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for any activity involving genetically modified organisms, including storage, irrespective of where the GMO was originally made.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 705, King's Buildings		**Phone: 650 7098**
E-mail: C.French@ed.ac.uk		
PROJECT TITLE: International Genetically Engineered Machine competition (iGEM) 2011 project.		

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).*

At this time, the project concept is still under development. The basic idea is to develop a scaffold system which can be used to co-display enzymes in the extracellular environment. Several different display systems will be tested, including P3-defective M13 filamentous bacteriophage (which remain attached to the cell surface rather than being released), cell-surface exposed proteins such as *Pseudomonas syringae* ice nucleation protein, and display systems in which proteins are attached to cell surface structures such as flagella and pili. As a demonstration system, enzymes will be attached and displayed which are able to degrade polysaccharides to sugars, and convert those sugars to other products. These may include amylase, endoglucanase, exoglucanase, β-glucosidase, xylanase, and xylose isomerase. In addition, intracellular enzymes may be expressed which are capable of converting assimilated sugars into useful products. These may include metabolic enzymes such as pyruvate decarboxylase, alcohol dehydrogenase, isoprene synthase, aldose reductase, and enzymes involved in synthesis of sugar-derived products.

Give brief details of Recipient/Host(s): *(specify if wild type or disabled)*	**Give brief details of Vector(s):**
Escherichia coli JM109 and similar disabled laboratory strains; possibly also *Escherichia coli* MG1655, a K12 laboratory strain (ACDP1) which is not specifically disabled but still fails to compete effectively with wild *E. coli* strains.	Plasmid vectors obtained from the Registry of Standard Biological Parts, such as pSB1C3; possibly also bacteriophage vectors such as M13mp9.

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)*

Scaffold proteins: M13 protein 8 is the coat protein of filamentous bacteriophage M13. Ice nucleation protein is a surface-exposed protein of *Pseudomonas syringae* which acts as a nucleus for ice crystal formation. Other potential scaffold proteins are normal components of *E. coli* cell surface structures such as flagella and pili. Proteins to be attached to the scaffolds are enzymes involved in degradation of polysaccharides, and minor transformations of the resulting sugars. Fluorescent proteins (commonly used reporter systems) may also be used to demonstrate correct fusion and display. Intracellular enzymes expressed will be involved in formation of non-hazardous products such as sorbitol, xylitol, sucrose, glycogen, ethanol, isoprene, etc.

Technique used to introduce insert or vector into host: Chemical transformation using calcium chloride or similar procedures.

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 genes by PCR and cloning of products in standard plasmid vectors	M13 protein 18	bacterio-phage M13	1	*Escherichia coli*	1	pSB1C3	<50 ml
as above	ice nucleation protein*	*Pseudo-monas syringae**	1	as above	as above	as above	as above
as above	amylase, xylose isomerase	*Escherichia coli* and/or *Bacillus subtilis*	1	as above	as above	as above	as above
as above	endo-glucanase, exoglucanase, β-glucosidase, xylanase	*Cellulo-monas fimi*	1	as above	as above	as above	as above
as above	pyruvate decarboxylase, alcohol dehyd-rogenase*	*Zymomonas mobilis**	1	as above	as above	as above	as above
as above	isoprene synthase	*Populus sp.*	1	as above	as above	as above	as above
as above	sucrose synthase, aldose reductase	various ACDP1 templates are possible	1	as above	as above	as above	as above
assembly and expression of combinations of the above	as above	as above	1	as above	as above	as above; possibly also use of M13 vector	as above

*we hope to obtain these parts in the form of pre-made BioBrick DNA components rather than preparing them from the original source organism.

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: Initial experiments, and possibly all experiments, will be performed using *Escherichia coli* JM109, a widely used disabled laboratory host strain which is not capable of competing effectively with wild-type strains of *E. coli*. Depending on the results, it may be useful also to test expression in *E. coli* MG1655, a commonly used strain of *E. coli* K12, which lacks the specific disabling mutations of JM109 and similar strains (Chart *et al*, 2001) but, like all K12 strains, has a defective outer membrane, and is also considered as ACDP1 (unlike most 'wild-type' strains of *E. coli*, which are considered ACDP2). *E. coli* K12 strains lack pathogenic characteristics and are not able to colonize the human intestine, the normal habitat of this species, under normal circumstances (http://epa.gov/biotech_rule/pubs/fra/fra004.htm). Are the cells to be used primary human cells and/or cell lines that are not fully authenticated and characterised? <u>No</u> (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*
ii) the inserted (donated) genetic material: The scaffold proteins to be used are components of bacteriophage, a parasite of bacteria, or of normal cell surface structures of *E. coli*, with the exception of the ice nucleation protein, which is a normal cell surface structure of certain strains of *Pseudomonas syringae*. Enzymes to be expressed will allow degradation of polysaccharides and conversion of sugars to non-hazardous products. Fluorescent proteins such as Yellow Fluorescent Protein and mCherry may also be used as markers. None of these genes should increase the pathogenicity of the host organism.	*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 non-transmissible plasmid vectors such as pSB1C3 will be used. For some experiments, bacteriophage vectors or 'helper' bacteriophage based on bacteriophage M13 may be used. These vectors were previously very widely used for the preparation of single stranded DNA for sequencing, and are not known to be associated with any hazards to human health.	*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.*	*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 evidence based*
iv) the resulting genetically modified micro-organism: The resulting genetically modified organisms are not expected to show any increase in pathogenicity compared to the host organisms used.	C*onsider all properties of the construct; take account of severity of consequences and likelihood of occurrence.*	
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*	

<table>
<tr>
<td>3. NATURE OF WORK TO BE UNDERTAKEN

Target genes will be amplified by PCR and cloned in plasmid vectors in BioBrick format, and then combined to form the desired expression constructs. These will be grown and tested for the ability to degrade various polysaccharides and produce various products as described above. Cell surface display will be tested using fluorescence microscopy and other imaging techniques. Enzyme activities may be tested in cell extracts which may be generated by sonication or detergent treatment.

<u>Additional control measures</u> required for specific risks:

Sonication, if required, will be performed in an enclosed cabinet to minimize risks of aerosol generation.</td>
<td>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:</td>
<td>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)</td>
</tr>
</table>

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)* The *Escherichia coli* strains used are not able to colonize vertebrate hosts (their normal environment) effectively in competition with wild-type strains (http://epa.gov/biotech_rule/pubs/fra/fra004.htm). ii) the inserted (donated) genetic material: The modifications to be introduced (described above) are not expected to enhance the ability of the organisms to survive in their normal environments, or to pose any hazard to plants or animals in the environment. iii) the vector: The plasmid vectors used are non-transmissible and should not be readily transferred to other organisms. In themselves, the vectors should pose no hazard to 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)* The resulting genetically modified microorganisms are not expected to pose any greater risk to the environment than the original 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: No extra control measures are required.	*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)* 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	**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
Chris French	Ph.D.	15 years postdoctoral	18 July 2000	1 Nov 2011
Eugene Fletcher	B.Sc. (Hons)	currently first year Ph.D.	18 July 2000	1 Nov 2011
Allan Crossman	none	undergraduate practicals	18 July 2000	1 Nov 2011
Mun Ching Lee	none	undergraduate practicals	18 July 2000	1 Nov 2011
Sylvia Ispasanie	none	undergraduate practicals	18 July 2000	1 Nov 2011
Yassen Abbas	none	none	18 July 2000	1 Nov 2011
Di Li	none	none	18 July 2000	1 Nov 2011
Lukasz Kopec	none	none	18 July 2000	1 Nov 2011
Clare Gibson	none	none	18 July 2000	1 Nov 2011
Fionn O'Mahoney	none	none	18 July 2000	1 Nov 2011

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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Name of reviewer: Date: Signature: Amendments:
Name of reviewer: Date: Signature: Amendments:

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 121-125ºC for at least 15 minutes or 126-130ºC for at least 10 minutes or 134-138ºC 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 121-125ºC for at least 15 minutes or 126-130ºC for at least 10 minutes or 134-138ºC 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.