iGEM 2013 Biosafety Form Part 2

Deadline: 30th of August 2013

Team name: TU-Munich

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.)

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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 poliovirus 1 strain Mahoney

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.

We are using an internal ribosome entry site (IRES) from poliovirus 1 because it allows for translation initiation in the middle of a messenger RNA (mRNA) sequence and thus enables to build constructs consisting of several parts controlled by one promoter.

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

We got it from Prof. Martin Fussenegger (ETH Zürich)
(http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3449837/pdf/10616_2004_Article_194745.pdf)

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 poliovirus 1 is a human pathogen but the IRES part we are using is itself not harmful

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?

We have all completed a biosafety course and we handle everything in the lab with the necessary precaution. We will not distribute any parts or organisms outside the lab, we are using moss that is not able to sporulate and have implemented a kill switch (see basic safety form)

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.

Internal ribosome entry sites are widely used as a means to accomplish polycistronic expression in eukaryotes

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?

Without IRES we would have to put a promotor in front of every protein we want to express which would make cloning of large constructs much more difficult. There are IRES elements found in cellular mRNAs (e.g. in Fibroblast growth factor) however, they are not as established in expression systems.

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?

not listed

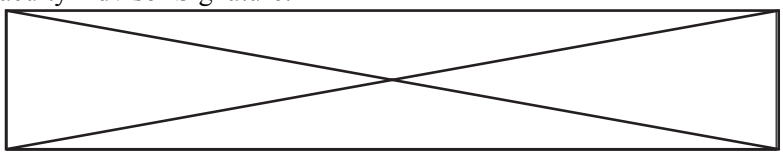
10. Please describe the BioSafety Level of the lab in which the team works, or description of safety features of lab (Refer to Basic Safety form, question 8. d.). If you are using organisms with a BSL level greater than you lab, please explain any additional safety precautions you are taking.

The lab we work in has BioSafety Level 1 and we are not using organisms with a BSL level greater than 1.

Faculty Advisor Name:

Prof. Dr. Arne Skerra

Faculty Advisor Signature:



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- Genetic parts derived from mammals

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- 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.)

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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.

Mus musculus (mammal)

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.

We are using the Ig-Kappa secretion signal as a genetic part. We are using this part as a signal peptide for our eucaryotic chassis Physcomitrella patens in order to secrete effector proteins

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

We acquired the part by gene synthesis

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?

No potential safety/health risks of the part itself. Composite parts containing this signal sequence could be possibly dangerous when the signal sequence is fused to a dangerous protein. However, we are not using the part in combination with possibly dangerous other parts.

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?

We have all completed a biosafety course and we handle everything in the lab with the necessary precaution. We will not distribute any parts or organisms outside the lab, we are using moss that is not able to sporulate and have implemented a kill switch (see basic safety form)

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.

It is a eukaryotic secretion signal which enables secretion of proteins that carry this signal sequence in mammals and other eukaryotes. Therefore the part itself is not dangerous.

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 need this part to be able to secrete our effector proteins. Since we are using Physcomitrella patens as a chassis, we have to use a eukaryotic secretion signal. The use of the Ig-kappa secretion signal from mouse (BSL 1) is widely established and does not pose any safety risks.

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?

not listed

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.

The lab we work in has BioSafety Level 1 and we are not using organisms with a BSL level greater than 1.

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Prof. Dr. Arne Skerra

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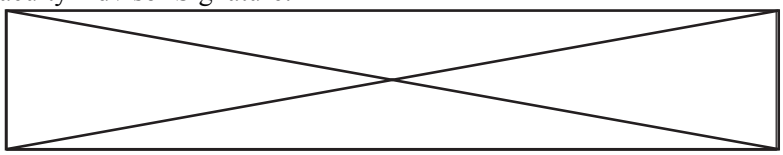

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1. Organism name and strain name or number.

Simean virus 40

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.

Part BBa_K801030 is a nuclear localization sequence (NLS) from Simian virus 40, it enables targeting of proteins into the nucleus

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

parts registry

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?

The part itself consists of 7 amino acids and does not pose any safety or health risks

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?

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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 part is based on the plasmid pGADT7 AD from Clontech, so the NLS is a commercially available and widely used. We therefore consider it not dangerous.

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?

Part is needed to target the nuclease of our killswitch into the nucleus of our moss. Without a localization signal, the killswitch would not work. The NLS from SV40 is used in almost all commercially available vector systems and has been in use for a long time which is why we chose it

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?

not listed

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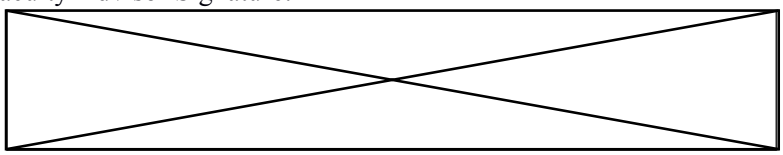

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This form must be completed separately for each organism or part above RG1. Please cite sources, including web links as applicable, to support your statements.

1. Organism name and strain name or number.

Staphylococcus aureus (Part: BBa_K729004)

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.

We are using the part BBa_K729004 (nuclease from Staphylococcus aureus) but have performed a PCR to remove bacterial signal peptides. It causes genomic degradation and we are using it in our kill switch to kill the moss and destroy all genetic material.

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

We acquired it from the parts registry

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?

The protein produced by the part is not dangerous(http://www.thermoscientificbio.com/uploadedFiles/Resources/en0181-usa-msds.pdf). However, S. aureus nuclease production contributes to disease pathogenesis in vivo.

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?

We have all completed a biosafety course and we handle everything in the lab with the necessary precaution. We will not distribute any parts or organisms outside the lab, we are using moss that is not able to sporulate and have implemented a kill switch (see basic safety form)

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.

S. aureus nuclease production contributes to disease pathogenesis in vivo so it could possibly enhance pathogenesis in other microorganisms as well (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2982853/). It is therefore important to ensure that no genetic material of our moss gets into the environment.

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 need this part in our kill switch to ensure that the moss completely killed and that all genetic material is destroyed.

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?

not listed

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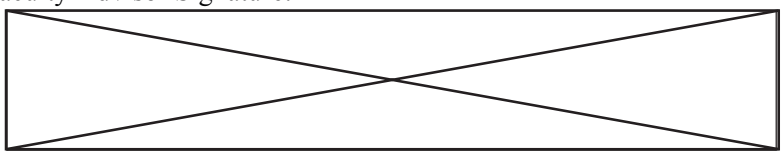

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1. Organism name and strain name or number.

Homo sapiens

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.

Proteinphosphatase 1 is able to bind the cyanotoxin microcystin and is used in our bioaccumulation project. Our moss could thus filter the toxin from the water.

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

iGEM Team Dundee 2013

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?

No safety risks

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?

We have all completed a biosafety course and we handle everything in the lab with the necessary precaution. We will not distribute any parts or organisms outside the lab, we are using moss that is not able to sporulate and have implemented a kill switch (see basic safety form)

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 part is human-derived and should thus not be dangerous.

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 need to use this part to filter the dangerous cyanotoxin microcystin from the water. Since proteinphosphatase binds microcystin with a very high affinity it is the best choice.

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?

not listed

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.

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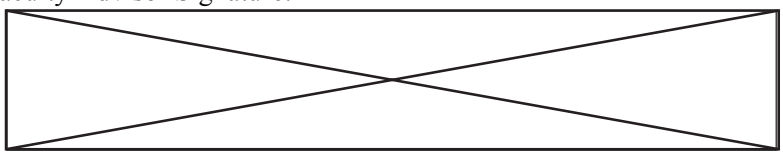

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1. Organism name and strain name or number.

Streptococcus pyogenes

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.

We are using the Spytag/Spycatcher part for posttranslational protein fusions (fusion of our effectors to membrane receptors).The two parts consists of a domain of streptococcus pyogenes fibronectin-binding protein FbaB. This domain was split and the fragments were rationally engineered

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

We acquired the part by gene synthesis
source paper with underlying principle:
http://www.pnas.org/content/early/2012/02/17/1115485109.full.pdf

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?

We believe that the part itself is not dangerous. Since the binding of tag/catcher is very specific, the catcher cannot bind to other proteins and only binds proteins that are fused to the tag

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?

We have all completed a biosafety course and we handle everything in the lab with the necessary precaution. We will not distribute any parts or organisms outside the lab, we are using moss that is not able to sporulate and have implemented a kill switch (see basic safety form)

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.

Since it was partly engineered, the parts itself is not dangerous in a way that it is connected to Streptococcus pyogenes. The fusion product tag-catcher itself is not dangerous but this depends on the proteins tag/catcher are fused to.

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 the Spytag/Spycatcher part for posttranslational fusion of our effectors to membrane receptors. There is no part from a less dangerous risk group that would accomplish this purpose this good

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?

not listed

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.

The lab we work in has BioSafety Level 1 and we are not using organisms with a BSL level greater than 1.

Faculty Advisor Name:

Prof. Dr. Arne Skerra

Faculty Advisor Signature: