IEF Electrophoresis

2016 SCST

Genetic Testing Super Workshop

Isoelectric Focusing (IEF)

- Can use polyacrylamide or agarose gels
- Based on molecules that can be positively or negatively charged (amphoteric)
- For example: proteins, enzymes, peptides
- The net charge of protein is the sum of the positive and negative charges of the amino acid side chains.
- When the net charge of the protein is 0 that is the proteins isoelectric point.
- When a protein has reached its isoelectric point, it will stop migrating through the gel at a certain pH point.

How does the pH gradient work in the gel matrix

- Carrier ampholytes are in the gel matrix that are low molecular weight and have closely related isoelectric points
- When electricity is applied to the gel the ampholytes forms a pH gradient in the gel.
- When an amphoteric protein from a sample is no longer charged the electrical current will not have an effect on it. If that protein were to diffuse back away from its pI point, it will gain a net charge again, and go back to that same pI and pH point in the gel. Thus, the term FOCUSING.

Four processes are involved:

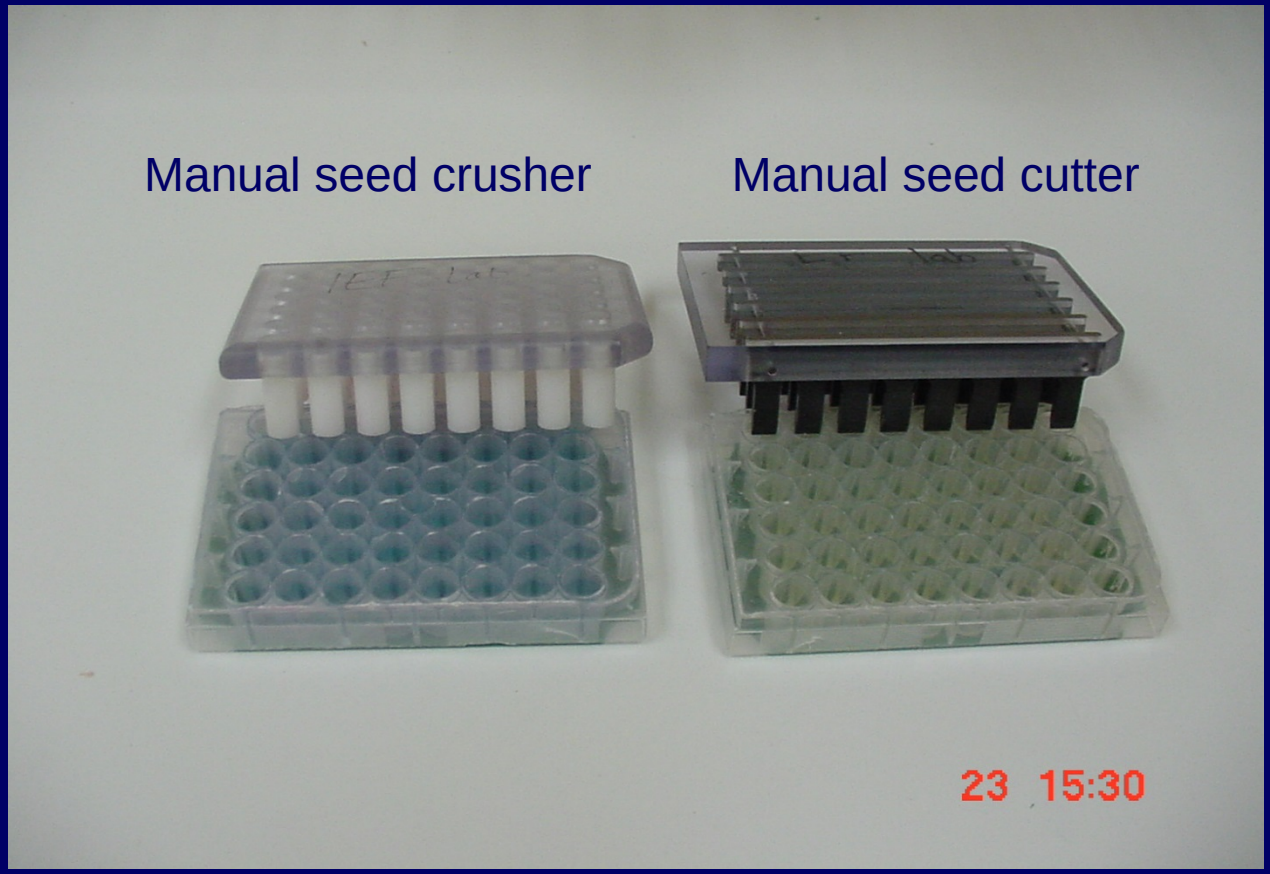
- Extraction
- Focusing
- Staining
- Analysis

Extraction Process

- Weigh 10 seeds of sample to determine extraction amount used
- Load a single seed into each well of a 48 well plate
- Crush seed
- Extract plates
- Vortex and let incubate overnight

Supplies needed for extraction process

Crushing seed



Crushed corn seed

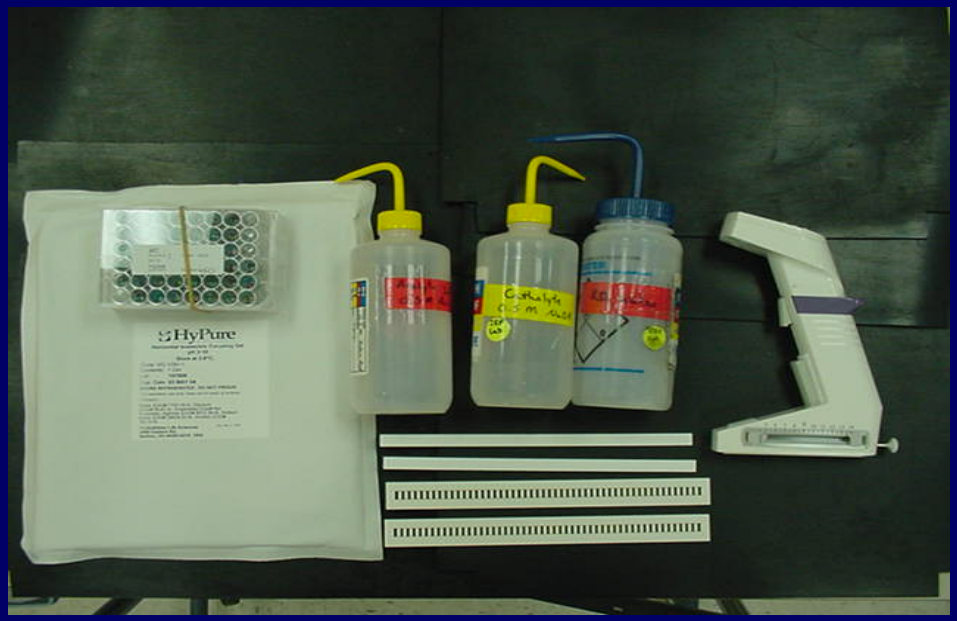
Vortexing plates after adding extraction solution

Supplies for making control samples

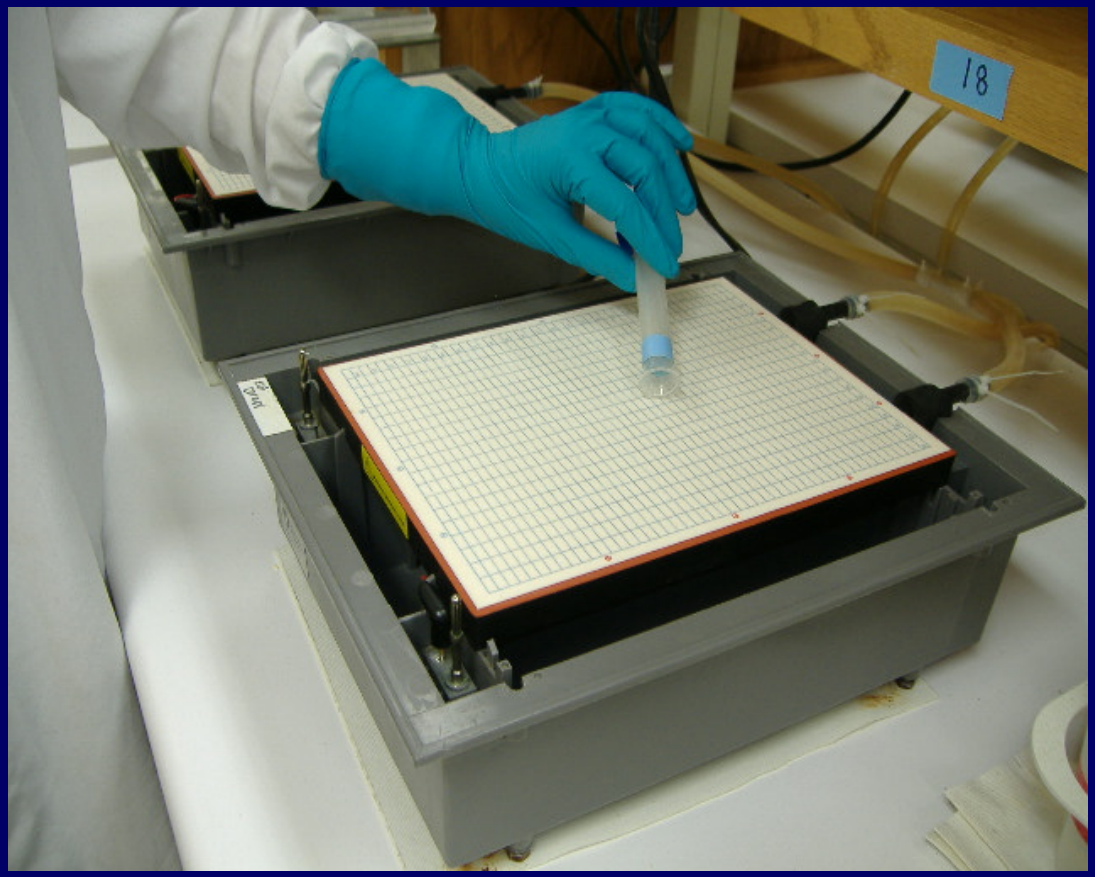
Focusing

- Label gel with date, sample #, and gel type
- Make sure data sheet matches the plates
- Load the gel
- Run the gel
- Place in TCA fixative solution
- Place in 2 water rinses

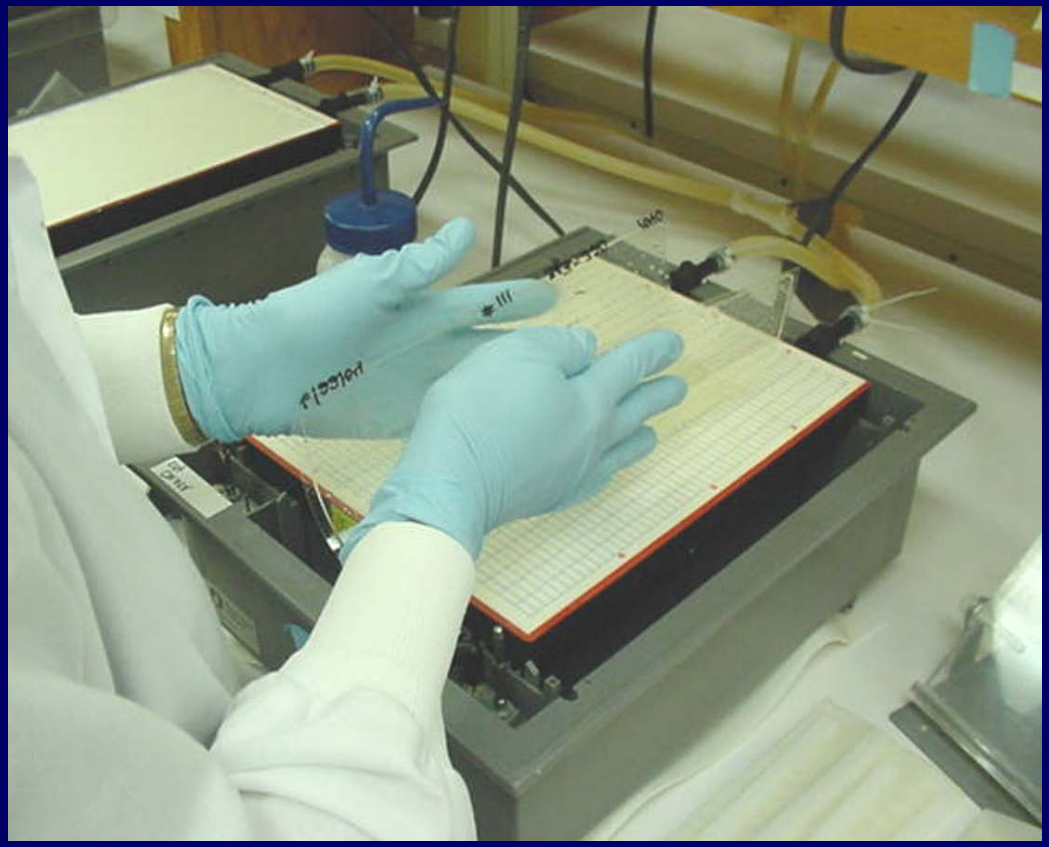
Supplies for focusing gels



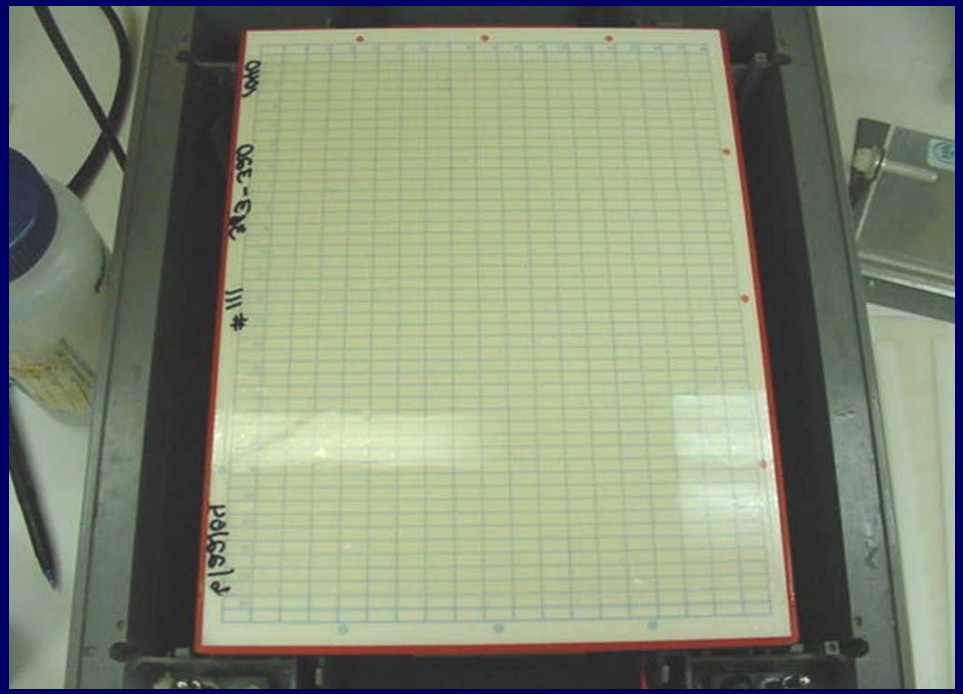
Pour ~2 ml water on plate

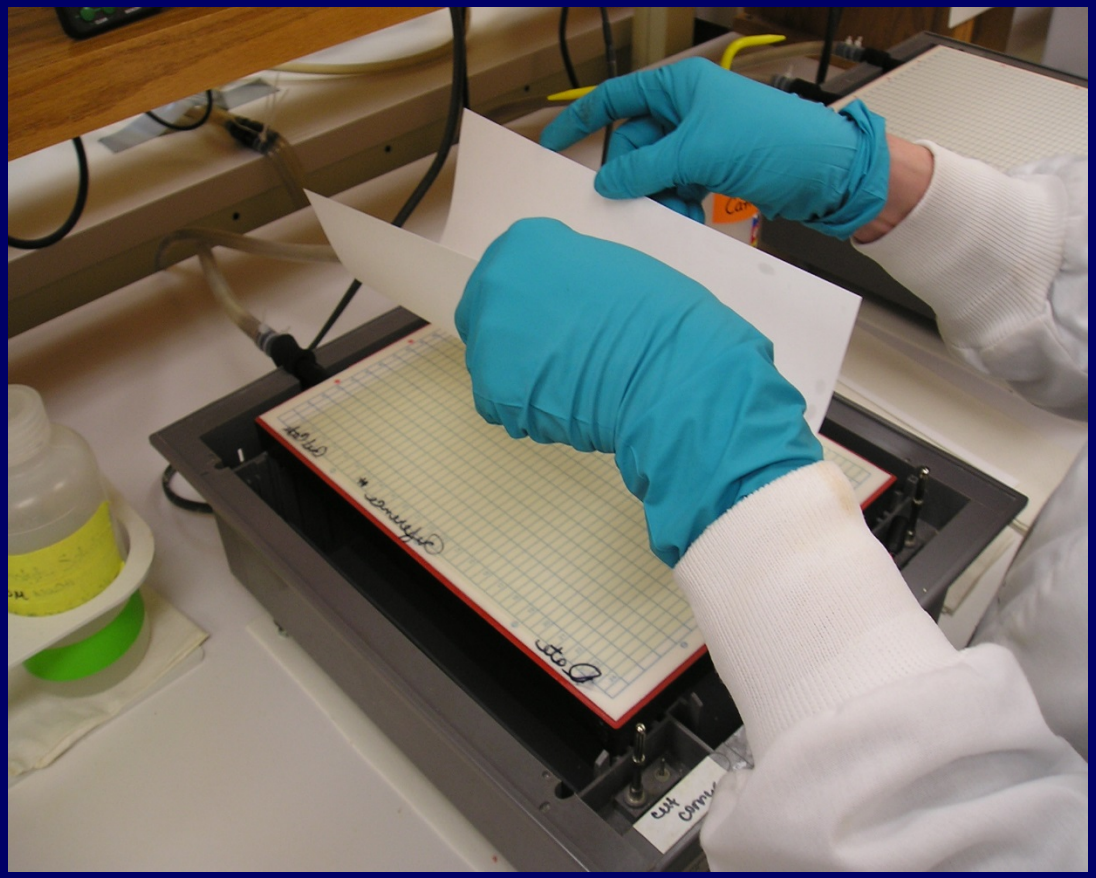


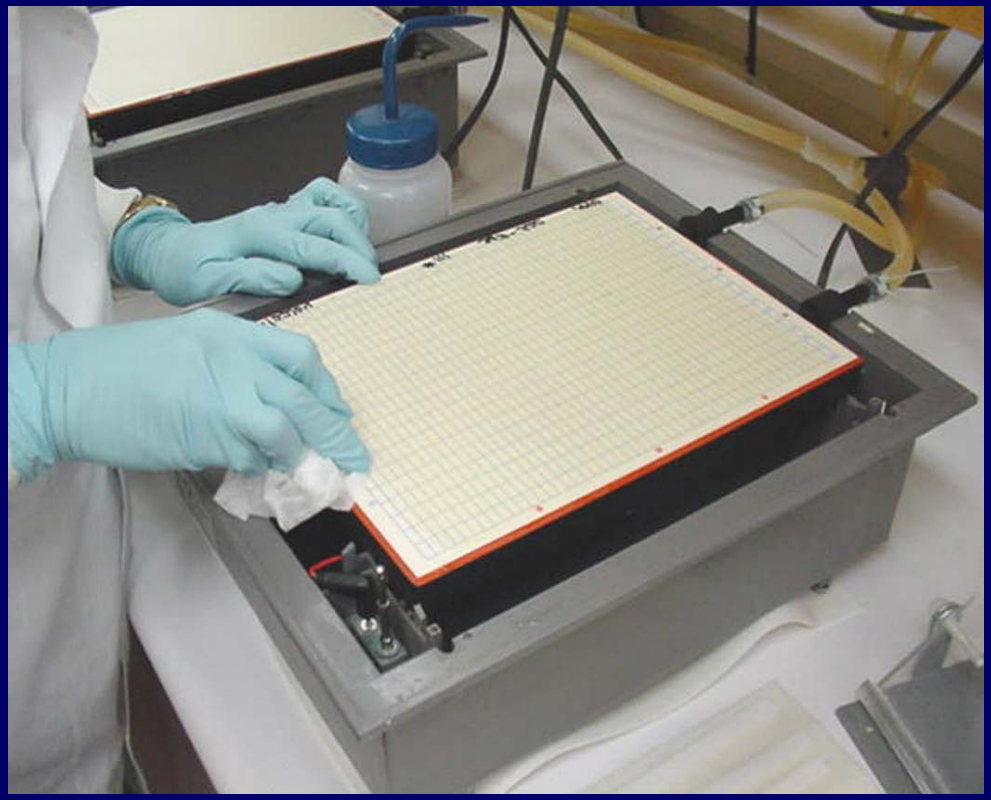
Circulating water bath

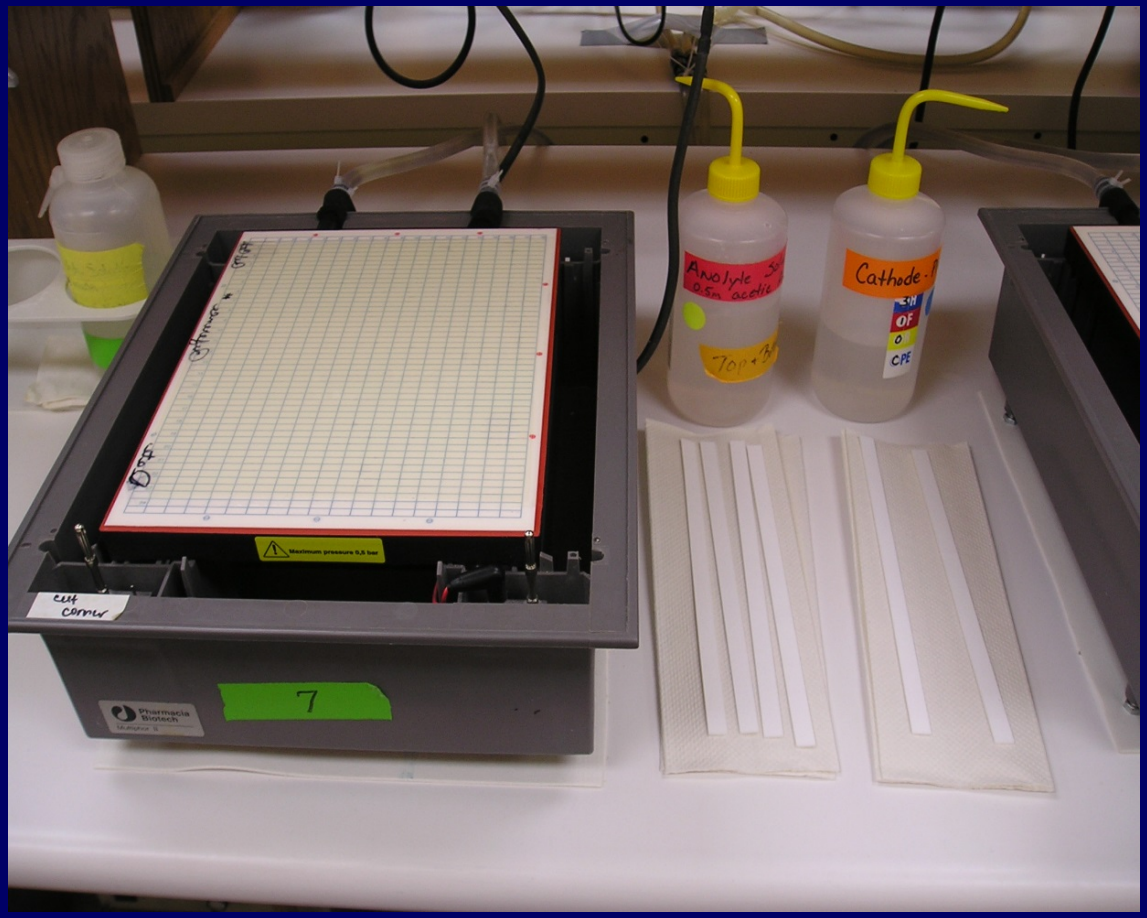


Place gel on multiphor using grid pattern on plate



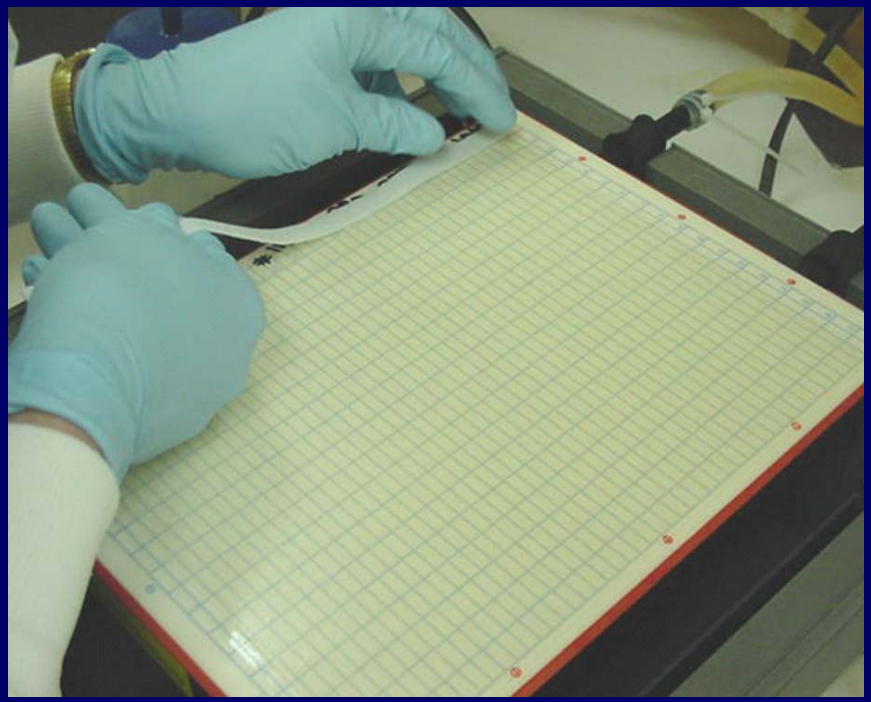




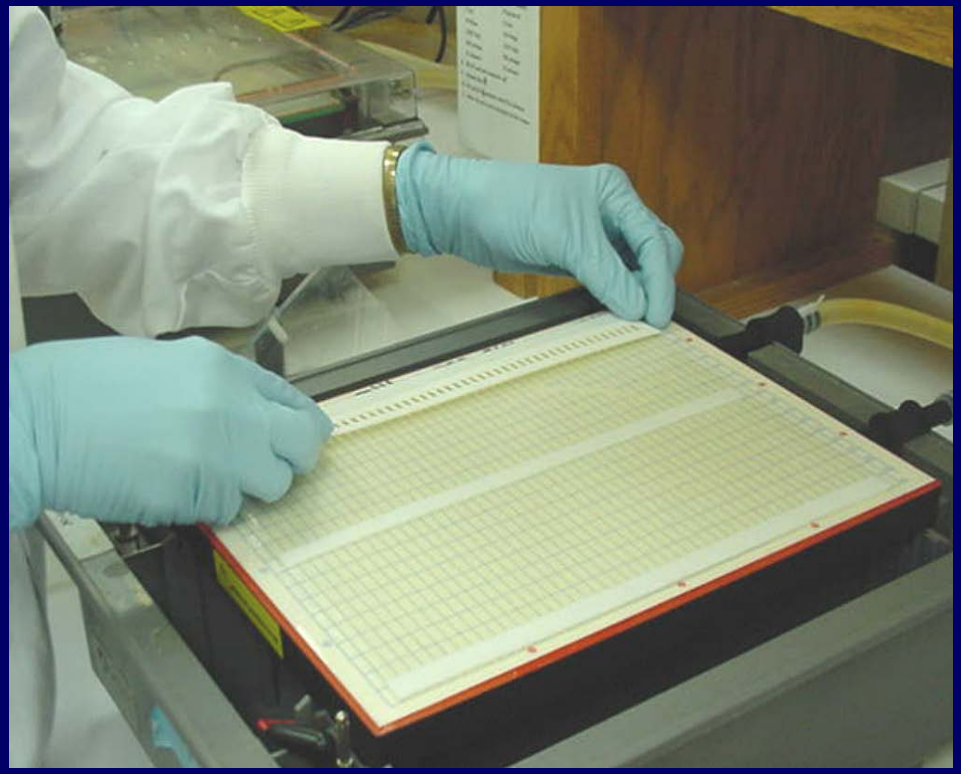

Anolyte
Cathode
7

Soaking wicks with anode and cathode solutions

Placing wicks on gel

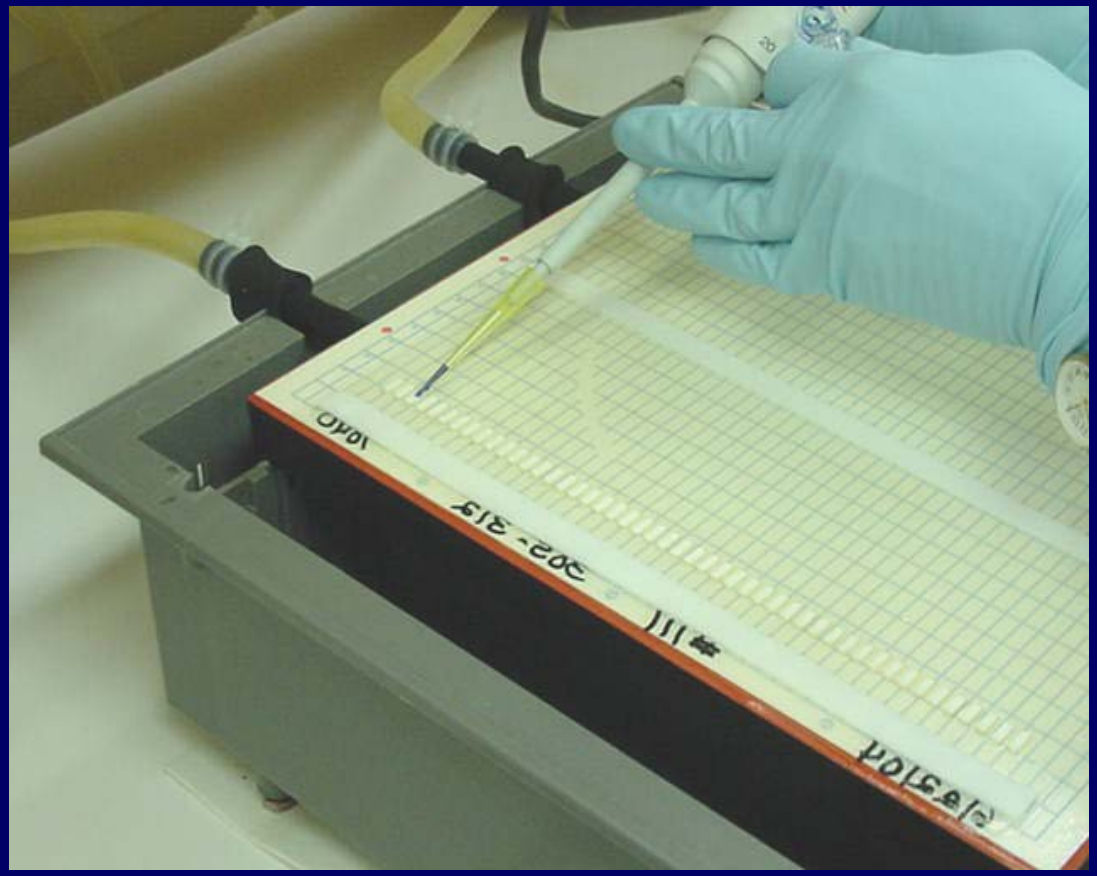


Placing template on gel

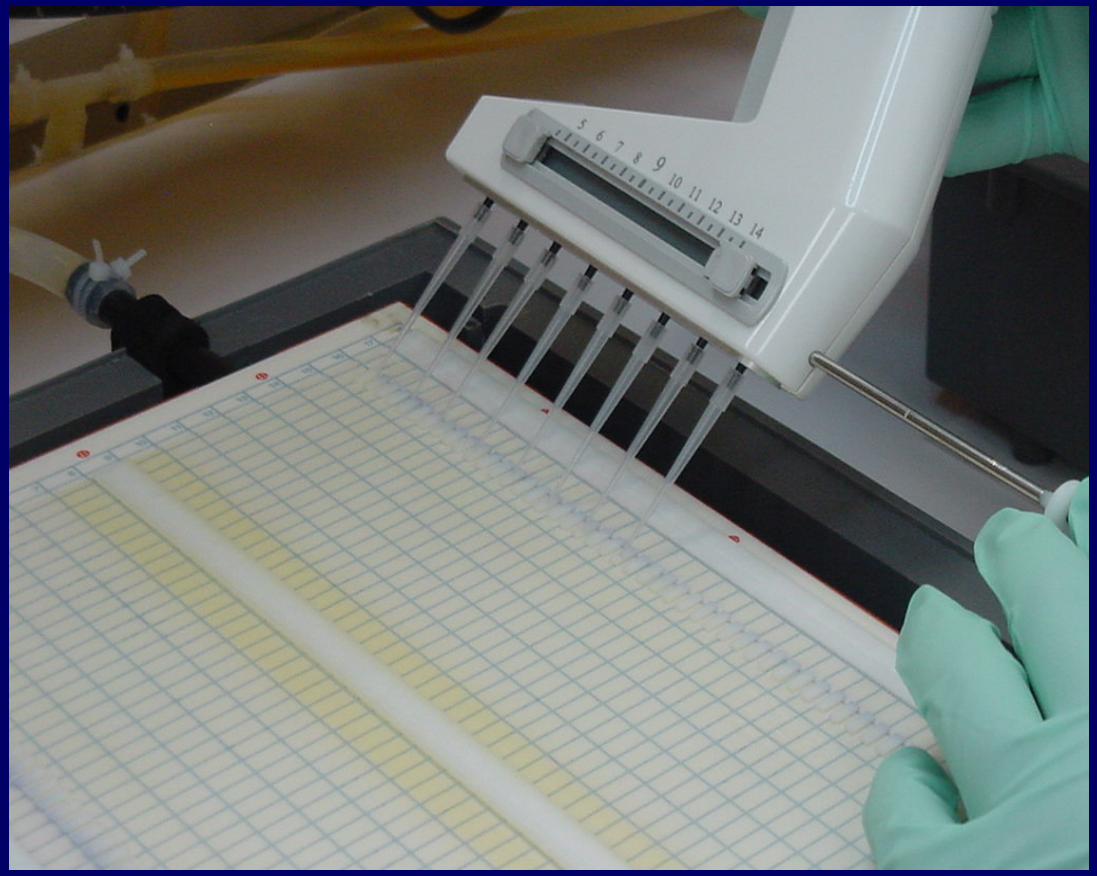


ELECTRODE

Pipetting sample onto template

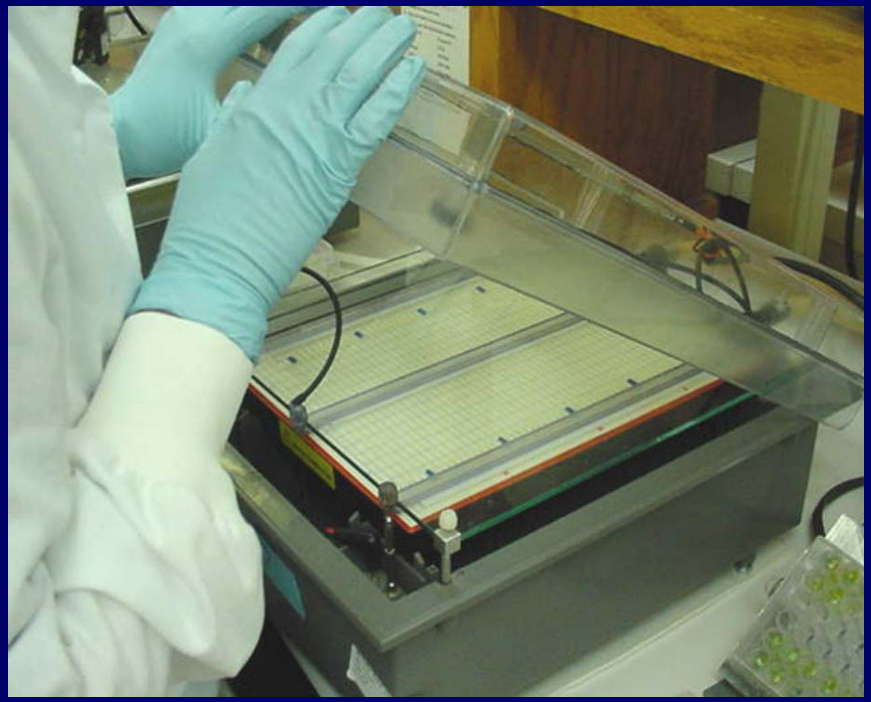


Pipetting sample onto template using multi-channel pipette.

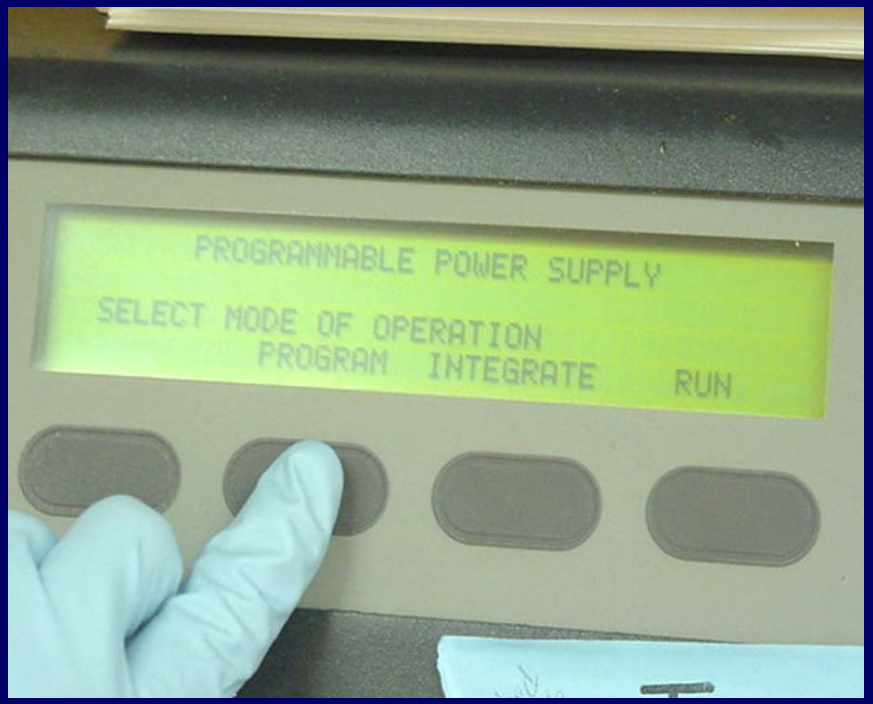


Placing anode and cathode wires onto gel.

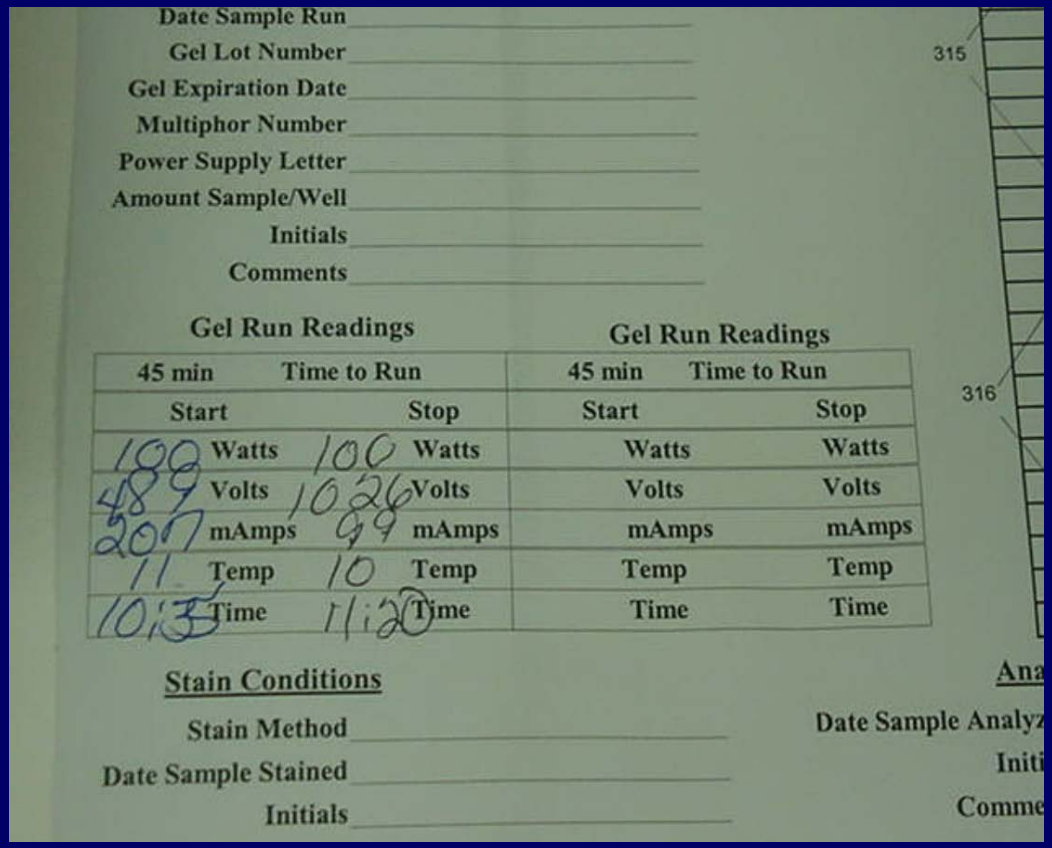
Putting lid on which completes the circuit



Starting up the power supply



Record readings on data sheet



Date Sample Run _____
Gel Lot Number _____
Gel Expiration Date _____
Multiphor Number _____
Power Supply Letter _____
Amount Sample/Well _____
Initials _____
Comments _____

315

316

Gel Run Readings				Gel Run Readings			
45 min		Time to Run		45 min		Time to Run	
Start		Stop		Start		Stop	
100	Watts	100	Watts		Watts		Watts
489	Volts	1026	Volts		Volts		Volts
207	mAmps	99	mAmps		mAmps		mAmps
11	Temp	10	Temp		Temp		Temp
10:35	Time	11:20	Time		Time		Time

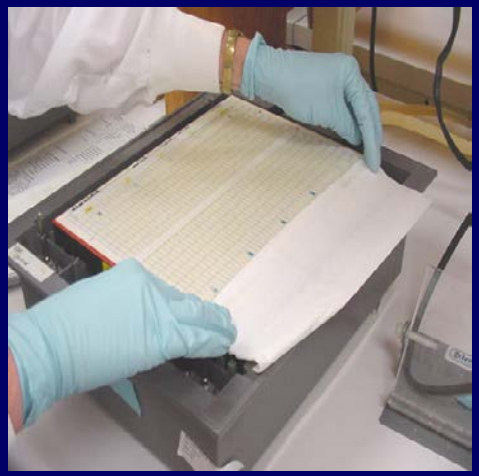
Stain Conditions

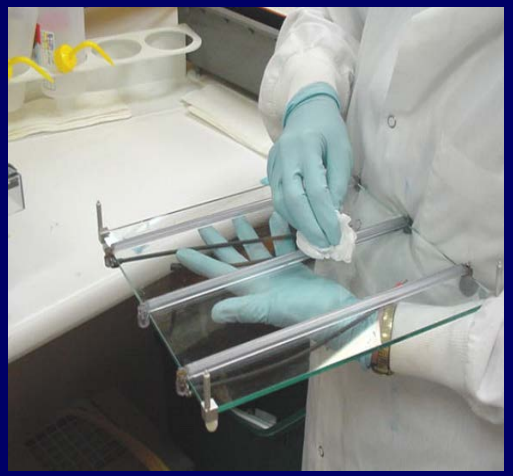
Stain Method _____
Date Sample Stained _____
Initials _____

Ana
Date Sample Analyz
Initi
Comme

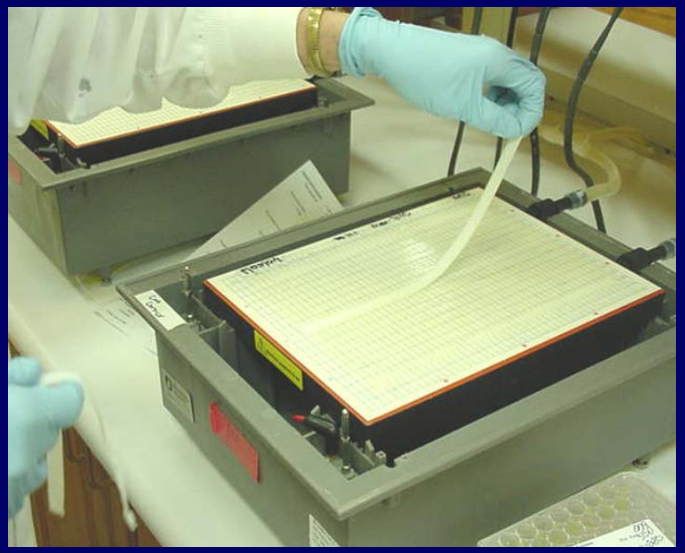
Gels running

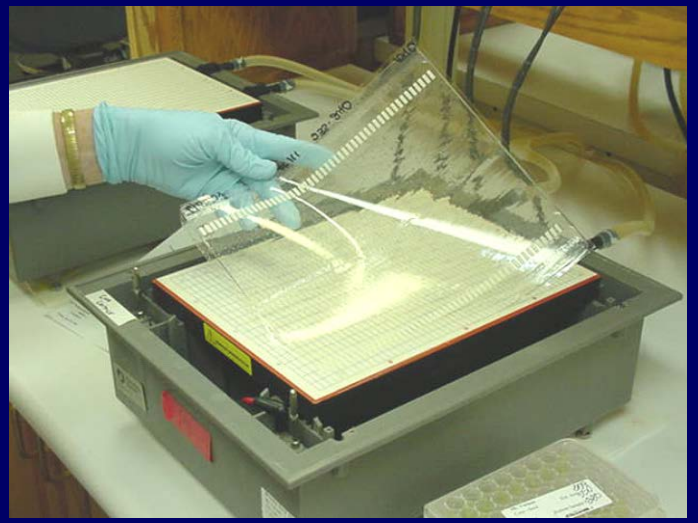
Blotting the gel. (with towel or blotting paper)





Taking gel off once it is done with the run





Gels rocking in TCA fixative step.

TCA fume hood

Gels rocking in water rinses

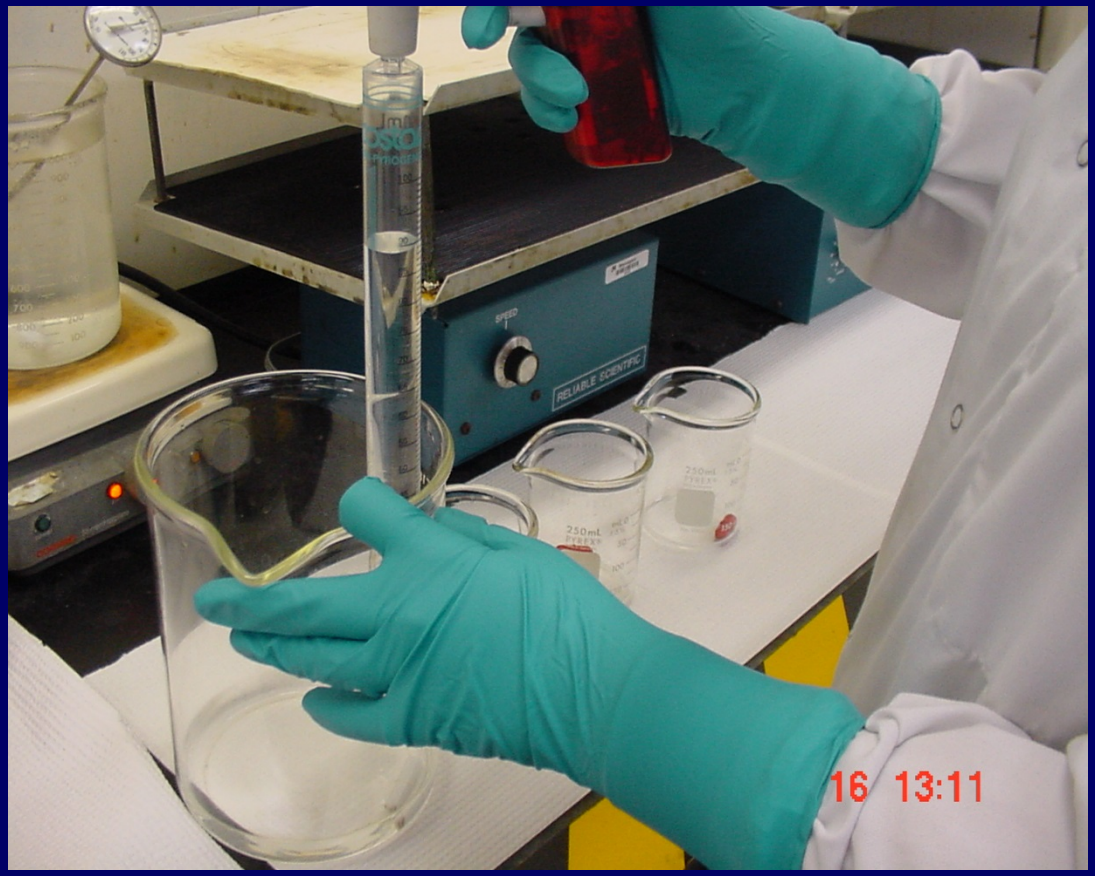
Once gels are out of water rinses, they hang dry overnight or go into a dryer

Staining Process

- Make all solutions for staining
- Place gels in dishes and heat
- Start staining
- When gel is stained, place in an acetic acid stop solution
- Rinse gel with water
- Blot dry

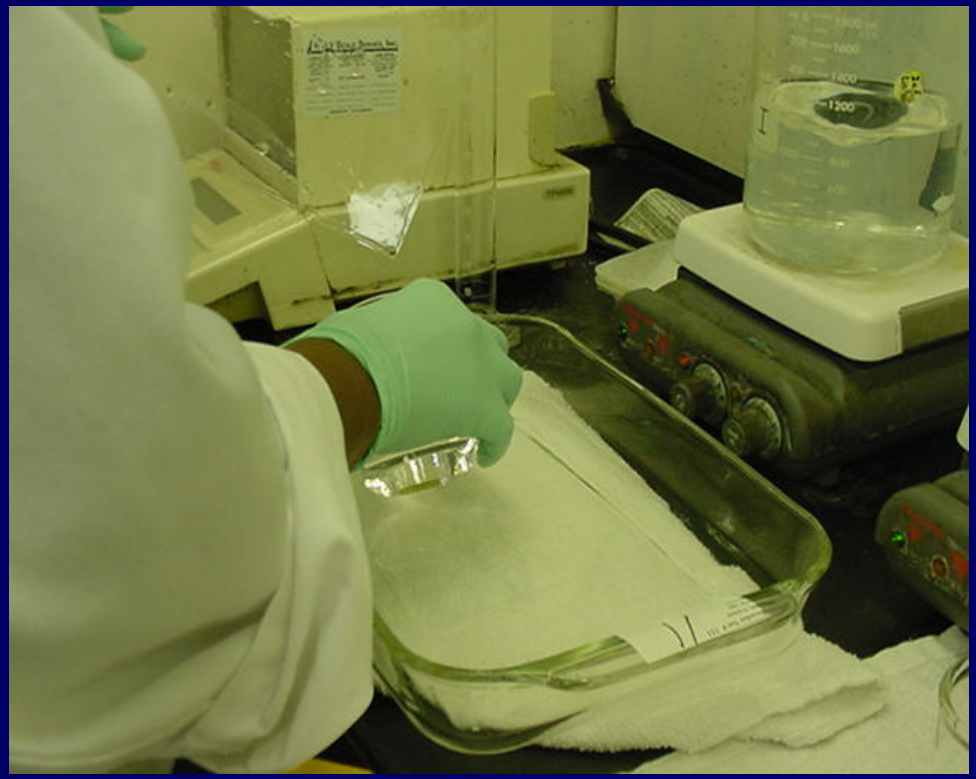
Sodium Carbonate solution

Chemical prep for staining



Staining hood

Pouring staining solution onto gel



Stained Gel

Pouring stop solution in waste container

Blotting gel after stop solution step

Gels that are ready for analysis

What can we see with IEF gels

- Can determine female selfing
- Can determine if a parent line is fixed or not (segregation present)
- Can determine off types
- Can have results within 24 hours (can crush and extract sample same day received and focus/stain/analyze the next day)

What can we see (cont.)

- Can compare foundation Gen0 against Gen1 and Gen2
- IEF gels will keep indefinitely. This is valuable for use as reference material.
- We have every gel that has been run on every material that has been tested since using IEF for genetic purity testing.

Nothing ever goes wrong!

- If you switch the anode and cathode solutions when loading your gels, the readings will be off. (Volts go down, amps go up)
- If you forget to put a gel in TCA soln you will have a practically blank gel when staining
- If a platinum wire breaks, an anode/cathode isn’t plugged in right, or a gel is taken off to soon-the gel will not run the whole way
- A gel can start sparking
- Power supply is set for the wrong wattage

Safety considerations

- Formaldehyde-regulated chemical
- TCA-pH 1
- We wear cuffed lab coats and long cuffed gloves

Questions? Comments?