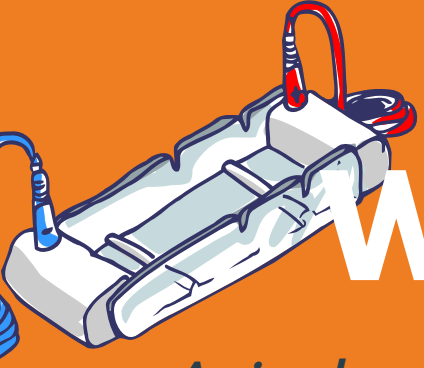


PROTOCOL
WHODUNNIT?

A simple gel electrophoresis practical using food dyes

sserc

As part of the Science benchmarks, 3rd level Outcome 14b states: "I have extracted DNA and understand its function. I can express an informed view of the risks and benefits of DNA profiling". As part of this area of the BGE curriculum, a simple gel electrophoresis to simulate the separation of DNA to generate a "genetic fingerprint" is an exciting practical to carry out. SSERC have previously published "Wizard Genes", where the powers possessed by a wizard are determined based on a "blood" sample. However, the dyes that are recommended in these protocols are no longer available, leading to disappointing results using supermarket-bought food dyes that are now more natural & less complex in their composition. This update presents the use of food dyes purchased from a high-street chain (Hobbycraft) that give good results.

TECHNICIAN - PREPARATION OF GELS

Gels can be made in advance, covered in water. Each gel will require about 16 cm^3 of molten agar.

Materials:

- balance
- weigh boat
- spatula
- 3 g agar
- 100 cm^3 boiling water
- magnetic stirrer and flea
- electrophoresis tank
- 6-well comb
- water

Method:

1. Add 3 g agar to a beaker, with a magnetic flea.
2. Add 100 cm^3 boiling water to the beaker and set the magnetic stirrer to a medium speed and high heat. Keep stirring until the agar solution goes clear.
3. Cool agar to approximately 60 °C.
4. Position a 6-well comb into the electrophoresis tank and place it on a level surface.
5. Pour the molten agae into the centre of each tank so that it flows between the teeth of the comb. The agar should be about 5 mm thick so that its surface is level with the plastic ridges which form the end channels. Avoid pouring the agar into the end channels. If agar solidifies in the end channels, this must be removed before running the electrophoresis.
6. Leave the gels to set and then remove the combs gently.
7. Cover the gels with water to stop them drying out.

TECHNICIAN - PREPARATION OF FOOD DYES

Materials:

- balance
- weigh boat
- spatula
- 24 g sucrose
- 80 cm^3 boiling water
- magnetic stirrer and flea
- 5x test tubes
- test tube rack
- 10 cm^3 syringe
- Colour Splash™ concentrated food colouring (25g packs) - blue, green, violet, yellow, red

This will make 10 cm^3 of each food dye, which will be used in various proportions to make the final samples.

Method:

1. Add 24 g sucrose to 80 cm^3 boiling water. Mix to dissolve using a magnetic flea on a magnetic stirrer.
2. Using the syringe, add 10 cm^3sucrose solution to 5 test tubes.
3. Add a food dye colour to each test tube. Each has a slightly different consistency, but add roughly 4 "drops" to each.

A NOTE ABOUT THE FOOD DYES

These Colour Splash™ food dyes were purchased from Hobbycraft (Figure 1). They can be purchased in 25 g packets, each costing £2.50. We purchased the following dyes, which contain the stated artificial colours.

Green	Tartrazine - E102 Brown - E155 Brilliant Blue - E133
Blue	Brilliant Blue - E133
Violet	Carmoisine - E122 Brilliant Blue - E133
Yellow	Tartrazine - E102
Red	Allura Red - E129

There is significant overlap in the colours present in each of the dyes. To make 4 combinations that each have a similar "green" appearance is a bit of a challenge, e.g. combining yellow and blue to achieve a green colour involves the same colours as the green food dye. The use of violet and red in these different samples is critical to achieve a range of bands during electrophoresis.

TECHNICIAN - PREPARATION OF ELECTROPHORESIS SAMPLES

Materials:

- automatic pipette and tips
- 5 microfuge tubes
- marker pen
- Dyes prepared, as above

Method:

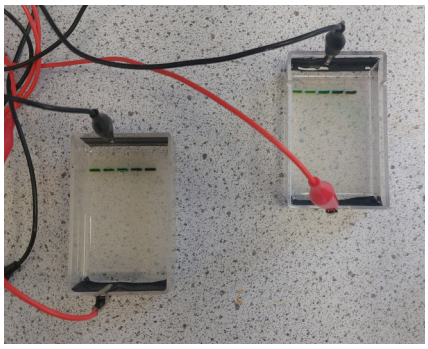
The table below shows the combination of food dyes (green, blue, violet, yellow and red) that can be used to generate 5 samples that all appear green, to be used for gel electrophoresis. These can be used in the "Wizard Gene" protocol or as a forensic-style investigation, where samples are labelled "Crime Scene", "Suspect 1", "Suspect 2", and so on. In the example shown below, sample 1 = "crime scene sample" and sample 2 = "suspect 1" are a match and pupils will suggest that Suspect 1 was present at the crime scene.

	Crime Scene Sample	Suspect 1 (match to crime scene)	Suspect 2	Suspect 3	Suspect 4
	500 µl green dye	500 µl green	300 µl yellow dye	200 µl blue dye	200 µl blue
			300 µl blue dye	200 µl yellow dye	200 µl yellow
			100 µl purple dye	50 µl red dye	50 µl red
					50 µl purple
Colours present in sample	E102, E155, E133	E102, E155, E133	E102, E155, E133 E102 E122	E102 E133, E129	E102, E155, E133 E102 E133

RUNNING THE GEL ELECTROPHORESIS PRACTICAL ACTIVITY

Materials (per pair):

- Microfuge tubes containing the 5 samples, as indicated above
- Electrophoresis tank, with 3% agar gel covered in water
- 20 cm^3 10 mM sodium hydrogencarbonate solution
- 2x carbon fibre electrodes
- Access to a 36 V transformer (shared between 4 electrophoresis gel tanks)
- Black card to place under the gel to see the wells clearly
- microsyringe and tips
- Discard container for used tips



METHOD

1. Remove the water from the prepared gel and replace with about 12 - 15 cm^3 sodium hydrogencarbonate buffer.
2. Place a piece of black card under the wells of the gel. This will make the wells more visible when you load the sample.
3. Add a fresh tip to the microsyringe and transfer 10 cm^3 of the "crime scene" sample into well 1.
4. Using a fresh tip each time, load the suspect samples into separate wells.

5 Add a carbon fibre electrode into the buffer solution at the top of the tank (within the tank channel) and to the bottom of the tank.

6 Attach the crocodile clip of the black lead from the negative terminal of the power supply to the "north" (well) end of the tank. Attach the crocodile clip of the red lead from the positive terminal of the power supply to the opposite end of the tank.

7 Run the electrophoresis at 36 V for about 20 minutes. Any longer than this can result in the dyes becoming too disperse and less intense. The gels are best run in 10 mM sodium hydrogencarbonate solution.

Figure 1: The range of food dyes available in Hobbycraft.

Figure 2: Preparing the sucrose solution for combining with food dyes. 10 cm^3 of sucrose solution is added to 5 test tubes, ready for 4 drops of food dyes to be added and mixed.

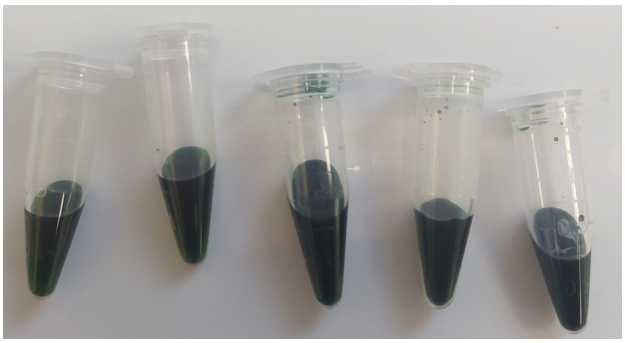


Figure 3: When prepared in the combinations suggested, all samples look very similar prior to electrophoresis.

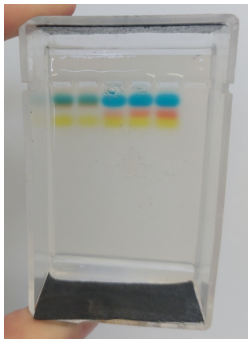
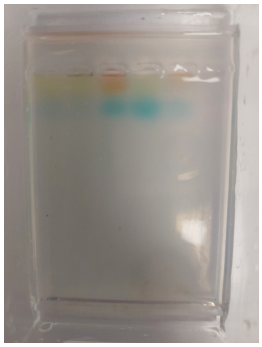


Figure 4: Electrophoresis tank set up with carbon fibre electrodes, each connected to the power supply. 10 µl of each sample are loaded into the wells. Electrophoresis should continue at 36 V for 20 minutes. The result shown on the right could be used to demonstrate that Suspect 1 (well 2) matches the "DNA" sample obtained from the crime scene (well 1). Alternatively, akin to the original "Wizard Genes" protocol, the gel results could be used to determine the powers of each wizard.



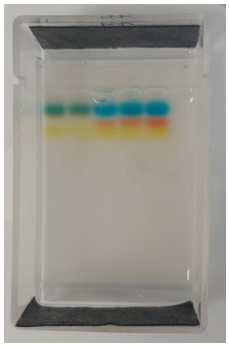
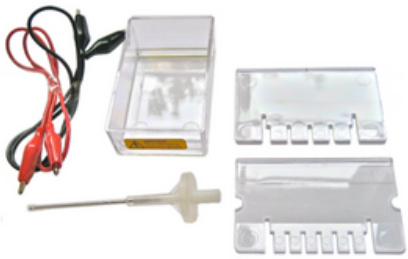


Figure 5: Comparison of Dr Oetkar Food Gels (left) and Colour Splash Food Colouring (right). For the Dr Oetkar Food Gels, the result shown was based on combinations of food dyes prepared from 10 cm^3 water, 3 g sucrose and 20 drops of each dye. For the Colour Spash result, dyes were prepared with 10 cm^3 water, 3 g sucrose and 4 drops of each dye. In both cases, gel were run in 10 mM sodium hydrogencarbonate solution.

SOURCING EQUIPMENT

Food dyes: These were purchased from Hobbycraft in the Baking section. Also available online. Each dye costs £2.50. They are also available on Amazon.

At SSERC, we use the NCBE (University of Reading) electrophoresis equipment.
Base unit: https://www.ncbe.reading.ac.uk/electrophoresis-base-unit/



The "base unit" consists of all the re-usable parts required for gel electrophoresis, except for the 36 V transformer. The "base unit" contains 8 sets of the equipment, i.e.

- 8x gel tanks
- 8x 4-toothed combs
- 8x 6-toothed combs
- 8x pairs of wires with crocodile clips
- 8x microsyringe (without tips)

The 36 V transformer - £48
The "base unit" - £67

REFERENCES

The original "Wizard Genes" protocols and Bulletin articles are available via the following links:

- Food dyes and Electrophoresis - click here
- Wizard Genes - Tech Guide (older dyes but rest of guide is still relevant) - click here
- "The Wonderful Wizardry of Finding a Gene", Bulletin Summer 2007 - click here
- Wizard Genes re-visited, Bulletin Winter 2017 - click here