University of Glasgow

Form 1: Proposal and Risk Assessment for Work with Genetically Modified Microorganisms and or Animal Cells Excluding Use of Viruses

A GM risk assessment is required for the possession or use of genetically modified organisms. Please complete this form by computer and send it as an email attachment to your GM Biological Safety Officer (GMBSO) to submit it to your GM Safety Committee (GMSC). GMBSO will advise Principal Investigators on all aspects of GM risk assessment and HSE notification. Guidance on completing this form is provided on the Health and Safety section of the CMVLS website and GM Risk Assessment section of the SEPS website.

Title of project	Glasgow University iGEM project: Recombinase switch control of Ecoli gas vesicles
GM reference	[ENTER DETAILS HERE]
Principal investigator	Sean Colloms
School / Institute	The University of Glasgow
Date of application	dd/mm/yyyy
Location of work (Building & room numbers)	Bower Building, Wilkins Teaching Laboratory

Summary Details

(Delete or enter not applicable (N/A) where appropriate)

GM activity class	1 / 2 / 3		
Connected programme?	Yes / No	**If Yes, and not parent, give parent GM reference and where relevant, the Biological Services risk assessment serial number**	

Section 1 Personnel

1.1: Briefly indicate your experience of working with microorganisms and genetically modified organisms and any training you have received
[ENTER DETAILS HERE]

1.2.1: Other workers on the project (if known)	1.2.2: Qualifications	1.2.3: Experience / Training
Jumai Abioye	Bsc Microbiology (UoG)	Extensive lab experience
Emma Smith	Bsc Genetics (UoG)	Extensive lab experience
Jake Casson		Completed 3 years of a molecular and cellular biology degree at the UoG, involving multiple laboratory sessions working with the organisms and equipment we will be using in this project.
Gintare Sendžikaitė		As above, but 3 years of a Genetics degree
Jacob Roberts		As above
Lydia Aldred		As above
Gemma Mclelland		As above
Martin Campbell		As above
Amy Ferguson		As above
Beth Grieg		As above
Aimee Bias (Dry lab)		No wet lab experience
Robbie Evans (Dry lab)		No wet lab experience

Section 2 Project

2.1: Brief description of the project, including the methods to be used and the purpose of the genetic modification (Preferably no more than 500 words unless the work is very complex)

The project is Glasgow University's entry to the iGEM (International Genetically Engineered Machine) competition. The competition involves the engineering of bacteria using "biobricks" - genetic building blocks that can be arranged in a modular fashion.
The project will be on creating *E.coli* which, upon activation of a recombinase switch by an external stimulus, will switch off flagella production (through knockout of the motA and/or fliC gene) and begin to produce gas vesicles to increase buoyancy. In anticipation of time constraints, we will be aiming to test the system using arabinose sugar as the stimulus, though an eventual use for this system would see salt or CO2 as the stimulus.
Gas Vesicle Proteins A and C will be inserted into the *E. coli* via plasmids, with the view to the *E.coli* beginning to express gas vesicles.
phi31C-integrase will also be inserted via plasmids, and when activated, will start the expression of the GvpA and C proteins.
GFP will be used as both an indicator for the successful activation of the recombinase switch, and as a visual indication of bacterial vertical distribution in culture.
The methods used will be standard biomolecular practices such as PCR, transformations, restriction digests, ligations and so on.

2.2: Will you cultivate on a large scale (eg 10 or more litres per culture) Yes / No

2.3: Host organism

Escherichia coli K-12

2.4: Vector system

Plasmids – biobrick vectors with p15A, pBR322, pSC101,pSB6A1,pSB3C5, pSB1C3; ampicillin, chloramphenicol, kanamycin antibiotic resistances, non mobilisable, non conjugative.

2.5: Origins, nature of modification(s), and intended function of the genetic material involved

GvpA, GvpC – from the cyanobacteria *Planktothrix rubescenes* - they are the main constituent proteins of gas vesicles.

GFP- Originally from the jellyfish *Aequorea victoria* – for both the testing of the recombinase switch and for visualisation of upward bacterial movement

phiC31 integrase - *Streptomyces* phage phiC31 – the source of the recombinase switch for turning on gas vesicle production.

We will also be knocking out the genes for fliC and/or motA, to remove/control the swimming ability of the host. Since these genes are present in *E.coli* there are less complications – they are present naturally in the organism.

None of the genetic material we use will be altered in any way (other than its original removal from its host organism), the lengths of genes we require will be inserted unaltered. The exception to this would be the removal of undesirable restriction sites to make the parts comply with iGEM standards, this would be done using mutagenic PCR in most cases, though we anticipate site-directed mutagenesis is likely required for one particular removal.

Section 3 Risk Assessment

This section should include clear and explicit justification of any statements made about the risks and be supported as far as is reasonable by adequate evidence and a logical explanation. **Level of risk** may be estimated using the matrix given at the end of this form and then stating the risk as either Effectively zero, Low, Low / Medium, Medium or High.

3A Risks to Human Health

3A.1: Characteristics of the host and any hazards associated with it

The host is a debilitated strain of the *E.coli* that usually populates almost all mammalian guts. The K-12 strain's characteristics are well documented, and it has a history of safe use in the GM field. It has limited capacity to colonise the mammalian gut, lacking some of the specific structures required for attachment. Even if attachment was successful, it does not possess any pathogenic properties. It does not survive well in an uncontrolled environment. In addition, it is not believed to have an adverse effects on the environment, animals or plants.

Level of risk	**Effectively zero**

3A.2: Characteristics of the vector system and any hazards associated with it

The plasmids used are both non-mobilisable and non-conjugatable, which should remove the risks of undesirable plasmid transfer to other species. They have low ORI numbers. Little hazard is associated with the material transferred by the plasmids, regardless – the genetic material on the plasmids has not been engineered to produce chemicals potentially harmful to the environment, plants, animals or humans.

Level of risk	**Effectively zero**

3A.3: Source and characteristics of the inserted gene product and any hazards arising directly from its use (including an estimation of the level of expression and biological activity of the recombinant gene product)

GFP – Fluoresces green light when exposed to light in the blue/violet wavelength range. It has a history of safe use in a number of fields, and should not pose a risk to human health.

GvpA and GvpC – originally taken from the cyanobacteria *planktotrix rubescens* (capable of forming algal blooms and producing toxins), the required genes will be sourced from the iGEM distribution of parts, contained on dehydrated plasmids. GvpA forms the main wall of the gas vesicle, and as such is expressed more than GvpC, which is required for added strength. The vesicles are susceptible to pressure changes. The organism in its entirety will never be present in the laboratory.

PhiC31-integrase – Is a recombinase switch which reverses a section of DNA (and a promoter region), upon a given stimulus.

For the purposes of the project, all of the above genetic material will be sourced from plasmids containing said parts – either from the iGEM distribution wells, or from other labs at the university.

Level of risk	**Effectively zero**

3A.4: Hazards arising from the alteration of any existing pathogenic traits, if applicable

N/A

Level of risk	**N/A**

3A.5: Potential hazards of sequences within the GMM being transferred to related micro-organisms

The material only causes the bacteria to float and/or glow. There are no harmful chemicals being produced

Level of risk	**Effectively zero**

3A.6: The overall likelihood that, in the event of exposure, the GMM could cause harm to human health

Extremely low risk – as mentioned previously, the host has limited capacity for colonisation.

Level of risk	**Effectively zero**

Assign the provisional containment level: (Delete as appropriate)	**1**

3B Risks to Environment

3B.1: What is the capacity of the GMM to survive, establish, disseminate with and or displace other organisms

The *E.coli* K-12 is a debilitated strain, posing little to no risk to human, plant or other animal health. The *E.coli*'s capacity to populate the mammalian gut is not present on this strain, and it will not survive long outside of a laboratory environment due to a lack of colonising ability.	
Level of risk	**Effectively zero**
3B.2 What is its ability to cause harm to animals	
Unlike other strains of *E.coli*, K-12 does not populate the mammalian gut, lacking the proteins required for fixing to the intestinal walls. Being unable to colonise, it presents negligible pathogenic risk to animals.	
Level of risk:	**Effectively zero**
3B.3: What is its ability to cause harm to plants	
E.coli K-12 is not known to cause harm to plants.	
Level of risk	**Effectively zero**
3B.4: What is its ability to cause harm to other organisms	
E.coli K-12 is not known to cause harm to other organisms.	
Level of risk	**Effectively zero**
3B.5: What is the potential for transfer of genetic material between the GMM and other organisms	
The chance of transfer is very low – the plasmids are both non-conjugatable and non-mobiliational.	
Level of risk	**Effectively zero**
3B.6: Is there any hazard as a result of phenotypic or genetic instability	
The genetic material and resulting structures/chemicals are not close in structure to any toxins, so even if there was some instability (and we don't anticipate this), the result is unlikely to be hazardous	
Level of risk	**Effectively zero**
3C Final Activity Class	
Assign the class: (Delete as appropriate)	**1**

Section 4 Control Measures to be Used

4: Provide details of the control measures to be used to protect human health and the environment and the means by which their use and effectiveness will be monitored. This must include details of the inactivation procedures to be employed for waste contaminated with GMM, the expected degree of kill and any appropriate validation procedures

4.1: Containment level 1

The containment level of the lab is designated 1, which is a suitable level for the type of organisms and genetic modifications being carried out. As stated previously, the non-pathogenic nature of the *E.coli* K-12 strain is well documented and is not in any doubt. In addition, none of the modifications being made are potentially toxic – while the original host of the GvpA and GvpC genes *P.rubecens* is capable of toxin production, the entire organism will never be present in the laboratory.

4.2: Controls

These controls are in place to stop the transport of any organisms outside the laboratory. These include:
Wearing labcoats inside the lab, and removing them when going outside
Wearing gloves when handling material, and regularly checking them for holes.
Magnetic doors – with alarms if the door is left open for too long.
Aseptic techniques – general lab safety, including hand washing before and after working with the organisms and leaving the laboratory.

4.3: Inactivation of genetically modified organisms

Disinfection
Any contaminated equipment/worktop space is disinfected with the detergent "Trigene", which is distributed throughout the lab in spray bottles (1% solution). Accurate degree of kill could be not obtained, but the chemical is widely used in the laboratory, with a history of effective use against the *E.coli K-12* strain.

The effective of trigene will be regularly tested by observing its effect on a culture of bacteria – it should stop all growth.
The trigene is expected to have a lab diluted shelf life of 6 months – they will be refilled before the beginning of the 10 week project to maintain maximum effectiveness.
Equipment is then autoclaved.

Autoclaving
All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.
Or
All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored using TST (Time, Steam, and Temperature) test strips (Albert Browne Ltd., TST class 6 emulating indicator 121ºC for 20 min).

4.4: Waste disposal

Plastics (pipette tips, etc), are autoclaved as detailed above.
Plates containing bacteria are placed into a biohazard bag, and autoclaved.

Section 5 Emergency Planning

5: Are there risks to human health and safety or to the environment that require an emergency plan for action in case of accidental release — Yes / No

If Yes, provide full details of the plan

Section 6 Declaration and Approval

Declaration	**I declare that this work will be conducted in accordance with University rules, practices and requirements on GM procedures. If at any stage there is any indication that hazards or risks could be significantly higher than originally assessed or that changes to controls are needed then the work will cease until the risk assessment has been revised and approval granted from the GM Safety Committee.**
Principal investigator	
As the principal investigator for this GM project you have a legal responsibility to ensure that all those involved or working on the project have an appropriate level of training and expertise to enable safe working. This includes ensuring that they read and understand this risk assessment and that all procedures they undertake including the control measures are in strict accordance with those approved for the project. To ensure the latter you are advised to check for compliance with procedures and make an appropriate record to be kept as part of the project file.	
Sign and date	
Declaration	**I declare that this risk assessment has been scrutinised and approved by the GM Safety Committee.**
To be signed by the GMBSO or in the event they are the principal investigator by another member of the GM Safety Committee.	
GMBSO	
Sign and date	

Risk Estimation Matrix

Consequence of hazard	**Likelihood of hazard**			
	High	**Medium**	**Low**	**Negligible**
Severe	High	High	Medium	Effectively zero
Modest	High	Medium	Medium / Low	Effectively zero
Minor	Medium / Low	Low	Low	Effectively zero
Negligible	Effectively zero	Effectively zero	Effectively zero	Effectively zero