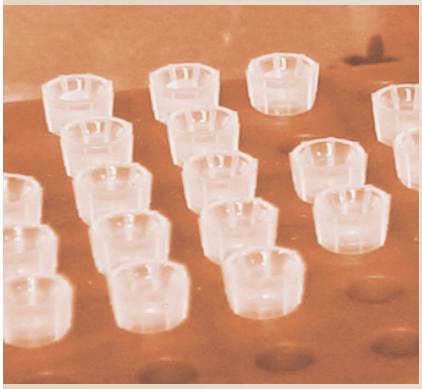
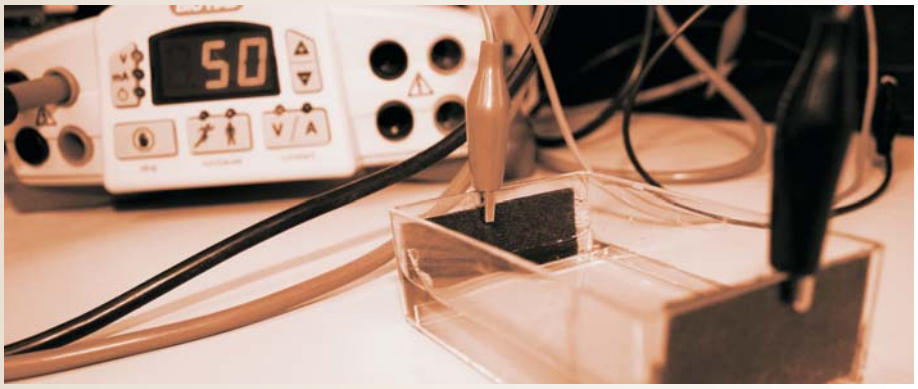
CRG
Centre de Regulació Genòmica





PROTOCOL FOR AGAR ELECTROPHORESIS USING FOOD COLORS SAMPLES

FECYT
Generalitat de Catalunya
Departament d'Economia i Coneixement
Secretaria d'Universitats i Recerca
Ajuntament de Barcelona
Institut de Cultura
CONSELL DE COORDINACIÓ PEDAGÒGICA BARCELONA

Centre de Regulació Genòmica

PROTOCOL FOR AGAR ELECTROPHORESIS USING FOOD COLORS SAMPLES

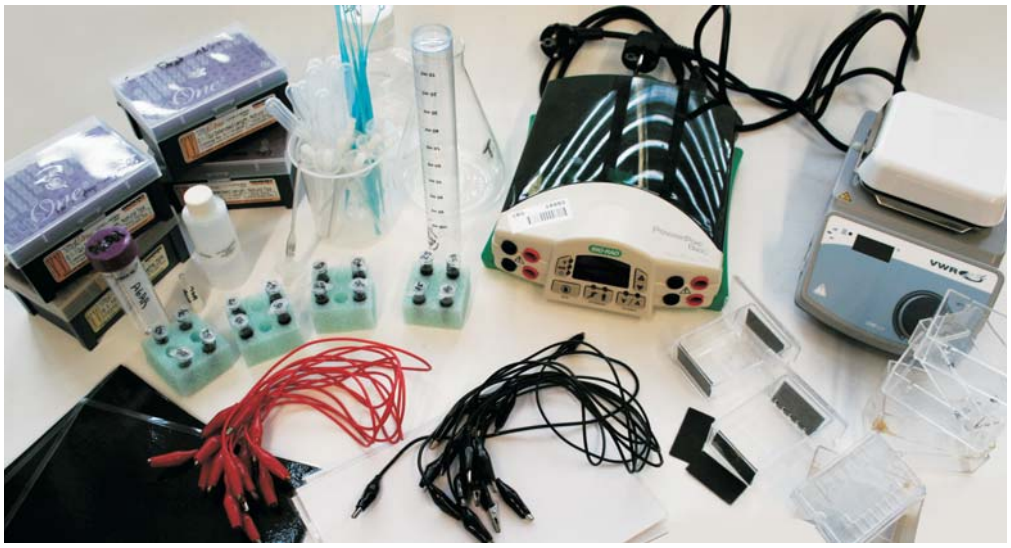
- Here you will find the complete protocol. In order to adapt it to your specific school schedules, it is advisable to prepare some steps before the class with the students (for example point 1 and/or 3)
- You can also divide this practical into different sessions for convenience and pause at the places 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 4 samples that are prepared in this kit are labelled as follows:

EC Crime Scene S1 Suspect 1, S2, and S3
A possible scenario would be to compare the DNA from the crime scene to the DNA of 3 suspects.
But you could also imagine that you compare the DNA from a patient who has a mutation on a specific gene and has developed leukaemia, to the DNA of other family members to see if they have the same mutation.

Centre de Regulació Genòmica

PROTOCOL FOR AGAR ELECTROPHORESIS USING FOOD COLORS SAMPLES



Material required for the class:

- 4 x 1 g of agar
- 10 ml of TAE 50x
- 1 Erlenmeyer of at least 100 ml
- 1 bottle/recipient of at least 100 ml
- 1 scale (not provide with the kit
- 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 4 wells
- 2 carbon papers aprox. 4 x 2 cm
- 2 electric cables: 1 black, 1 red
- 3 x 9 V batteries or a power supply for electrophoresis
- 1 syringe
- 4 x 10 ul tips
- 4 Eppendorf tubes with samples
- 1 piece of black and white paper (A5 laminated)
- 1 pipette
- 1 inoculation loop

Centre de Regulació Genòmica

PROTOCOL FOR AGAR ELECTROPHORESIS USING FOOD COLORS SAMPLES

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.

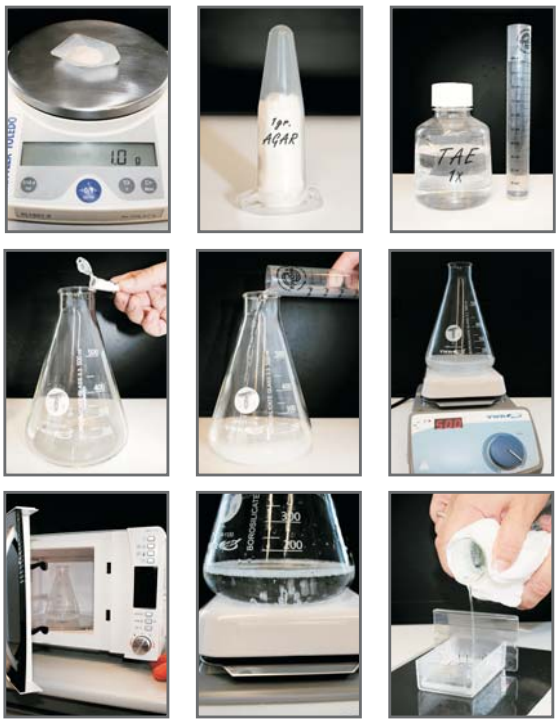

3 Prepare the agar gel at 1%

- If you prepare the solution for 6 gels:
  - Weigh out 0.3g of agar powder and
  - Mix it with 30ml of TAE (1x)
- If you prepare the solution for 4 students / 2gels
  - Weigh out 1g of agar powder and
  - Mix it with 100 ml 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.



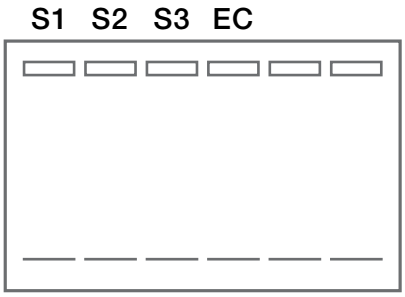
Stop point: you can cover your gel with TAE solution and put it in the fridge for days

Centre de Regulació Genòmica

PROTOCOL FOR AGAR ELECTROPHORESIS USING FOOD COLORS SAMPLES

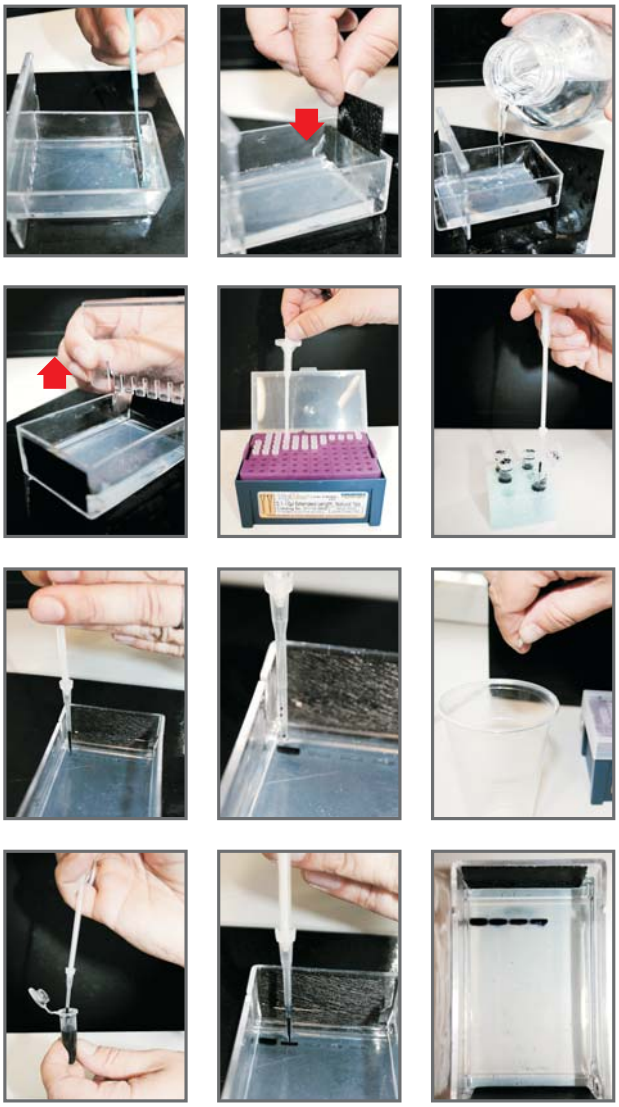
4 Diagram

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



5 Load the samples

- Cut off the gel that has gone over the little wells using an inoculation loop.
- 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
- Take out approximately 10 µl of the first solution (to the second line marked on the tip)
- Put the sample in the first well (or as was decided in point 4). Ensure your hand doesn't shake.
- Load the other samples into the other wells. Do not forget to change your tip each time

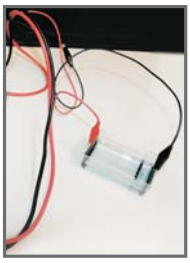


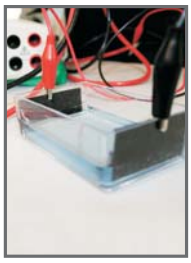
Centre de Regulació Genòmica

PROTOCOL FOR AGAR ELECTROPHORESIS USING FOOD COLORS SAMPLES

6 Run the gel

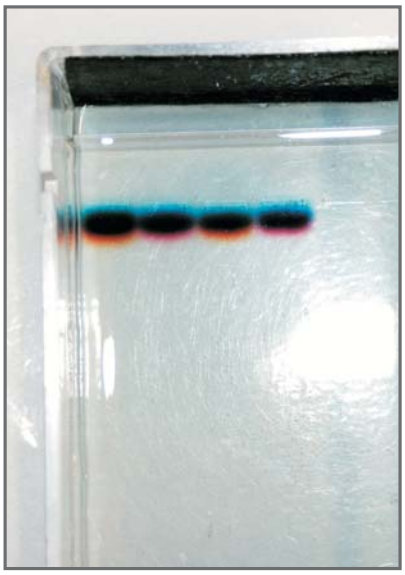
- Put the power supply at 50V
- Connect the cables, using the colour code:
  Black-negative side on the top, close to the samples
  Red-positive side, at the bottom, far away from the samples
- Turn on the power (run button)
- Wait approximately 15 minutes, until different bands appear





7 Result

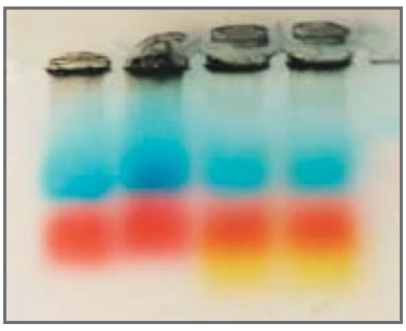
- Place your box on white paper so you can see the result more clearly.



Observation

The samples were composed of different colours, each colour moves at a different specific speed in the gel, attracted by the positive pole.

S1 S2 S3 EC



The samples of the pictures are not exactly in the same order that the one in the kit.

Conclusion

Electrophoresis is a technique that allows us to separate components by size.
We can see that some of the samples have 2 components and others have 3.

- The yellow component is the smallest and/or is the most negatively charged as it moves the furthest through the gel towards the positive anode.
- The blue component is the largest and/or has the least negative charge as it moves the least through the gel.

Sample ____ has the same components as the Crime Scene (EC) sample.

CRG Centre de Regulació Genòmica

PROTOCOL FOR AGAR ELECTROPHORESIS USING FOOD COLORS SAMPLES

Discussion: very challenging

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 move through more easily or not? What do you think it might be useful for?

4. In this experiment we model DNA fingerprinting with colours. What is DNA fingerprinting?

5. How do scientists extract your DNA? How do they make millions copies of it and cut it into fragments?

Discussion challenging:

1. The TAE Solution contains distilled water and salt. Why do we use a salty solution? Think of the electrical properties of salts.

2. List all the components that are conducting electricity, and that you used during this experiment.

3. A research is looking for two large bands of DNA. He runs a gel in a 1.2% agar gel. He finds a unique large band of DNA at the top of its gel. So he decides to run the same DNA sample in a agar gel at 0.7%. In this new gel, he observes two separated bands. Why can he see two bands of DNA in the 0.7% gel and not in the 1.2%?

Do it yourself!

If you want to construct your own DNA electrophoresis chamber you can find very simple ideas on the web, or ask us for some. You can easily switch the agar powder for the agar flakes use in Asian cooking. You can also exchange the TAE solution for water and salt solution. The colour samples are prepared with food colouring and droplets of glycerol and are easy to prepare at home.

References

- Carolina.com: references 211026: discover electrophoresis kit
- www.learn.genetics.utah.edu