# Welcome to our learning material!

This material package is designed specifically for upper secondary school students to support their study and review of key themes related to biology. The material will help you deepen and strengthen your knowledge of the topics in the Finnish upper secondary schoolbooks *Cell and Heredity* and *Biotechnology and its Applications*.

The material combines:

- clear theory – key concepts and phenomena are presented in an understandable format,
- versatile tasks – exercises that allow you to test and strengthen your own knowledge,
- correct answers – to support self-assessment or for use in teaching.

You can use this material for self-study or in teaching under the guidance of a teacher. During the tasks, we encourage you to reflect, apply your knowledge and make observations to make studying as meaningful and fun as possible.

The material has been created by the ABOA 2025 iGEM team, which will participate in the world's largest synthetic biology competition, iGEM, in 2025. We hope that this package will give you new insights and enthusiasm for learning – perhaps you will also be inspired to explore the fascinating world of synthetic biology in more detail!

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# Operation: A missing antibiotic

NOTE:
This is a fictional story, simplified for educational purposes. It is not a proper method for identifying antibiotic resistance and does not replace laboratory or treatment recommendations.

THE STORY:
A mysterious chain of infections has begun at a local hospital. The same unknown bacteria is found in the samples of multiple patients. The bacteria does not seem to react to common antibiotics. The hospital calls on you to help find the answer: what bacteria is it and what antibiotic can be used to fight it?

Your tasks will proceed similarly to a real investigation:
→ DNA (extract the bacteria's DNA)
→ PCR (multiply the bacteria's genome)
→ gelelectrophoresis (ensure that the PCR has succeeded)
→ figuring out the suitable antibiotic

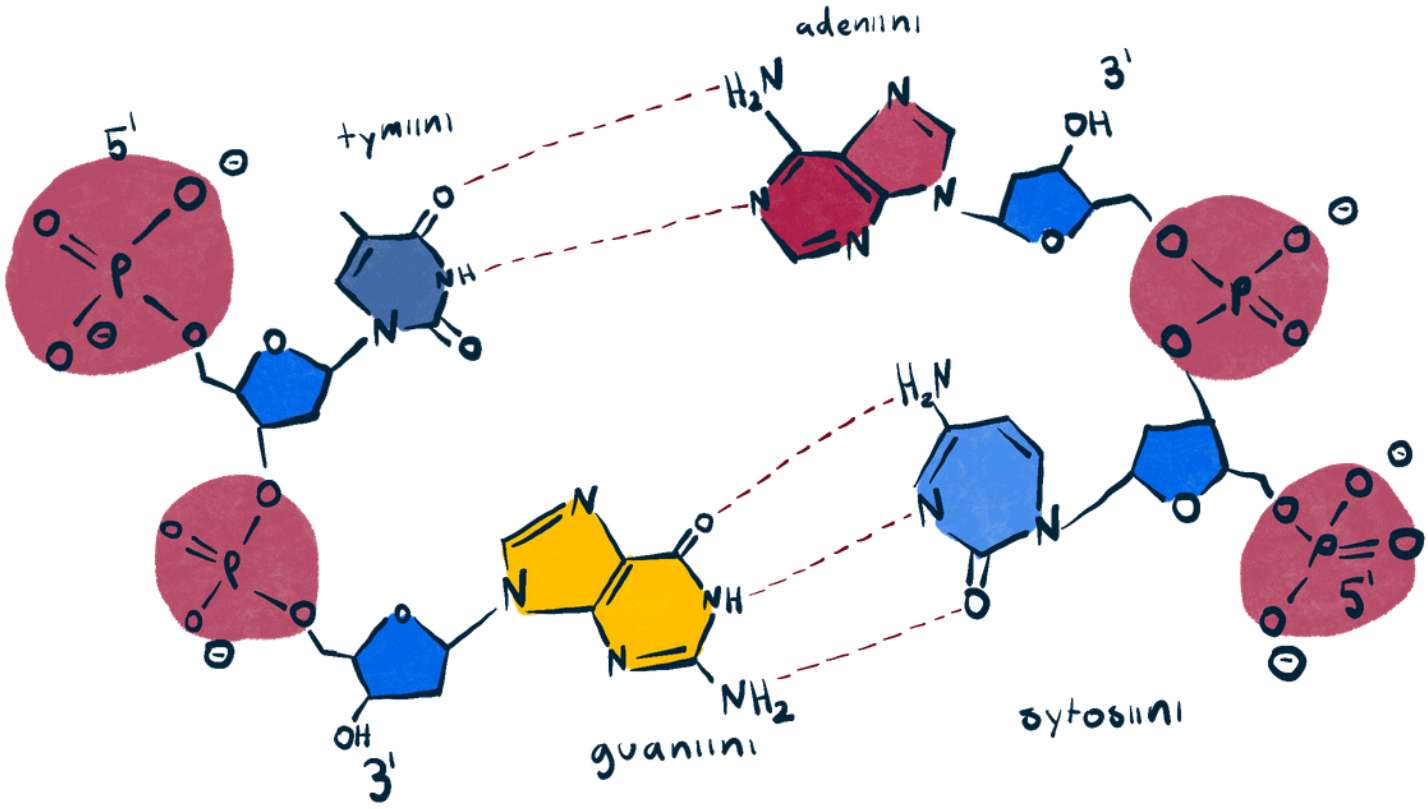
# 1. DNA

The researchers took a sample from a patient, from which they isolated the bacterial DNA using chemical methods. Before PCR, it is a good idea to review the mechanisms and function of DNA.

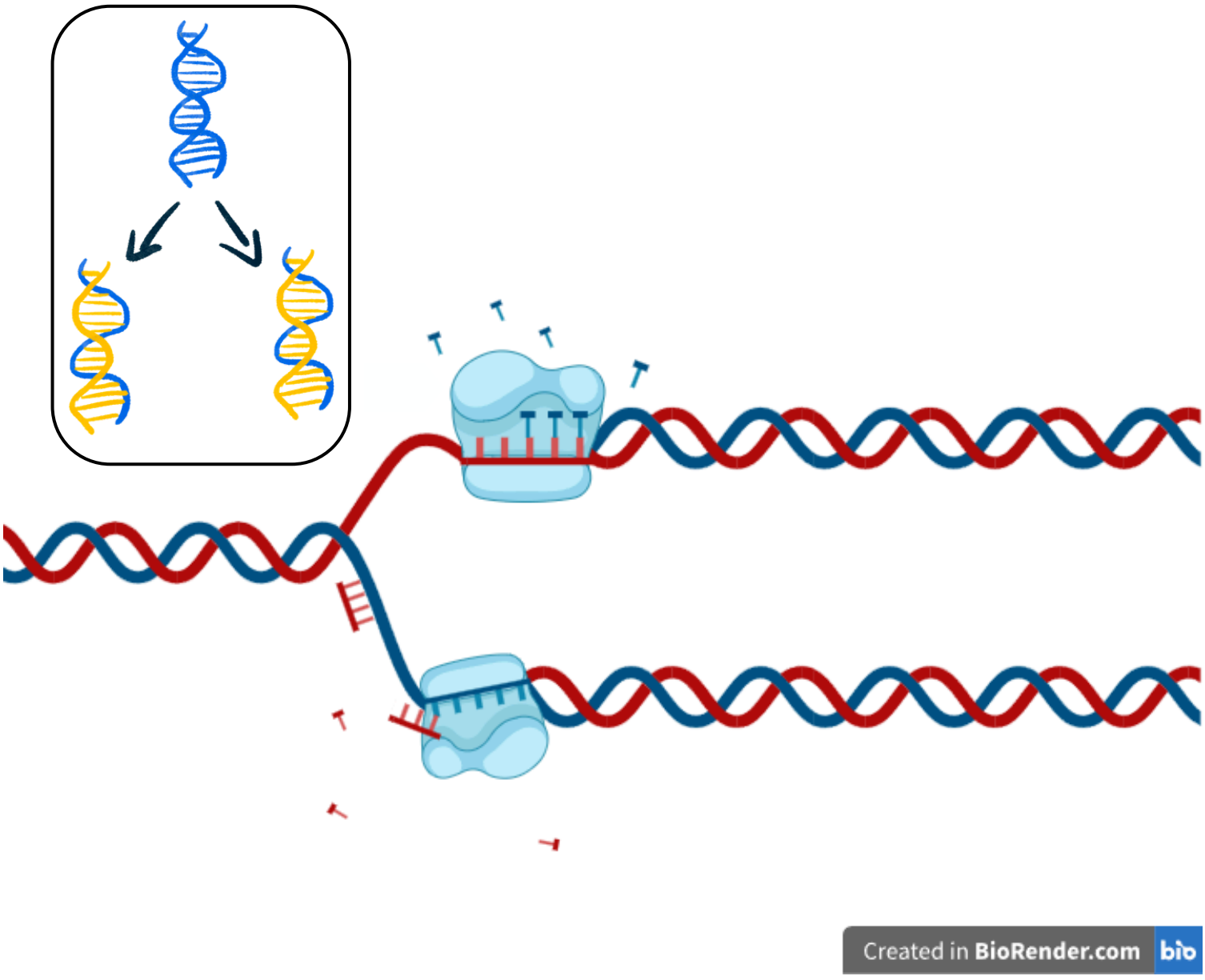
## Exercise 1. a) Fill in the gaps

DNA, also known as _____ , is a biomolecule and belongs to nucleic acids. All information about cell function is stored in the DNA. DNA is double-stranded in structure, meaning DNA has a so-called ladder structure and consists of nucleotides, i.e. small structural units that repeat.

A nucleotide has a sugar part, also known as deoxyribose, a base part which consist of either _____ (A), _____(C), guanine (___) or thymine (___) and a phosphate part. Nucleotides are attached to each other through the phosphate part, so that in one DNA strand, the sugar and phosphate parts alternate lengthwise. The base part is in turn attached to the sugar part. The base parts form _____bonds based on the base pair rule. This means that _____ forms bonds with thymine and cytocine forms bonds with _____. This allows the DNA strands to pair and the bases form the “rungs of the ladder.” The four bases form the genetic code of DNA, which allows information to be stored in the DNA molecule.



DNA synthesis refers to the replication, also known as multiplication, of DNA. In it, DNA duplicates, i.e., one double-stranded DNA molecule produces two double-stranded DNA molecules. DNA duplication is semiconservative, i.e., one of the strands originates from the original strand.




**Exercise 1. b) Mark the 3' ja 5' ends to all four strands of DNA in the picture. To what direction does the DNA replication always proceeds in?**

_____

**Exercise 1. c) Connect the enzymes needed in DNA replication and their functions.**

| | |
|---|---|
| DNA-polymerase | Cutting the hydrogen bonds in between DNA's base pairs. |
| Fixer enzymes | Building the DNA strand |
| Helicase | Fixing the replication mistakes |
| Ligases | The synthesis of RNA-primers |
| RNA-polymerase | Joining pieces of DNA together |

**Exercise 1. d) Fill in the DNA's base pairs**

1. ATGGGCGATAGCTAGCTA
→

2. CTGATCGGATCGATGGTA
→

1. Apply knowledge: What is the difference with DNA synthesis and transcription?

_____

_____

_____

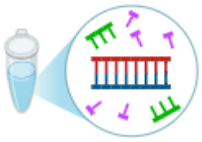
_____

## 2. PCR

A small amount of the hospital bacterium DNA has been isolated from samples taken from sick patients. The DNA must be amplified using PCR technology so that there is enough of it for research purposes.

PCR, or polymerase chain reaction, is a method that can be used to amplify DNA fragments. The PCR cycle has three stages: DNA denaturation, primer annealing, and elongation. In the first stage, the temperature is raised to 95°C for a minute or two to separate the DNA strands. Raising the temperature breaks the hydrogen bonds in the double strand, causing the strands to separate from each other. After this, the temperature is lowered to 45-65°C, at which point the primers attach to the 3' end of these individual DNA strands. Finally, in the elongation stage, the temperature is raised to 72°C, where the DNA polymerase enzyme is activated and forms double-stranded DNA using one of the existing DNA strands as a guide. There can be as many cycles as there are DNA nucleotides in the reaction, but usually the cycle is repeated at least 25-30 times to sufficiently amplify the DNA molecule. The DNA doubles after each cycle, so after x cycles there are $2^x$ copies of the DNA.

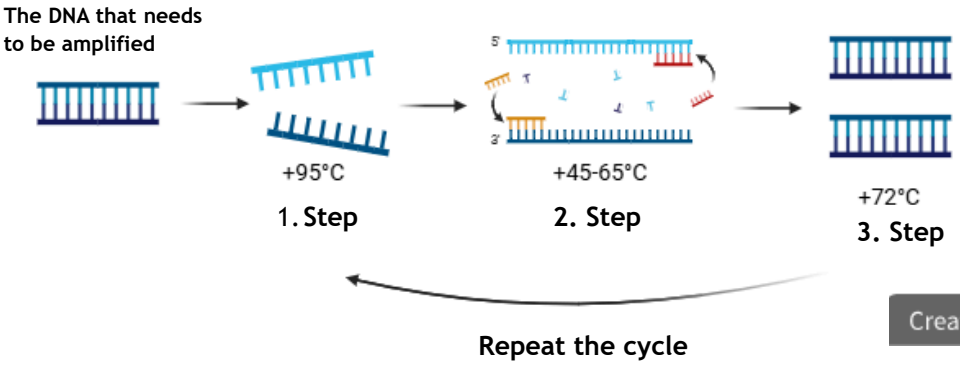
**PCR reaction**



**You'll need:**

- **DNA**
- **DNA-nucleotides**
- **Primers**
- **DNA polymerase enzyme**

**The PCR cycle:**




**Exercise 2. a) Choose the right things to add to a PCR reaction**

- ○ Template DNA
- ○ RNA
- ○ Proteins
- ○ Primers
- ○ Helicase
- ○ DNA nucleotides
- ○ DNA polymerase
- ○ Acid

**Exercise 2. b) What are the steps in a PCR cycle? What happens to DNA during these steps?**

_____

_____

_____

_____

**Exercise 2. c) Why are primers needed in the reaction?**

_____

_____

**Exercise 2. d) Fill in the opposite DNA strand and the 6 nucleotides long primers for the bacterium's DNA to use in the PCR reaction**


**2. Apply knowledge: The PCR reaction did not work and the bacterium's DNA did not replicate. What could be wrong with the reaction? Ponder, how can you ensure that there is no contamination in the sampel.**

There can be many reasons why a PCR reaction might fail. For example:

- The temperatures of the cycle may be wrong.
  - The optimal thermal cycle for a PCR reaction varies depending on the DNA fragment. For example, denaturation of long strands may require a higher temperature or a longer heating period.
  - Too low or too high a temperature may inhibit the polymerase's activity.
- Primers
  - The primers may have been planned wrong. The 5' ja 3' ends can be confused fairly easily when planning primers.
  - There are too little primers.
- Human mistake
  - The tubes may have been mixed up.
  - Pipetting errors because of which the tubes have a wrong amount of reagents

**Cross out two possible reasons that you feel like are the most common ones and ponder, how to solve them.**

## 3. Gel electrophoresis

You perform the PCR reaction again and check its success with gel electrophoresis.

**Exercise 3. a) Place the electrophoresis steps from first to last.**

- ☐ Negatively charged molecules move towards the plus pole, positive ones towards the minus pole.
- ☐ The substance to be examined is pipetted into a small well at the edge of the gel.
- ☐ The gel is stained or illuminated with UV light, which creates lines that show the size of the molecules.
- ☐ Smaller molecules pass through the pores of the gel faster and travel further than larger ones.
- ☐ An electric current is passed through the gel, i.e. a negative pole (–) is placed on one side and a positive pole (+) on the other.

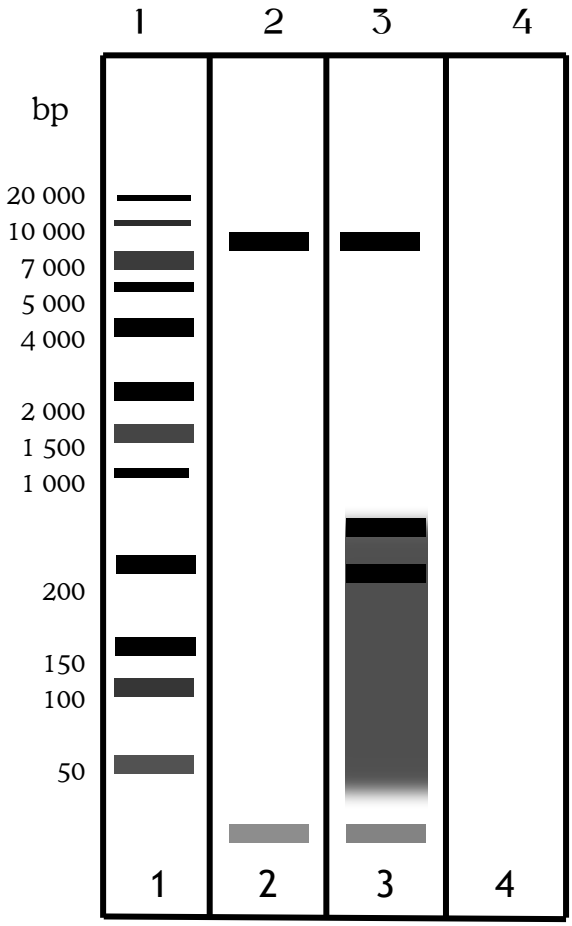
In a successful PCR reaction, only the desired DNA fragment is amplified. This can be checked at the end of the reaction by gel electrophoresis. A successfully amplified DNA sample will show a single clear line, and the size of this line corresponds to the size of the DNA that was intended to be amplified.

The size of the sample can be determined using a molecular size reference marker. The sizes of the lines of the reference marker are known in advance. It is loaded into one of the electrophoresis wells and by comparing its lines, the size of the other samples can be determined.

PCR is a sensitive reaction and can easily become contaminated. Therefore, it is important to ensure that no foreign DNA has accidentally entered the reaction. One way to do this is to use a negative control. A negative control is a sample that contains all the other things required for the PCR reaction, but no DNA.

**Exercise 3. b) Connect the right term to the right sample**

| | |
|---|---|
| negative control | contaminated bacterial DNA |
| bacterial DNA | molecular size reference marker |

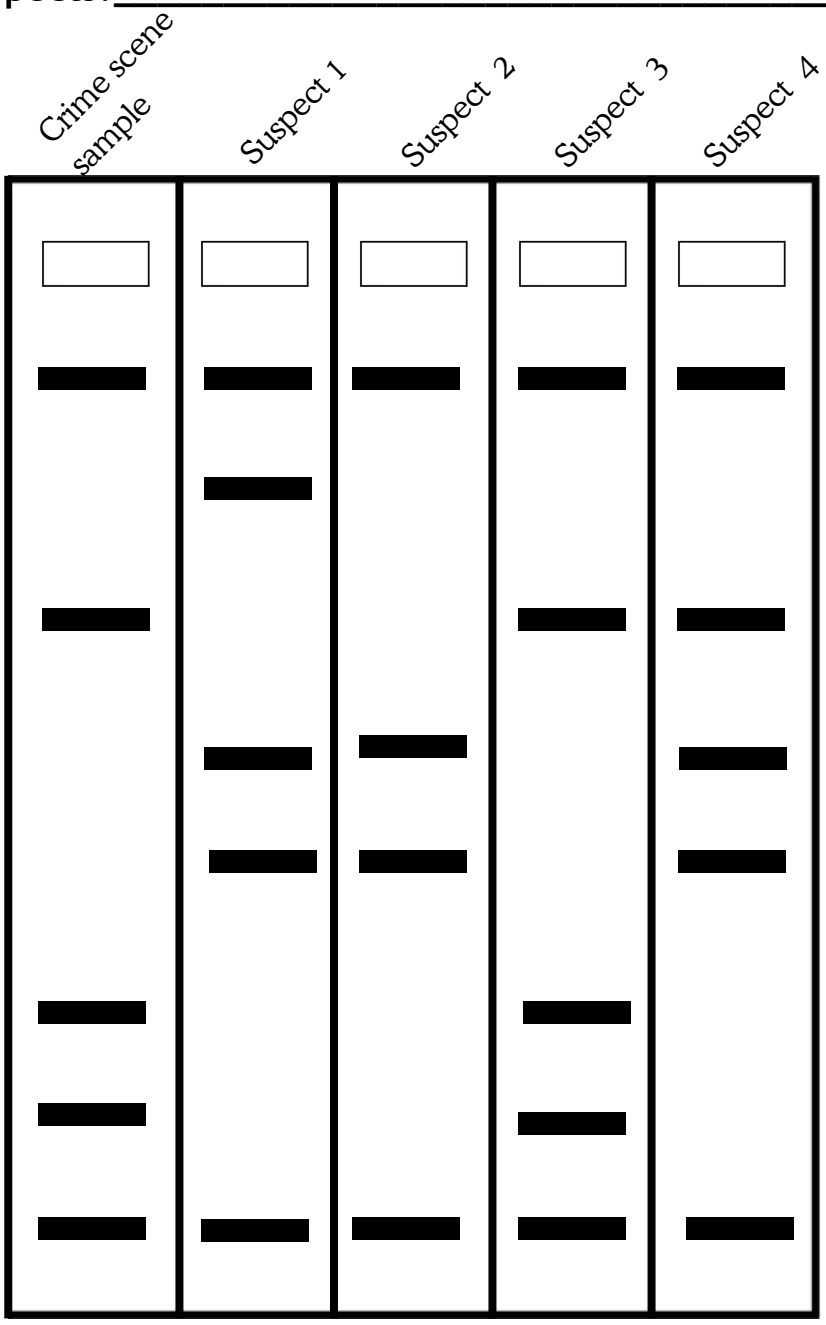



**Exercise 3. c) Estimate the size of the bacterial DNA you amplified.**

_____

PCR and gel electrophoresis can be used to determine a person's genetic fingerprint, which is unique to each individual. For example, forensic scientists use the method to match a suspect to a DNA sample found at a crime scene.

**3. Apply knowledge: Police compare a sample found at the crime scene to the DNA of 4 possible suspects. Does the sample belong to any of the suspects?**_____




## 4. Figuring out the suitable antibiotic

As a final step, you want to find out which antibiotic(s) are effective against the bacteria. You can find this out by adding tablets containing different antibiotics to the bacterial culture. If a clear inhibition ring, where no bacteria grow, forms around the tablet then the antibiotic in question is effective against the bacteria. The diameter of the inhibition ring can be used to determine the effectiveness of the antibiotic.

**Exercise 4. a) How are multi-resistant super bugs created?**

_____

_____

_____

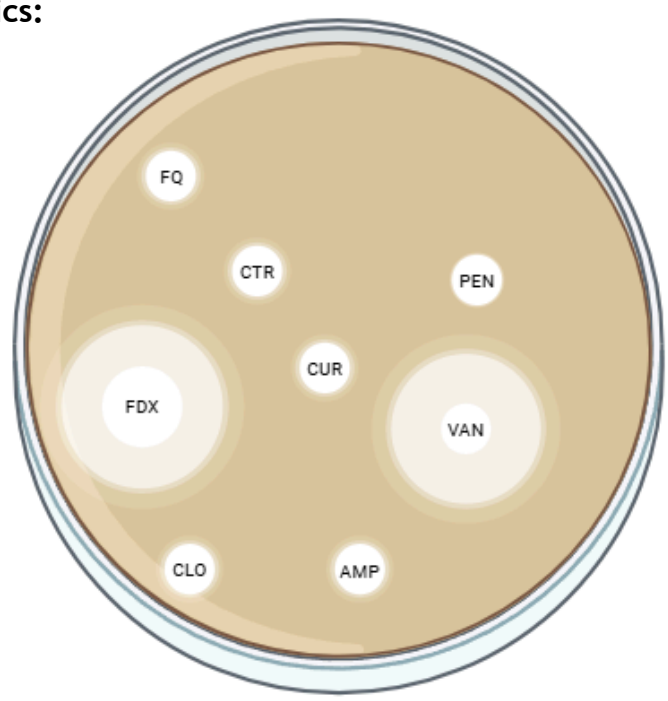
_____

_____

**Exercise 4. b) Below is a picture of a bacterial plate with antibiotic tablets added. Which antibiotics are effective against the bacteria found at the hospital?**_____

**Abbreviations of the antibiotics:**

VAN = vancomycin
PEN = penicillin
AMP = ampicillin
CLO = cloxacillin
CUR = cefuroxime
FDX cetriaxone
FQ = fluoroquinolone



**Exercise 4. c) Now you have the information you need to determine which bacteria it is. Compare the characteristics of the bacteria to the list of known bacteria. The list can be found on page 10. What bacteria is it?**

_____

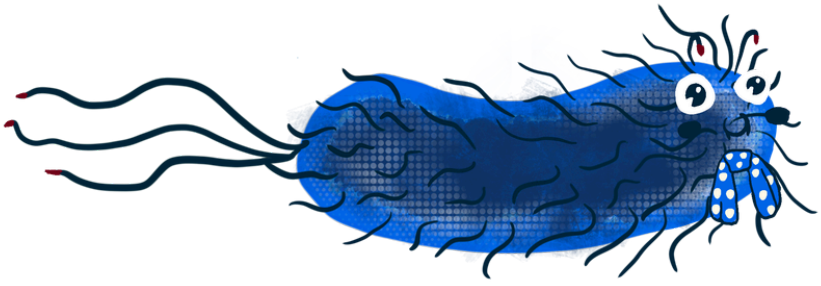
**Exercise 4. d) Fill out a report to send to the hospital, detailing your test results.**

**I have determined that the bacteria in question is _____.**
**I recommend _____antibiotic to treat the patients because**

_____
_____
_____
_____.

**4. Apply knowledge: Why is it important to finish the entire course of antibiotics?**

_____
_____
_____
_____
_____
_____



# BACTERIA AND THE ANTIBIOTICS THAT WORK ON THEM

| | Pennicillin | Ampicillin | Cloxacillin | Vancomycin | Cefuroxime | Fidaxomicin | Cetriaxone | Fluoroquinolc |
|---|---|---|---|---|---|---|---|---|
| E. Coli | | | | | | | | |
| S. Aureus | | | | | | | | |
| E. faecalis | | | | | | | | |
| Pneumokokki | | | | | | | | |
| CRE ** | | | | | | | | |
| C. difficile | | | | | | | | |
| VRE | | | | | | | | |
| MSRA | | | | | | | | |
| V. Lumina | | | | | | | | |

Will most likely work

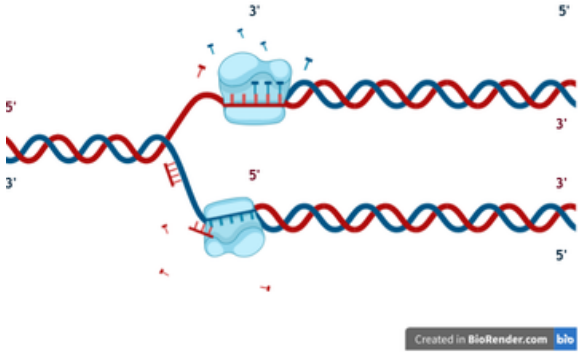
May work but is not optimal

** Always requires individual case investigation, no universally effective antibiotic

Note: This list is a simplification made for the purpose of this assignment and should not be taken as fact.

**Answers**

1. a) Deoxyribonucleic acid, adenine, cytosine, G, T, hydrogen, adenine, guanine.
1. b) DNA's replication always proceeds in 5' → 3' direction.




1. c) DNA polymerase → Building the DNA strand, Fixer enzymes → Fixing the replication mistakes, helicase → Cutting the hydrogen bonds in between DNA's base pairs., ligases → Joining pieces of DNA together, RNA-polymerase → The synthesis of RNA-primers
1. d) TACCCGCTATCGATCGAT ja GACTAGCCTAGCTACCAT
1. Apply knowledge:

DNA-synthesis (replication)

- Purpose: To copy the whole DNA molecule so that during cell division both daughter cells receive a complete genome.
- Product: Two identical double stranded DNA molecules.
- Enzymes: DNA-polymerase, helicase, ligase and others.
- Template Strand: Both DNA strands are used as a template.
- Bases: A, T, G, C.  A pairs with T and C pairs with G.
- Direction: New DNA is synthesized in the 5' → 3' direction.

Transcription

- Purpose: To make a RNA copy of genes in DNA for the purpose of protein synthesis.
- Product: One stranded RNA molecule (for example mRNA, tRNA or rRNA).
- Enzymes: RNA-polymerase.
- Template Strand: Only other DNA strand works as a template strand.
- Bases: A, U, G, C. Uracil (U) replaces thymine (T).
- Direction: RNA is also synthesized in the 5' → 3' direction.

2. a) Template DNA, DNA nucleotides, DNA polymerase, primers.
2. b) DNA's denaturation, annealing of the primers and elongation. When DNA denaturates the two DNA strands apart from one another and one stranded DNA molecules are formed. In annealing the primers find their corresponding place based on the base pair rule from the one stranded DNA molecules and attach. In elongation DNA polymerase builds a corresponding strand for the one stranded DNA. The polymerase uses the attached primer as a starting point and attaches nucleotides based on the baise pair rule.
2. c) DNA-polymerase can only add nucleotides to DNA srand's 3' end. Without primers DNA polymerase does not have a place to attach nucelotides to.
2. d) Corresponding strad: 5' TCTAGCATCGAAGTACTCTAGCATCGAAGTACTCTAGCATCGAAGTACGT 3'
Primers: 5' TCTAGC 3' ja 5' ACGTAC 3'

2. Apply knowledge:

- **PCR thermal cycling is incorrect:** Ensure that the temperatures in the different stages of the cycle are correct and tests that there is no fault in the PCR device, in which case the temperatures in the device are actually different from what is programmed for it.
- **Primers**: Carefully redesigns the primers and adds excess primers to the reaction (more than the desired amount of amplified DNA molecules).
- **Human error**: By labeling the tubes carefully and accurately and maintaining a clear work order. For example, by moving the tube to a different row of the tube rack after adding the reagent, so that you can track which tubes have had reagent added so far and which have not.

3. a) The substance to be examined is pipetted into a small well at the edge of the gel. → An electric current is passed through the gel, i.e. a negative pole (-) is placed on one side and a positive pole (+) on the other. → Negatively charged molecules travel towards the positive pole, positive ones towards the negative pole. → Smaller molecules travel through the pores of the gel faster and travel further than larger ones. → The gel is stained or illuminated with UV light, forming bands that show the size of the molecules.

3. b) 1- molecular size reference marker, 2- bacterial DNA, 3- contaminated bacterial DNA, 4- negative control

3. c) 9000 bp (bp = base pairs)

3. Apply knowledge: The sample belongs to the suspect number 3.

4. a) Multidrug-resistant hospital bacteria arise as a result of genetic changes (mutations and gene transfer) and strong antibiotic pressure. After the use of antibiotics, bacteria that are more resistant to the antibiotic may remain and continue to multiply. They are therefore a product of evolution: the hospital environment selects the bacteria that have the ability to survive many different antibiotics.

4. b) The bacteria is sensitive to fidaksomicin (FDX) and vancomycin (VAN).

4. c) C. difficile also known as Clostridioides difficile.

4. d) I have determined that the bacteria in question is **Clostridioides difficile/C. difficile**. I recommend **vancomycin or fidaxomicin** for the treatment of infected patients, as C. difficile is not resistant to these antibiotics according to the antibiotic test performed on a culture plate.

4. Apply knowledge: It is very important to finish the full course of antibiotics and to **not stop** taking them as soon as your symptoms improve. There are several reasons for this:

- Total destruction of bacteria
  - Antibiotics kill bacteria gradually. If you stop taking them too early, some bacteria may survive. These surviving bacteria can cause another infection.
- Preventing resistance
  - If bacteria survive, they can become resistant to the antibiotic in question. This means that the same antibiotic may not work the next time, and the infection may be more difficult to treat.
- Recurrence of infection
  - Interrupting the course of treatment can lead to a return of symptoms or a chronic infection.
- Protecting the environment
  - Antibiotic resistance is not just an individual’s problem: resistant bacteria can spread to others, making future infections more dangerous.