Paternity test with the blueGel™ electrophoresis chamber

This experiment builds a bridge between classical genetics with its Mendelian rules and the concept of alleles on the one hand and molecular genetics on the other. It deals with the genetic method of paternity analysis as it is used today. In this experiment, we help a pack of young dogs in their search for their father.

Biology > Microbiology & genetics > Classical genetics

Biology > Microbiology & genetics > Molecular Genetics

Difficulty level	Group size	Preparation time	Execution time
medium	2	30 minutes	40 minutes

This content can also be found online at:

https://www.curriculab.de/c/686683cf4fa225000266f3ff

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Teacher information

Application

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Gos d'Atura (Catalan herding dog)

A healthy dog usually has 78 chromosomes (39 pairs of chromosomes). With the exception of the sex chromosomes, each pair carries the same genetic information. Nevertheless, within the genes there are often small differences - so-called alleles -, which can be inherited either in a dominant or recessive way. Such genetic variations lead to heterozygous genotypes and make out the individuality of each dog. Differences of this kind can be used in paternity tests.

In a paternity test, the DNA of the puppy is compared with the DNA of the putative father. An important method for visualising differences in the DNA is gel electrophoresis, which is the perfect lab tool for such paternity tests.

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Other teacher information (1/4)

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Prior knowledge

Students should already be familiar with the properties of DNA know the the terms chromosome, genome, genotype, phenotype and allele and be able to apply them. Furthermore, they should already know how polymerase chain reaction (PCR) works and be familiar with the principle of gel electrophoresis. The students should also practise pipetting micro-quantities in advance.

Principle

The DNA samples from male dog (possible father) and puppies (offspring) are applied to an agarose gel and separated using gel electrophoresis. The band patterns are compared with each other to determine whether the puppy carries the same genetic traits. If the SeeGreen™ 3-in-1 agarose tablets are used, the SYBR-Green fluorescent dye contained in the tablets intercalates with the DNA. The dye is stimulated by the special light of the blueGel™ gel chamber, causing the DNA to become visible.

Other teacher information (2/4)

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Learning objective

The aim of this experiment is to familiarise students with how gel electrophoresis works. DNA is separated according to size and visualised using this method.

Tasks

The students prepare an agarose gel of the specified concentration and apply the various DNA samples. The separation can be observed live and documented independently using the camera on a smartphone or tablet. The learners can then determine whether the individual puppies carry the genetic material of the male dog.

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Other teacher information (3/4)

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blueGel™ electrophoresis device with dark hood and live image on mobile phone

Notes on sample preparation and performing the experiment

- The video on the left shows how to prepare a gel, the assembly of the system and how to perform DNA separation.
- When using SeeGreen™ tablets, please note that the tablet already contains TBE salt. No additional salt should be added. Only the amount of deionised water specified in the leaflet must be added to the tablet.
- The running buffer must also be a TBE buffer and should have a concentration of 1x. We recommend that teaching staff dilute the buffer in advance and distribute aliquots to the students.

Other teacher information (4/4)

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Test variants and notes

- We recommend using a microwave oven to boil the gel, but a hotplate can also be used.
- The experiment can also be carried out with gels of individual components (agarose, TBE or TAE) and the DNA bands stained with a methylene blue solution. However, it is not possible to observe the separation in real time in this case.
- As pipetting very small quantities is not easy, it is recommended that this procedure is practised with the students in advance.
- If you prepare the DNA samples more than 24 hours before electrophoresis, store the sample tubes in the refrigerator.

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Safety instructions

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- The general instructions for safe experimentation in science courses apply to this experiment.
- Please refer to the safety data sheets of the respective chemicals before using them.
- The SYBR-Green used in the SeeGreen™ tablets is a safe alternative to conventional ethidium bromide. It cannot penetrate the skin, but can penetrate the tissue through open wounds. The use of nitrile gloves is therefore recommended.
- Agarose gels containing the contents of SeeGeen tablets™ can be disposed of in the regular household waste.

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Student information

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Motivation / Theory (1/4)

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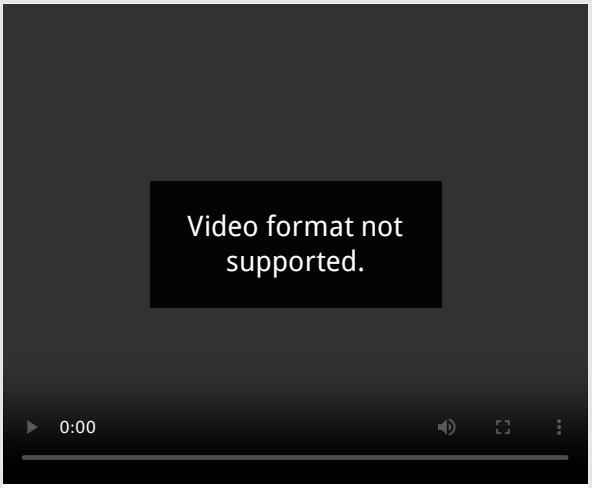

Young pack of dogs (Gos d'Atura, Catalan herding dog)

Molly, the Labradoodle, has surprised you with a litter of puppies! - But who is the father? Molecular biological methods such as polymerase chain reaction (PCR) and agarose gel electrophoresis can provide a clear answer to these questions.

The **DNA contains genes** which serve as blueprints for proteins and provide features such as how a dog looks like. The g**enotype** describes the genetic make-up, the **phenotype** the visible characteristics, which can also be influenced by environmental factors. In the experiment, the **coat structure on the face** ("furnishings") analysed by the **RSPO2 gene** is investigated. There are two alleles: a dominant allele (F), which leads to the development of the coat structure, and a recessive allele (f), which does not lead to any specific coat structure characteristics.

Theory (2/4)

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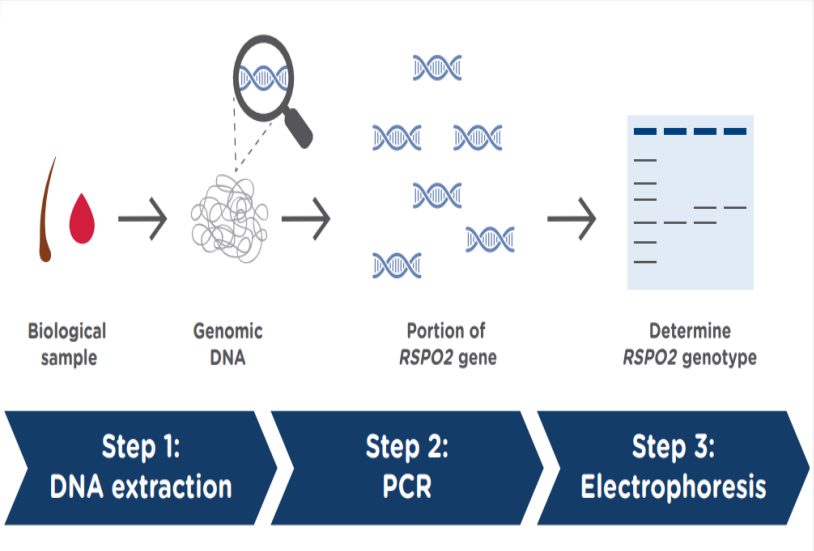

To determine the **genotype** of a dog, it is often not enough to simply observe the dog's looks. Therefore a **genetic test** is carried out. First, DNA is extracted from a biological sample (e.g. hair or blood). This DNA is amplified using **PCR (polymerase chain reaction)**. This is followed by an analysis using **gel electrophoresis**. with which DNA fragments are analysed according to their **length**. As the two alleles of the RSPO2 gene differ in length, it is possible to determine which alleles a dog carries.

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Theory (3/4)

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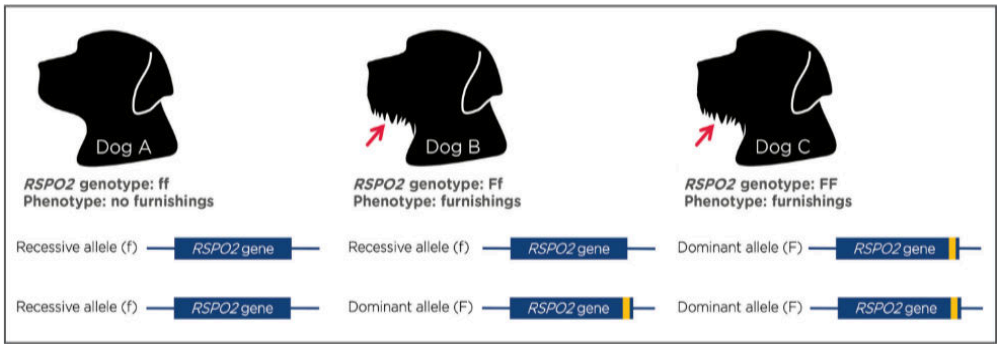

The RSPO2 gene determines the coat structure (furnishings) in dogs and has two variants (alleles): a dominant allele (F) with an insertion of 167 base pairs, which leads to the development of a special coat structure, and a recessive allele (f) without this insertion, which leads to the absence of the special coat structure. Dogs inherit one allele from each parent. Just one copy of the dominant allele (F) is sufficient for the coat structure to develop. Only dogs with two recessive alleles (ff) show no coat structure.

Theory (4/4)

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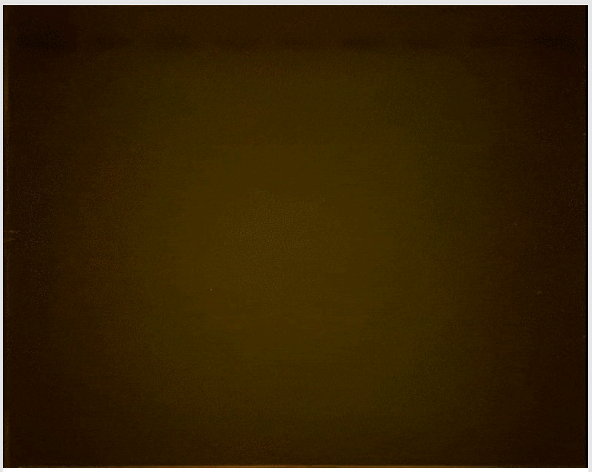

DNA bands of the dog samples in the agarose gel

Gel electrophoresis is an important procedure to make **DNA fragments** visible and **according to their size.** DNA molecules are **negatively charged** because they contain phosphate groups. When an electric field is applied, **they migrate through a gel in the direction of the positive pole (anode). Smaller fragments move faster and further** than larger ones, resulting in a typical b**and pattern**. DNA is dyed so that the bands become visible. Each band represents a piece of DNA of a certain size. Through the **comparison of band patterns** of various samples, for example, it is possible to determine whether a puppy is related to a particular dog.

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Tasks

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1. Pour a 2% agarose gel: note that the agarose tablet not only contains agarose, but also the fluorescent dye and TBE salt, so only deionised water needs to be added.
2. Load the DNA samples into the gel pockets and start electrophoresis.
3. Compare the banding patterns and try to determine whether the genotype of the puppies match that of the putative father.

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Equipment

Position	Equipment	Item no.	Quantity
1	blueGel™ gel electrophoresis chamber with power supply and built-in exposure unit	35017-99	1
2	Paternity analysis via DNA profiles, electrophoresis	15312-04	1
3	SeeGreen™ Agarose Tabs™, 3-in-1 agarose tablets, for gel electrophoresis, for 16 gels	35018-71	1
4	Microlitre pipette 2-20 µl, autoclavable	47141-10	1
5	Tips, plastic (PP) 2-200 µl, 1000 pieces	47148-01	1
6	Erlenmeyer flask, boro, wide neck, 100 ml	46151-00	1
7	Graduated cylinder, boro, tall form, 100 ml	36629-00	1
8	PCR single tubes, 0.2 ml, 100 pieces, in a bag	35928-01	1
9	Stand for 8 x 0.2 ml disposable reaction tubes	37652-01	1
10	TBE electrophoresis buffer, 1000 ml, 10-fold conc. solution	35018-73	1
11	Water, distilled, 5 litres	31246-81	1
12	Safety goggles "classic" - OneSize, unisex	39316-00	1
13	Gloves, rubber, size M, pair	39323-00	1
14	Graduated pipette, 25 ml, graduation 0.1 ml	36602-00	1
15	Pipette ball, flip model, pipettes up to 100 ml	36592-00	1

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Setup (1/2)

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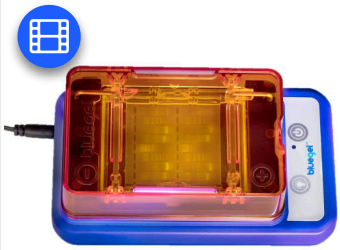

Video: Pouring a gel

- Dilute the 10x concentrated TBE running buffer concentrate to 1x with deionised water (approx. 30 ml buffer is required for one gel).
- Pour a 2% agarose gel (see also video). A 2% agarose gel is somewhat more difficult to cast, but is better suited for separating smaller DNA fragments.
- Remove one SeeGreen™ agarose tab from the pack and add it to the Erlenmeyer flask.
- Add 40 ml of deionised water to the tablet and boil both (microwave or hotplate). Do not exceed 60°C!
- Insert a gel tray into the casting platform and a comb into the gel tray (the comb is located underneath the casting platform).

Setup (2/2)

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Finished gel, ready for loading

- Now pour 20 ml of the liquid gel into the tray and allow the gel to harden (about 10 minutes). This means that two gels can be created with 40 ml of the liquid gel.
- Once the gel has cooled, carefully pull the comb vertically out of the gel.
- Now place the gel tray in the buffer chamber of the base unit.
- Add sufficient 1x TBE running buffer to the buffer chamber so that the gel is covered. Not too much, otherwise the electrophoresis run will take longer.

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Procedure (1/3)

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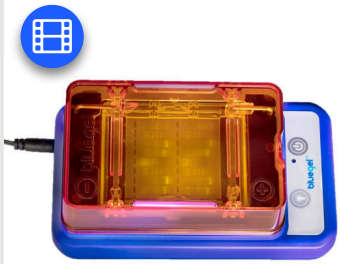

Video: Load and run the gel

Loading and running the gel (see also video):

- Set the 2 µl - 20 µl pipette to 15 µl and place a yellow pipette tip on the pipette.
- Load the DNA samples onto the gel in the following order: (see next slide). Please note that a new pipette tip must be used for each DNA sample to avoid cross-contamination of the samples.
- Now use the pipette to aspirate 15 µl of a sample into a tip and carefully transfer the contents into a pocket.

Procedure (2/3)

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DNA samples

Load the samples onto the gel in the following order:

- Tube L: 15 µl DNA ladder 1 ➔ Lane 1
- Tube A: 15µl DNA from Astro ➔ Lane 2
- Tube B: 15 µl DNA from Buster ➔ Lane 3
- Tube C: 15 µl DNA from Chewy ➔ Lane 4
- Tube D: 15 µl DNA from Daisy ➔ Lane 5
- Tube E: 15 µl DNA from Elsa ➔ Lane 6
- Tube F: 15 µl DNA from Flora ➔ Lane 7
- Tube G: 15 µl DNA from Ginger ➔ Lane 8

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Procedure (3/3)

Gel chamber with dark hood and smartphone

Running the gel

- Place the transparent cover on the base unit and start the run by pressing the "On/Off" button.
- Carefully place the dark hood over the lid (see also the photo on the left).
- Press the "Light" button to activate the blue light and you can now follow the separation of the samples. With the help of a smartphone or tablet, you can also film or photograph the separation.
- The separation is completed after about 20 minutes.

Report

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Task 1

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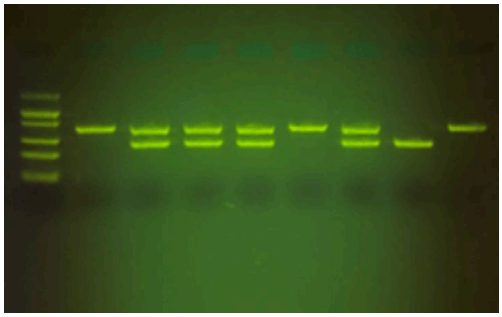
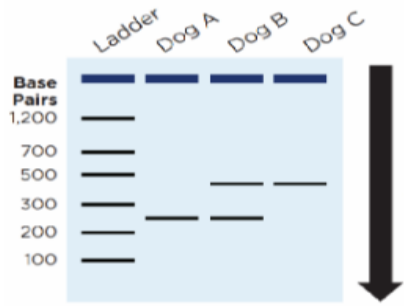
The dominant allele contains an **insertion of 167 base pairs**. Therefore the PCR product for **(f)** is approx. 250 bp long and for **(F)** approx. 400 bp long. The bands in the gel then show the genotype: a band at 250 bp means **(ff)**, one at 400 bp means **(FF)** and two bands (250 and 400 bp) mean (**Ff**). This means that the gel image can be used to show the **genotype** as well as the **phenotype** of a dog. Compare your results with this information.



DNA bands in the agarose gel



Task 2

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What happens during gel electrophoresis?

- ○ The higher the agarose concentration, the worse the current is conducted through the chamber.
- ○ Separation of DNA fragments according to their charge.
- ○ Separation of DNA fragments according to their size.
- ○ DNA fragments become visible without dye.

Check

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Task 3

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What is a genotype?

- ○ The behaviour of an animal in everyday life
- ○ The visible characteristics of an animal, for example coat colour or eye shape
- ○ The genetic make-up of an animal - i.e. which genes or alleles it possesses
- ○ The environmental conditions in which an animal lives

Check

Task 4

PHYWE

Which gene determines whether a dog has this particular coat structure?

- ○ RSPO2 gene
- ○ MC1R gene
- ○ OCA2 gene
- ○ MYO5A gene

Check

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Slide	Score / Total
Slide 22: Principle of gel electrophoresis	0/1
Slide 23: Untitled: Multiple Choice	0/1
Slide 24: Untitled: Multiple Choice	0/1

Total amount 0 / 3

Solutions Repeat

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