Risk Assessment made under the Genetically Modified Organisms (Contained Use) Regulations 2000

(Form GMM – for genetically modified micro-organisms and eukaryotic cell and tissue culture systems)

Department: NDCLS | **Supervisor: Dr Alison Banham** **Ref. No: CS19**

Project Title: Expression and silencing of mammalian genes and use of standard laboratory reporter genes in mammalian cells

Overview of Project:
(include aims and objectives)

Our aims are to express a variety of human/murine proteins and reporter genes (e.g. beta-galactosidase, green fluorescent protein, luciferase, renilla luciferase) in both mammalian cell lines and primary human cells. These include markers of leukocyte differentiation, lymphoma-associated antigens (e.g. PASD1, HIP1R, HIP1, JMJD3, MORC4), transcription factors (e.g. FOXP1, FOXP2, FOXP3, FOXP4, E4BP4, EBF1, BCL11A), and proteins involved in tumour angiogenesis (ELTD1) or Notch signalling (e.g. DLL1, NOTCH1), which have potential roles in lymphoma, leukaemia or other types of cancer. Proteins may be fused to a variety of tags to aid purification and/or detection e.g. Xpress, His, Myc, Flag, etc.

The proteins are generally expressed in mammalian cells for:
1) Testing the reactivity, affinity or specificity of antibodies for the recombinantly expressed protein. This is key because it ensures the correct post-translational modifications, which may not occur in bacteria.
2) We are also interested in assessing the functional affects of expressing proteins in mammalian cells e.g. whether particular proteins affect cell growth, survival, adhesion, cellular morphology, gene expression patterns, drug resistance and/or immunophenotype.
3) Studies may also be performed to investigate the physical interaction between proteins e.g. whether expressed proteins can coimmunoprecipitate.
4) Future studies may also include functional assays using recombinant proteins e.g. the use of proteins to stimulate or block cell signalling pathways for molecules involved in the Notch pathway.
5) Studies involving transcriptional regulatory proteins will include analysing affects of modulating their expression on the activity of both their own and target promoters. This will include the co-transfection of promoter-reporter constructs and control vectors containing green fluorescent protein.
6) Cell lines may also be transfected with constructs expressing recombinant proteins of interest to test their endogenous processing and immunological recognition or killing by T cells.
7) Modified mammalian cells may be introduced into animal models to study the *in vivo* functional affects of modulating the expression of genes of interest.

In addition we would like to introduce microRNAs, siRNAs and vectors encoding shRNAs to silence gene expression in both mammalian cell lines and primary human cells.

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

We use a wide range of human cell lines and those derived from other mammalian species. We may also transfect primary human peripheral blood cells or also primary human tumour cells from patients with haematological malignancy e.g. lymphoma, leukaemia or myeloma. Analysis of murine models of disease may require the expression of murine or human genes in both cell lines and in splenic lymphocytes and/or bone marrow cells.

The genes will be cloned and maintained in plasmids that are grown in K12-derived *Escherichia coli* prior to transfection of

Vector(s):
Expression vectors e.g.
pBK-CMV, pcDNA3, pcDNA4, pCEP4, pCMV5, pCEP4ER, pECE, pCR, pCMV

Reporters e.g.
pMaxGFP, pGL3, pGL4, pNFkB-TA-luc, pNFAT-TA-luc, pTA-luc

Gene silencing vectors e.g.
pcDNA6.2-GW/EmGFP-miR

Cloning and Entry (Gateway, Invitrogen) vectors e.g.
pGEMT-Easy, pENTR-D-TOPO,

mammalian cells. Strains include XL1Blue, XLOLR, DH5α, JM109, One Shot TOP10, One Shot OmniMAX 2T-1^R Phage resistant cells, One Shot® ccdB Survival™ 2 T1 Phage-Resistant (T1R) (resistant to the toxic effects of the *ccdB* gene for use in propagating vectors containing the *ccdb* gene, Gateway system, Invitrogen), SCS110 (*Dam* and *Dcm* deficient strain); and other common, disabled laboratory strains.	pDONR221

Normal/expected biological action of inserted DNA/RNA or transcribed/translated gene product:

Many genes will be of unknown biological function. In particular proteins are likely to have roles in transcriptional regulation, cell survival, cell proliferation, angiogenesis and may act as either tumour suppressor or oncogenes. FOXP3 has a potential immunomodulatory role due to its expression in regulatory T cells.

Technique used to introduce insert or vector into host:

Plasmids will be introduced into *E. coli* by standard transformation procedures.

Plasmids, siRNAs and microRNA precursors will be introduced into mammalian cells using either lipid-mediated transfection, calcium phosphate-mediated transfection or electroporation (Nucleofection, Lonza).

Assessed By: Dr Alison Banham

Signature: A. H. Banham

Date: 16-12-2009

Risk Assessment approved by Genetic Modification Safety Committee

Signature: Stephen Hyde

(Biological Safety Officer)

Date: 8/9/2011

Permission granted by Head of Department for project to be undertaken

Signature:

(Head of Department)

Date 8/9/2011

RISK ASSESSMENT FOR HUMAN HEALTH AND SAFETY

Human health hazard identification – (Identify any potential harmful properties of:)

i) the recipient micro-organism *(for micro-organisms also give ACDP hazard group)*

The recipient *E. coli* K-12 derivatives (ACDP Hazard Group 1) are disabled hosts that cannot colonise the human gut and have a history of safe use.

The recipient mammalian cell lines and primary cells pose little risk to human health (Hepatitis vaccination is provided for those working with human tissues). Human peripheral blood cells are purchased from the Blood Transfusion Service to avoid the risk of working with HIV infected material or the loss of histocompatibility barriers (no workers will culture cells from themselves or a first-degree relative). The risk from culturing human cell lines is through the presence of mammalian oncogenic viruses and all human lymphocytes are handled under the assumption that they might harbour Epstein-Barr virus.

ii) the inserted (donated) genetic material

The majority of the sequences are expected to pose little or no risk in humans. However, some inserted sequences may have harmful properties in humans via the ability to act as tumour suppressor and/or oncogenes e.g. truncated FOXP1, or by their ability to affect normal human defence mechanisms by mediating immunotolerance e.g. FOXP3. Consequently, gene transfer of some of the sequences to humans may be hazardous.

iii) the donor micro-organisms *(where used/appropriate)*

N/A

iv) the vector

The expression vectors are standard laboratory derived or commercial DNA plasmids and are considered non-pathogenic. The microRNA precursors are purchased from a commercial source and require *in vivo* processing for activity.

v) the resulting genetically modified micro-organism

Most resulting GMOs and mammalian cell lines are not expected to carry any significant risk compared to those of the un-modified recipients. Those mammalian cells containing candidate tumour suppressor genes or oncogenes may have a slightly increased risk to human health. However this should not need any greater containment than is already required under good class II standards of practise.

GUIDANCE

Potentially harmful effects include:

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

alteration of existing pathogenic traits – consider alteration of tissue tropism or host range, alteration in susceptibility to human defence mechanisms etc

adverse effects resulting from inability to treat disease or offer effective prophylaxis

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

Brenner Scheme values (*COMPLETION OPTIONAL and in any case for disabled E. coli only*) Access Expression Damage Overall **<u>Control measures</u> –** Assign provisional containment level: **Containment Level: 1 for both mammalian and bacterial cell culture with Good Microbiological Practice and Good Occupational Safety and Hygiene**	*Assign a provisional containment 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*
NATURE OF WORK TO BE UNDERTAKEN Give brief description of types of laboratory procedures including maximum culture volumes at any time (show as multiples of unit volumes) The procedures are standard laboratory practice for gene cloning and manipulation. Individual culture volumes will typically be ≤500mL. The procedures are standard laboratory practice for mammalian cell and tissue culture and will be performed using a Class II Microbiological Safety Cabinet. Individual culture volumes will typically be ≤100mL. Provide details of any non-standard laboratory operations N/A **<u>Additional control measures</u>** required for specific risks: After consideration of the procedures to be undertaken, no need was identified for additional control measures to protect human health and safety when handling the genetically modified bacteria or mammalian cells.	***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*

RISK ASSESSMENT FOR ENVIRONMENTAL HARM	***GUIDANCE***
Environmental hazard identification - Identify any potentially harmful properties of: i) the recipient micro-organism The disabled *E.coli* K-12 derivatives (ACDP hazard Group 1) are considered non-pathogenic, and cannot survive in the environment. ii) the inserted (donated) genetic material The inserted donor cDNAs are normal human genes. Some may be capable of encoding proteins that could have either tumour suppressor or oncogenic activity in humans. We have no reason to suspect that these will alter the tissue tropism, host range, infectivity or pathogenicity characteristics of the bacterial or mammalian cells. iii) the donor micro-organisms *(where used/appropriate)* N/A iv) the vector The expression vectors are standard laboratory derived or commercial DNA plasmids and do not pose special risk to the environment. v) the resulting genetically modified micro-organism ***E. coli*** Expression of inserted genes does not present a hazard. The vector is non-mobilisable. The resulting GMM would not survive outside laboratory conditions. Since it is non-colonising, and none of the inserted sequences would affect the level of risk, it would not be harmful to animals, plants or humans. **Mammalian cells** These cannot survive outside of laboratory conditions and thus do not pose a risk to the environment.	*Potentially harmful effects include: disease to animals 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* *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 Effectively zero **<u>Additional control measures</u>** required to reduce all risks to low/effectively zero: No additional risk management measure is necessary to protect the environment other than those needed to protect human health and safety.	*select from: Severe/Medium/Low/Negligible* *Select from: High/Medium/LowNegligible* *Select from: High/Medium/Low/Effectively zero*
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 environment risks	***GUIDANCE*** *Mark up table(s) by circling for each item the first correct answer reading across the table from left to right*

Consider also Tables 1b and 1c where appropriate **<u>Classification:</u>** **Class: 1 for both mammalian and bacterial work** **<u>Assign corresponding level of containment:</u>** **Containment Level: 1 for both mammalian and bacterial work** **Specify any other control measures required** None	*The highest numbered column in which a control measure is required indicates the Class of the activity – circle class on table 1a* *The class number indicates the minimum containment level required*

Table 1a: Containment Measures for Activities involving modified *E. coli* in Laboratories

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. Delete no or yes as indicated by risk assessment.

Containment Measures	**Containment Levels**			
	1	**2**	**3**	**4**
Isolated laboratory suite	**not required**	~~not required~~	~~required~~	~~required~~
Laboratory sealable for fumigation	**not required**	~~not required~~	~~required~~	~~required~~
Surfaces impervious, resistant and easy to clean	**required for bench**	~~required for bench~~	~~required for bench and floor~~	~~required for bench, floor, ceiling and walls~~
Entry to lab via airlock	**not required**	~~not required~~	~~may be required no / yes~~	~~required~~
Negative pressure relative to the pressure of the immediate surroundings	**not required**	~~may be required no / yes~~	~~required~~	~~required~~
HEPA filtered extract and input air	**not required**	~~not required~~	~~required for extract~~	~~required for input and extract~~
Microbiological safety cabinet/enclosure	**not required**	~~may be required no / yes~~	~~required~~	~~required (class 3)~~
Autoclave	**required on site**	~~required in the building~~	~~required in the lab suite~~	~~required in lab (double ended)~~
Access restricted to authorised personnel	**not required**	~~required~~	~~required~~	~~required~~
Specified measures to control aerosol dissemination	**not required**	~~required so as to minimise~~	~~required so as to prevent~~	~~required so as to prevent~~
Shower	**not required**	~~not required~~	~~may be required no / yes~~	~~required~~
Protective clothing	**suitable protective clothing required**	~~suitable protective clothing required~~	~~suitable protective clothing required~~	~~complete change of clothing and footwear~~
Gloves	**not required**	~~may be required no / yes~~	~~required~~	~~required~~
Control of disease vectors (eg rodents, insects) which could disseminate GMMs	may be required **no** / yes	~~required~~	~~required~~	~~required~~
Specified disinfection procedures in place	may be required **no** / yes	~~required~~	~~required~~	~~required~~
Inactivation of GMMs in effluent from handwashing sinks, showers etc	**not required**	~~not required~~	~~may be required no / yes~~	~~required~~
Inactivation of GMMs in contaminated material and waste	**required by validated means**	~~required by validated means~~	~~required by validated means~~	~~required by validated means~~
Laboratory to contain its own equipment	**not required**	~~not required~~	~~required~~	~~required~~
An observation window or alternative so that occupants can be seen	may be required **no** / yes	~~may be required no / yes~~	~~required~~	~~required~~
Safe storage of GMMs	may be required **no** / yes	~~required~~	~~required~~	~~secure storage required~~
Written records of staff training	**not required**	~~may be required no / yes~~	~~required~~	~~required~~

CLASSIFICATION	**CLASS 1**	~~CLASS 2~~	~~CLASS 3~~	~~CLASS 4~~

Table 1b: Containment Measures for Activities involving modified mammalian cells in Laboratories
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. Delete no or yes as indicated by risk assessment.

Containment Measures	**Containment Levels**			
	1	~~2~~	~~3~~	~~4~~
Isolated laboratory suite	**not required**	~~not required~~	~~required~~	~~required~~
Laboratory sealable for fumigation	**not required**	~~not required~~	~~required~~	~~required~~
Surfaces impervious, resistant and easy to clean	**required for bench**	~~required for bench~~	~~required for bench and floor~~	~~required for bench, floor, ceiling and walls~~
Entry to lab via airlock	**not required**	~~not required~~	~~may be required no / yes~~	~~required~~
Negative pressure relative to the pressure of the immediate surroundings	**not required**	~~may be required no / yes~~	~~required~~	~~required~~
HEPA filtered extract and input air	**not required**	~~not required~~	~~required for extract~~	~~required for input and extract~~
Microbiological safety cabinet/enclosure	**not required**	~~may be required no / yes~~	~~required~~	~~required (class 3)~~
Autoclave	**required on site**	~~required in the building~~	~~required in the lab suite~~	~~required in lab (double ended)~~
Access restricted to authorised personnel	**not required**	~~required~~	~~required~~	~~required~~
Specified measures to control aerosol dissemination	**not required**	~~required so as to minimise~~	~~required so as to prevent~~	~~required so as to prevent~~
Shower	**not required**	~~not required~~	~~may be required no / yes~~	~~required~~
Protective clothing	**suitable protective clothing required**	~~suitable protective clothing required~~	~~suitable protective clothing required~~	~~complete change of clothing and footwear~~
Gloves	**not required**	~~may be required no / yes~~	~~required~~	~~required~~
Control of disease vectors (eg rodents, insects) which could disseminate GMMs	**may be required no / yes**	~~required~~	~~required~~	~~required~~
Specified disinfection procedures in place	**may be required no / yes**	~~required~~	~~required~~	~~required~~
Inactivation of GMMs in effluent from handwashing sinks, showers etc	**not required**	~~not required~~	~~may be required no / yes~~	~~required~~
Inactivation of GMMs in contaminated material and waste	**required by validated means**	~~required by validated means~~	~~required by validated means~~	~~required by validated means~~
Laboratory to contain its own equipment	**not required**	~~not required~~	~~required~~	~~required~~
An observation window or alternative so that occupants can be seen	**may be required no / yes**	~~may be required no / yes~~	~~required~~	~~required~~
Safe storage of GMMs	**may be required no / yes**	~~required~~	~~required~~	~~secure storage required~~
Written records of staff training	**not required**	~~may be required no / yes~~	~~required~~	~~required~~

CLASSIFICATION	CLASS 1	~~CLASS 2~~	~~CLASS 3~~	~~CLASS 4~~

Table 1c: Containment Measures for Activities involving GMMs in Animal Units - TABLE 1a TO BE COMPLETED WITH THE FOLLOWING ADDITIONS/MODIFICATIONS:

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. Delete no or yes as indicated by risk assessment.

Containment Measures	**Containment Levels**				**Addition/ modification**
	1	**2**	**3**	**4**	
Isolation of animal unit (note 1)	~~may be required no / yes~~	**required**	~~required~~	~~required~~	~~modification~~
Animal facilities (note 2) separated by lockable doors	~~may be required no / yes~~	**required**	~~required~~	~~required~~	~~addition~~
Animal facilities (cages etc) designed to facilitate decontamination (waterproof and easily washable material)	~~may be required no / yes~~	**may be required no** ~~/ yes~~	~~required~~	~~required~~	~~addition~~
Floor and/or walls and ceiling easily washable	~~may be required no / yes~~	**required for floor**	~~required for floor and walls~~	~~required for floor, walls and ceiling~~	~~modification~~
Appropriate filters on isolators or isolated rooms (note 3)	~~not required~~	**may be required no** ~~/ yes~~	~~required~~	~~required~~	~~addition~~
Incinerator for disposal of animal carcasses	~~required to be accessible~~	**required to be accessible**	~~required to be accessible~~	~~required to be on site~~	~~addition~~
Appropriate barriers at the room exit, and at drains and ventilation duct work	~~required~~	**required**	~~required~~	~~required~~	~~addition~~
Animals kept in appropriate containment facilities, such as cages, pens, tanks or isolator	~~may be required no / yes~~	**may be required no** ~~/ yes~~	~~may be required no / yes~~	~~may be required no / yes~~	~~addition~~

CLASSIFICATION	**CLASS 1**	**CLASS 2**	**CLASS 3**	**CLASS 4**

Notes

1. "Animal unit" means a building, or separate area within a building, containing an animal facility and other areas such as changing rooms, showers, autoclaves, food storage areas etc.

2. "Animal facility" means a facility normally used to house stock, breeding or experimental animals or one which is used for the performance of minor surgical procedures on animals.

3. "Isolators" means transparent boxes where small animals are contained within or outside a cage; for large animals, isolated rooms may be more appropriate.

Subject: RE: Potential Class II Biological Risk assessment
Date: Thursday, September 8, 2011 10:42 am
From: Andrew Thompson <andrew.thompson@safety.ox.ac.uk>
To: Steve Hyde <steve.hyde@ndcls.ox.ac.uk>, Julie Hamilton <julie.hamilton@safety.ox.ac.uk>
Cc: Alison Banham <alison.banham@ndcls.ox.ac.uk>
Conversation: Potential Class II Biological Risk assessment
Category: Work

Hi Steve,

Although the group has considered the potential oncogenic nature of some of the genes expressed and has assigned the work to Class 2 I am content that the vector systems proposed will not pose any risk to workers and, just as the control table indicates, the work can be assigned to Class 1 - at no point in the construction or subsequent work with the final GMMOs would exposure result in transfer of harmful genes. If viral vectors were to be used or there was large scale expression of oncogenic proteins/DNA then this would not be the case but as it stands it's a Class 1! Just change that classification and the assessment is good to go.

Regards

Andrew

Andrew Thompson
Biological Safety Officer
University of Oxford

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> -----Original Message-----
> From: Stephen Hyde [mailto:steve.hyde@ndcls.ox.ac.uk]
> Sent: 08 September 2011 09:28
> To: Julie Hamilton
> Cc: Andrew Thompson; Alison Banham
> Subject: Potential Class II Biological Risk assessment
>
> Dear University Biological Safety Office,
>
> I'm hoping you can offer me some advice/assistance.
>
> One of my colleagues has sent me a draft risk assessment (attached)
> covering the expression of some human genes in bugs and mammalian
> cells. The initial scoring is for the mammalian cell part of project to
> be conducted at containment/class level 2.
>
> Before committing to additional paperwork, grief and expense of a class
> 2 project, I would welcome your thoughts on the scoring. Have we been
> over conservative? Do similar projects run at level 1?
>
> Kind regards
>
> Steve Hyde
> --
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