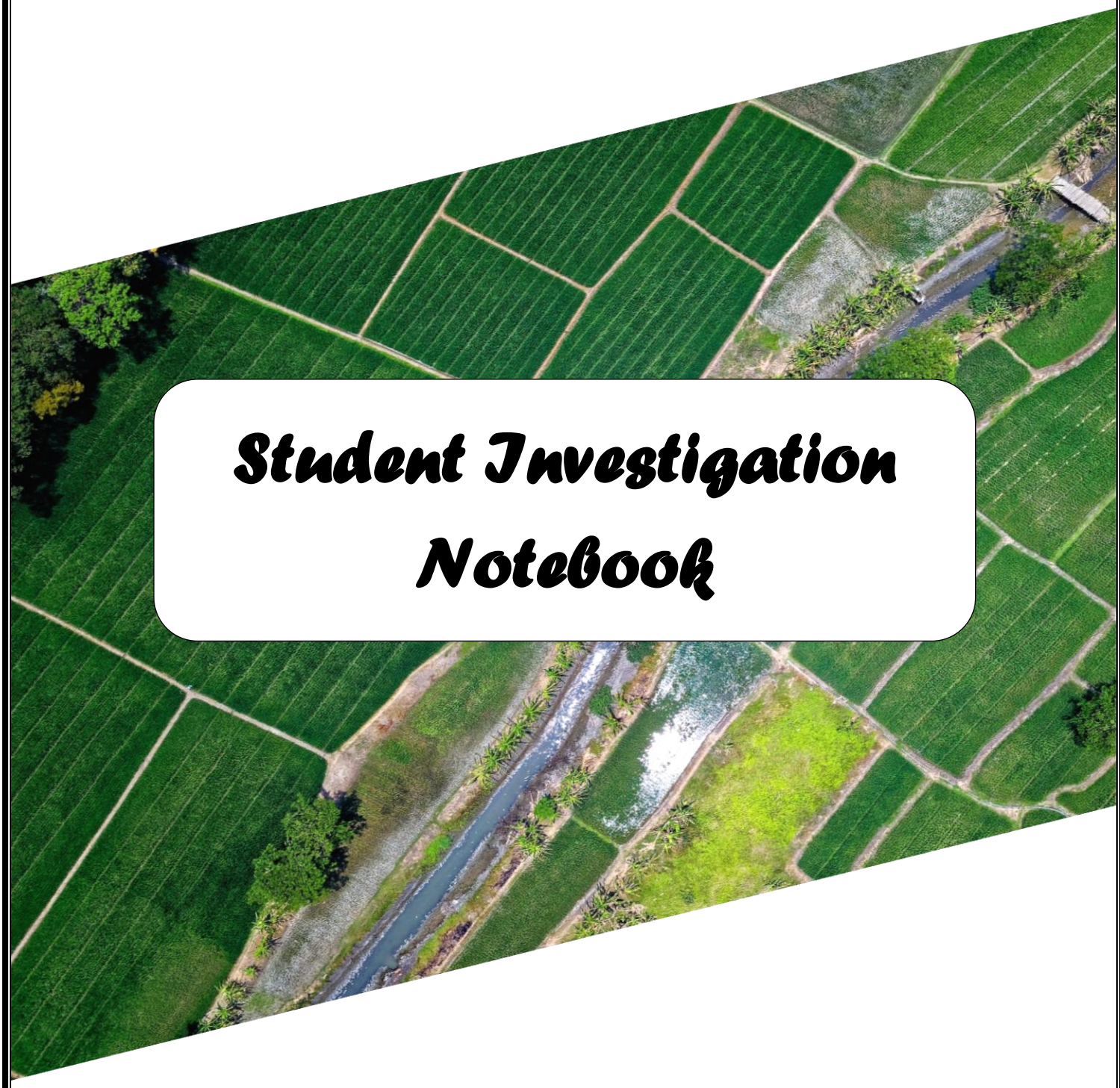
WHAT CAN WE DO?

ANTIMICROBIAL RESISTANCE IN AGRICULTURE AND BEYOND



Student Investigation Notebook

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Students,
All activities and investigations should be done with safety as a priority. You and your teacher are responsible for ensuring that all relevant safety rules including, but not limited to, those mentioned in this document are followed.

Getting to Know the Puppy Problem

Module 1, Activity #1

Students will…

- Obtain and evaluate information from a variety of sources.
- Write, categorize and prioritize questions generated by themselves and other class members.
- Ensure questions developed relate directly to the phenomenon.

Questions you have…

What are microbes?

Module 2, Activity #2

Students will…

- Access a variety of resources to develop claims about microbe structure
- Develop an initial model/explanation of their current understandings

Learn about microbes!

Using the resources provided by your teacher and others you find on your own, attempt to complete the table below. You need not create exhaustive lists of examples and characteristics! Focus on just a few for reach microbe type. You may or may not use all of the spaces provided in the table below.

Microbe type (common identification)	Specific examples (scientific names) and the diseases caused	General characteristics including shape (morphology), nutrition, reproduction and growth, metabolism, genetic properties, etc.

How Does AMR Happen?

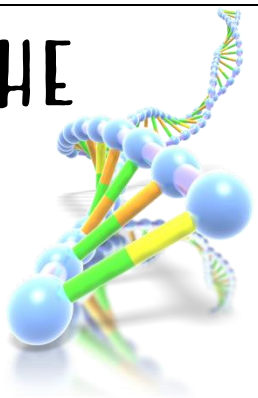
Module 2, Activity #3

Students will…

- Create a visual model of the process by which bacteria become resistant to antibiotics.
- Begin to understand the role humans play in the development of antibiotic resistant microbes.

Draw your model/concept map in the space below. Make sure you include arrows and "connection words" to help make sense of your ideas.

What Role Does DNA Play In The Life Of A Bacterium??



Module 2, Activity #4

Students will…

- Watch a brief video about the DNA structure, size and shape of bacterial DNA (https://www.youtube.com/watch?v=yQETaLlqub0)
- Watch another brief video about how "mega-plate" Petri dishes can be used to visualize bacteria becoming resistant to antibiotics (https://vimeo.com/180908160)
- Incorporate their learning into a "final" model of their understanding of the anchor phenomenon

Look back at your sticky notes from Activity #1. What additional questions do you have? What questions have you answered, so far? Keep track of your thinking in the space below…

Determining Resistance In Bacterial Populations

Module 3, Activity #5

Safety protocols must be observed throughout the following investigations. Specific techniques and protocols may be required in your school/district, and it is important to remember that many bacteria can cause disease, especially when present in larger-than-usual quantities, such as on a petri dish. Additionally, should any colonies of antibiotic resistant bacteria be present, special care should be taken to contain and properly dispose of these cultures. We suggest the use of appropriate personal protective equipment throughout the investigation, proper sterilization and transfer techniques, as well as proper disposal of waste. Please consult your science specialist or other documentation to ensure all safety precautions are taken.

Students will…

- Engage in a hands-on investigation to determine whether or not antibiotic resistance is present in a population of bacteria
- Use the information from the investigation to continue to refine their thinking around the anchor phenomenon

After incubating your plates for 24 hours, draw the results using the space below. Be sure to indicate WHERE your antimicrobial disc ◌ is located and record what antimicrobial substance you used.

Plate #1 antimicrobial used: _____

Plate #2 antimicrobial used: _____

Student reflections on Activity #5

Describe any zones of inhibition you noticed on your plate(s).

In what ways were you surprised by your results?

What additional variables would you like to manipulate if you were to do this experiment again?

How effective was the antimicrobial you selected?

What evidence do you have to support your claim?

Is there any additional information that you would like to have that would better support your claim?

Would you recommend this antimicrobial to a hospital? Why or why not?

Transforming bacterial DNA: ampicillin resistance

Module 3, Activity #6

Safety protocols must be observed throughout the following investigations. Specific techniques and protocols may be required in your school/district, and it is important to remember that many bacteria can cause disease, especially when present in larger-than-usual quantities, such as on a petri dish. Additionally, should any colonies of antibiotic resistant bacteria be present, special care should be taken to contain and properly dispose of these cultures. We suggest the use of appropriate personal protective equipment throughout the investigation, proper sterilization and transfer techniques, as well as proper disposal of waste. Please consult your science specialist or other documentation to ensure all safety precautions are taken.

Students will…

- Engage in a complex investigation to better understand the methods used by researchers to learn more about antibiotic resistant populations.
- Discuss how bacterial DNA transformation in a lab might lead to solutions for problems arising from antibiotic resistance in agriculture.

STUDENT INSTRUCTIONS

Genes control the traits that living organisms possess. Bacteria, such as E. coli, have genes on their chromosome and on a small circular piece of DNA called a plasmid. Genes can be transferred from one bacterium to another on the plasmid by a process known as transformation. In this experiment, a plasmid with a gene (DNA) for resistance to the antibiotic ampicillin will be used to transfer the resistance gene into a susceptible strain of the bacteria. The same technique is used to transfer genes (DNA) for production of insulin, growth hormones, and other proteins into bacteria. The transformed bacteria are used in fermentation to produce commercial quantities of the protein for treating diabetes and other conditions.

You will work with two other people while conducting this investigation.

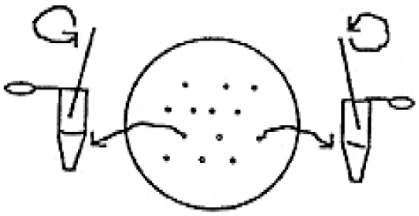
Day 1 (pre-lab)
- Collect cells
- $CaCl_2$ conditions the cells for following steps

Day 2
- Add plasmid DNA
- Heat shock
- Spread on plates
- Incubate

Day 3
- Analyze results

DAY 1 (pre-lab)

☐ Step 1. Use a separate sterile toothpick to transfer a colony of E. coli about the size of this 0 into each of two tubes of calcium chloride. Use the toothpick to stir the cells vigorously and thoroughly into the solution. The solution should appear milky. Close the caps of both tubes and discard the toothpicks into the container provided for that purpose. One person in the pair should label one of the tubes "B1". The other person should label the other tube "B2".

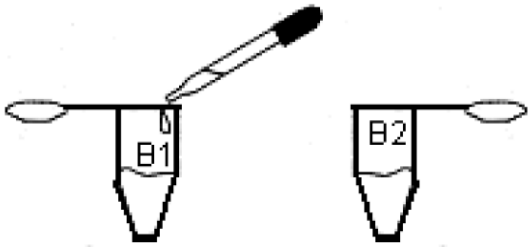


☐ Step 2. Place the tubes back in the ice and place the container of ice with tubes back in the refrigerator. (DO NOT FREEZE) (*The cold calcium chloride, in the tubes, conditions the surface of the bacteria for DNA uptake the following day.*)

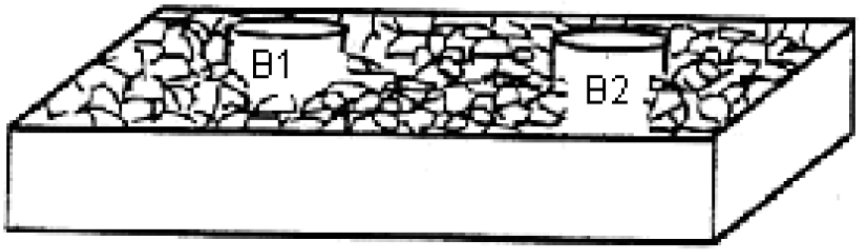
DAY 2

☐ Step 1. Finger flick tube to resuspend cells.

☐ Step 2. Open the tube labeled "B1" and with a sterile pipette add one drop of solution from the "P" tube. Close the tube. Do not add anything to the tube labeled "B2". (*The plasmid DNA, from the "P" tube, added to the tube has a gene for resistance to ampicillin.*)



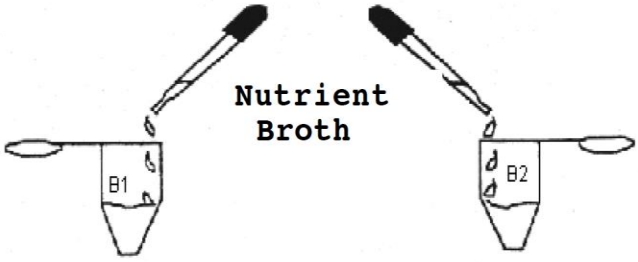
☐ Step 3. Place the tubes on ice for 15 minutes. (*The cells are kept cold to prevent them from growing while the plasmids are being absorbed.*)



☐ Step 4. Remove the tubes from the ice and immediately hold them in a 42°C water bath for 90 seconds. (*The marked temperature change causes the cells to readily absorb the plasmid DNA*).

Steps 3 and 4 can be repeated for up to a total of 3 times. This may improve plasmid incorporation into the competent cells.

☐ Step 5. Use a sterile pipette to add 5 drops of sterile nutrient broth to each of the tubes. Close the tubes. Mix by tipping the tube and inverting it gently (*The bacteria are provided nutrients to help them recover from the calcium chloride and heat shock treatments*).

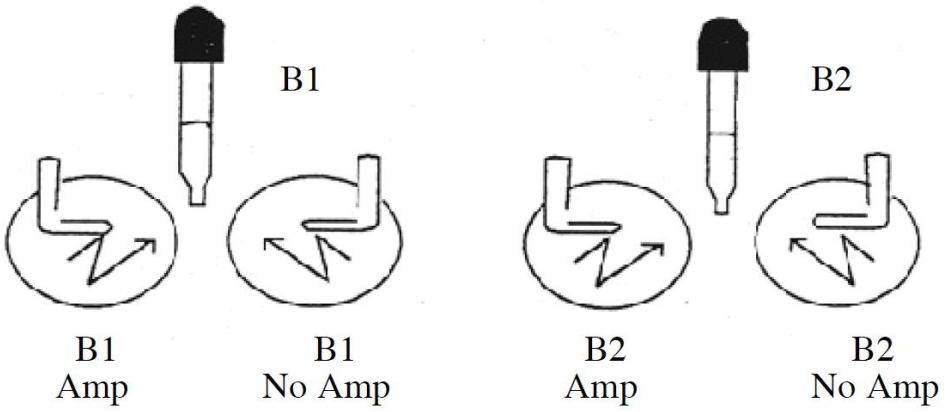


Note: For better results allow cell recovery at 37°C for any amount of extra time, 20 minutes preferred.

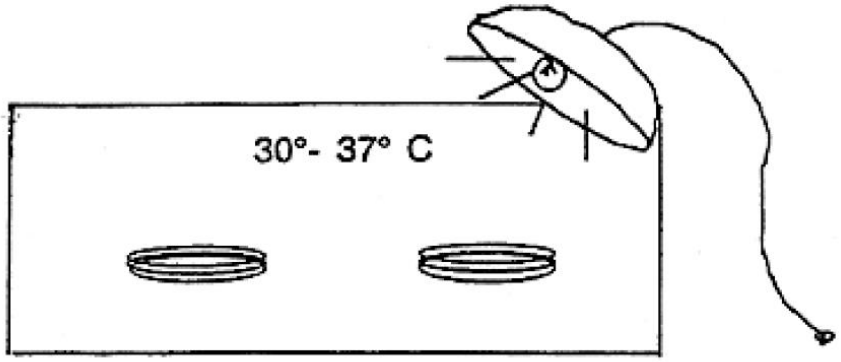
☐ Step 6. Label the underside of the four petri dishes with your name. On one "Amp" plate, print "B1" and on the other "Amp" plate print "B2". On one "No Amp" plate print "B1" and on the other "No Amp" plate print "B2".

☐ Step 7. Use a fresh sterile pipette to place 3 drops of cell suspension from the tube labeled "B1" onto the center of a petri dish labeled "Amp"/"B1" and 3 drops to the center of a dish labeled "No Amp"/"DNA". Use another fresh sterile pipette to place 3 drops of cell suspension from the tube labeled "B2" onto the center of the dish labeled "Amp"/"B2" and 3 drops to the center of the dish labeled "No Amp"/"B2".

Use a fresh sterile paper clip to spread the liquid evenly across the surface of each plate. Do not touch the part of the paper clip that comes in contact with the agar.

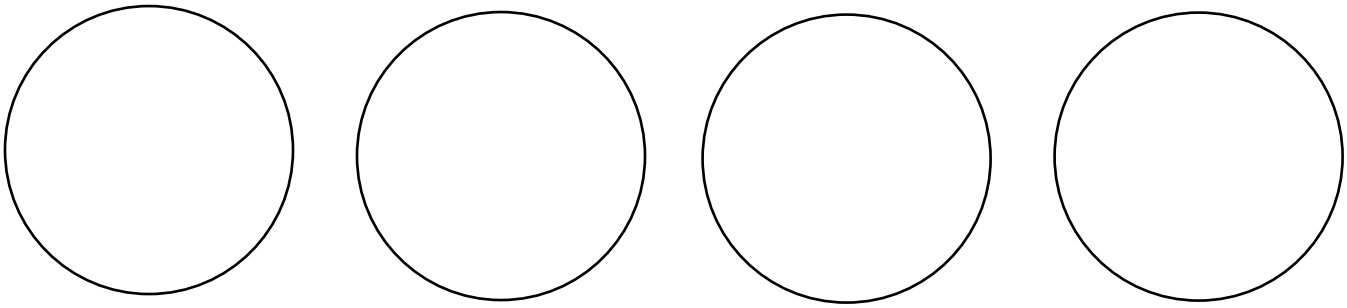


☐ Step 8. Incubate the plates upside down for 24 hours at 37o C.



DAY 3

☐ Step 9. Analyze the results of the transformation by placing the two plates labeled "Amp" and the two plates labeled "No Amp" together. Record your results below…



B2 (Amp) B1 (Amp) B2 (No Amp) B1 (No Amp)

On Health in action

Module 3, Activity #7

Students will…

- Thoughtfully discuss the major tenets of the One Health initiative and how it can be used to mitigate the effects of antimicrobials in agriculture and beyond
- Explore the antibiotic use pathways in each of the One Health domains and connections among them
- Negotiate peer-to-peer conversations in order to develop consensus understanding of the One Health initiative

Materials Needed:

- Technology access for all students
- Student notebooks or similar method to record findings
- Optional: poster paper, dry erase board

Supporting Resources provided by the Soupir Lab

One Health
https://www.cdc.gov/onehealth/index.html

https://blogs.cdc.gov/global/2016/11/01/one-health/

https://onehealthinitiative.com/

https://www.who.int/news-room/q-a-detail/one-health

https://www.cdc.gov/onehealth/images/multimedia/one-health-definition-graphic-with-bats.jpg

One Health - AMR
https://www.health.state.mn.us/onehealthabx/

https://www.ecdc.europa.eu/en/antimicrobial-consumption/facts/infographics

https://www.cdc.gov/globalhealth/infographics/pdf/World_Antibiotic_Awarness_Infographic-1st.pdf

https://www.ecdc.europa.eu/en/publications-data/antibiotic-resistance-how-does-antibiotic-resistance-spread

https://www.amr.gov.au/resources/infographic-how-antibiotic-resistance-can-spread

Record your thoughts, research, and ideas here…

Jyll & Jakk Dilemma

Module 4, Activity #8

Jyll thinks:

The fact that there are an increasing number of bacteria that are resistant to common antibiotics is a problem that we must address quickly! We need to invest in scientific research that gives us new ways to improve livestock health and quality without the use of any antibiotics. If we don't do this, more and more bacteria will be resistant to antimicrobials and more and more people and animals will die of infections.

Jakk thinks:

Antimicrobial resistance in bacteria is a naturally-occurring phenomenon. We should make sure the use of antibiotics in livestock is carefully monitored by expert agriculturists, but getting rid of them altogether isn't a realistic solution. The number of cases of people being infected with resistant bacteria from an animal source is very low and doesn't really pose a problem, anyway.

Student instructions: using what you know about antimicrobial resistance and the use of antibiotics in agriculture, decide who you agree with more – Jyll or Jakk. Providing evidence of your choice is important and will help you build an argument for your decision.

With which position do you agree most? ☐ **Jyll** ☐ **Jakk**

Supporting Evidence for Your Position

Evidence from within this unit of study	Evidence from outside this unit of study

Counter-argument Evidence

Evidence from within this unit of study	Evidence from outside this unit of study