GENETICALLY MODIFIED ORGANISMS (CONTAINED USE) REGULATIONS 2000

School of Biological Sciences

RISK ASSESSMENT FORM FOR ACTIVITIES INVOLVING THE USE OF GENETICALLY MODIFIED MICRO-ORGANISMS AND EUKARYOTIC CELL AND TISSUE CULTURE SYSTEMS

GMMO Form: SBS version No. 6 (March 2010)

<u>Notes</u>

(1) It is the responsibility of the Principal Investigator (PI) to undertake a risk assessment in relation to any genetic modification work they, or members of their research group, undertake. The risk assessment must be undertaken and be reviewed and approved by the School GM Safety Committee in advance of work starting. A risk assessment is required for any activity involving genetically modified organisms, including storage, irrespective of where the GMO was originally made.

(2) In the following form, the spaces expand as required. The spacing in the master version is not indicative of the length of answer expected. Unless given as an option, it is not acceptable to give one-word answers. Justification must be given for all answers/ statements.

(3) If it is likely that the work will require notification to the Health and Safety Executive (Class 2 or 3) you should contact the University Biological Safety Adviser for further guidance PRIOR to completing this form.

(4) Do not use this form for genetically modified plant pathogens or plant associated micro-organisms (there is a separate form available specifically for these).

SCHOOL: Biological Sciences **INSTITUTE/CENTRE:** **Cell Biology**	**PRINCIPAL INVESTIGATOR:** **Dr Chris French**	**GM RA Ref. No:** ***SBS_1209***
Contact Address: Darwin 705, KB	**Phone:** 650 7098	
E-mail: c.french@ed.ac.uk		
PROJECT TITLE: iGEM project 2012		

PREMISES WHERE THIS WORK WILL BE CARRIED OUT
Laboratory work: Darwin 705
Animal Work: *Include Home Office licence number where applicable*:

1.1 OVERVIEW AND SUMMARY OF PROJECT: *(include aims and objectives. This section should be completed in simple terms and provide enough basic information in order that a person with no experience of this area can understand the work).*

The International Genetically Engineered Machine competition (iGEM) is an annual synthetic biology competition run by researchers at Massachusetts Institute of Technology (MIT), in which teams of undergraduate biologists and engineers work over the summer vacation period to design and construct novel genetically engineered systems. The Edinburgh 2012 iGEM project has three aspects:

1-testing of novel selection and counter-selection systems which do not involve antibiotics, and hence which may be useful for organisms which are ultimately intended for release into the environment. This project will involve sugar-assimilation genes, and genes encoding enzymes which can activate substances to a more toxic form.

2-modification of the electron transport chain of cells to facilitate transfer of electrons to or from an electrode, for use in developing bio-electric devices. This will involve expression of outer membrance cytochromes from *Shewanella oneidensis*, together with functions required for maturation of c-type cytochromes.

3-testing of *Citrobacter freundii* as an alternative to *Escherichia coli* as a host strain for synthetic biology projects. This will require testing of various *Citrobacter* promoters, as well as expression and testing of widely used biological components in *C. freundii*. We will also seek to develop a disabled strain of *C. freundii* with reduced ability to compete in the environment.

Give brief details of Recipient/Host(s): *(specify if wild type or disabled)*

Escherichia coli, disabled laboratory strains (ACDP1)
Citrobacter freundii, wild type strains (ACDP1)

Give brief details of Vector(s):

Non-transmissible plasmid vectors from the Registry of Standard Biological Parts, such as pSB1C3 and derivatives thereof.

What is the normal/expected biological action of the inserted DNA/RNA or transcribed/translated gene product: *(if not known indicate the type of processes these may be associated with)*

1-genes encoding the ability to utilize sugars and other substrates: eg *cscA*, permitting utilization of sucrose in *E. coli* strains.

2-genes encoding enzymes capable of activating prodrugs to more toxic forms: eg *nfsI*, encoding nitroreductase, and *dhlA*, encoding haloalkane dehalogenase; also genes encoding other proteins which are toxic under particular circumstances, such as *sacB* (levansucrase) which is toxic to Gram negative cells in the presence of sucrose.

3-genes encoding outer membrane cytochromes and accessory proteins, such as MtrA, MtrB, MtrC and CymA of *Shewanella oneidensis*, and NapC of *E. coli*, as well as cytochrome c maturation (Ccm) proteins of *E. coli* and *S. oneidensis*.

4-standard reporter genes such as those encoding Green Fluorescent Protein and related fluorescent proteins.

5-possibly, genes encoding other functions used in previous (approved) iGEM projects, such as those involved in production of carotenoid pigments and terpenoid fragrances (eg limonene).

Technique used to introduce insert or vector into host:

Chemical transformation using calcium chloride or similar reagents.

Details of Host/Vector and Inserted Gene(s)
MAKE CLEAR THE INDIVIDUAL STEPS INVOLVED IN THE PROJECT

This section will include information on the cloning and expression steps. See the Table/s below. (Please delete tables that do not apply and to convert from landscape to portrait, if desired.) Add any necessary supplementary information below the relevant Table. See Guidance notes.

1.2 Bacterial Systems

Description of each step e.g. cloning target gene into plasmid vector	Target DNA/Gene	Source	Source ACDP	Host	Host ACDP	Vector	Scale
PCR and cloning of genes into pSB1C3 or similar plasmids	*cscA, nfsI, dhlA*	Originally from *Escherichia coli* Sakai, *Enterobacter cloacae* and *Xanotho-bacter auto-trophicus*; our source will be genes previously cloned in *E. coli* plasmids	Originai source ACDP2 (*E. coli* Sakai, *E. cloacae*) and ACDP1 (*X. auto-trophicus*); *E. coli* clones ACDP1.	*E. coli* disabled strains, eg JM109.	ACDP1	pSB1C3 or similar	up to 50 ml
As above	*napC, ccm* gene cluster	*E. coli* lab strains	1	*E. coli* disabled strains, eg JM109.	1	pSB1C3 or similar	up to 50 ml
As above	*mtrA, mtrB, mtrC, cymA,* possibly *ccm* genes	*Shewanella oneidensis* MR1	1	*E. coli* disabled strains, eg JM109.	1	pSB1C3 or similar	up to 50 ml
Combination of genes in artificial operons, and assays of cultures for various properties	as above	as above	as above	*E. coli* disabled strains, eg JM109.	1	pSB1C3 or similar	up to 500 ml
Expression testing in *Citrobacter freundii*	as above	as above	as above	*C. freundii*	1	pSB1C3 or similar	up to 50 ml

<table>
<tr>
<td>2. RISK ASSESSMENT FOR HUMAN HEALTH AND SAFETY
Identify any potential harmful properties of the following to human health and safety: (see side panel)</td>
<td></td>
<td rowspan="4">GUIDANCE

Potentially harmful effects include:

disease to humans – consider all properties which may give rise to harm e.g. infection, toxins, cytokines, allergens, hormones etc

alteration of existing pathogenic traits – consider possibility of increase in infectivity or pathogenicity, alteration of tissue tropism or host range, alteration in susceptibility to human defence mechanisms etc
note in particular if the insert codes for a pathogenicity determinant

adverse effects resulting from inability to treat disease or offer effective prophylaxis- consider antibiotic resistance markers introduced

possibilities for any disablement or attenuation to be overcome by recombination or complementation

adverse effects resulting from the potential for transfer of inserted genetic material to another micro-organism particularly if there were escape to the environment – consider likelihood of transfer, selection pressure, and whether the gene is present in the environment

consider also fitness – the modification may make the micro-organisms more hazardous but less fit, any claim must be</td>
</tr>
<tr>
<td>i) the recipient micro-organism:
Escherichia coli JM109 is a widely used host strain with disabling mutations which is incapable of colonizing the human intestine, its natural habitat. The same is true of other similar E. coli strains which may be used. Citrobacter freundii is a close relative of E. coli which is not normally associated with human disease in healthy subjects, and is ACDP1 (unlike wild strains of E. coli, which are ACDP2). (Note: like many bacteria capable of growing at human body temperature, some strains of C. freundii are capable of infecting compromised hosts under unusual circumstances, especially in hospitals; however, our strains are not clinical isolates and are not considered to pose a health risk). One aspect of the project will be to prepare a disabled strain of C. freundii.
Are the cells to be used primary human cells and/or cell lines that are not fully authenticated and characterised? No (If yes, give details)</td>
<td>Consider pathogenicity of host strain including virulence, infectivity and toxin production, for micro-organisms give ACDP hazard group)

These may carry contaminating infectious agents, consequently containment level 2 plus the use of a microbiological safety cabinet is required under the COSHH Regulations. This is separate to, and does not affect, the control measures determined in the GM risk assessment)</td>
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<td>ii) the inserted (donated) genetic material:
Genes encoding sugar uptake systems, respiratory proteins and their accessory proteins, and common reporter genes, are not expected to increase pathogenicity in any way. Genes encoding counterselection enzymes such as nitroreductase and dehalogenase are expected to be toxic to cells in the presence of counterselective agents.</td>
<td>Consider biological properties of the inserted gene which may give rise to harm such as toxins, cytokines, allergens, hormones etc.; take account of the level of expression and whether it is expressed in an active form)</td>
</tr>
<tr>
<td>iii) the vector:
Vectors used will be standard, widely used, non-transmissible cloning plasmids such as pSB1C3 (Registry of Standard Biological Parts). These encode resistance to antibiotics such as chloramphenicol. This is not expected to pose any risk to human health, nor should such resistance determinants be passed to other bacteria. Part of this project will include the development of standard cloning plasmids which do not include antibiotic resistance determinants, which will decrease this risk even further.</td>
<td>Identify type of vector and any hazards associated with it. If a viral vector is used give full details especially in relation to any disablement, consider all properties of the construct as in iv below.</td>
</tr>
</table>

iv) the resulting genetically modified micro-organism: Resulting genetically modified microorganisms should pose no greater risk to human health than the host strains.	*Consider all properties of the construct; take account of severity of consequences and likelihood of occurrence.*	*evidence based*
Brenner Scheme values *COMPLETION OPTIONAL and in any case only for disabled E. coli* Access: _______ Expression: _______ Damage: _______ Overall: _______		
<u>Control measures</u> – Assign provisional containment level **Containment Level: 1** **with Good Microbiological Practice and Good Occupational Safety and Hygiene**	*Assign a provisional containment level to control the hazards identified above taking account of severity of any consequence and likelihood of harm occurring. Select from 1, 2, 3 or 4*	

3. NATURE OF WORK TO BE UNDERTAKEN Genes will be cloned in BioBrick format by PCR, and introduced into standard vectors such as pSB1C3. The resulting BioBricks will be combined in various ways to generate test constructs. These will be tested for properties such as growth in the presence or absence of various substances, ability to transfer electrons to electrodes in a microbial fuel cell, half-cell or similar apparatus, ability to produce certain pigments or fragrances, enzyme activities, levels of reporter gene expression, etc. in volumes perhaps as high as 500 ml.	*Give brief description of types of laboratory procedures including maximum culture volumes at any time (show as multiples of unit volumes):*	***GUIDANCE*** *Consider any activities that may involve risks which require specific additional control measures such as:* *inoculation of animals or plants with GMMs* *the use of equipment or procedures likely to generate aerosols* *large scale work (>10 litres)*
<u>Additional control measures</u> required for specific risks: if cell lysates are prepared by sonication, this will be performed in an enclosed cabinet to minimize the risk of aerosol production.	*Provide details of any laboratory operations that may have additional risks:*	

4. RISK ASSESSMENT FOR ENVIRONMENTAL HARM	*GUIDANCE*
Identify any potentially harmful properties of the following <u>to the environment:</u> *(see side panel)* i) the recipient micro-organism: *(for micro-organisms indicate if subject to any DEFRA/SAPO controls)* *E. coli* host strains are unable to colonize the intestines, their natural environment. *C. freundii* strains are close to wild type and may be able to proliferate in the environment, but are not expected to pose any hazard to animals or plants or to the environment in general. One aspect of the project will be to prepare a disabled strain of *C. freundii*. ii) the inserted (donated) genetic material: Inserted genes are not expected to increase the ability of the organisms to survive in the external environment, or to cause harm to the environment or any other organism. iii) the vector: plasmids used are non-transmissible and gene transfer is not likely to occur. iv) the resulting genetically modified micro-organism: *(consider all properties of the construct, especially potential effects of gene transfer to, or recombination with, any wild type micro-organisms)* Resulting genetically modified microorganisms are not likely to pose any greater hazards to the environment than unmodified host organisms.	*Potentially harmful effects include:* *products of gene expression including allergenic and toxic effects* *disease to animals and plants* *adverse effects resulting from inability to treat disease or offer effective prophylaxis* *adverse effects resulting from establishment or dissemination of the GMMs in the environment and displacement of other organisms* *adverse effects resulting from the natural transfer of inserted genetic material to other organisms*
Where potentially harmful effects are identified estimate: i) consequence/severity of effects: NEGLIGIBLE ii) likelihood of effects being realised: *(taking containment and control measures assigned above into account)* NEGLIGIBLE iii) overall risk: *(consequence x likelihood, refer to risk matrix)* EFFECTIVELY ZERO	*Select from: Severe/Medium/Low/Negligible* *Select from: High/Medium/Low/Negligible* *Select from: High/Medium/Low/Effectively zero*
<u>**Additional control measures**</u> required to reduce all risks to low/effectively zero: None.	*Plant or animal pathogens will always require containment level 2 or higher*

5. CLASSIFICATION AND ASSIGNMENT OF FINAL CONTROL MEASURES

Consider each item on Table 1a - indicate whether or not it is required taking account of the provisional containment level assigned to protect human health and safety and any additional control measures necessary to control specific activities and environmental risks. *Note: some parts have already been completed for you, these are standard minimum requirements.*

Consider also Table 1c where appropriate

<u>Classification</u>

Class: 1

<u>Assign corresponding level of containment</u>

Containment Level: 1

specify any other control measures required: none

….. tick if some cells and/or cell lines require Containment Level 2 plus microbiological safety cabinet under COSHH Regulations (separate consideration to GM risk assessment)

GUIDANCE

Mark up table(s) by circling or highlighting/colouring for each item the first correct answer when reading across the table from left to right. Items should only be marked as required based only on risk assessment and not if they are used for other reasons such as product protection or convention

The highest numbered column in which a control measure is required indicates the Class of the activity – mark up class on table 1a

The class number indicates the minimum containment level required

Name of Assessor: *Dr Chris French*

Signature: Date:

Risk Assessment approved by Genetic Modification Safety Committee: Yes / ~~No~~

Signature: Date: 17 August 2012

(GM Biological Safety Officer)

Permission granted by Head of School for project to be undertaken: Yes / No

Signature: Date:

(Head of School)

APPENDICES

The following are to be attached:

1. Containment measures table(s) - Table 1a and Table 1c where appropriate
2. Personnel sheet - List of all persons working with the GMOs detailed above
3. Review sheet - Record of annual reviews of risk assessment
4. Waste disposal procedures

Table 1a: Containment Measures for Activities involving GMMOs in Laboratories

MARK UP THIS TABLE TO INDICATE WHETHER OR NOT THE LISTED CONTROL MEASUES ARE REQUIRED

Where an item is listed as "may be required" this indicates the item to be an option at that particular containment level and its requirement should be determined by the risk assessment for the particular activity concerned.

Containment Measures	**Containment Levels**			
	1			
Isolated laboratory suite	not required			
Laboratory sealable for fumigation	not required			
Surfaces impervious, resistant and easy to clean	**required for bench**			
Entry to lab via airlock	not required			
Negative pressure relative to the pressure of the immediate surroundings	not required			
HEPA filtered extract and input air	not required			
Microbiological safety cabinet/enclosure	not required			
Autoclave	required on site			
Access restricted to authorised personnel	not required			
Specified measures to control aerosol dissemination	not required			
Shower	not required			
Protective clothing	suitable protective clothing required			
Gloves	not required			
Control of disease vectors (e.g. rodents, insects) which could disseminate GMMs	may be required no			
Specified disinfection procedures in place	may be required **yes**			
Inactivation of GMMs in effluent from hand washing sinks, showers etc	not required			
Inactivation of GMMs in contaminated material and waste	required by validated means			
Laboratory to contain its own equipment	not required			
An observation window or alternative so that occupants can be seen	may be required no			
Safe storage of GMMs	may be required **yes**			
Written records of staff training	not required			
CLASSIFICATION	**CLASS 1**			

[Source: adapted from the ACGM Compendium of Guidance and Schedule 8 of the GMO (CU) Regulations 2000, as amended in 2005]

RECORD OF PERSONNEL INVOLVED **GM RA Ref. No: SBS_1209**

Name	Qualifications	Experience	Start date	Finish date
Dr. Chris French	Ph.D.	15 years post-doctoral	1 Aug 2012	10 Nov 2012
Dr. David Radford	Ph.D.	7 years post-doctoral	1 August 2012	10 Nov 2012
Eugene Fletcher	B.Sc. (Hons)	2 years Ph.D. training	1 August 2012	10 Nov 2012
Réka Nagy	Undergraduate student (BSc Molecular Genetics)	3 years undergraduate work	1 August 2012	10 Nov 2012
Jakub Krakowiak	Undergraduate student (BSc Biotechnology)	3 years undergraduate work, 2 months work in biological lab	1 August 2012	10 Nov 2012
Elitsa Peeva	Undergraduate student (BSc Pharmacology)	3 years undergraduate work, 18 weeks work in biological lab	1 August 2012	10 Nov 2012
Oscar Koch	Undergraduate student (BSc Biochemistry)	3 years undergraduate work	1 August 2012	10 Nov 2012
Parthenopi Vasiliadou	Undergraduate student (MEng Chemical engineering)	No biological experience	1 August 2012	10 Nov 2012
Kirsty McCarlie	Undergraduate student (MEng Chemical engineering)	No biological experience	1 August 2012	10 Nov 2012
Evgeniya Sotirova	Undergraduate student (BSc Computer Science and Mathematics)	No biological experience	1 August 2012	10 Nov 2012
Razvan Ranca	Undergraduate student (BSc Artificial Intelligence and Computer Science)	No biological experience	1 August 2012	10 Nov 2012
Melanie Dutton	Undergraduate student (BA Intermedia Art	4 months work in biological lab	1 August 2012	10 Nov 2012

REVIEW OF RISK ASSESSMENT	**GM RA Ref No: SBS_1209**
This risk assessment should be reviewed **annually** or more frequently if there is any change in the work, or if new information becomes available that indicates the assessment may no longer be valid. Reviews have been carried out on the following dates and either the assessment remains valid or it has been amended as indicated.	
Name of reviewer: Signature: Amendments:	Date:
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Name of reviewer: Signature: Amendments:	Date:
Name of reviewer: Signature: Amendments:	Date:

WASTE DISPOSAL PROCEDURES

Solids (e.g. plastic-ware such as pipettes, flasks, tubes etc and agar plates) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration or heat treatment or via the industrial (black bag) waste stream for landfill.

Liquids (e.g. samples, culture supernatants, tissue culture media) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 (either 121-125°C for at least 15 minutes or 126-130°C for at least 10 minutes or 134-138°C for at least 3 minutes), discharge to drains.

Sharps (in sharps bin, e.g. needles, syringes, scalpels) - dispose via clinical waste stream for heat treatment.

Waste residues that cannot be autoclaved, or cell culture waste will be inactivated by treatment with disinfectant: 1% Virkon solution for plastic-ware (soak for 2 hours), for treatment of minor contamination (10 min contact time) and surface disinfection (benches and floors); 2% for disinfection of liquid cultures and supernatants that cannot be autoclaved. Equipment that cannot be autoclaved will be disinfected as above; physically clean surfaces may be disinfected with 70% ethanol. Presept may be used as an alternative to Virkon; 1,000 ppm for general wiping of equipment and benches, 2,500 ppm free chlorine for discard containers/liquid cultures.

SPILLAGES

Particular care should be taken to ensure that others in the laboratory do not help with the clear up of accidental spillage (especially where there has been an accident that involves broken glass) unless they are aware of the potential risks and trained in safe working practices.
If spillage occurs, allow aerosols to settle and then working from the outside of the spill, apply powdered Virkon disinfectant to absorb liquids before mopping up with paper towels and disposing of all waste via autoclave waste stream.