UNIVERSITY OF ABERDEEN

Application to Foresterhill Genetic Modification Safety Committee for approval to carry out work with genetically modified micro-organisms (GMMs)

Important

Obtain a copy of the SACGM's Compendium of Guidance before completing this application. The Compendium provides guidance on risk assessment of GMMs and the containment measures required. Copies of the Compendium can be borrowed from Mrs Maureen Carr, School of Medical Sciences, IMS. The Compendium is also at http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/index.htm. Further guidance on genetic modification can be found at http://www.hse.gov.uk/biosafety/gmo/information.htm

- Your responses to the sections should not be limited by the sizes of the boxes on this form. Expand the table in the electronic version of form as necessary to accommodate your responses.
- Applications will be considered by a committee composed of both specialists and non-specialists in genetic modification.
  Your application should be comprehensible to non-specialist scientists.

1.	**Title of project**	Genetic manipulation of *E.coli* by the University of Aberdeen iGEM (International Genetically Engineered Machine Competition) team
2.	**Proposer (must be Project Leader)** This will normally be the most senior member of staff in the group who has involvement in, and responsibility for, the project.	Dr Ian Stansfield
3.	**School**	Medical Sciences
4.	**Building**	Polwarth
5.	**Laboratory** *Give details of all laboratories which will be used*	2.054 (the lab formerly used by the Wyeth pharmaceutical company)
6.	**List other facilities which will be used and confirm that those in charge of the facilities are aware of this application** *For example, biological service units, plant growth units, specialist equipment suites*	Some facilities in lab 2.01, IMS (Stansfield lab) may be used; this lab is aware of the project.
7.	**Names of those who will work on the project**	**Team Instructors** Ian Stansfield Al Brown **Team advisors (providing some day-to-day guidance for undergraduate members)** Claudia Rato da Silva Russell Betney Sam Miller Rosa Llanos de Frutos Mete Jacobsen **Technical Staff** Kelly Read Alison Davidson **Team undergraduate members**

		Liam Barry Stuart Campbell Calum De Burgh Risat Ul Haque Zuzana Hruskova Jennifer Klein-Albers Daniel Leirer Rory McDonald Katriona Mcmichael James Reid Nicholas Smart Annika Wipprecht
8.	**Previous experience of key individuals in genetic modification**	**Instructors** Ian Stansfield (13 years experience managing molecular biology, recombinant expression projects) Al Brown (>20 years experience managing molecular biology, recombinant expression projects) Sam Miller (10 years experience managing molecular biology, recombinant expression projects) **Technical Staff** Kelly Read , Alison Davidson; both Kelly and Alison have extensive experience over a number of years managing large groups of undergraduates engaged in recombinant DNA projects; both have extensive experience managing the safety culture of large laboratories with big groups of experimentalists. **Advisors** Claudia Rato da Silva Russell Betney Rosa Llanos de Frutos Mete Jacobsen All the above advisors have between 3 and 10 years post-doctoral experience managing recombinant DNA research projects. Undergraduate team members (section 7) have little or no experience of genetically manipulating microorganisms, and will be closely supervised by the personnel listed above.
9.	**Overview of the project** *Include* *(a) scientific goals,* *(b) details of recipient micro-organism (including strain number of micro-organisms),* *(c) details of vectors,* *(d) details of genes being modified,* *(e) an estimation of culture volumes which will be used*	**Microbial two-component glue to engineer self-healing pipes** **Layperson Summary:** This synthetic biology project is the University of Aberdeen entry into the Massachussetts Institute of Technology iGEM competition (International Genetically Engineered Machines). The project will engineer a bacterium from the human gut, *Escherichia coli*, to detect and fix leaking pipes. Engineered *E. coli* will detect leaks by homing in on a chemical signal released at the leak site. As the bacteria migrate towards the leak, they will synthesise two proteins, one on the outside of the cell, the other inside, which represent the two components of a biological, protein glue. The adhesive is only activated when the two components mix. The *E. coli* cells will be engineered to burst at the leak site, mixing the two glue components and creating a sticky protein plug to repair the pipe.

Scientific goals

Synthetic biology approaches are being used in a novel iGEM project that will engineer *E. coli* to detect and repair leaks and corrosion in pipes. The bacterium will respond by migrating to the site of the leak and plugging the breach using a protein-based glue. This solution could be used to seal leaks in pipes in inaccessible places, for example within a nuclear power station cooling system. The novelty of the biological engineering lies in the implementation of a two-component glue to seal the pipe, analogous to an epoxy resin that is only activated when two components, an adhesive and hardener, are mixed. In this project *E. coli* is engineered to synthesise the glue components (tropoelastin and lysyl oxidase) in different cellular compartments, which are then mixed following autolysis of the bacterium. The relative timings of the elastin glue synthesis and autolysis steps are carefully controlled, thus ensuring regulated lysis, but only after tropoeleastin/lysyl oxidase synthesis. As an alternative to tropoelastin, mussel foot adhesive protein will be expressed on the cell surface, to allow *E.coli* adhesion to the pipe breach site.

To achieve these aims, *E. coli* will be engineered to respond to an inducer molecule (IPTG) released from the site of a pipe breach. In this project, our model pipes will have a surface coating of the inducer and glucose, which are only released into the pipe in the case of a breach in the wall. Once the inducer IPTG is sensed by the bacterium, lacI repression will be relieved, inducing expression of tropoelastin (adhesive) within the *E. coli*, and the enzyme lysyl oxidase (the adhesive hardener) on the cell surface. This will be achieved by expressing lysyl oxidase as a translational fusion with the outer membrane protein OmpX. Motile *E. coli* will be used to allow chemotactic migration of the tropoelastin-expressing E. coli to the site of the pipe breach. In order that this sequence of events happens in close proximity to the pipe breach, quorum sensing will also be used to control synthesis of the glue proteins in response to a high concentration of bacteria. Thus IPTG and raised concentrations of the quorum sensing molecule homoserine lactone will be required to activate recombinant protein synthesis.

The expression of tropoelastin within the cytoplasm, and lysyl oxidase on the cell surface, will maintain the glue components in separate compartments. Their mixing at the site of pipe breach will be achieved by regulated autolysis, triggered by the lytic peptide T4 holin, initiating a cross-linking reaction and the formation of elastin. However, holin expression will be delayed relative to that of tropoelastin and lysyl oxidase, to allow the manufacture of glue components before cell lysis and glue mixing is initiated. This temporal delay will be achieved by using IPTG to induce a molecular inverter element that switches off a repressor of holin production. Slow decay in the levels of this repressor will trigger eventual holin synthesis thereby allowing cell lysis and tropoelastin/lysyl oxidase mixing.

Recipient microorganisms

Strains of *E.*coli to be used are all derivatives of the disabled K12 strain (e.g. DH5-alpha, JM-series, TG-1, C-600, XL1-Blue and K-12 strain AW405, a chemotactic, motile variant of K12 [Proc Natl Acad Sci 86:8358 (1989)), and RP437 , a K12-derivative chemotaxis-active strain; [F– thr-1 leuB6 his-4 metF159 thi-1 ara-14 lacY1 mtl-1 xyl-5 rpsL136 tonA31 tsx-78 eda-50 (J. Bacteriol July 1982, p. 106-113)]. The exception is BL21, which will be used for high-level expression of some genes (see below). BL21 [DE3] is considered inherently safe, in the sense that it is broadly

equivalent to K12, and offers no additional risks to the use of K12 derivatives (Chart et al., (2000) J.Applied Microbiol. 89, 1048-1058). It is believed to be unlikely to colonise and establish a persistent infection in the gut of a healthy individual. Overall, *E.*coli is considered inherently safe.

Strain details: JM109, DH5α, DH10B (F$^-$*mc*rA D(*mrr-hs*dRMS-*mc*rBC) ϕ80*dlac*ZΔM15 Δ*lac*X74 *de*oR *rec*A1 *end*A1 *ara*D139 Δ(*ara*, *leu*)7697 *gal*U *gal*K λ^- *rps*L *nup*G; XL1blue, XL1red (endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac mutD5 mutS mutT Tn10(Tet^R)

Vectors to be used
All vectors to be used are one of the following;
Either;
1. Standard cloning vectors based on pBR327, pAT153, pUC series, m13-series, pBluescript, pSELECT, pGEM. pT3/T7 and pET series vectors will be used for high level gene expression, which require separate expression of non-host T7 polymerase for expression.
Or;
2. Vectors designed for, and used in, the iGEM competition. These vectors are listed at the following web site;
http://partsregistry.org/Plasmid_backbones

..which is part of the iGEM competition web site (http://2009.igem.org/Main_Page). This site, and the link through to the Standard Registry of Parts, lists the complete catalogue of standard engineering biology parts. Some of these Parts (vectors) have been chosen to be used in this project;

(a) pSB1A3-1 is a high copy number plasmid carrying ampicillin resistance.
The replication origin is a pUC19-derived pMB1 (copy number of 100-300 per cell).
http://partsregistry.org/wiki/index.php?title=Part:pSB1A3

(b) pSB3C5 is a BioBrick standard vector with low to medium copy p15A replication origin (BBa_I50032) and chloramphenicol antibiotic resistance marker
http://partsregistry.org/wiki/index.php?title=Part:pSB3C5

(c) pSB3T5 is a BioBrick standard vector with low to medium copy p15A replication origin (BBa_I50032) and tetracycline antibiotic resistance marker
http://partsregistry.org/wiki/index.php?title=Part:pSB3T5

(d) pSB4C5 is a BioBrick standard vector with low copy pSC101 replication origin (BBa_I50042) and chloramphenicol antibiotic resistance marker
http://partsregistry.org/wiki/index.php?title=Part:pSB4C5

(e) pSB3C5 is a BioBrick standard vector with low to medium copy p15A replication origin (BBa_I50032) and chloramphenicol antibiotic resistance marker
http://partsregistry.org/wiki/index.php?title=Part:pSB3C5

(f) pSB4K5 is a BioBrick standard vector with low copy pSC101 replication origin (BBa_I50042) and kanamycin antibiotic resistance

marker (BBa_P1003).
http://partsregistry.org/wiki/index.php?title=Part:pSB4K5

Genes being modified
Heterologous genes will be genetically manipulated by introducing them into new plasmid combinations *in vitro*, and propagating the plasmids in *E.coli K-12* derivatives. The introduced genes will consist of defined coding and flanking non-coding sequences. Many of the genes listed will be expressed in *E.coli* at a range of expression levels.

Heterologous genes to be introduced and expressed are as follows;

- Green fluorescent protein from *Aequoria Victoria*, and derivatives such as mCherry designed to fluoresce at different wavelengths
- luciferase from firefly and *Renilla reniformis*
- lux gene operon from *Vibrio fisheri* bacterial species

- Phage lambda transcriptional repressor cI.
- Phage T4 holin and antiholin system, which is E.coli-lytic. This consists of the following T4 genes; T4 holin, T4 antiholin, and endolysin
- The bacteriophage T7 RNA polymerase gene
- Tetracycline-inducible repressor (tetR) from the Tn10-encoded tetracycline-resistance operon, in standard use throughout molecular biology and thus regularly propagated in *E.coli* . This protein binds tetracycline operators in a tetracycline-dependent manner.
- The quorum sensing luxI and luxR genes from the bacterium *Vibrio fisheri*. LuxI encodes the enzyme synthetase required to make the quorum sensing molecule homoserine lactone (HSL), and luxR is a transcriptional activator responsive to HSL levels.
- The human tropoelastin gene; tropoelastin has been safely expressed in E.coli in other published studies; Martin SL, Vrhovski B, Weiss AS. Total synthesis and expression in Escherichia coli of a gene encoding human tropoelastin. Gene. 1995 Mar 10;154(2):159-66. PubMed PMID: 7890158.
- The human lysyl oxidase gene: this human gene has been safely expressed at high level in Pichia pastoris yeast; Mithieux SM, Wise SG, Raftery MJ, Starcher B, Weiss AS. A model two-component system for studying the architecture of elastin assembly in vitro. J Struct Biol. 2005 Mar;149(3):282-9. PubMed PMID: 15721582.
- Mussel adhesive protein Mefp1 and Mgfp5; these proteins have been safely expressed in E.coli in other published studies; (1) Mol Cells. 2008 ;26:34-40. A novel expression system for recombinant marine mussel adhesive protein Mefp1 using a truncated OmpA signal peptide. Lee SJ, Han YH, Nam BH, Kim YO, Reeves P. (2) J Biotechnol. 2007 ;127:727-35. Recombinant mussel adhesive protein Mgfp-5 as cell adhesion biomaterial. Hwang DS, Gim Y, Kang DG, Kim YK, Cha HJ.

Homologous *E.coli* genes to be introduced and expressed are as follows

- β-galactosidase from *Escherichia coli*
- the lacI transcriptional repressor protein
- maltose binding protein from *E.coli* (*malE*).
- Outer membrane protein X (OmpX) and Outer membrane protein C

(OmpC)

Expression levels of expressed genes in K-12 derivatives;

1. The following genes (E.coli laci repressor, T4 phage holing, antiholin and endolysin, Vibrio fisheri luxR and LuxI quorum sensing operon genes, lambda cI transcriptional repressor, mussel adhesive protein, lysyl oxidase, tropoelastin) will be expressed to low to moderate level under control of promoters such as;

(a) Artificial, moderate strength constitutive promoters, http://partsregistry.org/wiki/index.php?title=Part:BBa_J23100

(b) the beta-lactamase gene P(bla) http://partsregistry.org/wiki/index.php?title=Part:BBa_I14018

(c) plux-lac hybrid promoter (sense TWO INPUTS, activation by AHL and repression by LacI.) http://partsregistry.org/wiki/index.php?title=Part:BBa_I751502

(d) plux promoter governing expression of the *Vibrio* fisheri lux operon for quorum sensing

2. A number of genes will be expressed at high level using the T7 polymerase promoter. These include;

Human tropoelastin, human lysyl oxidase, mussel adhesive protein, T4 holin, T7 polymerase, T4 endolysin

Cell culture volumes will be restricted to less than 500 ml.

Expression levels of expressed genes in strain BL21[DE3], and in K12-derivatives engineered to express genes under control of the T7 RNA polymerase;

Heterologous proteins will be expressed in mg amounts per 500 ml culture volume using the pET-series or pT7-series vectors, or low copy-number vectors carrying T7 promoter sequences. Cell culture volumes will be restricted to less than 500ml.

Potential for harm;

As commonly-used reporters, transcriptional repressors and RNA polymerase enzymes, expression of the genes listed is unlikely in the extreme to endow *E.coli* with any harmful properties. .None of the gene products is known to be toxic. Likewise expression of the human or mussel proteins is similarly unlikely in the extreme to endow *E.coli* with any harmful properties, since none of the proteins have known toxic properties.

Antibiotic resistance markers, reporter genes, RNA polymerases, fluorescent proteins and DNA repressor proteins listed likewise have no know harmful properties, and are in widespread established use in molecular biology.

Mitigation of the potential for harm. In this project, the genes to be expressed will be combined in such a way that a combination of high cell density (quorum sensing) and either lactose or IPTG will induce expression of either mussel foot protein, or a combination of tropoelastin and lysyl oxidase. The system therefore contains 'AND' gate function- both IPTG and high cell density are required for circuit switch on.

Coincident with this induction, the expression of T4 holin will be induced, resullting in the lysis of the host cell.

		It is considered unlikely that there would be a simultaneous coincidence of these two 'AND' gate requirements (IPTG or lactose, with high cell density) in the environment, in the event of accidental ingestion/release. However, in order that any potential for harm is reduced to a minimum, expression of mussel protein, lysyl oxidase and tropoelastin will only be attempted once it is established that the combined quorum sensing signal plus IPTG will successfully trigger cell lysis using T4 holin. Experimental data demonstrating the T4 holin-mediated lysis is operational will be presented to the ACGM Committee for review, before permission is then sought to move to the second stage of the project, where the mussel protein and or tropoelastin/lysyl oxidase expression will be placed under IPTG/quorum sensing control. In this way, circuit triggering using the AND gate inputs of cell density and lactose/IPTG will also trigger destruction of the recombinant cells. **Plasmid mobilisation;** Plasmids are all based on either the pAT153 vector, or pBR327 vector or later variants, which lack the relaxation site (*bom*) required for ColK mobilisation. Other vectors used (pUC series, m13-series, pBluescript) are non-mobilisable.
10.	**How might the GMM be a hazard to human health?** **Evaluate the severity of the harmful effects if they were to occur.** *Consider* *(a) hazards associated with the recipient organism including ACDP hazard group and the effects of any stable disabling mutations,* *(b) hazards arising directly from the inserted gene,* *(c) hazards arising from the alteration of existing pathogenic traits,* *(d) likelihood and effects of natural gene transfer to other organisms.* *If there are considered to be no harmful effects or only effects of low severity, explain how this conclusion has been reached.*	*E. coli* K12 and its derivatives are multiply disabled and are designated as Class 1 organisms. Good microbiological practice will be followed when using these organisms and over many years of use, no adverse effects have been noted. For protein expression in *E. coli* we will preferably use BL21[DE3] or B21[DE3]pLysS and its derivatives as expression strain of choice. This strain is essentially equivalent to K12. (Chart et al., (2000) J.Applied Microbiol. 89, 1048-1058.) Where this strain is used, culture volumes will be restricted to 500 ml. As the genes expressed have no known toxic effects, there is minimal risk arising from gene transfer to other bacteria. As BL21 is not known to be harmful, there is no known risk from these bacteria. In other experiments, K12 derivatives will be engineered to express T7 polymerase for high level expression of tropoelastin, lysyl oxidase and mussel adhesive proteins. This should pose limited risk since K12 is multiply disabled, and the proteins to be expressed have no known or reported harmful properties. Should transfer occur, the nature of most of the genes being manipulated (housekeeping or reporter genes) means deleterious consequences are unlikely in the extreme to result. Plasmids used are mobilisation defective. Where human genes are being overexpressed, their human nature means they should not generate an immune response, in the extremely unlikely event of survival of the disabled *E.coli* host in the body, None of the genes that are to be propagated or expressed in *E.coli* are known to have deleterious effects on human health. All genes occur naturally in normal cells and are involved in basic metabolic processes. It is highly unlikely that even if large amounts of GMM were ingested that the protein would be targeted in sufficient quantity to a location likely to cause detrimental effects.

11.	**Which containment level is necessary to protect human health?** *See HSE guidance on GMMs for requirements of containment levels 1, 2, 3 and 4* *Give details of any additional precautions which are necessary in addition to those of the assigned containment level*	Containment level 1
12.	**Is the required level of containment available in the laboratories and other facilities that will be used for the work?**	Yes
13.	**How might the GMM be a hazard to the environment?** **Evaluate the severity of the harmful effects if they were to occur.** *If there are considered to be no harmful effects or only effects of low severity, explain how this conclusion has been reached.*	Except for BL21 derivatives, the strains used are multiply-disabled and therefore pose no risk to the environment. BL21 is a *E. coli* B derivative and, as proteins expressed are non-toxic, it poses no risk to the environment. In fact BL21 is disabled to an extent it is considered essentially equivalent to K12. (Chart et al., (2000) J.Applied Microbiol. 89, 1048-1058.)
14.	**Are any additional containment measures required to protect the environment in addition to those necessary to protect human health?** *Give details*	No
15.	**Assign the work to an activity class** *Class 1, 2, 3 or 4* *The activity class is equivalent to the containment level except that if some additional precautions from a higher containment level are used, the work must be assigned to the activity class equivalent to that higher level.*	Class 1
16.	***For work provisionally assigned at activity class 2 or above*** **What factors must be taken into account with respect to health surveillance of people working on this project?** *Provide details of (a) factors that increase the susceptibility of an individual to infection by the*	N/A

	genetically modified micro-organism(s), and (b) symptoms of an infection by the genetically modified micro-organism(s).	

Note1: If the work is assigned to activity class 1, the GMM must present no or negligible risk either to humans or to the environment

Note2: Work assigned to activity classes 2 and above must be notified to the Health and Safety Executive after approval by a Genetic Modification Safety Committee and before work can begin. A notification fee will be payable.

ADDITIONAL INFORMATION

All GMMs in contaminated material and waste must be inactivated by "validated means", the method of inactivation chosen being appropriate to the level of risk.

1.	Will it be necessary for gloves to be worn to protect the laboratory workers from the GMM?	No
2.	Will a microbiological safety cabinet be required to protect laboratory workers from the GMM?	No
3.	Explain how GMMs in contaminated material and waste will be inactivated.	Autoclaving
4.	Explain how the means of inactivation will be validated	Small samples of the autoclaved material will be tested for presence of viable organisms at monthly intervals to verify efficacy of the autoclave cycle.
5.	What "degree of kill" is the means of inactivation expected to achieve? How has it been arrived at?	100%. Reasonable expectation of kill of microorganisms exposed to 126°C for 14 minutes (standard autoclave cycle, Prestige Autoclaves). While this autoclave cycle is non-standard (usual cycle is 121°C, 15 min.), our laboratory has experimentally tested the effectiveness of the Prestige cycle, and found it to completely kill *E.coli* cells, in standard 100 ml volumes in flasks placed in the middle of a full autoclave load.
6.	If autoclave facilities are to be used, where are they located?	In the lab (2.054) where the work is being carried out
7.	If chemical means of inactivation are to be used, what chemicals will be used and at what concentrations?	1 % Virkon (small volume spills) Virkon powder (large volume spills). 70% ethanol. Chemical means will only be used to disinfect surfaces and in the case of accidental spillage. Decontamination methods are specified in detail in our Local Rules for GM work.
8.	What will be the means of disposal of the inactivated waste?	Microbiological waste will be disposed of by CFA processing

	At Foresterhill liquid waste, after inactivation, will normally be disposed of to drain. Solid waste, after inactivation will normally be sent off site as part of the "orange bag" waste stream. The waste will be macerated to make it unrecognisable and further heat treated before being placed in landfill. Provide details of any alternative or additional means of disposal which will be used.	(solid waste) or discarded (autoclaved liquid waste).
9.	What disinfectant will be available for immediate use in event of a spillage? Please specify type and concentration.	Virkon; Powder will be used in liquid spillage situations involving larger volume (as specified in Lab 2.054 GM Local Rules)
10.	What disinfectant will be used to clean bench tops and laboratory equipment after use? Please specify type and concentration.	Ethanol; 70% v/v

Work must not commence until the proposer has received written approval from an authorised representative of the Foresterhill Genetic Modification Safety Committee.

APPLICANTS ARE STRONGLY ENCOURAGED TO ATTEND THE COMMITTEE MEETING AT WHICH THEIR APPLICATION IS CONSIDERED. FAILURE TO DO SO MAY DELAY APPROVAL AND PREVENT THE PROJECT STARTING.

Signature of Proposer:…… Date:……..

e-mail: ……i.stansfield@abdn.ac.uk …….. Telephone:……F 55806……

Submit the completed form to: Mrs Maureen Carr, School Co-ordinator, School of Medical Sciences, IMS, Foresterhill

Advice and assistance with genetic modification safety matters can be obtained from
Dr P Cash School of Medicine and Dentistry
Dr J M Collinson School of Medical Sciences
Dr J Crockett School of Medicine and Dentistry

Comments of Genetic Modification Safety Committee
Date considered
Signature of Biological Safety Adviser

Version: Foresterhill – September 2008