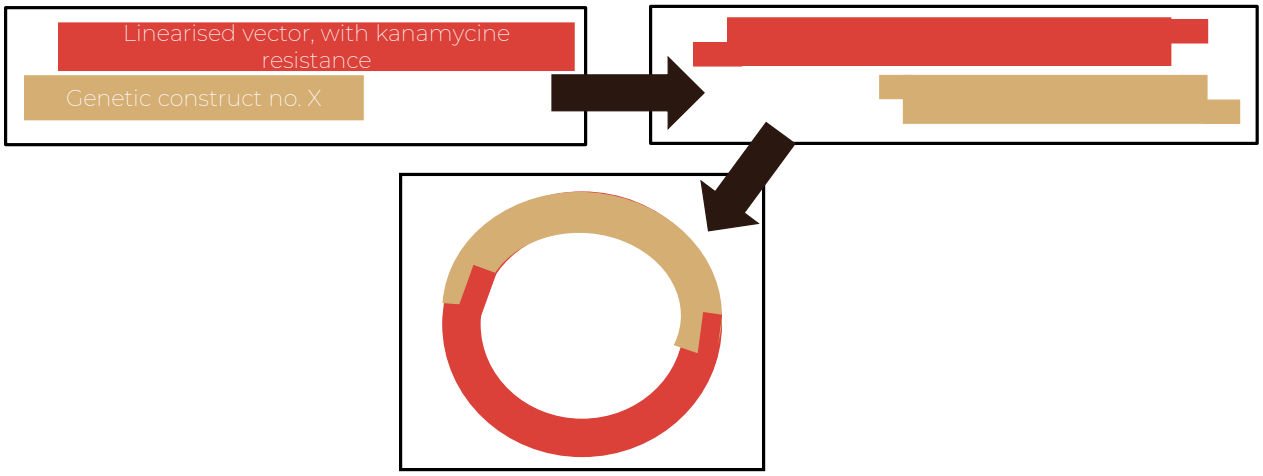
Who did it? A true crime lab story

Today you will be solving a laboratory issue that some students faced a couple of months ago, in the Time schedule & used protocols file you will find a general overview of the handling of the students, a description of the issue, some refreshers on the used techniques, detailed time schedules and the used protocols for every technique. By analyzing these documents and looking closely at some of the details, and by your knowledge of the techniques, you should be able to come up with one (or a couple) of explanations on what happened in the lab.

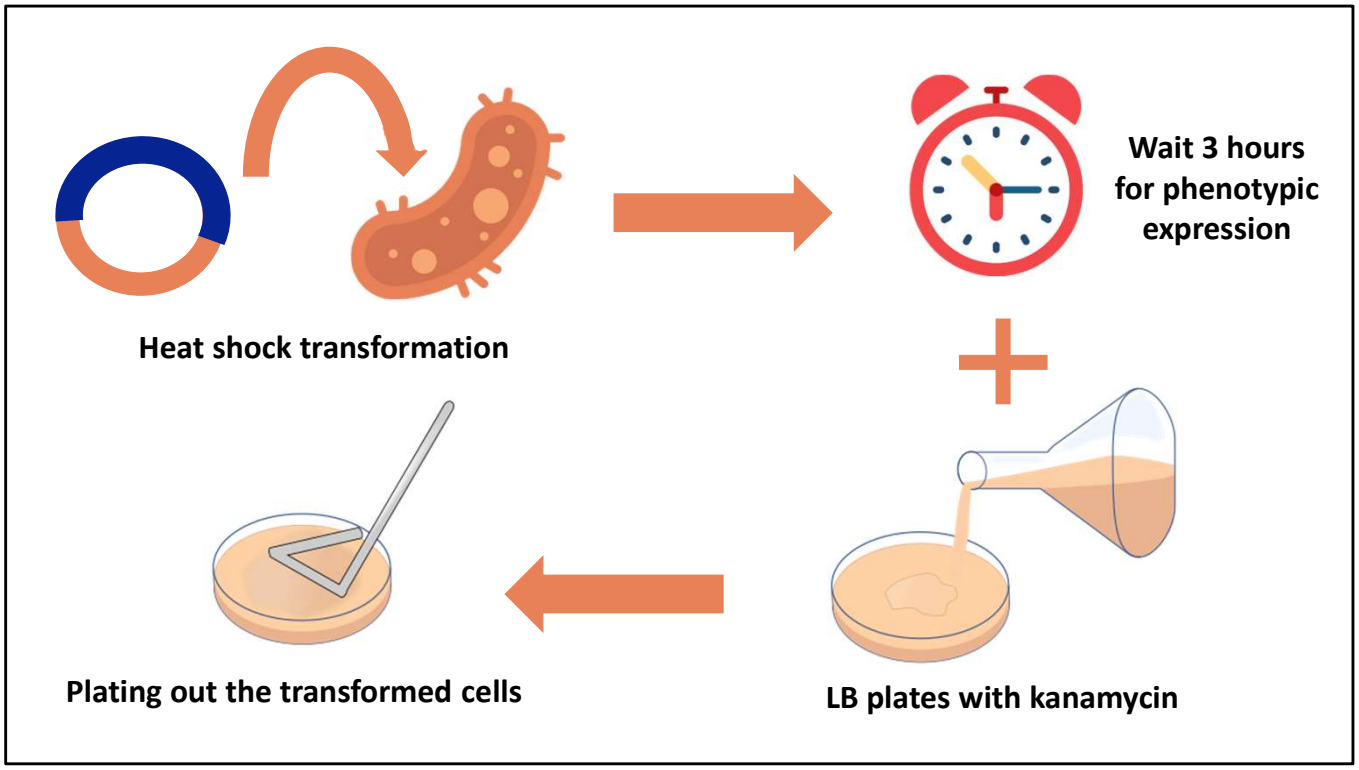
This story starts on Monday;

Jonas and Vita were at the lab all day and performed gibson assembly for 9 different genetic constructs.

A little recap of gibson:



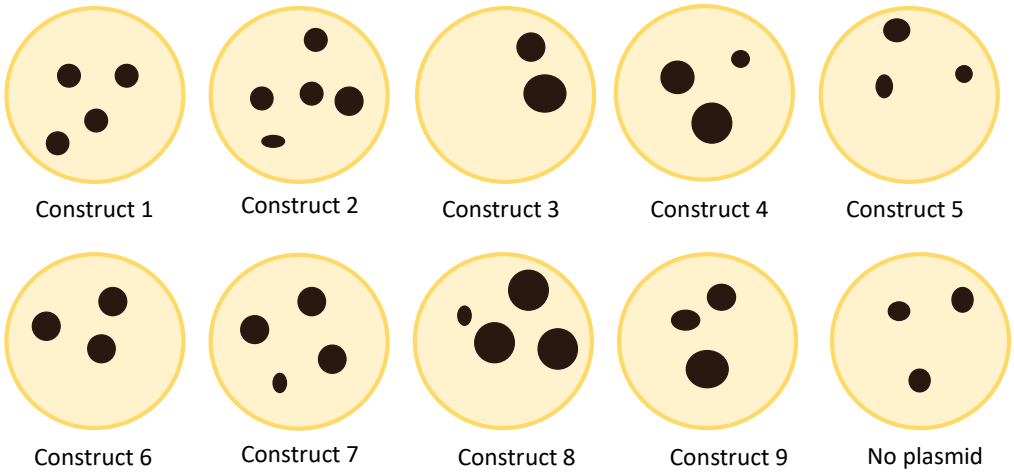
I'll start by walking you through the actions of the past days, you can always look at these slides in more detail later when conducting your investigation. The story starts with Jonas and Vita, who were performing the cloning pathway starting from gibson assembly reactions. This slide shows a general overview of how it works and in your time shedules you can find a protocol for it.



Gibson was followed by heat shock transformation, incubation and plating. They found that they had ran out of plates so they poured some new ones, again you can see all of the actions performed and when they were performed on the protocols and time sheets provided.

They transformed their vector by a heat shock transformation in a brand new batch of competent top 10 cells
They left the cells 3 hours of phenotypic expression, and in the meantime they poured some new kanamycine plates
They finally plated the cells and left for the day, they also plated a negative control: which consisted of only competent cells, without construct

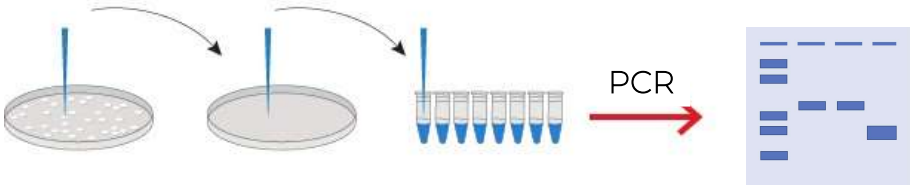
On Tuesday Jonas, Vita and Lander went to the lab and discovered the following…



When you do a transformation you need to let your cells grow overnight, so they left and came back the next day to this. As negative control they used their competent cells without any plasmid present.

The decision was made to perform a colony PCR!

What is a colony PCR?



- **Did the PCR reaction work?**
- **Is the insert present?**

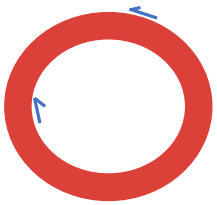
On the plates they performed colony PCR, you can look at this explanation and recap of PCR on this slide and the following slide. Again: protocol is provided.

Colony PCR is a technique to determine whether or not the plasmid presented during transformation is present in a given cell, the technique is done with a positive control next to it, which is most often the so called “empty vector” (the vector before a cloning step is done). The use of this positive control allows you to determine two things:

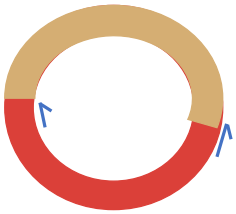
1. Your PCR reaction worked
2. The plasmid obtained through the cloning step is heavier than the empty vector, and thus your cloning and transformation might be succesful.

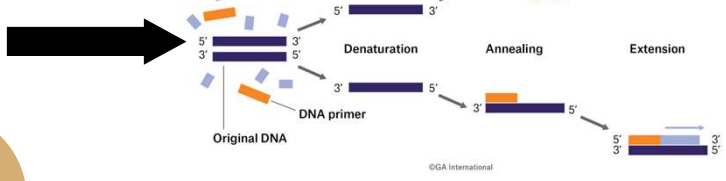
What is a colony PCR?

Positive control:



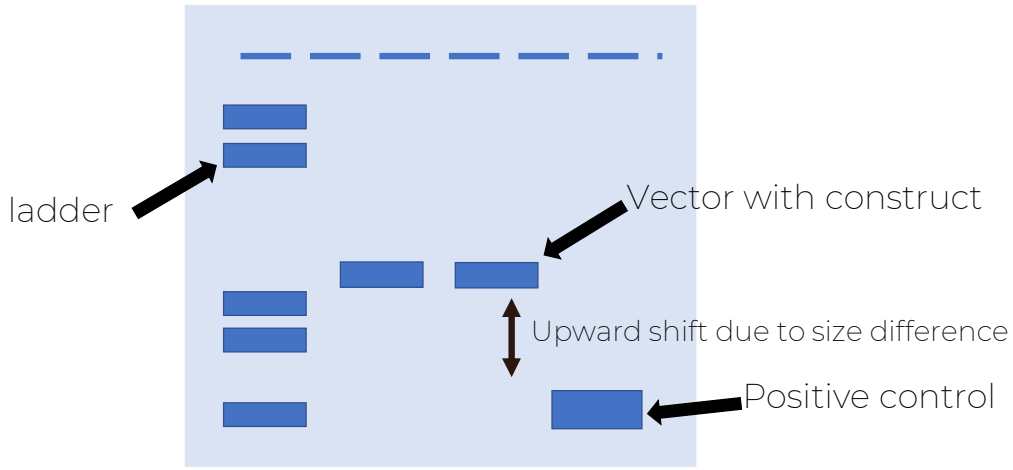
If the cloning went successful:





The positive control doesn't contain a construct, but it does contain vector

Visually you would expect a result like the following:

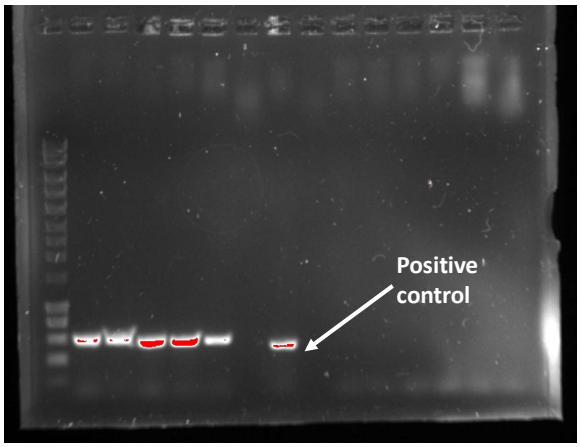


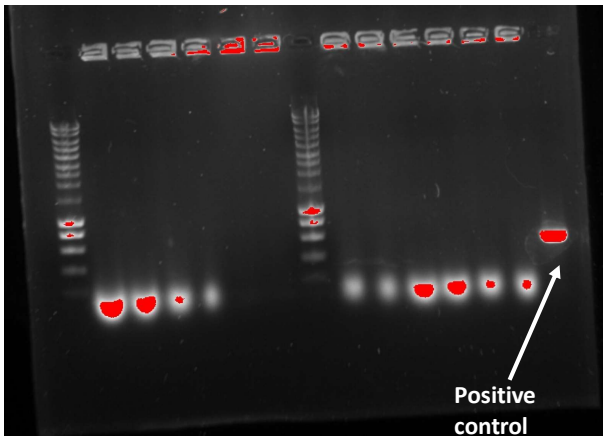
This slide can help you familiarise yourself with what an agarose gel of the colony pcr should look like

Gel results

But… in reality they obtained the following:

Expectations vs Reality





*Since the constructs are very small, the upward shift is minimal

But instead we saw this, for reference I gave a realistic example of the gels with a very small construct, so we've got a very minimal upwards shift. But what we saw that day in the lab looked like on the right. The stripes you see at the bottom are primer aggregates that you don't really have to worry about.

What do you think happened?

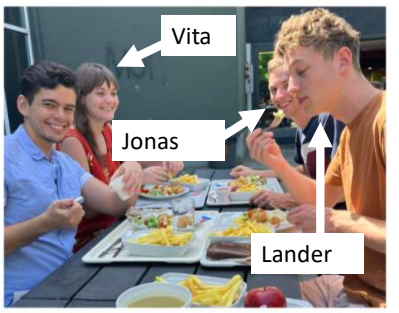
It’s up to you to figure it out!

- Did the PCR reaction work? How can this be determined?
    - Do the plates look like you would expect?
- Assuming the trio did a good job of pricking the colonies,what kind of bacteria give these type of results?

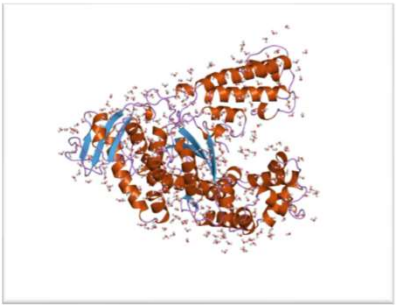
So now it’s up to you! Look through the evidence provided in these slides (the brown slides especially) and the time shedules and protocols to find out what went wrong in the lab to cause such a gel!

What the hell happened? Our approach!

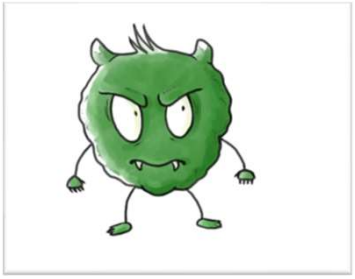
The suspects:



The scientists who maybe had too many fries and lunch and didn't prick the colonies right because they weren't thinking straight



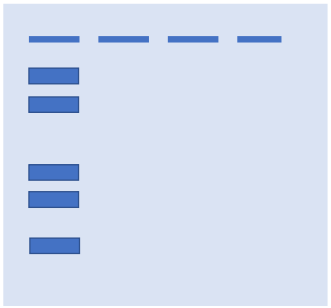
The PCR enzyme that maybe forgot to wake up that day and decided to amplify nothing



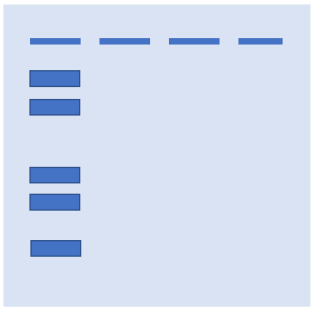
contaminants that came to ruin the day

What the hell happened? Our approach!

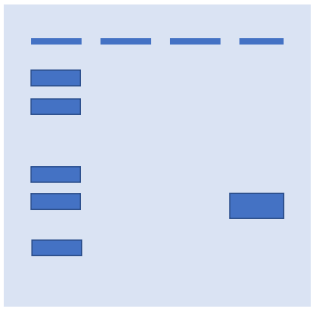
What the gell would look like:



The scientists



The PCR enzyme



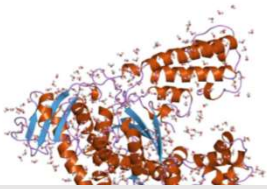
contaminants

What the hell happened? Our approach!

The suspects:

Innocent

The scientists who maybe had too many fries and lunch and didn't prick the colonies right because they weren't thinking straight



Innocent

The PCR enzyme that maybe forgot to wake up that day and decided to amplify nothing

contaminants that came to ruin the day

Where could the contamination have come from?

Hint: look in the time shedule!

Jonas

Vita

* The scientists were released from prison a few days later when, through repeating the experiment with propper antibiotics treatment it turned out that it was actually the entire stock of competent cells that was contaminated with kanamycine resistent bacteria, and their mistakes were pardonned.