First iGEM 2021 Meeting
Zoom link for meetings: https://zoom.us/j/2078779459?pwd=OW95b3h0UHRvZ2o0UjJ3YTNWb1lHQT09

1. Quick introductions
2. Overview of iGEM
    a. Teams
    b. Deliverables
    c. Wiki
    d. Byford - Set up Benchling and send join emails
    e. Byford - list of groups needed on website; send email with instructions for editing
    f. Byford - Grant application submitted 6/12/21 4:15pm EDT
    g. Members - pick 2-3 topics from iGEM to study over the next week
    h. Members - Think about local issues and the sustainability goals and come with at least 1 new idea
    i. Safety form due June 25
3. When do we meet next? Next Friday June 18 9pm Eastern / 9am Saturday June 19 Beijing
4. Projects
    a. Water sanitation - detect pollutants
    b. Process garbage using bacteria
    c. Increase amount of CO2 plant can absorb
        i. Produce more oxygen - link to NASA
        ii. Plant matter to sustain ecosystem - Biolab 2
    d. Bacterial insecticide (bt)
        i. Prevent spread through pollination
    e. Musical DNA
        i. Differentiate promoters by sound
5. Problems
    a. Microplastics in water
    b. Fragile/overripe fruits or vegetables
    c. Overfishing
    d. Biodiversity falling - less stable ecosystems
    e. What local issues are there?
        i. Invasive species (kudzu)
    f. Water pollution
        i. Brain-eating amoeba
        ii. Fish kills
        iii. Algae overgrowth
        iv. Coal ash (heavy metals)
    g.
6. Safety Grant
    a. Overall project: (150 words max)
        i. Bacteriophages have been used to transfer DNA from one bacterium to another in labs. It has also been shown that bacteriophages can participate in the transfer of DNA in the environment and that some can transfer genetic material between species. We are planning to assess the rate at which wild bacteriophages contribute to the horizontal spread of bacterial genes, particularly antibiotic resistance. Risk of transfer should be

directly related to the rate at which bacteriophages pick up DNA from the engineered bacteria. We would like to develop a set of simple inexpensive procedures or a test that can be used to assess a wide range of environmental conditions. The makeup of our team is particularly well suited to testing multiple areas since half of the team is based in North Carolina, USA and the other half is based in China. (140 words)

b. Technical advances in safety and security (1-3 sentences)
    i. We plan to develop a simple and inexpensive procedure, model, and/or test to measure the potential for horizontal gene transfer in a given environment. The procedures should be relatively quick and not require specialized equipment, making it accessible for a wide range of applications.

c. What research, designs, and/or experiments will you carry out in order to pursue these technical goals?

    i. Control Data: Using well characterized bacteriophages and E. coli (subtypes of K-12), we will establish the rate of plasmid transfer using a plasmid producing Red Fluorescent Protein and chloramphenicol resistance using standard transduction procedures and optimal conditions to provide a maximum expected rate.
    ii. Wild bacteriophages in ideal conditions: Isolate the bacteriophages from a wide range of settings and determine the percent of samples that have one or more bacteriophages that can infect one or more subtypes of *E. coli* and then determine the frequency that either the RFP or chloramphenicol resistance gene are transferred. Semi-quantitative PCR could give an estimate of the frequency that the genes are picked up by the bacteriophages. Procedures used for isolating wild bacteriophages are those recommended by the SEAPhages program sponsored by HHMI (https://seaphages.org/).
    iii. Known and wild bacteriophages in environmental conditions: Add standard amount of either known or wild bacteriophages that have been grown with bacteria containing the RFP/chloramphenicol resistant plasmid to water or soil collected from various locations. At time points, sample the water/soil and plate bacteria to assess chloramphenicol

resistance and RFP production. Bacterial and phage DNA can be isolated to detect evidence of RFP or chloramphenicol resistance genes. DNA-level detection is needed since the promoters are optimized for E. coli. All soil and water samples will be sterilized for 20 minutes at 15 psi and then tested for the presence of live bacteria and phage before disposal. Samples can also be treated with 10% bleach if needed. Once plated, wild samples will not be worked with on the open bench. After overnight growth, plates will be sealed (by the instructor) before colonies and plaques will be counted on sealed plates. No individual bacterial strains will be isolated from the wild samples; DNA will be isolated from samples using a commercially available kit. Potential environmental conditions include pond water, creek/river water, salt water, soils from various locations and of various types (rich and fertile, red clay, leaf litter, etc), variable light and humidity levels, etc. Procedures for working with wild phage are modeled on those in the SEAPhages program sponsored by HHMI (https://seaphages.org/).

iv. Known bacteria spilled in various (contained in the lab) environments: These experiments will add one more step to the process by mixing bacteria with the known plasmid with samples containing wild bacteriophages. Testing of the samples can be carried out as above. Additionally, samples can be tested for the persistence of the original plasmid by looking for both RFP production and chloramphenicol resistance.

v. Modeling: Data from the experiments, particularly parts i and ii, can be used to develop a predictive model for transmission rate. The model can be tested and improved using data from part iii.

vi. Additional analyses: The persistence of bacteria in various environmental conditions can be measured. It will also be possible to assess the uptake of plasmid DNA into plants grown in soil containing either bacteriophages, live cultures, or DNA. While this seems an unlikely pathway, it should be tested.

d. What will this grant allow us to do that we couldn't do otherwise?

i. This grant will allow us to pursue iGEM without constantly counting pennies. The team operates on a budget of under $1500 for supplies for the entire project. We currently

work with equipment that is old (our PCR machine requires mineral oil overlay) and without the use of commercial kits (plasmid preps, for example) because they are too expensive. With this grant, we can get a more efficient PCR machine (such as the one by miniPCR https://www.minipcr.com/product-category/minipcr-thermal-cycler/) and buy the petri plates, agar, enzymes, DNA isolation kits, and syringe filters with primary concern for getting the project completed with high quality materials instead of spending hours on ebay to find things that should work. In terms of the project, we will be able to test a significantly wider range of environmental conditions and increase the number of trials at each point which will increase the quality of the study overall. Any money not used on supplies will help defray the costs of the Jamboree, whether it is online or some version of an in-person event.

ii. If we don't receive the grant, we will probably continue with a much smaller version of the project. Unfortunately, we are severely limited in the amount of fundraising we are able to do because of regulations of the school. We can set up a fund-raising site, but we cannot send the link to anyone associated with the school. We cannot do any on campus fundraising activities at all. For equity reasons, the team is very reluctant to charge a participation fee since team members already pay all entry and travel costs themselves, including the instructor.

iii. Up to $2000 of the grant will be used to replace our old PCR machine and the rest of the money will be used for supplies to conduct the project. No money will be used for salary or overhead. Any not used for supplies will be applied to the team fee for the Jamboree.