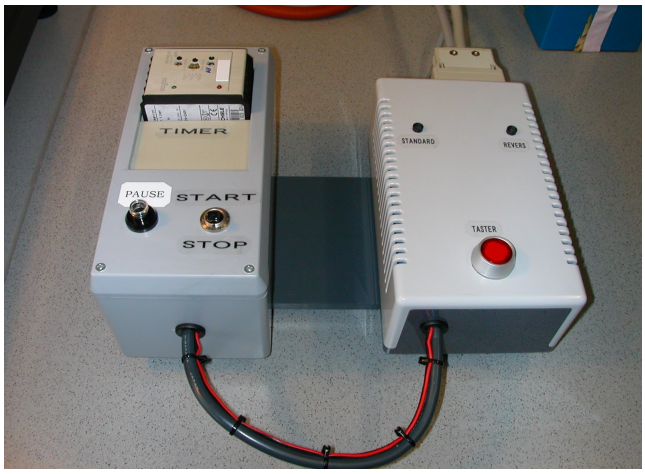
Manual Field Switch for CHEF Pulse Field Gel Electrophoresis

C. Lang & M. Lang, April 2004



Introduction

Pulse Field Gel Electrophoresis (PFGE) is an essential method for size separation of large DNA fragments for BAC cloning and many other purposes. Different electrophoresis systems are available, like FIGE (Field Inversion Gel Electrophoresis) (1) (2) or CHEF (Clamped Homogeneous Electric Fields) (3), all based on rhythmic and symmetrical oscillation of the electric field direction during gel electrophoresis.

The electric field oscillates around a certain axes at a certain angle, usually 90°-120° (oscillations must occur symmetrically regarding switch angle and switch time). Thus, over time the field vectors result in a single "main field direction", as described in Figure 1A. Some electrophoresis systems are able to freely re-orientate this main axis by a free determination of the extreme angles (4). However, not all commercially available CHEF systems have this option (5).

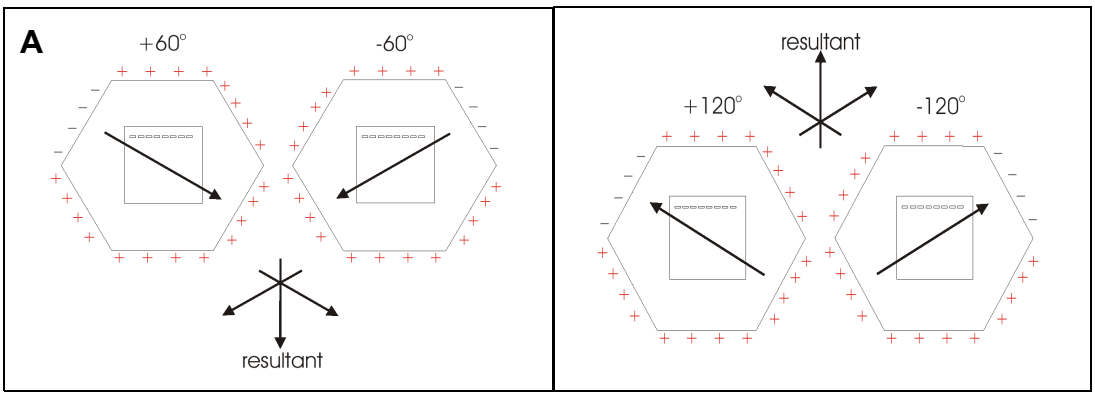


Figure 1: Field angles for a CHEF PFGE system, A and B display opposite main field directions

BAC cloning (6) sometimes requires a back and forth migration of DNA fragments inside an agarose gel (7). One possibility would be to take the gel out from time to time and to re-locate it in an opposite orientation. Another way is to re-orientate the field angles by 180°, so that mechanical stress for the gel is avoided (compare figure 1A and Figure 1B). But still not all electrophoresis systems possess this function (5).

Here we describe a simple machine that alternatively switches the electrical connections of oppositely located electrodes of the gel tank and the CHEF power module. This results in a complete field inversion of the main electric field direction without altering any other electrophoresis conditions which are controlled by the CHEF power module. The principle of this apparatus was described previously (1).

The machine was designed for a 24 electrode CHEF PFGE system (5). It enables both-, a manual and a timer dependent programmed switch.

Construction

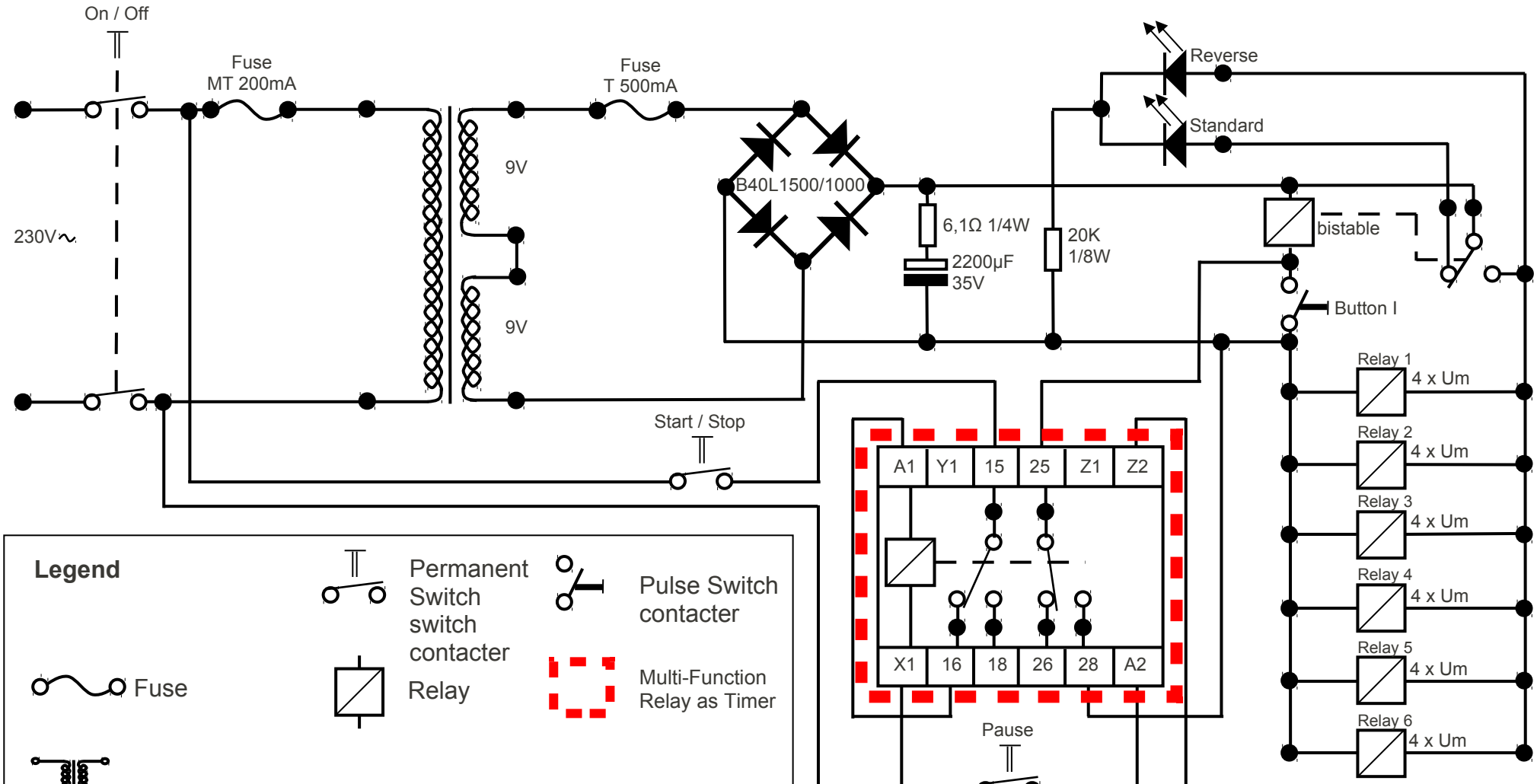
In the following, the electrical circuit and the interconnections are described that are relevant to mention for construction of the machine. First, the power supply of the Manual Field Switch itself is explained; later we describe the interconnections at the mono-stable relays. Those interconnect the electrical contacts of the CHEF power module with the electrodes of the gel tank.
Further, we describe a timer module that was incorporated into the switch apparatus. Finally, we show the plug type for the interconnections of the input and the output cable.

The power supply of the field switch

The entire power supply of the field switch was arranged according to Figure 2. The timer was added to the system later, after the manual switch had been tested. This is the only reason why we had put the timer into a separate case (compare to front picture).
When pressing push button 1, a bi-stable relay shortly gets electrical contact and switches. This leads to a supply of the six mono-stable relays with electricity so that they also switch the connections that are described later (see figure 3, 4). Pressing the push button once more leads to the reversal. The two alternative states of the mono-stable relays were called “Standard”, when electricity is not applied and the main field direction in the gel tank is not altered; and “Reverse”, when electricity is applied and main the field direction in the tank is reversed. Light Emitting Diodes (LED) indicate each status. The power button switches the machine “On” or “Off”. When the switch is plugged off, it remains in the “Standard” mode. This has the advantage that there is no need to disconnect the switch from the CHEF apparatus while it is not used.

Figure 2: (next page) Power supply electric circuit of the Manual Field Switch; The legend in this figure explains the major components of the circuit. The relays are presented in the “Standard”, when no electricity is applied. The 24 contacts of the six mono-stable relays switch as soon as the electric circuit is closed by the bi-stable relay. The bi-stable relay is induced to switch by a “sensory button switch” or alternatively by the timer module. Inducing the bi-stable relay once more results in the interruption of the electric circuit and to the reverse switch of the electric connections of the six mono-stable relays. The labelling of the timer module was taken from “Schiele, Multifunktionsrelais, MFN timetron® mit Relaisausgang”.

Manual Field Switch



The Interconnections

The arrangements and the connections of the mono-stable relays are presented in Figure 3 and Figure 4. Connections from the power supply were named S1-S24 and connections leading to the electrodes of the gel tanks were named E1-E24. The labelling of each channel 1-24 was defined by the position of each corresponding electrode in the gel tank (Compare to Figure 3). The interconnections were chosen in a pattern that always switches the electrical contacts of two oppositely located electrodes.

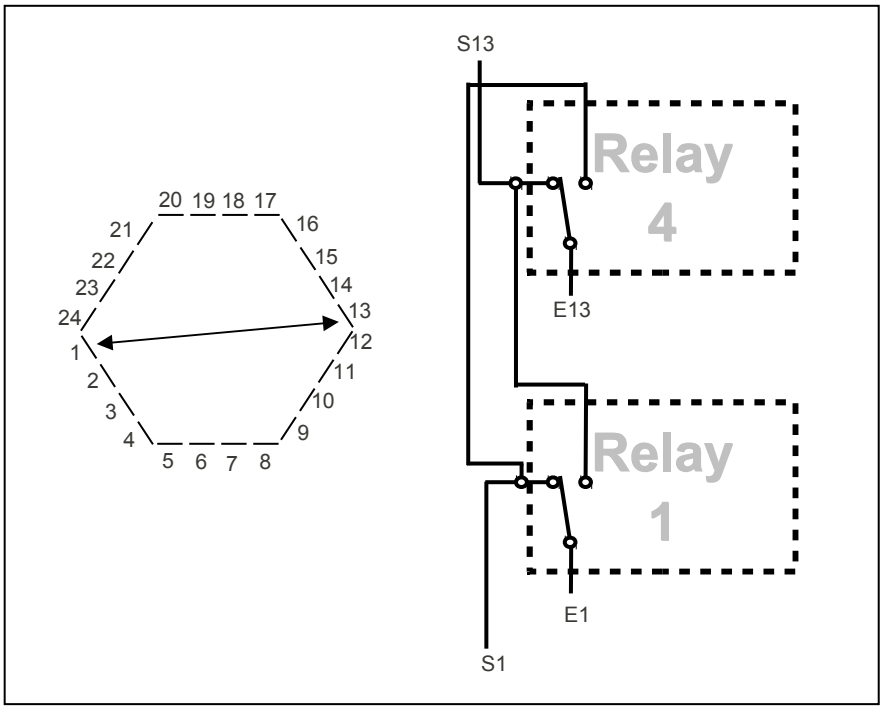
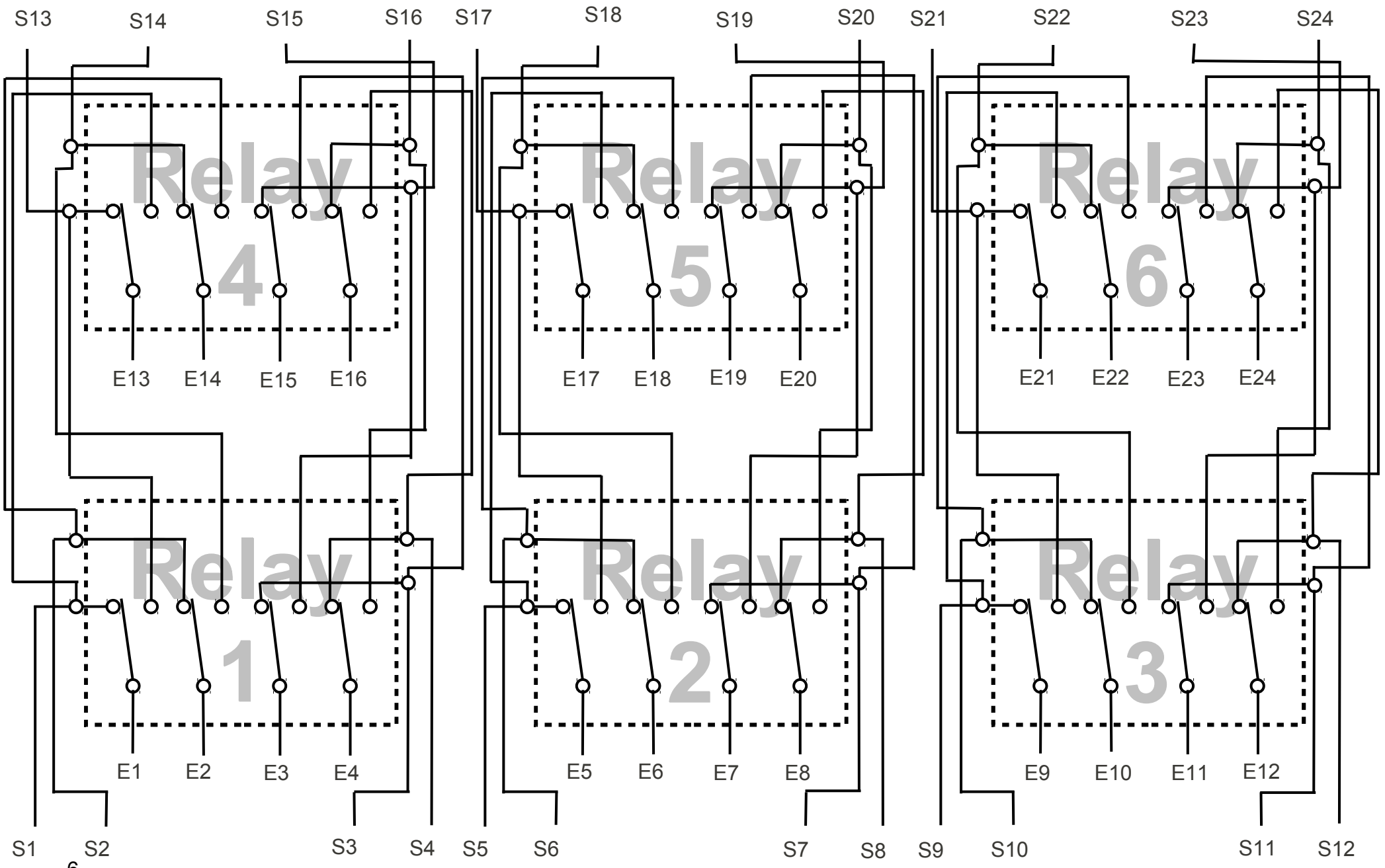


Figure 3: Concept of the field switch; A: The 24 Electrodes that are allocated as a hexagon inside the gel tank are shown. Each electrode is connected independently to the CHEF power module. The Manual field switch apparatus switches the electric contacts between the CHEF power module and two oppositely located electrodes. The switch for electrodes 1 and 13 is indicated by the arrow. B: The corresponding relay mechanism to switch electrical contacts of electrodes 1 and 13 is described. The indicated switch mode is in "Standard" or "off" status. The labels S1 and S13 indicate the electric connections that lead to the CHEF power module. The labels E1 and E13 indicate the electric connections that lead to the gel tank apparatus. If the relays switch connections, S1 will be connected to E13 and S13 will be connected to E1. Figure 4 describes the electrical connections for all 24 Electrodes.

Figure 4: (next page) The interconnections of the 24 electrodes with the CHEF Power module mediated by the mono-stable relays. This figure shows all the interconnections that were done corresponding to the concept that is shown in figure 3. The labels S1 – S24 indicate the electric connections that lead to the CHEF power module. The labels E1 – E24 indicate the electric connections that lead to the gel tank apparatus. For more detailed insight please read the instructions of "Matsushita Mono-stable Relay, NF4EB".

Manual Field Switch



The Switch Timer

Alternatively to press button 1, the timer module can regulate the switch apparatus automatically. The timer that we used is able to switch in intervals from 0,5sec to 300h. We used it in the AV-mode (initiation time lag mode). The switch time must be adjusted to the needs of the gel run conditions and must be synchronised to the run time of the CHEF Apparature. The timer is interrupted by two buttons, Start/Stop and Pause, to facilitate handling. We used the Multifunction Relay from Schiele (Compare Components for correct reference). However, any timer with similar characteristics could be taken for this purpose. For instructions, please read the information material for: “Schiele, Multifunktionsrelais, MFN timetron® mit Relaisausgang“.

The plugs

The manual field switch was built for a 24 Electrode CHEF-PFGE system (5). The electrodes of this system were interconnected with the power module by a 25 lead cable of which 24 leads were actually used. The connections of this system were of the type Sub D 25 pol. We applied the same type of plugs for the manual field switch. Specific connections were made after sorting the leads that were specific for each one electrode. The connection plugs of the machine are shown in figure 5.

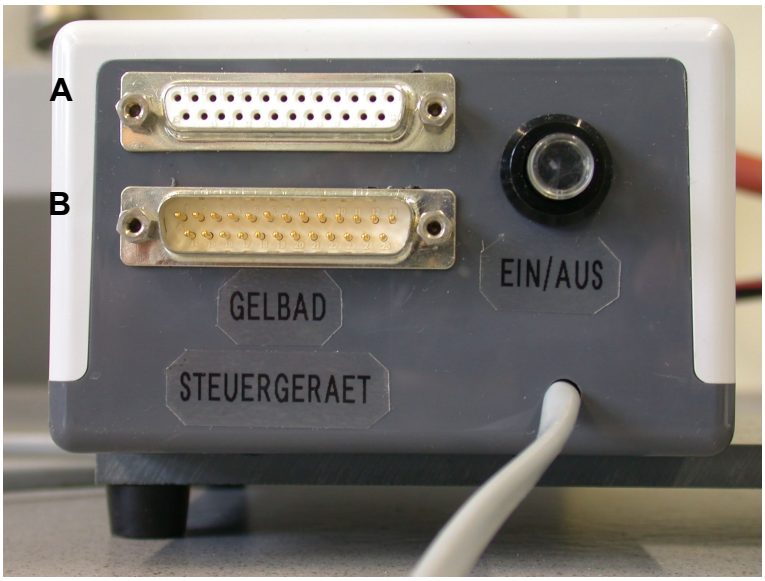


Figure 5: Field switch connections; The two 25x Sub D connections are shown. Of each contact 24/25 leads are actually used to connect each of the 24 electrodes. Connection A is the output connection that leads to the gel tank. Connection B is the Input connection and is connected by the cable from the power module.

Comment

We hope that these instructions will enable others to construct such an apparatus.
Alternatively to the relays, a microprocessor could also be used to build up a switch and surely this would enable a lot more options to switch around the electrode connections.
However, whenever someone realises that the used CHEF system cannot inverse the electric field, this machine might be a rather cheap solution to overcome the problem.

Components

Parts of the mashine that are self evident or that are not of particular importance for its function are not listed

Relay, bi-stable (Dold OB5693/OB 5694)

Relay, 6x, mono-stable, 4u (Matsushita, NF4EB)

Transformer, Block, 50-60Hz, prim. 2x115V/0,145A, 24VA, sek. 2x9V/1,25AT (Block Type: FL 24/9)

Multi-Function Relay (as Timer): (Schiele, MFN timetron® mit Relaisausgang)

LED, 2x

Resistor, 6,1Ω 1/4 W

Resistor, 20KΩ 1/8W

ELKO 2200µF, 35V

Rectification (Graetz Bridge), B40L1500/1000

Micro-Fuses, T500mA

Micro-Fuses, MT200mA

Pulse switches, 1x

Permanent switches, 3x

Cable Connection, Type Sub D 25 pol, 2x

References

(1) Carle G. F. and Olson M. V., Nucleic Acids Research Vol. 12, No. 14, pp. 5647-5664, 1984

(2) Carle G. F., Frank M., Olson M. V., Science, Vol. 232, pp. 65-68, 1986

(3) Chu G., Pulse Field Gel Electrophoresis, Theory and Practice, Apparatus for contour-clamped homogeneous electric fields, Methods 1: p 212-214

(4) For example Biorad, CHEF XA Mapper

(5) For example Biorad, CHEF DR III

(6) Shizuya H., Birren B., Ki U-Z., Mancino V., Slepak T., Tachiiri Y., and Simon M., Proc. Natl. Acad. Sci. USA, Vol. 89 pp. 8794-8797, 1992

(7) Amemiya C.T., Miyake T., Schoenborn M., Danke J., Hill-Force A., Lippmann W., Stuart A., and Tinnemore D., and Nefedov M.; BAC Cloning Manual: Pre-Run to Large-Scale Ligation and Transformation; p. 10-12.