# Final Safety Form

## 1. Your New Parts

**Complete the spreadsheet. Include all whole organisms that you will handle in the lab, whether you are using them as a chassis or for some other reason. Include all new or highly modified protein coding parts that you are using. If you submitted a Check-In for an organism or part, you should still include it in this spreadsheet.**

**You may omit non-protein-coding parts, and you may omit parts that were already in the Registry if you are using them without significant modifications.**

Spreadsheet available for download in http://2015.igem.org/Team:Brasil-USP/Notebook/Safety

## 2. What is your chassis organism?

**Check all species you are genetically modifying in your project.**

- [x] **E. coli (lab strains that are not harmful to humans)**
- [ ] Yeast (Saccharomyces)
- [ ] Lactobacillus
- [ ] B. subtilis
- [ ] Others (give species names):
- [ ] No chassis organism (please comment):

Comments:

## 3. Do you plan to experiment with any other organisms, besides your chassis?

**What organisms, and what experiments will you do? Please explain briefly. Please include the names of species / cell lines / strains.**

We are genetically modifying only Escherichia coli. To ensure a higher insert stability, we are using DH5-alpha on transformation experiments, and, for higher levels of protein expression we are using BL21.

Also, we are employing wild strains of Acidithiobacillus ferrooxidans (strain LR) and Acidithiobacillus thiooxidans (strain FG-01) on the devulcanization process.

**4. How will your project work?**

**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.)**

Our engineered organism is supposed to degrade rubber, mostly poly(cis-1,4-isoprene). The degradation process is based on two enzymes, latex clearing protein (Lcp) and rubber oxygenase (RoxA), that will be exported out of E. coli due to the insertion of a label. Besides, as a pretreatment to obtain devulcanized rubber, we are using wild strains of Acidithiobacillus sp. A kill switch mechanism (using hokD) is also included in the circuit design, ensuring that the GMO is safe for the environment and would not be able to survive if accidentally released. As we plan to use the developed bacteria inside bioreactors, the chance of spreading GMO is even lower; further, we will be able to easily ramp up the process. Producing combined rubber degradation enzymes in a simple organism has got great potential to assist a huge ambiental issue in a simple, easy and environmentally friendly manner.

**5. What risks does your project pose at the laboratory stage? What actions are you taking to reduce those risks?**

**If you are working in a biology lab, you cannot answer "no risks". Even the simplest experiment, with the safest bacteria, poses some small risk. The actions you take to reduce that risk would include safety level 1 procedures, wearing rubber gloves, sterilizing waste, etc.**

As most projects using genetically modified organism, our project might be an issue regarding the mammals health and other organisms, mostly for those working on the laboratory, if the GMO is not properly contained. To avoid any leakage or contact with these organism, some basic rules are always followed as laboratory procedures and they are: sterilization of waste, restriction of access, use of nitrile butadiene rubber or latex gloves, lab coats, long pants and any other personal protective equipment.

Because our bacteria will degrade latex, we chose to use nitrile gloves when manipulating any GMO that could degrade the latex gloves. Also, we designed the organism in such a manner that they can grow only in the presence of inducers (Lactose, IPTG, Rhamnose or Arabinose) - which are not commonly found in the environment.

**6. How would your project be used in the real world?**

**Imagine that your project were fully developed into a real product that real people could use. How would people use it? Check all appropriate boxes.**

☐ 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)

☐ Only in the lab (Examples: reporter strain for measuring the strength of promoters)

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

☐ In a consumer product that ordinary people buy (Examples: cells that clean your clothes, bread made with engineered yeast)

☐ In agriculture / on a farm (Examples: cells that guard against pests, engineered rice plants, cells that promote growth of crop plants)

☐ In a small enclosed device (Examples: a bio-sensing strip with cells that detect arsenic)

☐ In the natural environment (Examples: cells that remove pollution from lakes, engineered forest trees that can resist drought)

☐ To be used in the human body, or in food (Examples: anti-cancer bacteria, bread made with engineered yeast, engineered rice plants)

☐ Other (Examples: bacteria that live on Mars)

**Comments:**

Our project can be scaled up to be applied in bioreactors, degrading rubber and generating other products from the degraded material, e.g. fuel.

**7. What risks might your project pose, if it were fully developed into a real product that real people could use? What future work might you do to reduce those risks?**

If our engineered organism (without the kill switch mechanism) was released to the environmental it probably would be able to degrade any rubber based material. However, two points are important to highlight. The degradation process takes a considerable time to happen even using our engineered organism. Also, our kill switch mechanism ensures that degradation enzymes will only be expressed under certain conditions, mainly the presence of an inducer (Lactose, IPTG, Rhamnose or Arabinose) - which are not commonly present in the environment.

As a final action, devulcanization and degradation processes are taking place in bioreactors that are able to physically contain the bacteria.

**8. Any further comments about your project:**

**9. Comments about this form: Is it easy or difficult to use? Are the questions confusing?**

This form is easy to use, but we take the liberty of adding some extra information relevant to the project to avoid any confusion.