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Supplemental Materials

for

Simply InGEN[E]ious! How Creative DNA Modeling Can Enrich Classic Hands-On Experimentation

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Appendix 1: Evaluation instrument (multiple-choice questionnaire)

Cognitive knowledge questionnaire (adapted and extended from [Authors] 2016). Questions were split up into subcategories evaluating the modeling phase (M) or the laboratory activities (L).

Note. Correct answers are written in *italics*.

L1	**In an electric field positive charged particles migrate …**
☐	back and forth between both poles.
☐	to the positive pole.
☐	*to the negative pole.*
☐	not at all.

M2	**Which of the following components is <u>not</u> included in the DNA?**
☐	adenine
☐	*ribose*
☐	guanine
☐	deoxyribose

L3	**Which option is wrong? The migration rate of electrically charged molecules depends on …**
☐	the applied voltage.
☐	*the density of the sample.*
☐	the density of the agarose gel.
☐	the size of the molecules.

M4	**In 1962, James Watson and Francis Crick received the Nobel Prize for Medicine for the Discovery of …**
☐	DNA is located in the nucleus.
☐	components that form the DNA.
☐	gel electrophoresis.
☐	*the double helix structure of the DNA.*

M5	**Which option is wrong? The DNA of human is…**
☐	carrier of genetic information.
☐	a long chain molecule.
☐	*built of amino acids.*
☐	a super molecule.

L6	**With the aid of gel electrophoresis, you get information about …**
☐	*the molecular mass.*
☐	the number of bindings of a molecule.
☐	the components of a molecule.
☐	the atoms of a molecule.

M7	**Which base pairing is correct?**
☐	*Guanine pairs with cytosine.*
☐	Thymine pairs with cytosine.
☐	Adenine pairs with guanine.
☐	Cytosine pairs with adenine.

L8	**To add 20 µl to your sample, you use …**
☐	a Pasteur pipette.
☐	a graduated pipette.
☐	*a micropipette.*
☐	a measuring cylinder.

L9	**With the help of a centrifuge …**
☐	you can mix a sample.
☐	the molecules are set into motion.
☐	single molecules can be isolated.
☐	*solid substances can be separated from liquids.*

M10	**If you speak of 'the DNA backbone', you mean …**
☐	the annular structure of the DNA.
☐	the fatty acids bound to protect the DNA.
☐	the DNA-base pairings.
☐	*the alternating phosphate-sugar-chain as part of the DNA.*

M11	**The DNA bases are located …**
☐	*at the interior of the DNA molecule and are linked with sugar.*
☐	at the exterior of the DNA molecule and are linked with sugar.
☐	at the exterior of the DNA molecule and are linked with phosphate.
☐	at the interior of the DNA molecule and are linked with phosphate.

M12	**Matching DNA strands are …**
☐	shifted arranged.
☐	identical.
☐	independent of each other.
☐	*antiparallel.*

L13	**The electrophoretic separation of DNA-molecules is based on the DNA-component …**
☐	thymine
☐	*phosphate*
☐	sugar
☐	cytosine

L14	**Which option is wrong? In cold alcohol the DNA is …**
☐	insoluble.
☐	a filamentous structure.
☐	a white solid.
☐	*soluble.*

M15	**The molecular structure of DNA can best be compared with …**
☐	a cardboard tube.
☐	*a twisted rope ladder.*
☐	railway tracks.
☐	a twine.

L16	**A DNA size standard helps to …**
☐	*estimate the length of DNA fragments*
☐	extend DNA sequences.
☐	repair DNA fragments.
☐	stain DNA strands.

M17	**The abbreviation 'DNA' stands for …**
☐	Deoxynucleic acid.
☐	Oxyribonucleic acid.
☐	*Deoxyribonucleic acid.*
☐	Dideoxyribonucleic acid.

M18	**How many different repeating DNA-components exist?**
☐	2
☐	4
☐	*6*
☐	8

M19	**Give the opposite bases to the base sequence: AATGGG** *(Capital letters = initial letter of the base, e.g. 'A' for adenine)*
☐	TTGCCC
☐	*TTACCC*
☐	TTGAAA
☐	GGACCC

M20	**The cohesion of the two DNA-strands is based on the formation of …**
☐	atomic bonds.
☐	*hydrogen bonds.*
☐	disulfide bridges.
☐	ionic interactions.

L21	**The total length of human DNA per cell is about …**
☐	200 meters.
☐	*2 meters.*
☐	20 meters.
☐	2 centimeters.

M22	**The genetic information is encoded in the DNA by …**
☐	*the sequence of the single bases.*
☐	the formation of different chromosomes.
☐	the fusion of egg and sperm cells during fertilization.
☐	the turn of the DNA strand.

M23	**A section of DNA that provides the basic information for building a particular characteristic is called …**
☐	plasmid.
☐	genome.
☐	chromosome.
☐	*gene.*

L24	**The DNA is the carrier of genetic information …**
☐	*in all organisms.*
☐	in apes.
☐	in all organisms except bacteria.
☐	in vertebrates.

L25	**In which cell organelle is the DNA located?**
☐	in the ribosome
☐	*in the nucleus*
☐	in the cytoplasm
☐	in the vacuole

M26	**The analysis of a DNA-section revealed a proportion of guanine with 30 %. Following, the proportion of adenine is …**
☐	is not determinable.
☐	also 30%.
☐	70%.
☐	*20%.*

M27	**The proportion of sugar to phosphate in the DNA-molecule is …**
☐	2:1
☐	3:1
☐	*1:1*
☐	1:2

M28	**The DNA consist of the following chemical elements:**
☐	hydrogen, sulfur, phosphorus, carbon and nitrogen
☐	hydrogen, oxygen, phosphorus, sulfur and nitrogen
☐	*hydrogen, oxygen, phosphorus, carbon and nitrogen*
☐	hydrogen, oxygen, sulfur, carbon and nitrogen

M29	**The spatial structure of DNA …**
☐	is a left-handed double-helix.
☐	*is a right-handed double-helix.*
☐	has no direction of rotation.
☐	is an alternating right- and left-handed double-helix.

L30	**Which option is wrong? Visualizing DNA-molecules in gel electrophoresis is made possible by …**
☐	*the blue-colored loading buffer.*
☐	a molecular dye, that binds on the DNA.
☐	a dye, that glows under UV light.
☐	the addition of dye into the agarose gel.

A STEAM outreach lab module

SIMPLY INGEN(E)IOUS!

DNA AS A CARRIER OF GENETIC INFORMATION

Workbook

A practical training course for 9th graders

Group No.:

GLOSSARY

of essential working tools

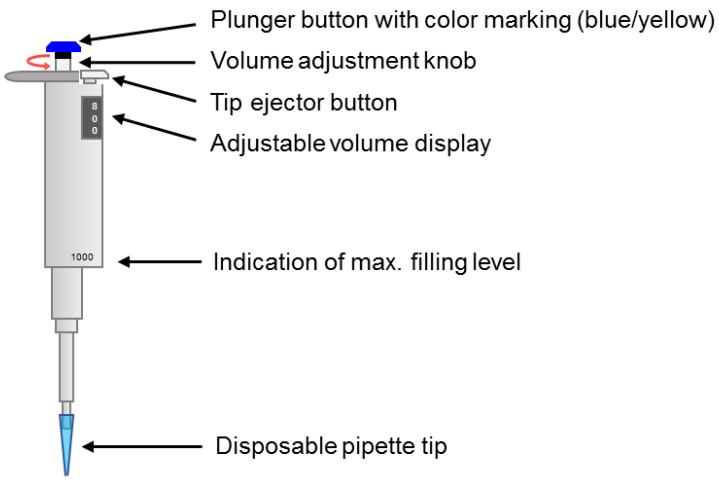
beaker	
centrifuge	
Eppendorf tubes	
Erlenmeyer flask	
graduated pipette	
micropipette	
Pasteur pipette	
pipette tips	 stored in 
pipette pump	
snap-cap vial	

Working with Micropipettes

Using **micropipettes** is one of the most important working techniques in gene technology labs. You need to understand the handling of such instruments for several experimentation steps we will do today. Named after a manufacturer, micropipettes are also called 'Eppendorf pipettes'.

With these special laboratory instruments, it is possible to pipette very low liquid volumes of 2 µl (≈0,0000676 floz) to 1000 µl (≈0,0338 floz), depending on the selected micropipette and the previously adjusted volume.

Elements of a micropipette:

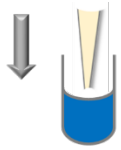
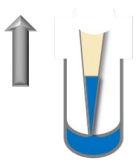
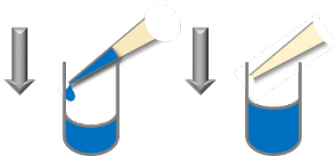


Please note:

Micropipettes are very expensive, sensitive instruments and must be handled carefully!

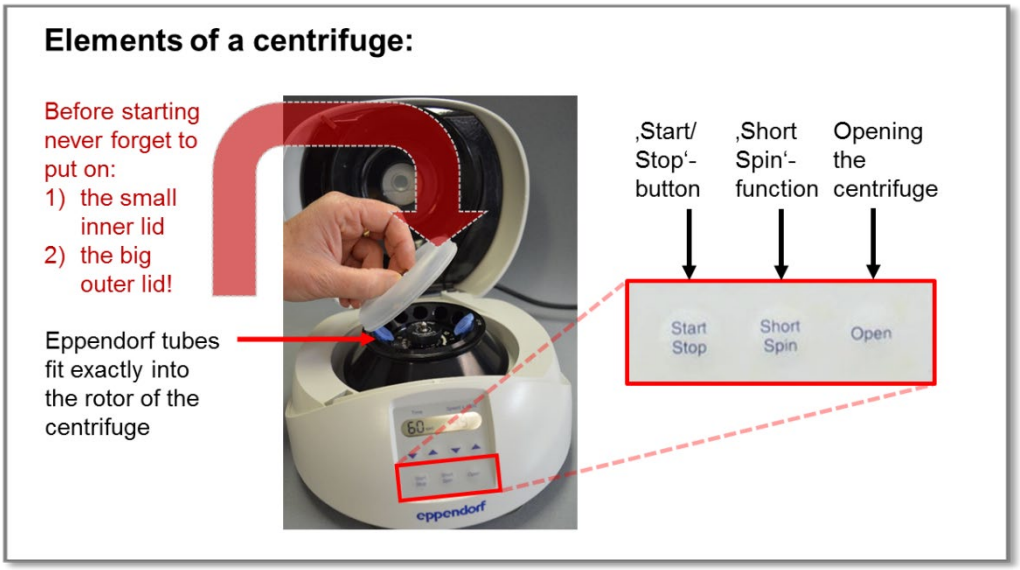
- **Micropipettes may only be used with a pipette tip attached!**
- **Always keep the tip of the pipettes downwards!**
- **Never use the same pipette tip for different substances!**
- **Discard used pipette tips into appropriate waste jar!**

How to handle a micropipette:

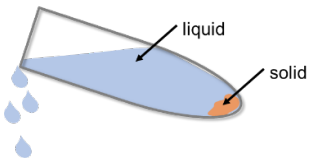
1		Turn the **volume adjustment knob** to select the desired amount of liquid. The volume is read in µl (said **'microliter'**) from top to bottom. **1000 µl ≈ 0.0338 floz** *e.g., 81.2 µl are indicated in the picture on the left (≈ 0.0027 floz)* Now put on a suitable **pipette tip**.
2		Press the plunger button down to the **first pressure point** and hold, then immerse the tip 0.2 inch in the liquid.
3		Slowly release the plunger button. The medium is sucked in.
4		To eject the medium from the pipette tip, press the plunger button down to the first pressure point again, then down to the **second pressure point** so that the tip is completely empty. Always place droplets on the vessel wall.

CENTRIFUGATION

Centrifugation is a separating-method using inertia. Thereby, a mixture of substances is accelerated in a uniform circular motion. Due to their inertia, higher density components migrate outwards and displace low density substances that get into the center.

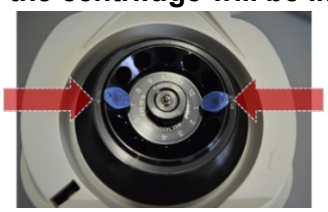


The separation process of a mixture of liquid and solid components (such as a suspension) is called **Decantation**. The liquid, from which the precipitate or sediment has settled out, *e.g.* after centrifugation, is poured off, leaving the other, solid component of the mixture behind:



Please note:

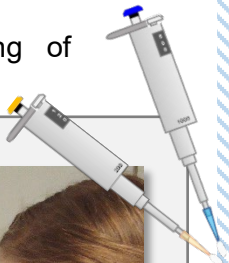
Always put two Eppendorf tubes containing an equivalent volume opposite to each other in the centrifuge, as otherwise the centrifuge will be imbalanced!



- **'Start'-button:** When this button is pressed, the centrifuge starts and runs to the previously set duration and rotational speed ('rounds per minute' = rpm).
- **'Short-Spin'-function:** When holding the 'Short-Spin'-button, the centrifuge starts and runs until the button is released. This function is used to move liquid droplets from the Eppendorf tube's edge down to the bottom of the tube.
- **'Stop'-button:** If you accidentally start the centrifuge without balancing, press the 'Stop'-button immediately.

PRACTICING WORKING TECHNIQUES

In this practical exercise, we familiarize ourselves with the handling of micropipettes and the centrifuge.



Task:

After getting into pairs, take an Eppendorf tube and alternately pipette the following amounts of color solution from the beaker into this tube.

2 µl
4 µl
10 µl
14 µl
20 µl
50 µl
150 µl
250 µl

How it works:

1) Choose the micropipette most suitable for the required volume!

2) Adjust the exact volume quantity and put on a pipette tip!

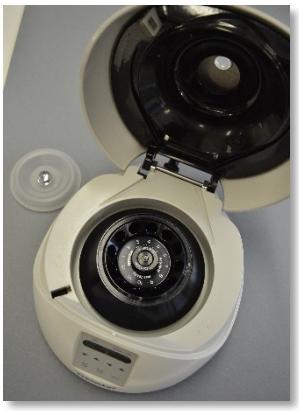
3) Press and hold the plunger button down to the first pressure point and immerse the pipette tip into the color solution!

4) Slowly release the plunger button; the color solution is sucked in.

5) To transfer the liquid completely into the Eppendorf tube, push the plunger button down to the second pressure point. Place small amounts of liquid directly on the vessel wall.

6) Throw away the used pipette tip into the waste container on the table!

Alternately repeat the steps until all of the above listed quantities of liquid are collected in the Eppendorf tube (2 µl to 250 µl)!



7) Mix solutions by pipetting up and down:
Test this technique now by repeatedly and slowly pushing the plunger button down to the first pressure point and releasing it again!

8) Place your Eppendorf tube into the centrifuge. Make sure that another vessel is exactly opposite to it. First place the inner lid on the centrifuge and then close the device. When using the 'Short-Spin'-function, the liquid droplets will be collected in the bottom of the tube; hold the 'Short-Spin'-button for 5 seconds!

9) Finally, pipette the entire liquid from the Eppendorf tube back into the beaker in one step (Math problem: Add the volumes!)

DNA-ISOLATION

FROM ORAL MUCOSAL CELLS

Materials:

- drinking cup
- distilled water
- 2 Pasteur pipettes (sterilized)
- snap-cap vial
- chronometer
- micropipettes
- graduated pipette (iced; 0.338 floz)
- pipette pump
- water bath (122°F)
- black placemat
- waterproof pens

Chemicals:

- lysis buffer (white)
- mild detergent (green)
- chilled Isopropyl alcohol (P)

Experimental procedure:

1) Briefly rinse your mouth twice with **distilled water** from the drinking cup.

2) Then chew for 2 minutes (do not swallow!) so that **saliva** containing **ablated oral mucosal cells** forms.

3) Fill the **Pasteur pipette** with distilled water from the drinking cup up to the mark in front of the suction head. Release the water into your mouth and **intensively swill it around for 40 seconds** (as if rinsing with water after brushing your teeth).

4) Afterwards, the 'rinse water' should be spat into the **snap-cap vial**. Make sure you **label** your vial with your group number to avoid confusion.

5) Use a micropipette to add **2000 µl lysis buffer (white)** to the contents of the vial. Then put on the snap-cap and carefully (!) tilt it back and forth **five times** to mix it with the saliva.

Explain the role of the lysis buffer:

_____

_____

6) Use a second Pasteur pipette to add **5 drops of mild detergent (green)** to the solution in the snap-cap. Close the cap and carefully tilt it back and forth **once again**.

Explain the role of the mild detergent:

_____

_____

7) Place the snap-cap vial in a 122°F **water bath for 10 minutes** and then return it to your workplace.

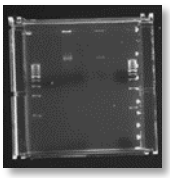
Explain the role of the warm water bath:

8) Take an **iced graduated pipette and pipetting pump with 0.17 floz of chilled alcohol (P)**. The alcohol must be carefully and slowly run down the inside wall of the snap-cap vial to build a second phase over the saliva sample. This is known as over-coating with alcohol.

Explain why you over-coat with alcohol:

9) Close the snap-cap vial and leave it on the **black placemat** for **5 minutes** without touching it.

Observation:



AGAROSE GEL ELECTROPHORESIS

A METHOD TO VISUALIZE DNA-MOLECULES

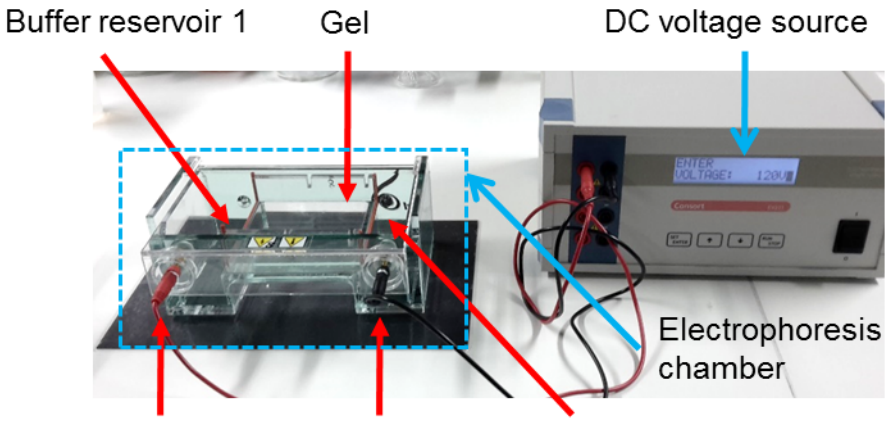
Agarose:

A carbohydrate from the cell wall of red algae. After boiling in water and cooling down, it forms a solid **gel** ('carrier material').

Electrophoresis:

The migration of electrically charged particles through a substance which serves as a carrier material in an electric field.

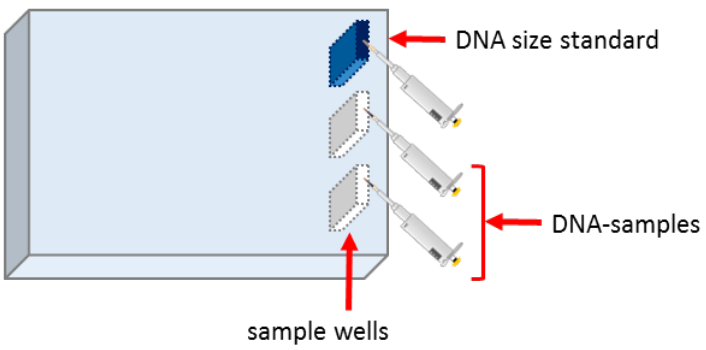
Elements of the apparatus:



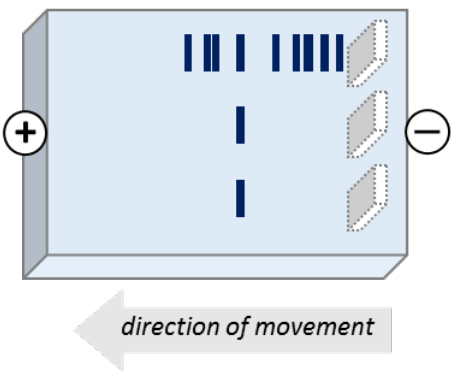
Please note:

- The method distinguishes only between **shorter and longer DNA molecules**.
- For exact length determination, a so-called **'DNA size standard'** is necessary for comparison!
- The **migration rate of electrically charged molecules** depends on:
  - the applied voltage
  - the density of the gel
  - the size / length of the DNA molecules

1. Loading DNA-samples



2. After gel electrophoresis



DNA GEL ELECTROPHORESIS

Materials:

Agarose-Gel (Preparation)

- heating plate
- scales
- Erlenmeyer flask
- spatula
- aluminum foil
- heat-resistant gloves
- rubber gloves
- chronometer

Gel electrophoresis

- electrophoresis chamber
- Eppendorf tubes
- micropipettes
- tweezers
- centrifuge
- ice bath
- waterproof pens

Chemicals:

- agarose
- molecular dye 'SYBR-Green'
- electrophoresis buffer (TBE; blue)
- chilled Isopropyl alcohol (yellow)
- loading buffer (red)

Gel station: Preparing an agarose gel (two groups prepare this for the entire class)

1) Weigh out **0.012 oz agarose** into an Erlenmeyer flask, suspend it with **1.7 floz electrophoresis buffer (TBE)** and seal the Erlenmeyer flask with a piece of aluminum foil.

2) The agarose suspension is **boiled** on the heating plate and then stored in a drying oven (140°F) until use.

Caution very hot! **When transporting the hot Erlenmeyer flask, always wear a heat-resistant glove!**

3) To avoid contamination, wear **rubber gloves** when working with the gel!
Your laboratory supervisor should add **5 µl of the molecular dye 'SYBR-Green'** to the boiled agarose solution. Distribute the dye by gently swirling the Erlenmeyer flask.

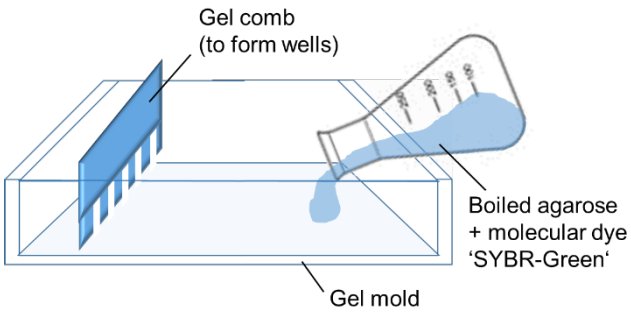
Explain the role of the molecular dye 'SYBR-Green':

_____

_____

4) Pour the agarose solution into the **gel mold** of the electrophoresis chamber and insert the **gel comb**.
The agarose solution **solidifies** as it cools down (ca. 30-40 min).

5) For proper electrophoresis the mold with the prepared gel needs to be **turned** so that the gel comb is in line with the (-)-electrode. Now carefully (!) **remove the gel comb**.



6) Finally, the right buffer reservoir is filled with an **electrophoresis buffer** until the gel is flooded. Then fill the left buffer reservoir up to the 'max filling' line.

DNA-sample processing and gel electrophoresis

1) Use the tweezers to transfer some of your **DNA** from the snap-cap vial into the **Eppendorf tube (1)**. It is easier when the snap-cap vial is held slightly inclined.

2) The transferred DNA is dissolved with **100 µl electrophoresis buffer (blue)**.
Pipette up and down for at least **1 minute** to mix it. Please keep the pipette tip in the liquid to avoid bubbles!

3) Transfer **20 µl of the DNA-solution** into another **Eppendorf tube (2)**.
The following instructions concern **the sample processing**.

- ☞ First add **100 µl of chilled alcohol (yellow; stored on ice)** and pipette up and down again for **1 minute**. Then, the sample should be **centrifuged for 1 minute** (14.5 rpm).
- ☞ After centrifugation, a **solid sediment (pellet)** can be seen at the bottom of the Eppendorf tube. Before doing anything else, the **alcohol** must be **completely (!) decanted** onto a piece of paper.
- ☞ Leave the Eppendorf tube **open** for **2 minutes** so that the remaining alcohol evaporates.
- ☞ Add **50 µl electrophoresis buffer (blue)** into the Eppendorf tube with the pellet and pipette up and down again for **1 minute**.

Explain the purpose of the sample processing:

_____

_____

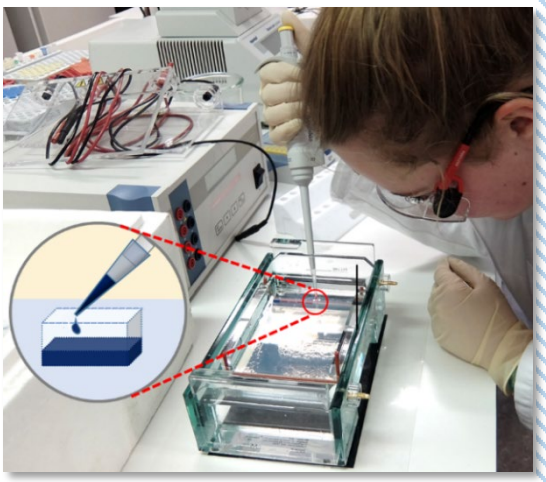
4) After transferring **20 µl of the processed sample** into another **Eppendorf tube (3)**, add **5 µl of loading buffer (red)**. Slightly mix the solutions by snapping on the closed Eppendorf tube, then centrifuge for **5 seconds ('Short Spin'-function)** to collect liquid droplets from the tube's edge. Now your DNA-sample is ready for electrophoresis! Store it **on ice** until application.

Explain the role of the loading buffer:

_____

_____

5) At the gel station carefully (!) inject **20 µl of the chilled DNA-sample** into an empty well on the agarose gel (*Note your well number!*).



6) Additionally, the laboratory supervisor should inject a **'DNA size standard'** into a different well for length determination. The supervisor should **start** the gel electrophoresis with a voltage of **120 V**.

7) The electrophoresis should be **terminated** as soon as the first dye of the loading buffer reaches the leading edge of the gel (ca. 2 hours). The laboratory supervisor should remove the gel from the apparatus. Finally, the gel is **observed under UV** and the findings are **analyzed** in plenary.

DNA-MODELING

After it had been proven by experiments that DNA carries the genetic information, important questions about the structure of the DNA and the encryption of the genetic code were raised. At the beginning of the 1950s there was a race between several groups of researchers who wanted to answer these questions.

Tasks:

1) Read the **information text** about DNA-structure which you find in the modeling box!
2) **Underline** the chemical **components** that compose the **DNA**!
3) Answer the **following questions about DNA structure** with the help of the text!

(1) How many strands does the DNA consist of?

(2) Please tick as appropriate! The DNA-strands are …

- ☐ independent of each other.
- ☐ antiparallel.
- ☐ shifted arranged.
- ☐ identical.

(3) Explain the cohesion of the DNA-strands!

(4) The DNA backbone: Name the associated components and describe their set up!

(5) Which DNA backbone's compound are the bases linked to?

(6) Name the bases and indicate possible base pairings!

(7) Give the opposite bases to the base sequence 'GATCTA'!

(8) Check! The bases are located …

- ☐ at the interior of the DNA-molecule.
- ☐ at the exterior of the DNA-molecule.

(9) The DNA forms a right-handed double-helix. Explain what this means!

(10) Describe how genetic information is encoded in the DNA!

4) Slip into the role of the scientists Watson & Crick and **build a DNA-model** with the help of the answered questions! Work in pairs.
You will find a selection of material in the box (glue, straws, scissors, pipe cleaners, colored beads and cardboards etc.).

Some clues:

- First consider which materials could represent the single components of the DNA (several options are possible)!
- Decide upon appropriate material and start modeling. Build just a short sequence of the giant molecule!
- Try to visualize as many characteristics of the DNA-structure as possible in your model! Do not hesitate to change and test your DNA-model's properties; this is part of the modeling process!

5) Finally, draw a **simplified sketch** of your model and label the components!
You will be given an extra sheet of paper for this purpose.

Following in the footsteps of two GEN(E)iuses:

How Watson and Crick solved the molecular puzzle of DNA structure

Source: http://cshlarchives.blogspot.de/2013/03/dna-letters-1951-1953.html

In 1953 two until then little-known scientists won the race to encode the structure of DNA: James Watson and Francis Crick (see picture). Although they did not perform their own experiments, they understood how to link and correctly interpret the findings of other researchers.

This is what Francis Crick wrote to his twelve year old son on March 19th 1953:

'My Dear Michael,

Jim Watson and I have probably made a most important discovery. We have built a model for the structure of dex-oxi-ribose-nucleic-acid (read it carefully) called D.N.A. for short. …'

After numerous discussions and exchanges of ideas, the two researchers caused a sensation: They succeeded in translating the available DNA data into a spatial model which they had formed from the simplest materials, such as wire, cardboard and staples.

In the letter to his son, Francis Crick describes the structure of their *'very beautiful'* DNA-model in detail. According to this, the simplest way to imagine the DNA is like a twisted rope ladder. The alternating spars consist of firmly linked phosphate groups and a sugar called deoxyribose. These phosphate-sugar-chains are also called the DNA backbone, because they are at the exterior of the DNA-molecule. At the interior of the giant molecule are the four different bases adenine (A), thymine (T), guanine (G) und cytosine (C). The bases form pairwise the ladder rungs of the DNA, whereby the individual bases are firmly attached to the sugar components of the lateral spars. The complete DNA-molecule is composed of two single DNA strands, each consisting of one of the two sugar-phosphate-chains and each with one base attached to the sugar components. Both single strands are linked to each other via hydrogen bonds between the complementary base pairs. It is crucial that there are only two possible base pairings. Adenine always pairs with thymine and guanine always pairs with cytosine.

The special nature of the DNA structure is that the base sequence of one single strand (*e.g.* TTCAG) automatically specifies the base sequence of the complementary single strand (→ AAGTC), because there are only two possible base pairings. Furthermore, the resulting base sequence is a code: Certain sections form different genes which carry the basic information for the expression of different hereditary characteristics.

In summary, the DNA is an aliphatic giant molecule consisting of two opposing DNA single strands. If you look at the vertical single strands from above, you see that they are helically wound around each other in a clockwise direction. This is also called a right-handed double helix structure.

In 1962, the scientists Watson & Crick awarded the Nobel Prize for Medicine for their discovery of the double-helix structure of DNA.

A STEAM outreach lab module

SIMPLY INGEN(E)IOUS!

DNA AS A CARRIER OF GENETIC INFORMATION

Suggested solutions

for experimental tasks and modeling

A practical training course for 9th graders

DNA-ISOLATION

FROM ORAL MUCOSAL CELLS

Materials:

- drinking cup
- distilled water
- 2 Pasteur pipettes (sterilized)
- snap-cap vial
- chronometer
- micropipettes
- graduated pipette (iced; 0.338 floz)
- pipette pump
- water bath (122°F)
- black placemat
- waterproof pens

Chemicals:

- lysis buffer (white)
- mild detergent (green)
- chilled Isopropyl alcohol (P)

Experimental procedure:

10) Briefly rinse your mouth twice with **distilled water** from the drinking cup.

11) Then chew for 2 minutes (do not swallow!) so that **saliva** containing **ablated oral mucosal cells** forms.

12) Fill the **Pasteur pipette** with distilled water from the drinking cup up to the mark in front of the suction head. Release the water into your mouth and **intensively swill it around for 40 seconds** (as if rinsing with water after brushing your teeth).

13) Afterwards, the 'rinse water' should be spat into the **snap-cap vial**. Make sure you **label** your vial with your group number to avoid confusion.

14) Use a micropipette to add **2000 µl lysis buffer (white)** to the contents of the vial. Then put on the snap-cap and carefully (!) tilt it back and forth **five times** to mix it with the saliva.

Explain the role of the lysis buffer:

The lysis buffer (dishwashing detergent-salt solution) causes the dissolution of nuclear and cell membranes (more precise: The phospholipid bilayer is destroyed by the detergent component; the saline component increases solubility of DNA in the fluid).

15) Use a second Pasteur pipette to add **5 drops of mild detergent (green)** to the solution in the snap-cap. Close the cap and carefully tilt it back and forth **once again**.

Explain the role of the mild detergent:

Enzymes in the mild detergent degrade proteins, fats and carbohydrates.

16) Place the snap-cap vial in a 122°F **water bath for 10 minutes** and then return it to your workplace.

Explain the role of the warm water bath:

The heat improves the effect of the detergents and the enzymes.

17) Take an **iced graduated pipette and pipetting pump with 0.17 floz of chilled alcohol (P)**. The alcohol must be carefully and slowly run down the inside wall of the snap-cap vial to build a second phase over the saliva sample. This is known as over-coating with alcohol

Explain why you over-coat with alcohol:

The DNA is insoluble in alcohol (more precise: At the interface alcohol molecules affect DNA molecules' hydration shell. In consequence, DNA becomes macroscopically visible through interaction between single DNA molecules).

18) Close the snap-cap vial and leave it on the **black placemat** for **5 minutes** without touching it.

Observation:

At the phase boundary between the upper alcohol layer and the lower saliva layer whitish threads and/or lumps become visible.

DNA GEL ELECTROPHORESIS

Materials:

Agarose-Gel (Preparation)

- heating plate
- scales
- Erlenmeyer flask
- spatula
- aluminum foil
- heat-resistant glove
- rubber gloves
- chronometer

Gel electrophoresis

- electrophoresis chamber
- Eppendorf tubes
- micropipettes
- tweezers
- centrifuge
- ice bath
- waterproof pens

Chemicals:

- agarose
- molecular dye 'SYBR-Green'
- electrophoresis buffer (TBE; blue)
- chilled Isopropyl alcohol (yellow)
- loading buffer (red)

Gel station: Preparing an agarose gel (two groups prepare this for the entire class)

7) Weigh out **0.012 oz agarose** into an Erlenmeyer flask, suspend it with **1.7 floz electrophoresis buffer (TBE)** and seal the Erlenmeyer flask with a piece of aluminum foil.

8) The agarose suspension is **boiled** on the heating plate and then stored in a drying oven (140°F) until use.

Caution very hot! **When transporting the hot Erlenmeyer flask, always wear a heat-resistant glove!**

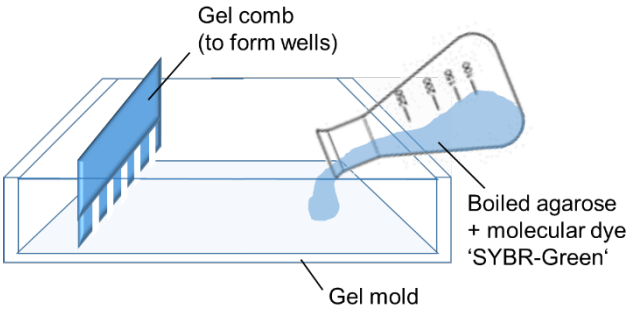
9) To avoid contamination, wear **rubber gloves** when working with the gel!
Your laboratory supervisor should add **5 µl of the molecular dye 'SYBR-Green'** to the boiled agarose solution. Distribute the dye by gently swirling the Erlenmeyer flask.

Explain the role of the molecular dye 'SYBR-Green':

The dye (detection reagent) binds specifically to double-stranded DNA-molecules. These dye-DNA particles become visible by emitting green light (at 521 nm) under UV light.

10) Pour the agarose solution into the **gel mold** of the electrophoresis chamber and insert the **gel comb**.
The agarose solution **solidifies** as it cools down (ca. 30-40 min).

11) For proper electrophoresis the mold with the prepared gel needs to be **turned** so that the gel comb is in line with the (-)-electrode. Now carefully (!) **remove the gel comb**.



12) Finally, the right buffer reservoir should be filled with an **electrophoresis buffer** until the gel is flooded. Then fill the left buffer reservoir up to the 'max filling' line.

DNA-sample processing and gel electrophoresis

8) Use the tweezers to transfer some of your **DNA** from the snap-cap vial into the **Eppendorf tube (1)**. It is easier when the snap-cap vial is held slightly inclined.

9) The transferred DNA is dissolved with **100 µl electrophoresis buffer (blue)**.
Pipette up and down for at least **1 minute** to mix it. Please keep the pipette tip in the liquid to avoid bubbles!

10) Transfer **20 µl of the DNA-solution** into another **Eppendorf tube (2)**.
The following instructions concern **the sample processing**.

- ☞ First add **100 µl of chilled alcohol (yellow; stored on ice)** and pipette up and down again for **1 minute**. Then, the sample should be **centrifuged for 1 minute** (14.5 rpm).
- ☞ After centrifugation, a **solid sediment (pellet)** can be seen at the bottom of the Eppendorf tube. Before doing anything else, the **alcohol** must be **completely (!) decanted** onto a piece of paper.
- ☞ Leave the Eppendorf tube **open** for **2 minutes** so that the remaining alcohol evaporates.
- ☞ Add **50 µl electrophoresis buffer (blue)** into the Eppendorf tube with the pellet and pipette up and down again for **1 minute**.

Explain the purpose of the sample processing:

During sample processing interfering substances are removed so that the DNA-sample is prepared for further investigation. Such reactions are also referred to as reprecipitation.

11) After transferring **20 µl of the processed sample** into another **Eppendorf tube (3)**, add **5 µl of loading buffer (red)**. Slightly mix the solutions by snapping on the closed Eppendorf tube, then centrifuge for **5 seconds ('Short Spin'-function)** to collect liquid droplets from the tube's edge. Now your DNA-sample is ready for electrophoresis! Store it **on ice** until application.

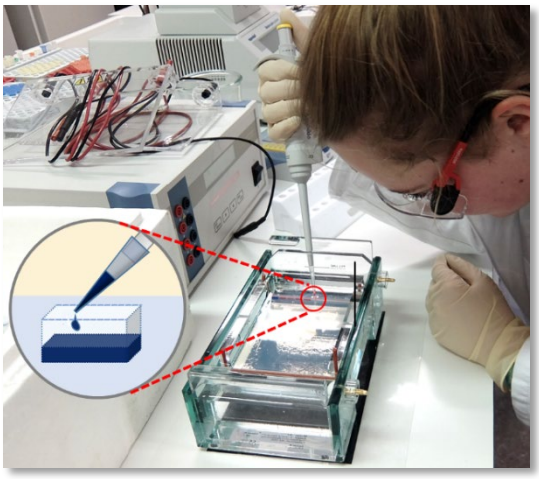
Explain the role of the loading buffer:

The loading buffer contains glycerol and sucrose which cause the DNA-sample to sink into the gel wells due to their relatively high density. The included blue dye allows to control the movement of the DNA.

12) At the gel station carefully (!) inject **20 µl of the chilled DNA-sample** into an empty well on the agarose gel (*Note your well number!*).

13) Additionally, the laboratory supervisor should inject a **'DNA size standard'** into a different well for length determination. The supervisor should **start** the gel electrophoresis with a voltage of **120 V**.

14) The electrophoresis should be **terminated** as soon as the first dye of the loading buffer reaches the leading edge of the gel (ca. 2 hours). The laboratory supervisor should remove the gel from the apparatus. Finally, the gel is **observed under UV** and the findings are **analyzed** in plenary.



DNA-MODELING

After it had been proven by experiments that the DNA carries the genetic information, important questions about the structure of the DNA and the encryption of the genetic code were raised. At the beginning of the 1950s there was a race between several groups of researchers who wanted to answer these questions.

Tasks:

6) Read the **information text** about DNA-structure which you find in the modeling box!

7) **Underline** the chemical **components** that compose the **DNA**!

8) Answer the **following questions about DNA structure** with the help of the text!

(11) How many strands does the DNA consist of?

The DNA consists of two single strands.

(12) Please tick as appropriate! The DNA-strands are …

- ☐ independent of each other.
- ☒ antiparallel.
- ☐ shifted arranged.
- ☐ identical.

(13) Explain the cohesion of the DNA-strands!

The DNA single strands are linked to each other via hydrogen bonds between the individual base pairs.

(14) The DNA backbone: Name the associated components and describe their set up!

It consists of phosphate groups and a sugar called deoxyribose. These components are arranged alternately resulting in a phosphate-sugar-chain.

(15) Which DNA backbone's compound are the bases linked to?

The single bases are firmly attached to the sugar components of the DNA backbone.

(16) Name the bases and indicate possible base pairings!

The bases are adenine (A), thymine (T), guanine (G) and cytosine (C). Only two possible base pairings exist: (A) pairs with (T) and (G) pairs with (C) (complementary base pairing).

(17) Give the opposite bases to the base sequence 'GATCTA'!

The base sequence of the complementary DNA strand is CTAGAT.

(18) Check! The DNA bases are located …

- [x] at the interior of the DNA-molecule.
- [ ] at the exterior of the DNA-molecule.

(19) The DNA forms a right-handed double-helix. Explain what this means!

If you look at the vertical single strands from above, you see that they are helically wound around each other in a clockwise direction.

(20) Describe how genetic information is encoded in the DNA!

The base sequence is a code: Certain sections form different genes which carry the basic information for the expression of different hereditary characteristics.

9) Slip into the role of the scientists Watson & Crick and **build a DNA-model** with the help of the answered questions! Work in pairs.
You will find a selection of material in the box (glue, straws, scissors, pipe cleaners, colored beads and cardboards etc.).

Some clues:

- First consider which materials could represent the single components of the DNA (several options are possible)!
- Decide upon appropriate material and start modeling. Build just a short sequence of the giant molecule!
- Try to visualize as many characteristics of the DNA-structure as possible in your model! Do not hesitate to change and test your DNA-model's properties, this is part of the modeling process!

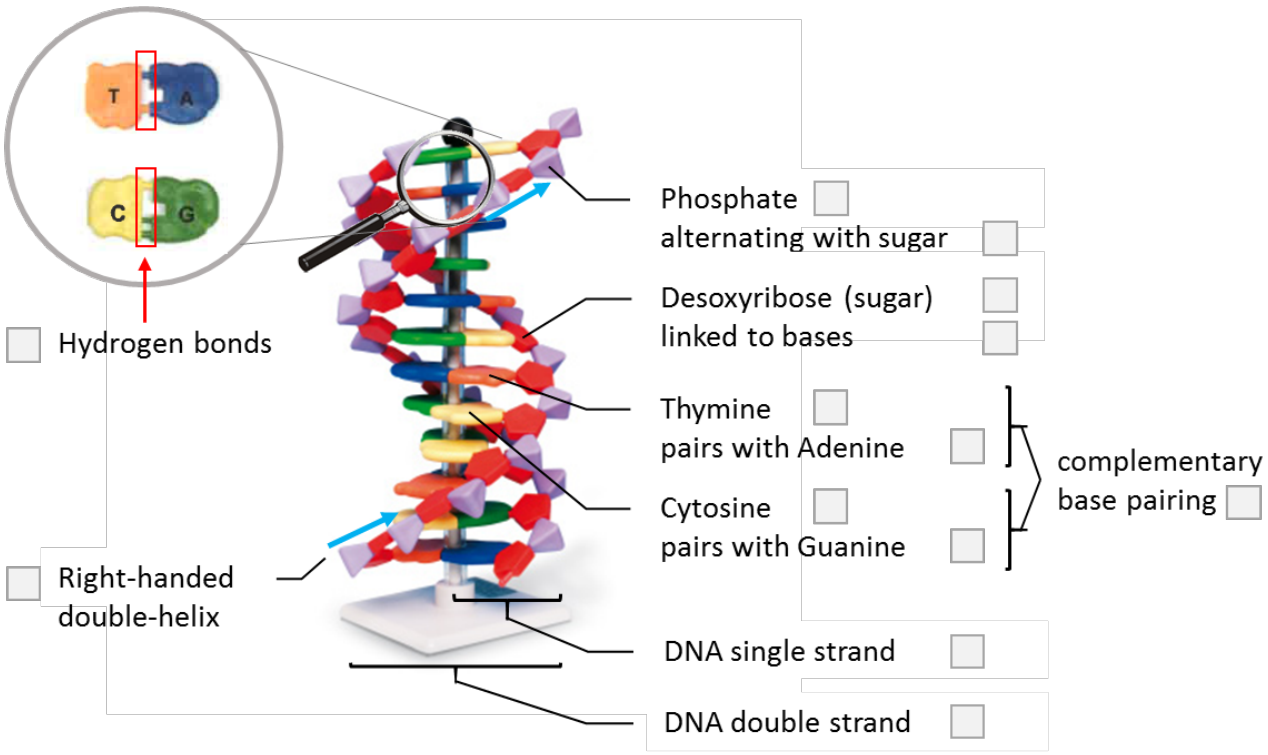
10) Finally, draw a **simplified sketch** of your model and label the components! You will be given an extra sheet of paper for this purpose.

DNA-MODEL EVALUATION

Tasks:

1) **Look** closely at your DNA model and **compare** it with the distributed school model (similarities / differences)!

2) **Check** the image below to identify which DNA components are represented in both your elaborated model and the school model!



Additional task for young researchers:

3) Search for a picture of Watson & Crick's original DNA-model on the internet and compare it to yours!

Appendix 5: Instruction poster 'Gel electrophoresis' with removable magnet applications

Simply inGEN(E)ious! Visualize the invisible with

AGAROSE GEL ELECTROPHORESIS

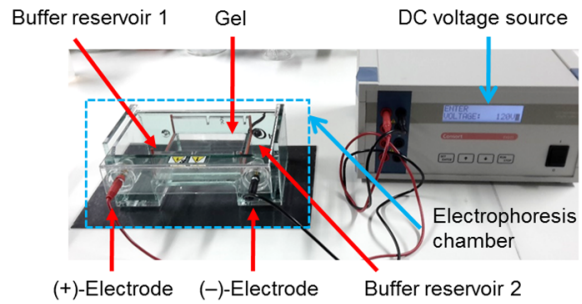
Agarose:

A carbohydrate from the cell wall of red algae. After boiling in water and cooling down, it forms a solid **gel** ('carrier material').

Electrophoresis:

The migration of electrically charged particles through a substance which serves as a carrier material in an electric field.

Elements of the apparatus:



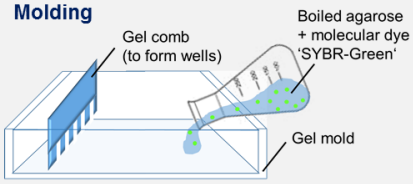
Preparing an agarose gel:

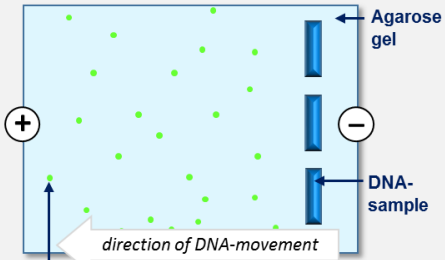
Weighing — Suspending — Boiling

What makes DNA visible in gel electrophoresis?

Addition of the molecular dye 'SYBR-Green'

Molding





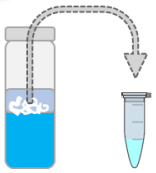
Molecular dye ‚SYBR-Green' — binds to DNA-molecules and glows under UV light

The DNA consists of 5 different chemical elements:

1,0	12,0	14,0	16,0	31,0
1 H 2,2	6 C 2,5	7 N 2,2	8 O 3,5	15 P 2,1

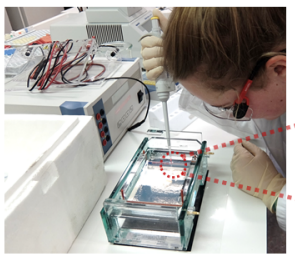
Before starting the gel electrophoresis:

Why is the DNA-sample not applied directly?

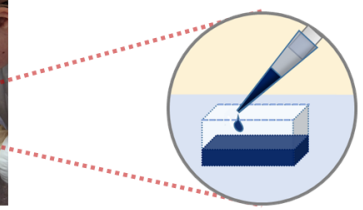


Processing the DNA-sample:
Interfering substances are removed so that the DNA-sample is prepared for further investigation

Addition of the loading buffer:
Weights the sample + dye controls the movement



Application of the samples:



A giant molecule carries our genetic information

The DNA has a relatively large molecular weight, since it is a chain molecule of up to one hundred thousand repetitive single components.

Which component(s) cause(s) the DNA-movement in the electric field?

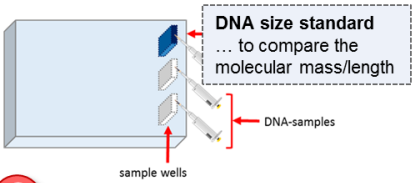
Only **six different components** form the DNA-molecule:

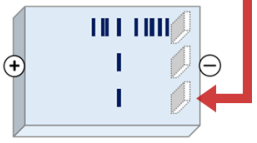
A T G C = the four bases of the DNA

= sugar

P = phosphate

Negative charges!





On what does the migration rate of the DNA depend?

- … on the applied voltage.
- … on the density of the gel.
- … on the size/length of the DNA molecules.

Simply inGEN(E)ious! Visualize the invisible with

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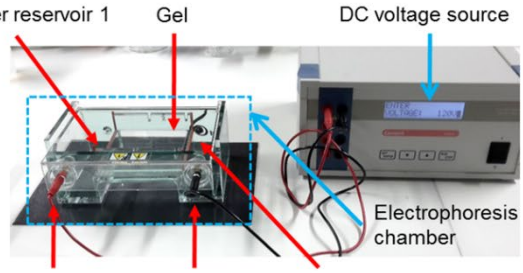
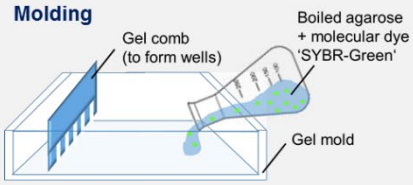
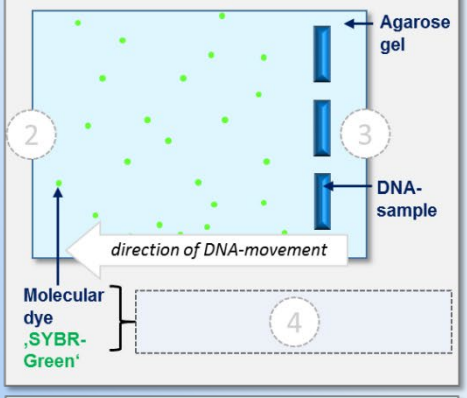


Preparing an agarose gel:

What makes DNA visible in gel electrophoresis?

1



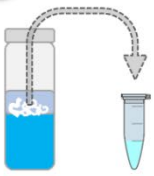


The DNA consists of 5 different chemical elements:

10

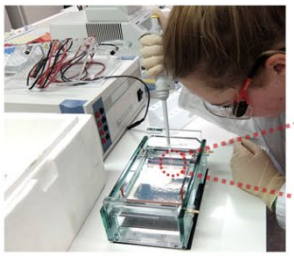
Before starting the gel electrophoresis:

Why is the DNA-sample not applied directly?

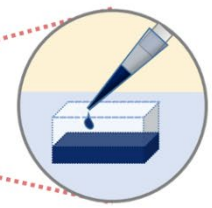


5

6



Application of the samples:



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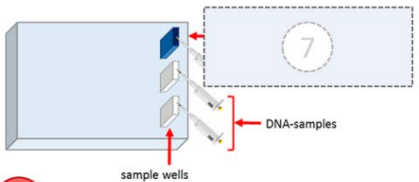

After gel electrophoresis:

8

On what does the migration rate of the DNA depend?

9

Magnet applications:

Can be cut out, laminated and used with magnetic tape on a metallic board onto the poster version with blank spaces.
(Recommended print sizes for the posters: DIN A0 → 33.1 x 46.8 inches)

1 **Addition of the molecular dye 'SYBR-Green'**

2 +

3 −

4 **binds to DNA-molecules and glows under UV light**

5 **Processing the DNA-sample:**
Interfering substances are removed so that the DNA-sample is prepared for further investigation

6 **Addition of the loading buffer:**
Weights the sample + dye controls the movement

7 **DNA size standard**
… to compare the molecular mass/length

8 **Negative charges!**

9
- … on the applied voltage.
- … on the density of the gel.
- … on the size/length of the DNA molecules.

10

1,0	12,0	14,0	16,0	31,0
1 H 2,2	6 C 2,5	7 N 2,2	8 O 3,5	15 P 2,1