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: CS18**

Project Title: Expression of human genes in *E. coli*

Overview of Project:
(include aims and objectives)

Our aims are to overexpress a variety of human proteins in *Escherichia coli*. These include markers of leukocyte differentiation, lymphoma-associated antigens, transcription factors, and proteins involved in tumour angiogenesis or Notch signalling which have potential roles in either lymphoma, leukaemia or other types of cancer.
The proteins are generally expressed in bacteria for the purposes of purifying recombinant proteins that will subsequently be used for antibody production. Future studies may also include functional assays using recombinant proteins e.g. the use of proteins to stimulate cell signalling pathways for molecules involved in the Notch pathway. Proteins identified by expression cloning from lambda phage libraries may also be expressed in *E. coli* to confirm their molecular weight and recognition by the antiserum used for cDNA library screening. Proteins may be fused to a variety of tags to aid purification, examples include glutathione-S-transferase, TrpE, His, Myc, Flag, etc.

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

E. coli K12 derivatives (e.g. XL1Blue, DH5α, Rosetta, XLOLR)
E. coli BL21 (DE3) – B strain derivative that lacks the major protease encoded by the *lon* gene.

Vector(s):

Common, commercially available vectors e.g. pBK-CMV, pGEX, pET, pGMT7

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.

Technique used to introduce insert or vector into host:

Vectors are introduced by standard transformation procedures.

Assessed By: Dr Alison Banham

Signature: A. H. Banham Date: 16-12-2009

Risk Assessment approved by Genetic Modification Safety Committee

Signature: Stephen Hyde Date: 8/9/2011

(Biological Safety Officer)

Permission granted by Head of Department for project to be undertaken

Signature: Date 8/9/2011.

(Head of Department)

RISK ASSESSMENT FOR HUMAN HEALTH AND SAFETY

GUIDANCE

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

Potentially harmful effects include:

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

E. coli K-12 derivatives and BL21(DE3) (both ACDP Hazard Group 1) are disabled hosts that cannot colonise the human gut and have a history of safe use.

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

ii) the inserted (donated) genetic material

The inserted mammlian DNAs are derived from human genes expressed in normal somatic cells. Some may be capable of encoding proteins that could have either tumour suppressor or oncogenic activity in humans. Generally we do not express the full-length protein in bacteria and thus for most proteins only specific domains, that will not be functional in isolation, will be expressed.

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

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

N/A

adverse effects resulting from the potential for transfer of inserted genetic material to another micro-organism

iv) the vector

The expression vectors are standard laboratory derived or commercial DNA plasmids and are considered non-pathogenic.

v) the resulting genetically modified micro-organism

Most resulting GMOs are not expected to carry any significant risk compared to that of the unmodified recipients. These are disabled hosts that cannot colonise the human gut and have a history of safe use.

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:

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

Containment Level: 1

with Good Microbiological Practice and Good Occupational Safety and Hygiene

NATURE OF WORK TO BE UNDERTAKEN

GUIDANCE

Give brief description of types of laboratory procedures including maximum culture volumes at any time (show as multiples of unit volumes)

Consider any activities that may involve risks which require specific additional control measures such as:

Transformed *E. coli* are grown in small-scale culture of up to 20ml (2x 10ml) prior to protein expression. For protein purification our standard protocol uses 1 x 200ml culture but we may use larger volumes eg 2 x 1 litre if only low levels of the recombinant protein is expressed.

inoculation of animals or plants with GMMs

the use of equipment or procedures likely to generate aerosols

Provide details of any non-standard laboratory operations N/A **<u>Additional control measures</u>** required for specific risks:	*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 and BL21(DE3) (both ACDP hazard Group 1) are considered non-pathogenic, and have limited survival in the environment. ii) the inserted (donated) genetic material The insert donor cDNAs are normal human genes. Some may be capable of encoding proteins that could have either tumour suppressor or oncogenic activity in humans. Generally we do not express the full length protein in bacteria and thus for many proteins only specific domains, that will not be functional in isolation, will be expressed. We have no reason to suspect that these will alter the tissue tropism, host range, infectivity or pathogenicity characteristics of the vector. 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 environment. v) the resulting genetically modified micro-organism Insertion of the viral and mammalian sequences into the *E.coli* hosts is not expected to result in harmful physiological or pharmacological properties. The resulting GMO's are not expected to carry any additional risks compared to that of the unmodified recipients. The inserted sequences are unlikely to affect the GMO's pathogenicity or normal human defence mechanisms.	*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.	*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	***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 environment risks **Consider also Tables 1b and 1c where appropriate** **<u>Classification:</u>** **Class: 1** **<u>Assign corresponding level of containment:</u>** **Containment Level: 1** specify any other control measures required	*Mark up table(s) by circling for each item the first correct answer reading across the table from left to right* *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 GMMs 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~~**