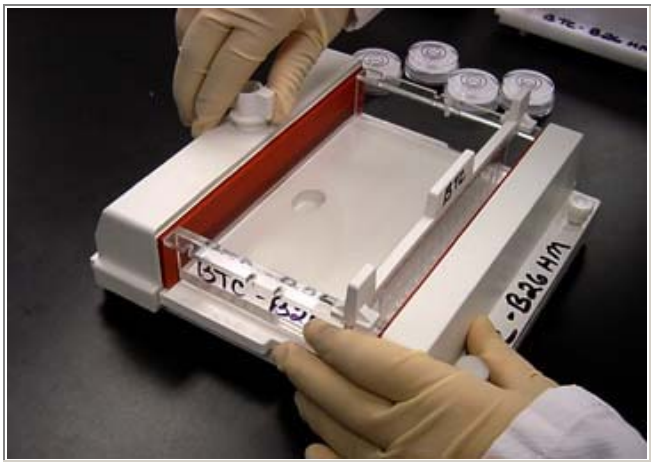
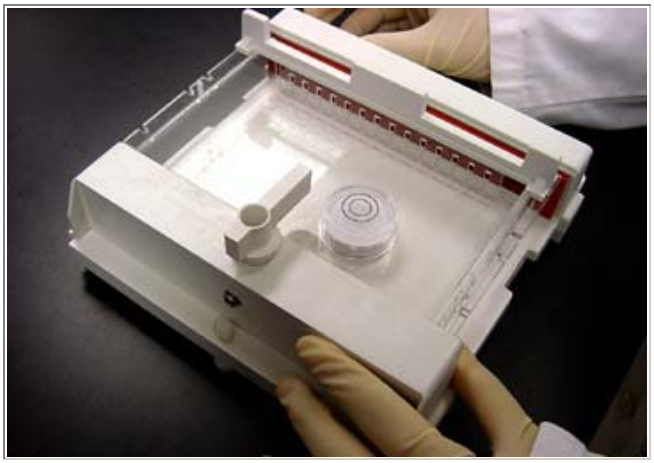
Agarose Gel Electrophoresis

The protocol below illustrustrates Agarose Gel Electrophoresis using the BTC equipment (BioRad).

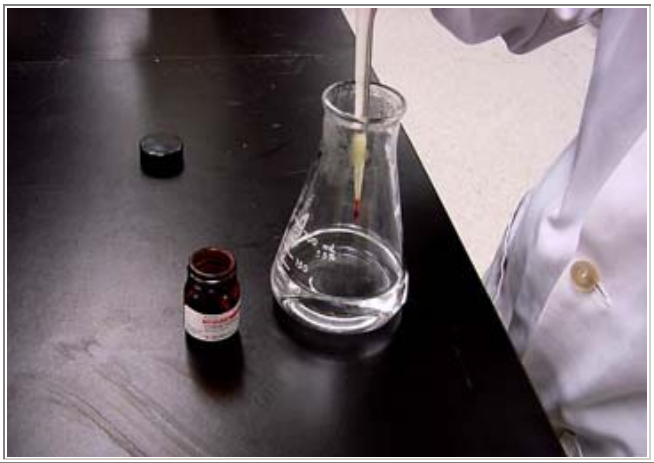


Seal the edges of the gel casting tray according to the manufacturer's guidelines.

Level the gel tray and insert the appropriate size comb.

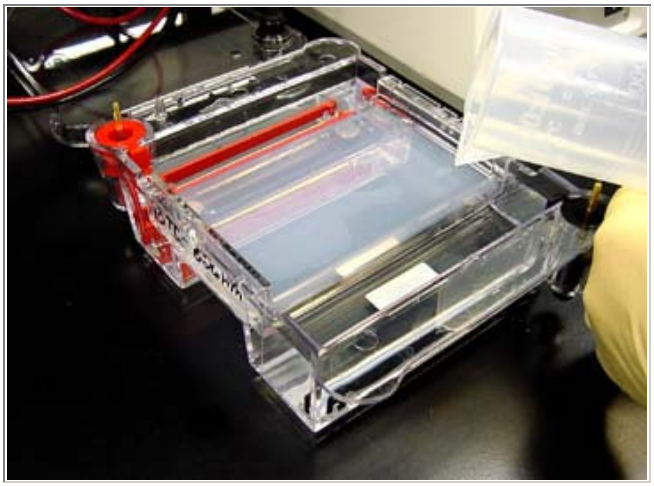


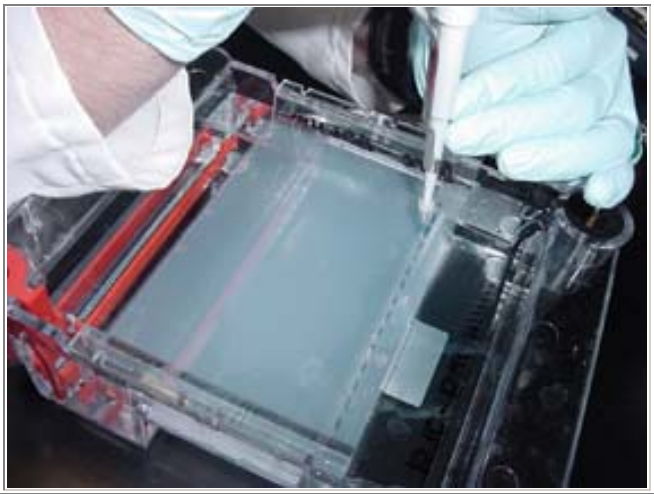
Weigh out the required amount of agarose for the size and strength of gel required. [For the BTC workshops, 100 ml of 1% agarose is used for most applications.]

Add the agarose and buffer to a microwave- or hotplate-safe flask. Usual buffers - TAE or TBE. Check your downstream application for specific choices. [For BTC workshops, most applications require TAE.]	
	Bring the agarose/buffer solution to a boil to melt the agarose. If using a hotplate, a magnetic stir bar will help prevent burning of the agarose.
Once the agarose solution has cooled enough to permit holding the flask, add the required amount of Ethidium Bromide. [For BTC workshops, 10 µl of 10 mg/ml solution for 100 ml of agarose.] Caution: Ethidium Bromide is a carcinogen. Dispose of the pipette tip appropriately.	

Pour the agarose solution into the gel tray ensuring there are no bubbles, especially around the comb.

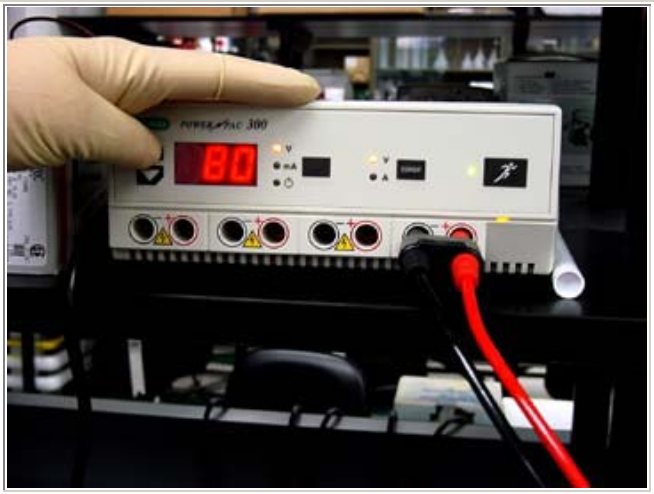
Once the gel is set and cool, transfer it to the electrophoresis chamber and add the buffer to cover. [For BTC workshops, 600 ml of TAE is required.]

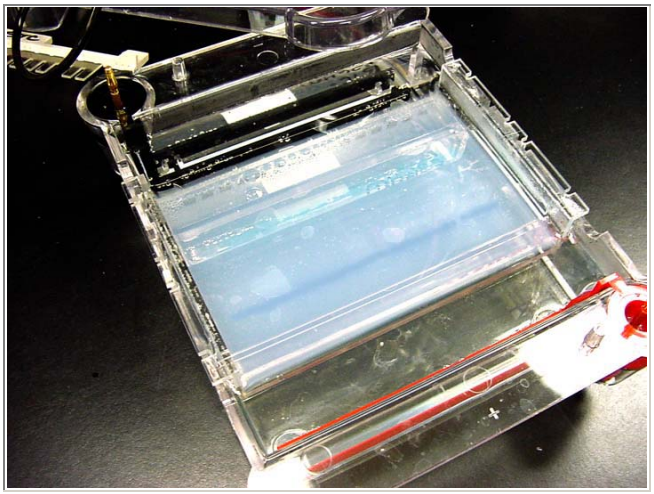




Add the required volume of 6X loading dye to your samples. [BTC workshop - 20 µl samples require 4 µl of loading dye.] Transfer 20 µl to each well of the gel.

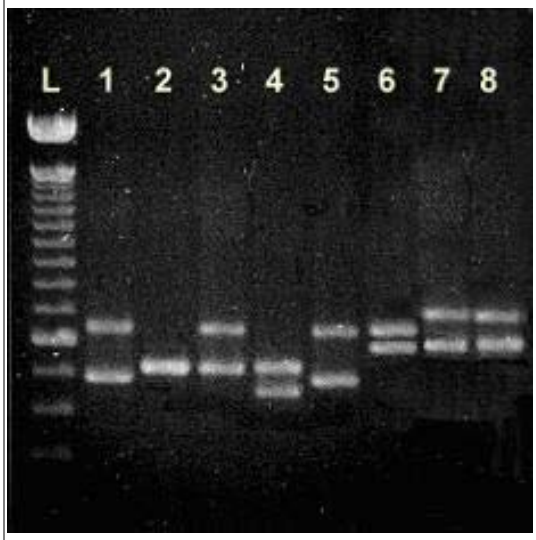
Apply voltage at approximately 10 volts/centimetre of gel length.





Run until the DNA is well isolated - approximately one hour. The Bromphenol Blue band in the dye should run as far in the gel as a 100 bp fragment.

Visualize your DNA bands using a UV transilluminator. Take a poloroid picture. Caution: Wear protective face shield and eyewear to prevent UV burns.



WhoDunnit?

Sample #1 is the PCR amplification of DNA from blood found at the crime scene. Samples #2-8 are PCR from DNA from the suspects who all had access.

Sample #3 is from a child. Who might his parents be? How might all these people be related? [The region amplified by PCR is a region between genes that has a repeat of the nucleotides: in this case, CA. Each person

will have a different number of CAs, thus a different length of band.] The answer link. If this link is not working, a workshop is in progress.	
	A DNA fragment can be isolated from the gel for further applications or the gel can be set up for a Southern transfer.
Credits: Models, reagents, text and layout - Jennifer Sparling, Lesley Ing, and Vicky Lau (summer volunteers from St. Mary's High School, Calgary, AB - With thanks and hope you had fun!); Wendy Hutchins – photos	