

Biometra

Rotaphor[®] Type VI

Instruction Manual



Chapter 1

Unpacking

1.1 Electrophoresis Chamber

Lower part with data and power cable

- Gel support tray
- 4 corner insulators
- Safety lid with Rotor V2.1

Note: Each Rotaphor is checked individually and therefore scratches on the gel support tray may occur during this testing.

1.2 Computer

- Computer in a mini tower
- Power cord
- Manuals of the computer components
- Windows XP licence
- Electrode power cables

Optional:

- Keyboard
- Mouse
- Speakers
- Monitor

1.3 Accessories

1.3.1 Gel Casting Frame

- 20 x 20 cm frame
- Comb with 18 wells
- Four locating screws

1.3.2 Block Former

- Insert mould with 20 holes
- block remover

Please remove all adhesive tape and proceed to the next page.

Chapter 2

Setting up

2.1 Short Description

2.1.1 Electrophoresis Chamber

The lower part of the electrophoresis chamber contains:

- An efficient cooling coil
- A pump to circulate the electrophoresis buffer
- Power and data connectors
- Mains and data cable
- Levelling feet
- Temperature sensor
- Temperature control valve

Place the chamber, with cables and connectors for the cooling circulation at the rear, on a suitable work surface.

The gel support tray consists of:

- 4 cylindrical pillars to maintain the correct distance between electrodes and tray
- 4 edge insulators to achieve the rotary symmetry of the electric field in the rectangular chamber
- 4 holes to keep the gel in position during electrophoresis
- 8 slots to allow buffer circulation
- 4 threaded holes to secure the casting frame

Insert the gel support tray into the lower part of the electrophoresis chamber.

The safety lid consists of:

- ROTOR V2.1 (A field correcting electrode array)
- Stepping motor
- Gear box
- Light barrier
- Connectors fitting to the lower part of the chamber

Place the lid on the lower part of the chamber. The electrical connectors will fit correctly together.

Warning: Placing the lid on an uneven surface or closing the lid with the gel casting frame mounted, can damage the electrodes!

2.1.2 The Computer

The included Computer is a standard PC operated with Microsoft Windows XP.

2.2 Installation

2.2.1 Cooling

Connect silicon tubing (best with temperature isolation) to the coolant in and outlet and secure the connections with hose clamps. Connect the opposite remaining ends of the tubing in the same way to a thermostatic circulator.

It is possible to cool several ROTAPHOR devices with one thermostatic circulator, if you connect them with tubing 'in line'. 5 chambers can be connected in this way but the coolant has to be as cold as -5°C . We recommend a thermostatic circulator able to cool efficiently from -10°C to $+20^{\circ}\text{C}$. Ideally it should contain at least 5 l of methanol/water or glycerine/water coolant. Please take into consideration that the maintenance of the electric field produces about 30 W heats (210V, 130mA, 0.025M TBE) per ROTAPHOR that needs to be removed from the chamber.

2.2.2 The Computer

Connect the following cables to the sockets at the back of the computer:

- Keyboard connector (encoded violet)
- Mouse connector (encoded light green)
- Monitor connector (VGA connector – it fits only at one place)
- Loudspeaker connector (marked)
- Cable of the electrophoresis chamber (placed in an extension bay – **Chamber**)
- Data cable of the electrode power supply (placed in an extension bay – **Supply**)

Connectors on most cables ensure error-proof fitting. Secure connectors with screws as far as possible.

2.2.3 Power supply

Connect your power supply to the sockets at the back of the electrophoresis chamber using the electrode power cable. The red banana plug corresponds to the anode (+), the black to the cathode (-). Theoretically, any power supply providing 250V, 400mA is sufficient for virtually all possible applications, but we do explicitly **not** recommend it, since only the electrode power supply from BIOMETRA has a suitable interface. This interface enables the computer to control the field strength automatically and to switch of electrode voltage if no electrophoresis is running.

2.2.4 Final Settings

Use the levelling feet to maintain a horizontal position of the electrophoresis chamber. Because it is very important for optimal results, measure the horizontal position of the electrophoresis chamber at the top of the safety lid at the 3 possible sides with a large spirit level and correct the position, if necessary, with the levelling feet.

Connect the power cords to the rear of the computer, the monitor and the power supply. Finally, connect the power leads to a properly grounded wall socket.

Chapter 3

Handling the Electrophoresis Chamber

The following sections describe how to use the features of your Rotaphor.

3.1 Sample Preparation

Large DNA molecules must be prepared in agarose inserts as described by various authors. When the agarose blocks are prepared with the mould provided, they will match the size of teeth on the provided comb. Before filling up the holes of the mould, moisten the mould by immersing it into water (this avoids air bubbles at the corners). Dry the surface and seal one side of the mould with water resistant adhesive tape. After solidification of the low melting point agarose inserts (15 min 4° C), remove the tape and push the blocks out of the mould by using the plexiglas insert remover“ provided with the apparatus. For convenience, cut the blocks with a scalpel into two identical parts and transfer them into a container filled with NDS-buffer.

3.2 Buffer Preparation

Best results are obtained with 0.025 M TBE (pH 8.5) prepared in the following way:

3.2.1 Stock Solution of TBE Buffer

To prepare 1 l stock solution (usually known as 10 x TBE), dissolve the following substances in 900 ml deionized (or distilled) water:

- 108 g Tris
- 55 g Boric Acid
- 40 ml 0.5 M EDTA pH 9

Adjust volume to 1000 ml

Store this stock solution at 4°C and do not keep it for more than two weeks.

The electrophoresis buffer is obtained by diluting the stock solution 1:40 with distilled water (0,025 x TBE - 60 ml / 2400 ml). Pour 2.4 l of this buffer into the electrophoresis chamber.

Do not use more than 2.4 l of electrophoresis buffer !

3.2.2 Stock Solution of Loenning Buffer

To prepare 1 l stock solution, dissolve the following substances in 900 ml deionised (or distilled) water:

- 44 g Tris
- 42 g NaH₂PO₄ * 1 H₂O
- 20 ml 0.5 M EDTA pH 7.5

Adjust volume to 1000 ml

Store this stock solution at room temperature

The final electrophoresis buffer is obtained by diluting the stock solution 1:30 with distilled water (80 ml / 2400 ml). Pour 2.4 l of this buffer into the electrophoresis chamber. This buffer withstands only an electrophoresis duration of less than 30 hours but small DNA fragments (20-100kb) resolve very well. The current with this buffer is very high. Therefore do not apply voltages above 130V, otherwise the rotor electronics might be damaged or the fuse will blow.

3.3 Setting up the Electrophoresis Chamber

Open the electrophoresis chamber and take out the gel support tray. Pour 2.4 l of the selected buffer into the electrophoresis chamber and switch on the computer if not already done. Start the thermostatic circulator and allow the apparatus to cool to the desired temperature as displayed in the main window. In our experience, the optimum temperature range for most applications is 10°C to 15° C (see below).

3.4 Precautions Using the Rotor V2.1

There are some rules to be born in mind whenever using the Rotor V2.1:

3.4.1 Voltage

The Rotor V2.1 is able to withstand a maximum voltage of 255 Volts. Voltages higher than this will activate the fuse at the back of the controller and thereby disconnect the electrodes from voltage. In addition, it is possible to damage the Rotor V2.1 by applying voltages higher than 255 V.

*Therefore, never apply voltages higher than 255 V
to the Rotor V2.1.*

3.4.2 Buffer Concentration

The Rotor V2.1 is designed to work with the buffer concentrations described above. Working with buffer concentrations higher than this, leads to a high current. Consequently, the electronics generates a considerable amount of heat, especially at high voltages, which may not be carried off properly. At 80°C, a fuse inside the rotor, or the fuse at the back of the controller will disconnect the rotor from the electricity.

*Therefore, never apply buffer concentrations that
lead to a power consumption above 40 W*

3.4.3 General Precautions

Some ions, e.g. chloride, will lead to corrosion or other structural changes of the platinum electrodes. These ions are a normal component of tap water.

Therefore, use only deionized or double distilled water and chemicals of analytical grade.

Check quality of water and chemicals when electrodes do not shine metallically after a run.

The electronics of the Rotor V2.1 is only water resistant, and *not waterproof*. Particular care should be taken to minimize the periods of time the electronics are subjected to conditions of high humidity (e.g. insufficient cooling of buffer during electrophoresis or storing a buffer filled ROTAPHOR with the lid closed) as this may, over a considerable period of time, lead to some corrosion of metallic components of rotor electronics and chamber. Your Rotaphor will be destroyed in that way.

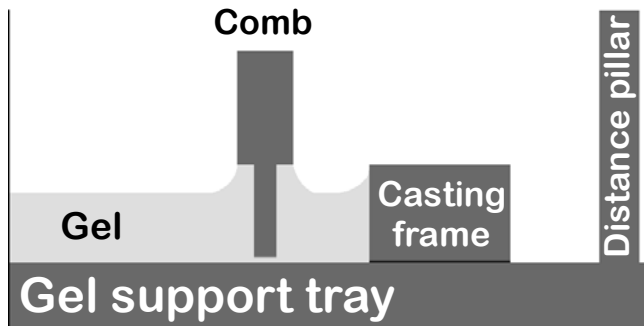
Please do not submerge the rotor electronics under water.

3.5 Preparation of Agarose Gels

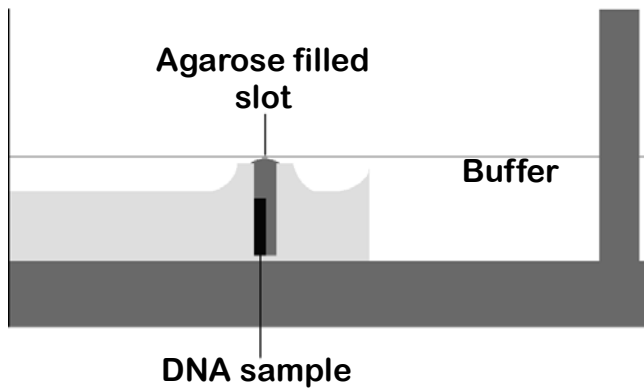
Place the gel support tray on an even horizontal surface (check with spirit level), take away the corner insulators and secure the gel casting frame to the tray with the four screws provided. To make 1% gels of 7 mm thickness, dissolve 3.0 g agarose (e.g. BIOMETRA GTG-agarose Rotaphor Grade, order no R2244) in 300 ml of electrophoresis buffer. Agarose gels from 0.5% to 1.5% can also be employed; the choice of concentration depends on the length of the nucleic acid molecules which are to be separated and the band sharpness desired. Heat the suspension to boiling in a microwave oven, ensuring that all agarose particles are properly dissolved. Then, for convenience, seal the edges of the frame with agarose and let cool down the rest of the suspension to about 50°C before pouring the gel. Then pour the gel, and remove the remaining air bubbles from the 4 cylindrical holes and from the gel surface with a pipette. At last position the comb. It fits the two cylindrical holes on top of the gel casting frame.

Let the gel solidify for 45 min without moving it around! When the gel is set, carefully remove the comb and cut the gel from the casting frame with a scalpel. Then remove the four screws and lift the frame very carefully. Failure to take care at this point may result in the gel becoming detached from the gel tray during electrophoresis. Avoid an uneven gel surface (see below). Remove all loose agarose particles from the tray and close the holes of the 4 screws in the tray at the lower side with water resistant adhesive tape, to avoid any possible distortion of the electric field due to electricity flow through these holes.

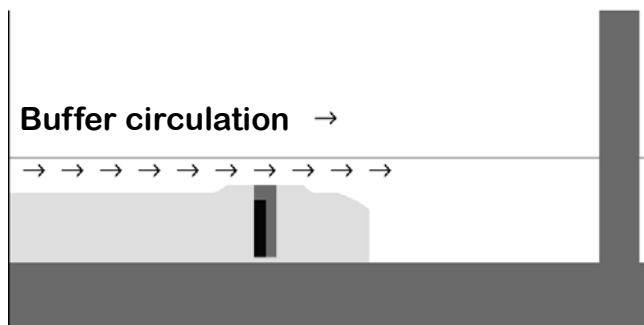
Note: Pulsed field gel electrophoresis is not compatible with ethidium bromide in the gel during the run. DNA becomes stiff and moves very badly if the dye is intercalated. Consequently, the separation range is narrowed dramatically.



Adhesion pulls up the agarose solution at the casting frame and at the comb. This causes an uneven gel surface.



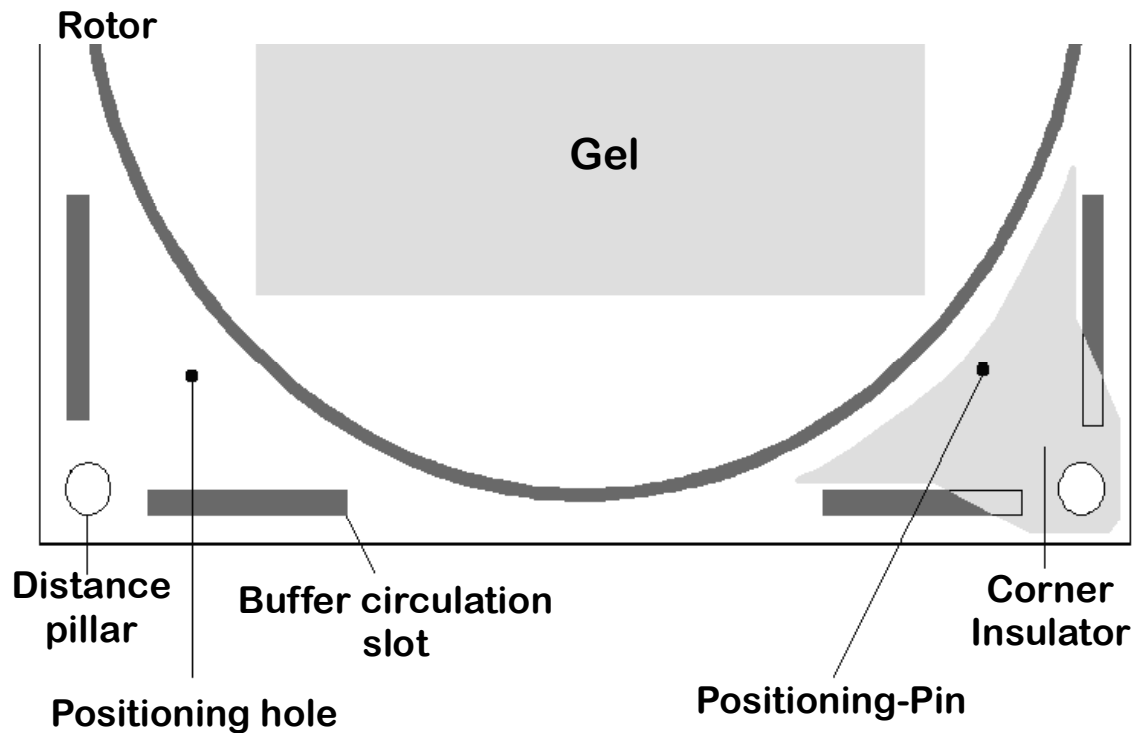
Especially after filling and capping the slots, the buffer circulation above the gel is blocked by this agarose rampart..



Therefore, cut away the agarose rampart with a scalpel to allow free buffer circulation.

3.6 Loading the Gel

To load the gel, cut the agarose blocks containing the sample to the desired size and insert them into the wells using a metal spatula. Make sure that the inserts attach to the front wall (direction of electrophoretic separation) of the well and finally “seal” the wells with some hot 1% agarose. Now put the corner insulators over the distance pillars and press them down until they sit on the gel support tray (see below). The corner insulators are held in place by a plastic pin which has to be on the lower side.



Mount the 4 corner insulators as shown in the right part of the figure. There should be no space between gel support tray and the corner insulators.

3.7 Loading the Electrophoresis Chamber

After the buffer has reached the desired temperature, remove the safety lid. This action prevents all current flow, to the electrodes as well as to the motor. Place the gel support tray with the gel and the corner insulators into the electrophoresis chamber and lower it carefully until the tray rests firmly on the inner edges of the tank. The sample wells have to be in the back of the chamber near the electrical connectors. This will ensure the nucleic acids migrate in the correct direction once the power supply is switched on and electrophoresis has commenced. The gel should be covered by about 5 mm depth of buffer when the corner insulators are in place. Any additional buffer required should be added at this point. Be sure, however, that the buffer level does not reach the rotor electronics! Place the safety lid on the lower part of the electrophoresis chamber and take care that all plugs fit into their respective sockets. Now the lid is safely interlocked. Wait for 5 to 20 minutes until the temperature of the gel is approximately equal to that of the electrophoresis buffer.

3.8 Start and Termination of Electrophoresis

After setting the parameters and pressing the “Start Electrophoresis” button, the anode and cathode can easily be distinguished, at least when TBE is employed as electrophoresis buffer, because more bubbles develop at the cathode than at the anode. DNA molecules move towards the anode, therefore the anode should be the electrode at the front. The electronics of the Rotor V2.1 interrupts current flow when the polarity is wrong!

The electrophoresis is terminated automatically but it might be interrupted by pressing the “Interrupt Electrophoresis” button at any time. Remove the safety lid and the corner insulators. Then lift the gel support tray out of the electrophoresis chamber. Cut off the gel’s

“feet” with a scalpel and slide it gently from its support. Proceed as usual with staining, destaining, nicking, blotting and hybridization.

Note that special care has to be taken to prepare the gel for blotting when very long DNA molecules have been separated. We have found that vacuum blotting gives an excellent sharpness after transfer but the sensitivity of hybridization is better with capillary blotting.

After each electrophoresis experiment, the chamber should be emptied in the following way:

Insert one half of a tubing connector of the appropriate diameter into a long piece of silicone tubing and press the other half into the outlet of the pump. The free end of the tubing should hang into a large container standing on the floor. Switch off the pump at the controller, when the tube is filled with buffer. Remove the buffer by gravity siphoning into the container. Moving around the upper end of the tubing helps cleaning the chamber and the inlet of the pump from agarose particulate matter. At the end of the draining procedure the instrument should be tilted to remove the last small volumes of buffer.

In order to prepare the tray for the next gel, remove the agarose plugs from the 4 cylindrical holes with a spatula or by vigorously blowing air into the holes. Check that the inlet of the buffer circulation pump is free of all particulate matter. If the instrument is not to be used immediately, let it air dry with the lid removed from the main chamber body. If the instrument is not to be used for more than 1 week, pipette 0.5 ml glycerine into the pump inlet for pump maintenance.

Chapter 4

Experimental Considerations

4.1 Introduction

The search for a theoretical basis regarding the separation of very large DNA by pulsed field gel electrophoresis revealed that no simple theory describes all the characteristics of this method. We will therefore use a relatively simple model to define parameters for separations of the desired size range. In all cases, the following parameters have to be considered:

- Size of the nucleic acid molecules
- Interval
- Interval Inverse
- Angle between the orientations of the electric field
- Field strength
- Temperature
- Agarose concentration
- Ionic strength of the electrophoresis buffer
- Duration of electrophoresis

5.2 Included Standard Parameter Lists

The controller software includes numerous parameter lists covering most of the separation needs. After selection of such a parameter list, an image of a separation of different size markers will be shown that was generated using the respective parameters. These images are intended to give you a realistic idea of the inherent separation performances and should act as guidelines for initial separation conditions for a given DNA size range. If you need to fine tune the separation conditions, we recommend that you start with a standard list and apply subtle changes.

Note: An alteration of only one parameter mentioned above will lead to a different behaviour of DNA molecules in the gel with obvious consequences for their separation. If, for instance, the voltage is increased by 2 Volts at the separation of *S.pombe*, the upper bands fade or become invisible (see below).

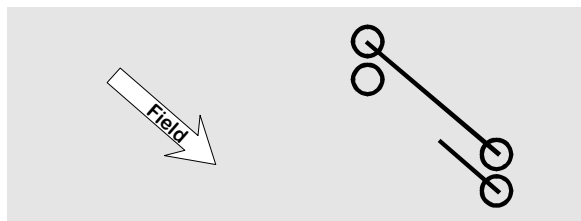
5.3 Conventional Gel Electrophoresis with the Rotaphor

It is also possible to run a conventional electrophoresis with the Rotaphor. In this case, place the gel support tray (with gel and corner insulators) into the buffer filled electrophoresis chamber and wait until buffer has reached the predetermined temperature. Stop buffer circulation by turning of the pump by unchecking the box Pump in the main window. Otherwise the buffer circulation will wash your samples out of the wells. Enter the DNA (in sample buffer e.g. 10% glycerine) directly into the wells of the gel. Close the lid very carefully, choose for start and end angle 0° (if you want to apply this electrophoresis as 2nd dimension after a PFGE, please enter 90° for start and end angle. Please note that the DNA moves from right to left in this second dimension). Enter also a suitable duration and field strength and start electrophoresis. Since inhomogeneous temperatures impair the results, the buffer circulation pump will start after 30 minutes automatically.

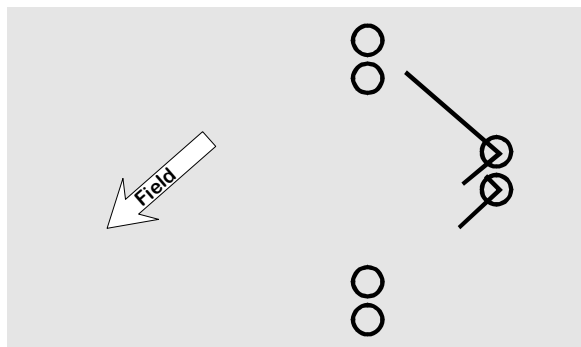
5.4 Consequences of Parameter Modifications

Using a simple theory developed by southern and colleagues, the consequences of modifications made to parameter lists shall be discussed. This theory explains relatively well the observed changes of DNA mobility if applied only qualitatively and if parameters are not changed dramatically.

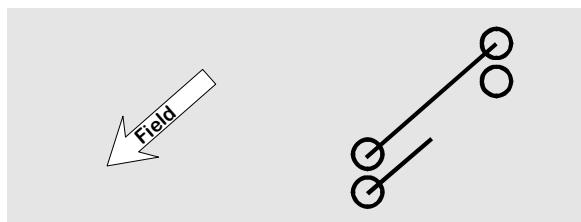
5.4.1 Angles between 0° and 90°



Two DNA molecules are shown, a large DNA (~300kb) and a small one (~50kb). These move, driven by the electrical field, through the gel pores.

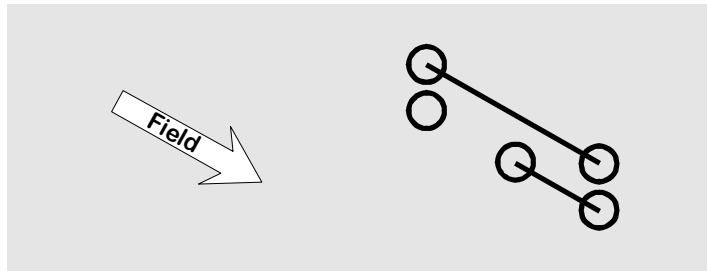


After changing the field angle by 90° or less, the DNA molecules will move into the new direction. After direction change, the leading end is still the leading end.

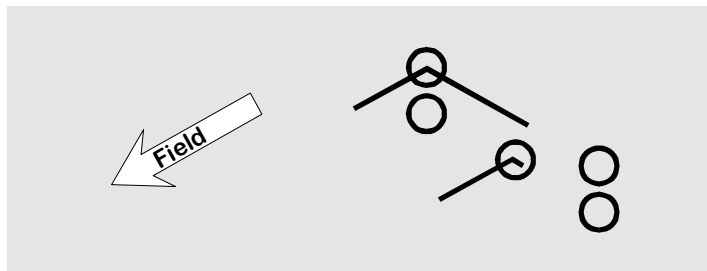


Since velocity of DNA molecules with a size above 50 kb is virtually identical, no separation of the small and large molecules will be observed.

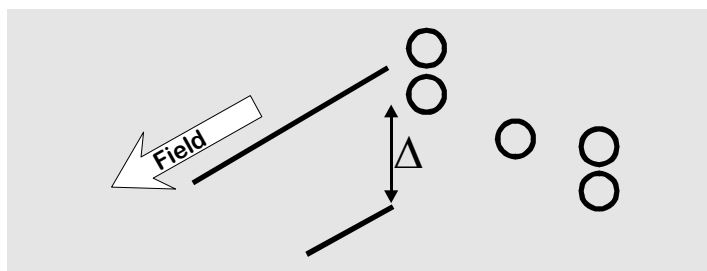
5.4.2 Angles between 91° and 160°



Looking at the pulsed field gel electrophoresis, the situation is identical at the beginning.



However, the angle between the field vectors is more obtuse at PFGE. Consequently, the force in backwards direction is larger than in forwards direction. Therefore, the trailing end becomes the leading end and the whole molecule is forced through the pore which harboured this end when the field was last changed. Since the trailing end of a short molecule is not



as far behind as the trailing end of a larger DNA molecule, the shorter molecule has moved further than the larger molecule. The position difference Δ results in the observed separation of the respective molecules.

This simple theory allows the prediction of the consequences of parameter changes, which fit the observed behaviour very closely, at least if only subtle changes are applied.

1. Duration

The duration of an electrophoresis is directly proportional to the distance a given DNA molecule travels during a run, if no exponential voltage ramps are used. The separation distance Δ increases proportional to the travelling distance, and therefore also proportional to the duration. In reality, it has to be taken into account that a gel has a limited size and bands which have left the gel are lost.

2. Interval

According to the theory shown above, the separation distance Δ increases with the number of pulses. Since separation takes place only, if the lagging

ends of all molecules that should be separated have left the pore of the pulse before, the product of interval (time) and field strength (voltage) needs to be adequately high. Otherwise, the larger molecules are trapped in a pore, do almost not move and are not separated. It might therefore be concluded that increasing interval and/or voltage increases the upper border of the separation range. Since in both cases the number of pulses per run is reduced (higher voltages result in shorter duration), the separation distance between two given molecules is also reduced.

3. Angle

According to the above theory, the angle should be not relevant for the separation quality if it is larger than 90° . Only the duration needed for the same travelling distance should increase with the angle. This fits more or less the observed situation, but more obtuse angles result in sharper bands. It therefore needs to be decided whether short runs or sharp bands are most relevant.

4. Voltage

Most of the influence of the field strength has already been discussed together with the interval, but there is an observation which needs to be mentioned: If the DNA molecules are larger than 2000 kb a phenomenon called “trapping“ leads to impaired results. Trapping means that a voltage dependent fraction of the molecules is “trapped” each pulse in the gel. If huge amounts of such DNA are applied to the gel, these trapped molecules may be seen after staining as smeary ladder. Consequently, the amount of DNA in the “real band” is reduced every pulse resulting in complete disappearance of the “real band” if the voltage is too high for the separation problem. Especially if the size of the molecules becomes > 4 mbp, the voltage needs to be reduced to very low values ($\sim 40V$). This leads to very long durations and limits therefore the upper size of DNA molecules that may be separated by PFGE.

5. Temperature

The temperature has a relatively small influence on the separation. Only the duration needs to be increased with lower temperatures. If the temperature is lowered from 13 to $8^\circ C$, the molecules travel about 30 % less down the gel in the same time. Since the non separated molecules travel also faster at high temperatures, the upper part of the gel is wasted at elevated temperatures. Additionally, high temperatures lead to blurred bands.

Chapter 6

Maintenance

The ROTAPHOR electrophoresis chamber is designed to operate with aqueous solutions and not with organic solvents !! Do not run the buffer circulation pump without buffer in the electrophoresis unit since the pump is not designed to run dry. The pump motor with the impeller is fitted to the pump hose through a bayonet attachment and can be changed if necessary. It is not essential to clean the electrodes or any other part of the lid routinely, but it is essential to empty the chamber after each run. Do not close the ROTAPHOR until chamber, tray and lid are dry. Very high humidity (e.g. generated by buffers in the closed chamber at room temperature) leads to condensation on the metallic parts of the electronics and can therefore lead to early age-hardening. If the ROTAPHOR electrophoresis chamber is not to be used for a long period of time, it should be rinsed several times with tap water and finally once with deionised water. To dry, leave the electrophoresis chamber upside down (without the lid) for a suitable period of time and then pipet 0.5ml glycerine into the pump inlet, before storing it away.

Chapter 7

Glossary

Corner Insulators Plastic pieces, that prevent the buffer to fill the corners of the electrophoresis chamber. They are necessary to make the homogeneity of the electric field independent of the rotor angle.

Duration Time until the end of electrophoresis

Rotor V2.1 Rotatable field adjusted electrode array

Interval Time between two rotor movements (in other designs described as 'pulse time')

kb Kilo bases (size of DNA molecules).

PFGE Pulsed Field Gel Electrophoresis

Rotor Angle Angle between the two opposite positions of the electrodes

Spirit Level Instrument to check whether a device takes up a correct horizontal position

Startposition Position of the rotor where the light barrier is interrupted

Chapter 8

Trouble Shooting

8.1 Rotaphor does not function

a) No action at all

- + The pilot lamp does not light green when computer is turned on.
 - There is no power in your outlet. Try another outlet.
 - Mains cable is not connected to the rear of the controller. Check connection.
 - Mains cable is defective. Replace with new power cable.

b) Computer works but chamber shows no reaction.

- Check that all connectors are correctly fitted.
- Check that the lid has been correctly positioned.

c) Rotor does not stop at the light barrier.

- In very brightly lit areas, the light barrier can not work. Operate only in normally lit areas and also avoid areas which may be subjected to direct sunlight.
- The position indicator (metal needle) on the rotor is damaged. Repair or replace needle.
- The message “Rotor Position inexact” is often displayed. In this case select a slower rotor speed.

d) The computer crashes regularly.

- The computer may be subjected to considerable surges or electrical ‘spikes’. This often occurs when numerous pieces of equipment are operating simultaneously in the same working area. Change location of the instrument or filter the supply power.

e) There is no power at the electrodes

- The power supply is not working
- The fuse at the back of the chamber is blown. Replace by a new 250mA/250V fuse.
- Polarity is wrong. Change the connectors at the power supply.
- There is no contact between rotor electronics and the metal rings in the lid. Withdraw rotor and bend metal pieces in a way that they make a good contact with the metal rings in the lid.

f) The 3 way valve of the temperature control does not work

- + The TEMPERATURE “LED” does not light green (main window).
 - Temperature chosen was too high. Lower maximal temperature.
- + The TEMPERATURE LED lights green.
 - Relay becomes faulty with age. Contact your dealer for replacement.
 - Check whether the connector of the cable to the chamber is inserted correctly at the rear of the computer.

g) The buffer circulation pump does not work

- The checkbox “Pump” in the main window is not checked.
Click the checkbox to start buffer pump.
- Relay becomes faulty with age. Contact your dealer for replacement.
- Check whether the connector of the cable to the chamber is inserted correctly at the rear of the computer.

h) Platinum electrodes do not have shiny metallic finish after electrophoresis.

- + The water is insufficiently deionized. Use distilled or double distilled water to prepare buffer.
- + Chemicals are not of the required quality. Use only analytical grade chemicals.

i) Build-up of condensation on the lid during or after the run.

+ Buffer temperature is too high.

– Selected temperature limit is too high. The buffer temperature should be always 5°C lower than the air temperature around the ROTAPHOR.

– 3 way valve is malfunctioning (you hear no “clack” from the chamber when the valve is switched on / off.

– Thermostatic circulator does not work or chosen temperature limit is too high.

– After completion of electrophoresis, unit was not sufficiently dried.

If there is any problem not mentioned here, please contact your dealer.

8.2 Experimental Problems

a) Very large DNAs do not enter the gel.

+ Field strength is too high. Use lower field strength (see explanation above).

+ Incorrect buffer concentration used. Use correct buffer.

+ Incorrect buffer pH used. Use correct buffer.

b) Lanes are distorted.

+ The electrophoresis chamber is not exactly horizontal or the lid is incorrectly positioned on the chamber.

– Check level with large spirit level without the lid in place on top of distance pillars!

– Check current from power supply. The current should be the same in both rotor positions.

+ Corner insulators have not been in place during electrophoresis or have been mounted incorrectly.

+ Incorrect volume of buffer used. Check that volume is 2.4 liters.

+ Poor grade agarose employed in experiments.

+ Poor circulation of electrophoresis buffer

– the pump does not work (as described above)

○ the fuse inside the computer is blown. Replace with an 800mA / mtr fuse

- The inlet of the pump may be blocked with debris
- Check the connector at the rear of the computer.
- If you did not apply glycerine before long time storage, crystals have formed in the pump. Try to solve these blocking crystals with water over some days or contact your dealer.
- Inlet of the pump is blocked. Clean it and take care when preparing the gel that all loose agarose particles are removed before the gel tray is placed in the chamber.

Air bubbles sucked under the tray by very intensive buffer circulation do not affect the separation.

c) Different velocity of same DNA size

- + Different amounts of DNA may lead to very different velocity of the same DNA size. This effect influences even adjacent lanes !!! The more DNA is applied, the slower it moves.

d) Very blurred bands and / or areas where no DNA can be detected

- + Poor circulation of electrophoresis buffer.
 - Buffer pump is not working (see above)
 - Inlet of the pump is blocked (see above)
 - The gel surface is very uneven and blocks the buffer circulation above the gel.
- + The chamber is used the first time or it was stored longer than 2 weeks. This phenomenon is seen sometimes. To prevent this problem, fill the chamber with desired buffer, insert tray, close lid and cool to 12°C. Incubate for 1 hour, change the buffer and start your electrophoresis.