Protocol for a school visit

The high school visit done during our project was a highly enriching experience, not only for the students, but also for us as a team. We created two lecture plans of different lengths. The planned lectures differed in scope. They contained various parts of common molecular biology experiments: a restriction digestion followed by an agarose gel analysis. Here we want to share our tips and plans regarding school visits with other iGEM teams. These protocols were refined over weeks to get it to a point of perfection. It is easy to follow, and the teenagers will have an exciting time and learn about molecular biology. Taking our protocol as a base for your own project is a fantastic opportunity to save time while planning your own school visit. This saved time can be dedicated to contacting schools and for work in the dry or wet lab.

Four-Hour-Plan

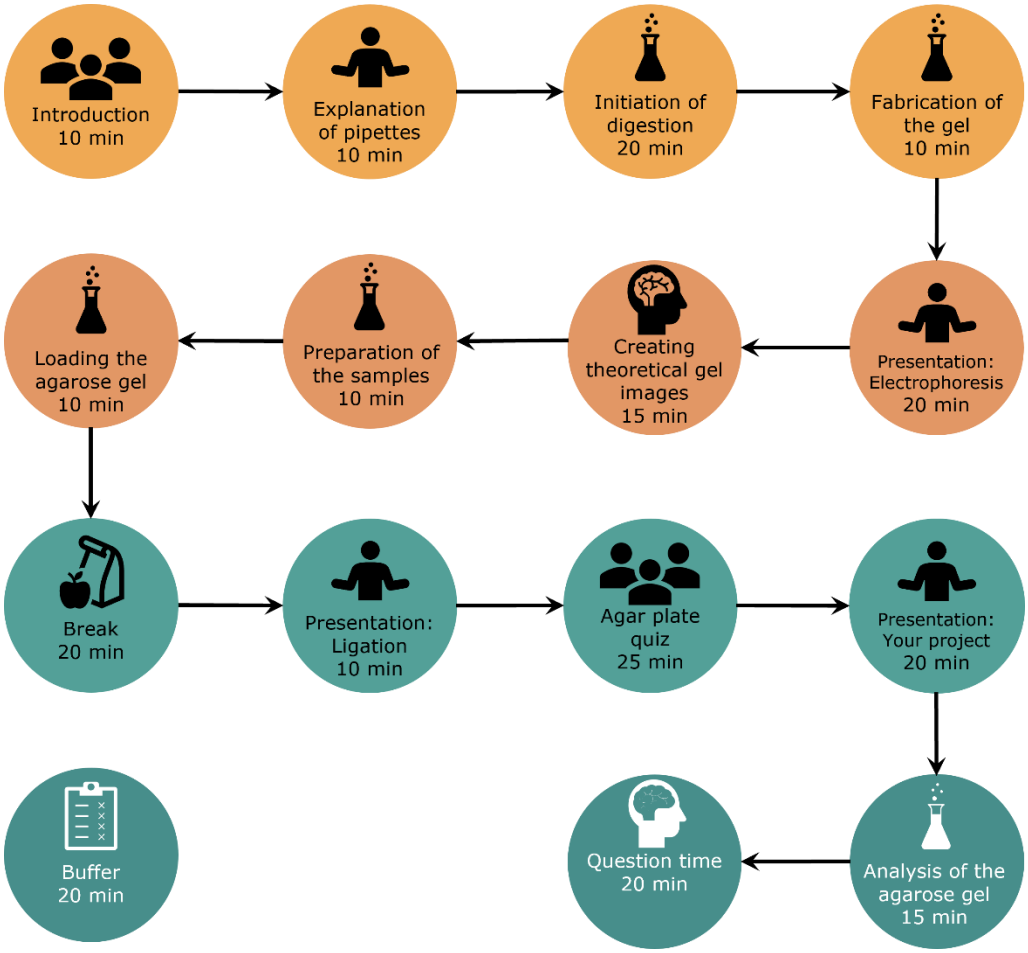

Figure 1: Simplified illustration of the schedule for a four-hour school visit

The initial plan outlines a four-hour instructional visit. A simplified overview is shown in Figure 1. The first 10 minutes are occupied for the team to briefly introduce themselves and mention our participation in the iGEM competition. Each team member shared a few sentences about themselves to create a relaxed atmosphere, allowing the students to perceive us on the same level and encouraging interaction. A more detailed explanation of who we are and what our project is, was provided later in the visit, as the experiments require a lot of time they should start as soon as possible. Students are divided into smaller groups, which are overseen by us. In these groups, the handling of a pipette is explained in 10 minutes and each student gets the chance to practice using a pipette.

With the knowledge of how pipettes work, the preparation of reaction mixtures for the plasmid digestions begins. Each group is responsible for

two reactions to ensure there is a backup if one digestion fails. Our team decided to prepare four digestion reactions considering the class size. In each digestion, our team's constructed plasmid YSD was modified and used. The plasmid map is shown in Figure 2.

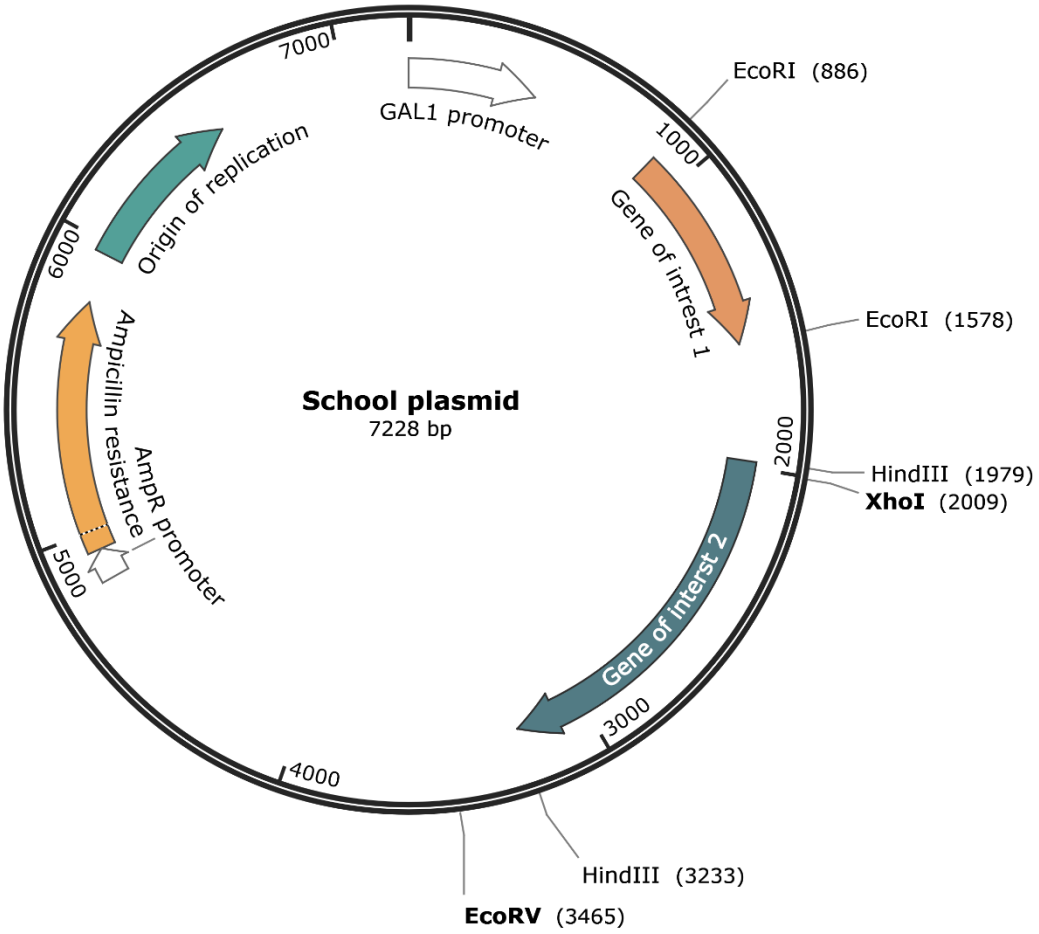

Figure 2 School plasmid vector

The plasmid was digested in four individual reactions. For the first reaction the restriction enzymes XbaI and EcoRV were used. The second reaction was digested only with EcoRI, the third used the restriction enzymes XbaI and HindIII and the last reaction with EcoRI and EcoRV. Our team refined the laboratory protocol for digestion by multiple tests to eliminate sources of errors due to incorrect pipetting. A detailed list of experimental setups and quantities used is listed in Figure 3.

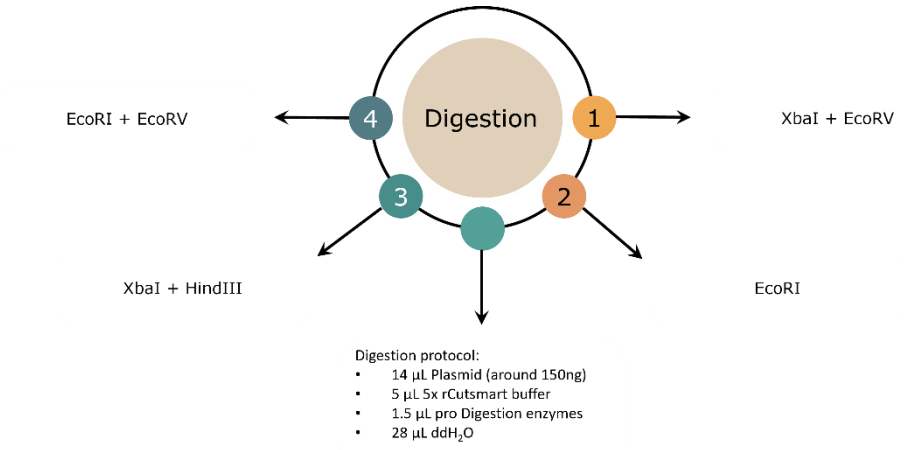

Figure 3 Overview of the Digestion

For pipetting the reactions, we aimed for a time limit of 20 minutes. The reactions were then incubated for 45 minutes in a heat block at 37 °C.

While waiting for the digestions, the students have ten minutes to prepare a 1% agarose gel. Here we also worked in duplicates to have an extra gel in case an error occurs. Subsequently, both agarose gels are given 45 minutes to solidify before loading. While waiting for the samples and gel to set, the lesson continues with a twenty-minute presentation on electrophoresis. After the presentation, students have knowledge of how electrophoresis works and can apply their knowledge for the next fifteen minutes by creating a theoretical gel image that fits our experiment. Next, the digested reactions are treated with loading dye, with ten minutes allocated for this step. The solidified gels then can be placed in the gel chambers and the samples are loaded into the wells. The applied voltage depends on the gel sled used, with a recommended voltage range of 90-120 volts. Ten minutes are allocated for this process.

As students have been actively engaged for some time, they are granted a twenty-minute break afterward. Following we prepared a brief ten-minute presentation on ligation. As time is running out, we wanted to include something special in the class. Therefore, we decided to create an agar plate quiz. Standard 1 agar was used for this purpose. Different plates were placed in various locations so that we have various plates with microbial growth to be assigned to separate locations later. A friendly competition between the distinct groups is encouraged. Students are expected

to discuss among themselves which area they expect to have the most microorganisms. This activity is allocated for twenty-five minutes. The final presentation in the instructional visit covers our BeeVAX topic. This presentation can introduce the respective iGEM team as well as provide a brief explanation of iGEM itself. This presentation takes twenty minutes. After the presentation, the gel should have run enough to be removed from the chamber and viewed under UV light. This is the moment when the students see the results of their hours of work. The results can now be compared with the theoretical gel images and checked for accuracy within the groups. If the gel does not match the expectation, the students can discuss why this might have happened. This entire process should take fifteen minutes.

The last item on the teaching agenda is a Q&A session with the students, where they can ask us anything. As working with students always comes with some challenges, we have also decided to allocate a twenty-minute buffer to ensure that if some points take longer than planned, it does not cause any problems.

Two-Hour Plan:

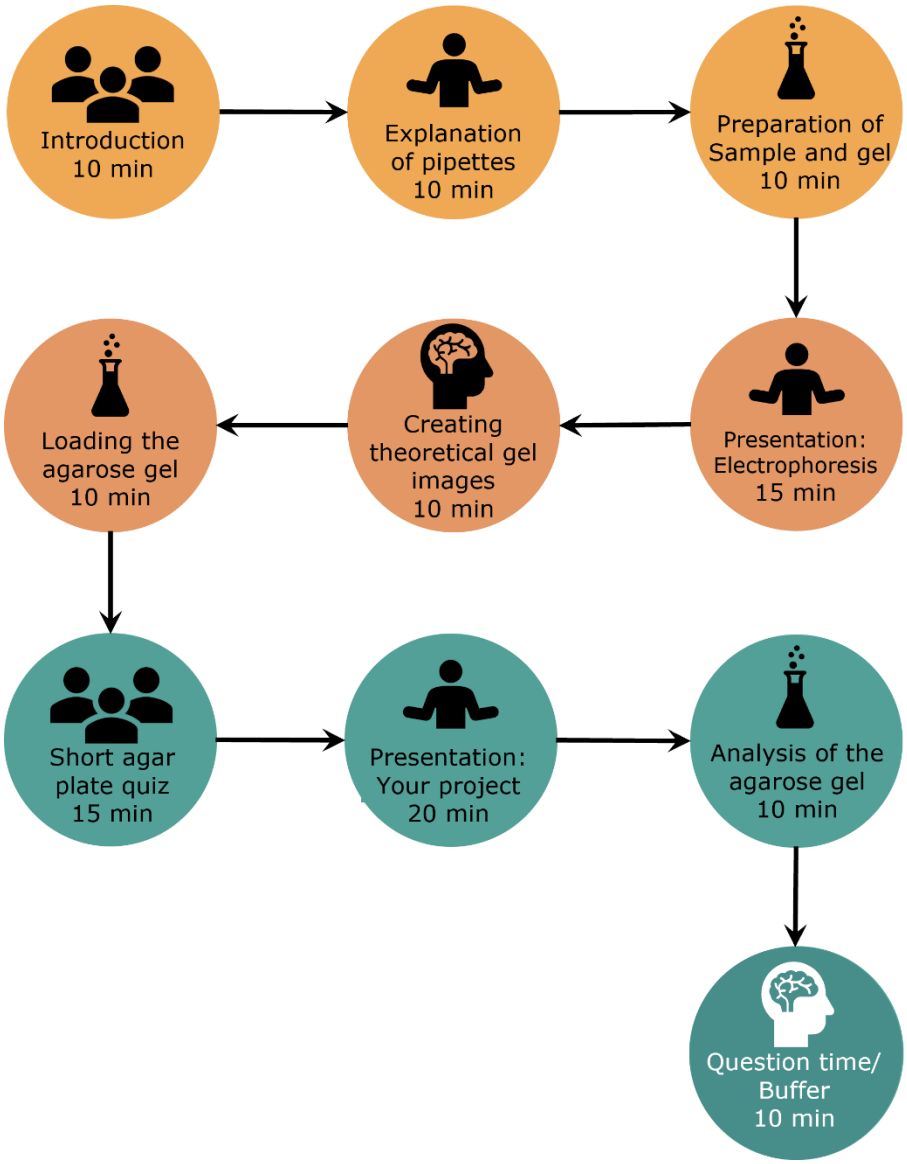

Figure 4 Simplified illustration of the schedule for a two-hour school visit

The plan for a two-hour instructional visit is quite similar. Due to time constraints, the digestion must be prepared in advance by the iGEM team. Otherwise, the structure is identical. As shown in Figure 4, the agarose gel is prepared simultaneously with the samples. Teams are divided to either work on the agarose gel or treat the samples with loading dye. The subsequent presentation, as well as the creation of theoretical agarose images, will be reduced by 5 minutes each. Similarly, a shorter version of the agar plate quiz with the students will be conducted, reducing it from twenty-five to fifteen minutes. Unfortunately, due to time constraints, the second

presentation on ligation had to be completely omitted. However, there were no deductions for the presentation on iGEM and this year's team project. Following this, only ten minutes will be available to analyze the gel image and the subsequent ten-minute Q&A session will also serve as a buffer.

Preparations:

Agar plate quiz:

Get samples from diverse places with variable numbers of microorganisms on ten standard 1 agar plates. We used our sterile fume hood, lab work bench, our office, toilet room, saliva, our refectory, a laptop keyboard, a used water bottle, a door handle, and a smartphone.

Testing of the digestion:

If you are using a different plasmid and enzymes than we are, perform the laboratory experiment multiple times in your lab to verify its accuracy and make any necessary adjustments.

For the 2-hour version: Preparation of the Digestion:

Since the digestion is not initiated at school, it should be conducted in advance in the laboratory.

What will you need:

Table 1 Checklist of materials needed for the school visit.

Materials	**Present**
Pipettes and pipette tips • 4x 2-20 • 4x 10-100 • 2x 1-10	
Gel chamber	
Gel sled	
Gel comb	
Power supply	
Agarose	
TAE buffer	
UV table	
Midori green	
Gloves in S/M/L sizes	
4x Loading dye	
4x Plasmid	
2x Restriction enzymes	
4x Reaction buffer	
4x ddH2O (deionized water)	
Eppendorf tubes (1.5mL)	
Thermoblock	
Laptop	
Worksheets	
Microwave	
Heat-resistant gloves	
Beakers	
Permanent marker	
Analytical balance (Fine balance)	
Graduated cylinder	
Paper towels	
Agar plates for the quiz	
Agar plates for the students	