Ref:

UNIVERSITY OF LEICESTER

Application to carry out activities involving genetically modified micro-organisms

- Please return your completed form to Dr Sarah Nelson, Safety Services Office, Block L, Freemans Common (sc128@le.ac.uk).
- Please complete the form in **TYPESCRIPT**. Please note that you are not limited by text box size, these can be expanded.
- Note that for the purposes of the Contained Use Regulations, "micro-organism" is defined as a microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material including animal or plant cell cultures, viruses and viroids.
- For help in completing this form, see the University Genetic Modification guidance document or the ACGM Compendium, which can be viewed at http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/

1a). Title of project

iGEM 2015: Synthetic biology solution for therapeutic modulation of NAD+ levels

1b) Proposer/lab PI details

Name of Proposer	Email address	Telephone number	Department
Dr Richard Badge	rmb19@le.ac.uk	2525042	Genetics

Name of laboratory PI (if different from above)	Email address	Telephone number	Department
NA	NA	NA	NA

2. Construct(s) – please specify each host/vector/insert combination

(Part 2 for guidance on inserts and vectors)

host	**vector**	**Insert/gene affected**
E.coli DH5α	Standard iGEM Registry vectors (See attached list and maps)	**nadD, nadE and PncB are NAD+ biosynthetic enzymes** **ccdA and ccdB are bacterial antitoxin / toxin genes** **ccdB – (Biobrick BB_K581008)**

3. Summary of intended work

Give a brief summary of the aims of the project, and the procedures and materials to be used (including how the GMM will be used). **The biological functions of the insert(s) *must* be explained.**

1) **NAD+ level modulation has been shown to produce therapeutic benefits on muscle fatigue in neurodegenerative diseases such as Parkinson's Disease. A future route (NOT the topic of this application) to the delivery of NAD+ could be via NAD+ excreting commensal bacteria colonising the gut of patients. As a first step the current project aims to modify the enzymes encoded in *E. coli* by the nadD, nadE and PncB genes so that they are expressed in the periplasm rather than cytoplasm. If successful, the increase in extracellular NAD+ will be monitored by standard colorimetric assays. The genes are all enzymes involved in *E. coli* metabolism and none are known to be toxic to humans.**
2) **"Kill Switch" – all iGEM systems are required to address the issue of release of GMOs, by incorporating a kill switch that enables the GMO to be killed outside of the lab. In the current project the aim is to combine an established bacteria specific toxin / antitoxin system (ccdB (Biobrick BB_K581008) / ccd A) with a temperature sensitive ribosome binding site (Biobrick BB_K115002), such that at low temperature the antitoxin is not translated. The effectiveness of this system will be**

assessed by low temperature culture and viability assays. The genes are all bacterial and none are known to be toxic to humans.

4. Describe the validated waste treatment procedures, for both liquid and solid waste.
Procedures in case of spillage should also be included. *Specify named disinfectants and state working concentrations. (*Disinfection MUST be by validated means. See p*art 3.5 for guidance on waste disposal)*

Solid waste:

Solid microbiological waste is disposed of by autoclaving at 130ºC for 20 min at 1.8 Bar

Liquid waste:

Liquid microbiological waste is treated with disinfectant (Titan Chlor-Tabs, 1000ppm) for 24hrs at RT, then disposed to sink with copious water rinsing.

Disinfectant to be used, exposure time and working concentration:

Titan Chlor-Tabs, 24hrs, 1000ppm

Source of validation data: ***(e.g. manufacturer's data or own studies)***

Manufacturer's data, supplied with product.

5. Does the work involve a recipient micro-organism that is inherently safe? **Yes**

Examples of inherently safe micro-organisms:

- *E. coli K12 (this does not include the strain BL21 which is not a derivative of K12- See section 8 of the University guidance document https://swww2.le.ac.uk/offices/safety-services/documents/pdfs/gmguide0211.pdf)*
- *Yeast*
- *Mammalian cells*
- *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.)*

6. Is the gene(s) non-harmful? **Yes**

Types of gene that might give rise to a harmful phenotype:
1. A gene encoding a product that could directly cause harmful effects, e.g. a toxin gene or an oncogene
2. A gene encoding a product that could act alongside the existing characteristics of the recipient to endow the GMM with altered pathogenic properties; e.g. a pathogenicity gene or a viral envelope gene with altered receptor-binding activity.
You should also consider whether the gene(s) could cause harm if transferred, by natural gene transfer processes, to another host organism following breach of containment.

If you have answered YES to both the above questions, do you believe you have sufficient information at this stage to classify the project as Activity Class 1, as defined in the Contained Use Regulations 2000? **If YES, sign declaration A below, complete the rest of this form and submit it to the Biological and Chemical Safety Officer. If NO, please sign declaration B below, complete the rest of this form and form GMM-B in addition and submit both forms to the Biological and Chemical Safety Officer.**	**Yes**

Please note:

- To make this classification you need to be confident that **under no circumstances will the GMM be likely to cause disease to humans, animals or plants or cause harm to the environment.** You need to be satisfied that harm would not arise even if a worker was accidentally inoculated or there was a total breach of containment resulting in the GMM spilling into the environment.
- If you are uncertain whether the proposal meets the above criteria, you should proceed to the detailed risk assessment (form GMM-B), then sign declaration B.

- The person signing agrees to take responsibility for the work and act as Deputy Biological Safety Officer in connection with the proposed project.

DECLARATION

SIGN AT "A" or "B".
This must be signed by the laboratory PI who will have responsibility for this work

A	**This project meets the above criteria for classification as Activity Class 1 and is being submitted to the Genetic Modification Sub-Committee on that basis, without further risk assessment.**
Signature of Laboratory PI:	**Date:**
R.M. Bge	**03/08/15**
B	**Activity Class from form GMM-B: Please Select**
Signature of Laboratory PI:	**Date:**

Laboratories in which the work will take place: (lab numbers, building)

Lab LG15, Adrian Building

Names & job titles of all workers on this project:

Name	Job Title
Dr Richard Badge	Lecturer
Professor Raymond Dalgleish	Professor
Liam Crawford	iGEM Team Member (UG)
Payal Karia	"
Amy Evans	"
Ross Campbell	"
Cameron Grundy	"
Joss Auty	"
Charlie Kruczko	"

Anticipated start date of project:

1st August 2015

Office use only:
This proposal has been considered by the GMSC of which I am the authorised representative and whose views are accurately set out below

Name:	Signed:	Date:

I agree to act as Biological safety Officer in connection with this proposed work

Name:	Signed:	Date:

GMSC Comments: