Safety Form

Part 1: Overview

1. Please upload a photo or two of your lab to the iGEM 2020 server (include your team name in the file name), preferably showing the relevant safety features and paste the link here: *(Instructions on how to upload an image to our servers can be found **here**)*

2. Describe the goal of your project: what is your engineered organism supposed to do? Please include specific technical details and names of important parts.

(Even though your project might change, please describe the main project idea you are working on right now. See the example answers for help.)

The goal of the project is to design, build, and test a bacterium which produces PDU bacterial microcompartments, to which zinc-finger-domain-containing, double-stranded DNA scaffolds will be attached through interactions between shell and zinc finger fusion proteins. Two such scaffold sequences will be present, where one contains ZFc, PBSII, and Zif268 recognition sequences, while the second contains ZFc, ZFa, and ZFb recognition sequences. Furthermore, the cell will also be producing the following fusion proteins: (4-Coumarate-CoA ligase)-Zif268, (Stilbene synthase)-PBSII, pduD-ZFc, (Acetyl-CoA carboxylase)-ZFa, and (Acetyl-CoA carboxylase)-ZFb. These proteins will complex with the DNA scaffolds, allowing the enzymes to be recruited to the inside of forming BMCs while also remaining properly oriented relative to one another. Also of note, the PDU BMC shell consists of the proteins pduA-B-J-K-N-U and T, and the double stranded scaffolds will be produced by HIV-Reverse Transcriptase and Murine Leukemia Reverse Transcriptase which will be selectively acting on ncRNAs encoded by r_oligo genes corresponding to each scaffold sequence, in addition to its reverse complement.

Part 2: Identifying possible risks

iGEM has developed a **Risk Assessment Tool** to help you identify possible risks to you, your colleagues, communities, or the environment. We encourage you to use this tool before filling in this part of the form. What are you using / planning to use? Some organisms and parts present risks beyond what is ordinary for lab work in synthetic biology. As your project progresses, you should consider the risks presented by each organism and part you plan to use.

3. Which whole organisms, including viruses and cell lines, are you planning to use or using in your project?

Please provide as much detail as possible (such as strain information). If you are not using an organism, please note this.

New England Biolabs BL21(DE3) *E. coli*

4. What risks could these organisms pose to you or your colleagues in the laboratory, or to your community or the environment if they escape the lab?

If you are not using an organism, please note this.

This strain of E. coli is non-pathogenic, and is risk group 1. There is a small risk of toxicity for those who are immunocompromised, which can be mitigated by common lab safety procedures; ie. wearing gloves, sanitizing the workspace often, hand washing, and preventing the organism from leaving the lab by adequately sanitizing the workbench after using the organism.

5. What organisms are you using as chasses in your project?

For the purposes of iGEM, a chassis is the organism in which you are putting your parts, or which you are modifying in your project. Many teams will use a common lab organism as a chassis. Some teams may use a more exotic organism. Some project may not involve a chassis.

Escherichia coli BL21(DE3)

6. What risks could your chassis pose to you or your colleagues in the laboratory, or to your community or the environment if they escape the lab?

If not using a chassis organism, please note this.

This strain of E. coli is non-pathogenic, and is risk group 1. There is a small risk of toxicity for those who are immunocompromised, which can be mitigated by common lab safety procedures; ie. wearing gloves, sanitizing the workspace often, hand washing, and preventing the organism from leaving the lab by adequately sanitizing the workbench after using the organism.

7. Which chemicals are you using in your project that might be hazardous?

- Carcinogens
- Other controlled chemicals

(If you selected any of the above, please list the chemicals)

Ethidium bromide, Acrylamide, Phenol, Chloroform, Isoamyl alcohol, HCl, NaOH, Sodium dodecyl sulfate, TRIzol™ Reagent, 100% Ethanol.

Parts

This part of the form is for you to tell us about the parts you are planning to make or have developed during your project. It summarises information that might already have been submitted through **Check-in forms**. If you submitted a **Check-In form** for a part, you should still include it in this section. You may omit parts that were already in the Registry if you are using them without significant modifications.

For more information on virulence factors see the **Safety Policy** page and the **White List**.

8. As part of your project, are you planning to make / have made new parts or substantively changed existing parts in the Registry.

- **Yes** *(All relevant new or revised parts should be described on a spreadsheet)*
- No

9. Part information is submitted in a spreadsheet.

Instructions for parts spreadsheet: *Remember to change the filename of your spreadsheet! Put your team's name in place of "TeamName". Please visit this page to download a blank copy of the spreadsheet for this question.*

See attached spreadsheet

10. What experiments will you do with your organisms and parts?

Please explain briefly. We are particularly keen to understand the boundaries or scope of your project. You should include the names of species / cell lines / strains. You should include experiments involving parts taken from other organisms, even if they are being synthesized rather than isolated from nature – you need not include any parts already in the registry.

All of the experiments will be performed in BL21(DE3) *E. coli.* This same chassis will be used in both the assembly of the device components and the experimental testing of the device. Device assembly will primarily use PCR, Golden Gate Assembly and Site Directed Mutagenesis for sequence manipulation and gel electrophoresis (both PAGE and Agarose), qPCR, phenol chloroform extraction, and spin column kits for purification and screening. In order to test the device, we plan on measuring the rate, concentration, and efficiency of resveratrol production by HPLC analysis of the culture medium (TB medium) and comparing these measurements to a standard curve generated from a mix of medium and resveratrol.

11. What risks could arise from these experiments?

For example, could they produce aerosols making it more likely that you could inhale something? Or are you using needles and could accidentally stick yourself? Could you produce something that is not inactivated using standard lab protocols? If you are not conducting any experiments, please note this.

Exposure to the dangerous chemicals previously listed could pose a risk to researchers, but would not have any impact outside of the lab. This could occur via skin contact, inhalation, or eye contact, but all of these risks can be prevented by using personal protective equipment and safe lab practices. As for the resulting device, the chassis organism could be inhaled or ingested if safe procedures are not followed, but would not cause damage because the device requires the presence of certain enabling chemicals to function, the chassis is non-pathogenic, and the organism can be killed by mild antibiotics.

12. Are you collecting any data about people, such as their opinions, quotations, health information, gender, behavior, attitudes, or concerns?

This includes surveys and interviews carried out as part of human practices work, whether anonymous or not.

- No
- **Yes** ***(Please read iGEM's policy on Human Subjects Research very carefully. For good reasons, many countries require formal approval for Human Subjects Research, as well as consent procedures for participants. You may need formal permission from a Research Ethics Committee, Institutional Research Board, or***

equivalent. Remember compliance with relevant laws and regulations is a requirement for participation in iGEM.)

13. Imagine that your project was fully developed into a real product that real people could use. How would people use it?

Check all appropriate boxes and expand in the comments section. (Note: iGEM teams should not release modified organisms into the natural environment but you could imagine such a release if your project was fully developed.)

- Our project is foundational / we do not have a specific real-world application in mind *(Examples: library of standardized promoters, system for communication between cells)*

- In a factory *(Examples: cells that make a flavor chemical for food, cells that make biofuel)*

Our device can be used in bioreactors, and is a foundational advance.

14. What safety, security or ethical risks would be involved with such a use?

Virtually all modern life science and biotechnology carries with it some risks. These can be identified and managed helping to ensure your work makes a positive impact on the world. Basic risks are managed for you in many institutions. As you think about how your project might enter the real world, being a responsible biological engineer will require you to think about and manage these risks yourselves. You can find some great resources on the ***Safety and Security*** *and* ***Human Practices*** *hubs on our wiki. It is even possible that software projects could also pose relevant risks.*

Our project compartmentalizes biosynthetic pathways, which has the potential for numerous applications. Regarding the ethical risks: toxic chemicals, bioweapons, or environmentally hazardous chemicals could potentially be produced using our device without killing the chassis organism due to the increased compartmentalization of the pathway reactants and products. Additionally, greater efficiency of drug production would hopefully lower costs; however, one ethical risk is that pharmaceutical companies would keep the difference and not actually lower the price of a drug for consumers. To mitigate these risks, our Human Practices committee is working on a Code of Ethical Principles and talking to experts about how to best support access to health care outside of our wetlab work.

Part 3: Managing the risks you have identified

It is impossible to remove all risk from any biological activity. Instead, we attempt to manage these risks - to reduce them to an acceptable level. In this section, you should outline what steps have been taken (or you are planning to take) to reduce any risks you identified in Part 2 of the form, in particular Question 10.

15. How will experts overseeing your project help to manage any of the risks you identified in this form?

For example, who is responsible for the safety and security of biology labs at your institution? What role has any institutional biosafety officer played in reviewing your work? What skills do these experts have that might help? For example, do they have a long history of working with an organism or part that might pose a risk? How familiar are they with the experimental procedures and practices you will be, or are, using?

We have complied with our institution's regulations for BSL2 safety, and we have reviewed the requirements for safety checks for both iGEM and our University. For iGEM, we have filled out a Check-In form for the use of HIV Reverse Transcriptase, as well as accounted for the risk of that part by modifying it for safe use in our project. For our university, the Institutional Biosafety Committee has approved the parts of our proposed work that are not automatically exempt from oversight. We have also continuously reviewed the project and any concerns with our advisor, Professor Keith Kozminski, an expert researcher in the field of synthetic biology.

16. What rules or guidance cover your work which could help to manage any of the risks you identified in Part 2 of this form (in particular Question 10)?

For example: In your country / region, what are the laws and regulations that govern biosafety or biosecurity in research laboratories? Please give a link to these regulations, or briefly describe them if you cannot give a link.What are the guidelines for laboratory biosafety and biosecurity? Please give a link to these guidelines, or briefly describe them if you cannot give a link.

Our University has safety regulations similar to the iGEM White List and Check-ins, and we have gone through our Institutional Biosafety Committee to get our plans approved. Each member of our team has completed institutional online safety training modules to ensure they are prepared if/when we are able to do lab work.

Here is a link to the UVA Biosafety guidelines: http://ehs.virginia.edu/Biosafety.html

17. Have your team members received any safety training?

For the purposes of iGEM, biosafety and biosecurity training covers the procedures and practices used to manage risks from accidents or deliberate misuse of your projects to your team, colleagues, communities and the environment. All team members are expected to be aware of these risks and to work to manage them.

- **Yes, we have already received safety training.**
- **Yes, we have already received security training.**
- We plan to receive safety and security training in the future. Please specify approximately when:
- We will not have safety and security training. *Please explain in detail how team members will be aware of and manage risks to the team, colleagues, communities, and the environment in the absence of training. If it is not relevant because there is no lab component to your project, please note this:*

18. Please select the topics that you learned about (or will learn about) in your safety training.

- **Lab access and rules** ***(including appropriate clothing, eating and drinking, etc.***
- **Responsible individuals** ***(such as lab or departmental specialist or institutional biosafety officer)***
- **Differences between biosafety levels**
- **Biosafety equipment** ***(such as biosafety cabinets)***
- **Good microbial technique** ***(such as lab practices)***
- **Disinfection and sterilization**
- **Emergency procedures**

- **Transport rules**
- **Physical biosecurity**
- **Personnel biosecurity**
- **Dual-use and experiments of concern**
- **Data biosecurity**
- **Chemicals, fire and electrical safety**
- We will not have safety training

19. Which work areas do you use / are you using to handle biological materials?

Please check all the containment provisions you are using. If you are using more than one space please check all that apply and note this in the other work area box.

- **No lab work** ***(e.g. software project)***
- **Open bench**
- Biosafety cabinet *(please note there are important differences between biosafety cabinets and laminar flow hoods / clean benches. iGEM encourages the use of biosafety cabinets but discourages the use of laminar flow hoods or clean benches. This* ***Factsheet*** *from the University of Massachusetts Amherst helps explain the differences.)*
- Specialist greenhouse
- Specialist animal house
- Specialist insect facility
- **Chemical fume hood** *(Please note – this is designed to manage risks from hazardous chemicals. It is different from a biological safety cabinet designed to manage risks from hazardous biological agents and a clean bench or laminar flow hood designed to prevent contamination of your experiment.)*

- Other work area. *Please describe:*
- Unknown. *Please comment:*

20. What is the biosafety Level of your work space?

*Help about **Risk Groups and Safety Levels***

If you are working in a biosafety cabinet it may be a biosafety level 2 space (then select Level 2), but biosafety cabinets are sometimes also used in a biosafety level 1 laboratory to provide a sterile work space (then select Level 1). If in any doubt, please discuss this with a biosafety professional or your instructors, supervisors or lab techs to make sure you understand how the equipment you use helps to manage risks.

- Not applicable as we have no lab component
- We have several different lab spaces with different biosafety Levels. Please describe:
- Level 1 *(low risk)*
- **Level 2** *(moderate risk)*
- Level 3 *(high risk)*
- Level 4 *(extreme risk)*
- Other biosafety level. *Please describe:*

Alert
iGEM teams should not use Risk Group 3 or 4 organisms, and they should not work in Safety Level 3 or 4 labs. If you are planning to work at Safety Level 3 or 4, contact safety (AT) igem (DOT) org right away!!

21. What other risk management tools will cover you work?

Please select as many as are relevant, including those that happen automatically at your institution and are not specifically connected to your project. If in any doubt, please

discuss this with a biosafety professional or your instructors, supervisors or lab techs to make sure you understand how the equipment you use helps to manage risks.

- **Accident reporting** ***(measures to record any accidents)***
- **Personal Protective Equipment** ***(including lab coats, gloves, eye protection, etc)***
- **An inventory control system** ***(measures to track who has what materials and where they are)***
- **Access controls** ***(measures to control who can access your work spaces, or where materials are kept)***
- **Medical surveillance** ***(measures to find out if you get sick because of something you were using)***

[Not part of a teaching laboratory protocol in the strict sense.]

- **Waste management system** ***(measures to make sure waste is not hazardous before it leaves your institution)***
- **Special procedures or protocols that address safety or security**
- Others *Please describe:*

22. How will the rules, training, containment and other procedures and practices help to manage any of the risks you identified in Part 2 of this form (in particular Question 10)?

Please give details of any steps you have taken to manage any risks identified. This might include how any of the following have helped manage risks: the rules you identified, the training you have had, the equipment you have used, the spaces you have worked in, and the procedures and protocols you have followed. It might also include things you deliberately didn't do. For example, if you are not conducting any experiments, especially on grounds of safety, security or as a responsible scientist / engineer, please note this. Examples might include, making sure you only use non-pathogenic strains of an organism, deciding that animal use experiments are not yet warranted, or avoiding plant infection experiments because the affected plant is found in your country. Please also

consider waste treatment – how will you know that any waste produced in your project will be successfully inactivated?

- Continual disinfection of any surfaces touched by either those in the lab, or different objects being placed on different surfaces.
- Constant usage of gloves, lab coats, eye goggles, face masks and other types of protective gear.
- Continuous hand washing.
- Autoclaving materials in order to sterilize them.
- Autoclaving potential hazardous waste in order to sterilize it, so it can be discarded through our hazardous waste protocol.
- Using non-pathogenic strains of E. coli that are typically unable to infect humans.

Part 4: Compliance with iGEM's rules and policies

23. Are you planning to/ have released any organism or product derived from your project?

For the purposes of iGEM, release includes putting any engineered organism or product from one into the environment, yourselves or volunteers (including by eating or drinking), or into a device that will be placed in the environment.

- **No**
- Yes
  STOP: *Release is not allowed in iGEM. For more information see the* ***policy page****. Please contact the Safety and Security Committee by emailing safety AT igem DOT org*

24. Are you planning to use, or using any animals (including insects and invertebrates) not on the Whitelist?

- **No**

- Yes

  STOP: *Before you acquire or use any animal NOT on the **Whitelist**, you must - submit a **Check-In form**. Check-Ins allow the iGEM Safety and Security Committee to help you ensure that you will work safely and responsibly with these organisms. The Safety and Security Committee will base its review on the information you provide – please provide as much information as possible and use references as appropriate.*

25. Are you planning to use / have used any vertebrates (e.g. rats, mice, guinea pigs, hamsters) or higher order invertebrates (e.g. cuttlefish, octopus, squid, lobster)?

- **No**

- Yes

  STOP: *Before you acquire or use any vertebrate you must submit an **Animal Use form**. The use of animals is not allowed in iGEM projects without a special exception from the Safety and Security Committee. For more information see the **policy page** and the **White List**. Please contact the Safety and Security Committee by emailing safety AT igem DOT org*

26. Are you planning to use, or using any parts not on the Whitelist?

- **No**

- Yes

  STOP: *Before you acquire or use any part that is NOT on the Whitelist, you must - submit a **Check-In form**. Check-Ins allow the iGEM Safety and Security Committee to help you ensure that you will work safely with these riskier parts. The Safety and Security Committee will base its review on the information you provide – please provide as much information as possible and use references as appropriate.*

27. Are you planning to carry out any of the activities not on the Whitelist?

These include experiments likely to bias the inheritance frequency of a genetic marker in an organism's progeny, such as through the creation of a gene drive, experiments likely to confer resistance to the World Health Organization's list of Critically Important Antimicrobials, and experiments likely to increase the hazard posed by your project.

- No

- **Yes**

  STOP: *Before you carry out any of the activities NOT on the **White List**, you must - submit a **Check-In form**. Check-Ins allow the iGEM Safety and Security Committee to help you ensure that you will work safely with these riskier organisms/parts. The Safety and Security Committee will base its review on the information you provide – please provide as much information as possible and use references as appropriate.*

28. Are you planning to use, or using any parts or organisms obtained from outside the lab or regular suppliers?

- No

- **Yes**

  STOP: *Before you acquire or use any organism/part that has come from outside the lab or regular suppliers, you must - submit a **Check-In form**. Check-Ins allow the iGEM Safety and Security Committee to help you ensure that you will work safely with these riskier organisms/parts. The Safety and Security Committee will base its review on the information you provide – please provide as much information as possible and use references as appropriate.*

29. What else can you tell us about any risks associated with your project, how you are managing them, or your compliance with iGEM's safety and security rules and policies?

This can include any improvements you would like to see to our safety and security efforts, or anything that has not been sufficiently clear, or where additional guidance would be useful, or where you see important uncertainties.

We are consistently reviewing our procedures and protocols with our advisors throughout the course of our project to ensure that any new risks that may arise can be dealt with.