iGEM 2013 Basic Safety Form

Team name: Arizona_State

Deadline: 30th of August 2013

Submission method: email form to the correct email list for your region:

safety_forms_asia@igem.org
safety_forms_europe@igem.org
safety_forms_north_america@igem.org
safety_forms_latin_america@igem.org

Students can complete this safety form, but it must be read and signed (electronic or hard copy) by your team's faculty advisor. Your advisor must verify the information contained in this form and sign it.

The iGEM Safety Committee must be able to easily reach the advisor with questions or other follow-up communication. If you have made changes to your project (new coding regions or organisms) you must re-submit your safety form before wiki freeze (date TBD).

Key points to remember as you complete the safety assessment process:

- For help in completing questions 1 and 2, you may find it useful to consult the Risk Groups section of the Safety Resources List [2013.igem.org/Safety].
- The iGEM Safety Committee will be reviewing your project. To avoid temporary suspensions, answer these questions completely and accurately.
- The Safety Committee needs to be able to communicate with your faculty advisor about any safety concerns. If we cannot reach your advisor in a reasonable amount of time, you may be subject to restrictions at the Jamboree.
- Your safety page, wiki project page and poster should be consistent with each other. If you change your project, submit an updated Basic Safety Page to the iGEM Safety Committee before the wiki freeze. (Your faculty advisor must also read and sign the updated page.)
- We understand that projects may still be changing at a late date. However, large discrepancies between what you submit on the Basic Safety Page and what you present at the Jamborees may result in restrictions at the Jamboree.

Basic Safety Questions for iGEM 2013

a. Please describe the chassis organism(s) you will be using for this project. If you will be using more than one chassis organism, provide information on each of them:

	Species	Strain no/name	Risk Group	Risk group source link	Disease risk to humans? If so, which disease?
Ex	*E. coli* (K 12)	NEB 10 Beta	1	www.absa.org/riskgroups/bacteriasearch.php?genus=&species=coli	Yes. May cause irritation to skin, eyes, and respiratory tract, may affect kidneys.
1	E. coli (K 12)	NEB 10 Beta	1	www.absa.org/riskgroups/bacteria search.php?	Yes. May cause irritation to skin.
2					
3					
4					
5					
6					
7					
8					

*For additional organisms, please include a spreadsheet in your submission.

2. Highest Risk Group Listed:

1 (●) Greater than 1 (○)

If you answered 1+, please also complete the iGEM Biosafety form part 2 for any organisms in this category.

3. List and describe *all* new or modified coding regions you will be using in your project. (If you use parts from the 2013 iGEM Distribution without modifying them, you do not need to list those parts.)

	Part number.	Where did you get the physical DNA for this part (which lab, synthesis company, etc)	What species does this part originally come from?	What is the Risk Group of the species?	What is the function of this part, in its parent species?
Ex	BBa_C0040	Synthesized, Blue Heron	Acinetobacter baumannii	2	Confers tetracycline resistance

1	BBa_K1190002	Bought from ATCC by the Anderson Lab, ASU Biodesign Institute	Listeria monocytogenes	2	Breaks dendritic cell phagolysosomes for MHC I antigen presentation
2	BBa_K1190001	Synthesized, IDT	Homo sapiens	2	Human immune cell stimulating factor
3	BBa_K1190000	the Anderson Lab, ASU Biodesign Institute	Homo sapiens	2	Cell surface protein that distinguishes melanocytes from other tissue
4					
5					
6					
7					
8					

*For additional coding regions, please include a spreadsheet in your submission.

4. Do the biological materials used in your lab work pose any of the following risks? Please describe.

a. Risks to the safety and health of team members or others working in the lab?

All the organisms utilized by ASU iGEM in the Anderson Lab and the Haynes Lab comply with biosafety level 1 (BSL1) and do not possess the potential to cause disease in individuals. All organisms, despite having no association with disease, are treated as potential pathogens, thus personal protective equipment such as gloves, laboratory coats, and protective eyewear/goggles are used to prevent contact

b. Risks to the safety and health of the general public, if released by design or by accident?

The recombinant DNA (rDNA) experiments conducted in the laboratory provide ampicillin, kanamycin and/or chloramphenicol resistance to E.coli BL21(DE3) and E.coli NEB-10 Beta to select for plasmids. Under the circumstances that any of these genetically altered organisms were released they would have minimal potential for pathogenesis. In accordance with Arizona State University's Environmental Health

c. Risks to the environment, if released by design or by accident?

Our project involves developing a modular bacterial lab-strain, and eventually probiotic bacteria currently being consumed by humans, cancer vaccine platform. None of the genes themselves have had any previous literature detailing detrimental environmental effects. Because the introduced genes themselves are either from human tumor cells or are the LLO gene, we do not believe they pose any significant

d. Risks to security through malicious misuse by individuals, groups, or countries?

There is a potential for malicious misuse of the LLO toxin as a biological weapon. However, the construct that we have built in the iGEM lab is not functional as a stand alone biological weapon because it lacks invasive proteins, such as invasin or internalin, is only active in acidic phagosomal conditions, and prematurely folds the proteins needed for pore-forming activity in neutral pH conditions.

5. If your project moved from a small-scale lab study to become widely used as a commercial/industrial product, what new risks might arise? (Consider the different categories of risks that are listed in parts a-d of the previous question.) Also, what risks might arise if the knowledge you generate or the methods you develop became widely available? (Note: This is meant to be a somewhat open-ended discussion question.)

We would need to evaluate the risk of side effects the vaccine has on the patient as a whole, not at just the single cell level, including measures to prevent autoimmunity and sepsis. We would also have to evaluate cross-reactivity with other drugs and radiation treatments. If the methods we develop become widely available, more drugs could be produced that target specific immune cells and stimulate stronger

6. Does your project include any design features to address safety risks? (For example: kill switches, auxotrophic chassis, etc.) Note that including such features is not mandatory to participate in iGEM, but many groups choose to include them.

We are utilizing the pOSIP plasmid to chromosomally integrate transformed genes into NEB 10 Beta cells and then remove antibiotic resistance genes to prevent horizontal gene transfer of LLO or any antibiotic resistance genes. We are also developing a pH-based gene switch to create a secondary safety mechanism so that LLO is only translated under acidic conditions and translation is repressed by

7. What safety training have you received (or plan to receive in the future)? Provide a brief description, and a link to your institution's safety training requirements, if available.

All iGEM team members have completed training in: Biological Safety, Bloodborne pathogen safety, recombinant DNA safety, and fire safety. https://cfo.asu.edu/ehs-biosafety-training

8. Under what biosafety provisions will / do you work?

a. Please provide a link to your institution biosafety guidelines.

http://www.asu.edu/uagc/EHS/documents/biosafetymanual.pdf

b. Does your institution have an Institutional Biosafety Committee, or an equivalent group? If yes, have you discussed your project with them? Describe any concerns they raised with your project, and any changes you made to your project plan based on their review.

Yes. The Institutional Biosafety Committee sees no foreseeable public health threat associated with the organisms and recombinant DNA project utilized by our team.

c. Does your country have national biosafety regulations or guidelines? If so, please provide a link to these regulations or guidelines if possible.

Yes. http://oba.od.nih.gov/oba/rac/Guidelines/NIH_Guidelines.htm

d. According to the WHO Biosafety Manual, what is the BioSafety Level rating of your lab? (Check the summary table on page 3, and the fuller description that starts on page 9.) If your lab does not fit neatly into category 1, 2, 3, or 4, please describe its safety features [see 2013.igem.org/Safety for help].

2.

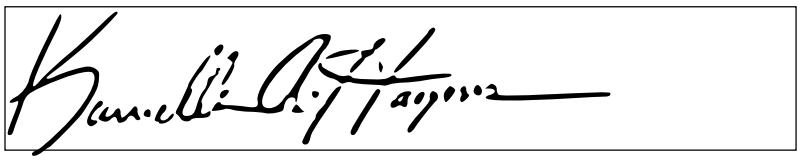
e. What is the Risk Group of your chassis organism(s), as you stated in question 1? If it does not match the BSL rating of your laboratory, please explain what additional safety measures you are taking.

1.

Faculty Advisor Name:

Dr. Karmella Haynes

Faculty Advisor Signature:



Safety Form 1

4.

a. All the organisms utilized by ASU iGEM in the Anderson Lab and the Haynes Lab comply with biosafety level 1 (BSL1) and do not possess the potential to cause disease in individuals. All organisms, despite having no association with disease, are treated as potential pathogens, thus personal protective equipment such as gloves, laboratory coats, and protective eyewear/goggles are used to prevent contact with bacteria and yeast samples in the lab, to prevent splashes, and to avoid sources of ultraviolet radiation.

b. The recombinant DNA (rDNA) experiments conducted in the laboratory provide ampicillin, kanamycin and/or chloramphenicol resistance to E.coli BL21(DE3) and E.coli NEB-10 Beta to select for plasmids. Under the circumstances that any of these genetically altered organisms were released they would have minimal potential for pathogenesis. In accordance with Arizona State University's Environmental Health & Safety policy, "Nothing in the trash, nothing down the drain", we autoclave and bleach sterilize all waste from recombinant DNA experiments. This reduces the likelihood of accidental release.

c. Our project involves developing a modular bacterial lab-strain, and eventually probiotic bacteria currently being consumed by humans, cancer vaccine platform. None of the genes themselves have had any previous literature detailing detrimental environmental effects. Because the introduced genes themselves are either from human tumor cells or are the LLO gene, we do not believe they pose any significant environmental hazarsds. In addition, because we are attempting to develop a bacterial-based vaccine, we envision this system to be used only in basic and clinical laboratories.

d. There is a potential for malicious misuse of the LLO toxin as a biological weapon. However, the construct that we have built in the iGEM lab is not functional as a stand alone biological weapon because it lacks invasive proteins, such as invasin or internalin, is only active in acidic phagosomal conditions, and prematurely folds the proteins needed for pore-forming activity in neutral pH conditions.

5. We would need to evaluate the risk of side effects the vaccine has on the patient as a whole, not at just the single cell level, including measures to prevent autoimmunity and sepsis. We would also have to evaluate cross-reactivity with other drugs and radiation treatments. If the methods we develop become widely available, more drugs could be produced that target specific immune cells and stimulate stronger immune responses. These new methods need to be carefully fine-tuned in order to prevent overstimulation or unintended side effects. Researchers, because the system is modular, could potentially try using other antigens similar to MelanA or Flu M1, and if they don't think about their antigens carefully, they may pick a non-cancerous antigen found naturally in the body, like Her2, which could induce autoimmunity. However, the human immune system's central tolerance may minimize the capability for vaccine-induced autoimmunity. Because this system is

meant to be a vaccine, it would not be available to patients directly; it would be administered by a physician. They would need to consider the correct dosage amount depending on the stage of the disease in the patient. Because we are hoping to port the system into a probiotic bacterial strain, overuse could disrupt the human microbiome, so a precise dosage is important in administering the vaccine.

6. We are utilizing the pOSIP plasmid to chromosomally integrate transformed genes into NEB 10 Beta cells and then remove antibiotic resistance genes to prevent horizontal gene transfer of LLO or any antibiotic resistance genes. We are also developing a pH-based gene switch to create a secondary safety mechanism so that LLO is only translated under acidic conditions and translation is repressed by neutral and basic conditions.

iGEM 2013 Biosafety Form Part 2

Deadline: 30th of August 2013

Team name: Arizona State

Submission method: email form to the correct email list for your region:

safety_forms_asia@igem.org
safety_forms_europe@igem.org
safety_forms_north_america@igem.org
safety_forms_latin_america@igem.org

You must submit this form if you are working with any of the following:

- Organisms classified above Risk Group 1 (RG1) (or, if your country rates organisms with 4 being the *least* dangerous, organisms more dangerous than Risk Group 4)
- Coding regions derived from organisms above RG1
- Mammalian cells or organisms
- Genetic parts derived from mammals

If you are only working with organisms/parts that are rated Risk Group 1 (the safest risk group), and have filled out the Basic Safety Form, you do not need to submit this form. You may use your own country's standards or WHO standards to determine which organisms/parts require this form. Please see *2013.igem.org/Safety* for more information on how to determine the Risk Group of your organism and Biological Safety Level of your lab.

The following are *exempt* and do not require you to submit this form:

- *Pseudomonas aeruginosa* and any genetic parts derived from it.
- Any parts included in the 2013 official iGEM distribution kit. (Note: many Registry parts are not in the distribution kit, and these parts still require a Beyond the Basics Form if they come from an organism above RG1, or from a mammal.)

Please complete this form and have your team faculty advisor sign it by the deadline. While students can complete this form, the faculty instructor needs to read your answers and sign it (electronically or hard copy). The Safety Committee will review your submissions and may request further information if your project raises safety concerns. Projects that raise the most serious concerns will be required to complete an extended biosafety form. (We expect that this will only happen only in a very small number of cases).

Please note:

- Although this form is required only for organisms/parts above RG1, that does not mean that RG1 organisms are totally safe. Good judgment and proper lab practices are necessary at all times.
- Consult with your faculty advisor, and with the biosafety committee at your institution. This form does not replace local institutional review. You must receive approval from your government or institution as may be required under local law.

This form must be completed separately for each organism or part above RG1. Please cite sources, including web links as applicable, to support your statements.

1. Organism name and strain name or number.

Human Immature Dendritic Cells

2. Organism Risk Group:

1 ◉ 2 ○ Greater than 2 ○

3. If you are using this organism as a chassis, write "chassis". If you are using a genetic part from the organism, give the name of the part and a brief description of what it does and why you are using it.

N/A

4. How did you physically acquire the organism or part?

Cultured by The Anderson Lab at the ASU Biodesign Institute

5. What potential safety/health risks to team members, other people at your institution, or the general public could arise from your use of this organism/part?

Human immature dendritic cells do not pose any significant health or safety risk to team members or the public. They are present in all humans and do not have any pathogenic characteristics.

6. What measures do you intend to take to ensure that your project is safe for team members, other people at your institution, and the general public?

While these cells are non-pathogenic, all cells, despite having no association with disease, are treated as potential pathogens, thus personal protective equipment such as gloves, laboratory coats, and protective eyewear/goggles are used to prevent contact with dendritic cell samples in the lab and prevent splashes. PPE is also used to protect the user from exposure to fetal bovine serum in the growth

7. If you are using only a part from the organism, and you believe the part by itself is not dangerous, explain why you believe it is not dangerous.

N/A

8. Why do you need to use this organism/part? Is there an organism/part from a less dangerous Risk Group that would accomplish the same purpose?

We are using dendritic cells to determine whether or not they will uptake the bacterial vaccine and present cancer antigens on MHC Class I proteins on their surface, which should activate human T cells and confirm that our system is functional in vitro.

9. Is the organism/part listed under the Australia Group guidelines, or otherwise restricted for transport? If so, how will your team ship this part to iGEM and the Jamborees?

We are not shipping this organism to iGEM or the jamboree.

10. Please describe the BioSafety Level of the lab in which the team works, or description of safety features of lab (Refer to Basic Safety form, question 8. d.). If you are using organisms with a BSL level greater than you lab, please explain any additional safety precautions you are taking.

2.

Faculty Advisor Name:

Dr. Karmella Haynes

Faculty Advisor Signature:

iGEM 2013 Biosafety Form Part 2

Deadline: 30th of August 2013

Team name: Arizona State

Submission method: email form to the correct email list for your region:

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You must submit this form if you are working with any of the following:

- Organisms classified above Risk Group 1 (RG1) (or, if your country rates organisms with 4 being the *least* dangerous, organisms more dangerous than Risk Group 4)
- Coding regions derived from organisms above RG1
- Mammalian cells or organisms
- Genetic parts derived from mammals

If you are only working with organisms/parts that are rated Risk Group 1 (the safest risk group), and have filled out the Basic Safety Form, you do not need to submit this form. You may use your own country's standards or WHO standards to determine which organisms/parts require this form. Please see *2013.igem.org/Safety* for more information on how to determine the Risk Group of your organism and Biological Safety Level of your lab.

The following are *exempt* and do not require you to submit this form:

- *Pseudomonas aeruginosa* and any genetic parts derived from it.
- Any parts included in the 2013 official iGEM distribution kit. (Note: many Registry parts are not in the distribution kit, and these parts still require a Beyond the Basics Form if they come from an organism above RG1, or from a mammal.)

Please complete this form and have your team faculty advisor sign it by the deadline. While students can complete this form, the faculty instructor needs to read your answers and sign it (electronically or hard copy). The Safety Committee will review your submissions and may request further information if your project raises safety concerns. Projects that raise the most serious concerns will be required to complete an extended biosafety form. (We expect that this will only happen only in a very small number of cases).

Please note:

- Although this form is required only for organisms/parts above RG1, that does not mean that RG1 organisms are totally safe. Good judgment and proper lab practices are necessary at all times.
- Consult with your faculty advisor, and with the biosafety committee at your institution. This form does not replace local institutional review. You must receive approval from your government or institution as may be required under local law.

This form must be completed separately for each organism or part above RG1. Please cite sources, including web links as applicable, to support your statements.

1. Organism name and strain name or number.

Listeria monocytogenes

2. Organism Risk Group:

1 ○ 2 ◉ Greater than 2 ○

3. If you are using this organism as a chassis, write "chassis". If you are using a genetic part from the organism, give the name of the part and a brief description of what it does and why you are using it.

N/A

4. How did you physically acquire the organism or part?

The Anderson Lab at the ASU Biodesign Institute ordered the gene sequence for Listeriolysin O (LLO) from ATCC.

5. What potential safety/health risks to team members, other people at your institution, or the general public could arise from your use of this organism/part?

There is a risk of toxicity via human cell damage. Listeriolysin O (LLO) is a virulence factor protein naturally found in Listeria monocytogenes. The pathogenic function of LLO occurs when Listeria enters the host's macrophage immune system cells, and then enters the phagolysosome organelle. Listeria secretes LLO protein, the protein ruptures the phagolysosome, and live Listeria escapes the

6. What measures do you intend to take to ensure that your project is safe for team members, other people at your institution, and the general public?

The LLO in our E. coli chassis is expressed without the secretion tag found naturally preceding the LLO coding sequence in Listeria monocytogenes. Once our bacteria is engulfed by a macrophage, an acidic phagolysosomes must first degrade the membrane of the E. coli before LLO can be exposed and subsequently break open the phagolysosome for proper antigen presentation. This prevents the bacteria

7. If you are using only a part from the organism, and you believe the part by itself is not dangerous, explain why you believe it is not dangerous.

The LLO-based BioBrick does not contain the secretion tag. Therefore, we believe that LLO functions only after the chassis (E. coli) is degraded by the phagolysosome. LLO should result in the release of the therapeutic payload (anti-cancer antigens), and not live E. coli cells. We are performing assays to confirm that E. coli will not secrete LLO prior to deeming that there is no safety risk to this part.

8. Why do you need to use this organism/part? Is there an organism/part from a less dangerous Risk Group that would accomplish the same purpose?

8. Phagolysosome lysis, induced by LLO after the E. coli membrane is degraded inside a dendritic cell, is necessary for our cancer antigens can be released from the E. coli chassis into the macrophage cytoplasm, which results in presentation on MHC Class I complexes and subsequent cytotoxic T cell activation, needed for destroy tumor cells. Without LLO, the antigens would be confined to MHC Class II

9. Is the organism/part listed under the Australia Group guidelines, or otherwise restricted for transport? If so, how will your team ship this part to iGEM and the Jamborees?

This part and organism is not restricted under those guidelines.

10. Please describe the BioSafety Level of the lab in which the team works, or description of safety features of lab (Refer to Basic Safety form, question 8. d.). If you are using organisms with a BSL level greater than you lab, please explain any additional safety precautions you are taking.

2.

Faculty Advisor Name:

Dr. Karmella Haynes

Faculty Advisor Signature:

Statement from the advisor: This information is correct to the best of my knowledge. I approve of the *complete* safety form submission, which includes both this form plus the continuation of answers for questions 5, 6, 7, and 8 on the attached document (saved in my files on Sept. 27, 2013).

Biosafety Form 2 (LLO)

5. There is a risk of toxicity via human cell damage. Listeriolysin O (LLO) is a virulence factor protein naturally found in Listeria monocytogenes. The pathogenic function of LLO occurs when Listeria enters the host's macrophage immune system cells, and then enters the phagolysosome organelle. Listeria secretes LLO protein, the protein ruptures the phagolysosome, and live Listeria escapes the macrophage cell. In combination with other expressed toxins such as invasin or internalin, this process allows listeria to escape macrophages and continue infecting the host. LLO could potentially lyse human blood cells if unintentionally ingested by team members, other people at ASU, or the public.

6. The LLO in our E. coli chassis is expressed without the secretion tag found naturally preceding the LLO coding sequence in Listeria monocytogenes. Once our bacteria is engulfed by a macrophage, an acidic phagolysosomes must first degrade the membrane of the E. coli before LLO can be exposed and subsequently break open the phagolysosome for proper antigen presentation. This prevents the bacteria from continuously escaping macrophages inside the body and spreading unintentionally. The ASU team is conducting assays of this process on blood agar plates to confirm that LLO is not secreted from E. coli. We will also include the following warnings on the Registry page: (1) Do not add a secretion tag to LLO, (2) Do not express LLO along with BioBricks or in chassis that enable active cell invasion (e.g., BBa_BBa_I10001) Before this project is developed any further as a therapeutic, we will carry out experiments to confirm that live E. coli do not escape the macrophages, and that the E. coli only enter macrophages, and not other types of cells. We ask that this part not be distributed on the registry until we have completed this testing and verified the safety of this part.

7. The LLO-based BioBrick does not contain the secretion tag. Therefore, we believe that LLO functions only after the chassis (E. coli) is degraded by the phagolysosome. LLO should result in the release of the therapeutic payload (anti-cancer antigens), and not live E. coli cells. We are performing assays to confirm that E. coli will not secrete LLO prior to deeming that there is no safety risk to this part.
If the LLO protein were to escape the E. coli chassis in the bloodstream before the bacteria was engulfed by a macrophage, then LLO, in the absence of the Listeria internalin cofactors, would be disrupted by the neutral pH of blood [PNAS USA 2005 Aug 30; 102(35):12537-42, PLoS Pathogens 2011 Nov; 7(11):e1002356. doi: 10.1371/journal.ppat.1002356]. We believe that if an unintended subject ingested the E. coli, their macrophages and dendritic cells would engulf the bacteria and LLO would break open the phagolysosomes of those cells, resulting in unintended immunization against cancer. Although the system is designed as a prophylactic vaccine and we envision seeing no immediate negative consequence of unintended vaccination, it may lead to a dangerous autoimmune response against self-antigens in at-risk subjects that are unintentionally vaccinated.

8. Phagolysosome lysis, induced by LLO after the E. coli membrane is degraded inside a dendritic cell, is necessary for our cancer antigens can be released from the E. coli chassis into the macrophage cytoplasm, which results in presentation on MHC Class I complexes and subsequent cytotoxic T cell activation, needed for destroy tumor cells. Without LLO, the antigens would be confined to MHC Class II presentation, which would only activate B cells. Because cells in the body, such as tumor cells, only contain MHC Class I proteins on their surface, B cell activation is not sufficient to trigger an anti-tumor response because tumor cells would still be indistinguishable from healthy cells. B cells produce antibodies, but they will not be able to recruit cytotoxic T cells to tumor cells without the previous existence of T cells that recognize tumor antigens. MHC Class I activation is the only method to activate cytotoxic T cells to recognize antigens on the surface of tumor cells.