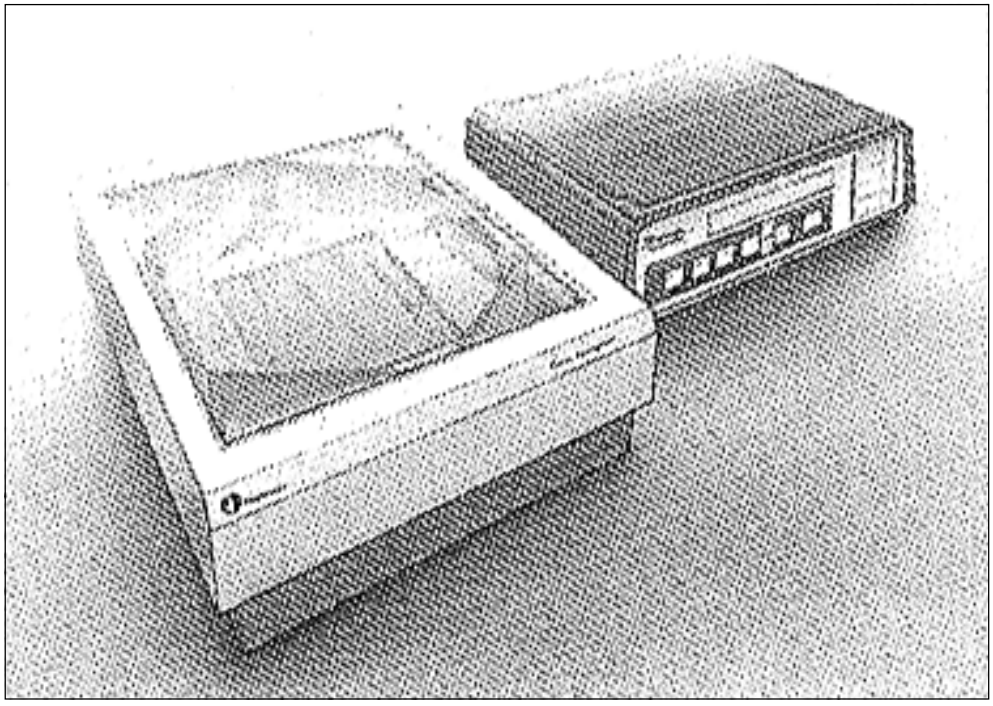
Gene Navigator™ System



User manual

56-1089-80
Edition AF

Amersham Biosciences

Important user information

Reading this entire manual is recommended for full understanding of the use of this product.

Meaning: Consult the instruction manual to avoid personal injury or damage to the product or other equipment.

WARNING!
The Warning sign is used to call attention to the necessity to follow an instruction in detail to avoid personal injury. Be sure not to proceed until the instructions are clearly understood and all stated conditions are met.

CAUTION!
The Caution sign is used to call attention to instructions or conditions that shall be followed to avoid damage to the product or other equipment. Be sure not to proceed until the instructions are clearly understood and all stated conditions are met.

Note
The Note sign is used to indicate information important for trouble free or optimal use of the product.

Should you have any comments on this manual, we will be pleased to receive them at:

Amersham Biosciences AB

S-751 82 Uppsala
Sweden

Amersham Biosciences AB reserves the right to make changes in the specifications without prior notice.

Warranty and Liability

Amersham Biosciences AB guarantees that the product delivered has been thoroughly tested to ensure that it meets its published specifications. The warranty included in the conditions of delivery is valid only if the product has been installed and used according to the instructions supplied by Amersham Biosciences AB.

Amersham Biosciences AB shall in no event be liable for incidental or consequential damages, including without limitation, lost profits, loss of income, loss of business opportunities, loss of use and other related exposures, however caused, arising from the faulty and incorrect use of the product.

Trade marks

Gene Navigator® is the exclusive trade mark of Amersham Biosciences AB. In view of the risk of trade mark degeneration, it is respectfully suggested that authors wishing to use these designations refer to their trade mark status at least once in each article.

Contents

1. Introduction

Congratulations! You are about to use Gene Navigator® system from Amersham Biosciences Gene Navigator represents a state of the art system and is designed to provide safe, reliable performance of pulsed field gel electrophoresis (PFGE). Gene Navigator is also flexible. Point electrodes generating non-homogeneous fields and HEX electrodes generating homogeneous fields can be used with optimal resolution.

Gene Navigator permits radical extension of the molecular weight separation range by enhancing the effective use of the PFGE technique. As a result, very large DNA molecules can be separated. The largest chromosome separated with Gene Navigator system so far comes from a fungus and is 12.6 million base pairs in size.

This manual will help you get the most out of your Gene Navigator system.

Recommended running parameters and the latest preparation protocols for the most commonly used samples are included for the benefit of newcomers to PFGE.

Experienced users will find that Gene Navigator is easy to adapt to the running conditions required by different samples. The system consists of an electrophoresis unit, a power supply, a control unit and a cooling bath, which enable you to optimize running conditions and fine-tune all separations (Fig. 1).

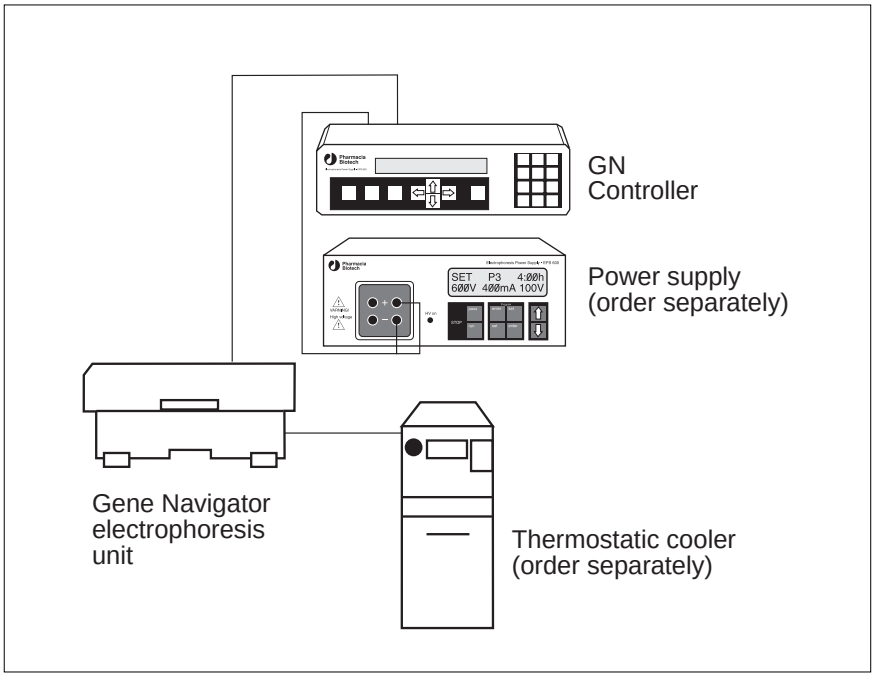


Fig.1. Gene Navigator® system is shown here using two electrophoresis units.

Please do not attempt to use the system until you have at least read the operating instructions in Chapter 5.

2. Important safety information

To avoid any risk of injury, the instrument should only be operated by properly trained personnel and always in accordance with the instruction provided. Read this entire manual before using the instrument.

WARNING! This instrument is designed for indoor use only.

WARNING! Do not operate the instrument in extreme humidity (above 95% RH). Avoid condensation by equilibrating to ambient temperature when taking the unit from a colder to a warmer environment.

WARNING! Always check the wires for damage before using the unit.

WARNING! Always check that the electrodes are properly connected before closing the lid.

WARNING! Always connect the lid according to the mounting instruction.

WARNING! Always connect the cables to the power supply BEFORE turning the power supply ON.

WARNING! Always TURN OFF the power supply before removing the lid.

WARNING! Do NOT use concentrated acids, bases or halogenated and aromatic hydrocarbons.

WARNING! Never exceed maximum allowed voltage, current or power.

WARNING! The instrument must be connected to a grounded mains socket.

WARNING! Changing of the pump unit must always be done by Amersham Biosciences AB Service Engineer.

1. The contact points on the right (east) wall of the electrophoresis unit and the corresponding right side of the safety lid should be kept dry. If they are wet when the safety lid is installed (see below) a fuse may blow out.
2. **NEVER** run with more than 450 V or 500 mA, as this will burn out the control unit.
3. **NEVER** run the circulation pump dry!
4. **NEVER** leave buffer in the unit for long periods. Evaporation may cause the buffer to crystallize and hinder the smooth operation of the pump. If the unit is to be stored unused for a long time, it should first be rinsed several times with tap water, then rinsed twice with distilled water, and finally placed upside down and left to dry thoroughly.
5. **NEVER** use organic solvents with Gene Navigator electrophoresis unit!

3. Description of the system

Gene Navigator system is made up of the following components:

- Gene Navigator electrophoresis unit for pulsed field gel electrophoresis. Includes pump for buffer circulation, cooling coil, safety lid with cable, insert moulds, gel staining tray, silicone tubing, hose connectors and hose clamps.

  Gene Navigator electrophoresis unit is available in two versions: 100-130 V, 60 Hz (18-1019-18) and 200-260 V, 50 Hz (18-1019-19)

  Note: The HEX electrode kit (18-1019-20) and the point electrode kit (18-1019-23) can both be used with either of the above electrophoresis units.

- GN control unit (18-1026-17). A controller for switching the electric field and programming up to 9 run procedures. Includes connecting cable with banana plugs. For either 100-130 V, 60 Hz or 200-260 V, 50 Hz.

- Power supply EPS 600 (19-0600-00).
- Power supply EPS 200 (19-0200-00).

- HEX electrode kit (18-1019-20). Includes a Hexagonal electrode, gel supporting tray and gel casting frame, and three double combs 1 mm (18-1019-25) and 2mm (18-1019-26) thick and a preparative comb (18-1019-27).

- Point electrode kit (18-1019-23). Includes 6 cathode electrodes and 2 anode electrodes, gel support tray, gel casting frame (for 20 x 20 cm gels), and a 22-well comb (2 mm thick).

- MultiTemp III thermostatic circulator for 100-120 V, 60 Hz (18-1102-77) or for 220-240 V, 50 Hz (18-1102-78).

4. Installation

4.1 Gene Navigator electrophoresis unit

Unpack the instruments and place Gene Navigator electrophoresis unit on a bench so that the cooling coil connections are at the rear and the handle on the lid is on the right. Make sure that the unit is equipped with the properly rated pump for the mains power source, by checking the label on the pump.

Note: Do not plug in the pump until you have buffer in the electrophoresis unit.

Cut off a 0.5 meter long section of silicone tubing and set it aside. You will need it to empty the buffer at the end of a run. See section 5.7 of this manual.

Attach short pieces of silicone tubing to the cooling coils. Place a male connector on one end of tubing, and a female connector on the other end. Use the hose clamps to secure the connections.

To connect MultiTemp III thermostatic circulator, repeat the above procedure, but use longer pieces of tubing. When you have made all the necessary connections, adjust the level of the coolant (usually tap water) to about 1 cm below the lid of MultiTemp III.

WARNING! The contact points on the right (east) wall of the electrophoresis unit and the corresponding right side of the safety lid should be kept dry. If they are wet when the safety lid is installed (see below) a fuse may blow out.

Place the EPS 600 or EPS 200 power supply on the bench or on a shelf close to Gene Navigator electrophoresis unit.

If you use a different power supply check that its ground current leakage circuit is compatible with the controller. To do this, run an application and check if the power supply cuts the current.

4.2 GN Controller

Carefully unpack GN control unit and place it on a work bench so that you can look at the rear panel.

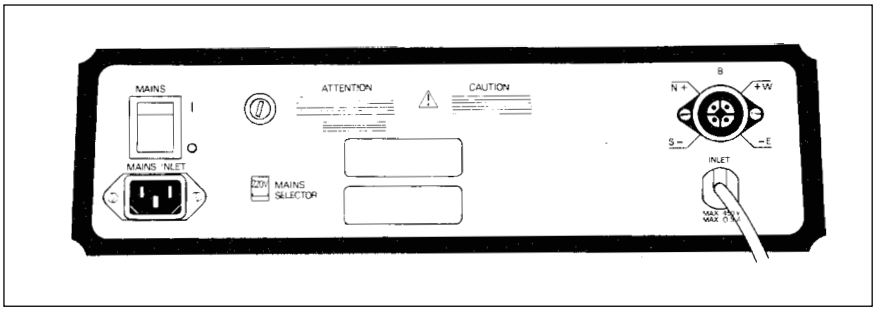


Fig. 2. Rear panel.

To begin, set the mains selector switch at the correct voltage (115 V or 220 V). Check that the power rating on the power supply is appropriate. Screw in the correct fuse to match the selected voltage. Plug the mains power cable into both the mains power source and the mains inlet socket on the rear panel.

It may be a good idea to conduct a ‘‘trial run” through the control unit’s operational functions and programming steps once, before connecting the separation unit’s 4-pole plug or the power supply’s inlet plugs (see Section 10.2).

To perform this trial run, turn GN Controller ‘‘On” by pushing the mains toggle switch located just above the mains inlet plug. Proceed to section 10.2. You can practice programming GN Controller, and become familiar with the various operational modes and procedures, without activating the procedure that you have programmed.

When you are ready for an actual run, return to the rear panel and complete the hook-up as follows:

1. Connect the (DC inlet) plugs to the outlet on your power supply. The red plug is the anode (+) and the black plug is the cathode (-).

WARNING! NEVER run with more than 450 V or 500 mA, as this will burn out the control unit.

2. Connect the 4-pole plug from the electrophoresis unit to the 4-pole socket above the DC inlets. Secure the connection by turning the locking ring clockwise.

5. Operation

5.1 Preparing the tank

You will need 2.5 l of buffer for the electrophoresis unit, pH ca 8.3. We recommend using TBE because of its buffering capacity.

Prepare 10 litres of 5x TBE (0.5M TBE) stock by dissolving:

Tris base	540 g
Boric acid	275 g
EDTA	37.2 g or
	200 ml 0.5 M pH 8.0

The buffer will last longer if you autoclave and store it cold. If you store the diluted buffer a long time some buffer salt may precipitate.

We recommend using either 0.15x TBE (0.015M) or 0.5x TBE (0.05M) as buffer depending on application. These buffers differ in migration speed, current flow and heat generation.

For your first trials we recommend 0.5x TBE.

Start the thermostatic circulator well in advance of the run (ca 30 min) to cool the circulating buffer. To reach a typical running temperature of 12°C to 15°C in the buffer, MultiTemp III thermostatic circulator should be set at 8°to 10°C. A uniform temperature is critical for reproducibility. If you have a high ambient temperature, it may be wise to cover the cooling tubing with insulation tubing .

Before you pour the buffer, level the electrophoresis unit. Adjust the unit by turning the appropriate feet.

Pour buffer into the tank (2.5 L), before inserting the gel support tray.

Note: To make sure that the pump works properly, check that the tubing underneath the tank is filled with cooling solution.

5.2 Preparing the gel

The protocol below describes how to prepare 15 x15 cm or 20 x 20 cm gels. The gels are made in the same buffer you use for electrophoresis.

Amersham Biosciences supplies high gel strength Agarose NA (17-0554-01/02/03) and low melting Agarose Prep (80-1130-07) for pulsed field gel electrophoresis. See information on chemicals and consumables, Section 9.1.

Melt the agarose by boiling it in a microwave oven or in a water bath. To obtain optimal resolution in reasonable time a 1.2% agarose concentration should be used for most applications.

For a 15 x 15 cm gel:	dissolve 1.3 g agarose in 110 ml 0.5x (or 0.15x) TBE, pH 8.3.
For a 20 x 20 cm gel:	dissolve 2.4 g of agarose in 200 ml of buffer, 0.5x(or 0.15x) TBE, pH 8.3.

5.3 Casting gels

Make sure that the conical holes in your tray are free from agarose. When the gel is cast, the holes will prevent the gel from floating around when the buffer is being circulated during a run.

Place the gel support tray on a levelling table. For proper buffer circulation the slits must be parallel to the East (right) and West (left) walls of the electrophoresis chamber.

If you will be using the point electrodes, the 20 x 20 cm or 15 x 15 cm casting frame should be positioned ‘‘diagonally”, with the corners pointing toward the middle of the sides of the running tray (see Fig. 3, below).

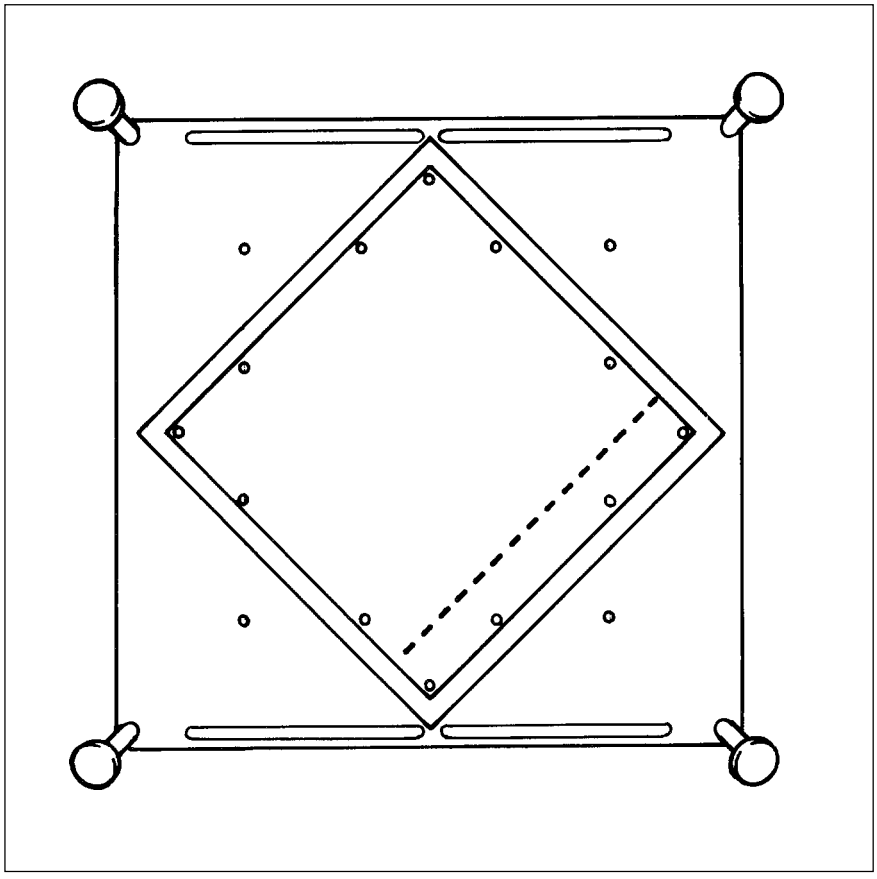


Fig. 3. Gel casting frame position for separations with point electrodes.

If you will be using the HEX electrode the 15 x 15 cm frame should be placed on the red square on the running tray, Fig. 4.

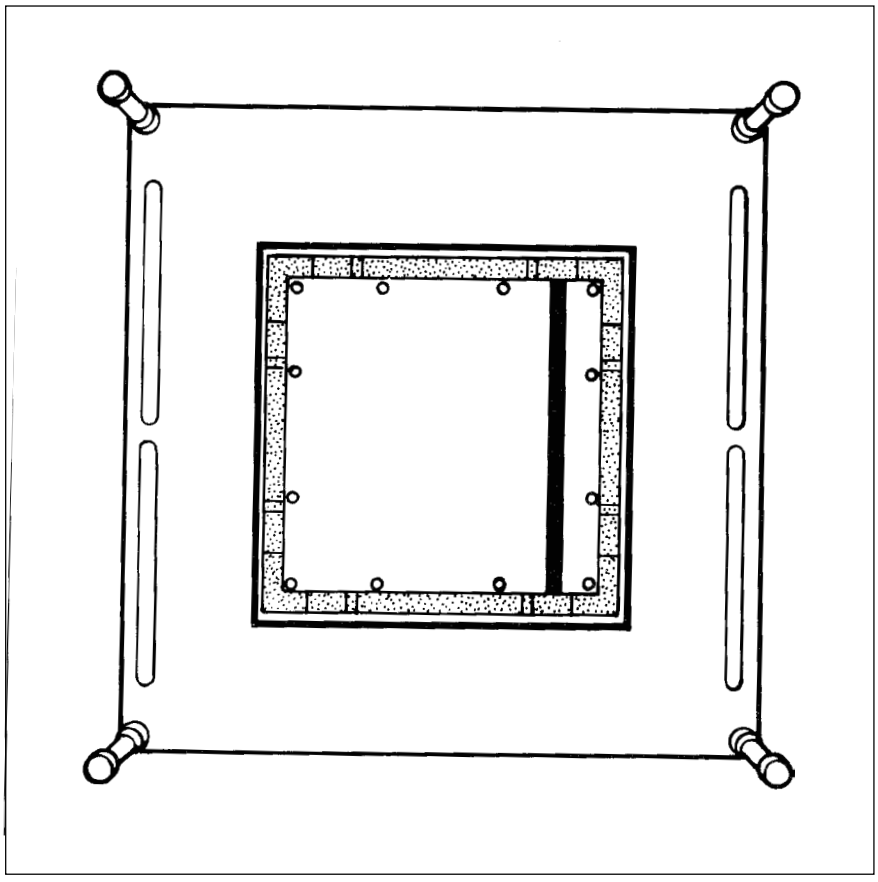


Fig. 4. Gel frame position for HEX electrode. The rubber casting frame should be placed on the red square marked on the running tray.

In either arrangement, the conical holes or depressions at the bottom of the tray must be clearly inside (not immediately underneath) the gel casting frame. The red square will help you position the frame correctly. Make sure the ‘‘ridged” side of the rubber frame is placed down, resting on the tray, and that the comb position marks are on top. The ridge is designed to make the frame self-sealing and no clamping is needed.

The position of the comb is marked by the thick red line inside the square on the gel support tray.

Before pouring the gel into the frame, place the plastic clips and then the comb inside the rubber frame in the same position that will be used when the gel is in place. Be sure that the ‘‘teeth” of the comb touch the bottom of the tray. Check that the clips are fixed on the edge of the frame (see Fig. 6). Leave the clips in position on the comb, remove the comb and set it aside momentarily.

Pour the gel into the frame, making sure that the holes (or depressions) inside the frame are filled (Fig. 5). If necessary, suck out air bubbles with a Pasteur pipette.

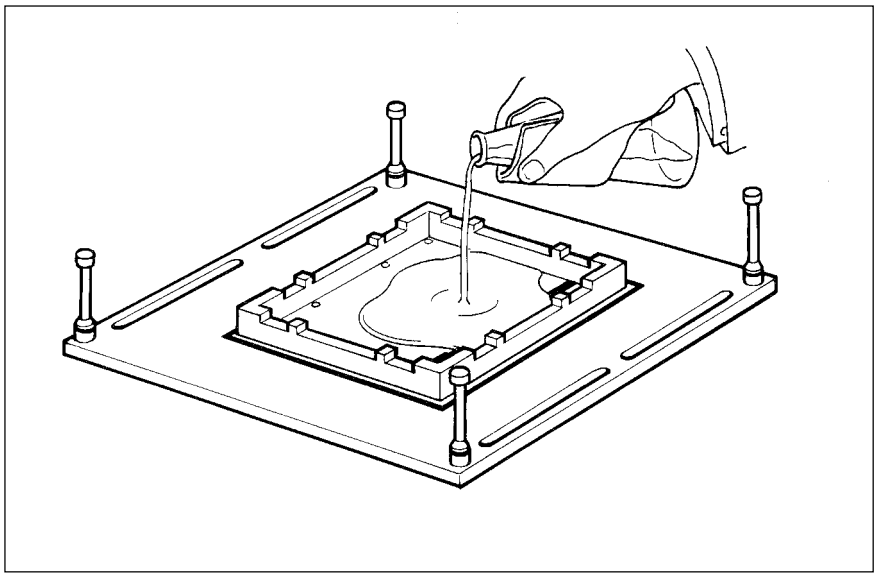


Fig. 5. Pouring gel into the rubber casting frame which is positioned for separations using the HEX electrode.

Place the comb in the gel. The plastic clips should now rest on the top of the gel casting frame, holding the comb firmly in place (Fig. 6 below).

If you use point electrodes (Fig. 3) place the comb parallel to the side of the gel that will be facing the southeast corner (nearest right corner) of the tank (the walls of the tank are marked North, South, East and West), and about 2 cm away from the edge of the gel.

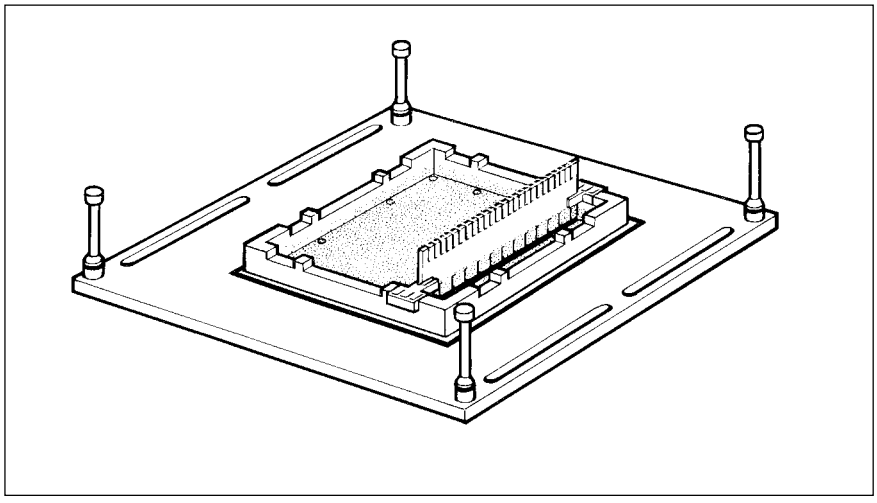


Fig. 6. Setting the comb into the gel. Note the placement of the clips.

5.4 Sample loading

Liquid samples should be loaded after the gel tray and electrode(s) have been placed in the tank as is also the case with submarine gels.

Sample blocks should be loaded before the gel is submerged. The blocks can be removed from the sample tube by using a flame-sterilized Pasteur pipette with a bent tip. The tip can easily be bent over a flame. Blocks should be cut on a sterile surface (for example, Parafilm®), preferably with a pair of nuclease-free microscope cover slips (Fig. 7) or a sterilized razor blade. If you cool the blocks on ice before cutting them, it will be easier to make symmetric slices.

Note: The slice must be slightly narrower than the width of the combs; if not, reduce the width of the sample blocks. This will improve loading.

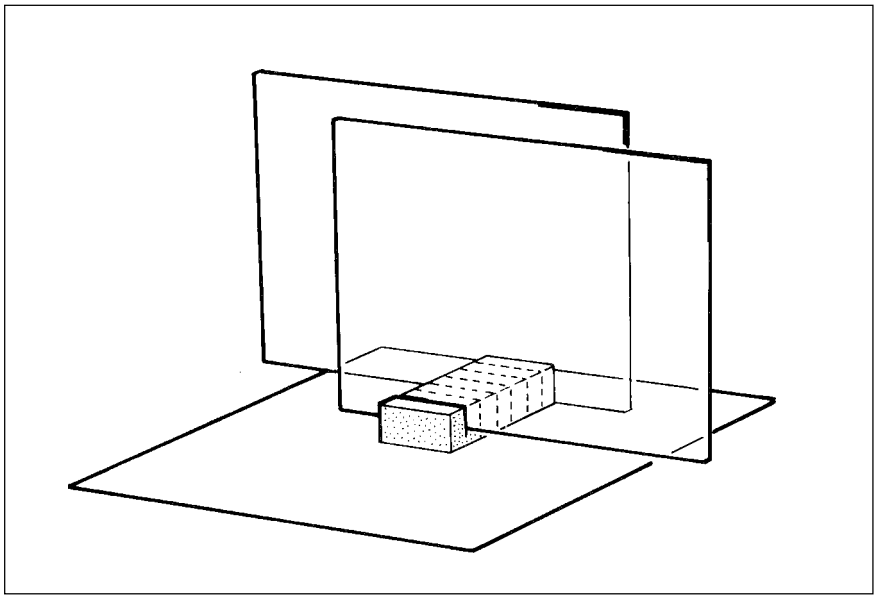


Fig. 7. To cut sample blocks use microscope cover slips.

Transfer a slice to the gel while it is attached to one of the glass cover slips. Use the other cover slip to push the slice into the well. If necessary, use a bent Pasteur pipette to carefully push the slices farther down, or suck them down with a syringe if the slices cover the whole well (Fig. 8). The slices should be placed in contact with the front wall of the well. (The front wall faces the direction in which samples will move). This positioning will help produce sharper bands. Also make sure that the sample block slices are not wider than the well.

Parafilm® is a trademark of American Can Company

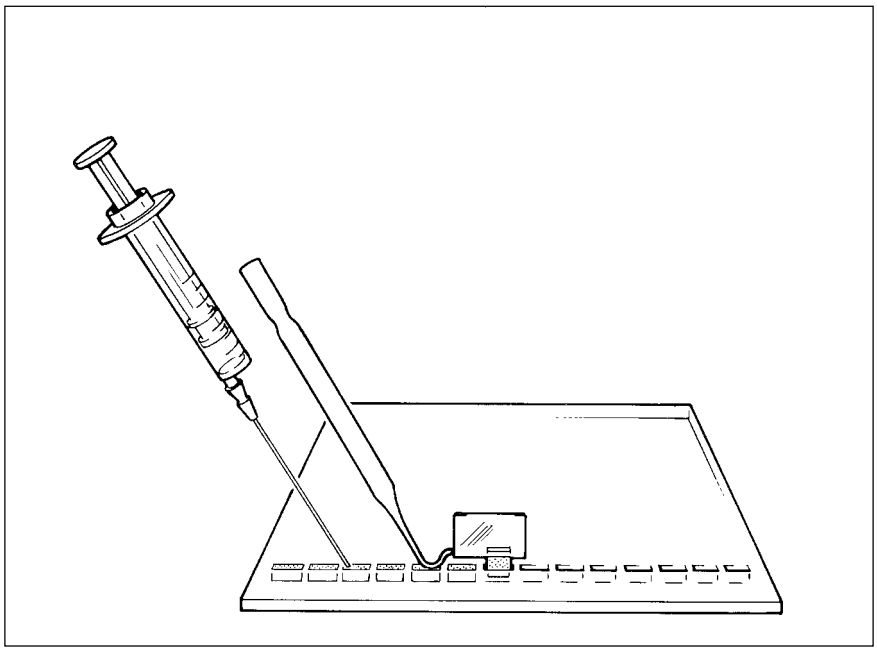


Fig. 8. Loading sample into wells. Three different methods that can also be used in combination.

Between 0.5 and 1.0 microgram of DNA is usually loaded into each well. A higly concentrated sample loaded as a thin slice gives better separation (enhanced resolution) than a less concentrated sample loaded as a thicker slice. Therefore, we recommend using a 1 mm comb instead of 2 mm comb if the sample is sufficiently concentrated.

To seal the wells you may use a little low-melting-point agarose (Agarose Prep 80-1130-07). This step may not be absolutely necessary, but it will ensure that the blocks or block slices stay in position during subsequent handling of the gel. (This is especially important when thin slices are loaded in the gel.)

We recommend that newcomers to PFGE use Amersham Biosciences markers *. These are delivered with a Certificate of Analysis and have been thoroughly tested with Gene Navigator system.

5.5 Running

Place the gel support tray, with the gel attached, into the tank. Make sure that the cut out corner of the gel support tray is placed in the upper left corner of the electrophoresis tank.

To avoid entrapping air bubbles beneath the gel support tray, place one side of the tray down first and then lower the remainder carefully into place. If the buffer does not cover the gel, add a little more.

*[1]Yeast DNA PFGE markers, Order no. 27-4520-01, 5 blocks
Lambda DNA PFGE markers, Order no. 27-4530-01, 5 blocks

If you are using the point electrodes, the gel will be in a ‘‘diagonal” position. In this case, the wells should be nearest the southeast (front, right) corner of the tank (see Fig. 3). When you use the HEX electrode, however, the gel will be in a ‘‘squared” position, and the sample should be nearest the east side (Figs.9 and 10 below).

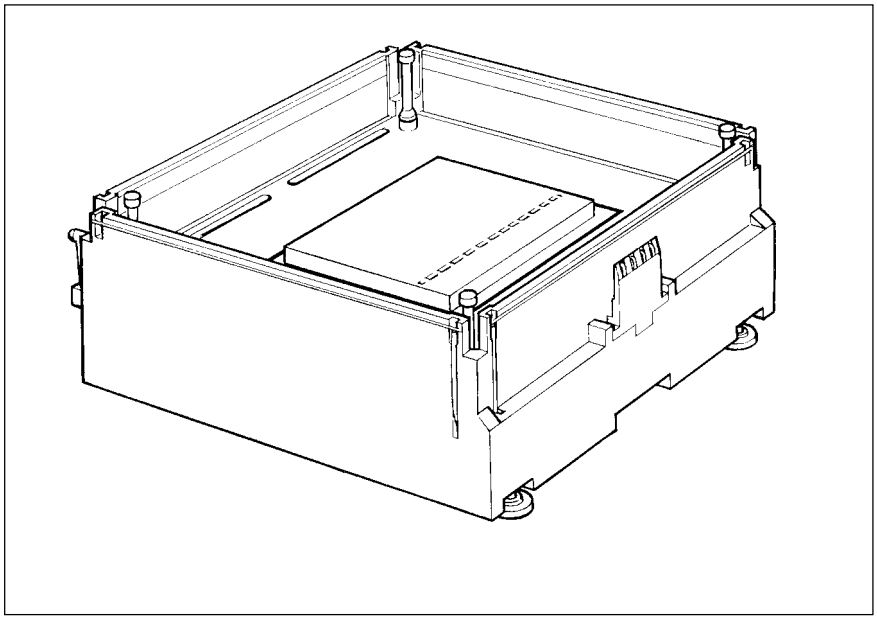


Fig. 9. Setting gel support tray in tank. The gel support tray can be placed in only one way due to its cut out corner.

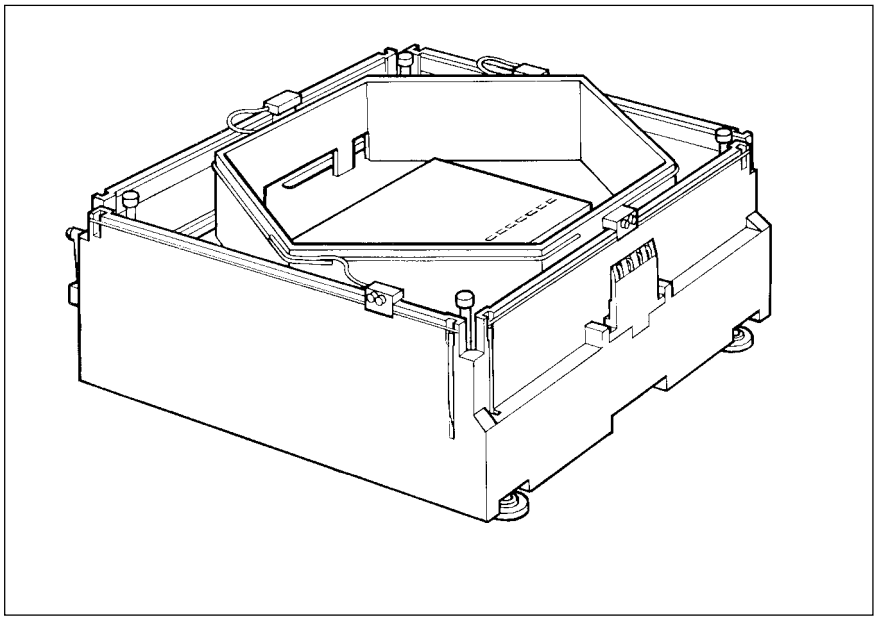


Fig. 10. Position of HEX electrode and gel support tray in the electrophoresis tank.

Lower the tray carefully, until it is resting securely on the inner edge of the tank. If the hinged gate over the pump is open when you lower the gel support tray, the gate will close automatically.

To start circulating the buffer, plug the pump into the mains power supply outlet. Always make sure that there is buffer in the tank when the pump is running.

If you are using the HEX electrode, make sure that the hook on the electrode rests on the center of the east (or right hand) power rail when you place the electrode in the tank. Then press each of the 3 power connectors that are attached to the electrode into place on the north, south and west power rails.

When the HEX electrode is used there are no separate electrodes to position. All are fixed within the HEX electrode.

When you use the point electrodes, however, you must press them over the power rails in the following manner:

The point electrode configuration is known as the double inhomogeneous (DI).

DI Configuration

North wall:	1 anode (+, orange) in position 90.
West wall:	1 anode (+, orange) in position 90.
South wall:	3 cathodes (-, black) in positions 5, 95, 180.
East wall:	3 cathodes (-, black) in positions 5, 95, 180.

Straighter lanes can be achieved by either moving the electrodes to other positions or putting a fourth cathode inside the unit.

Place the safety lid carefully on the unit, making sure that the pins on the west (left) side of the unit are in position before you lower the east (right) side and secure the safety electrical coupling.

The electrical connection to the electrophoresis unit will break when you lift the latching mechanism on the right-hand side (east wall) of the lid. Nevertheless, for absolute safety you should always turn off the power supply before removing the lid. Also, make sure that the maximum values set for the power supply do not exceed 450 V and 500 mA before you plug the controller's DC-inlet banana plugs into the power supply.

When using GN Controller you can either store all run parameters in a memory in the controller or work in BASIC mode where the procedure cannot be stored. Programming GN Controller is described in detail in Section 6.

Before starting a run you should check the performance of the electrodes and the levelling of the electrophoresis unit. This is most easily done by using BASIC mode. Program 4.0 s in N/S and E/W and set the voltage. Activate the power supply and read the current in each field. The current difference should not exceed more than 10 mA at approximately 200 V or 3 mA at approximately 30 V. This is called ‘‘current levelling”and is the easiest way to check the system. When both electrical fields are working correctly you can also see the electrodes ‘‘firing” by observing bubbles.

To make a run using only one phase, which is similar to one set of pulse times and run times, use either BASIC or EDIT. To program several phases use EDIT mode, where you can store your run procedure.

To stop the run manually, press the ON/OFF button on the front panel of GN Controller.

WARNING! Turn the power supply OFF before opening the lid.

5.6 Removing the gel

To remove the gel, slide a razor blade or a microscope coverslip beneath it to cut off the agarose in the holes and release the gel from the bottom of the tray, then slide the gel into a staining dish. Some users think that it is easier, however, to first slide the gel onto a plastic staining tray (delivered with the electrophoresis unit), which is 20 x 25 cm long, and then place this tray directly into the staining dish. This plastic tray is also used to pour off excessive buffer from the gels.

The ‘‘old” buffer from the tank can now be reused for staining. To pump the buffer from the tank into a container, lift the left (west) side of the tank slightly and raise the collapsible leg. Let the tank rest on this leg, thereby tilting it so that the buffer shifts toward the right side, where the pump hole is located.

Use the piece of silicone tubing that you cut earlier (see Section 4.1) to lift up the hinged gate on the right side of the tank. Cut a blue plastic pipette tip (1000 µl) from about 1 cm from the wide end and put the widest part of the piece in the tubing This will make it easier to attach the tubing to the outlet of the pump (Fig. 11). Place the tubing in the outlet. The running buffer will now be pumped out of the tank, through the tubing, and into the staining dish or a bottle. Pump out one liter of buffer into the dish, then store the remainder into a bottle for destaining.

Ethidium bromide is used for staining. Add 0.5 ml of stock solution (1 mg EtBr/ml water stored in a dark bottle at 5°C) and agitate to homogeneity. Staining takes up to 30 minutes.

Complete destaining will take several hours.

WARNING! Ethidium bromide is carcinogenic. Observe all safety precautions when using it.

For information on blotting and hybridization, see Amersham Biosciences blotting protocols, and especially the protocols for VacuGene™ XL, for information on blotting and hybridization.

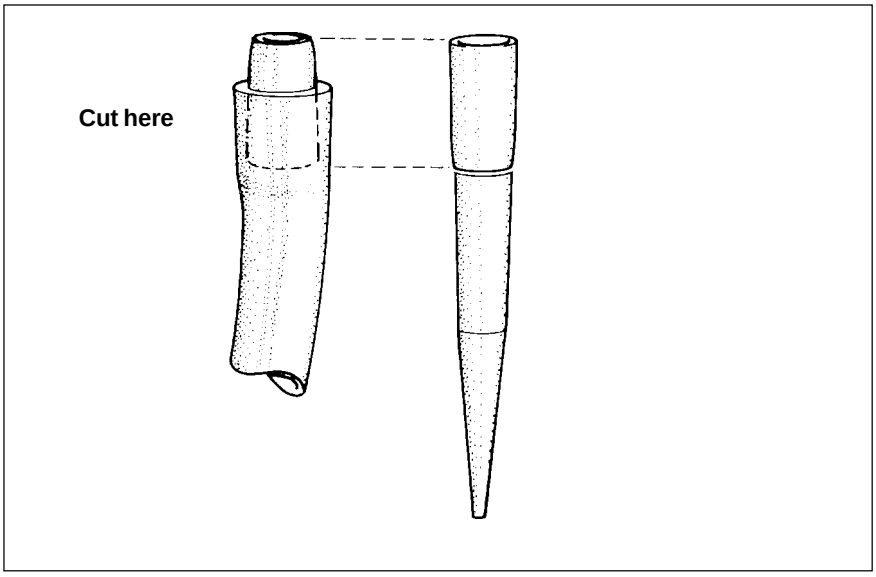


Fig. 11. How to cut a pipette plastic tip to fit the tubing used with the pump of Gene Navigator electrophoresis unit.

5.7 Photographing stained gels

For routine work, Polaroid 667 film or a similar film can be used together with a Kodak Wratten No. 9 filter. Typical exposure times are 5 to 10 seconds at f 16. If negatives are required use Polaroid 665 or 55 film. If high resolution is required use Polaroid 55 film, which has a longer exposure time.

When photographing a separation of very large molecules, for example *Neurospora crassa* chromosomes, you may occasionally need to use a magnifying lens to get the best possible picture.

A gel documentation system, such as the Amersham Biosciences ImageMaster VDS System, can also be used for documentation and analysis.

WARNING! When photographing gels stained with ethidium bromide use gloves, protective clothing and face shield. Ethidium bromide is a carcinogen.

UV-irradiation enhances transfer during vacuum and Southern blotting, but do not irradiate a gel more than necessary if you want to store it for a long time or if UV nicking is undesirable.

6. Programming the controller

6.1 GN Controller

The front panel of GN Controller consists of:

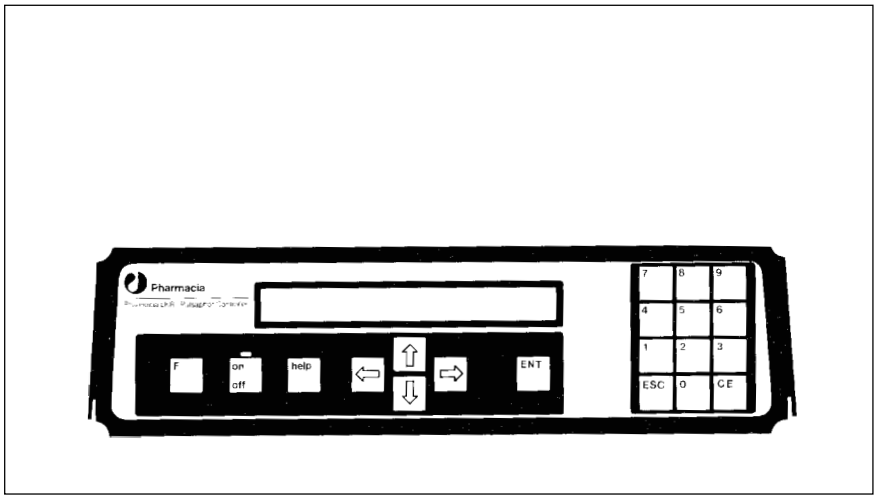


Fig. 13. Front panel.

A **Liquid Crystal Display (LCD)**, which shows the menu selections and program parameters that have been chosen. The contrast of the LCD can be adjusted by using the F Key. See Section 6.2, Contrast and reset commands.

Number keys, 0 through 9, which are used to enter run parameters, such as run time and pulse times, or to number procedures when they are called for (INPUT) or stored (STORE). The Number Keys can also be used instead of Arrow Keys to reach a command in a menu. The commands in a menu are numbered from left to right, i.e. the leftmost command is called number 1, etc.

An **F -key (Function key)** which permits two functions: resetting of all run- and pulse-time parameters or adjusting the contrast of the LCD. See Section 6.2, Contrast and reset commands.

Left and **Right arrow keys ← and →**, which will move the cursor horizontally between menu selections on the display (LCD).

After pressing the F key (see Example 1 below), use ← and → keys to position the cursor over either CONTRAST or RESET. Upper case characters indicate where the cursor is placed on the screen.

The [on off] **key** is used to start an electrophoresis run after all parameters have been set. It is also used to freeze, or temporarily halt, a run that has begun, or to end a run that has been completed (when the buzzer sounds).

ENT is used to enter a command (in upper case letters) in a menu or a new parameter value. When questions are prompted on the LCD, press ENT to respond ''yes''. ENT also moves the cursor one position to the right, similar to the right arrow key, when you are in the basic or edit input modes.

CE will clear the last entry and return the old pulse- or run-time parameter that was typed over. This works before ENT has been pressed.

ESC is used to exit from a sub-menu and move back to an earlier menu, or to the main menu. Also, when questions are prompted on the LCD, press ESC to indicate a ''no'' response.

Up and **down arrow keys, ↑ and ↓**, are used to move to the previous phase or the next phase in a procedure. When revising a previously stored procedure it is very useful to either INSERT or DELETE phases.

help provides further instructions. Press help a second time, or the ESC key, to return to the position in the menu at which help was requested.

The menu structure described in Section 6.5, Edit mode, will clarify how different commands are related to each other.

The menu structure is further explained in Fig. 16. Please note that the SETUP command is general when used from main menu, whereas it is only valid for the particular procedure of interest when used from the EDIT menu.

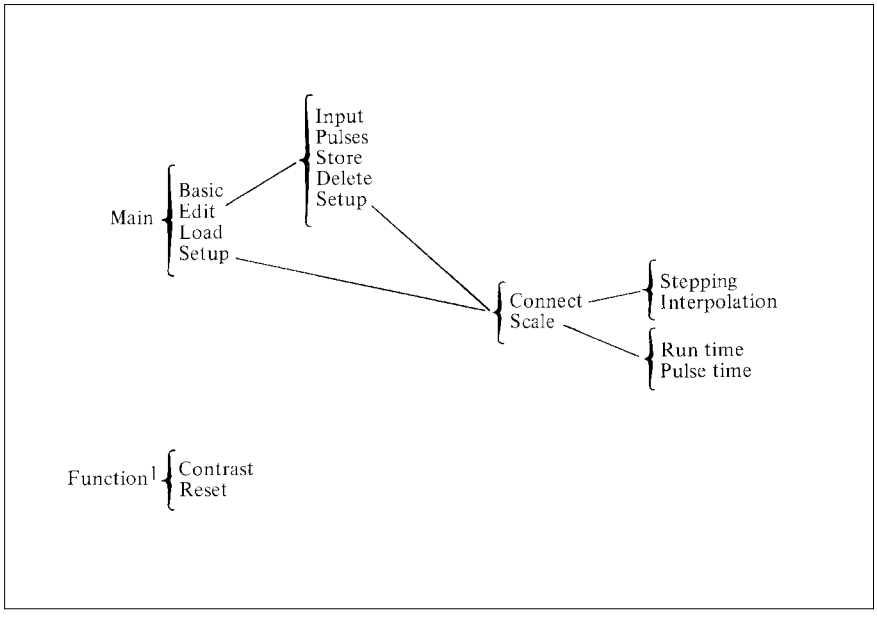


Fig. 16. Menu structure for GN Controller. (ESC lets you return to a previous higher menu.)

6.2 Contrast and reset commands

In the display, the choosen command is written in capital letters.

6.2.1 Contrast

When you turn the unit on (with the switch on the rear panel) for the first time, the LCD will indicate the software version number. Press any key to move to the main menu, which looks like this:

```
Main> BASIC     Edit   Load  Setup
Operate the controller in a basic way
```

To adjust the contrast on the LCD, press the [F]-key (F means function). You will see the following on your LCD:

```
Function> CONTRAST          Reset
Adjust the contrast of the display
```

The upper case characters in CONTRAST indicate that the cursor is in that position. Execute your menu choice by pressing [ENT]. Now the LCD will prompt:

```
Function contrast>
Use arrow keys to adjust contrast
```

Adjust the background and foreground clarity of the LCD by repeatedly pressing either the [←] or the [→] key. Either key will both lighten and darken the LCD if you continue pressing it.

If you turn the mains switch on and find the LCD blank, push [F] followed by [2] and [ENT].

Note: This will reset the contrast and erase all previously stored procedures as well as reset all default values. If you want to keep your stored procedures, type [F], [1] and [ENT]. Use the arrow keys.

When the contrast is satisfactory, press [ESC] twice to return to the main menu.

Note: You can press [F], which is the Function key to adjust the contrast from any menu position. You need not begin from the main menu, as in this example.

6.2.2 Reset

To reset the instrument, press the Function key [F]. Now press [→] or [2] to move the cursor to RESET. The following will appear on the LCD:

```
Function> Contrast          RESET
Reset the instrument
```

Again, the upper case characters indicate the position of the cursor. Now execute RESET by pressing [ENT]. The following warning message will appear on the LCD:

```
WARNING!  All procedures will be ERASED!
Press ESC to abort.  Press ENTER to RESET
```

If you press [ENT], the instrument will reset itself and return you to the initial screen showing the software version number. Strike any key to move into the main menu.

As indicated above, all procedures that have been created and stored will be erased. All set parameters will be changed to default.

If you press [ESC], you will return to the Contrast/Reset menu. Press [ESC] again to return to the main menu.

6.3 BASIC mode

When you turn the unit on (with the switch on the rear panel) for the first time, the LCD will indicate the version number of the installed software. (The same information will be displayed whenever you reset the unit.) Press any key to move to the main menu, which looks like this:

```
Main>      BASIC      Edit  Load  Setup
Operate the controller in a basic way
```

BASIC mode is the simplest way to program one set of pulse and run time parameters just before starting a run.

To program and store a run procedure to use later, select the Edit mode (see Section 6.5).

The upper case letters indicate that the screen cursor is on BASIC. If the cursor indication is not in the BASIC position, use an arrow key to move the cursor to BASIC. Then move into BASIC by pressing the [ENT] key.

As an alternative, you may use the numeric keys to move directly into the main menu choices. If you do so, you will not need to press [ENT]. Press [1] to move into BASIC, [2] to move into EDIT, [3] to move into LOAD, [4] to move into SETUP mode.

When you have entered the BASIC mode, the following default parameter settings will be displayed on the LCD:

```
N/S 000.0   e/w 000.0   time 000:00
Start by ON       Freeze by OFF          End by ESC
```

Now you must fill in the numbers for the North/South (N/S) and East/West (E/W) pulse times, as well as the total run time. The default scale unit for pulse time is seconds (SSS.s) and for run time (HHH:MM). When you start a run with the run time set at zero, the run will continue until you press off or until the power supply turns off.

As you press the digits on the numerical keyboard, the parameter numbers will move from right to left. For a North/South pulse time of one minute (60.0 seconds), therefore, you press [6], [0], [0] on the numerical keypad if default units are used (SSS.s, see Section 6.4) or, for example, 00.01.00 if the HH:MM:SS setup is used.

If you type a N/S pulse time of 60 seconds, and then either press [ENT] or [→] to move to the E/W pulse time, the LCD will appear as below (default setup):

```
n/s 060.0   E/W 060.0   time 000:00
Start by ON        Freeze by OFF          End by ESC
```

The time entered for the N/S Pulse will automatically be repeated as the E/W pulse time. If you want both pulse times to be the same, press [ENT] again to move to the (run) TIME parameter setting.

Now indicate the desired (run) TIME (Hhh:mm). If it is 20 hours, for example, press [2], [0], [0], [0]. Then execute with [ENT]. Your BASIC run parameters are now set and you are ready to begin a run.

If you type a desired time and then change your mind before moving to the next field, you can clear the field by pressing [CE]. Then you can start anew. Once you have pressed the [ENT] or any arrow key, the [CE] key will no longer clear the field.

When the time parameters are correctly indicated in all three fields, press [ENT] before starting the run.

Note: The pulse and run time parameter SCALE units can be changed from the default shown above. You can choose between five different SCALE units (Hh:mm:ss, Ssss, Sss.s, Ss:ss, and S:sss) for pulse time setting, and three different SCALE units (Days/hh:mm, Hhh:hh, and Hh:mm:ss) for run TIME SCALE SETUP. To change the default SCALE, press ESC to go to the main menu and select the SETUP procedure. See Section 6.4.2.

Start the run by pressing the [on/off] key on the front panel on the control unit. This activates the BASIC mode you have just programmed. The green LED indicates that the unit is ‘‘ON”. The LCD will then resemble the screen below, with the clock counting elapsed time.

```
Basic        N/S    Run time
             060.   0000:00     of 020:00
```

Fluctuations back and forth between the N/S and E/W pulses will be displayed during the run. You will also be able to read how much of the total run time has elapsed.

If you press [on off] while a run is in progress, the green LED will go off and the LCD will indicate the following:

Basic frozen: Continue by On End by ESC

1. If you press [on off], the run will continue from the point at which you stopped.

   Note: If a power failure should occur during a run, when the power returns the run will continue from where it was frozen.

2. If you press [ESC] instead of [on off], the BASIC programming screen will be displayed again.

   Now press [on off] again and the run will start from the beginning.

3. If you press [ESC] twice, you will return to the main menu.

When the set (run) time has elapsed, a buzzer will sound and the LCD will show:

Basic expired: Restart by On End by ESC

Note: This buzzer will continue to sound at 10 minute intervals until any key except the on and off key is pressed. Pressing the on and off key again will restart the the sequence.

6.4 Setup

SETUP is used to set default values for either connect (the stepping or interpolation run mode) or the pulse and run time scale units. From the main menu:

Main> Basic Edit Load SETUP Check or change instrument modes

Select SETUP and one of the following configurations will appear:

Setup> CONNECT Scale Select how to connect programmed points

```
Setup>      Connect     SCALE
Set time scales
```

6.4.1 Connection

Press the ENT button to select CONNECT, and one of the following configurations will appear on your LCD:

```
Setup connection = Stepping
STEPPING Interpolation
```

```
Setup connection = Interpolation
Stepping     INTERPOLATION
```

Use the ← and → arrow keys to select either STEPPING or INTERPOLATION. Press ENT to execute your choice, and you will return to the SETUP menu. Press ESC to return to the main menu.

Note that the system is delivered with STEPPING as default. It will remain so unless you change it. You cannot use interpolation when you are running in BASIC mode, because interpolation requires at least two phases, and the BASIC mode can only consist of one phase.

You can use interpolation when working in the EDIT mode because up to 6 different phases, each with its own pulse time and (phase) time values, can be programmed. In the EDIT mode, the total run time becomes the sum of the programmed phase time values.

If you work mostly in the EDIT mode and wish to use interpolation most of the time, you can save time by changing the default SETUP (SETUP in the main menu) to INTERPOLATION. Empty procedures (in EDIT mode) will indicate the INTERPOLATION as the default.

If you change SETUP in the EDIT mode you will only alter the setup for that particular running procedure.

When you are working with several phases, in the EDIT mode, stepping gives a stepwise increase or decrease in pulse time. One phase is one step.

In the BASIC mode the pulse time values are constant throughout the run.

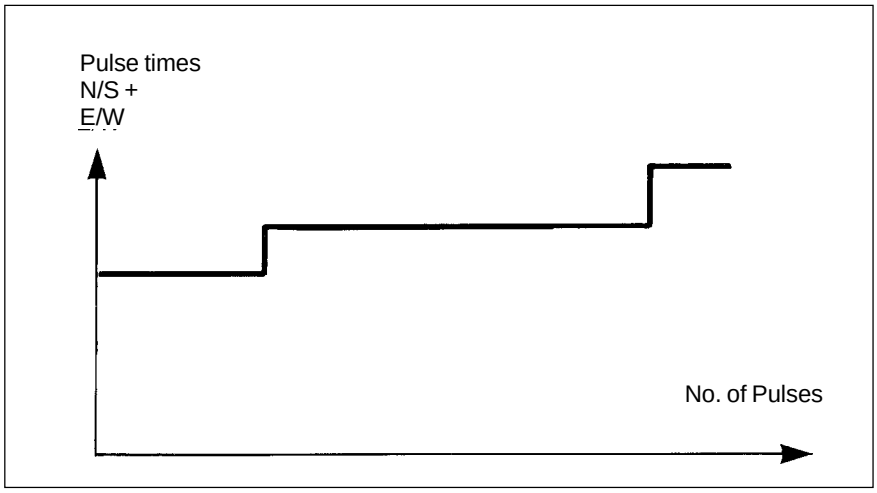


Fig. 14. Stepping.

If STEPPING is chosen in the EDIT mode, each phase of the run can have different constant values. This will not influence the first phase. The curve would still appear as illustrated above.

A multi-phase run using INTERPOLATION mode asks GN Controller to interpolate the set pulse times during the run. In this mode the following phase will influence the separation performance during the previous phase, as the two pulse times will be interpolated.

The control unit interpolates the difference between the N/S and E/W pulse times of the first phase and those of the next higher phase. It then automatically applies as many pulses as are possible within the limits of the phase time that has been programmed.

The pulse always stops when the programmed phase time ends. The separation will continue with the next phase and new pulse time values.Then GN Controller begins interpolating anew, using the programmed values for the next higher phase.

When interpolation is used, therefore, the pulse time values must always be increasingly higher with each new phase. Interpolation means that pulse time is linearly increasing with increased number of pulses (see Section 6.5.1, Edit setup). Fig. 15, below, illustrates the results of a 4-phase interpolated run.

Note: During the last phase, pulse times are held constant since there is no reference (5th phase) for interpolation.

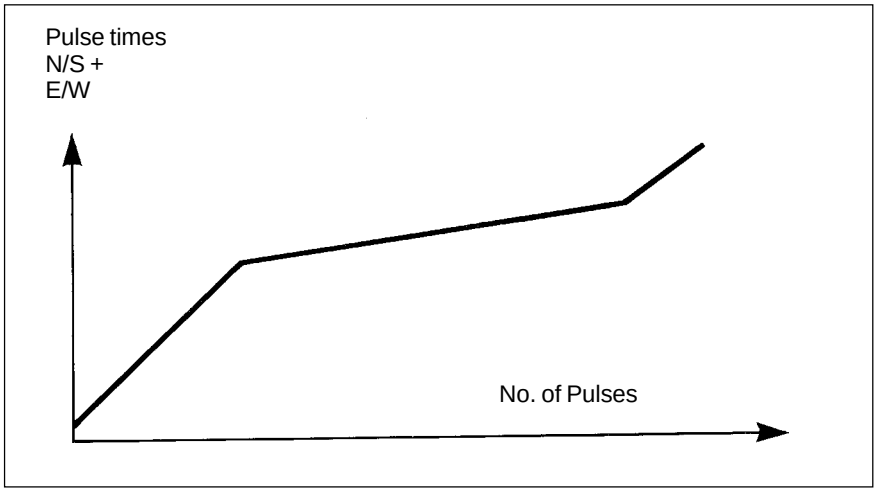


Fig. 15. Interpolation.

6.4.2 Scale

To change scale in the SETUP mode, use → to move the cursor to SCALE.

```
Setup>      Connect     SCALE
Set time scales
```

Press ENT to select SCALE and the setup scale menu appears:

```
Setup scale>      RUN TIME      Pulse Time
Set run time scales
```

Press ENT when the cursor is on RUN TIME and the following choices appear:

```
Setup scale  run time = Hhh:mm
Days/hh:mm      HHH:MM      Hh:mm:ss
```

The first line indicates the default scale units or the previously chosen scale units. Use ← and → keys to select the run time scale of your choice, followed by ENT . Return to the setup scale menu. Use → key to move the cursor to the PULSE TIME position. The LCD will appear as:

```
Setup scale>      Run Time      PULSE TIME
Set pulse time scales
```

Press ENT when the cursor is on PULSE TIME, and the following will appear:

```
Setup scale  pulse time = Sss.s
Hh:mm:ss        Ssss        SSS.S        Ss.ss        S.ssss
```

The first line indicates the existing default pulse time. Use the right and left arrow keys to move the cursor position to the desired scale unit, then press ENT .

When both the CONNECTION and SCALE have been entered, press ESC twice to return to the main menu.

Note: The setup scale that you select from the main menu is a global parameter. It will become the default scale for future procedures in both the BASIC and EDIT modes. When working in the EDIT mode, however, you will have the option of customizing the setup for each individual procedure, if you wish.

6.5 Edit mode

From the main menu:

```
Main>      Basic      EDIT      Load      Setup
Edit the experimental conditions
```

Select EDIT. The LCD now displays the following:

```
Edit> Select procedure number (1-9): [C]
Existing procedures =
```

You can now create up to 9 different procedures or sets of customized run parameters (each with up to 6 phases) that can be stored and quickly and easily repeated. INPUT is a command that makes it possible to either create or call a procedure for revision. The figure [C] above represents a blinking cursor on your LCD.

Create a procedure by pressing a number between 0 and 9. Zero will always take you to the next available new procedure, in this case number 1. If you press the 0 key, followed by ENT , the following LCD message will appear:

```
Edit 1 > SETUP    Input    Pulses    Store  Delete
Check or change procedure modes
```

6.5.1 Edit setup

As mentioned in Section 6.4, you can enter the setup from the main menu to change the default connection (stepping or interpolation) and time parameter scale units. Unless changed, these defaults will appear in both the BASIC and EDIT modes. The only exception is interpolation, which can only exist in EDIT mode.

In the EDIT mode, however, the default setup can be changed, or customized, for each procedure. To do this, press ENT on SETUP from the above Edit menu.

Now you can customize both the connection and scale for this particular procedure. For the scale, follow the same steps outlined in the SETUP mode. Interpolation will only affect procedures containing more than one phase; thus it is only used in the EDIT mode.

```
Setup connection  =  Stepping
STEPPING                Interpolation
```

When using interpolation, GN Controller calculates and inserts pulses based on the difference between the pulse times (both N/S and E/W) of the first (1 of 2) phase and those of the second (2 of 2) phase, etc.

Note: Running in INTERPOLATION mode the pulse times of each successive pulse must be at least 2 milliseconds longer than the **previous pulse**. GN Controller automatically calculates the number of pulses to be inserted, at equal intervals, based on each phase time. The result of interpolation is always a straight line that increases over the total number of pulses, as illustrated in Fig. 15.

A new interpolation begins as soon as each phase ends. The new interpolation is then based on the difference between the N/S and E/W pulse times of the second (2 of 3) and the third (3 of 3) phases. To end the procedure, the final phase time is set with all zeros. This means that the final phase of an interpolation is not actually run.

As a result, an interpolation run can consist of no more than 5 phases. The parameter values for phase 6 will resemble those shown below:

```
phase       n/s        e/w        PHASE TIME
6 of 6      030.0      0.30.0          00:00:00
```

If, for example, the total run consists of only 3 phases, the run will be terminated by entering a zero phase time in the 4th phase. Pulse times will still be needed in the 4th phase.

When the setup selections have all been entered, press ESC to return to the edit procedure menu.

6.5.2 Edit input

```
Edit 1 > SETUP    Input     Pulses     Store     Delete
Setup procedure
```

Select INPUT by pressing → and ENT . Now the following screen appears:

```
phase      N/S      e/w      phase time
1 of 1     000.0    000.0    000.00
```

Now you are positioned to indicate the parameter values desired for the first phase of the first procedure, or run.

The phase field (which now indicates phase 1 of 1) shows how many phases you have created and which one you reside in at the moment. The cursor begins on the N/S pulse value. Use the number keys to indicate the desired N/S pulse time, we shall use 60 seconds for purposes of illustration, then press ENT or → to move to the E/W pulse parameter. Now indicate 60.0 seconds, for example.

The digits move from right to left as they are indicated. In this setup scale (000.0 seconds), you press 6 , 0 , 0 for 60 seconds. You can also change the default scale, and customize it for each procedure, if desired, by selecting SETUP from the edit menu (as noted earlier in this section).

When the E/W pulse time has been entered, indicate the desired time for the first phase. If, for example, you indicate 2 hours and 30 minutes, the cursor will move to the phase field, as below, when the phase time has been indicated and entered:

```
PHASE      n/s      e/w      phase time
1 of 1     060.0    060.0    002:30
```

If you wish to have only one phase in this procedure, press the ESC key and you will be informed that procedure 1 consists of 1 phase with a run time of 2 hours and 30 minutes. Now you can press ESC once more and move to STORE to save the newly created single-phase procedure (see Store procedure below).

Note: When you have programmed the last phase use ESC to store the procedure. Do not use INSERT when you want to finish programming a procedure!

If you want to create a second phase in the same procedure, however, press ENT from the phase field, instead of ESC , and the following phase menu will be displayed:

Phase	Select action:		Insert
1 of 1	None	INSERT	Delete:

If you enter on INSERT from the above menu, you will move into phase 2 of the same procedure, as shown below:

phase	N/S	e/w	phase time
2 of 2	060.0	060.0	002:30

All of the values from phase 1 are automatically duplicated, but can easily be changed, by writing them over, if desired. Just use arrow keys to move through the fields, as before.

When the desired phase time has been typed in, press ENT to move into the phase field (PHASE capitalized). Press ENT again and the phase menu (see below) is displayed again. Press ESC again and you will go into phase 2 of 2. Repeat the same procedure each time you want to create a new phase. Continue in the same way to create up to 6 phases in the Stepping mode, or 5 phases in the Interpolation mode.

When you have entered all of the parameter values for the final phase of this procedure, press ESC from the phase field and review the total run time. Then press ESC again to return to the edit menu, from which you must move to STORE to save the new procedure. All of the phases just created will be erased if you return to the main menu before the procedure has been stored!

Phase	Select action:		Insert
1 of 1	NONE	Insert	Delete

Instead of INSERT, you may select NONE from the above phase menu to move back into the phase parameter field you just left. You can then check and change any or all of the parameter settings previously entered.

The NONE option takes you back into the previous phase, analogous to the ESC key that moves you back to a previous menu in the menu structure. When editing a procedure, ESC moves you out of the procedure and back to the edit menu.

From the phase menu you may also choose to delete a phase.

Phase	Select action:		Insert
1 of 1	None	Insert	DELETE

When a number of phases have been entered, move quickly between them using ← and →. Push ENT on any phase field (PHASE capitalized) to bring up the phase menu (as shown above). Then use → to move the cursor to DELETE.

When ENT is pressed the indicated phase will be instantly deleted.

When you have indicated and entered the phase time for the final phase of your procedure, press ESC instead of entering again. Now the LCD will indicate the procedure number, the total run time (or sum of all the phase times in the procedure), and the total number of phases, as shown below:

Procedure 1 Run time: 007:30 Phases:3

IMPORTANT! The procedure you have just created will be erased if you push ESC to return to the main menu before first having stored the procedure. To store the procedure, press ESC once more to return to the edit menu (see Edit store section below).

6.5.3 Edit pulses

Edit 1 > Setup Input PULSES Store Delete Check number of pulses

Use ← or → to move the cursor to pulses and press ENT . Now the screen will display the number of pulses that will result from the pulse and run TIME values entered in the first phase of the procedure you are editing. This display may look like this:

Procedure 1	Phase 1 of 3	Pulses 600

Use ← or → to move between all phases in the procedure. GN Controller will calculate and display the number of pulses that will occur in each phase of the scheduled run.

The number of pulses can be adjusted prior to the run by returning to edit input (see Section 6.5.2) and changing the pulse and run time values, as desired, in each phase.

6.5.4 Edit store

Edit 1 > Setup Input STORE Delete Store procedure

Use → to move the cursor to STORE and press ENT to bring forward the following:

```
Edit 1 > Store as procedure [1-9]: [C]
Existing procedures =
```

As before, when you entered on INPUT, the [C] above is a blinking cursor. If you had previously stored any procedures, the numbers that you assigned them would appear after ‘‘Existing procedures =”.

Store the procedure by pressing any number between 1 and 9, since all 9 positions are available. When the number has been pressed and entered (in the example below we pressed number 1) you will return immediately again to the edit menu. Now you can safely escape to the main menu.

When next you re-enter the EDIT mode from the main menu, the following will be displayed:

```
Edit 1 > Store as procedure [1-9]: [C]
Existing procedures=     1
```

Now you can either re-enter the existing procedure, by pressing 1 , or INPUT a new procedure by pressing another number.

Remember that pressing 0 always takes you to the next available number, in this case 2. If you have previously created and stored several procedures, the numbers of the existing procedures will all be displayed.

If you press 1 , to re-enter the procedure just stored, you will return to the edit menu. Select INPUT to re-enter the first phase of that procedure.

Select INPUT from the edit menu to get back into the phases of the stored procedure just selected.

When you have re-entered an existing procedure, use up and down arrow keys to move quickly from phase to phase. You can enter any changes in phase parameters that you wish.

Once more, if you return to the main menu without having stored the changes, they will be erased. You will still have saved the old information but not the changes.

To insert a phase into an existing procedure, via the INPUT command, move to the phase that will be placed before the one you will add. Capitalize the word PHASE by using the arrow keys and press ENT . You will be asked if the new phase shall be inserted (INSERT), the old phase shall be deleted (DELETE), or if you want to go back to a previous phase (NONE). Press ENT on INSERT.

To delete an existing phase, you must again move to the phase field (PHASE capitalized) and press ENT . Now move the cursor position to DELETE and press ENT again. That phase will be deleted.

When editing a procedure, therefore, you can add phases, delete phases, or change the parameters in any or all phases. When the editing is complete, you must escape to the edit menu only, and then press ENT on STORE before returning to the main menu.

By doing this you will replace the previous procedure with the newly edited version by storing the edited version under the same procedure number. You also have the option, however, of keeping the original procedure, while creating a revised version of it with a new procedure number.

6.5.5 Edit delete

Any procedure that has been stored in GN Controller can easily be deleted from its memory. This is done from the edit menu, compared to deleting a phase which is done from the edit input menu.

Move the cursor to DELETE and push ENT. The numbers of all stored procedures will appear following ‘‘Existing procedures =”. When you press one of these numbers on the keypad, followed by ENT, that procedure will be permanently removed from the memory.

Example: Edit procedure

When several procedures have been created (INPUT) and stored (STORE), you may find it faster to edit an existing stored procedure than to delete an existing one and begin creating a new one again. The following example describes how EDIT can be used to quickly change, to whatever extent is required, a procedure that has been previously stored.

Let us say you have created and stored 9 different procedures. You cannot, therefore create another procedure unless you first delete or edit an existing one. Instead of deleting a procedure, however, you can revise one of these 9.

You decide to edit the procedure that you have earlier created and stored as number 3. It contains 6 phases and was created in the STEPPING mode.

The new procedure will be in INTERPOLATION mode, and can, therefore, only have a maximum of 5 phases. The 6th phase will be programmed with the requisite higher pulse times, so that the interpolated insertions can occur, but the phase time will be set at zero. The run procedure will conclude after phase 5.

You press ENT on EDIT from the main menu and then press 3 to call up the procedure that was previously stored as number 3. Now the edit menu will appear, as below:

```
Edit 3 > SETUP    Input        Pulses        Store        Delete
Check or change procedure modes
```

Move the cursor to SETUP and press ENT. Now change the connection setup for this procedure, number 3, from STEPPING to INTERPOLATION.

Then move the cursor to INPUT and press ENT. Now you are in position to change any or all of the parameters in phase 1 of 5. When

you have pressed ENT on the phase TIME parameter, you will automatically advance to phase 2 of 5.

Continue in this manner until you reach the final phase TIME parameter in phase 6 of 6. Now you will replace the previous phase TIME with all zeros, and press ENT . You are now in the phase field (Phase capitalized). Press ENT again to move to the following menu:

Phase	Select	Action	Delete
6 of 6	NONE	Insert	Delete

Select NONE only if you want to return to the phase parameters and recheck them. The up and down arrow keys move you quickly through the phases. Be sure to press ENT after all parameter changes you make.

If no rechecking is required, or when all of your value changes have been entered in all phases, escape back to the edit menu and press ENT on STORE.

IMPORTANT! Be careful NOT to press ESC one time too many, because you will have erased the above created procedure in the EDIT mode.

After entering on STORE in the edit menu, you have an option. If you store the newly created procedure as number 3, you will replace the previous procedure with the version you just created. If you select another number, this procedure will replace the one previously stored on that number.

To begin the run, press ENT on LOAD from the main menu to load a previously stored procedure.

Note: When you load a procedure which is using INTERPOLATION mode, error messages will warn you if the time differences between two subsequent pulses are below 2 milliseconds. See Section 7.3 for a list of all system error messages.

6.6 Loading a procedure

From the main menu:

Main>	Basic	Edit	LOAD	Setup
Load the experimental conditions				

Select LOAD and press ENT . The LCD now displays the following:

```
Load> Select procedure number [1-9]: [C]
Existing procedures = 1 2 3 4
```

The [C] above represents a blinking cursor. Any numbers following the ‘‘Existing procedures =” indicate procedures that have been created and STORED. To be able to run a procedure load it by simply pressing the appropriate number, followed by ENT.

If any of the programmed pulse or run times now require adjusting, a message on the LCD will indicate what changes are required. If this occurs, note the message carefully and then press ESC. If there is more than one error in the procedure, a new message may appear. Note it, and then continue pressing ESC until this menu appears:

Procedure 1 Start by ON Freeze by Off End by ESC

Now press ESC once again to move back into the EDIT mode, where you can re-enter the procedure and make the required changes. When completed, return to LOAD and repeat the above steps. If all programmed values are acceptable, the procedure number will move you directly to the above menu.

Begin the programmed run by pressing on/off key. Temporarily stop, or freeze, the run at any time by pressing the button once more.

To terminate a run, press on/off and then ESC.

You can also escape from LOAD while a procedure is running, to edit another procedure or even change the one that is currently running.

When the run is completed, you can use the switch on the rear panel to turn off the instrument.

7. Evaluation and presentation of data

7.1 Electrical parameters

The parameters for a typical experiment are shown in Table 1, below. These parameters will give good overall separation for 40-2000 kb. The values described below are typical when using 2.5 liters 0.5 x TBE buffer.

Table 1. Typical current values for different electrode configurations

Parameter electrode	Point electrode (20 x 20 cm gel)	HEX electrode (15 x 15 cm gel)
Set voltage	300-330 V	165-170 V
Starting 0.5 x TBE current ca.	100-140mA	80-100 mA

The current usually increases 5-10% during a run

If more than 2.5 liters of TBE buffer are used, the current will be higher. Similarly, other buffers (or concentrations) will result in different current values, depending on conductivity of the buffer.

Table 2. General run parameters

Size (Mb)	0.01-0.05	<0.1	0.1-2	2-6	6-12
% agarose	1.2	1.2	1.2	0.6*	0.6*
Buffer (xTBE)	0.15	0.15/0.5	0.5	0.5	0.50
Temperature/°C - in MultiTemp II (set value)	8-9	8-9	8-9	8-9	8-9
- in elpho. unit	12-14	12-14	12-14	12-14	12-14
Voltage/V (HEX)	450	ca 300	165-200	40-100	25-50
Voltage (point)	450	ca 370	300-330	60-100	ca 30
Pulse time (s)	0.3-1.0	1-10	10-120	3-75 (min)	50-100 (min)
Run times (h)	1-4	1-6	17-24	24h-3 days	3-6 (days)

* 0.6 % agarose will give a faster separation at the cost of resolution

An agarose concentration of 1.2 - 1.5% has been found to give better resolution than a 1% concentration, without significantly increasing the run time. Concentrations around 2% improve resolution even more, but at the cost of the speed of separation.

Changes in the temperature of the running buffer will affect the separation pattern. The pulse times shown in Table 2 will produce a resolution window within the range specified at the head of each column, but one pulse time usually does not cover the entire size range in a sample, depending on its diversity of molecules.

The information in Table 2 is valid when the running buffer is 0.5 x TBE; if the buffer concentration used is lowered the resolution window will be shifted to slightly lower sizes, i.e. optimal resolution will appear in a slightly lower size range.

7.2 Relationship between pulse time and resolution

The pulse times below are based on the suggested experimental conditions and electrical parameters outlined in Section 6. If the field strength is changed, the pulse time should be scaled accordingly. For an efficient separation range of 100 kb, for example, this means that if the voltage is reduced by 50% the pulse time should be increased by a factor of 2.

For separation of very large DNA (> 2000 kb) it is important to lower the voltage. Recommended running parameters are shown in Table 2. For molecules below 100 kb, higher voltages can be used without problems.

Table 3. Relationship between pulse time and resolution for a HEX electrode

Pulse time	Efficient separation	Maximum resolution	Run time	Set voltage (V)
0.3 s	1-10kb	1-10kb	1	450 (0.15x TBE)
0.5 s	5-30kb	10-25kb	2	450 (0.15x TBE)
0.8 s	30-50kb	35-50kb	3	450 (0.15x TBE)
5 s	20-100kb	60-90kb	4	300
25 s	40-400kb	200-300kb	6	300
45 s	40-600kb	400-550kb	10-24	300
100 s	40-1000kb	700-900kb	17-40	165-200
125 s	100-1600 kb	800-1200kb	17-40	165-200
20 min	1-2.5Mb	1.6-2.5Mb	100-140	165-200
30 min	1.6-3Mb	2.5-3Mb	100-140	165-200
40 min	1.6-6Mb	2.5-6Mb	140	30
75 min	2-9 Mb	3-6Mb	140-170	30
90 min	2-10Mb	6-9Mb	185	30
100 min	3-13Mb	7-10 Mb	190-200	30 (0.6% agarose)
180 min	3-13Mb	10-13Mb	190-200	27 (0.6% agarose)

The buffer used is 0.5x TBE and agarose concentration is 1.2% **unless a different value is stated.**

Tables 2 and 3 can be used as rough guidelines for separations in 0.5x TBE (and also for separations in 0.15x TBE). The figures in Table 3 are sometimes limited by the size of the sample at hand and do not reflect exact limits. Run times for certain samples can be reduced considerably, depending on how complex the separation pattern is. The figures mentioned here are presented only as a guide for the first trial. The optimal voltage for each sample size can be found in Table 2. In Table 3 the lower voltages are valid when the HEX electrode is used.

When a certain pulse time is selected, molecules smaller or larger than the chosen range will not get the optimal resolving conditions. The pulse time makes it possible, therefore, to focus on the size range of interest. This size range is called the ‘‘resolution window”. The range in the resolution window is a function of field strength, current and pulse time.

Changes in run time can also affect the degree of separation. Generally, longer than overnight runs are required to separate large fragments (see Table 4).

The design of Gene Navigator makes it possible to generate almost any field shape. The HEX electrode gives a homogenous field resulting in straight lanes when the pulsed field technique is used. The diode-equipped point electrodes reduce undesirable field distortions. The DI configuration works well for a broad range of separations.

To create more unusual field shapes (DI configuration), both the anode and cathode electrodes can be set at other positions on the north and south sides.

For maximum resolution up to 2000 kb, allow at least 17 hours of run time. Larger DNA’s such as the chromosomes of *Schizosaccharomyces pombe* need longer run times. For most purposes, however, an overnight run will provide satisfactory resolution. When determining the quality of sample preparation, begin with a short run. When it is clear that the samples are of good quality, you can begin fine tuning the separation for the best possible result.

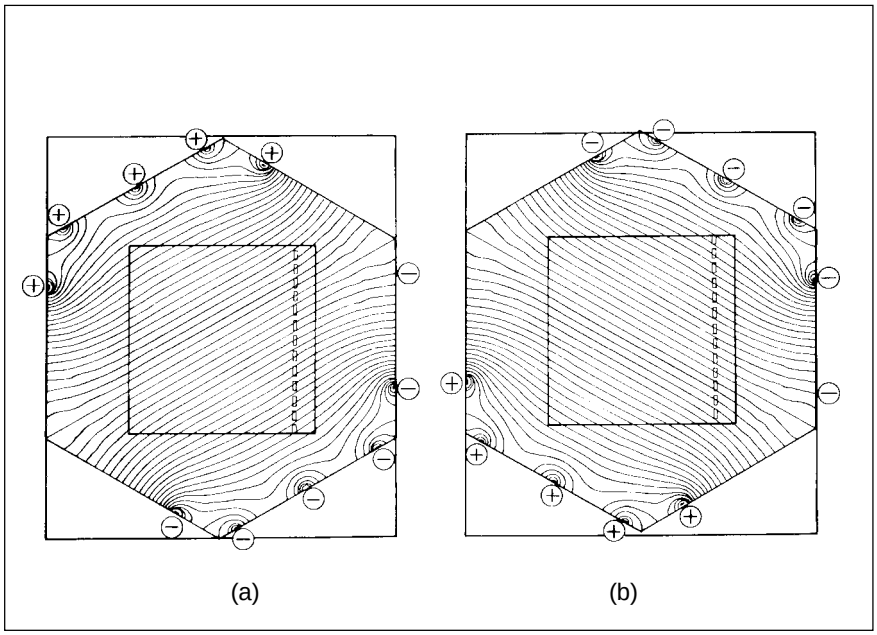


Fig. 17. Two different fields are applied, using the HEX electrode. The figures show isofield lines. (a) North/South field (b) East/West field.

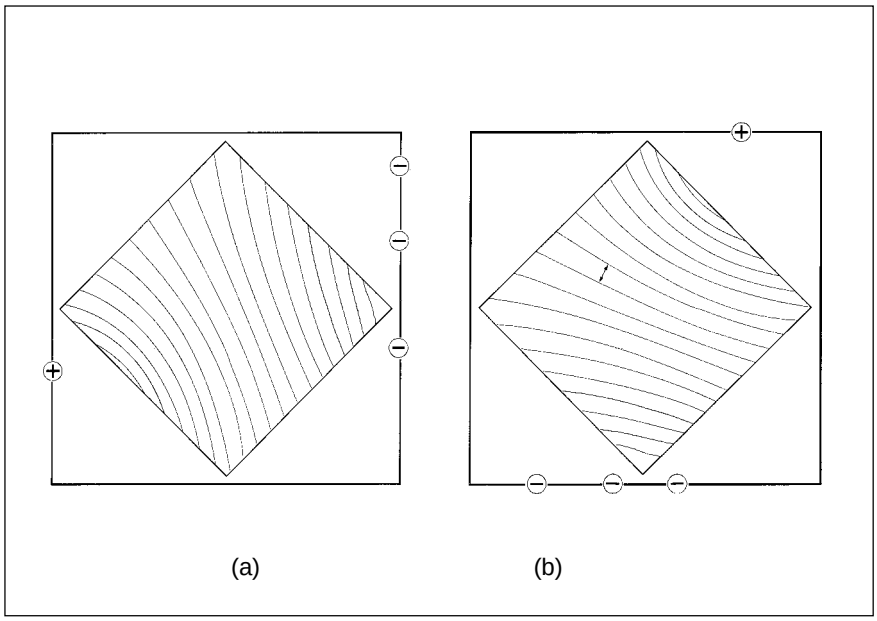


Fig. 18. Two different fields are applied using the point electrodes (DI configuration). The figures show isofield lines. (a) North/South field (b) East/West field

Example: Fine-tuning a separation of *Saccaromyces cerevisieae* chromosomes (YPH 148).

In an earlier run, a 90 second pulse time with 17 hrs run time resolved all except the two largest bands. Another run with 125 s pulse time resolved the larger bands but not all the mid range-bands.

After a trial using 90 s and 125 s pulse time in a stepped program the optimal program looked like this:

Phase	**Pulse time**	**Run time**
1	90 s	6:00 hrs
2	105 s	5:00 hrs
3	125 s	6:00 hrs
		17:00 hrs

Table 4. Examples of run parameters

DNA sample	DNA size range (Kb)	Pulse Time	Run Time (hours)	Voltage (HEX Electr./ Volts)	Buffer (xTBE)
molecular weight DNA standards	0.5-50	300-800 ms	1-2	450	0.15
Lambda ladder	50-1100	90 s	24-40	165-200	0.5
Saccharo-myces cerevisieae	90-2500	90/105/125 s	5/6/6 =17	165-200	0.5
Hansenula wingei	1000-3000	40/20 min	80/60 = 140	165	0.5
Schizo-saccharomyces pombe	3000-6000	75 min	160 (min run time 80 h)	35 V	0.5
Neurospora crassa	4000-12600	180/75 min	100/90 =190	25-30	0.5

Note: / means that several phases are used in stepping mode. Several phases will give a better overall separation over a wider size range than one phase can provide.

If several phases are used, for example 180 minutes pulse time and 75 minutes pulse time, the order in which they are applied becomes important.

If you start with the longer pulse time, the resolution window for the overall separation shifts to a higher molecule size, compared to a start made with a shorter pulse time.

7.3 System messages

Minute or second cannot be greater than 59	Press a key

When time values are being entered, the minutes and seconds cannot exceed 59. If you enter a higher number in such a field, the above message will appear after you press ENT . Just return to the field and enter a corrected value.

Odd milliseconds are rounded to even! Press a key

This indicates that you entered a value of, for example, 0.001 ms. When this scale is used for either a pulse or time field, the last figure must be an even number.

> Total run time too large!
> Decrease phase times. Press a key

This indicates that the sum of all the phase times in the procedure exceeds 48 days, 23 hours and 59 minutes. You will not be able to return to EDIT and store the procedure until the total run time is reduced. Some phase times must be shorter.

> N/S Pulse delta of Phase x too small!
> Increase the N/S Pulse time of Phase x

This message only appears when INTERPOLATION is being used and not until you attempt to load the procedure. Either increase the indicated pulse time or decrease the phase time of the previous phase.

Note: This message will appear if the time difference between two subsequent pulses is less than 2 milliseconds, i.e. the time difference is too short for the INTERPOLATION to occur. The remedy is to a) increase pulse time in the latter phase, b) decrease the pulse time of phase x, or c) increase the run time of phase x.

> No phases defined
> Press a key

This message, which occurs in the EDIT mode, indicates that the user has failed to program a phase or pulse time somewhere in the current procedure. Use the up and down arrow keys to move through the phases, checking that parameter values have been entered.

> A procedure is running in load!
> Press a key

When working in the BASIC mode, this message indicates that the user has started a run and then tried to return to the main menu without first having stopped the run. You must first press the on and off key, to stop the run, and then press ESC.

8. Maintenance and trouble shooting

8.1 Maintenance

We recommend a mild detergent and a soft brush to clean the electrophoresis unit. Rinse with distilled water several times before using it. Check the pump housing monthly to remove any particles capable of reducing the efficiency of the pump.

If the unit is to be stored unused for a long time, it should first be rinsed several times with tap water, then rinsed twice with distilled water, and finally placed upside down and left to dry thoroughly.

Due to the unusually high current which is used, the platinum wires must eventually be replaced. In continuous operation, the anodes will last about six months and the cathodes for about 18 months. The condition of the electrodes can be revealed by visual inspection.

8.2 Trouble shooting

Problem	Correction
Current differs by more than 10 mA between the two fields and Curved results are obtained using HEX electrode	1. The electrophoresis unit is not levelled. 2. Check that the tray is resting properly in the tank. 3. The electrodes are burned out or faulty. Check that all electrodes fire - watch for bubbles. 4. The electrodes are not properly hooked onto the wall of the electrophoresis unit. 5. Check circulation of the coolant and buffer. Remove and clean circulation pump if necessary.
One or both fields are not working	1. Check the reading on power supply; check that the two fields are ‘‘firing”, i.e. bubbles are formed at the electrode. If the electrode wiring is worn out parts of the wiring is extremely thin or ‘‘cut off”. Exchange electrode. 2. Check that the contacts work between lid and elpho unit. Check the metal tongues.

Problem	Correction
Circulation pump does not work	1. The electrophoresis unit has been left with buffer in the pump - the pump does not work due to crystallization. Check the pump housing and remove any particles, rinse with a mild detergent and a soft brush.

WARNING! Changing of the pump unit must always be done by Amersham Biosciences AB Service Engineer.

High background	1. Insufficient washing of samples. 2. Sample contaminated with other material such as RNA or endonucleases. 3. Overloading of samples. 4. Incomplete destaining.
Smeary lanes and bad resolution	1. Improper preparation of DNA samples. Protein has not been dialyzed away from the DNA samples. The sample contains too much RNA. RNAse the sample inserts. 2. Restriction enzyme digestion of DNA samples is incomplete because Proteinase K was not completely inactivated. 3. Too much sample was loaded. Load thinner slices to improve resolution. 4. The voltage/current used was too high. 5. Gel concentration too low. 6. Impure enzymes (Amersham Biosciences enzymes recommended).
Bad resolution	1. Check samples; they may be contaminated with endonucleases from hands or other sources. 2. You are not using the optimal pulse time. 3. The voltage is too high. 4. The insert or insert slices were not loaded flat against the wall in the well.

Problem	Correction
(Bad resolution cont.)	5. Air bubbles in the well. 6. The insert was crushed when it was pushed into the well. Use a bent glass Pasteur pipette or a syringe to suck the inserts down into the well. 7. Buffer breakdown. Change according to recommendation. 8. Distorted wells. Cast a new gel.
No bands	1. Check samples; poor lysis of cells will result in stained material only in the wells. 2. The DNA is severely degraded due to wrong treatment or storage buffer.
Separation has gone too far Abnormal mobility	1. Wrong buffer concentration. 2. You forgot to turn on the buffer circulation or pump is not working due to formation of salt crystals in the pump. 3. Voltage/current too high.
High currents (cf. table 1)	1. Check buffer concentration and level - it should be 2.5 litres of 0.15x or 0.5x or 1.0x TBE. (Check the actual amperage.) 2. Check electrodes.

9. Ordering information and technical data

9.1 Ordering information

Accessories

Product	Code no.
EPS 600 power supply	19-0600-00
EPS 200 power supply	19-0200-00
MultiTemp III thermostatic circulator for 100-120 V, 60 Hz or for 220-240 V, 50 Hz	18-1102-77 18-1102-78
MacroVue UV-20 Transilluminator, 115 V or 230 V	80-6245-11 80-6245-30
Image Master VDS, DU 115 VAC, 60 Hz DE 230 VAC, 50 Hz DJ 100 VAC, 50/60 Hz	80-6246-82 80-6247-01 80-6247-20
Image Master VDS Analysis Kit	80-6309-71
Levelling kit, including horizontal table and spirit level	18-1016-88
Staining kit, 25 x 35 cm	18-1018-09
Gel support tray, for 15 x 15 cm gels	18-1019-21
Gel casting frame, for 15 x 15 cm gels	18-1019-24
Double sided comb making 12 or 23 wells, 1 mm thick, for 15 x 15 cm gels.	18-1019-25
Double sided comb making 12 or 23 wells, 2 mm thick, for 15 x 15 cm gels.	18-1019-26
Double sided preparative comb making one or two preparative wells	18-1019-27
HEX electrode	18-1019-22
Gel casting frame for 20 x 20 cm gels	80-1251-34
Comb with 22 wells, 2 mm thick, for 20 x 20 cm gels	80-1102-56
Point electrode (-) cathode	18-1019-28
Point electrode (+) anode	18-1019-29
Tube connector set	18-1104-26

CAUTION! Only spare parts approved or supplied by Amersham Biosciences AB may be used for maintaining and servicing of Gene Navigator™ System.

Chemicals and consumables

Agarose NA, 10/100/1000 g	17-0554-01/02/03
Agarose Prep, 50 g, low melting agarose (Suitable to make inserts)	80-1130-07
EDTA, 100 g	17-1324-01
Boric acid, 500 g	17-1322-01
Bromophenol Blue, 10 g	17-1329-01
Tris, 500 g	17-1321-01
Ethidium bromide solution, 10 ml	17-1328-01

For other Pulse Field Gel Electrophoresis products, see the Amersham Biosciences BioDirectory.

9.2 Technical data

Gene Navigator electrophoresis unit

Electrical requirements:	100-130 V, 200-260 V; 50 or 60 Hz
Power consumption:	4 VA
Cooling requirement:	Minimum 6 l/min
Maximum input:	450 V, 500 mA
Dimensions (W x D x H):	395 x 380 x 196 mm
Weight:	5.5 kg
Environment:	+ 4 - 40 °C 20 - 95% relative humidity
Chemical:	The wetted parts are resistant to solvents commonly used in electrophoresis and solutions containing inorganic and organic acids, alkalis and alcohol's.
Safety standards	This products meets the requirements of the Low Voltage Directive (LVD) 73/23/EEC through the harmonised standards EN61010-1 **Note:** The declaration of conformity is valid for the instrument when it is • used in laboratory locations • used in the same state as it was delivered from Amersham Biosciences AB except for alterations described in the user manual. • used as a "stand alone" unit or connected to other CE labelled Amersham Biosciences AB instruments or other products as recommended.

GN control unit

Electrical requirements:	100-130 V, 200-260 V; 50 or 60 Hz
Power consumption:	30 VA
Maximum input:	450 V, 500 mA
Dimensions (W x D x H):	364 x 280 x 110 mm
Weight:	5 kg
Environment:	+ 4 - 40 °C 20 - 95% relative humidity
EMC standards	This product meets the requirement of the EMC directive 89/336/EEC through the harmonised standards EN 50081-1 (emission) and EN 50082-1 (immunity). **Note:** The declaration of conformity is valid for the instrument when it is • used in laboratory locations • used in the same state as it was delivered from Amersham Biosciences AB except for alterations described in the user manual. • used as a "stand alone" unit or connected to other CE labelled Amersham Biosciences AB instruments or other products as recommended.
Safety standards	This products meets the requirements of the Low Voltage Directive (LVD) 73/23/EEC through the harmonised standards EN61010-1

10. Short instructions and reference list

The short instructions consists of two parts:

Gene Navigator short instruction which makes it possible for a person who is not experienced with pulsed field electrophoresis to perform a run, and GN Controller short instruction which explains the programming of the controller in a way that is easy to understand. Read them carefully to avoid any mistakes.

For further instructions please read chapters 5 and 6.

10.1 Gene Navigator System

This short instruction applies primarily to separations using the HEX electrode.

I. Materials required

Chemicals

1. 250 ml 0.5M (5x) TBE buffer
2. 1.3 g agarose NA (17-0554-01/02/03)
3. Sample DNA, immobilized in gel inserts 2x5x10 mm
4. Ethidium bromide (EtBr) solution 0.5 ml (1.0 mg/ml)
5. Pasteur pipettes, 3 pcs

Equipment

6. Gene Navigator with HEX electrode kit (18-1019-20) consisting of Hexagonal electrode array, 15x15 cm gel support tray, 15x15 cm gel casting frame and three double combs.
7. Disposable pipette tips, 5 blue
8. Gilson pipette P1000
9. GN control unit (18-1026-17)
10. Hose clamps, 10 pcs (18-1104-27, included in Gene Navigator electrophoresis unit)
11. Tube connections, male and female (18-1104-26, included in Gene Navigator electrophoresis unit)
12. Gel knife for cutting gels (80-1106-37) or a razor blade
13. Power supply; EPS 600 (19-0600-00) or EPS 200 (19-0200-00).
14. MultiTemp III thermostatic circulator (18-1102-78; 220-240 V/50 Hz and 18-1102-77; 110 V/60 Hz).
15. Staining box, stainless steel (18-1018-09)
16. MacroVue UV-20 Transilluminator (80-6245-11, 115 V and 80-6245-30, 230 V)
17. ImageMaster VDS, (80-6246-82, 115 VAC, 60 Hz; 80-6247-01, 230 VAC, 50 Hz; 80-6247-20, 100 VAC, 50/60 Hz)
18. Levelling kit with spirit level (18-1016-88)

Gene Navigator gel preparation and electrophoresis using the HEX electrode

Notes

1. Do not plug the buffer circulation pump into the mains before the start of the run.
2. Before connecting the controller make sure that the current on the power supply does not exceed 450 V and 500 mA

Procedure

1. Check that you have everything on the checklist.
2. Dissolve 1.3 g agarose in 110 ml 0.05 M (0.5x) TBE buffer. Melt the agarose in a water-bath or a microwave oven while supervising it to make certain that the solution does not boil over. Mix the solution occasionally to avoid local gelling.
3. While the solution is cooling to 60°C, place the gel support tray on the levelling table. Level the table by placing a spirit level on the gel support tray.
4. Place the gel casting frame on the tray. The conical holes or depressions at the bottom of the tray must be clearly inside (not immediately underneath) the gel casting frame. The red square will help you position the frame correctly (Fig. 4). The ridged side of the frame must be turned downwards.
5. When the temperature of the gel solution has decreased to 60°C, pour the gel and make sure that the holes are filled. If necessary use a Pasteur pipette to suck air bubbles from the holes and in the gel. The gel should be about 5 mm thick.
6. Place the comb parallel to a slotted side on the gel support tray (see Fig. 6). The position of the comb is marked by the thick red line inside the square on the gel support tray.
7. Allow the gel to set for 30 minutes. Read Gene Navigator short instructions while the gel is setting.
8. Remove the comb by tilting it backward and forward to free the comb from the agarose, then lifting it at one end. Carefully pull the rubber frame free from the gel and remove it.
9. Start MultiTemp III with a set value of 9°C.
10. Inserts can either be cut on a sterile surface or on a nuclease-free plastic film. Microscope coverslips are preferred for cutting (see Fig. 8) but a scalpel can be used instead. Place the inserts in contact with the front wall of the well to get sharper bands. A flame sterilized Pasteur pipette with a bent tip is recommended for loading the inserts before submerging them in the gel.
11. Seal the loaded wells with 0.5% Agarose Prep (low melting point) at ca 40°C to keep the inserts in place during handling of the gel.
12. Fill the Gene Navigator with 2.5 l 0.5M (0.5x) TBE.

13. Carefully place the gel support tray with the attached gel into the tank. Make sure that the gel wells are on the right side (see Fig. 10).
14. Start circulating the buffer by connecting the buffer circulation pump to the mains.
15. Position the hexagonal electrode array as shown in Fig. 11. The hexagonal electrode holder/connector is hooked to the same wall that holds the lid power connection.
16. Connect the three remaining connectors to the walls and place the lid on the unit.
17. Set the voltage limit appropriately for resolution of the desired fragment size (see section 7.1, table 2) and the current limit to a maximum of 500 mA.
18. Read section 10.2 of the short instruction for GN control unit.
19. Program the control unit. Suitable run times and pulse times for different samples are presented in Tables 2, 3 and 4.
20. On the power supply switch high voltage on.

    Then start the run by pressing [on off], on the controller. (To interrupt the run press the key again. Another press continues the run.)
21. When the run time has elapsed, turn off the power on the power supply and press [on off] on the controller, if the time has not run out, before removing the lid.

    WARNING: Before taking off the lid always cut the current by pressing [on off] on the controller if the run is not yet finished.
22. Remove the gel support tray.
23. To remove the gel, cut through the agarose in the tiny holes by sliding a razor blade under the edges of the gel.
24. The buffer used during the run can also be used for staining. To empty the tank, raise the leg on the bottom left side of the unit. Fix a piece of silicone tubing tightly to the outlet from the buffer circulation pump and fill the staining box with approximately 1 litre. The rest of the buffer can be emptied into a separate bottle for destaining.
25. Add 0.5 ml Ethidium bromide stock solution (1 mg/ml water stored at 4°C in a dark bottle) to the buffer solution in the staining box. Mix!

    WARNING! Ethidium bromide is a carcinogen. Use gloves, protective clothing.
26. Stain the gel for 30 minutes.
27. Destain (if necessary) for 30 minutes to several hours.

Recipes for stock solutions

A. 0.5 M (5x) TBE buffer; pH ca 8.3
Tris, 500 g (17-1321-01)
Boric acid (17-1322-01)
Na-EDTA 37.2 g or 200 ml 0.5 m pH 8.0
Dissolve in distilled water and make up to 10 l.

B. 1.0 mg/ml Ethidium bromide
Dissolve 10 mg Ethidium bromide (17-1328-01) in 10 ml distilled water and store in a dark bottle at 4°C

Warning! Ethidium bromide is a carcinogen so wear gloves at all times and wash off any solution which comes in contact with your skin.

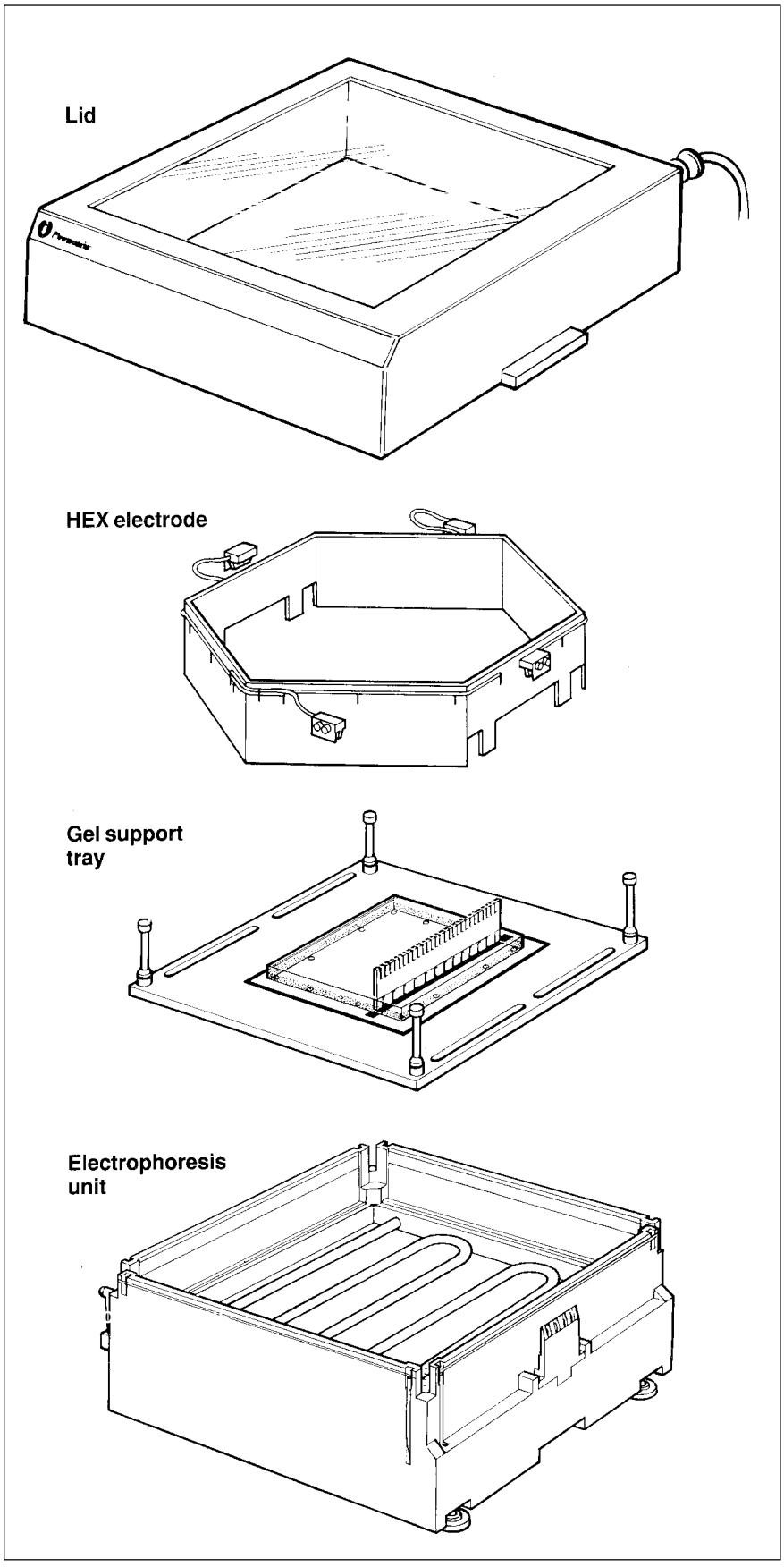
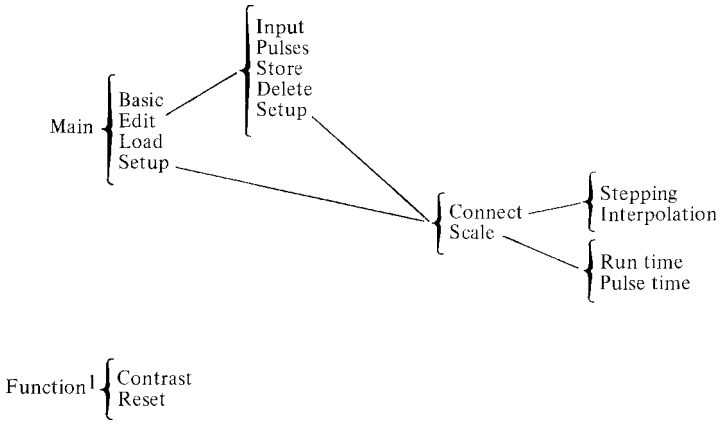


Fig. 20. Gene Navigator assembly.

10.2 GN Controller

Menu structure



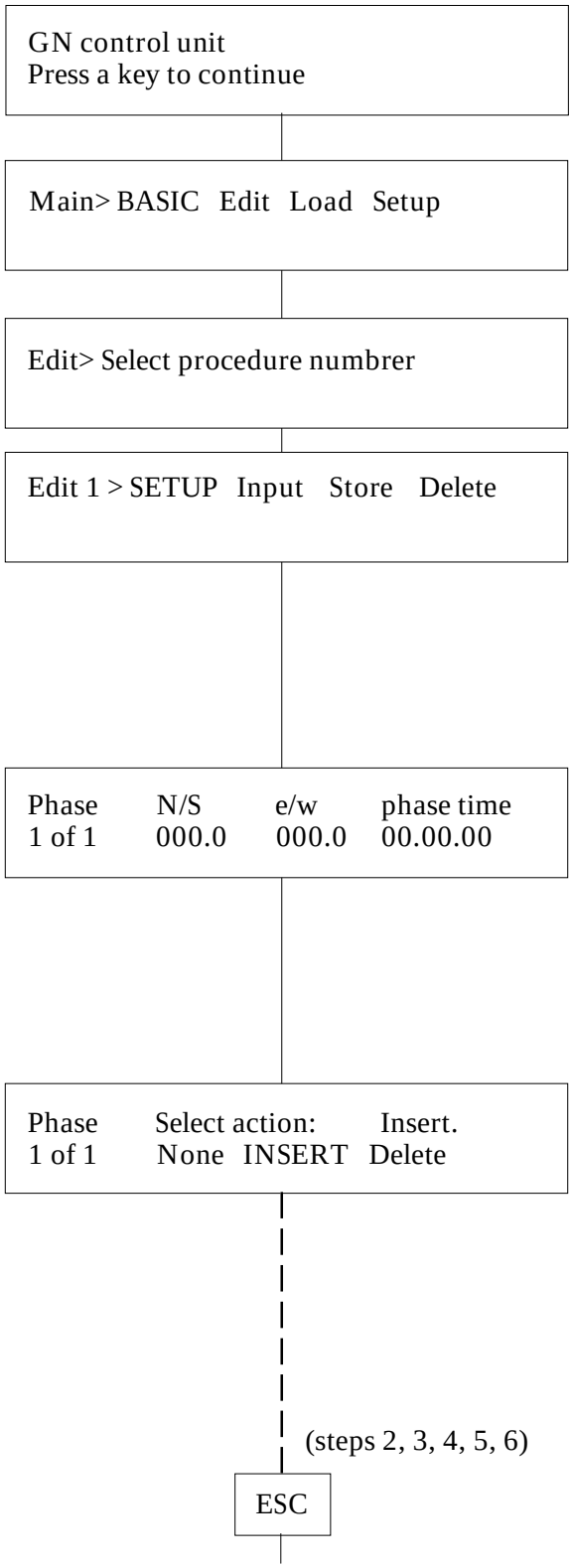
Function is the same as the F-button.

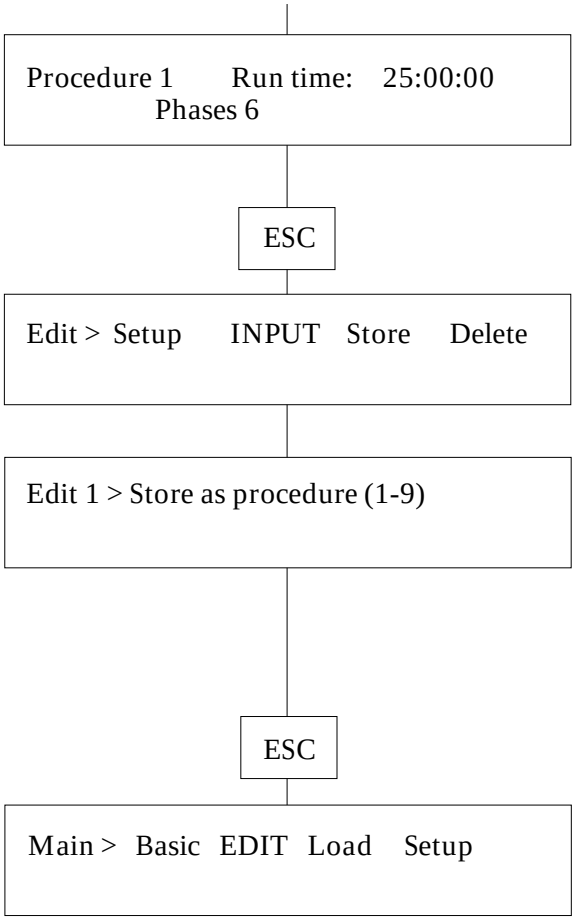
Programming the BASIC mode

All parameters can be reset by pressing the F button followed by → and ENT . An alternative is F , 2 , ENT .

Display	Comments
GN control unit Press a key to continue	Press a key
Main> BASIC Edit Load Setup	BASIC is already lighted. Just press ENT .
N/S 000.0 e/w 000.0 time 00.00.00 Start by ON Freeze by OFF End by ESC	Enter pulse time 90 s N/S by pressing 9 , 0 , 0 , ENT .
n/s 090.0 E/W 090.0 time 00.00.00	Enter the same value for E/W as for N/S by pressing ENT .
n/s 090.0 e/w 090.0 TIME 00.00.00	Enter time 24 hrs by pressing 2 , 4 , 0 , 0 , 0 , 0 , ENT .
N/S 090.0 e/w 090.0 time 24:00:00	Press on off to start, interrupt or continue the run. Pressing ESC ends the run.

Programming the phases (EDIT mode)





Both the total run time of all phases and the number of programmed phases are displayed.

Press →, ENT to store the procedure.

Enter the number under which you want to store the procedure by pressing the chosen number, followed by ENT .

To run the program the instrument must be in LOAD mode.

10.3 Reference list

This list describes some general applications of PFGE. In these applications, types of PFGE other than HEX and DI are also used. These references give you valuable information on sample preparation and running parameters.

Organism reference

Amoeba	24
Bacterial DNA	37-40
Blotting megabase DNA	Amersham Biosciences VacuGene Protocols 1 to 4
Candida	43-45
Circular DNA	20, 36, 37, 39
Crithidia	35
Genomic DNA	48-73
Human chromosome x and y	64, 70
Human chromosome 3	72
Human chromosome 4	68
Human chromosome 7	62, 65, 69
Human chromosome 9	71
Human chromosome 11	59
Human chromosome 17	55
Leishmania	17-20, 26, 36
Method	1-14, 48, 50, 54
Neurospora crassa	46
Plant	15,16
Plasmodium	21-23
Saccharomyces /Schizo-saccharomyces	41, 42
Theory	1, 2, 5, 10-12
Trypanosome	25-35
Virus	47

References

Reference 1. Pulsed-field electrophoresis: application of a computer model to the separation of large DNA molecules. Proc.Natl.Acad.Sci. 84 (1987) 8011-8015.
Author Lalande, M.
Keyword PFG/Theory/Method

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Author Corcoran, L.M.
Keyword PFG/Review/Mapping

Reference 3. Separations of open-circular DNA using pulsed-field electrophoresis. Biochemistry 84 (1987) 4054-4057,
Author Levene, S.D., Zimm, B.H.
Keyword PFG/Circular DNA/Topology

Reference 4. Preparation and manipulation of large DNA molecules: advances and applications. TIBS 12 (1987) 284-287
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Keyword PFG/Pulsaphor/Preparation/Method

Reference 5. Pulsed field gel electrophoresis and the technology of large DNA molecules, - Genome Analysis: a practical approach (Ed. Kay Davies). IRL Press Inc., McLean, Va.
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Author Smith, C.L., Cantor, C.R.
Keyword PFG/Preparation

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Applications of DNA probes (1986) 87-92
Author Smith, C.L., Cantor, C.R.
Keyword PFG/Mapping

Reference 8. Optimized conditions for pulsed-field electrophoretic separations of DNA. Nucleic Acids Research 16, no 15 (1986)
Author Birren, B.W., *et al.*
Keyword PFG/Method

Reference 9 Estimation of circular DNA size using gamma-irradiation and Pulsed Field Gel Electrophoresis. Analytical Biochem (US) 1989, vol.177, no 1, p. 110-114
Author Beverley, S. M.
Keyword *E.coli/Leishmania*/method

Reference 10. High-resolution separation and accurate size determination in pulsed-field gel electrophoresis of DNA. 1. DNA size standards and the effect of agarose and temperature. Biochemistry(US) 1988. Vol 27, no 26 p9204-9210
Author Mathew, M.K., Smith, C.L., Cantor, C.R.
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Pulsed field gel electrophoresis protocol

Box _____ Run date _____ Run no _____

Exp comments/aim .....
.....
.....

Gel: % Agarose Cooler Temp: °C Buffer: 0.5x 0.15 x TBE
Gel: 15 x 15 20 x 20 Comb: 1 mm 2 mm prep.

Pulse time			Fresh buffer		
Phase	N/S=E/W	Run time (hrs)	Run time	Current:	

Electrodes: Hexagonal Electrode no. .....
Point electrodes Position: + ..., - .....)

Voltage: .....V Start Current I_i = .../.....mA
Current levelling: Y / N Final Current I_f = .../.....mA

Sample			Insert		
Lane	Type	Date	Size	Amt	Enzyme / Comments

Insert size standard = 100 ul(2x5x10mm)

1 2 3 4 5 6 23

This is one of the layouts we use for protocols. It is especially good for trouble shooting.

Pulsed field gel electrophoresis protocol

Box _____ Run date _____ Run no _____

Exp comments/aim .....
.....
.....

Gel: % Agarose Cooler Temp: °C Buffer: 0.5x 0.15 x TBE
Gel: 15 x 15 20 x 20 Comb: 1 mm 2 mm prep.

Pulse time			Fresh buffer		
Phase	N/S=E/W	Run time (hrs)	Run time	Current:	

Electrodes: Hexagonal Electrode no. .....
Point electrodes Position: + ..., - .....)

Voltage: .....V Start Current I_i = .../.....mA
Current levelling: Y / N Final Current I_f = .../.....mA

Sample			Insert		
Lane	Type	Date	Size	Amt	Enzyme / Comments

Insert size standard = 100 ul(2x5x10mm)

1 2 3 4 5 6 23

Notes:

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