DNA Extraction and Gel Electrophoresis

INTRODUCTION

DNA extraction and separation by agarose gel electrophoresis is a simple and exciting process that anyone can perform. However, the high cost of specialized equipment and chemicals often hinder such an experiment from being carried by high school students. Here, we describe a cost effective way of extracting and electrophoresing DNA using products found in local grocery stores.

The first step in obtaining DNA involves breaking open the cell's membrane by using physical or chemical means. For example, you could use ultrasonic waves or vibrations (sonification). Alternatively, our method involves homogenization using a mortal and pestle and detergent to disrupt the cells' membrane. Why do people want to obtain genomic DNA? There are a number of reasons. For example, this DNA could be used to clone new genes or look for special regions of interest. You could also obtain a number of different genomic DNA and make comparisons with them to identify genetic diseases.

Electrophoresis is a way of separating molecules based on charge and size. In our case, we want to separate different sizes of genomic DNA molecules obtained from fruits, vegetables and yeast. Generally, agarose (polysacchardie polymer) or acrylamide (neural toxin) is used to form electrophoresis gels. At the currect concentration, both types of gels contain appropriate sized pores which allow molecules such as DNA to pass through. Current is then applied to push the molecules through. Because DNA is negatively charged, it will travel towards the positive electrode when placed in an electric field. We used agar agar as our matrix support to resolve DNA as it is cheaper than agarose and safer than acrylamide.

Upon completion of electrophoresis, the location of the bands need to be visualized. A common way to detect the bands is to stain the gel with ethidium bromide, using ultraviolet light to view the DNA. Unfortunately, both ethidium bromide and ultraviolet light are carcinogenic and are therefore, not ideal for use among high school students. We suggest using methylene blue to visualize the bands. This stain gives good results under suitable staining and destaining conditions.

MATERIALS AND METHODS

General DNA Extraction Procedure

1. Slice up DNA source of choice (fruit, vegetable or yeast).
2. Using a mortar and pestle, grind up about 30 mL DNA while gradually adding 10mL of prepared detergent buffer solution. Grind for at least 5 minutes with all of your weight and strength to ensure that you break open the cell membrane and reach a creamy soup consistency. If the sample is too thick after grinding, add more saline solution to achieve the optimal thickness so that the liquid portion of the sample is able to pass through the filter, while the larger cellular material remains behind on the filter. If the DNA sample is frozen, it is considerably easier to grind.
3. Filter your sample's juice through a coffee filter into a small beaker. You can also use 3-4 layers of cheesecloth instead of coffee filters. Coffee filters, however, are better because they are cheaper, more accessible, and easier to cleanup. Let the solution drip into the beaker until all of the liquid has passed through the filter. If this takes too long, simply squeeze all of the juice from the sample through the filter.
4. Gradually add ice cold 99% propanol by drizzling it down the side of the beaker. The isopropanol will form a clear layer on top of your sample. Add 2 volumes (this means approximately two times the volume of the sample present) of ice cold 99% isopropanol down the side of the beaker with a straw or pasteur pipette. It is helpful to tilt the beaker as you do this to increase the surface area of the juice layer in contact with the alcohol. Also, do this step slowly to enable the alcohol to form a layer on top of the juice layer. If the alcohol does not form a separate layer, the alcohol will be too dilute to precipitate the DNA. As you let the beaker sit, the DNA should precipitate. The longer you wait, the more DNA you should see.

5. DNA precipitates, resembling a thread of translucent white snot, at the interface between the juice and alcohol. After a considerable amount of time, the DNA may eventually float to the top of the alcohol layer.
   Note: If the DNA does not clump together, students can draw up the solution with a pasteur pipette or an eye dropper. They could then transfer the DNA to a new test tube and continue the normal procedure. If you have access to a centrifuge, you could spin down the precipitate before pouring out the isopropanol.
6. Remove the DNA with a wooden popsicle stick or glass rod. DNA adheres well to the wooden stick. Ideally, you'll have 0.02 to 0.10 mL of DNA.
7. Transfer the DNA into a clean test tube.
8. Rinse the DNA with 70% ethanol to remove excess salts. Decant the ethanol (i.e. pour off the liquid portion).
9. Dissolve your DNA into 0.5mL to 1mL of distilled water for about an hour. An alternate way to increase the concentration of dissolved DNA is to place the DNA smaple with the added 0.5 mL to 1 mL distilled water into a 55^{o}C water bath for 20-30 minutes.
10. Take about 85uL of DNA sample and add it to a new test tube. Also add 15uL of stop loading buffer to the sample.
11. Load the above sample (100uL) into the gel covered in electrophoresis buffer.
12. Run sample at 85V for about 1 hour.
13. Stain with 0.02% methylene blue dye overnight.
14. Visualize DNA bands!

In step 2, a variety of different buffers or method can be used to break open the cells' membrane. The recipe for each buffer are summarized below in table 1.

Table 1. Buffer recipe / Method used for disturbing cell membranes

Method	Recipe / Instructions
Saline Method	0.9% saline Following step 3, add 1.5mL of 10% dish washing liquid and swirl gently. We recommend using Palmolive brand in the unconcentrated clear form.
Regular Buffer Method	1.5g table salt 5.0g baking soda 5mL dish soap (clear Palmolive) Add distilled water into the container until it reaches a volume of 120mL
Acidic Buffer Method	1.5g table salt 5.0g baking soda 5mL dish soap (clear Palmolive) Add distilled water into the container until it reaches a volume of 120mL 5mL of vinegar
Basic Buffer Method	1.5g table salt 5.0g baking soda 5mL dish soap (clear Palmolive) Add distilled water into the container until it reaches a volume of 120mL 5mL of vinegar 10mL 0.4M NaOH
Proteinase Method	1.5g table salt 5.0g baking soda 5mL dish soap (clear Palmolive) Add distilled water into the container until it reaches a volume of 120mL 2.5mL Complete eye solution 2 protein tablets (Complete brand)
Meat Tenderizer Method (used for 1.5g raw wheat germ as DNA source)	100mL hot water 5mL detergent 3g meat tenderizer

Electrophoresis Box

You will need:

- a 12cm X 16cm X 4cm plastic container
- 20cm-25cm of stainless steel wire (20 guage)
- two 5cm stainless steel screws
- five to seven 9Vbatteries clipped together
- two alligator clips
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Assembling the electrophoresis box is quite simple. First, take a stainless steel screw and attach a 10 cm wire to the end of it while wrapping it around the bottom threads of the screw. Repeat with the other screw. Once the wires are firmly attached, place the screws at alternate corners of the plastic container so that they are diagonal from one another. The wires should rest flushed against the bottom edge of the walls in the plastic container. Secure the screws with electrical tape.

When you are ready to place your gel into the electrophoresis box, connect your 9V batteries in series. With an alligator clip, attach the battery's positive terminal to one of the screws. Using another alligator clip, attach the battery's negative terminal to the other screw.

Mold for Casting Gels

To create a mold for electrophoresis gels, use a simple square soap dish approximately 1.5cmx5cmx8cm. Then attach a "comb" near one end of the soap dish to create wells in the gel. A manicurists toe spacer, popsicle sticks and masking tape can be used together to create a comb. Two popsicle sticks on both sides of the toe spacer are used to balance the spacers along the side of the soap dish. Ensure that there is at least 3mm distance between the casting tray and the bottom of the toe spacer to allow the formation of proper wells. Also, before using the toe spacers, it is recommended to cut them in order to prepare a 3mm x 8mm well. Line the bottom of your soap dish with saran wrap for easier removal of gel. When handling the agar agar gel, be extra careful not to break it as it is extremely flmisy.

Gels

To make the gel, it is recommended to use agar agar in the powder form rather than the granular flakes of agar agar. Ensure that it does not contain any additional ingredients such as glucose as these ingredients may interfere with the formation of the gel matrix. 1% Agar agar. Agar agar in the noodle form is also available, but are much more difficult to use. They are messy, need to be cut up and strained after heating. Weigh out ??grams of agar agar and transfer it to an appropriately sized glass Erlenmeyer flask. The flask should be large enough so that the agar agar solution forms a 2 cm layer at the base. This is required to ensure a large surface area in contact with the hotplate and to bring the solution to a boil quickly. It is recommended to use a hot plate and a stir bar to heat the agar solution evenly. An alternative is to use a microwave, but with this method one must use a low heat level at short intervals while swirling the flask in between heating. If this procedure is not carried out carefully, the solution will likely overboil and create a large mess. If the agar agar solution is not completely dissolved, the undissolved bits of agar will be stained blue during the staining procedure, making it difficult to view the bands of DNA. Also, undissolved agar prevents the formation of pores, causing the bands to be faint and smeared. Once dissolved, the agar agar solution should be poured into the plastic-lined soap dish. Remember to also insert the toe spacers to form the wells. It will take approximately 20 minutes for the gel to solidify. During this time do not disturb the gel. An indicator that the gel is ready to be loaded is that there will be resistance while carefully pulling the toe spacers out of the soap dish. The gel should be poured to a 0.5 cm thickness. If the gel is too thin, causing it to float, the gel should be gently rubbed against the bottom of the electrophoresis box to create a static cling. For storage purposes, the wells of agar agar gel are often broken if immersed in running buffer overnight. It is preferred to wrap the gel in plastic wrap and place it into a fridge.

DISCUSSION AND RESULTS

Detergent Buffer (To break up cell) and DNA Source

Several different methods of extracting DNA were attempted, including: the saline method, the buffer method, the acidic buffer method, the basic buffer method, the proteinase method, the meat tenderizer method, and the heating method. The different types of detergents were tested systematically against a series of different fruits and vegetables and are summarized in the Table 2.

Both the regular buffer method and acidic buffer method allowed us to obtain large quantities of DNA. The regular buffer method was effective with onion, split peas, frozen corn, bean sprouts, kiwis and bananas. The acidic buffer method was especially effective with onion, split peas, frozen corn, yeast, lima bean bacteria, bean sprouts, and bananas. The basic buffer method and proteinase method also allowed us to collect DNA. Overall, we recommend the acidic buffer method to extract DNA from variou food sources.

Running Buffer

The preferred Magyver running buffer was made of: 0.05 g/L salt, volumized to 1 L with distilled water with a final pH of 7.5 ± 0.5. Baking soda (sodium bicarbonate) was used to change the pH of distilled water from pH 5 to the recommended pH. A superieur alternative to baking soda is an alkaline buffer made by Seachem. This product is commonly sold by pet food stores to buffer aquarium water. In this experiment, it was found the Seachem product demonstrates a superior buffering capacity compared to baking soda. To obtain the optimal pH approximately 3 g/L of baking soda or 0.48g/L of alkaline buffer solution is required in the running buffer. Litmus paper and a pH meter were used to determine the pH of the running buffer.

Modified running buffers were also used but found to be unsuccessful. A buffer made of 0.03 g/L salt in distilled water resulted in the disappearance of the tracking dye, the ethidium bromide, the standard, and the samples. A concentration of salt higher than 0.05g/L (we used 0.10 g/L salt) in the running buffer caused the tracking dye to turn from blue to yellow. As well, the DNA standard and samples were not visible under UV light with an increased salt concentration.

Table 3. Summary of different running buffers, staining conditions, and their effects.

Gel	Baking Soda (g/L)	Alkaline Buffer (g/L)	Water	Salt (g/L)	Other	PH	Staining	Comment
1.0% Agarose	---	---	---	---	1X TBE as RB	---	Stain with 20-30 drops of 5% MB in 100mL distilled water for 3 hr. Destain 2hr, during which changed distilled water 3 times.	EtBr bands present. Clear MB-stained bands for sample and standard present (Repeated experiment three times, achieved same results)
1.0% Agarose	---	---	---	---	1X TBE as RB. 5 drops of 5% MN in 1liter of RB	PH8 with MB before and after gel electrophoresis	---	EtBr bands for sample and standard not visible
1.2% Agar Agar	---	---	---	---	1X TBE	---	---	EtBr bands present for sample and standards (Repeated experiment twice, achieved same results)
1.0% Agar	---	---	---	---	1X TBE	---	---	EtBr bands present

Agar								for sample and standards (Repeated experiment twice, obtained same results)
1.0% Agarose	1	0	Distilled	0.05	10-15 drops/L Methylene Blue	PH 7.5	---	Samples including positive control did not show MB bands
1.0% Agarose	0	0	Distilled	0	1X TBE with 10-15 drops/L Methylene Blue	----	---	Samples including positive control did not show MB bands
1.0% Agarose	1	0	Distilled	0.05	----	PH 8.0	---	EtBr Bands present both times these conditions used
1.0% Agarose	1	0	Distilled	0.05	----	PH 8.0	---	EtBr Bands present but they were not tight bands
1.1% Agar Agar	1	0	Distilled	0.05	----	PH 8.0	---	EtBr Bands present but they were not tight bands
1.1% Agar Agar	1	0	Distilled	0.05	----	PH 8.0	---	EtBr Bands present
1.0% Agar Agar	1	0	Distilled	0.05	----	PH 8.0	---	Samples including positive control did not show EtBr bands. (Repeated experiment twice)
1.0% Agar Agar	1	0	Distilled	0.05	10-15 drops/L of Methylene Blue in gel and Running Buffer	PH 5.0 without MB PH 7.0 with MB	---	Samples including positive control did not show EtBr bands. (Repeated experiment twice)
1.0% Agar Agar	4	0	Distilled	0.05	10-15 drops/L MB only in RB	PH 8.0	---	Samples including positive control did not show EtBr bands. Experiment repeated, bands present.
1.0% Agar Agar	3	0	Distilled	0.05	10-15 drops/L MB in RB	PH 8.0	---	EtBr Bands present. (Repeated experiment twice)
1.2% Agar Agar	3	0	Distilled	0.05	10-15 drops/L MB only in RB	PH 8.0	---	Samples including positive control did not show EtBr bands.
0.8% Agar Agar	4	0	Distilled	0.05	---	PH 8.0	---	Samples including positive control did not show EtBr bands.
1.0% Agar Agar	3	0	Distilled	0.05	---	PH 8.0	---	EtBr Bands present
1.0% Agar Agar	3	0	Distilled	0.05	10-15 drops/L MB only in RB	PH 8.0	---	Samples including positive control did not show EtBr bands.

1.2% Agar Agar	0	0	Distilled	0.05	10-15 drops/L MB only in RB	---	---	Samples including positive control did not show EtBr bands in 3 of 4 gels. Bands present in samples and standard of fourth gel.
1.0% Agar Agar	0	0	Distilled	0.05	---	---	---	Samples including positive control did not show EtBr bands.
0.8% Agarose	0	0	Distilled	0.05	1X TBE used to only make gel	---	---	Tight EtBr bands present in samples and standard
0.8% Agarose	0	0	Distilled	0.05	---	---	---	EtBr Bands present in samples and standard.
0.8% Agar Agar	0	0	Distilled	0.05	1X TBE used to only make gel	---	---	EtBr Bands present in samples and standard.(repeat results twice)
0.8% Agar Agar	0	0	Distilled	0.10	---	---	---	Sample including positive control did not show EtBr bands. Tracking dye turned yellow.
0.8% Agar Agar	0	0	distilled	0	1X TBE in gel and as RB	---	Old stain with unknown concentration used for 1hr-did not work	Tight EtBr bands present in sample and standard
0.8%Agar agar	5	0	Distilled	0.05	Agar hydrated overnight	PH 8	---	Tight EtBr bands present in sample and standard
0.8%Agar agar	0	0	Distilled	0.05	0.3mL fo 0.4M NaOH for RB and to make gel. Agar hydrated overnight	PH 9.5	---	EtBr Band present for standard. Tracking dye turned yellow. Running buffer didn't bubble
0.8% Agar Agar	4	0	Distilled	0.05	Agar hydrated overnight	PH 7.5	---	EtBr Bands present for both standard and sample.
0.8%Agar agar	2	0	tap	0.05	---	PH8.0	---	Gel dark brown before electrophoresis. Light brown after run gel. EtBr Bands present in samples and standard
1.0%Agar agar	4	0	distilled	0.05	---	PH 7.8	---	Samples including positive control did not show EtBr bands. Gel dark brown before electrophoresis. Light brown after run

								gel Repeat experiment, achieved visible EtBr bands for sample and control
1.0%Agar agar	1	0	distilled	0.05	---	PH 7.8	---	EtBr Bands present for sample and standards
1.0% Agar Agar	1	0	tap	0.05	8 drops 5%MB in 1L of RB	PH 7.8	---	No EtBr bands visible for samples and standard (Repeat experiment three times, achieved same results)
1.0% Agar Agar	2	0	tap	0.05	8 drops 5%MB in 1L of RB	PH 7.8	---	No EtBr bands visible for samples and standard
0.8% Agar agar	1	0	tap	0.05	---	PH7.8	---	Samples including positive control did not show EtBr bands.
0.8%Agar Agar	1	0	tap	0.05	MB only added to RB	PH7.7 (with and without dilute MB in RB)	---	Samples including positive control did not show EtBr bands.
1.0% Agar Agar	5	0	Distilled	0.05	---	PH 8 without MB. PH 9 with MB	Stain with 40 drops of MB in 200mL distilled water for 30 min. Destain overnight	Bands stained with EtBr visible. Stained gel too dark to detect bands. Destain O/N, but no bands clearly visible.
1.0% Agar Agar	3	0	Distilled	0.05	No MB in RB	PH 8.0	Stain O/N with agitation with 0.02% MB in distilled water. No destaining required	Clear bands present after MB staining (Repeated experiment and achieved same results)
1.0% Agar Agar	3	0	Distilled	0.05	No MB in RB	PH 8.0	Stain O/N with agitation with 0.002% MB in distilled water. No destaining required	Very faint MB bands are present. Need a darker stain solution to make bands darker (Repeated experiment and achieved same results)
1.0% Agar Agar	0	0.48	Distilled	0.05	60µL of 5% MB per liter of RB	PH7.8	Stained with 0.05% MB for 50 min. Stain was too dark. Need less concentrated staining solution.	Bright bands stained with EtBr. (Repeated experiment four times and achieved same results)
1.0% Agar Agar	0	0.48	Distilled	0.05	No MB in RB	PH7.8	Stained with 0.05% MB for 50 min. Stain was too dark. Need less concentrated staining solution.	Bright bands stained with EtBr. (Repeated experiment and achieved same results)
1.0% Agar Agar	3	0	Distilled	0.05	No MB in RB	PH8.0	Stained with 0.025%MB O/N	Bright bands stained with EtBr. Clear,

							with agitation.	distinct bands detected with MB

Note: -EtBr is Ethidium Bromide, MB is Methylene Blue, O/N is over night and RB is Running Buffer.
-Unless otherwise noted, RB was used to prepare the gel
-Solutions made with Alkaline Buffer by Seachem are buffered at pH 8.

We found 1.0% agar agar gel as the most effective solid matrix support for DNA to travel through. 0.8% agar agar gel also worked well but is very fragile and is easily damaged. Below is an additional table summarizing various combinations of gels and running buffer.

Table 4. Comparison of DNA electrophoresis in various gels.

Gel	Baking Soda (g/L)	Alkaline Buffer (g/L)	Salt (g/L)	Water	Other	PH	Comment
0.8% agar agar	0	0	0.05	Distilled water			-did not work -standard stayed in well
1.0% agar agar	0	0	0.05	Distilled water			-did not work -standard disappeared
0.8% agar agar	0	0	0.03	Distilled water			-did not work -not many bubbles present during electrophoresis -tracking dye and bands disappeared
0.8% agar agar	-	-	-	-	1X TBE		-good results with positive control and samples -bands were very tight
0.8% agar agar	0	0	0.10	Distilled water			-tracking dye turned yellow -bands not visible under UV light
0.8% agar agar	Yes	No	0.05g/L	Distilled water		8.0	-tight bands
0.8% agar agar	1.71	0	0.05g/L	Distilled water		8.0	-tight bands, good results
0.8% agar agar	9.39	0	0.05	Distilled water		8.3	-standard had a very faint band -bands from samples were not present
1.2% agar agar	Yes	0	0.05	Distilled water		6.0	-standard and sample bands were not visible -tracking dye turned yellow
1.2% agar agar	Yes	0	0.05	Distilled water		7.0	-very faint bands present for standard and samples
1.2% agar agar	Yes	0	0.05	Distilled water		8.0	-faint bands present for standard and samples
1.2% agar agar	Yes	0	0.05	Tap Water		7.0	-tracking dye turned yellow -standard and samples' bands were not visible
1.0% agar agar	Yes	0	0.05	Distilled water	-added 10 drops methylene blue	8.0	-bands were not visible

					to buffer -very thin gel		
0.8% agar agar	Yes	0	0.05	Distilled water	-added 10 drops of methylene blue to buffer -very thin gel	8.0	-bands were not visible
1.0% agar agar	Yes	0	0.05	Distilled water	-stained for 20 min then destained	8.0	-bands visible under UBC light and were very tight -staining did not work
1.0% agar agar	-	-	-	-	1X TBE	-	-excellent results -tight bands were visible under UV light from positive control and samples
1.0% agar agar	Yes	0	0.05	Distilled water	-stained for 2 hours - destained for several days	8.0	-tight bands were visible under UV light from positive control and samples -staining did not work; no bands were visible
1% agarose	-	-	-	-1X TBE			-excellent results -tight bands were visible under UV light from positive control and samples
1% agarose	Yes	0	0.05	Distilled Water		8.0	-excellent results -tight bands were visible under UV light from positive control and samples -stained bands were also visible
1% agarose	Yes	0	0.05				

Staining Solution

To visualize the DNA bands in the gel, the gel was stained in a methylene blue solution. Methylene blue consists of the salt methylene blue chloride. In water, the salt disassociates into a positively charged methylene blue ion that is colored blue and a negatively charged chloride ion, which is colorless. This blue chromophore is then able to bind to the positively charged DNA in the gel. (Reference: http://www.personal.psu.edu/faculty/k/h/khb4/enve301/301labs/Labs2_Simple_staining.html)

Methylene blue is a convenient stain to use in the lab because it is chemically safe, readily available, reusable, and detects the presence of more than 20ng DNA/band. (Reference: Molecular Research Cernter at wysiwyg://25/http://www.mrcgene.com/met-blue.htm)

Methylene blue can conveniently be found at most pet stores. It is generally sold as an aquarium disinfectant at a 5% concentration. The best gel staining results were obtained using 0.02% methylene blue in distilled water overnight at room temperature. Using this protocol, destaining with distilled water was not required. Methylene blue staining solutions at higher concentrations were found to stain the gel too dark, making it difficult to differentiate the background from the DNA bands even after destaining with distilled water. Also, if the agar agar powder was not completely dissolved in the gel, the non-dissolved flakes throughout the gel were stained dark blue, preventing the formation of distinct, visible bands. Generally, it is recommended to not add methylene blue to the running buffer or gel since it provides no clear advantage in the detection of the DNA bands. Attempts to incorporate methylene blue in the running buffer at various concentrations were only successful once, but this was found to not be reproducible. If methylene blue is added to the running buffer, it is recommended that the stain is completely homogenous in the buffer, otherwise the gel will potentially be stained nonspecifically.

A stain of corn DNA in agar agar gels stained with methylene blue are shown in Figures 1 below.

Figure 1. Corn DNA stained

A comparison of the methylene blue stain versus the ethidium bromide stain is demonstrated in Figures 2 and 3.

Figure 2 and 3: 1.0% Agarose gel stained with ethidium bromide (figure 2) and methylene blue (figure 3). Loaded 60µL of a 110µL sample made of 100µL sample and 10 µL loading buffer. Lane 1 loaded with split pea DNA, lane 2 loaded white onion DNA, yellow onion DNA, and corn DNA extracted using the basic buffer method. The gel was run at 81 volts for 45 minutes

Tracking dye

Dyes of various concentrations and composition were tested to determine its suitability as tracking dye. After every run, each band was compared to the DNA loading buffer standard's band. Two dyes produced excellent results. In particular, Club House brand's red and blue dyes produced very tight bands. Club House blue dye ran closely behind the standard. However, Club House red dye ran much further than the DNA loading buffer, possibly due to a smaller sized particle. The figure below shows the distance that each tracking dye traveled. (Show figure)

The Club House blue tracking dye was prepared from 0.5mL glycerine, 0.1mL distilled water and 10 drops of dye. To ensure good results, wipe the outside of the pipette tip with a tissue before loading the wells. This prevents the dye from nonspecifically staining parts of the gel.

We also tested other dyes. The green fabric dye was found to be not suitable because it ran towards the negative electrode; it must, therefore, be positively charged. The hot blue fabric dye separated into two bands, suggesting that this dye is made up of two differently sized molecules. In addition, the bands smeared across the gel during the run. The cold blue dye was difficult to see, even at concentrations higher than recommended. It was found to be a large molecule because it migrated the same distance as the largest fragment in the lambda Hind 3 sample.

Attempts to find a tracking dye with different brands and colours were unsuccessful. These included Club House brand yellow, green and blue food colouring. Strawberry Kool-Aid also didn't work when we used concentrations of 2mL glycerol, 2mL distilled water and 2 grams of Kool-Aid crystals. Finally, food club dyes including red, yellow, green and blue food colouring were also unsuccessful.

Findings mentioned above are summarized in the Table 5 below.

Table 5. Summary of tracking dye composition and its effectiveness.

Brand	Colour	Composition	Comments
Club House	Red	2mL glycerol 2mL distilled water 25-30 drops food colouring	Did not work
Club House	Yellow	2mL glycerol 2mL distilled water 25-30 drops food colouring	Did not work
Club House	Green	2mL glycerol 2mL distilled water 25-30 drops food colouring	Did not work
Club House	Blue	2mL glycerol 2mL distilled water 25-30 drops food colouring	Did not work
Kool-Aid	Strawberry	2mL glycerol	Did not work

	flavour	2mL distilled water 25-30 drops food colouring	
Food Club	Red	2mL glycerol 2mL distilled water 25-30 drops food colouring	Did not work
Food Club	Yellow	2mL glycerol 2mL distilled water 25-30 drops food colouring	Did not work
Food Club	Green	2mL glycerol 2mL distilled water 25-30 drops food colouring	Did not work
Food Club	Blue	2mL glycerol 2mL distilled water 25-30 drops food colouring	Did not work
Fabric Dye	Hot Blue	0.5mL glycerol 0.1-0.5mL distilled water 0.03g dye	Did not work Separated into two streaks
Club House	Red	0.5mL glycerol 0.1-0.4mL distilled water	Very tight bands Ran ahead of DNA loading buffer
Club House	Blue	1mL glycerol 0.5mL distilled water 15 drops dye	Very tight band Ran slightly behind DNA loading buffer
Club House	Blue	Unknown concentration!?	Very tight band Ran almost right beside DNA loading buffer
Fabric Dye	Cold red	0.5mL glycerine 0.1mL distilled water	Did not work

CONCLUSION