has one fuse cassette, located on the right. The 230 V model has two fuse cassettes.

- 3 Insert the screwdriver above the arrow on one fuse cassette, catch the cassette end, and slowly slide it completely out of the module.
- 4 Pull the fuse out of its cassette and inspect. If the fuse element is burned or broken, replace the fuse with an identical type. If the fuse appears to be intact, check it with a multimeter. (A reading of 1Ω or less indicates the fuse is still usable.)
- 5 After placing a good fuse into the cassette, slide it into the power module, making sure the arrow on the cassette points to the right (in the same direction as the guide arrows on the inside of the compartment door.)
- 6 Repeat steps 3, 4, and 5 for the second cassette.
- 7 Close the fuse compartment cover and gently press it into the power module until it snaps shut.
- 8 Plug the power cord in and turn the mains power switch on.

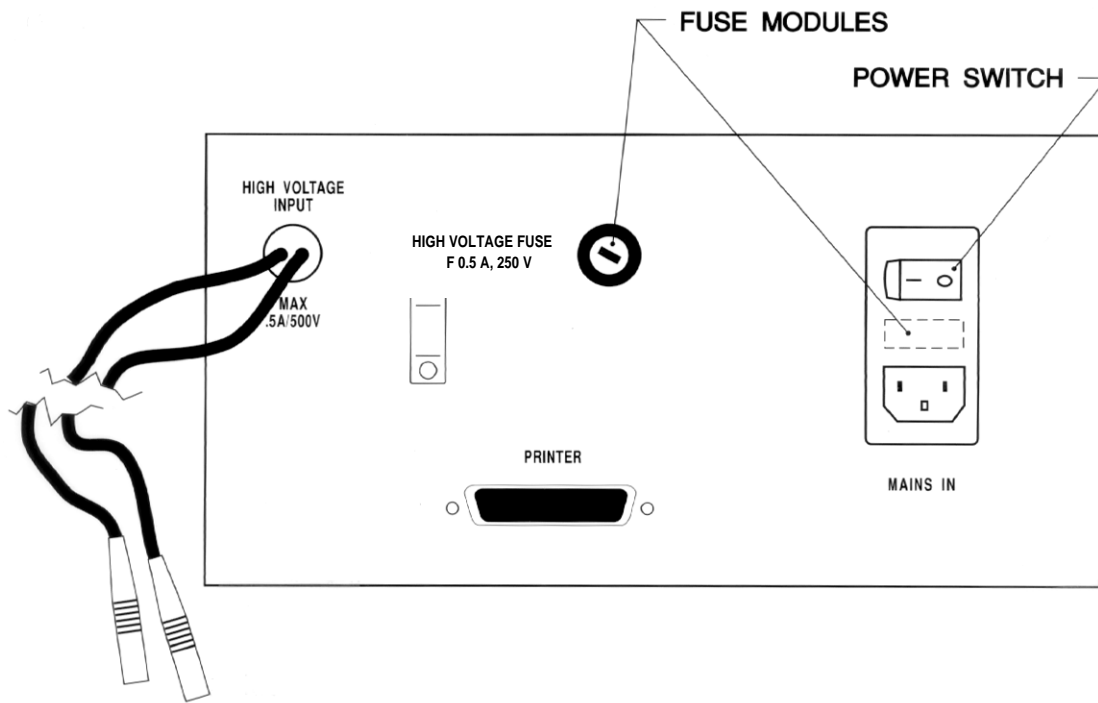


Figure 3. Back panel

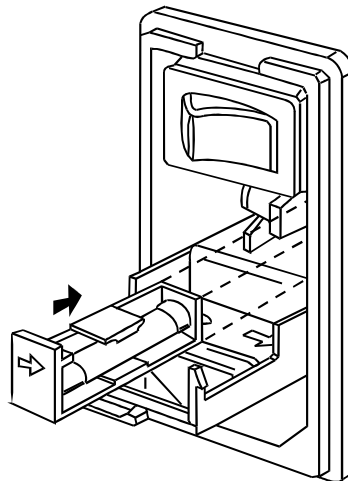


Figure 4 Mains power module—replacing fuses

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16. Customer Service Information

Technical service and repair

Amersham Biosciences offers complete technical support for all our products. If you have any questions about how to use this product, or would like to arrange to repair it, please call or fax your local Amersham Biosciences representative.

Important: Request a copy of the Amersham Biosciences Health and Safety Declaration Form before returning the item. No items can be accepted for servicing or return unless this form is properly completed.

Ordering information

| | Qty. | Code No. |
|--|------|------------|
| PC 500 SwitchBack Pulse Controller, 0-500 V $\overline{\sim}$, 500 mA. | | |
| 115 V $\overline{\sim}$ | 1 | 80-6073-92 |
| 230 V $\overline{\sim}$ | 1 | 80-6074-11 |
| Fuses | | |
| 115 V $\overline{\sim}$ | | |
| input: T 0.25A, 250V, 5x20 mm | 5 | 80-6106-98 |
| output: F 0.5A, 250V, 5x20 mm | 5 | 80-6335-74 |
| 230 V $\overline{\sim}$ | | |
| input: F 0.16A, 250V, 5x20 mm | 5 | 80-6270-76 |
| output: F 0.5A, 250V, 5x20 mm | 5 | 80-6335-74 |

Companion Products

| | | |
|--|---|------------|
| HE 100 SuperSub Submarine Electrophoresis Unit, complete. | 1 | 80-6043-71 |
| MultiTemp III Thermostatic Circulator | | |
| 115 V $\overline{\sim}$ | 1 | 18-1102-77 |
| 230 V $\overline{\sim}$ | 1 | 18-1102-78 |
| λ DNA PFGE markers | | 27-4530-01 |
| Yeast DNA PFGE markers | | 27-4520-01 |

| | | |
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