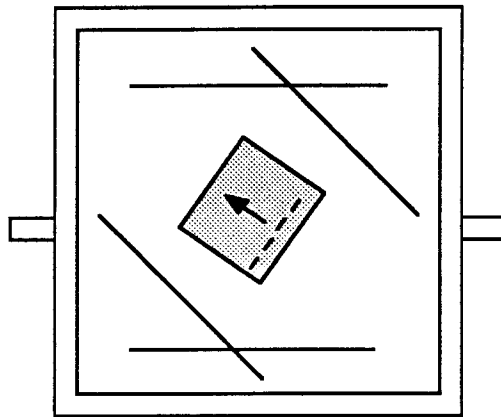


## The ED Pulsed-Field Electrophoresis Instrument



## **Introduction:**

The new instrument described here named ED (Electrophoresis Device) utilizes the pulsed electrophoresis effect to optimally resolve megabase as well as small kilobase sized DNA molecules. The instructions and blueprints supplied here enable the researcher to build and operate a sophisticated instrument, with little cost or effort. The instrument can be configured for pulsed electrophoresis with zero to two field gradients as well as providing any desired angle between the sets of electrodes. ED utilizes the same diode isolated array as described in Schwartz and Cantor, Cell 37, pp. 67-75, 1984. Switching can be accomplished through many means, but we like using Chronrol timers hooked up to heavy duty AC relays. Cooling and buffer circulation is accomplished using an external heat exchanger hooked up to a water bath and magnetic drive pump.

We are providing these plans and information with the hope that:

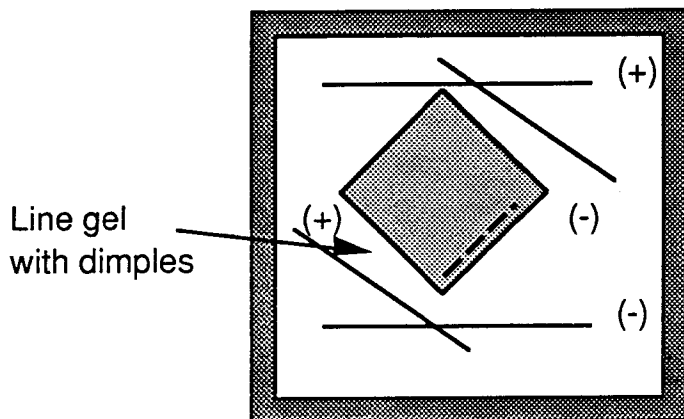
- 1) Any PFG data that is published or presented using ED must be referenced accordingly: Schwartz, D.C., Smith, L.C., Baker, M. and Hsu, M. "ED: pulsed electrophoresis instrument", *Nature*, 324, 575-576 (1989).
- 2) The plans may be copied and distributed; however, proper references must accompany all such material.
- 3) We would be very interested to learn of any improvements to the instrumentation.

This document was produced by Cynthia Smith.

## ED Box Set-Up

### Electrical System:

There are four arrays of electrode posts, with 5 posts per array. Two are positive, two negative. They should be arranged in the box as follows:



The electrode arrays have banana plugs at the ends - these plug into the jacks at the corners of the electrophoresis tank. On the switch box, there are jacks corresponding to each side of the tank; connect the switch box jacks with the remaining jacks on the corners of the tank using the patch cords. The other jacks on the switch box should be connected to the power supply. The power supply that you use can make a difference in the resolution that you get. Your power supply should be voltage regulated. This is important - power supplies that are not regulated do not handle pulsing well and the gels obtained are smeary. Also, be sure before you plug in the unit that your power supply can handle at least 250 milliamperes. It is possible to blow out your power supply if it cannot handle enough current.

One other note: The unit is not fail-safe, i.e. you can be shocked. Please be certain that the power is off before you lift the lid to the tank.

### Coolant:

Hook up the cooling system as per the plans. The water bath should be adjusted such that the box temperature is approximately 12°C.

### Gels:

1. Plates. We use 8 x 8 inch glass plates (window glass, single thickness) in our boxes for the best results. We have also used the glass plates left from silica gel TLC plates (20 x 20 cm).

2. Agarose. We recommend FMC brands of agarose. For most purposes we use 1% Sea-Kem (high-gelling temperature agarose.) Use 150 mL of 1% agarose made up in a buffer of 1/2X TBE.

To make 5X TBE (1 liter):

51.50g Tris base

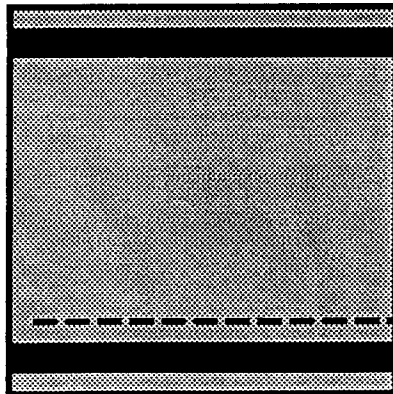
27.50g boric acid

4.65g EDTA (disodium dihydrate)

Final pH should be 8.0 to 8.1 and conductivity 2.1 to 2.2mS.

3. Positioning in box. To keep the gel from floating off the glass plate, tape it down with some tape. We use polyethylene plastic tape (3M brand). You can use labelling tape

for short runs, but it will disintegrate over a few days. One piece at the top and one at the bottom (below the wells) is usually sufficient.



On the base plate of the box, there should be three small indentations on the diagonal of the box. Line up the gel plate along these indentations with the wells at the southeast side of the box.

4. Running buffer. We use 1/2x TBE. You can also use 1X TBE, but we prefer the lower concentration because it cuts down on the wear and erosion of the electrodes.

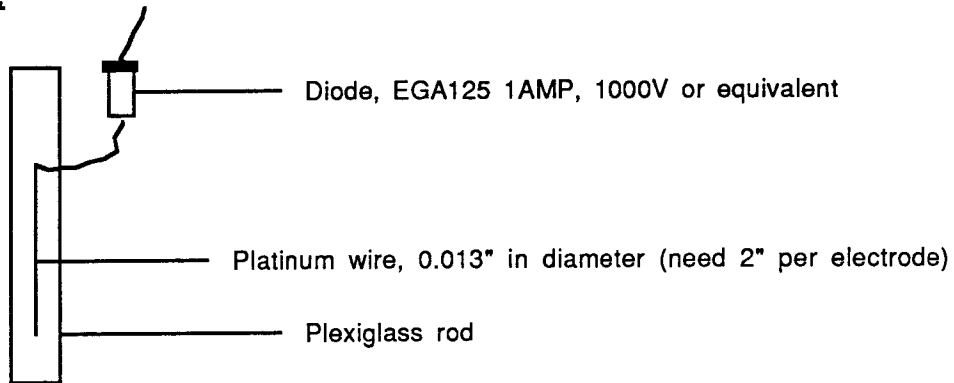
Generally, you should use 2 1/2 to 3 liters of buffer in the tank. More or less can cause distortion of the lanes. You may want to experiment a little to find the ideal level.

#### Running Conditions:

Higher field strengths run gels faster but produce smeary bands. In fact, large molecules remain trapped in the gel. A good yeast molecular karyotype (200-2000 kb) can be produced using a 140 second pulse time with 150 volts applied. Mixed pulse times will optimally resolve a broader size range, however, use the longest pulse time first followed by shorter times.

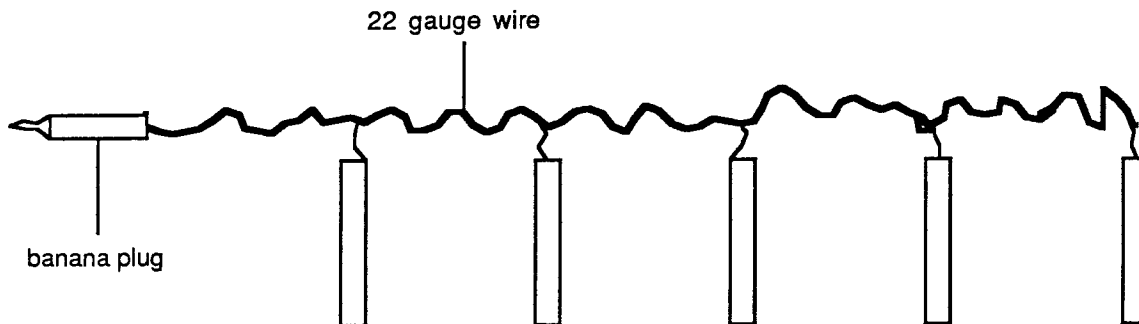
# Electrical Assembly

## Electrodes:



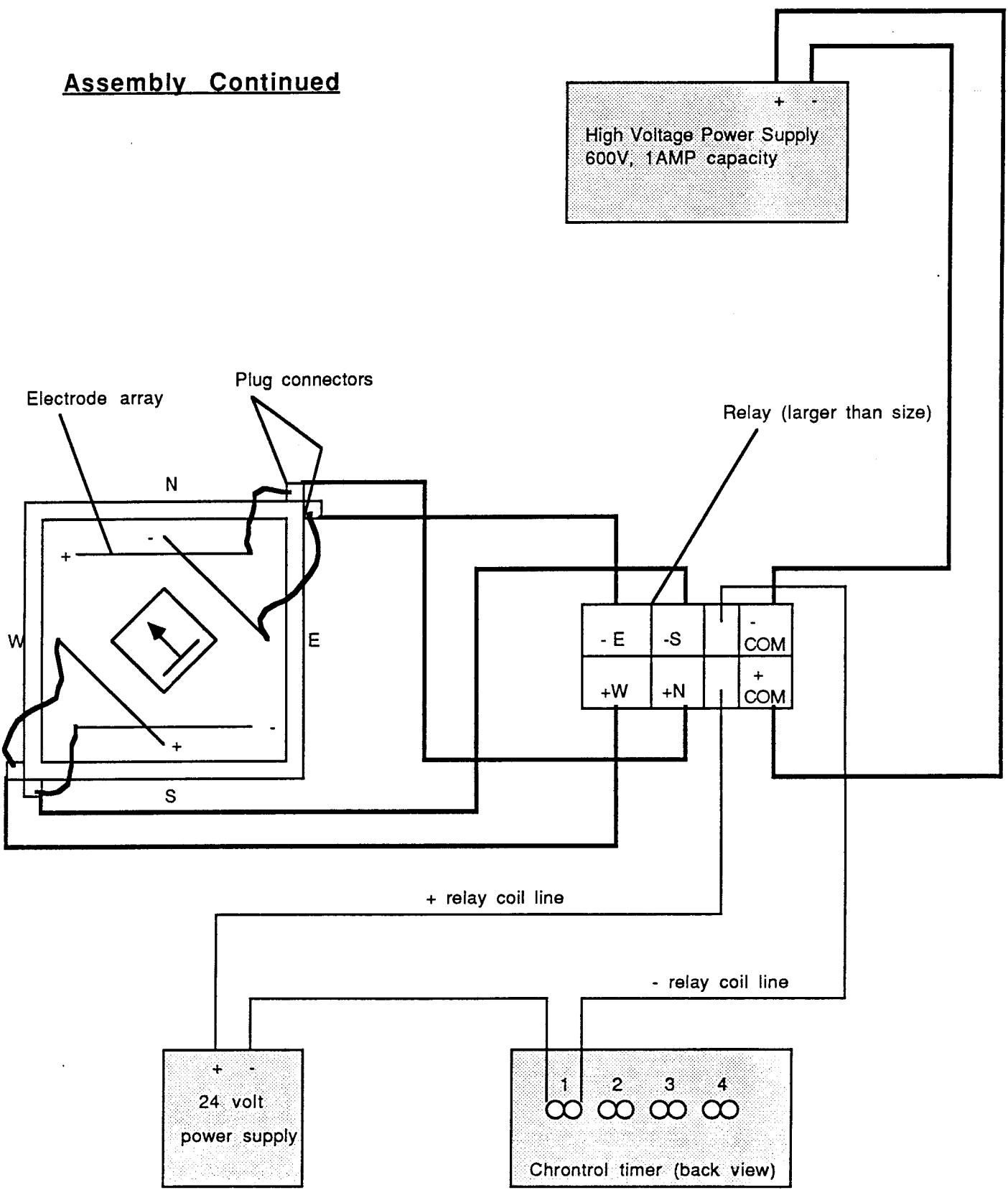
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## Electrode Array:

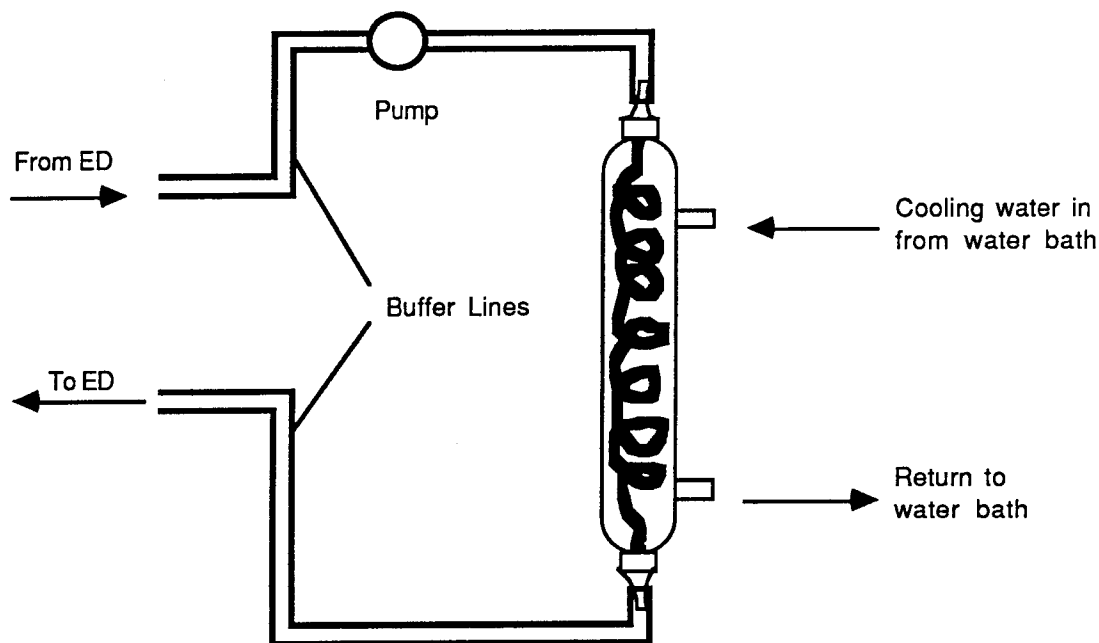


Four arrays are needed. Two arrays have diodes pointed up (negatives) and two have diodes pointed down (positives).

Assembly Continued



## Coolant/Heat Exchange System



### Coolant Parts List

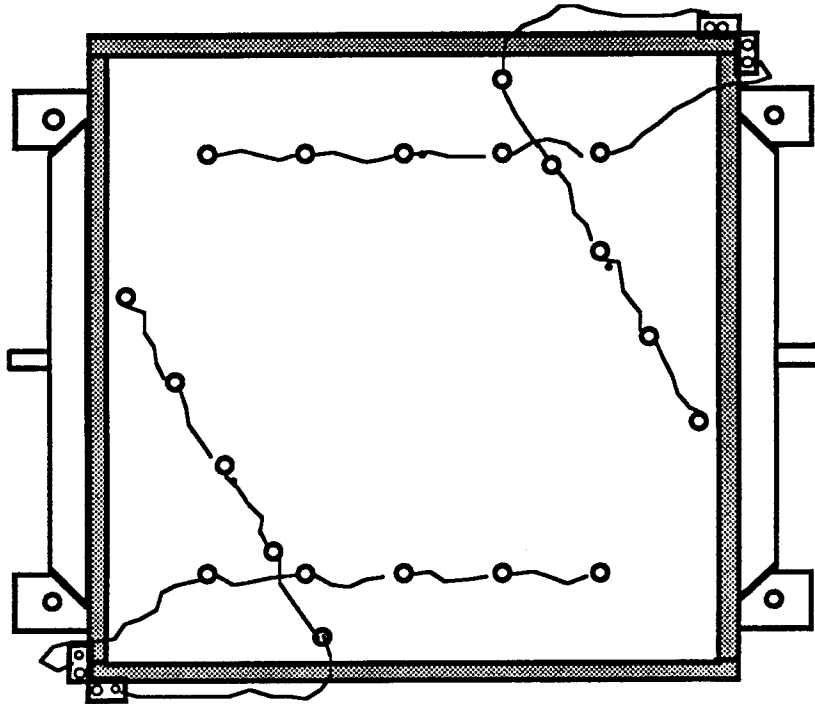
1) 800 mL spiral condenser. You will need one that you can fit the tubing over the end. Since most condensers come with ground glass joints, we had the ends fitted with circulation nipples by a glass blower.

2) Tubing to fit the condenser and ED .

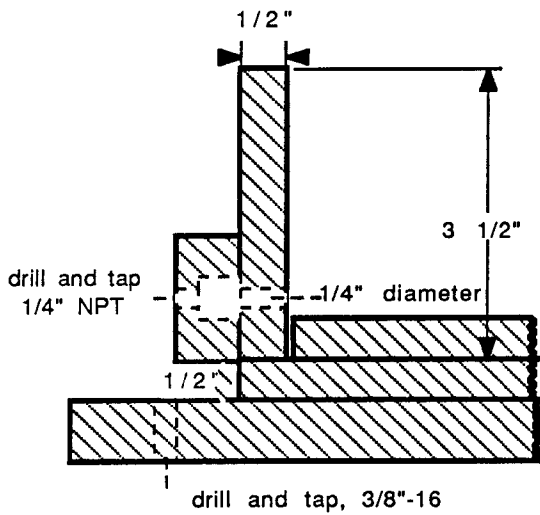
3) Centrifugal circulating pump. We use model #MD-6UNL from American Scientific Products. Any circulating pump designed for continuous use is suitable, but be certain there are no internal metal parts. Most magnetic drive pumps have plastic parts.

4) Refrigerated circulating water bath. Any good quality bath is suitable. We keep ours filled with 30% ethylene glycol instead of <sup>just</sup> water.

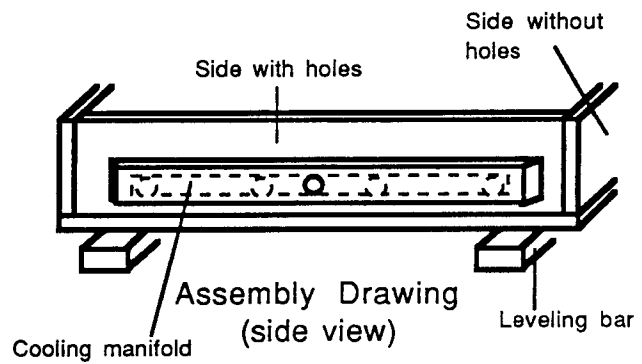
# ED Construction Plans

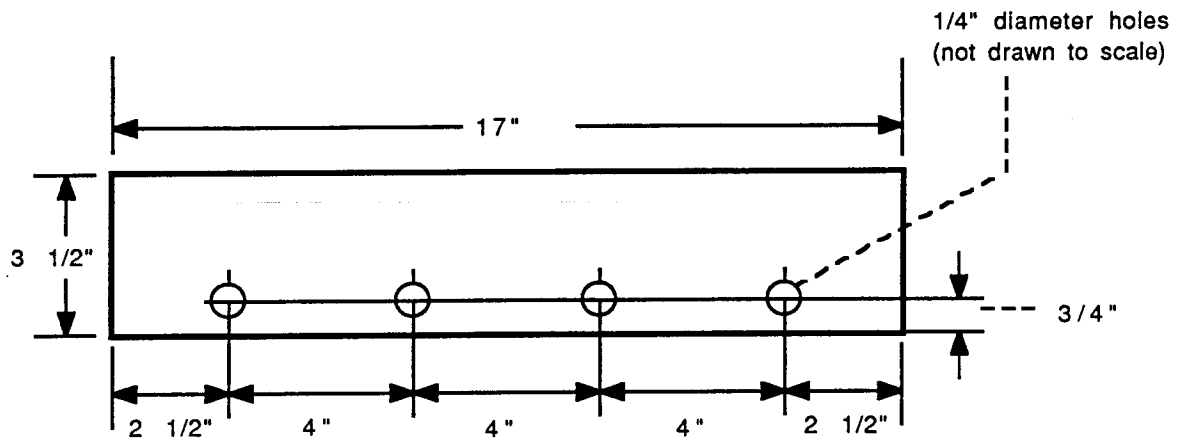


Assembly - top view

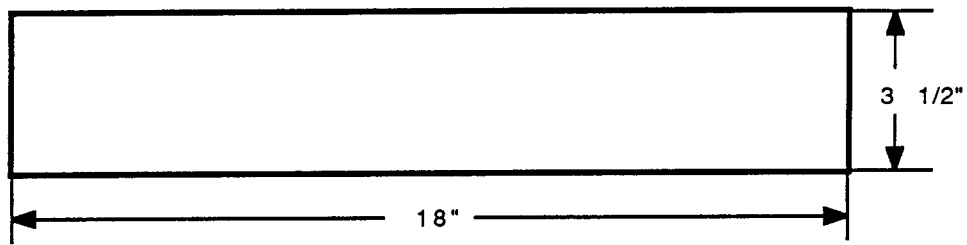


cross section of box through leveling bar

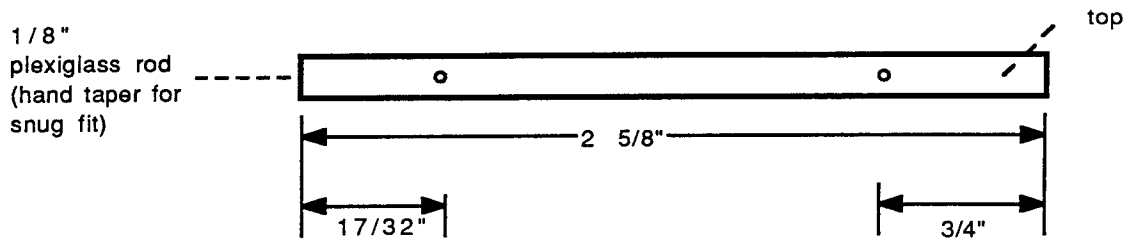




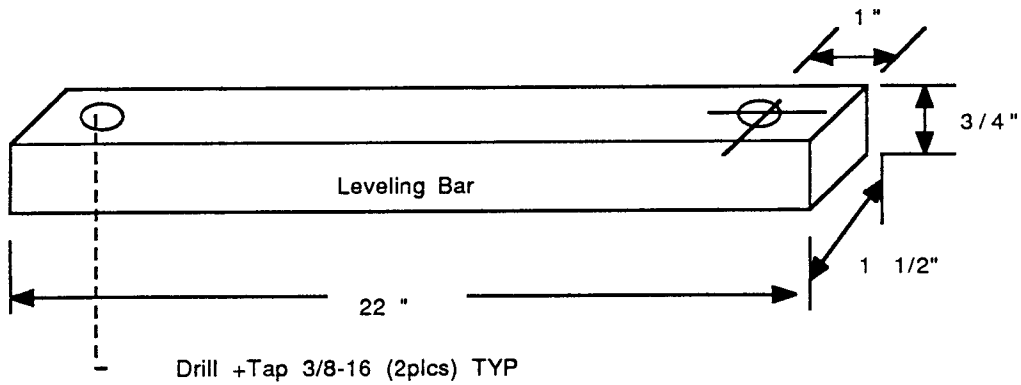
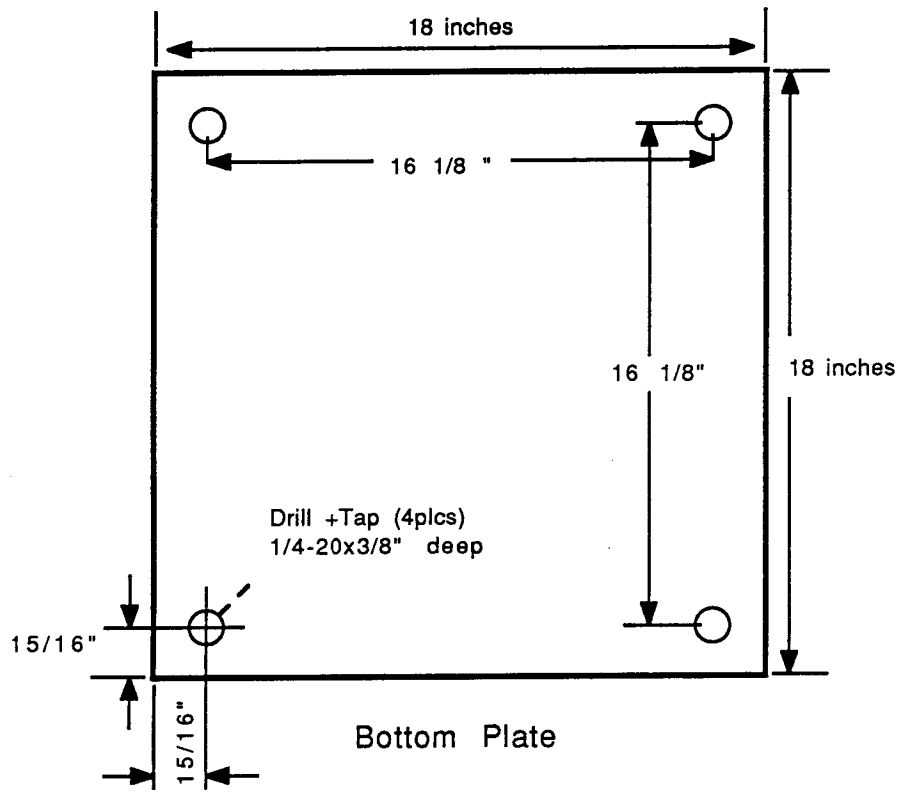
Side of box - need two (1/2" plexiglas)



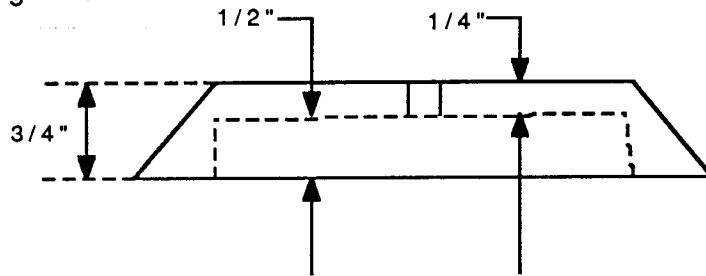
Side of box - need two (1/2" plexiglas)



electrodes - need 20 per unit

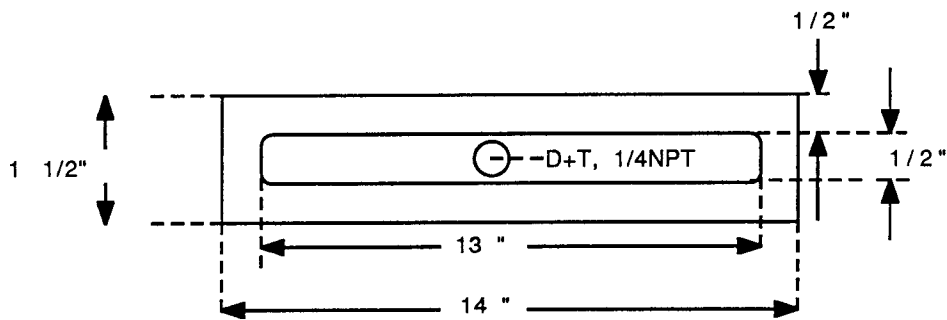


### Cooling Manifold

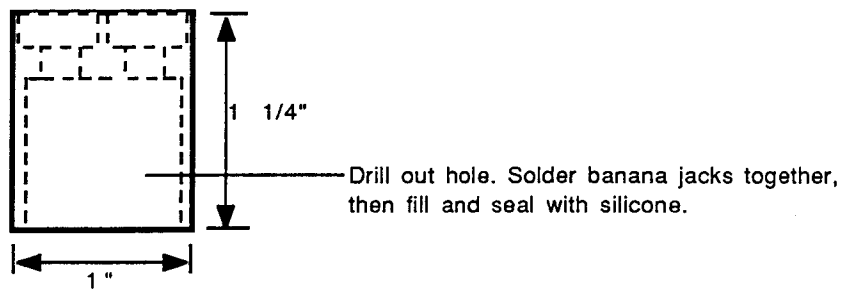
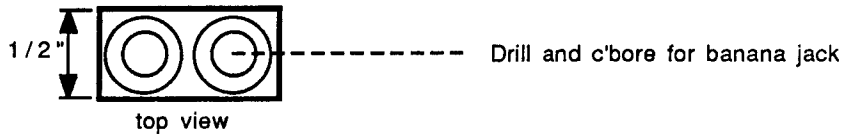


Cooling Manifold (top view)

Note: fit center hole with plastic circulation nipple



### Electrode Array Plug Connector (four required, 2 with red jacks, 2 with black jacks)

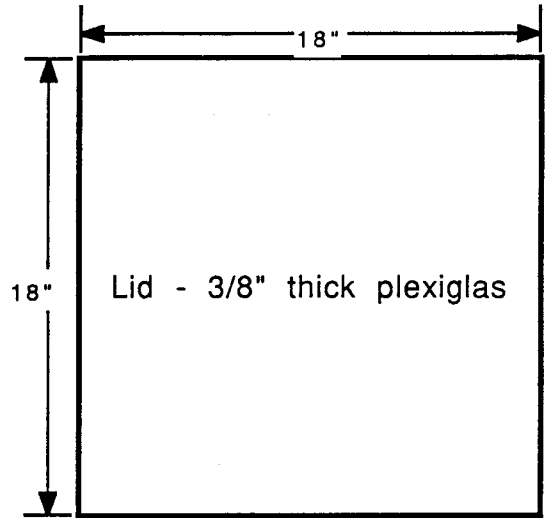


corner pieces

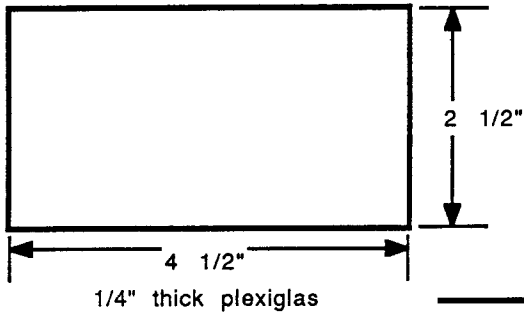
soft foam 1/8" x 1/2" tape to eliminate evaporation



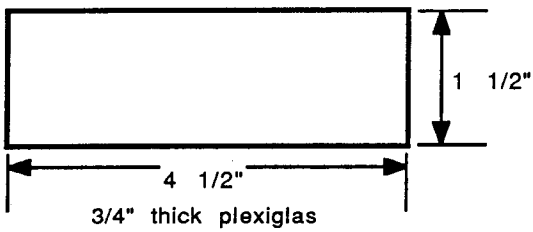
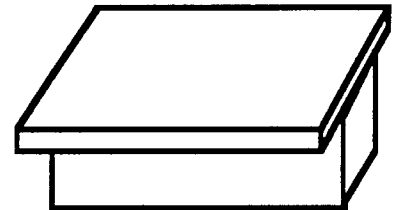
Lid Assembly



Lid - 3/8" thick plexiglas



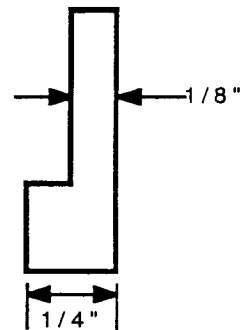
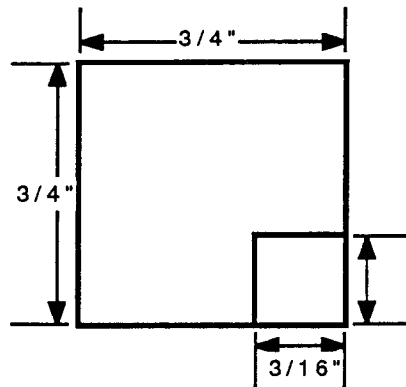
Lid Handle Assembly (optional)



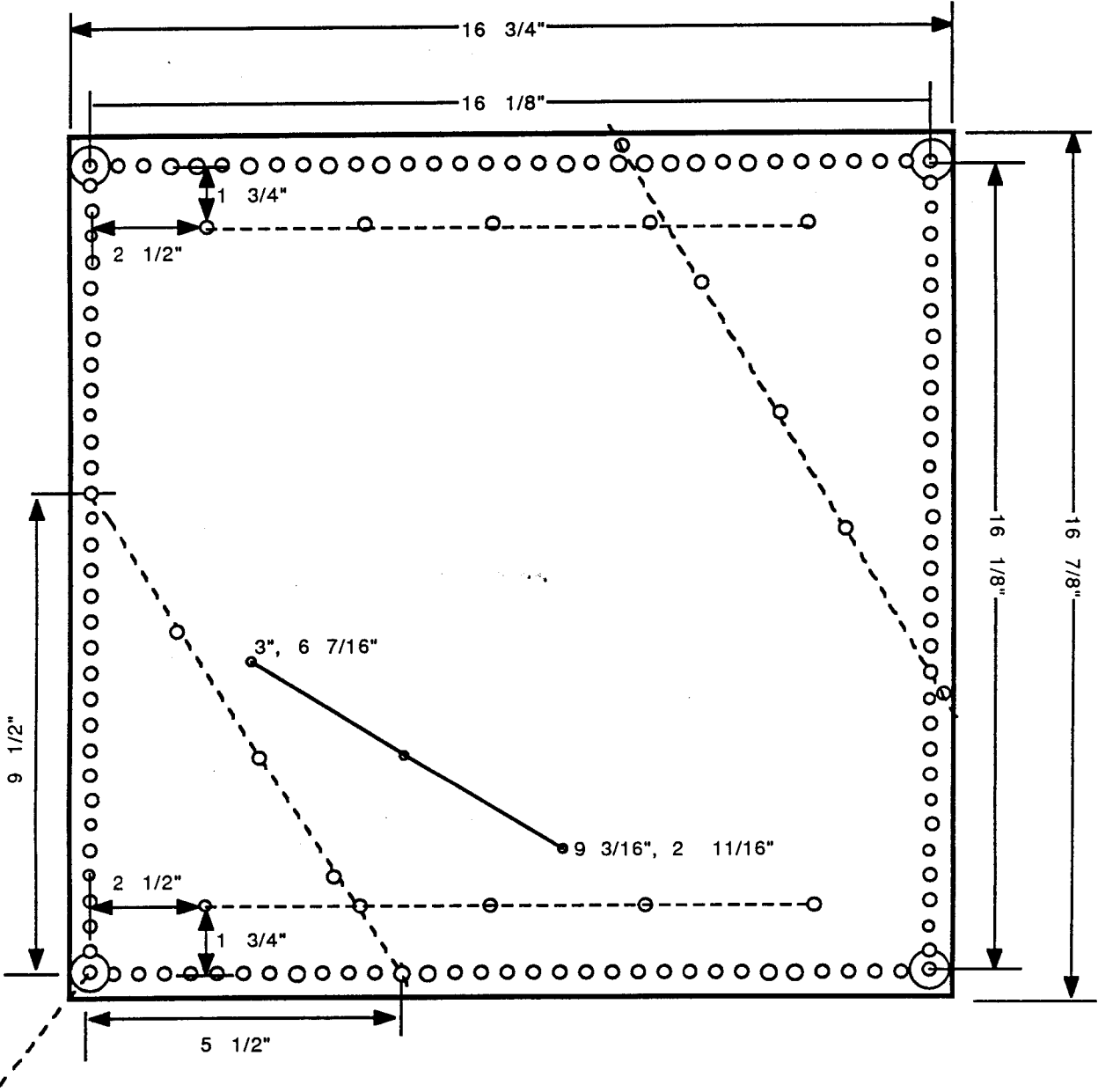
position handle in center of lid

Lid Corner Pieces

four required



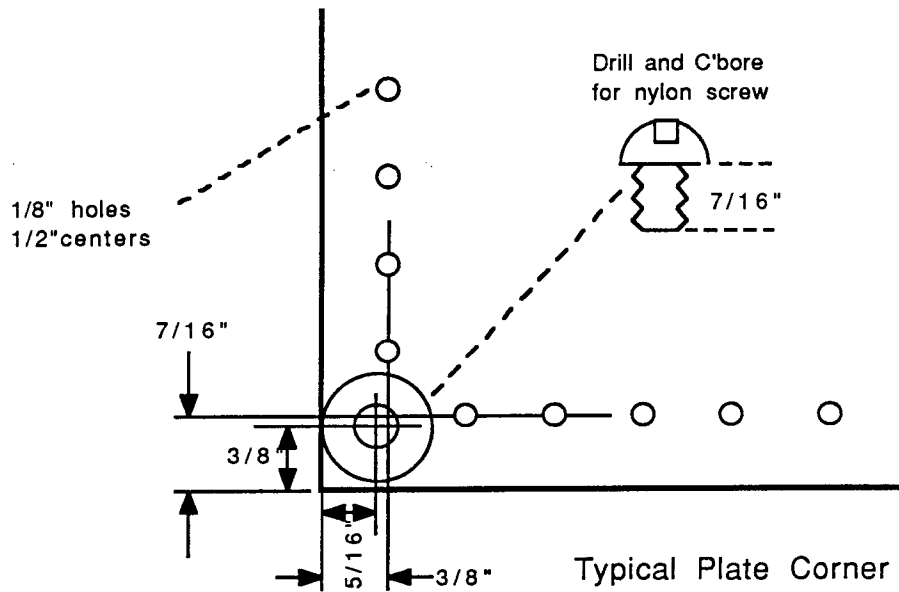
### Removable Electrode Holder Plate



Reference Hole (center taken, see detail next page)

Electrode Holder Plate Notes:

- 1) All holes on the outer edge of the plate are spaced on  $1/2$ " centers and are  $1/8$ " diameter.
- 2) All lines are 11" long. The holes on the lines are spaced  $2\ 3/4$ " center to center.
- 3) Line pairs are  $12\ 1/2$ " apart.
- 4) Measurements are made from center of holes on the edge of plate.
- 5) Holes on guide line in the center of plate should be dimpled, not drilled through.



## Electrical Assembly Parts List

- 1) Platinum wire, 0.013" diameter, approximately 40 inches. (2 inches per electrode, 20 electrodes)
- 2) Diodes (rectifiers) EGA125, 1 AMP, 1000V or equivalent. Need 20. (Can be obtained from Allied Electronics, stock# 858-0147 1N4007 Motorola rectifiers)
- 3) High voltage relay. Need one. Potter and Brumfield DPDT Relay # PRD-11DYO (24 volt). (Allied Electronics # 886-1010)
- 4) Low voltage (24 volt) power supply (one). Condor # B24-1.2. (Allied Electronics # 744-1412)
- 5) Chronrol timer, model CD-4S (dry contact model). Can be obtained from any large scientific supply company. (Can substitute model CD-2S)
- 6) Hook-up wire, 22 gauge (16 feet) and 18 gauge (about 15 feet, varies)(Can be obtained from Allied Electronics)
- 7) High voltage power supply. The power supplies that we use are purchased from Electronic Measurements Inc. Model # TCR 600-s-1-1D (1AMP, 600V) You will need to fit this model with a cord and grounded plug.
- 8) Banana plugs, 4 red, 4 black. (Allied Electronics)
- 9) Grounded plug and cord for 24 volt power supply.