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)

Notes

(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for any activity involving genetically modified organisms, including storage, irrespective of where the GMO was originally made.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:** ICB	**PRINCIPAL INVESTIGATOR:** Chris French	**GM RA Ref. No:** *(School BSO will complete this)* ***SBS_10??***
Contact Address: Darwin 702, KB	**Phone:** 650 7098	
E-mail: C.French@ed.ac.uk		
PROJECT TITLE: iGEM Project 2010		

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

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).*

This risk assessment concerns the University's entry in the International Genetically Engineered Machine Competition (iGEM) 2010. At the time of writing, three projects themes are under consideration. The first is an enabling technology: development of a standard method for markerless incorporation of BioBricks™ (modular DNA parts with standard ends) into the genome of *Escherichia coli* and related bacteria. The second is related to biofuel production, and concerns upregulation of fatty acid biosynthesis, coupled with expression of microbial enzymes which can convert fatty acids to wax esters, triglycerides or alkenes. The third concerns development of a system for optical communication between cells, where one type of cell emits light (bioluminescence) and another type detects this light and alters its behaviour in some predetermined way. This would allow cells to communicate rapidly in a non-chemical way, even if they were located in separate chambers, and could, for example, be a versatile replacement for quorum sensing to enable synchronization of cell behaviour.

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

Disabled strains of *Escherichia coli* such as JM109, DH5α and BW25113 (host of the KEIO mutant collection) For integration experiments, these may be expressing lambda recombination functions from a temperature-sensitive non-transmissible plasmid (pSC101-BAD-gbaA).

Give brief details of Vector(s):

Standard non-transmissible plasmid vectors such as pSB1C3. In some cases, constructs will be integrated onto the host chromosome using the Lambda-Red recombination system.

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. Bacteriophage lambda recombination functions, which allow gene cassettes to be integrated onto the host chromosome.
2. Proteins involved in fatty acid biosynthesis and its regulation (eg FadR, FadD), thioesterases, and enzymes converting fatty acids to further products (eg wax ester synthase/diacylglycerol acyltransferase (WS/DGAT, ArfA) of *Acinetobacter*; *Micrococcus* enzymes (Mlut_13230/OleA, 13240, 13250) converting fatty acids to alkenes.
3. Light emitting enzymes and accessory proteins (bacterial luciferase and its associated fatty acid reductase, flavin reductase, and wavelength-altering accessory proteins; firefly luciferase; *Renilla* luciferase; and mutants thereof with altered spectral characteristics) and light-detecting proteins (eg: Cph8, a fusion of *Synechocystis* Cph1 with *E. coli* EnvZ; YcgEF of *E. coli*; LOV-TAP fusion of *Avena sativa* (plant) LOV domain and *E. coli* trp repressor) and standard reporter genes (eg green and red fluorescent proteins, β-galactosidase).

Technique used to introduce insert or vector into host:
Chemical transformation (eg $CaCl_2$) or electroporation.

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
Amplification of target genes by PCR, insertion into standard plasmid vectors, growth of cultures for characterization	genes encoding enzymes of fatty acid biosynthesis	*Escherichia coli*	1	*Escherichia coli*, disabled	1	pSB1C3 or similar plasmid vector	<100 ml
As above	genes encoding transformation of fatty acids, eg WS/DGAT, Mlut_13230, 13240, 13250	*Acinetobacter baylii, Micrococcus luteus*	2, 1	as above	1	as above	<100 ml
As above	bacterial luciferase and accessory proteins, firefly luciferase, *Renilla* luciferase, and mutants thereof	*Xenorhabdus luminescens, Vibrio harveyi, Photobacterium phosphoreum, Photinus pyralis, Renilla sp.*	2, 1	as above	1	as above	<100 ml
As above	light sensing proteins (and fusions with regulatory proteins)	*Synechocystis, Avena sativa, Escherichia coli*	1	as above	1	as above	<100 ml

2. RISK ASSESSMENT FOR HUMAN HEALTH AND SAFETY Identify any potential harmful properties of the following to <u>human health and safety:</u> *(see side panel)*		***GUIDANCE*** *Potentially harmful effects include:*
i) the recipient micro-organism: Disabled laboratory strains of *Escherichia coli* (JM109, DH5a, and KEIO host strain BW25113). These strains have disabling mutations and are not expected to compete effectively with wildtype strains or colonize the mammalian intestine. 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)	*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)*	*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*
ii) the inserted (donated) genetic material: Upregulation of fatty acid synthesis will impose a severe metabolic burden on cells, reducing their fitness. Expression of light-emitting or light-sensing proteins or standard reporter genes is not expected to increase competitiveness or pathogenicity. Expression of bacteriophage recombination functions is also expected to reduce fitness by causing genomic instability.	*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)*	
iii) the vector: Standard plasmid vectors such as pSB1A3 and pSB1C3 confer ampicillin or chloramphenicol resistance. They are non-transmissible.	*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.*	

iv) the resulting genetically modified micro-organism: Resulting GMO are not expected to pose any hazard to human health beyond that of the unmodified hosts.	*Consider all properties of the construct; take account of severity of consequences and likelihood of occurrence.*	*hazardous but less fit, any claim must be evidence based*
Brenner Scheme values *COMPLETION OPTIONAL and in any case only for disabled E. coli* Access: _____ Expression: _____ Damage: _____ Overall: _____		
Control measures – 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 Preparation of DNA constructs, transformation of cells, preparation of plasmid DNA, characterization of transformed cells. Cell extracts may be prepared for enzyme activity assays. **Additional control measures** required for specific risks: If sonication is used for cell lysis, it will be performed in an enclosed cabinet to ensure that aerosols are contained..	*Give brief description of types of laboratory procedures including maximum culture volumes at any time (show as multiples of unit volumes):* *Provide details of any laboratory operations that may have additional risks:*	***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)*

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 have disabling mutations and are not expected to compete effectively with wildtype strains or colonize the mammalian intestine. ii) the inserted (donated) genetic material: Upregulation of fatty acid synthesis will impose a severe metabolic burden on cells, reducing their fitness. Expression of light-emitting or light-sensing proteins or standard reporter genes is not expected to increase competitiveness. Expression of bacteriophage recombination functions is expected to reduce fitness by causing genomic instability. iii) the vector: Plasmid vectors confer antibiotic ressistance but are non-transmissible, so should not spread to other organisms in the environment. 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 GMO are not expected to pose any hazard to the environment beyond that of the unmodified hosts.	*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	***GUIDANCE***
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: ….. 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)	*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: *(insert PI's name here)* Dr. Chris French Signature: Date:	
Risk Assessment approved by Genetic Modification Safety Committee: Yes / No Signature: Date: (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: …….**

Name	Qualifications	Experience	Start date	Finish date
Dr. Chris French	Ph.D.	15 years post-doctoral	12 July 2010	Not specified
Marta Bereska	none	undergraduate practicals	12 July 2010	5 Nov 2010
Maria Kowal	none	undergraduate practicals	12 July 2010	5 Nov 2010
William Rostain	none	undergraduate practicals	12 July 2010	5 Nov 2010
Richard Partride-Hicks	none	undergraduate practicals	12 July 2010	5 Nov 2010
Hannah Fraser	none	undergraduate practicals	12 July 2010	5 Nov 2010
Sarah Hunt	none	none	12 July 2010	5 Nov 2010
Meng Lu	none	none	12 July 2010	5 Nov 2010
John Wilson-Kanamori	none	none	12 July 2010	5 Nov 2010
Donal Stewart	B.Sc.?		12 July 2010	5 Nov 2010
Matthew Coombes	???		12 July 2010	5 Nov 2010

REVIEW OF RISK ASSESSMENT **GM RA Ref No: ……**
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: Date: Signature: Amendments:
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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^{o}C for at least 15 minutes or 126-130^{o}C for at least 10 minutes or 134-138^{o}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^{o}C for at least 15 minutes or 126-130^{o}C for at least 10 minutes or 134-138^{o}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.