1. **Your Training**

Have your team members received any safety training yet?

Yes, we have already received safety training.

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

All students have undergone lab safety training before beginning the project. This covered aspects of personal safety, fire safety, proper handling of chemicals and waste disposal training. There are separate bins for solid biological waste, liquid biological waste, chemical waste and sharps. Our lab adheres strictly to the UK health and safety executive containment 2 standard, as research groups within the lab work with Cat 2 organisms. Cat2 organisms must be handled with extra caution and biological waste must be labelled and handled with caution. All items are taped closed and labelled prior to being autoclaved and properly disposed of. Work surfaces are to be kept clean and tidy at all times, decontaminating after any spillages and at the end of the day.
While working at the benches we ensure gloves, lab coats and safety glasses are worn at all times. Personal safety precautions include no open toed and long hair being tied back when near and open flame. Extra safety precautions are taken when required e.g. full face shields are worn when analysing gels to prevent exposure to ultraviolet radiation. On exiting the lab, lab coats must be removed and hands must be washed. Eating and drinking is prohibited in the lab and any food or drink must be stored outside of the laboratory in designated areas.

Please give a link to the laboratory safety training requirements of your institution (college, university, community lab, etc). Or, if you cannot give a link, briefly describe the requirements.

https://www.lifesci.dundee.ac.uk/services/healthandsafety/training/training_home.html#biol

The University regulations state that comprehensive risk assessments must be carried out prior to the start of any laboratory project and any accidents or spillages of micro-organisms must be reported immediately. We were given a general lab safety induction by the Health and Safety board at the University of Dundee's College of Life Sciences, which included guidance in waste disposal of biohazardous material. Documents describing Standard Operating Procedures and risk assessments were made available to the whole team online.

2. **Your Local Rules and Regulations**

Who is responsible for biological safety at your institution? (You might have an Institutional Biosafety Committee, an Office of Environmental Health and Safety, a single Biosafety Officer, or some other arrangement.) Have you discussed your project with

them? Describe any concerns they raised, and any changes you made in your project based on your discussion.

The College of Life Sciences at Dundee University has a health and safety management committee. Additionally it has a dedicated Health and Safety coordinator and information officer. We have discussed out project with the Molecular microbiology Head of Division and the Health and Safety information officer; they have raised no concerns regarding our project.

What are the biosafety guidelines of your institution? Please give a link to these guidelines, or briefly describe them if you cannot give a link.

http://www.hse.gov.uk/biosafety/biologagents.pdf

3. The Organisms and Parts that You Use

***Escherichia coli* K-12**

Risk Group 1: http://www.absa.org/riskgroups/bacteriasearch.php?genus=escherichia
non-pathogenic, non-toxigenic, non-colonising chassis

Pseudomonas aeruginosa PA01
Risk Group 2 http://www.absa.org/riskgroups/bacteriasearch.php?genus=pseudomonas

PqsR: *A LysR-type transcriptional regulator protein. Coding sequence amplified from gDNA. PqsR is cytoplasmic membrane bound transcription regulator which binds PQS and activates expression of numerous genes including pqsABCDE. These parts will be constructed in a plasmid and the pqsABCDE gene will be coupled to a fluorescent output to allow us to measure any exogenous PQS.*

$P_{pqsABCDE}$: *PQS synthesis operon promoter. Seaquence amplified from gDNA*

Xanthomonas campestris (Xcc 8004)

Risk Group 1: http://www.absa.org/riskgroups/bacteriasearch.php?genus=xanthomonas

RpfC: *A membrane bound histidine sensor kinase. Coding sequence amplified from gDNA RpfC is a membrane bound histidine kinase that binds exogenous DSF and responds by phosphorylating the cytoplasmic response regulator RpfG. Upon phosphorylation this activates RpfG to degrade the intracellular second messenger cyclic di-GMP. The transcription factor Clp, which was originally repressed by cyclic di-GMP, becomes activated and binds to the manA promoter activating it's gene expression. These parts will be constructed in a plasmid and the manA gene will be coupled to a fluorescent output to allow us to measure any exogenous DSF.*

RpfG: *A cytoplasmic response regulator. Coding sequence amplified from gDNA*

Clp: *A transcription factor. Coding sequence amplified from gDNA*
P_{manA} *A mannan endo-1,4-beta-mannosidase/cellulase synthesis promoter. Sequence amplified from gDNA*

Burkholderia cenocepacia (J2315)

Risk Group 2: http://www.absa.org/riskgroups/bacteriasearch.php?genus=Burkholderia
BCAM0227: *A membrane bound histidine sensor kinase. Coding sequence synthesised by Dundee Cell Products*

> *BCAM0227 is a membrane bound histidine sensor kinase which senses BDSF. Upon activation by BDSF, BCAM0227 phosphorylates the response regulator protein BCAM0228. This promotes transcription by the cbld gene. These parts will be constructed in a plasmid and the cbld gene will be coupled to a fluorescent output to allow us to measure any exogenous BDSF.*

BCAM0228: *A response regulator protein. Coding sequence amplified from gDNA*

P_{cbld}: *A putative minor pilin initiator promoter. Sequence amplified from gDNA*

4. Risks of Your Project Now

Please describe risks of working with the biological materials (cells, organisms, DNA, etc.) that you are using in your project. If you are taking any safety precautions (even basic ones, like rubber gloves), that is because your work has some risks, however small. Therefore, please discuss possible risks and what you have done (or might do) to minimize them, instead of simply saying that there are no risks at all.

We ensure that the team, while working in the lab, follow the BioSafety Level 2 procedures as described above. Special care was required when making the gDNA from Pseudomonas aeruginosa *and* Burkholderia cenocepacia*. However these parts are then put into E.coli chassis and safe to handle, therefore the risk is reduced.*

However, our parts do not pose any health and safety concerns.

Risks to the safety and health of the general public (if any biological materials escaped from your lab):

*Our project is composed of three individual detection systems for 3 different organisms (*Psuedomonas*,* Burkholderia *and* Stenotrophomonas *species). Each system will detect quorum signalling molecules produced by each of the individual organisms when present at threshold level. The detector will only function when species are present at this threshold*

level, as in the case of a patient with CF. We are hoping that these detection systems will be used in the form of a contained device, that will be operated by trained medical staff according to standard operating procedures. Additionally we have chosen a chassis that is not harmful.

Risks to the environment (from waste disposal, or from materials escaping from your lab):

We do not intend to use these parts in the environment and so there are no direct risks. In order to prevent any biological materials leaving the lab we make sure to work aseptically and dispose of our waste according to Cat 2 regulations. Additionally we have chosen a chassis that is not harmful.

Risks to security through malicious mis-use by individuals, groups, or countries:

We have identified no potential risks though malicious mis-use. Our device is merely a bio-sensor with a luminescent protein output.

What measures are you taking to reduce these risks? (For example: safe lab practices, choices of which organisms to use.)

In order to reduce above risks all biological materials are handled and disposed of according to regulation. We have chosen a chassis that is not harmful.

5. Risks of Your Project in the Future

What would happen if all your dreams came true, and your project grew from a small lab study into a commercial/industrial/medical product that was used by many people? We invite you to speculate broadly and discuss possibilities, rather than providing definite answers. Even if the product is "safe", please discuss possible risks and how they could be addressed, rather than simply saying that there are no risks at all.

We hope to see our device being used as an integral part of the detection system of bacteria colonising CF patients lungs.
This may lead to widespread use of our device by many different professionals, e.g scientists, nurses, clinicians and maybe even demand as a home kit. We would have to ensure that all organisms are well contained in the device and we will be exploring the safety issues which would arise if our device was to be used as a home kit. To do this comprehensively, we plan on discussing our ideas further with the CF clinic team, medical laboratory staff and local biotech companies.

Does your project currently include any design features to reduce risks? Or, if you did all the future work to make your project grow into a popular product, would you plan to design any new features to minimize risks? (For example: auxotrophic chassis, physical containment, etc.) Such features are not required for an iGEM project, but many teams choose to explore them.

Our project has the overall aim of building a device that will physically contain the biological detectors. Our final device would contain an integrated disposal system for cartridges containing modified E. coli to be ejected in a sealed container.