BIOTECHNOLOGY LAB SKILLS

A Visual Guide for

Teachers and Students

Micropipetting Skills

- When using micropipettes, you need to decide which one is appropriate for your task.
- Check the number on the top of your pipette.
- The number in large type is the name of the pipette (e.g. P10, P20, P200, P1000).
- The smaller numbers (if given) indicate the range of values (maximum and minimum) for which this pipette is best suited.

P-10 is accurate between .5-10 ?L.

P-20 is accurate between 2-20 ?L.

P-200 is accurate between 20-200 ?L.

P-1000 is accurate between 200-1000 ?L.

One Stop or Two?

- When withdrawing a sample:
  - Push down plunger to the first stop, place tip in liquid and release plunger SLOWLY.
  - Check sides of tip to be sure no extra fluid is hanging on to the sides (If there is, simply wipe it on the inside of the tube as you withdraw your pipette.)
- When expelling a sample:
  - Place tip where desired and push down SLOWLY. When you feel slight resistance, you've reached the first stop. Continue pressing and proceed to the second stop.
  - Be sure to release your new sample into the liquid already in the tube, not on the side wall.
  - Remove tip from liquid and release the plunger. Again, check to be sure no extra fluid is hanging on before you remove the tip.

Mixing Your Samples

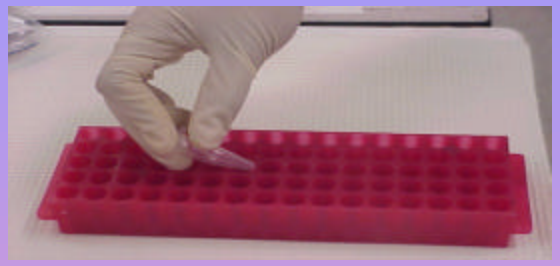
- You can mix your samples in 3 ways:

  - RACKING: “Rack” tube by capping and dragging it along an empty rack several times.

  - VORTEXING: Use a vortexer for a few seconds to vigorously shake the sample.

  - PIPETTING: Pipette up and down a few times before expelling to the second stop (be careful to leave all sample in the tube when finished).

- Teachers report racking works well in the classroom.



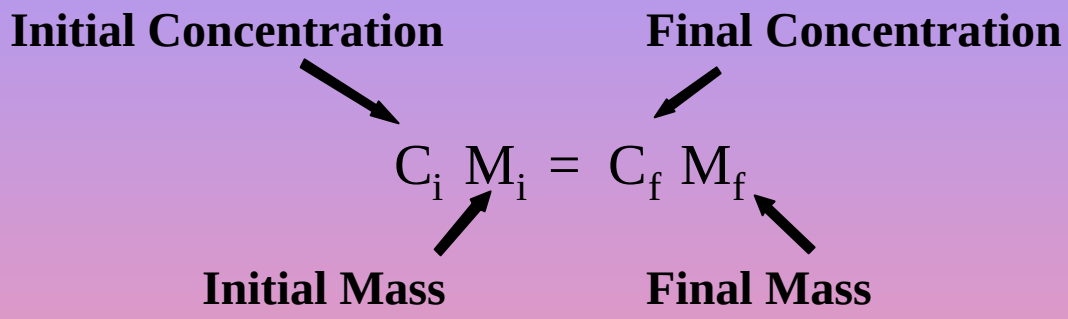
RACKING

VORTEXING

PIPETTING

CALCULATING CONCENTRATIONS

- This formula is a way to determine the concentrations needed when making gels, buffers, and other reagents.



- EXAMPLE:

  Make a 2% w/v (weight/volume) gel that will fit in a 50 ml gel tray.

  C_i = 100% (from the stock container) M_i = ?

  C_f = 2% M_f = 50 g

  (100%)(M_i)= (2%) (50 g)

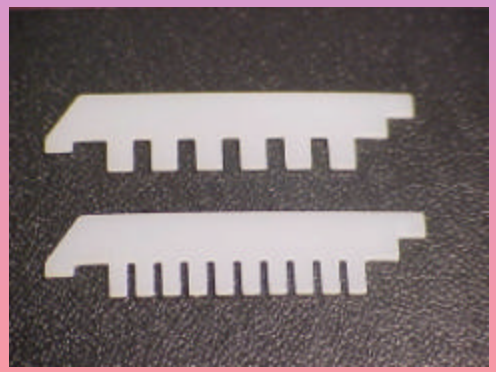
  M_i = 1 g of agarose

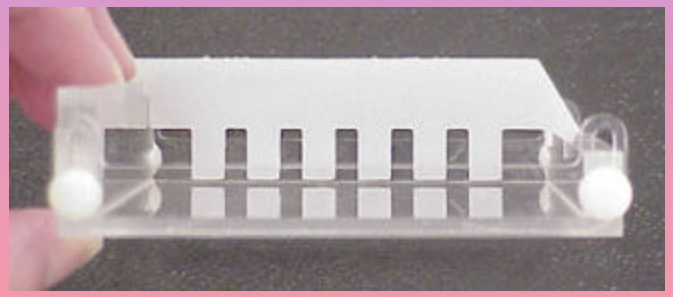
  50 g – 1 g = 49 ml of buffer

- *Note 1 g = 1 ml *Use $C_iV_i=C_fV_f$ when calculating volumes

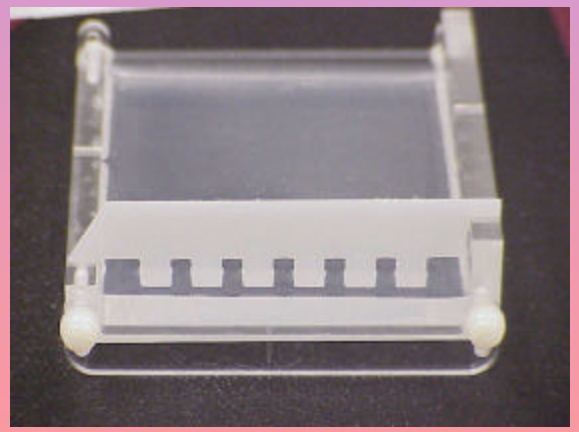
Before Pouring Your Gel

- COMBS come in many sizes (6, 8, 10, 12, 20 wells).
- Place comb into tray before pouring gel.
- GATES should be raised before pouring the gel.
- Gates should be lowered after the gel has solidified.





Gates up



Gates down

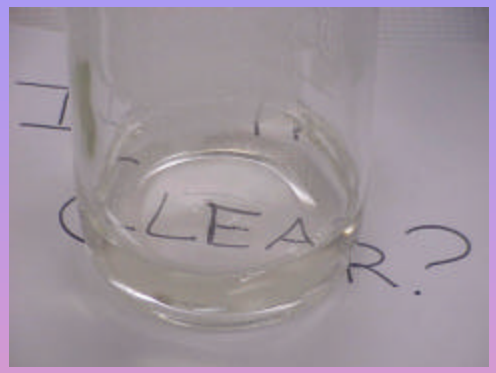
Tips for Making Agarose Gels

- Use a bottle or Erlenmeyer flask to mix agarose powder and buffer. Be sure to have a lid or rubber stopper to cover bottle/flask.
- Label the bottle/flask with initials, date, and contents.
- Microwave on high in 45 seconds increments with the cap/rubber stopper LOOSELY on top of the bottle.
- Caution: Agarose will boil easily and may boil over. Watch it carefully when it's in the microwave and when you take it out.
- At each time interval, remove agarose from the microwave with hot pads and swirl.

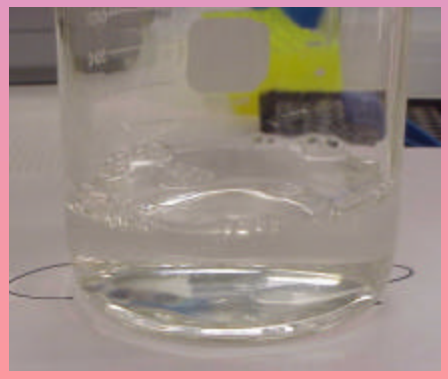
Tips for Making Agarose Gels

- Agarose should be transparent after heating. If not, put back in the microwave for 10 second increments until agarose is completely transparent.
- When the agarose is boiling and you see tiny bubbles it is not yet uniformly melted. Continue heating in 10 second increments.
- When bubbles are large and rolling, your agarose is ready to be cooled.

Not Transparent



Transparent



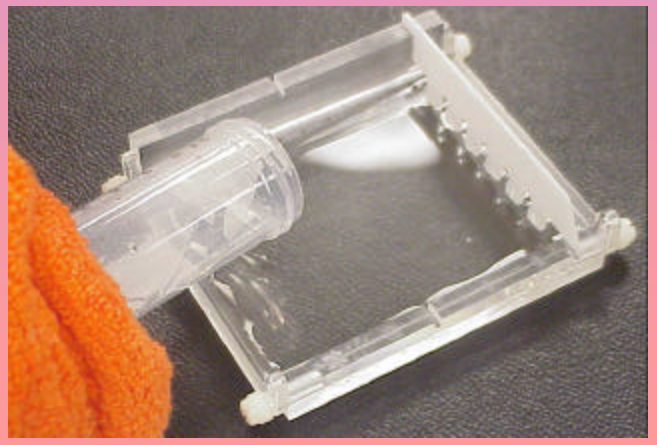
Large, rolling bubbles

Tips for Cooling Agarose Gels

- To cool agarose faster, put ice water in a bucket and swirl bottle/flask (with lid still on) continuously within bath.
- If you see agarose starting to solidify again it must be reheated and cooled again.
- Pour gel when bottle/flask is cool to the touch.

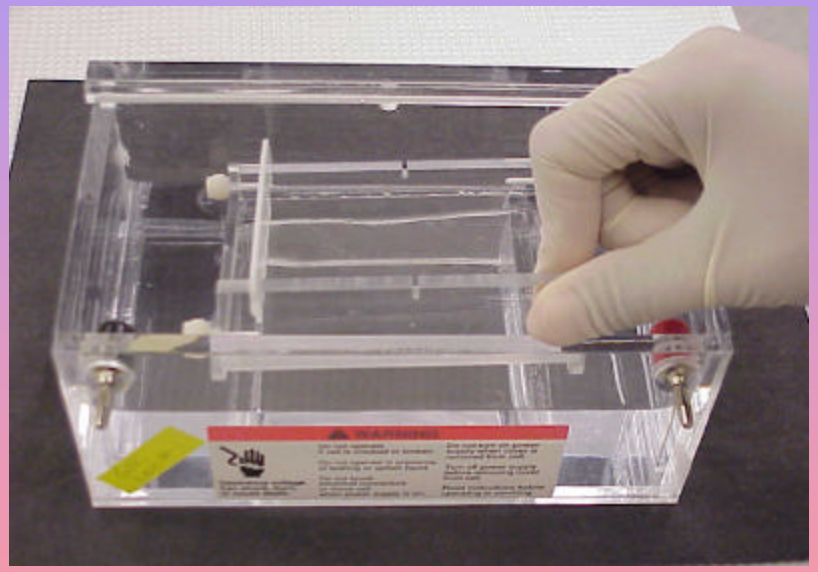
Tips for Pouring Agarose Gels

- Agarose gels are approximately 40-50 ml. You need to check your kit for the exact amount.
- Use graduated glassware or plastic ware to measure out the cooled agarose before pouring it into the gel tray.
- Your gel will take about 20 minutes to solidify. Do not disturb it!
- After 20 minutes, if you gently blow on it and the gel ripples it is not yet solidified. If it does not ripple, the gel is ready to be put into gel box.
- Gels should also be slightly opaque when solidified.



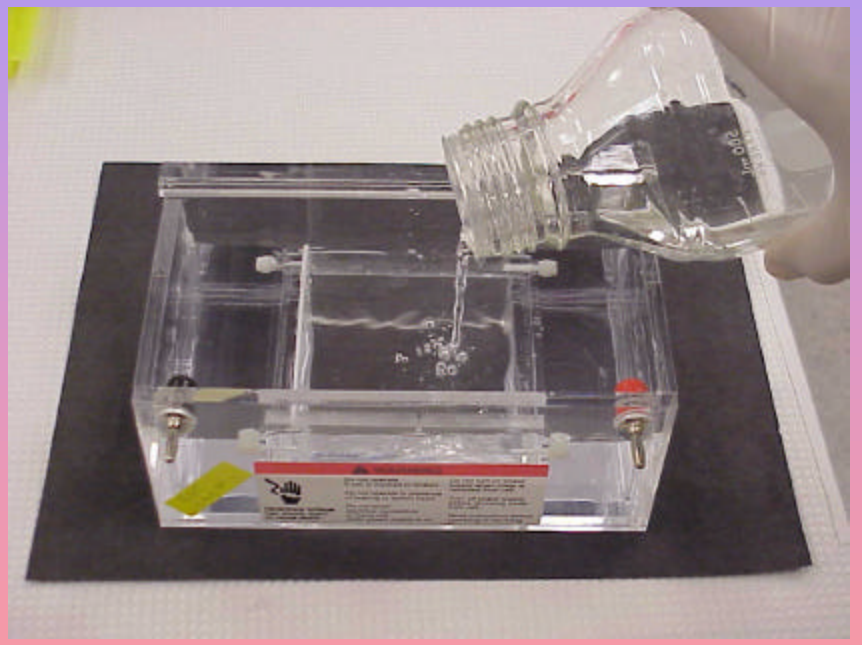
Making and Using Buffer

- Buffer should be .5 X TBE or 1 X TAE. Use the formula $C_iV_i = C_fV_f$ to make buffer that is the proper concentration.
- Be sure that gates are <u>down</u> before placing gel into gel box.
- Place solidified gel into gel box by holding onto the edge of the gel tray, being sure not to let the gel slide off, gently place it into the gel box.



Placing Gel for Electrophoresis

- Pour buffer gently on top of gel. Buffer should just cover the gel so that the top of the gel looks like a flat, glassy surface.
- Pull out comb after gel is placed inside the gel box and covered with buffer. This is an easier, smoother way of pulling out the comb, as opposed to pulling out the combs on the countertop. However, either way does work.

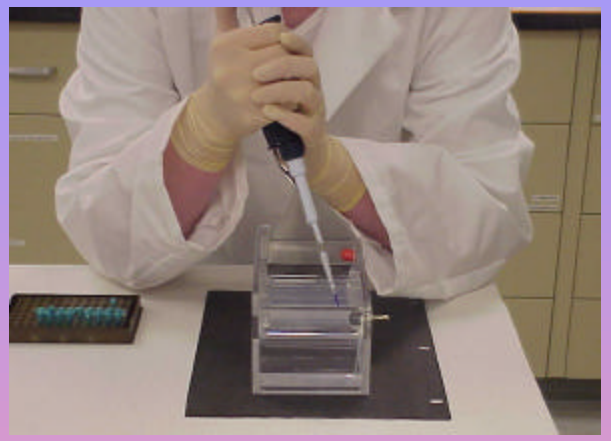


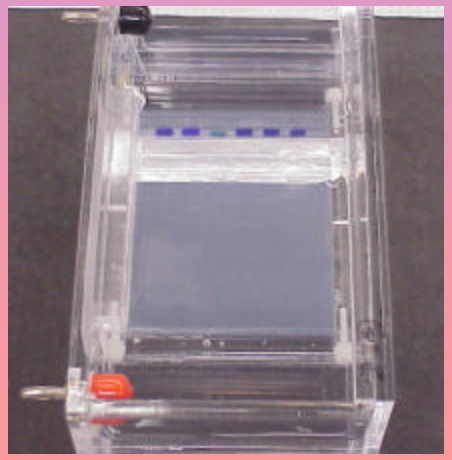
Storing the Gel

- Gels that have not undergone electrophoresis can be stored in a ziplock bag with .5X TBE buffer or 1X TAE buffer (which ever was used to make the gel) for up to 2 days in the refrigerator.
- After your gel has undergone electrophoresis, you can store it in 70% ethanol for up to 24 hours in the refrigerator. This will “fix” the DNA on your gel.

Loading Techniques

- Keep two hands on the pipette. One to press the plunger and one to steady your aim.
- Place both elbows firmly on the table. This provides stability.
- Look down at the wells from above.
- Place tip directly above and almost touching target well. Try not to puncture the well. Loading dye is dense and will help your sample sink into the target well.
- After loading be sure to write down exactly what sample is in each well.
- Loading dye runs ahead of your DNA sample to give you an idea of how far your sample has run. It does not stain DNA.





Running the Gel

- Your gel box should not be plugged into the power supply until after all samples are loaded. (Power is never turned on until both boxes are plugged in, if you are using 1 power supply for every 2 boxes.)
- Gels are usually run at 125 V for 45-50 minutes.
- If you are pressed for time, voltage can be increased to 150 V if you are using TAE buffer. However, this may cause smearing.
- If you need to leave your gel unattended for a short period of time, (<30 min) you can turn down the voltage to 50-75 V.
- Red is the anode, black is the cathode. Remember DNA is negatively charged so your wells should be closest to the BLACK electrode and DNA will run toward the RED electrode.
- When plugging in leads, be sure that the black lead goes into the black plug and the red lead goes into the red plug.

Staining the Gel

- Staining may only be done by the teacher or another trained adult. Ethidium Bromide (EtBr) is a known mutagen and suspected carcinogen.
- When staining:

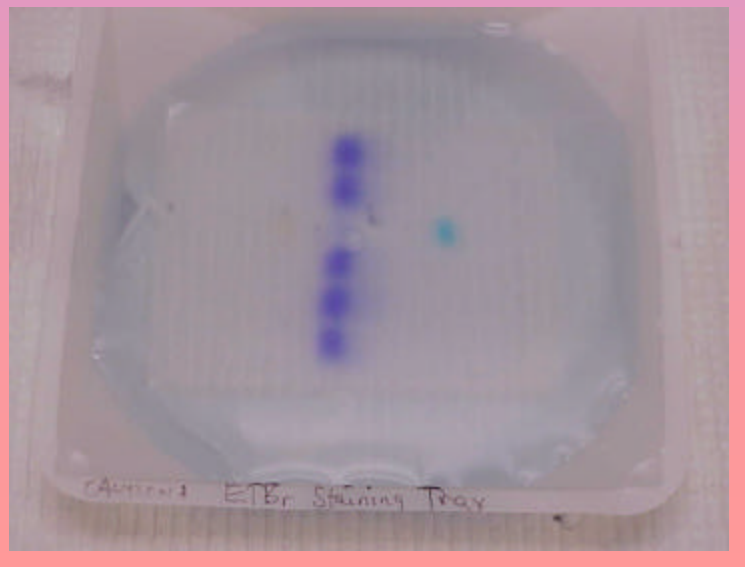
  - cover your workspace with a disposable material.

  - be sure to wear gloves and goggles.

  - label the area so others are aware of the hazard.

  - use equipment (trays/bottles/spatula) that is dedicated to EtBr staining only.

- Gels are usually stained for 20 minutes, but they can remain in the stain for up to 2 hours.
- Pour used EtBr back into bottle (it's reusable) and destain gel with water for no more than 5 minutes. The water can then be poured down the sink with running water.



Visualizing the Gel

- The mini-visionary comes in several parts.
- Before you take it out of the box make a note of how it was packed.
- When assembled correctly it should look like the pictures to the right.
- The mini-visionary comes with color coded cables. Use these to help you put everything together.

Photographing the Gel

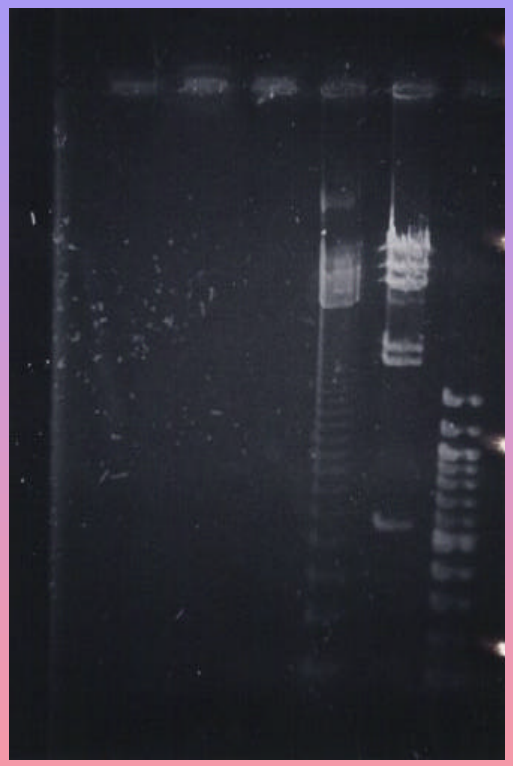
- Place gel on the FotoPhoresis UV light stage.
- Light will not turn on unless either the lid or the camera are firmly in place.
- Place camera above the stage (with lid open).
- Turn on FotoPhoresis unit (front).
- Turn on MiniVisionary (back). Exposure should start at about 20. Adjust it up or down to control brightness of the image.
- Turn on Image Viewer (back).
- Turn on Printer (front).
- Press “capture.”
- Press “print.”
- You will continue to print multiple copies of the same picture until you press capture to capture a new image.

Gel Troubles?

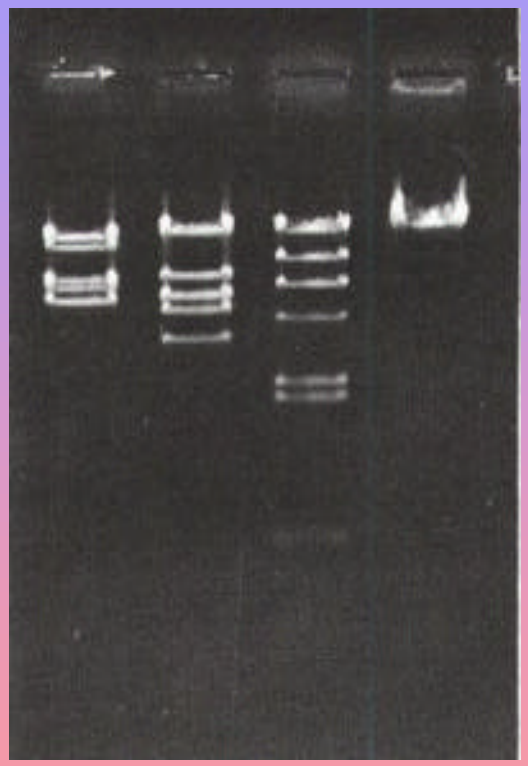
- Unusual gel results can occur as a result of the following conditions:
  - Making your gel with water or the wrong concentrations of buffer.
  - Overloading the well.
  - Running your gel too fast or too slow.
  - Puncturing the gel when loading.
  - A bubble forms in a lane of your agarose gel.
  - Pulling combs out of gel before it has solidified.

Gel made with water and agarose instead of buffer.

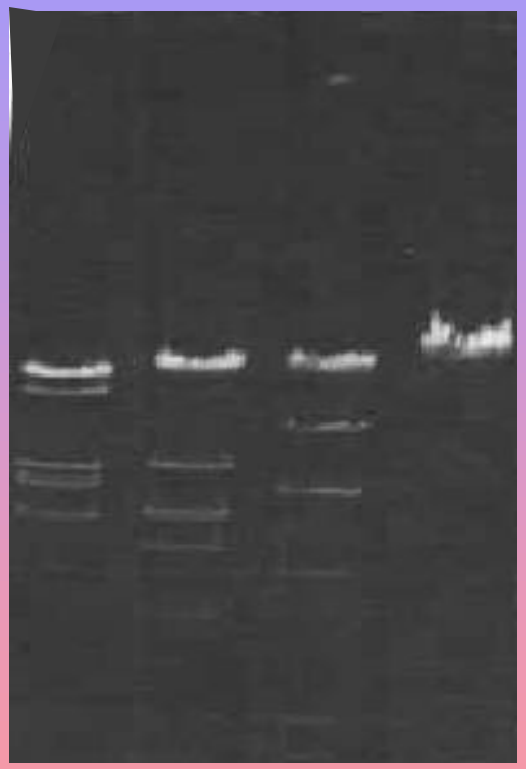
More Gel Troubles



*Gel loaded with too much sample.

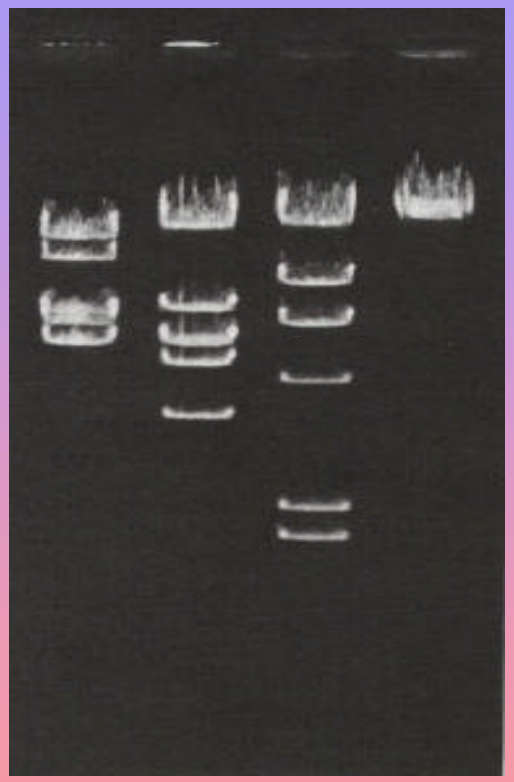


*A gel run too short so there is poor separation between the bands.

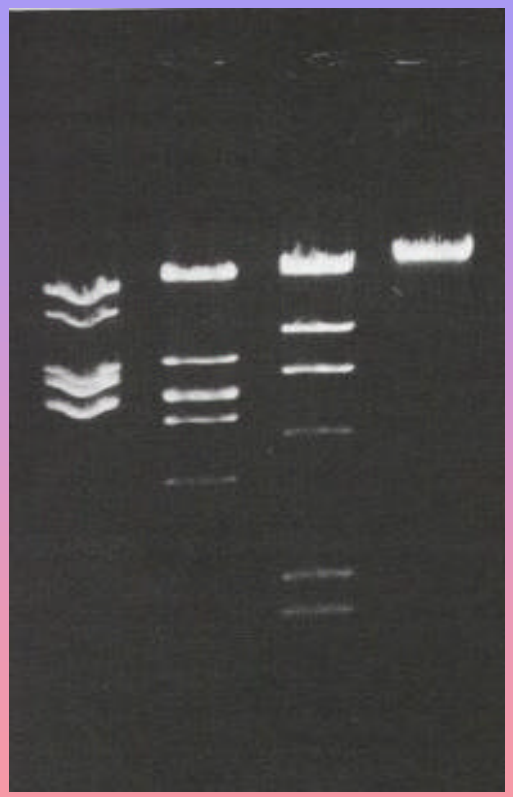


*A gel run too long.

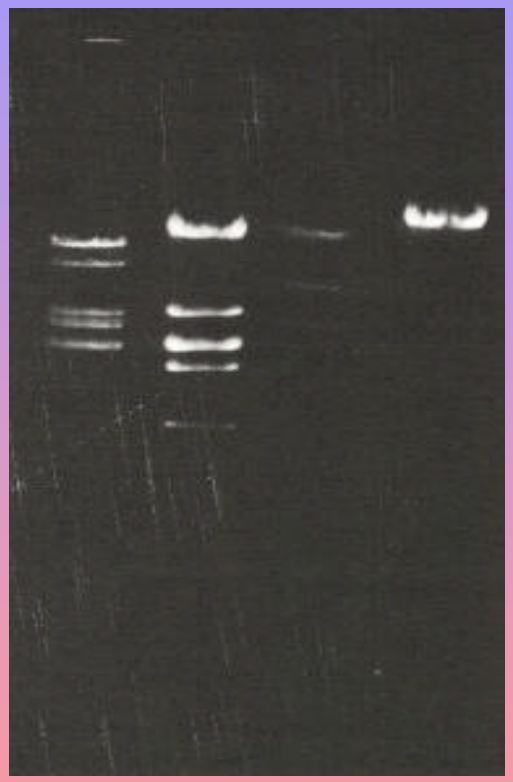
More Gel Troubles



*Punctured wells



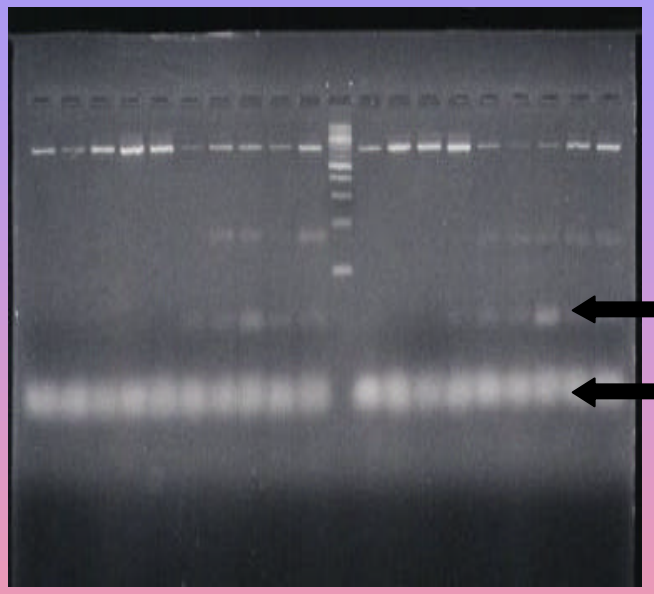
*A bubble set in the agarose in lane 1.



Comb pulled out too early.

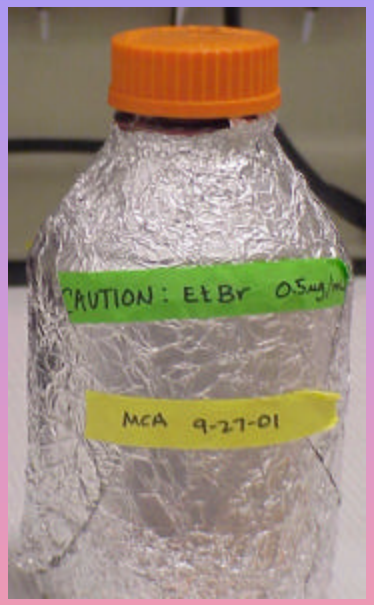
More Gel Troubles

- PRIMER DIMER is when the two primers anneal to each other and amplify in PCR. On a gel, they appear as diffuse bands below the 100 base pair marker.
- UNINCORPORATED PRIMER is caused by an excess of primer in your PCR mix.
- BIG FRAGMENTS: If the wells are illuminated, the DNA fragments may be too large to run across the gel. It is possible PCR did not occur properly.
- NO BANDS: A lack of bands may occur if some reagent was left out of your PCR mix, or if your DNA was not extracted or added properly. Running a control with every gel will help to verify what the problem may be.



Disposing of Gels and Ethidium Bromide Waste

- Wear gloves and goggles.
- Ethidium Bromide gels must be double bagged before you throw them away.
- Liquid Ethidium Bromide can be put back in the bottle after using. Be sure to have bottle covered with aluminum foil and properly labeled. (It degrades when exposed to light.)
- Carefully clean up your work area and throw away contaminated consumables.



Where to go For More Info

- BABEC (Bay Area Biotechnology Education Consortium)

  PCR Outreach Coordinator:650.554.2990 or

  babec_pcr@yahoo.com
- SCCBEP(Santa Clara County Biotechnology Education Partnership)

  Katy Korsmeyer - kuokaty@scientist.com
- G ≡C (Gene Connection)

  Pat Seawell - geneconn@aol.com
- PROBE (PROgram in Biotechnology Education)

  Mary Wuerth - mtwuerth@aol.com
- SF BASE (San Francisco Biotechnology Alliance for Science Education)

  George Cachianes - gcachia@hotmail.com
- EBBEP (East Bay Biotechnology Education Partnership)

  Shary Rosenbaum - sharyrosenbaum@hotmail.com

Acknowledgements

- Gel photographs with an asterisk in the caption are from Micklos, Dave and Greg Freyer. DNA Science: A First Course in Recombinant DNA Technology. New York: Cold Springs Harbor Laboratory Press, 1990. pg. 274-275.
- Other gel photographs were taken in Maria Abilock's Foothill College Biotechnology course.
- All other photos were taken at Applied Biosystems in Foster City, CA under the mentorship of Frank Stephenson and Maria Abilock.
- Any teacher may use any original parts of this presentation to make modifications that meet their needs.
- We'd like to thank Applied Biosystems, Frank Stephenson and BABEC's Maria Abilock for the opportunity to work in biotechnology and to create this presentation.

- After viewing this presentation, please take a moment to fill out the survey that is with the CD and mail to:
  - Kim Burlinson
    20 High School Court
    Los Gatos, CA 95030

Kim Burlinson, Los Gatos High School, kburlinson@lghs.net

Lata Mistry, Castro Valley High School, lmistry@hotmail.com