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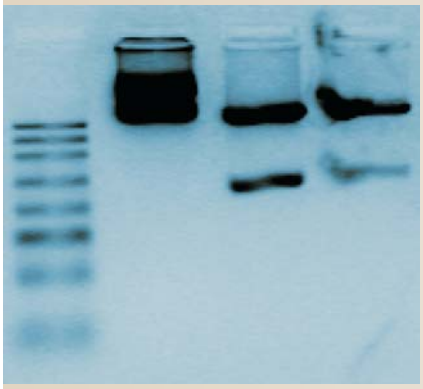

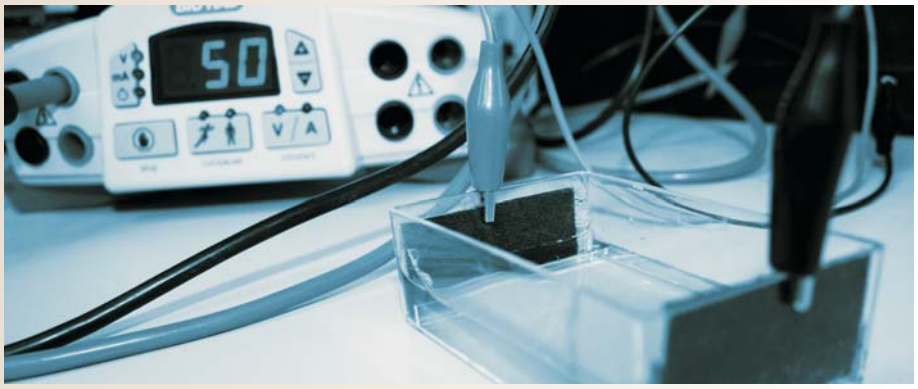

PROTOCOL FOR THE AGAR ELECTROPHORESIS GEL WITH DNA SAMPLES

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- Below you will find the complete protocol. In order to adapt it to your specific school schedules, it is advisable to prepare some steps before the class with the students (for example points 1 and/or 3)
- You can also divide this practical into different sessions for convenience and pause at the places indicated.
- The complete practical lasts between 45 min and 1h 30.
- The protocol below describes the requirements for 8 gels (16 students working in pairs), please adjust the volumes and quantities according to the size of your group and the material you have at your disposal.
- The DNA samples that are prepared in this kit are labelled A, B, C, D, E and F

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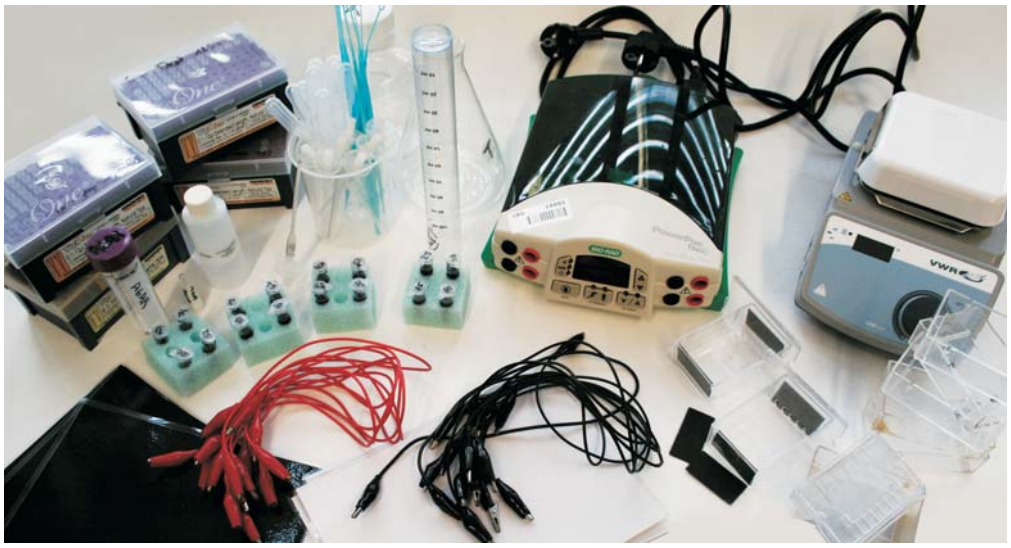

Material required for the class:

- 4 x 1g of agar
- 10 ml of TAE
- 1 Erlenmeyer of at least 100 ml
- 1 bottle/recipient of at least 100 ml
- 1 set of scales (optional)
- 1 microwave or hot plate
- Paper tissues or gloves (not provide with the kit)
- 500 ml of distilled water (not provide with the kit)

Material needed for each pair of students:

- 1 Gel chamber
- 1 comb with at least 6 wells
- 2 carbon papers of approx. 4 x 2 cm
- 2 electric cables: 1 black, 1 red
- 3 x 9 V batteries or a power supply for electrophoresis
- 1 syringe
- 6 x 10 µl tips
- 1 “quickstrip” with 5 or 6 40 µl of DNA samples ready to load (i.e., amplified by PCR, digested by restriction enzyme, with some colorant and glycerol) – from Edvotek reference 109, 130 fingerprinting or 115- gene mutation
- 1 piece of black and white paper (A5 laminated)
- 1 ruler
- 1 plastic gel staining tray
- 1 pipette

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1 Prepare the TAE 1x solution

- If you prepare the solution for the class:
  Dilute 10 ml de TAE (50x) in 490 ml of distilled water.
- If you prepare the solution for 4 students / 2 gels:
  Dilute 2 ml de TAE (50x) in 98ml of distilled water.

2 Prepare your electrophoresis box:

- Put the comb with 6 wells in the slot.
- Place your box on the black paper.


3 Prepare the agar gel at 0,8%

- If you prepare the solution for 6 gels:
  - Weigh out 1g of agar powder and
  - Mix it with 125ml of TAE (1x)
- If you prepare the solution for 4 students / 2gels
  - Weigh out 0,3g of agar powder and
  - Mix it with 42ml of TAE (1x)
- Heat it in the microwave (+/-2 minutes) or on a hotplate (+/- 20 minutes), until the solution becomes transparent.

CAREFUL! THE SOLUTION WILL BE VERY HOT, use special gloves or a tissue

- Pour the solution into the electrophoresis box, until it reaches the height of the 2 little walls.
- Wait around 5 minutes until the gel becomes solid and appears more opaque.

Stop point: you can cover your gel with TAE solution and put it in the fridge for several days

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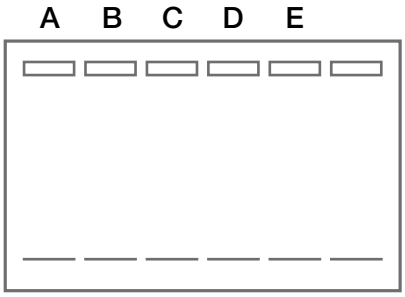
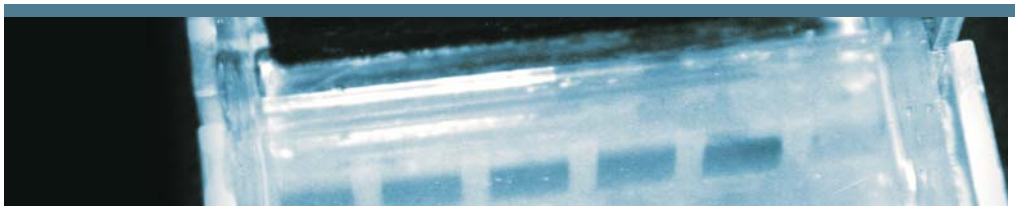
4 Diagram

- Draw a diagram of what you will run and in which order.



5 Load the samples

- Cut the gel at both ends at the level of the “little walls” using a ruler.
- Put carbon paper over each end of the box.
- Pour TAE 1x solution over the gel to cover it.
- Remove the comb slowly, and in a vertical position.
- Get the syringe and put a 10 µl tip on the end.
- Collect as much as you can of the first sample (around 40 µl).
- Put the sample in the first well (or whichever you decided on in point 4). Ensure your hands don’t shake.
- Load the other samples in the other wells. Do not forget to change your tips each time.



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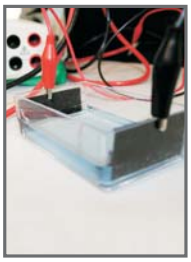
6 Run the gel

- Put the power supply at 50V.

  CAUTION! The plastic box cannot withstand more than 50 V, you must not use a higher voltage!

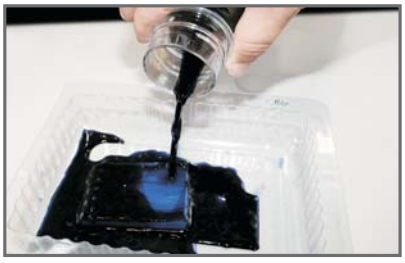
- Connect the cables, using the colour code: **Black-negative** side on the top, close to the samples. **Red-positive** side, at the bottom, far away from the samples.
- Turn on the power (run button).
- Wait approximately 20 minutes, until you see the front blue line in the middle of the gel.



7 Stain your gel

- Prepare the Flash blue stain 1x solution:

  Dilute 20 ml of Flash blue (concentration 10x in 180 ml of distilled water. (If you prepare the solution for all the class). Or Dilute 5 ml of Flash Blue (10x) on 45 ml of distilled water (if you prepare the solution for 4 students / 2 gels)
- Carefully take your gel out of the box, and put it in a plastic gel staining trail.
- Submerge your gel in Blue stain solution for 8 minutes.



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8 Destain your gel

- Remove the blue solution (you can put it back in the bottle and reuse it if needed).
- Submerge the gel under distilled water for 20 minutes, changing the water every 5 minutes.

Stop point: staining and destaining steps can be longer or shorter depending on the concentration of the solution. One option is to stain overnight in a 10times less concentrated solution of Methylene Blue

9 Reading the result

- Put your gel on a white paper so you can see the result more clearly.

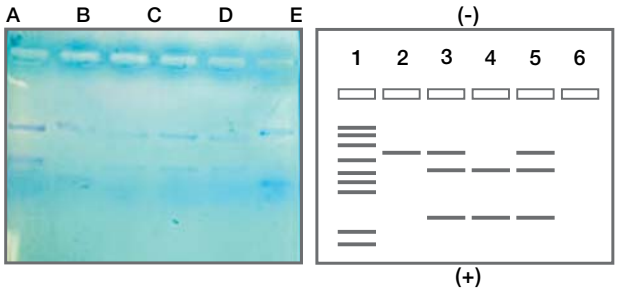
Observation

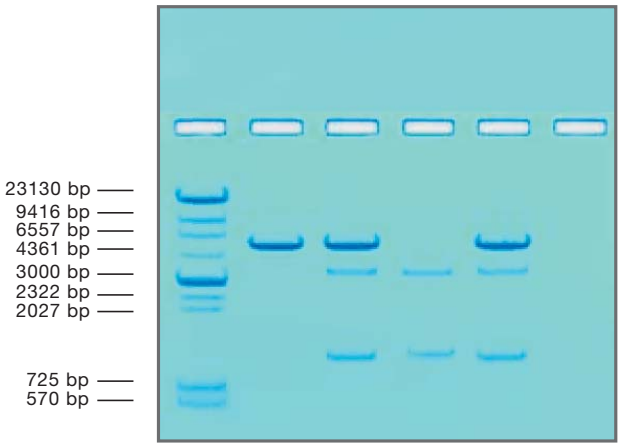
DNA is negatively charged.

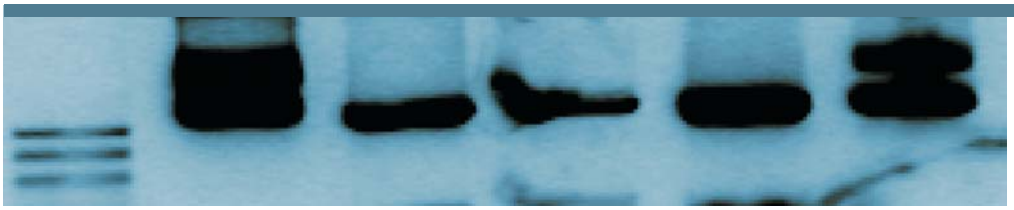
DNA sizes affects its mobility through the agar gel in the electrical field.

Longer molecules migrate more slowly because they experience greater resistance within the gel. Smaller molecules migrate faster because they experience less resistance.

The first line, A, is Standard DNA Fragments or DNA ladder, the references to which you can compare your results.







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Conclusion

Electrophoresis is a technique that allows us to separate DNA samples by size.

We can see that some of the lengths of DNA strands are different. Some of the samples have DNA strands with 1 different length while others have 2 or 3.

If we compare the migration of the sample with the first line or DNA ladder references (ref130-115), we can "measure" the DNA strands.

The Methylene blue binds to DNA molecules and allows us to see the DNA.

Ref 109: samples A and B have the same digested pattern as E and F; the DNA from Suspect 2 is similar to the Crime Scene DNA.

Ref 130: sample B has the same pattern as the Crime Scene. The DNA amplified by PCR from Suspect 2 and the Crime scene are similar for these DNA regions and specific primers.

Ref 115: Patient 1 (B) is homozygote and does not have the mutated gene on both alleles.

Patients 2 and 4 (C and E): are heterozygotes and have 1 mutated allele.

Patient 3: is homozygote and has both alleles mutated.

Discussion

1. What is the TAE solution? What does it contain?.

2. What special properties does agar gel have?.

3. What do you think would happen if you used a gel with 2% agar? Would the samples will move through more easily or not? What do you think it might be useful for?.

4. What is the Methylene blue used for? Can you list the advantages and disadvantages of is use, comparing it to the use of Ethilium Bromide and SYBR Green?.

5. The DNA samples are prepared by a biological supply company. In order to prepare them, they use different techniques. Could you briefly explain them?.
   a. DNA extraction.
   b. PCR.
   c. Digestion by restriction enzymes.

6. Blue colours and glycerol are finally added to the DNA samples in order to load them in the gel.
   Could you explain why?

7. These DNA samples are not human DNA, they are plasmids prepared specially for educational use. Plasmids are used a lot in biotechnology: find out one or more of their applications.

References

Carolina.com: references 211026: discover electrophoresis kit
Edvotek.co.uk: references 109, 130 and 115