

# UNIVERSITY OF EXETER

## Risk Assessment Of Genetically Modified Micro-organisms Where The Primary Risk Posed Is To Human Health Rather Than To The Environment

This risk assessment form is for use in cases where the primary risk posed by the Genetically Modified (GM) micro-organism is to human health rather than to the environment. Please obtain an alternative form if the primary risk posed by the GM micro-organism is to the environment.

Part 1 of this form should be completed for all projects. Parts 2 and 3 are for risk assessments where the issues involved are complex and require detailed consideration.

Please type in the boxes - you can replace the notes in the boxes.

Provide justification, explanation and detail in all parts of this form.

Carry out your risk assessment in accordance with Section 2 of the ACGM Compendium of Guidance, particularly Section 2A. These are accessible on the Biology fileserver in: G:\Safety\Genetic Modification\ACGM Compendium of Guidance\

This risk assessments should be reviewed whenever there is a significant change in the activity involving GM.

### Part 1

#### (a) Title of project and principal investigator

Exeter iGEM project 2012: engineering polysaccharide biosynthesis PI: John Love

#### (b) Scientific goals of project

To demonstrate production of novel and/or useful polysaccharide structures in *Escherichia coli* by engineering polysaccharide biosynthesis. The ability to synthesise designed polysaccharide structures has wide implications for vaccine research and in materials science.

#### (c) An overview of the different types of GMM that will be constructed

Depending on the success of the project, the GMMs constructed will consist of standard laboratory *E. coli* supplemented with expression plasmid(s) containing one or more genes coding for polysaccharide biosynthetic machinery (e.g. glycosyltransferases, polysaccharide transport proteins). In some instance, fusion proteins may be generated in which a reporter gene (e.g. GFP or derivatives, iLOV domain) is fused to the protein or protein domain of interest in order to characterise the cellular operation of the protein.

The only vectors that will be used will be well-characterised vectors that (i) are either non-mobilisable (e.g. pUC series and their derivatives) or mobilisation-defective (e.g. pBR322 and its derivatives) in bacteria; and (ii) contain only selective markers that are already in routine use in standard cloning vectors; and (iii) contain no recognisable harmful sequences. This specifically excludes cloning any sequences that are known or suspected to be (i) oncogenic, (ii) encode a toxin or allergen or (iii) encode a product that could be detrimental if delivered to a target tissue.

#### (i) List of recipient strain(s)

*E. coli* strains: XL10-Gold, DH5 $\alpha$ , BL21 (DE3), K-12, TOP-10, NovaBlue (and derivatives of these).

XL10-Gold, DH5 $\alpha$ , TOP-10 F', NovaBlue and K-12 all contain disabling mutations and are highly unlikely to survive in the environment.

BL21 (DE3) contains several disabling mutations, and is therefore considerably attenuated, and unlikely to be competitive in the environment. It has been used for many years in laboratories worldwide. To my knowledge there have been no reports that have shown harm to humans from this strain. The behaviour of the strain is extremely well characterised, and it meets the US NIH requirements for safety.

**(ii) List of vector(s)**

Vector list: *E. coli* vectors of the pET, pCDF, pACYC and pCOLA series

*E. coli* vectors of the pBAT series

*E. coli* vectors of the pENTR series

*E. coli* vectors of the pETG series

*E. coli* vectors of the pBAD series

*E. coli* vectors pSB1A3, pSB1C3, pSB1K3.m1, pSB1T3

All of these vectors are based on the pBR322 vector except for pCDF and pSB series, and as such are mobilisation defective. pCDF, pACYC and pCOLA are also mobilisation deficient. pSB series are derived from pUC vectors and are non-mobilisable and have been extensively utilised in the undergraduate iGEM competition.

**(iii) List of function of inserted gene(s)**

The functional genes will code for enzymes involved in polysaccharide biosynthesis (e.g. glycosyltransferases) which are responsible for catalysing the addition of one or more sugar groups onto an elongating sugar chain, or are involved in transportation of polysaccharide units out of the cell. Other functional genes will be standard laboratory reporter genes for assessing the cellular performance of the genes of interest.

**(d) An indication of the most hazardous GMM**

The most hazardous GMM is likely to be an *E. coli* with the capacity to generate novel extracellular polysaccharides. This should not provide any fitness advantage to the bacterium, and no increase in pathogenicity is likely. Consequently, it is unlikely that these modifications will be materially alter the pathogenicity of the GMM. The use of non-mobilisable vectors and *E. coli* strains unable to survive out of the laboratory mitigates the risk of these traits transferring to pathogenic strains.

**(e) Are you confident that for all of the GMMs covered by this assessment there are no harmful properties associated with the recipient strain, the vector, or the inserted material?**

Yes

**(f) Are you confident that none of the final GMMs could be hazardous to humans or the environment?**

Yes

If the GMM meets BOTH of the above criteria, you may believe that you have sufficient information at this stage to classify the project to **class 1**, as defined in the Contained Use Regulations 2000. In order to do this you should be confident that even in the event of a total breach of containment the genetically modified organism would be of no or negligible risk to human health or the environment.

If you are assigning the work to **class 1** according to the above streamlined procedures, sign the form below and submit it to the GM Safety Officer (GMSO) who will arrange for it to be considered by the local Genetic Modification Safety Committee (GMSC). Work should not be commenced until this form has been reviewed by the GMSC.

If you have any uncertainty as to whether the proposal meets the above criteria you should undertake a more detailed risk assessment by completing **Part 2** and **Part 3** of this form.

**Signature of Proposer:**

**Date: 15/06/2012**



### **Background notes**

It is not appropriate to consider non-disabled pathogens of plants, humans, animals or insects as inherently safe recipient micro-organisms. Examples of inherently safe recipient micro-organisms which, depending on the nature of the insert, would in most cases be expected to form the basis of extremely safe GMMs are as follows:

- (i) *Escherichia coli* K12, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*
- (ii) Defective retrovirus produced from packaging line in which the helper genes are located in two separate blocks of DNA (thus eliminating the possibility of a reversion to replication competence by a single recombination event) and which are self-inactivated to prevent insertional activation.
- (iii) E1 deleted adenovirus

The types of gene which when cloned into particular recipient micro-organisms might give rise to a harmful phenotype can be divided into two types. First the gene might encode a product that could act directly to cause harmful effects e.g. a toxin gene or a cytokine. Second the gene might encode a product that could act alongside the existing characteristics of the recipient micro-organism so as to endow the GMM with altered pathogenic properties e.g. a pathogenicity gene or an engineered viral envelope gene with an altered receptor binding capacity.

## **Part 2 – Continuation Form for Use in Cases Where a Detailed Risk Assessment is Necessary**

Projects that have a broad scope will involve the construction of several GMMs. Part 2 is designed for the detailed assessment of a single GMM. In general, as long as the scope of the work has been satisfactorily outlined in Part 1, it is sufficient to complete Part 2 for the most hazardous GMM being constructed. This will be the one identified in Part 1(d). In cases where there are two or more hazardous GMMs, with quite different properties, two or more copies of Part 2 may need to be completed.

In the format shown here, hazards to human health are considered first and used as the basis for assigning provisional containment prior to addressing environmental issues. In cases where environmental issues are of prime concern and there is little possibility of harm to humans e.g. work with plant or animal pathogens, please use the alternative form in which environmental issues are considered first.

### **(a) Hazards to human health**

#### **(i) Hazards associated with the recipient micro-organism (e.g. bacterial host or viral vector)**

Factors to consider include whether the recipient micro-organism is listed in ACDP hazard groups 2, 3 or 4. Other relevant factors may be the micro-organism's mode of transmission, disease symptoms, host range, and tissue tropism as well as an indication as to whether vaccines or chemotherapeutic agents are available. Information should also be provided on any disabling mutations and whether there is any possibility of any disabling mutations being complemented or reverting.

#### **(ii) Hazards arising directly from the inserted gene product (e.g. cloning of a toxin gene or oncogene)**

Consideration should be given to whether the inserted DNA encodes a toxin, an oncogenic protein, an allergen, a modulator of growth or differentiation (hormone or cytokine) or any other protein, which may result in potentially harmful biological activity. Where the function of the inserted gene is unknown, it may help to describe the function of any known homologues. Please note that even a normal human gene may be harmful if overexpressed, especially if the overexpression is in tissues that do not normally express the protein.

#### **(iii) Hazards arising from the alteration of existing traits (e.g. alteration of pathogenicity, host range, tissue tropism, mode of transmission or host immune response)**

One factor to consider is whether the inserted gene encodes a pathogenicity determinant, such as an adhesin, a penetration factor or a surface component providing resistance to host defence mechanisms. Another important consideration is whether the inserted gene encodes a surface component, envelope protein or capsid protein that might bind to a different receptor to that used by the recipient micro-organism. Consideration should also be given to whether the inserted DNA (or the plasmid sequence) encodes resistance to a drug or antibiotic that might be used for the treatment of a laboratory-acquired infection.

#### **(iv) The potential hazards of sequences within the GMM being transferred to related micro-organisms**

Factors to consider include whether widespread dissemination of the inserted gene as a result, for example, of either gene transfer or recombination of the GMM with a wild-type micro-organism, would be a matter of concern. If this is the case an important consideration will be whether, in the event of a breach of containment the GMM could survive in the environment for long enough for such a gene transfer to take place.

**(b) Assignment of a provisional containment level that is adequate to protect against hazards to human health**

This step will involve considering the containment level necessary to control the risk of the recipient micro-organism (i.e. the ACDP Hazard Group of the recipient micro-organism) and making a judgment about whether the modification will result in a GMM, which is more hazardous, less hazardous, or about the same. Sometimes it may help to compare the GMM with the relative hazard presented by other organisms that would fall within the same ACDP Hazard Group as the GMM.

**(c) Identification of any hazards to the environment**

**(i) Hazards associated with the recipient micro-organism (e.g. bacterial host or viral vector)**

Factors to consider include whether the recipient micro-organism is capable of infecting any plants, animals or insects in the environment and whether there is any possibility of any disabling mutations being complemented or reverting. In particular it should be ascertained whether the recipient micro-organism is a pathogen that is controlled by DEFRA.

**(ii) Hazards arising directly from the inserted gene product**

Factors to consider include whether the sequence encodes an insect or animal toxin or a product which can cause silencing of a gene encoding a crucial metabolic enzyme in susceptible hosts.

**(iii) Hazards arising from the alteration of existing traits (e.g. alteration of pathogenicity, host range or tissue tropism)**

One factor to consider is whether the inserted sequence encodes a pathogenicity determinant, such as an adhesin, a penetration factor or a surface component providing resistance to host defense mechanisms. Another important consideration is whether the inserted gene encodes a surface component, envelope protein or capsid protein that might bind to a different receptor to that used by recipient micro-organism.

**(iv) The potential hazards of sequences within the GMM being transferred to related micro-organisms**

Factors to consider include whether widespread dissemination of the inserted gene as a result, for example, of either gene transfer or recombination of the GMM with a wild-type micro-organism, would be a matter of concern. If this is the case an important consideration will be whether, in the event of a breach of containment the GMM could survive in the environment for long enough for such a gene transfer to take place.

**(d) Consideration of the nature of the work to be undertaken and a detailed review of the control measures**

**(i) Are any of the work procedures likely to generate aerosols?**

If so, should the work be undertaken in a safety cabinet or isolator?

**(ii) How will waste materials be disposed of?**

Include both solid and liquid laboratory waste and waste from experiments with infected animals.

**(iii) Will it be necessary to use sharps?**

Does work involve glass Pasteur pipettes?

**(iv) If the work involves the experimental infection of animals is it known whether the animal will shed the GMM?**

**(v) If the work involves the experimental infection of plants what is known about the likely route of transmission of the GMM?**

For example, is the micro-organism insect-borne or carried in run-off water? This will have important implications for the type of glasshouse used.

**(vi) In the case of organisms whose multiplication involves a complex life-cycle will the work involve the propagation of organisms that are in stages in that life-cycle that are particularly hazardous?**

Examples include the propagation of the infective stages of parasites or the release of spores from fungi. Consideration should be given to all potential routes of transmission including those that might not be used naturally.

**(vii) Have any disinfectants been validated under the actual conditions of use?**

For example, if disinfectant is being used for the treatment of virus in tissue culture medium, is it known that the disinfectant is effective in the presence of high levels of protein?

**(viii) Does the nature of this work preclude it being undertaken by any workers who have a serious skin condition (e.g. eczema) or other health problems that might make them more susceptible to infection (e.g. some kind of immunological defect)?**

**(ix) Will workers receive any vaccinations or health surveillance?**

**(e) Consideration of whether there is a need to assign additional measures over and above the provisional level of containment.**

It should be noted that the containment measures set out in Part II of Schedule 8 of the Contained Use Regulations will include some measures that are required where and to the extent that the risk assessment shows they are required

Additional measures may be necessary in any of the following circumstances:

- (i) to take full account of any properties of the GMM that may be hazardous to human health.
- (ii) to protect the environment.
- (iii) to provide additional safeguards for particular work procedures.

**Part 3. Final assignment of containment measures and risk class**

**The following aspects of this project are assigned to class 1.**

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**The following aspects of this project are assigned to class 2.**

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**The following aspects of this project are assigned to class 3.**

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**The following aspects of this project are assigned to class 4.**

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**Signature of Proposer:**

**Date:**