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: CS17**

Project Title:

Expression in bacteria of human MHC-I and associated proteins

Overview of Project:
(include aims and objectives)

The aim is to express human MHC-I and associated proteins, such as β2 microglobulin (β2m) in *E.coli* for the purpose of generating antigenic peptide-specific monomers/tetramers. Plasmids encoding human MHC-I and associated proteins will be transformed into *E.coli* and protein expressed as inclusion bodies, which will be refolded under appropriate conditions subsequently.

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

E.coli K12 derivatives
E.coli BL21(DE3)

Vector(s):

pCGMT7, pET vectors

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

T7 promoter drived the expression of LacZ and/or MHC-I/β2m genes. The LacZ gene produces β-galactosidase that converts lactose into glucose and galactose. MHC-I and β2m complex present antigenic peptides to cognate human T cells.

Technique used to introduce insert or vector into host:

The insert is cloned into the expression vectors using standard cloning techniques, in which K-12 derivative *E.coli* are used. The resulting plasmids are introduced into *E.coli* BL21(DE3) for protein expression by standard transformation procedures.

Assessed By:

Signature: Demin Li Date: 1 / 9 / 2009

Risk Assessment approved by Genetic Modification Safety Committee

Date: 23 / 9 / 2009

Signature: 

(Biological Safety Officer)

Permission granted by Head of Department for project to be undertaken

Date: 23 / 9 / 2009

Signature: 

(Head of Department)

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)*

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 DNAs are derived from human genes expressed in normal somatic cells, and pose no hazard.

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.

v) the resulting genetically modified micro-organism

No significant hazards identified above, the resulting GMOs are therefore not expected to carry any additional risk to that of the un-modified recipients.

Brenner Scheme values (*COMPLETION OPTIONAL and in any case for disabled E. coli only*)

Access Expression Damage Overall

Control measures – Assign provisional containment level:

Containment Level: 1

with Good Microbiological Practice and Good Occupational Safety and Hygiene

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

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)

K-12 bacteria are cultured in Luria-Bertani (LB) media at volume no more than 20ml. BL21(DE3) bacteria are cultured in low salt LB media with the maximum volume of 6 x 1 litre a time, followed by centrifugation and sonication to extract the expressed protein.

Provide details of any non-standard laboratory operations

N/A

Additional control measures required for specific risks:

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 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 DNAs are normal human genes, therefore posse no additional hazard, and should not alter the tissue tropism, host range, infectivity or pathogenicity characteristics of the vector. iii) the donor micro-organisms *(where used/appropriate)* Human. 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 No significant hazards identified above, the resulting GMOs are therefore not expected to carry any additional risk to that of the un-modified recipients.	*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 **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	***GUIDANCE*** *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~~