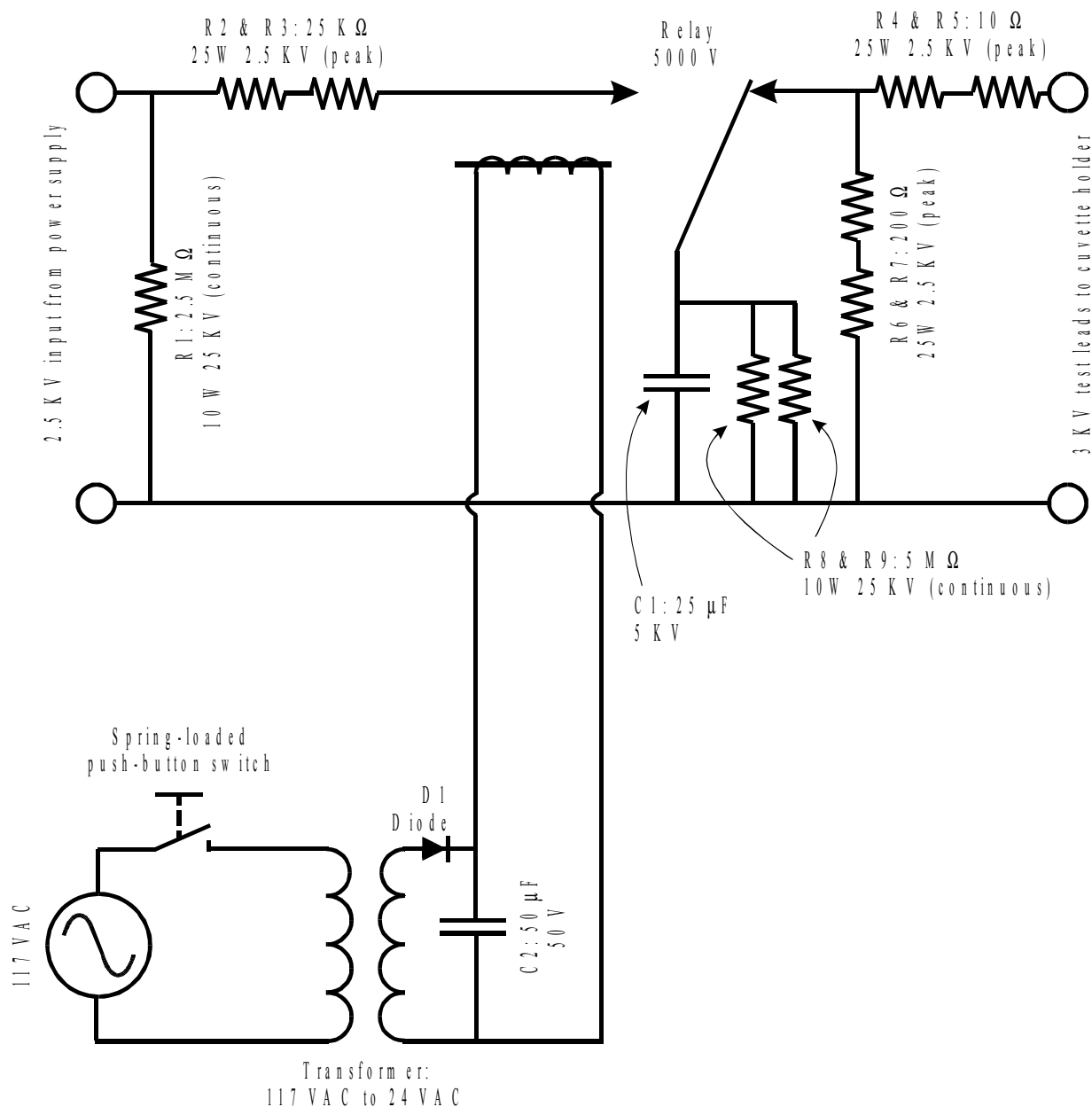


HOMEMADE ELECTROPORATION APPARATUS

This homemade electroporation unit, which cost under \$500 in 1991, is dedicated to electroporation of *E. coli*. It's relatively cheap because it gets its power from an external high-voltage power supply. It is used with the BioRad Pulser Chamber with standard 1- or 2-mm cuvettes. The device is designed throughout to be safe at 5 KV—twice the intended maximum voltage. Here is a circuit diagram:



PRINCIPLE OF OPERATION

The 2.5-KV input (1.25 KV for 1-mm cuvettes) is through 3-KV test leads with male banana plugs that plug into a high-voltage power supply—the kind used for sequencing gels. Resistor R1 is a bleeder to ensure that some current flows through the input even when no current is flowing into the capacitor C1; this is necessary because most high-voltage power supplies switch off automatically when no current is drawn on their output. The spring-loaded push-button switch is normally open, so that the solenoid in the relay is not actuated and the armature is in the “right-hand” position as depicted; the current flowing from the power supply in these circumstances is therefore limited by resistor R1 to $2.5 \text{ KV}/2.5 \text{ M}\Omega = 1 \text{ mA}$ (0.5 mA for 1.25 KV input). When the user presses the push-button switch (and keeps it pressed), the solenoid in the relay is actuated, pulling the armature to the “left-hand” position and allowing current to flow into the large capacitor C1. At the beginning of the charging process, when C1 is fully discharged, current flows into C1 at the maximum rate that the current-limiting resistors R2 and R3 will allow: $2.5 \text{ KV}/50 \text{ K}\Omega = 50 \text{ mA}$ (a current well within the capacity of a laboratory power supply; the current is 25 mA at 1.25 KV). When C1 is fully charged (the push-button switch still depressed), however, no current flows through it, and the current is therefore limited by the combined effects of resistors R1–R3, R8 and R9. The effective resistance of these resistors taken together is $1.26 \text{ M}\Omega$, limiting the current to an asymptotic value of $2.5 \text{ KV}/1.26 \text{ M}\Omega = 1.98 \text{ mA}$ (about 1 mA for 1.25 KV input).

The user then releases the push-button switch, de-actuating the relay’s solenoid and allowing the armature to spring back to the “right-hand” position. This disconnects the capacitor C1 from the power supply and connects it to the output: two female jacks for the male banana plugs on the leads from the BioRad Pulser Chamber (cuvette holder). Ordinarily, the resistance across the output is very high, since the suspension of electrocompetent cells (and DNA) in the cuvette has very low ionic strength. The two bleeder resistors R8 and R9 similarly have a very high resistance (combined resistance $2.5 \text{ M}\Omega$). Therefore, the overwhelmingly major path for discharging the capacitor C1 is through resistors R6 and R7, whose combined resistance amounts to $400 \text{ }\Omega$. The time-course for discharge of C1 in this RC circuit is

$$V = V_0 \exp\left(-\frac{t}{R \cdot C}\right) = 2500 \times \exp\left(-\frac{t}{400 \cdot 25 \times 10^{-6}}\right) = 2500 \times \exp\left(-\frac{t}{0.01}\right)$$

where V is the voltage remaining across the capacitor C1 (and therefore across the output) t seconds after the push-button is released; and V_0 is the starting charge on the capacitor, which is 2.5 KV for a 2-mm cuvette. The $400\text{-}\Omega$ combined resistance of R6 and R7 therefore determines a “time constant” for discharge of the capacitor of 0.01 seconds = 10 ms. This means that the cells in the cuvette experience a voltage that decays from 2.5 KV to $2.5/e = 0.92 \text{ KV}$ in 10 ms. This is pretty close to the optimal time-constant for electroporation of *E. coli* cells with an initial voltage gradient of 2.5 KV per 0.2 cm = 12.5 V/cm. If the smaller 1-mm cuvettes are used, the input voltage should be reduced to 1.25 KV in order to maintain the same initial voltage gradient of 12.5 V/cm.

SAFETY CONSIDERATIONS

Since the voltages in this device are lethal, it's important to use components that won't fail under normal circumstances, to mount the components firmly on the chassis, and to connect them with heavy-duty wire. The above circuit is designed to be safe even at 5 KV—twice the intended maximum operating voltage. In some cases, this is achieved by ganging two resistors (R2/R3, R4/R5 and R8/R9), each of which is individually rated at 2.5 KV peak.

We use a sealed, high-voltage relay to switch between charging and discharging modes of the unit. That way, the operator does not have to touch a high-voltage switch.

High-voltage capacitors like C1 are notorious killers. If for some reason they become disconnected after charging, they can hold a lethal charge for years! To guard against this possibility, we connect two bleeder resistors, R8 and R9, **DIRECTLY AND INDEPENDENTLY** across the terminals of the capacitor C1. These bleeders ensure that even if C1 becomes disconnected from the rest of the circuit, it will discharge with a time constant of $R \cdot C = 2.5 \text{ M}\Omega \times 25 \text{ }\mu\text{F} = 62.5 \text{ sec}$. Thus, within a few minutes, the charge remaining on C1 will be harmless. Since the two bleeders are connected independently to C1, the capacitor will be discharged within a few minutes (time constant 125 sec) even if one of the bleeders fails.

The two 10- Ω resistors R4 and R5 limit the current that flows to the output if, for some reason, the output leads are shorted. Even with these current-limiting resistors, the peak wattage immediately following a short can theoretically be as high as 313 KW! However, this current would be extremely short-lived (time constant 500 μs), and it's likely that R4 and R5 are sufficient to prevent a major melt-down of the components.

SOURCES OF COMPONENTS

Component	Specifications	Supplier	Cat. No.	Each
R1	2.5 M Ω , 10 W, 25 KV continuous	Caddock	MG810 (?)	\$45.57
R2, R3	25 K Ω , 50W, 2.5 KV peak	Allied (Ohmite)	850-F25K	7.37
R4, R5	10 Ω , 50 W, 2.5 KV peak	Allied	880-0708	4.84
R6, R7	200 Ω , 50 W, 2.5 KV peak	Allied	880-0724	4.84
R8, R9	5 M Ω , 10 W, 25 KV continuous	Caddock	MG810 (?)	45.57
C1	25 μ F, 5000 V	NWL		120.00
C2	50 μ F, 50 V	Radio Shack	272-1015	.69
Relay	SPDT, 5 KV, 24 VDC solenoid	Allied (Magnecraft)	W158 HVX-1 (24 VDC)	115.00
D1	Rectifying diode 1N 4001	?	?	.25
Switch	SPST, normally open, spring-loaded push-button	Radio Shack	275-1566	1.89
Case	Fully enclosed, with handle	Allied (Hammond)	1401K	50.66
Transformer	24 V	Radio Shack	273-1366	4.99

Suppliers:

Allied Electronics

Caddock Electronics, 1717 Chicago Ave., Riverside, CA

NWL Electronics, 8050 Monetary Dr., Riviera Beach, FL 33404; (407) 848-9009