UNIVERSITY OF ABERDEEN

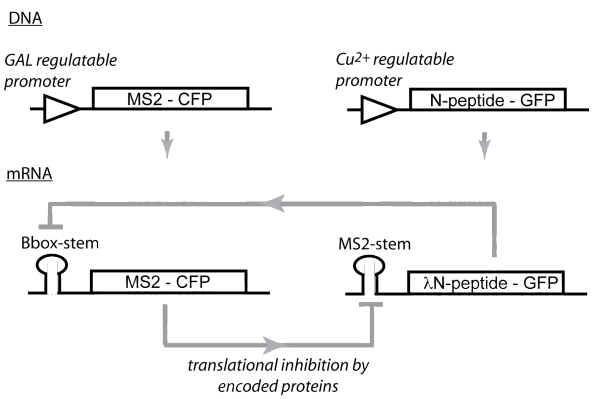
Application to Foresterhill Genetic Modification Safety Committee for approval to carry out work with genetically modified micro-organisms (GMMs)

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**	IMS
5.	**Laboratory** *Give details of all laboratories which will be used*	2.01 (Stansfield lab) and 2.50 (Fungal Lab)
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*	Equipment rooms associated with these two labs. FACS facilities will be used in analysis; Dr Raif Yucel
7.	**Names of those who will work on the project**	**Team Instructors** Ian Stansfield, Yasushi Saka, Duncan Shaw, Carol Munro **Team advisors (providing some day-to-day guidance for undergraduate members)** Claudia Rato da Silva Russell Betney Rosa Llanos de Frutos Mete Jacobsen **Technical Staff providing day-to-day guidance and support** Yvonne Knox, Linda Key, Susan Budge **Team undergraduate members** Stephen Lam Joseph Hoare Justyna Kucia

8.	**Previous experience of key individuals in genetic modification**	**Instructors** Ian Stansfield, Yasishi Saka, Duncan Shaw and Carol Munro (all > 15 years experience managing molecular biology, recombinant expression projects) **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 genetic toggle switch using translational control** **Scientific goals :** 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 the yeast *Saccharomyces cerevisiae* to detect two environmental signals and to respond by fixing the cell in one of two stable gene expression states, a so-called genetic toggle switch. The toggle switch behaviour will be implemented at the level of translational control of gene expression. Engineered *S.cerevisiae* will detect the presence of copper Cu^{2+} ions and a sugar (either galactose or glucose) using native yeast inducible promoters. These promoters will direct the expression of two mRNAs that each encode a fluorescent protein (CFP or GFP) fused to an RNA binding protein (either MS2 bacteriophage coat protein, or phage lambda N-peptide; Figure 1, below).  The relative strengths of the two promoters, and the affinities of the RNA binding proteins for their mRNA stem loops, will determine into which expression state (fluorescent cyan or green) the population will be stably maintained. If time permits, one of the fluorescent proteins will be replaced by the gene

encoding carbonic anhydrase, that catalyses the production of the bicarbonate anion from carbon dioxide, resulting in the regulated production of an anti-acid in response to different sugar concentrations.

Genetic manipulation summary:
All genes and promoters to be used are either naturally derived from *S.cerevisiae*, or are non-toxic proteins generally regarded as safe and in frequent use in molecular biology genetic manipulations in many labs, such as GFP and CFP (Ref 1), or MS2 and N-peptide phage proteins (Ref 2,3).

1. Cubitt AB, Heim R, Adams SR, Boyd AE, Gross LA, Tsien RY. Understanding, improving and using green fluorescent proteins. Trends Biochem Sci. 1995 20:448-55.

2. Keryer-Bibens C, Barreau C, Osborne HB. Tethering of proteins to RNAs by bacteriophage proteins. Biol Cell. 2008 ;100:125-38

3. Coller JM, Gray NK, Wickens MP. mRNA stabilization by poly(A) binding protein is independent of poly(A) and requires translation. Genes Dev. 1998 ;12:3226-35.

Gene constructs will be assembled in standard laboratory strains of *E.coli* (see below). These manipulations will not result in the intentional expression of the fusion proteins in *E.coli*.

Gene constructs will then be transferred into yeast, where expression levels will be within the normal range of expression directed by ordinary yeast promoters.

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 . 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 *en*dA1 *ar*aD139 Δ(*ar*a, *l*eu)7697 *ga*lU *ga*lK λ^- *rp*sL *nup*G; XL1blue, XL1red (endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac mutD5 mutS mutT Tn10(Tet^R)

S.cerevisiae strains to be used have no disabling mutations as such, other than auxotrophic mutations, but this micro-organism is considered inherently safe. Numerous different strains will be employed; all are considered inherently safe.

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 or pGEM.

2. **Yeast shuttle Vectors;** genes will be introduced on either YCp-, YRp-, YIp- or YEp-based vectors carrying either the *URA3, TRP1, LEU2, LYS2, ADE2, CAN1* or *ADE3* selectable genes. Expression of the genes will be driven by the genes' own promoter, or one of the following yeast homologous promoters; *TEF1, YEF3, PGK, GAL1,10, HSP26, TRP1, CYC1* or *ADC1* with transcriptional terminators.

Or;
3. 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.

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*
- Phage lambda translational repressor N-protein and derived peptide.
- Phage MS2 translational repressor coat protein.
- 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. Tet operators within artificial regulatable yeast promoters may be used. - β-galactosidase from *Escherichia coli* <u>Homologous *S.cerevisiae* genes and promoters to be introduced and expressed are as follows</u> - yeast *CUP1* promoter, yeast *GAL1,10* promoter, yand other native, constitutive yeast (*S.cerevisiae*) promoters, yeast carbonic anydrase gene, transcriptional terminators from a range of native yeast genes.. **Expression levels of expressed genes in K-12 derivatives;** Gene constructs will be assembled in standard laboratory strains of *E.coli* (see below). These manipulations will not result in the intentional expression of the fusion proteins in *E.coli*. **Expression levels of expressed genes in *S.cerevisiae*** Gene constructs will then be transferred into yeast, where expression levels will be within the normal range of expression directed by ordinary yeast promoters. **Potential for harm;** As commonly-used reporters, fluorescent proteins, translational repressors and native yeast enzymes, expression of the genes listed is unlikely in the extreme to endow either *E.coli* or *S.cerevisiae* with any harmful properties. .None of the gene products is known to be toxic. Antibiotic resistance markers, reporter genes, fluorescent proteins and RNA or DNA binding/repressor proteins listed likewise have no known harmful properties, and are in widespread established use in molecular biology. **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,*	*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. Should transfer occur, the nature of all of the genes being manipulated (housekeeping or reporter genes) means deleterious consequences are unlikely in the extreme to result. Plasmids used are mobilisation defective. No genes are being expressed at unusually high levels, it it is considered very unlikely they will generate toxicity, in the extremely unlikely event of survival of the disabled *E.coli* host in the body,

	(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.*	None of the genes that are to be propagated 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. *Saccharomyces cerevisiae* strains almost exclusively carry multiple auxotrophic mutations and are designated as Class 1 organisms. Good microbiological practice will be followed when using these organisms and no adverse consequences have been reported from the genetic manipulation of this strain over many years of use in multiple laboratories. There is extremely low likelihood of transfer of genes from *S,cerevisiae* to other micro-organisms. Should transfer occur, the nature of the genes being manipulated (housekeeping or reporter genes) means deleterious consequences are unlikely in the extreme to result. Plasmids used are mobilisation defective.
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.*	The strains used are multiply-disabled and therefore pose no risk to the environment.
14.	**Are any additional containment measures required to protect the environment in addition to those necessary to protect human health?**	No

	Give details	
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 genetically modified micro-organism(s), and (b) symptoms of an infection by the genetically modified micro-organism(s).*	N/A

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? *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.*	Microbiological waste will be disposed of by CFA processing (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 2.01/2.50 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

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