Wizard Genes re-visited

Delegates have recently reported difficulties in sourcing dyes to run the SSERC "Wizard Genes" experiment, published in 2007 [1] and updated in 2011 [2]. With the general trend in replacing synthetic food dyes with more natural alternatives, most commercially available food colourings simply do not electrophorese in the same way as was the case previously.



Figure 1 - 4 different green dye mixtures following electrophoresis in a 2% agar and 1 x TBE gel and buffer.

For instance, whilst black food colourings used to be a mixture of 3 or 4 differently coloured dyes, most food dyes now only contain "vegetable carbon" which is too



Figure 2 - 4 different green dye mixtures following electrophoresis in a 2% agar and 10 mM sodium hydrogencarbonate gel and buffer.

large to pass though agar gel and becomes "stuck" in the well during electrophoresis. Similarly, green colourings which were formerly a mixture of blue and yellow artificial dyes are typically now made from a *Spirulina* concentrate.

Never to shy away from a challenge, our technicians were dispatched to supermarkets to assess food colourings from 3 major supermarkets, and these were tested alongside commercially available dyes from Timstar, and Scientific and Chemical.

Mindful of the need to ensure gels were robust but would run fully in a period of 40 minutes, we tested all dyes in 2% agar gel. For the purposes of demonstrating this experiment, NCBE (http://www.ncbe.reading.ac.uk/) gel tanks and 36 V transformer systems (with carbon electrode material)

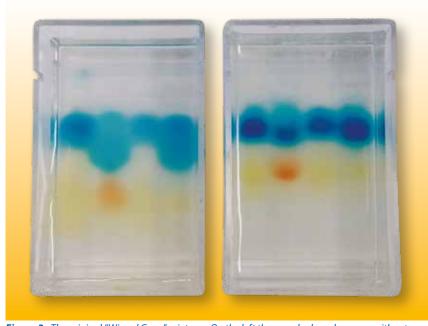


Figure 3 - The original "Wizard Gene" mixtures. On the left the samples have been run without buffer in just tap water (2% agar gel in water). The gel on the right uses a 10 mM sodium hydrogencarbonate solution as a buffer (2% agar gel in 10 mM sodium hydrogencarbonate).

Tube label	Food dyes present		
1	60 g sucrose + 0.27 g Quinoline Yellow (E104) + 0.067 g Green S (E142) made up to 100 cm³ with water.		
2	60 g sucrose $+$ 0.1 g Brilliant Blue (E133) $+$ 0.133 g Quinoline Yellow (E104) $+$ 0.033 g Allura Red (E129) made up to 100 cm^3 with water.		
3	60 g sucrose + 0.213 g Quinoline Yellow (E104) + 0.053 g Green S (E142) + 0.1 g Carmiosine (E122) made up to 100 cm³ with water.		
4	$60 \mathrm{g}$ sucrose + 0.1 g Brilliant Blue (E133) + 0.1 g Brilliant Blue (E133) + 0.133 g Quinoline Yellow (E104) + 0.033 g Green S (E142) made up to $100 \mathrm{cm}^3$ with water.		

Table 1 - Recipes to produce 100 cm³ of each "wizard blood" sample (each group requires 0.5 cm³ of each of the 4 samples in separate labelled tubes).

were used. Liquid and gel food colourings were prepared by adding 2 cm³ of the colour to a pre-prepared tube containing 2 cm³ of a 3:2 ratio of water:glycerol (to allow the sample to sink into the well). Solid colourings were prepared by adding 0.1 g of dye to 2 cm³ of a 3:2 ratio of water: glycerol. Wells were loaded with 20 µL of each dye mixture. Following the principles of the original experiment, all the food colouring mixtures suggested below start as a similar shade of green, but produce different banding patterns after electrophoresis.

In our experiments, the best results were always obtained using 1 x TBE buffer [3] solution (and gel). The presence of the buffer stops the dye bands from dispersing and gives clear, distinct results. Here is our suggestion of the best 4 dye mixtures:

- *Mixture 1* Tesco green food colouring.
- Mixture 2 Tesco yellow food colour: Tesco blue food colour: Tesco red food colour (2:1:0.5 ratio).
- *Mixture 3* Dr Oetker™ green gel.
- Mixture 4 Dr Oetker[™] green gel colour and Dr Oetker[™] purple gel colour (0.5:0.5 ratio).

As a cheaper alternative, the experiment was repeated using a 10 mM sodium hydrogencarbonate solution and gel. Although there was still a significant degree of dispersal and the bands appear "fuzzier", this still bore good results. The following 4 dye mixtures gave good results in this solution:

- *Mixture 5* Tesco green food colour.
- *Mixture 6* Timstar green powder food colour 0.1 g.
- Mixture 7 Timstar green power food colour 0.06 g and Timstar red powder food colour 0.03 g.
- *Mixture* 8 Dr Oetker™ green gel food colour.

It is still possible to purchase the original food colouring component dyes as a pack of powdered dyes from Scientific and Chemical (catalogue number EMZ 180 010, £64 for the complete set). To make up 4 green "blood" samples equivalent to the "wizard blood" samples in the original 2007 bulletin article [1], use the data in Table 1 as a quide. In this case, the sucrose



Figure 4 - An array of different colours that can be produced by electrophoresing different supermarket food colourings in agar gels using TBE buffer.

ltem	Supplier(s)	Order code	Cost (as at August 2017)
Agar	Timstar Scientific and Chemical	AG 1092 AG005	£8.39 for 100 g £7.99 for 100 g
TBE buffer	National Centre for Biotechnology Education (NCBE) Timstar	BT110100	£11.00 for 50 cm ³ 10 x concentrate (makes 500 cm ³ 1 x TBE) £21.86 to make 5 dm ³ of 1 x buffer
Powdered food colour (green)	Timstar	FC160115	£5.10 for 100 g
Powdered food colour (red)	Timstar	FC160100	£5.10 for 100 g
Food dyes set	Scientific and Chemical	EMZ 180 010	£64.00

Table 2 - Table of suppliers and cost for non-supermarket items.

solution increases sample density to allow easy loading. It is possible to run these on agar gels in just water, and this produces good results with a 4-tooth comb. As a slight improvement to the original protocol, running samples in either 10 mM sodium hydrogencarbonate solution produces really clear bands and could be better if using a 6- or 8-tooth comb (see Figure 3).

As an aside, the array of different colours that can be achieved using different supermarket dyes run on 1.5% agar gels in 1 x TBE buffer is striking. Figure 4 is a good example of several different supermarket dye mixtures. We have only included these as an interesting aside worthy of further investigation - because the mixtures are not all green, they are not suitable for the Wizard Genes practical.

References

- [1] The wonderful wizardry of finding a gene (2007), SSERC Bulletin, **221**, 2-4.
- [2] Food dyes and electrophoresis (2011), SSERC Bulletin, **237**, 2-3.
- [3] To prepare a 5 x stock solution of TBE buffer Prepare a 5 x stock solution take 54 g of Tris base and 27.5 g of boric acid and make to 1 dm³ in distilled water.



Why do I have to log in?

We have recently surveyed a large number of teachers and technicians, asking questions about our professional learning and our advisory service. The results are still being processed but we're very encouraged by what we have seen so far. We do take criticism very seriously and would like to address a comment about our website that appeared on several occasions.

Some respondents found it a nuisance to have to log in to access certain resources. The parts of the website that are passwordprotected include the health and safety advice and the most recent bulletins. SSERC is funded by local authorities as a shared service that gives advice and runs training based around effective, engaging, safe, exciting practical work. Local authorities can reasonably expect something for their money that somebody who did not fund SSERC could not access. This is why some of our material is not in the public domain. Classroom resources and bulletins over one year old are accessible to all, but if you are not logged in, the useful search facility is unavailable.

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