Insert BA Categorisation (Hazard Group 1 or 2/ or GMO Class 1):
GMO Class 1

Loughborough University

Health & Safety Unit Use Only	
Ref No:	
Department Use Only	
Ref No:	CBE/BRA/101

RISK ASSESSMENT OF WORK WITH BIOLOGICAL AGENTS

Please note the following before completing this form:

1. University Health and Safety Policy requires that risk assessment of all work with biological agents (BAs) must be carried out in advance of work commencing. A key requirement of The Control of Substances Hazardous to Health Regulations (COSHH) is to assess the risks associated with any work activity involving the use of biological materials may contain biological agents.
2. YOU SHOULD COMPLETE ALL OF PART A, THE APPROPRIATE SECTIONS OF PART B, AND ALL OF PART C. WHERE HAZARD GROUP 2 BIOLOGICAL MATERIAL IS INTENDED TO BE USED THE RISK ASSESSMENT MUST BE REVIEWED BY THE DEPT/SCHOOL BIOLOGICAL SAFETY ADVISOR AND EXPLICIT APPROVAL IS ALSO REQUIRED FROM THE UNIVERSITY BIOLOGICAL SAFETY OFFICER. THIS FORM SHOULD BE SUBMITTED TO THE HEALTH, SAFETY & ENVIRONMENT UNIT FOR REVIEW VIA YOUR DEPARTMENTAL BIOLOGICAL SAFETY ADVISOR.
3. It is the responsibility of the Principal Investigator/Supervisor to ensure compliance to these requirements and that this risk assessment remains valid.
4. This risk assessment form **IS NOT** for assessing the risks associated with **Genetically Modified Organism activities.**

Date Submitted:	24 March 2015	Date Approved:	
Version Number:	1	Supersedes *(insert version number if applicable)*	N/A

PART A: Please provide the following general information:

School/Department			
Centre for Biological Engineering			
Title of Project			
UK RMP Automation of iPS Cells			
Project Reference Number:	UKRMP		
Person responsible for this work (*Principle Investigator*)			
Name:	David Williams	Position:	Professor, Healthcare Eng.
Department:	Centre for Biological Engineering	University School:	Wolfson School of Mechanical and Manufacturing Engineering
Person conducting this assessment			
Name:	Amit Chandra	Position:	Research Associate
Department:	Centre for Biological Engineering	Date Risk Assessment Undertaken:	24 March 2015
Proposed Project Start Date:	31 March 2015	Proposed Project End Date:	31 January 2018

Review History: *required at least once a year or immediately following any significant change to the project. Significant revisions must be detailed on a revision form. The person responsible must ensure that this RA remains valid.*

	Review 1	Review 2	Review 3	Review 4	Review 5
Due Date					
Date Conducted					

A1 PROJECT SUMMARY

A1.1 Scientific Goals of the Project.

This provides a useful background for the reviewer and reader. It need only be brief and should provide an overview of the scientific goals.

The UK RMP programme is looking at the scalable culture of pluripotent stem cells and comparability between sites. In this workpackage, pluripotent stem cells (induced at the Anne McLaren Laboratory for Regenerative Medicine, Cambridge University) have been adapted to expand using enzymatic dissociation from the adherent tissue culture surface. These cells will be transferred to Loughborough University and culture on the automated cell culture platform (Compact Select) and then returned to Cambridge University for characterisation. The results will be compared to manual culture expansion at Cambridge University.

A1.2 Description of the Experimental Procedures

Describe laboratory procedures to be used and highlight any non-standard laboratory operations. This may need cross reference to supporting documentation i.e. protocols.

Human induced Pluripotent Stem Cells (hiPSCs) induced from commercially available human fibroblast and keratinocyte cell lines (Life Technologies) were cultured at the containment level 2 Anne McClaren Laboratory, Cambridge University. These were reprogrammed as per the published Kyoto protocol with an episomal non integrating vector without viral intervention. The resulting pluripotent stem cell is considered safe for clinical use if induced in relevant conditions. These hiPSCs are currently growing in E8 medium on a vitronectin matrix. The dissociation from the cell culture surface is performed enzymatically using CTS TryplE Select.

These cells will be brought to Loughborough University in T25/T75 flasks in culture. They will be manually cultured at Loughborough University CBE containment level 2 laboratories till there are enough cells to seed a T175 flask and import into the Compact Select. During the time the cells are cultured manually, they will be observed to ensure Compact Select protocols are developed for:

a. Media exchange
b. Passage.

On the compact Select, the cells will be taken up to two passages. The cells will be pooled and returned to Cambridge University for analysis of pluripotency by Cambridge University protocols.

PART B: Please provide information in one or more of the following sections, as appropriate. Only sections which you complete should be submitted:

Section 1: *micro-organisms (prions, viruses, bacteria, fungi, parasites in ACDP category 2 and pathogens controlled by the Department for the Environment, Food and Rural Affairs).* ***[Work with ACDP category 3 and 4 pathogens is not currently permitted in the University.]***

Section 2: *cell cultures, tissues, blood, body fluids or excreta*

Section 3: *plants and plant material*

Section 4: *animals and animal tissues*

SECTION 2: CELL CULTURES, TISSUES, BLOOD, BODY FLUIDS OR EXCRETA

B2.1 HAZARD & RISK IDENTIFICATION : NATURE OF CELLS, TISSUES OR BODY FLUIDS

*This information gives an indication of the **potential** harm that the biological material may cause*

B2.1.1 List all cells or tissues to be used. *For cells indicate if primary, continuous or finite.*

Indicate in the adjacent box if Not Relevant (N/R)			
Cell or tissue type and ID	Organ Source	Species	From where will it be obtained?
Human induced pluripotent stem cells Line 1	Reprogrammed fibroblasts (C-013-5C,HDFa, GIBCO)	Human	Anne McLaren Laboratories, Cambridge University
Human induced pluripotent stem cells Line 1	Reprogrammed keratinocytes (C-020-5c,HEKn – APF, GIBCO)	Human	Anne McLaren Laboratories, Cambridge University

B2.1.2 List all blood, body fluids or excreta to be used

Indicate in the adjacent box if Not Relevant (N/R)		N/R
Material type	Species	From where will it be obtained?
N/R		

B2.1.3 Has any material listed in section B2.1.1 been genetically modified in any way?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes
If Yes, complete Genetically Modified Organisms (GMO) Risk Assessment Form	

B2.1.4 Will material be screened for infectious agents? (if from a cell culture collection answer B2.1.6 instead)

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes
If Yes, provide details of the types of screening and agents screened for: The cells have been screened for Mycoplasma at University of Cambridge and all are negative. The cells will come to Loughborough University and will be quarantined immediately. A sample of the cells will be screened for mycoplasma and microbial infection at Loughborough University following the internal test procedure. Based on the test results and the providence the quality manager will release the cells from quarantine.	

B2.1.5 Will any clinical history (if relevant) be provided with this material?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	N/R
If yes give details: N/R	
If yes, will a policy of rejection of samples from diseased patients be adopted? Explain N/R	
If yes, how will the information be disseminated in the course of the project? N/R	
If yes, will this information be anonomised? N/R	

B2.1.6 If obtained from a cell culture collection, is safety information provided?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If Yes, summarise here:	

B2.1.7 Has any of the material listed in section B2.1.1 been identified in the list of cross-contaminated or misidentified cell lines, available on HPA website
(http://www.hpacultures.org.uk/media/E50/3B/Cell_Line_Cross_Contaminations_v6_0.pdf

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If Yes, provide details of the route of provenance back to the originator of the cell line, together with a Certificate of Analysis; identifying the methods used to qualify the cell type.	

B2.2 RISK TO HUMANS

B2.2.1 What is the likelihood of infection of this material? Indicate as None, Low Risk, Medium Risk, High Risk, Known Infected*

Cell type and ID	Risk Category	Justification for Selection
Human induced pluripotent stem cells Line 1	Low risk	Primary cells obtained with certificate of analysis in xenofree media, reprogrammed using non integrating Episomal vector without viruses. All media is xenofree and lines been cultured for about 5-6 months antibiotic free.
Human induced pluripotent stem cells Line 1	Low risk	Primary cells obtained with certificate of analysis in xenofree media, reprogrammed using non integrating Episomal vector without viruses. All media is xenofree and lines been cultured for about 5-6 months antibiotic free.
		If none proceed to section B2.2.4

see The Managing the risks in laboratories and healthcare premises – available at
http://www.hse.gov.uk/biosafety/biologagents.pdf

B2.2.2 If low, medium or high risk (section B2.2.1), name and classify the Biological Agents this material could be infected with. List the biological agents and indicate the ACDP hazard group classification*

Name of Agent	Classification
None	

see The Approved List of Biological Agents – available on the Health & Safety website or
http://www.hse.gov.uk/pubns/misc208.pdf.

B2.2.3 Describe the route(s) of infection (in humans) for these adventitious agents (place a 'X' in the relevant box)

Percutaneous	Mucocutaneous	Inhalation	Ingestion	N/R
				X
Details:				

B2.2.4 Are there any other biological hazards (other than adventitious infectious risk) associated with the materials e.g. aggressive tumourogenic cell lines

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If Yes, describe:	

B2.3 HUMANS AT INCREASED RISK OF INFECTION

B2.3.1 Do any of the agents listed in section 2.1 present an overt risk to humans at increased risk (including immunocompromised workers, pregnant workers, breast feeding mothers)?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, Occupational Health must be consulted:	

B2.4. PROPAGATION OR CONCENTRATION OF ADVENTITIOUS AGENTS

B2.4.1 Will any culturing of this material take place?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes
If yes, identify the cells and the conditions these will grow: hiPS cells will initially be expanded in E8 media with a vitronectin matrix using CTS TryplE Select as the enzymatic dissociation agent.	

B2.4.2 If culturing, will CD4+ cells be present. Describe what cells and for how long these cultures will be allowed to grow

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, explain:	

B2.4.3 If culturing, what is the maximum volume of culture grown?

Indicate in the adjacent box if Not Relevant (N/R)	
Per Flask Initial seeding density of 5000 cells/cm^2 (Approx. 0.32 cm^2 for a 96-well plate) up to maximum confluence of 80%.	Per experiment Not yet determined. A maximum of 16 T175 flasks per experiment. Maximum volume in a T175 flask is 40ml. Thus a maximum of 640ml per experiment.

B2.4.4 Will the cells be manipulated in any way that could result in a concentration of any adventitious biological agent present?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes

If yes, explain: Biological agents may potentially multiply after infection of one or more cell cultures.

B2.5 WORKING WITH MATERIAL DONATED BY YOURSELF OR COLLEAGUES:

Workers **MUST NEVER** culture, deliberately transform or modify their own cells or cells from their co-workers or workers otherwise associated with the experimental work. *NOTE: This presents a particular hazard since any self-inoculation injury could have potentially serious consequences as cells would essentially circumvent the normal protection of the immune system.*

B2.5.1 Will any cells be donated by persons working in or has access to the lab?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, explain what precautions are to be taken to prevent that person being exposed to the cells:	
If yes, where will this material be collected:	
If yes, provide justification for not using a safer source:	
If yes, how will confidentiality be assured:	
If yes, has Ethics Committee approval been obtained:	

B2.6 ENVIRONMENTAL CONSIDERATIONS:

B2.6.1 Are any of the agents capable of causing disease or other harm in animals, fish or plants?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, describe:	

B2.6.2 Will there be any other environmental risks?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, describe:	

B2.7 OTHER HAZARDS

B2.7.1 Are there any other hazards associated with this work? For example, hazardous chemicals (especially carcinogens, mutagens, substances toxic to reproduction, cytotoxins), cryogenic gases, ionising radiation.

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes
If yes, identify these: Cryogenic gas (gaseous nitrogen for low-temperature storage)	
If yes, have these been risk assessed and any necessary approval obtained? Yes	

B2.7.2 Are there any conditions associated with the hazards described in B2.7.1 that require special attention in Section C of this risk assessment? For example, material incompatibilities with disinfectants such as Virkon or hazardous product decomposition associated with high temperatures (ie autoclaving).

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No

If yes, provide details and ensure that appropriate control measures are addressed in Section C:

PART C: CONTROL MEASURES

C1. CONTROL MEASURES

The risk of exposure must be prevented or adequately controlled to minimise the chance of harm arising. COSHH Regulations require minimum containment measures for laboratories handling organisms from the different ACDP hazard groups (http://www.hse.gov.uk/pubns/misc208.pdf)
The hazard group number typically indicates the level of containment (includes physical measures & working practices) that must be used for its handling).

C1.1 Preventing Exposure

C1.1.1 Substitution with a Safer Alternative

Is substitution with a safer alternative practical, by for example, replacement of a clinical strain or pathogen with one that is lab adapted? Provide reasons for your answer:

Replacement of the chosen cell type is unlikely; the chosen cell types are safe for clinical purposes.

C1.1.2 Isolation/Segregation

(i) Is/Are the laboratory(s) to be used for this work to be shared with other workers not directly involved in this activity?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes

If yes, provide details:
Yes, the labs used in this project are the CBE laboratories at Loughborough University, Garendon Building, and these facilities are shared with multiple users.

(ii) Is access to the laboratory(s) to be used for this work restricted?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes

If yes, provide details:
Access is provided only to trained individuals or individuals that are formally permitted to enter the lab (such as outside contractors on a permit-to-work basis).

C1.2 Controlling Exposure

C1.2.1 Are sharps (needles, blades, scissors, forceps, glass or capillary tubes) to be used at any stage during this activity?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No

If yes, list the sharps:

If yes, justify there use – is there an alternative?

If yes, describe there use and disposal:

If yes, describe any additional precautions employed to reduce risk:

C1.2.2 Containment and Ventilation

(i) Is the use of BSC required for the protection of the worker i.e. do the work procedures generate aerosols or splashes that pose a risk to workers?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes
If yes, specify the type(s) and when they will be used: The BSCs used are Herasafe KS type, and will be used for all procedures involving biological material.	
(ii) Are there any requirements for room ventilation e.g. negative pressure, temperature control?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes
If yes, specify: The CBE is air-controlled to ensure safe working environments.	

C1.2.3 Transport and Storage within the laboratory

How and where are materials to be stored? During culturing, the hiPSCs will be stored in one or more incubators (including the Compact Select).
How will this material be transported within the laboratory e.g. between BSC and incubator? Detail the containment measures which will be used to prevent or contain accidental splashes or spills. The hiPSCs will always be kept in tissue culture flasks, sealed vials or tubes. While moving, other present coworkers are cautioned about the work to prevent accidental tripping, and the number of containers handled at any given time will be small enough that stacking of these containers does not cause any additional risks.

C1.2.4 Local transport out of the laboratory

How will this material be transported on-site (e.g. research material between labs on campus or movement of waste containing viable agents e.g. to a remote autoclave? Detail the containment measures which will be used to prevent or contain accidental splashes or spills This material will not be moved locally out of the laboratories prior to sterilization.

C1.2.5 Shipment of Biological Material

Will this material be shipped elsewhere in the UK or abroad?						
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)						Yes
If yes, give details to support compliance to the relevant regulation (e.g. category of material, correct packaging instruction):						
Description of material to be shipped (*indicate in available boxes*). Is this:						
Category A		UN2814		UN2900		*Packaging instruction 602 or 620 must be followed*
Or?						
Category B	x	UN3373				*Packaging instruction 650 must be followed*
Or?						
Non-hazardous						*Should be packaged to protect sample*

C1.2.6 Receipt of material

If material will be received from other sites or organisations, what precautions are being taken to ensure that the material is shipped correctly? Prior to accepting the material, the container is checked to ensure all proper containment requirements are applied; the material is transported as sealed tissue culture flasks in secondary packaging.

C1.2.7 Centrifugation

(i) If material is to be centrifuged will sealed buckets and rotors be used?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes
(ii) Where will these rotors/buckets be opened? The rotors/buckets will be opened in the centrifuge.	
(iii)Describe the procedures in place to deal with leaks and spillages in the centrifuge Centrifuge spills will be handled as outlined in SOP038 – Biological Spill Response, supported by other SOPs as needed, depending on the chosen centrifuge.	

C1.2.8 Incubators

If incubators are to be used, what type of incubator (e.g. shaking, static) is used and describe procedures to prevent and contain spillages. Only static incubators are used, stacking of well plates will be minimized to prevent spills. Any spills will be handled as outlined in SOP038.

C1.2.9 Disinfection

Specify the type and concentration of disinfectants to be used: IMS (70% solution in water) Virkon (1% solution in water)	
Have these disinfectants been validated for use with the recipient biological material?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes
If yes, describe the procedure: These materials are validated by the supplier and are supported by literature. Furthermore, IMS and Virkon are the current disinfectants of choice for all work in the CBE laboratories.	

C1.2.10 Personal Protective Equipment (PPE)

(i) What type of lab coats will be worn and where will they be stored? Side fastening white lab coats are used, when not worn these are stored in the first change room. (See SOP037 – Use of Personal Protective Equipment)
(ii) What type of gloves will be worn and where will they be stored? Nitrile gloves are worn for regular lab work, and are available in the first change room and the separate change rooms within the lab. Specialized cryostorage gloves are used when handling the liquid nitrogen storage system, and specialized autoclave gloves are used when handling the autoclaves. Both of these gloves are located in the autoclave room. (See SOP037 – Use of Personal Protective Equipment)
(iii) Describe any other PPE to be used: Eye protection will be used (safety glasses, goggles, or faceshield) where appropriate. Aprons will be used when handling the autoclave or liquid nitrogen storage system. (See SOP037 – Use of Personal Protective Equipment)

C1.2.11 Hygiene Measures

Describe the hygiene facilities available and where they are located Regular washing facilities are available in the first change room, in the change rooms inside the lab, and in H34. Eye wash facilities are available in the change rooms inside the lab.

C1.2.12 Vaccination

Are effective vaccines available against any of the agents listed in Section1, 2, 3, or 4 of Part B?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If yes, describe:	

C1.2.13 Waste Treatment before Disposal

How must waste to be treated before disposal and how has it been validated as being effective?		
Type of Waste	Treatment before disposal	Validation of this treatment
Liquid waste	Liquid waste is sterilized using Virkon, see SOP003 – Disposal of biological (healthcare) waste	Virkon is used as indicated by the supplier.
Solid waste	Solid waste is sterilized by autoclaving, see SOP003 – Disposal of biological (healthcare) waste, SOP54 – Use and maintenance of Systec Autoclave	The autoclave is used according to SOP054

C1.2.14 Autoclave sterilisation

If waste is treated by autoclave sterilisation then this section must be completed. If this section is not relevant then hatch the box			
Type of Waste	Composition of waste	Autoclave cycle (temp, cycle time)	Treatment monitor
Liquid waste	N/R	N/R	N/R
Solid waste	Culture medium components	121 degrees Celsius, 15 min.	Autoclave process/temperature reports, autoclave tape
Location of autoclave	Servicing details	Location of back-up autoclave	Designated area for storage of unsterilized waste
The autoclave is located in the CBE autoclave room.	Annual	Autoclave room	Second change

C1.2.15 Liquid Waste Disposal

How will liquid waste be disposed of?
To the drain?
As solid waste?
Other? Liquid waste is collected in aspiration bottles and sterilized with Virkon. See SOP003 – Disposal of biological (healthcare) waste.

C1.2.16 Solid Waste Disposal

Describe the waste category and disposal route. *(For guidance refer* to http://www.environment-agency.gov.uk)

Colour Code	Categorisation	*Hatch relevant box(es)*	Disposal Method
Yellow	Sharps (not contaminated with cytotoxic/cytostatic material)		Yellow Sharps bin>autoclave sterilisation if known or potentially infected >clinical waste disposal (incineration)
Purple/Yellow Special case, contact DSO	Sharps (contaminated with cytotoxic/cytostatic material)		Purple/Yellow lidded Sharps bin>clinical waste disposal (incineration @ 1000C)
Yellow	Human body parts, organs, including blood bags and blood preserves and excreta (unless identified as medium or high risk or known infected in Section 2.2.1 of this RA in which case they must be pre-treated before disposal)	X	Yellow rigid one way sealed tissue bins>clinical waste disposal (incineration)
Yellow	Animal body carcasses or recognisable parts ((unless identified as medium or high risk or known infected in Section 2.2.1 of this RA in which case they must be pre-treated before disposal		Yellow rigid one way sealed tissue bins > clinical waste disposal (incineration)
Special Case – Contact DSO	Potentially or known infected lab wastes (including sharps) of HG2, GM Class 2, DEFRA Cat 2 or higher, that have not been pre-treated before leaving the site.		This is not a route of preférence and is subject to special requirements
Orange	Infected or potentially infected lab wastes that have been pre treated before leaving the site	X	Disinfection or sterilisation (as identified in C1.2.14) in the laboratory suite > orange clinical waste bags > clinical waste disposal (incineration)
Yellow	Infected or potentially infected animal or human body parts, organs or excreta that have been pre treated before leaving site	X	Disinfection or sterilisation (as identified in C1.2.14) in the laboratory suite > yellow one way sealed tissue bins > clinical waste disposal (incineration)

C1.2.17 Work with Animals or Vectors (if none proceed to Section C1.2.18)

(i) Are animals or vectors to be infected with any of these biological agents?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If yes, describe the procedure and describe where this aspect of the work will be conducted:	
(ii) Is shedding of infectious materials by the infected animals possible or expected?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If yes, describe the routes of shedding, risk periods for such shedding and the additional precautions required to control exposure:	

(iii) Who will perform the inoculations of animals/vectors? What training have they received?	
Indicate in the adjacent box if Not Relevant (N/R)	N/R
Provide details of the training required:	

C1.2.18 Bioreactor/Fermenters (if none proceed to Section C1.2.19)

Will a bioreactor/fermenter be used to culture a biological agent?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If yes, describe the size, and type of the bioreactor/fermenter.	
(ii) Are any supplementary containment measures required, for example, the use of a BSC or spill tray.	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If yes, describe:	

C1.2.19 Other Control Measures Required?

None.

C1.3 Emergency Procedures

C1.3.1 Describe the procedures in place for dealing with spillages *(specify disinfectants and any special containment for large volumes)*

Within the BSC: Spills within BSCs will be dealt with as required depending on the nature of the spilled material, as dictated by SOP038 – Biological Spill Response, and any other SOPs relevant to the used materials and equipment.
Within the laboratory but outside the control measure e.g. BSC, spill tray Spills within the laboratory will be dealt with as required depending on the nature of the spilled material, as dictated by SOP038 – Biological Spill Response, and any other SOPs relevant to the used materials.
Outside the laboratory e.g. during transport Not applicable: the cells used in this project will not leave the laboratory prior to sterilization.
Describe the procedures in place for an accidental exposure (if necessary describe different procedures for different types of exposure e.g. eye splash or percutaneous inoculation) Accidents will be handled according to SOP038– Biological Spill Response, with follow-up procedures outlined in the Code of Practice and the Policy on the reporting of accidents, dangerous occurrences and occupational ill health.

C2 ASSIGNMENT OF CONTAINMENT LEVEL

The laboratory Containment Level is directly related to each of the 4 Hazard Groups; organisms categorised as

HG1 (lowest hazard rating) should normally be handled in CL1 facilities (minimum level of containment), and likewise up to HG4 (highest hazard rating) in CL4 facilities (maximum level of containment). Where the identity or presence of a biological agent is not known the following rules apply: a) where uncertainty exists over the of pathogenic biological agent – minimum of CL2; b) where the presence of a pathogenic biological agent is known or suspected – minimum of Containment Level appropriate to the agent, where the assessment is inconclusive but where the activity might involve serious risk – minimum CL3

C2.1. What containment level is required for this work? (see COSHH Schedule 3, Part II for a list of criteria)

Containment level 1 is required for this work (Hazard group 1).

C2.2. Describe extra controls or derogation from certain controls

N/R

C3 FACILITIES

C3.1 Where will this work take place?

Room(s)	Building	Campus	Person in Control of area
H21 H22	Garendon building, centre for biological engineering	Loughborough University, holywell park	Katie Glen/Qasim Rafiq/Andy Picken/Pete Mitchell/Elizabeth Ratcliffe (Lab Leaders), Carolyn Kavanagh/Kulvindar Sikand (Lab managers), Chris Hewitt (Biological safety officer), Robert Temple (Department safety officer)

C4 PERSONNEL

C4.1 Names of Personnel involved in the Project

Surname	Initials	University ID	Position
Chandra	A	5002714	Research Associate
Sebastian	S	5023802	Research Associate

C4.2 Information, Instruction and Training

Describe the training that will be given to all those affected (directly or indirectly) by the work activity. Instruction should include the 'Local Rules' or 'Local Codes of Practice' which focus on the working instructions to be followed by all persons involved in the work activity to control or prevent exposure to hazardous biological agent(s). These should be written and readily available to all workers working at Containment Level 2. A formal record of training should be kept for all individuals working at Containment Level 2.

All individuals working in the CBE laboratories will have received training regarding the general use of the lab, dealing with risks and risk assessments, emergency and spill procedures, as well as the code of practice. Furthermore, any work in the lab must be risk assessed and necessary training or authorization must be obtained before work with such requirements may be started.

C4.3 Relevant Experience/Training:

Surname	Experience/Training
Chandra	10 years in cell culture at the CBE. This includes use of the Compact Select for hiPSC.
Sebastian	5 years of cell culture. Use of pluripotent stem cells in manual culture.

C4.4 Other people who may be at risk from the activity e.g. cleaners, maintenance workers or other workers in shared laboratory

Details:

Other lab users may be subject to low risk in the event of spills or accidents. There are no cleaners or maintenance workers in the laboratory on a routine basis. If there are any external workers, they are controlled by a permit to work.

C5 OCCUPATIONAL HEALTH

C5.1 Vaccination

Is an effective vaccination available for any of the pathogens associated with this work? Advice can be obtained from the Occupational Health Adviser (OHA) if required. All workers involved with handling unscreened blood, blood products and other tissues are recommended to have Hepatitis B immunization

No vaccination is required for this project. Both researchers have been vaccinated against Hep B for working in a containment level 2 laboratory.

C5.2 Health Surveillance

Is health surveillance required? (Health surveillance is typically applied if working with a hazardous substance that:

a) produces an identifiable disease or adverse health effect that can be related to exposure; b) there is a reasonable likelihood that the disease or effect may occur under the conditions of work, and c) there are valid techniques for detecting indications of the disease or effect). No.

C6. NOTIFICATIONS: Human Tissue Act

C6.1.1 Relevant material covered by the Human Tissue Act

Are any of the cells, tissues or fluids to be used covered by the Human Tissue Act?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No

C6.1.2 Does This Work Have Ethical Approval? If Yes, Provide Details

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)			N/R
Approval number:			
Date obtained:		Ethics committee name:	

C6.1.3 Are other registrations/notifications required for this work? For example HSE notification under COSHH, Home Office notification under anti-terrorism, crime and security act etc

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If Yes, give details:	

7. LICENSING REQUIREMENTS FOR ANIMAL PRODUCTS

C7.1.1 Are there any licensing requirements for this work?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R

The regulations covering the import of animal products (including tissue cultures, tissues, body fluids or fractions thereof) are in a state of flux. Current procedures to be followed:

- If you wish to import any animal products that you know are not infected with an animal pathogen, or have good reason to expect that they are not infected with an animal pathogen, from within or outside of the EC you must apply for a Research Sample Licence using the Defra form IAPPO1. Follow this link to download the form http://www.defra.gov.uk/corporate/docs/forms/ahealth/iapppo1.htm

- If you wish to import such an animal product but it is known or suspected of being infected with an animal pathogen then you must use DEFRA form IM137. Follow this link to download the form http://www.defra.gov.uk/corporate/docs/forms/ahealth/inttrade/im137.htm

- If you wish to import an animal pathogen listed under the Specified Animal Pathogens Order then you must use DEFRA form PATH1. Follow this link to download the form http://www.defra.gov.uk/corporate/docs/forms/ahealth/path1.htm

In all cases the instructions for their submission is stated on the forms themselves.

ALL APPLICATIONS SHOULD BE REVIEWED BY THE DEPARTMENTAL SAFETY OFFICER AND THE UNIVERSITY BIOLOGICAL SAFETY OFFICER BEFORE SUBMISSION.

8. DECLARATION

*The declaration must be signed **before** submitting this assessment to the Departmental Safety Officer and University Biological Safety Officer*

I, the undersigned:

- confirm that all information contained in this assessment is correct and up to date
- will ensure that **suitable and sufficient instruction, information and supervision** is provided for all individuals working on the activity
- will ensure that no work will be carried out until this **assessment has been completed and approved** and that all necessary control measures are in place
- that all information contained in this assessment must remain correct and up to date (the assessment should be **reviewed once a year** and whenever any **significant changes** to the work activity occur)
- will re-submit the assessment for approval if any significant changes occur

Name: Person conducting assessment	**Signature:**	**Date:**
Amit Chandra	A. Chandra	31 Mar 2015
Name(s): All named persons involved in the project *(add additional rows below, as required)*	**Signature:**	**Date:**
Sujith Sebastian	Sujith	31/03/2015
Name: Principal Investigator/Supervisor/Line Manager	**Signature:**	**Date:**
David Williams		31 Mar 2015

9.APPROVAL

For work involving **Hazard Group 1** biological agents: Review and approval is required by authorised and designated members of CBE staff before the work begins

For work with **Hazard Group 2** biological agents: Explicit approval is required from the Departmental Biological Safety Advisor and the University Biological Safety Officer before work begins.

If the biological agent has been **Genetically Modified** this form, (approved by the relevant authority, as above) should be submitted with the GMO risk assessment to the Departmental Biological Safety Advisor and both forms forwarded to the LU GM Safety Committee for final approval.

Name: Authorised CBE Personnel (please indicate position)	**Signature**	**Date**
R. Bayley, Research Associate	R. Bayley	31.03.15
Name: Departmental Biological Safety Advisor	**Signature**	**Date**
R I Temple	R I Temple	02/04/2015
Name: University Biological Safety Officer (or Deputy)	**Signature**	**Date**
C. M. Moore.	C. M. Moore	2.4.15

Loughborough University

Health & Safety Unit Use Only	
Ref No:	
Department Use Only	
Ref No:	CBE/GMO/101

RISK ASSESSMENT of WORK with

GENETICALLY MODIFIED ORGANISMS

The requirements of Genetically Modified Organisms (Contained Use) Regulations 2000 are reflected in the University Health and Safety Policy which requires that risk assessment of all work with Genetically Modified Organisms ***must*** *be carried out in advance of work commencing and, in addition,* ***must be scrutinised and approved*** *by the University's relevant Safety personnel. The tables at the end of this document are drawn from the current legislation and the appropriate table* ***must*** *be completed as part of the assessment. Finally,* ***WORK MUST NOT BEGIN*** *until the proposal has been* ***approved*** *and clearance has been given via Health and Safety.*

Date submitted	25 March 2015	**Date approved**	

Please provide the following general information:

School/Department	Wolfson School of Mechanical and Manufacturing Engineering

Principal investigator	D. J. Williams	**Position**	Professor, Healthcare Engineering
E-mail address	d.j.williams@lboro.ac.uk	**Phone no.**	01509-227668

Please give a brief and descriptive title for this risk assessment

Title	UK RMP Automation of iPS Cells

Please provide a brief description of the nature of the work, identifying any GMMs produced *(e.g. virus vector with insert)*, and their use to transform cells. Please identify the components of the project for which this risk assessment is carried out.

The UK RMP programme is looking at the scalable culture of pluripotent stem cells and comparability between sites. In this workpackage, pluripotent stem cells (induced at the Anne McLaren Laboratory for Regenerative Medicine, Cambridge University) have been adapted to expand using enzymatic dissociation from the adherent tissue culture surface. These cells will be transferred to Loughborough University and culture on the automated cell culture platform (Compact Select) and then returned to Cambridge University for characterisation. The results will be compared to manual culture expansion at Cambridge University.

Human Induced Pluripotent Stem Cells (hIPSCs) induced from commercially available human fibroblast and keratinocyte cell lines (Life Technologies) were cultured at the containment level 2 Anne McClaren Laboratory, Cambridge University. These were reprogrammed as per the published Kyoto protocol with an episomal non integrating vector without viral intervention. The resulting pluripotent stem cell is considered safe for clinical use if induced in relevant conditions.

Donor	Human
Name of gene/nucleic acid sequences	Epi 5 Episomal iPSC Reprogramming Kit (A15960, Lifetechnologies) Oct4, Sox2, Nanog, Lin28,KLf4, LMyc, mp53DD, EBNA
Vector	Episomal Vector
Host	Human. The cells used are: 1. Reprogrammed fibroblasts (C-013-5C,HDFa, GIBCO) 2. Reprogrammed keratinocytes (C-020-5c,HEKn –APF, GIBCO)
ACDP category* of host (where appropriate)	

*The ACDP categorisation of biological agents can be found in the *Approved List of Biological Agents* published by the Health and Safety Executive.

Note: The questions in this proforma are designed to ensure that all the relevant issues have been addressed for the majority of Risk Assessments for work involving Genetic Modification at the University of Loughborough. However in the interests of streamlining the majority of applications, and because not all possible applications of genetic modification may have been anticipated, there may be instances in which answer of these questions alone may not be sufficient for a full risk assessment. The Genetic Modification Safety Committees reserve the right to request additional information. For a more complete description of the requirements of a Risk Assessment, refer to ACGM notes and newsletters, and the Guidelines to the 2000 Regulations. Less detail will be required for commonly used and familiar host/vector systems than for those less widely known or characterised. References may be helpful in some instances.

It may be appropriate to write the assessment to cover a range of closely related GMOs, e.g. a defined family of genes, a range of vectors with similar properties, complete and partial sequences, with and without expression; however the assessment and containment conditions proposed must reflect the greatest potential hazard of any of the range of GMMs covered by the assessment.

Do not feel constrained by the box sizes, in some cases considerably greater amounts of information may be required. The box sizes should expand to accommodate your text. To add further rows to a table, use tab key when cursor is in the last box.

Any potentially confidential information should be highlighted, e.g. by use of **red text**. This will include all personal information, and possibly e.g. commercially sensitive information, which the applicant wishes **NOT TO APPEAR ON THE PUBLIC REGISTER**. NB There are tight restrictions on what will be accepted as confidential. The remainder of the risk assessment must be understandable without the confidential information.

It may be possible for outside bodies to access information in this form under the Freedom of Information Act, unless it can be categorised as an exemption. Furthermore, work with organisms listed in Schedule 5 of the Anti-terrorism, Crime and Security Act 2001, or genetic material from those organisms, may be notifiable to the Home Office.

Characteristics of the Donor, Insert, Vector and Host

Name (species/strain if appropriate) **and characteristics of the source of the nucleic acid sequences ("the donor)**

Episomal vectors were developed By Shinya Yamanaka (eg. Addgene plasmid # 41813) A portion of this plasmid was derived from a plasmid made by pCEP4 is from Invitrogen. CAG Promoter was from Dr. Jun-ichi Miyazaki of Osaka University Graduate School of Medicine. The plasmid is cited as "Efficient selection for high-expression transfectants with a novel eukaryotic vector. Gene 108:193-200, 1991. Niwa, H., Yamamura, K. & Miyazaki, J."

For this work Episomal vector kit (A15960, Life technologies) containing above was purchased

Note: Species from which the nucleic acid sequences were obtained, whether a pest or pathogen, tissue (normal, tumour, healthy or diseased), health status of the donor, etc.

Name, description and function of the gene/nucleic acid sequences involved ("the insert")

Episomal non integrating vector (A15960, Lifetechnologies)

Oct4,Sox2,Lin28,l-Myc,Kf4,mp53DD, EBNA1

These are core pluripotency genes highly conserved and involved in early embryonic development. There is high transfection efficiency due to oriP/EBNA-1 mediated nuclear import and retention of vector DNA allowing iPSC derivation in single transfection.
The Episomal vectors are removed from iPSCs due to silencing of viral promoter driving EBNA-1 expression and the loss of the episomes due to defect in vector synthesis and partitioning.

Note: Biological function of the intact, natural gene; whether protein-coding sequence complete, partial, unknown, or known to be absent in construct; whether or not interrupted by introns etc; whether wild type or mutant; known, suspected or intended function of mutants; any other biological activities e.g. antisense, ribozyme, replication origin, mobilisation functions, etc. Genomic or cDNA library (consider the properties of the library as a whole; separate assessment is required for the specific clones you intend to isolate from the library).

Name and characteristics of the "vector"

pCE-hOCT3/4; pCE-hSK,pCE-hUL; pCE-mp53DD,pCXB-EBNA1
Episomal vectors

Note: Name of parental plasmid, bacteriophage, etc; characteristics, i.e. mobilisable, mobilisation defective, non-mobilisable; host range; presence of drug resistance markers or other sequences of potential clinical or environmental significance. Whether constructs transferred into host cells e.g. as non-mobilisable DNA; presence of replication origins, conditional (e.g. SV40, EBV) or otherwise. Involvement of viral vectors (e.g. retrovirus, baculovirus); name, characteristics, whether replication defective and the basis of this (e.g. deletion); host range; pathogenicity; potential for complementation by products expressed in the host, or by superinfection, etc.

Name and characteristics of the "host"

Human cell lines. Human. The cells used are:

1. Reprogrammed fibroblasts (C-013-5C,HDFa, GIBCO)
2. Reprogrammed keratinocytes (C-020-5c,HEKn –APF, GIBCO)

Note: Species/strain etc, whether disabled/ highly disabled; presence of other agents which may e.g. assist transmission; or affect pathogenicity; any history of safe use; whether an intact multicellular organism is produced at any stage (e.g. transgenic animals, plants); if host is (a) cell line(s) derived from multicellular organisms, the species, any potential for harm to humans or the environment; presence of other agents which are themselves transmissible or may assist the mobilisation of the transferred sequences e.g. as a result of recombination.

Characteristics of the Genetically Modified (Micro)Organism

Will there be expression of the protein (or other functional product) encoded by the insert, in the genetically modified organism?

Reprogramming process leads to expression of pluripotency genes(endogenous), No vector gene expression detected in mRNA in stable lines.

Note: Provide details, e.g. of the promoter, level of expression, secretion, presence of introns within the coding region which might preclude expression of a functional product in E. coli, or other specific hosts, etc.

Specify any known or expected characteristics of the GMO which pose a risk to human health and safety and assess the severity and likelihood of such effects

Effects on human health (include colonisation, infection, allergy, toxin-mediated disease)
None.
Humans at increased risk of the above effects (e.g. immunocompromised, pregnant or breastfeeding women)
None.

Note: Characteristics which might increase the pathogenicity of the GMO relative to the unmodified host, or decrease susceptibility to control measures, e.g. alteration in susceptibility to clinically relevant drugs or to immunological or other natural defences; any other potentially significant biological activities of encoded products, e.g. potential toxicity, allergenicity, growth promotion/inhibition, oncogenicity, other pharmacological activity, etc.

Does this project involve work with animals? Provide details

No.

Either use of transgenic animals or work with GMMs in animal models

Quantity of organisms to be used

The genetic modification has already taken place at the Anne McLaren Laboratory at Cambridge University. The modified cell line will be transferred to Loughborough University. A maximum of 16 T175 flasks per experiment will be used. Maximum volume in a T175 flask is 40ml. Thus a maximum of 640ml per experiment. Initial seeding density of 5000 cells/cm^2 (Approx. 0.32 cm^2 for a 96-well plate) up to maximum confluence of 80%.

Specify volumes and concentrations/culture density

Interim Assignment of Containment Conditions to Protect Human Health

Using the appropriate table(s) in Annex 1 of this form please select your control measures (you may place a **X** alongside each appropriate control measure to indicate that you have considered each one) and assign an interim level of containment for the work, i.e. ACGM containment level, (taking into account the hazard grouping of any biological agent). Please justify your decision to use this level of containment.
NB CLASSIFICATION OF THE PROJECT IS DEPENDENT ON ONLY THOSE CONTROL MEASURES THAT ARE SHOWN BY THE RISK ASSESSMENT TO BE NECESSARY TO PROTECT HUMAN HEALTH OR THE ENVIRONMENT. MEASURES THAT RESULT FROM CONVENTION, CONVENIENCE OR ARE REQUIRED FOR PRODUCT PROTECTION ARE NOT RELEVANT TO THE CLASSIFICATION See ACGM Newsletter 27/ACGM Compendium of guidance for further information

Interim containment level and corresponding Class (classes) of GMO(s) involved in the work (& explanation)

GMO class 1 is fine

Note: You will need to consider the containment level necessary to control the risk of the host and then make a judgement as to whether the modification will result in a GMO more hazardous/less hazardous/about the same

Please provide the following information for the Committee:

Are any of the work procedures likely to generate aerosols? If so, is the work to be undertaken in a safety cabinet?

Yes. Some aerosols may be generated during culture, manipulation and pipetting of the cells, risk is minimised by careful handling. All manual preparation work will be conducted in a class 2 biological safety cabinet. The manipulation inside the Compact Select is contained by an air curtain.

A Class II Biological Safety Cabinet or laminar flow protected automated processing platform will be used for all cell culture and viral transfection work to protect against aerosols or splashes. Post-transfection virus stocks will also be handled within the BSC.
All work will be carried out using aseptic techniques, maintaining a sterile environment and also protecting the operator and other users of the laboratory from biological agents using a class 2 biological safety cabinet / equivalent automated processing cabinet.
Procedures to be carried according to the following SOPs:

1) SOP009, "Use and Maintenance of HERASAFE KS Class II BSC"
2) SOP104, "Use and Maintenance of HERASAFE KS Class II re-circulating BSCs"
3) SOP035 "Use and Maintenance of the CompacT SelecT"

Identify any use of sharps in the work; justify their use and specify control measures

No sharps to be used.

Protective equipment and clothing to be used

Side fastening white lab coats are used, when not worn these are stored in the first change room. (See SOP037 – Use of Personal Protective Equipment)

Nitrile gloves are worn for regular lab work, and are available in the first change room and the separate change rooms within the lab. Specialized cryostorage gloves are used when handling the liquid nitrogen storage system, and specialized autoclave gloves are used when handling the autoclaves. Both of these gloves are located in the autoclave room. (See SOP037 – Use of Personal Protective Equipment)

Eye protection will be used (safety glasses, goggles, or faceshield) where appropriate. Aprons will be used when handling the autoclave or liquid nitrogen storage system. (See SOP037 – Use of Personal Protective Equipment)

Transport and storage arrangements

The cells will be shipped between Cambridge University and Loughborough University in sealed tissue culture flasks which will be secondary contained and provided with heating material.

These cells will be shipped as per Category B UN3373 requirements where Packaging instruction 650 is followed.

Cells will be stored in closed flasks within a designated incubator (H21) and automated platform incubator (H21).

Specify arrangements for safe storage; whether, and if so how, materials are likely to be transported between buildings, on public roads, or posted)

Disinfection

Virkon (1% w/v) is the sole disinfectant used in the CBE laboratories other than 70% IMS which is used for general disinfection cleaning (SOP004) where Virkon cannot be used; for example stainless steel surfaces.

For hazard group 1 and 2, biological agents it is normally sufficient to rely on the manufacturer's data providing the recommended concentrations and contact times are used. Virkon will be used per the manufacturer's instructions as follows:
Laboratory surface disinfection; wipe surface with 1% concentration and dry with a paper towel or allow to air dry.

- Laboratory benches and equipment will be cleaned with 70% IMS wipes. BSC cleaning will use 70% IMS spray, it will be wiped on with a paper towel to ensure all the surface is covered and allowed to air dry or wiped with a paper towel to dry. Bio-hazard disinfection; 5% concentration for ≥60m.
- Aspirated liquid waste disinfection: Autoclavable and volume graduated aspiration waste reservoir bottles will be prepared containing known volumes of virkon concentrate calculated on the volume of waste that will be generated during the operational procedure to ensure 1% disinfection treatment. A 24h contact time will be used for disinfection, after this time aspiration bottles will be emptied down the sink with copious amounts of water.

All solid waste is autoclaved on-site and incinerated using the GM waste route.

Specify disinfectant(s) to be used, and their dilution. Have these been validated for use with the relevant organism?

Inactivation of GMMs in waste, and subsequent disposal

Liquid waste is treated with virkon at 1% (v/v) dilution for 24h.

Disposable solid waste, such as tissue culture plastic and other consumables (e.g. serological pipettes in autoclavable plastic discard jars), and including sharps in the appropriate biohazard sharps bins, which is or may be contaminated with GMOs is also inactivated by autoclaving at 121°C for 15 minutes, before removal as "clinical waste" by specialist contractors, with final disposal by incineration.

Autoclave treatment is validated according to SOP024 "Maintenance of Systec VX-95 Autoclave CBE044". Annual validation is conducted by an external contractor, validation of individual cycles is performed using autoclave tape monitors.

Decontamination will be performed in accordance with procedures outlined in SOP003 "Disposal of Biological Waste".

Expected degree of kill: Autoclaving achieves effectively 100% kill of all GMMs

The Contained Use Regulations 2000 require that GMMs in contaminated material and waste are inactivated by validated means. You must specify the METHOD of inactivation of the GMMs, the expected DEGREE OF KILL of the GMM achieved by that method, and the VALIDATION of that method.

Monitoring of Containment and Control Methods

Monitoring of containment at point of use

It is the responsibility of the person undertaking GM work to check the correct operation of equipment. Culture vessels, tubes etc should be inspected to ensure leak-free operation. The work area and equipment should be checked for spillages before and after use and decontaminated if necessary. The bench will be swabbed with disinfectant before and after work and the disinfectant dated.

Safety cabinets and the automated platform are tested and maintained on a regular schedule; class 2 cabinets have a KI discus test and maintenance every 12 months; the automated platform has a service test every 12 months.

Monitoring of waste inactivation methods

Autoclaves serviced and calibrated every 12 months.
Safety cabinets and automated platform are inspected and serviced every 12 months

Emergency procedures - Is an emergency plan required? Provide details (or attach)

No, due to small scale of work and the low level of potential hazard.

Note: In the event of a reasonably foreseeable accident where the health and safety of people outside the premises is liable to be seriously affected or where there is a serious risk of damage to the environment then an emergency plan is required. This plan may need to be communicated to the emergency services and other relevant bodies. In most cases this will only be required for Class 3 and 4 projects (See ACGM Newsletter 27/Compendium of Guidance for further information). However, details of accident/spillage procedures should be provided for all projects.

Occupational Health issues

None. The material is GMO Class 1

Specify any requirements for immunisation, chemoprophylaxis or health monitoring, and any special requirements for record keeping

Environmental Considerations

ANSWERS MUST BE JUSTIFIED IN SOME DETAIL, i.e.- IT IS NOT ACCEPTABLE TO SIMPLY STATE THAT THERE IS NO RISK TO THE ENVIRONMENT.

Risk to animals, fish, plants etc

If the recipient microorganism is controlled by DEFRA, do you have a DEFRA licence? (delete as appropriate)	**No.**

Approval will not be granted until a copy of the DEFRA licence (if applicable) has been submitted to both the local GMSC and the Advisory Group for the Control of Biological Hazards

Identify any identifiable potential hazards to the environment, which might occur if the genetically modified organism were to be accidentally released. Classify the potential hazard as Severe, Medium, Low or Negligible.

Negligible

Note Potential hazards might be identified, and their severity assessed, dependent upon: the host species, the vector or the insert; or phenotypic changes caused by the genetic modification; the presence of host or susceptible species in the environment; the potential for survival, multiplication and dissemination in the environment; the stability of the GMO in the environment; the possibility of gene transfer to other species, etc. Refer to ACGM Compendium of guidance for further information

In view of the characteristics of the GMO, specify the likelihood of accidental release and occurrence of the above mentioned potential harmful effects, if the work were to be performed at the interim containment level specified above. Classify this as High, Medium, Low or Negligible.

The containment conditions specified should prevent release, therefore the risk is negligible.

Note: This includes the wider as well as the local environment in which the activity is to be carried out. Consideration should be given to any potential exposure of the environment to the GMMs and the magnitude and duration of such exposure. Refer to ACGM guidance for further information

Grade the overall Risk to the environment (= Potential harm x Likelihood) as High, Medium, Low or Effectively Zero.

Low

Additional Containment

If, in considering the potential for harm to the environment, you have concluded that the Risk to the environment is high or medium, then the containment conditions previously specified may need to be modified to reduce the risk to an acceptably low level. Use these considerations to revise your provisional containment level so that all risks are controlled to low or effectively zero.

Additional containment provisions for environmental protection

None

Assign your final containment level.

Containment Level 1 is sufficient for this work but to maintain quality , all work will be at Containment Level 2	
Are all hazards now controlled by this proposed level of containment?	Yes

Final classification of the activity, i.e.Class 1/2/3/4. Is the activity notifiable to HSE?

Class 1 Activity is not notifiable to the HSE.

Where the containment and control measures fall between two levels, e.g. where level 1 is appropriate with some control measures from level 2, the classification for the activity is equivalent to the HIGHER containment level. All Class 2,3 and 4 projects are notifiable to the Health and Safety Executive through the Health and Safety Unit

Do you intend to apply <u>all</u> control measures from your highest selected level of containment (See Annex 1)**? If not, please justify the exclusion of any control measures not used.**

Yes

Formal application to the Health and Safety Executive is required for derogation from the full containment level for all Class 2, 3 and 4 projects.

EC Regulation requires notification of transboundary movements of Class 3 GMMs to the Biological Clearing House and European Commission** ***(transboundary movements are those entering or leaving the EC)**. If your work involves Class 3 GMMs please indicate below whether they will be subject to transboundary movements.**

Not required.

Workers Involved in the Project and Facilities Used for the Work

Please indicate the areas where work will be carried out (including Room No. and Designation):	
Room No. and designation	**ACGM Categorisation**
Laboratories H20-H22, Centre for Biological Engineering, Holywell Park, Loughborough University	CL2 Facilities

Workers initially involved in work:	**Post/experience/training:**
Chandra	Documented in personal training file. Manual and automated cell culture expertise, worked in the CBE for 5 years.
Sebastian	Documented in personal training file. Manual cell culture expertise, worked in the CBE for 0.5 years.
Training and assessment of competence for existing and future personnel ***Specify arrangements for provision for existing and future personnel***	

Authorisation and Notification

The work proposed should be discussed with the Departmental Biological Safety Officer.

Signature of proposer A. Chandra **Date** 31 Mar 2015

Please print name A. Chandra

Other Signature (s) (if required – please state position) R. Bayley **Date** 31.03.15

Please print name RACHEL BAYLEY (CBE LOCAL REVIEWER)

Signature of Biological Safety Officer or authorised Deputy C. M. Moore **Date** 2.4.15

Please print name C.M. MOORE

NB The Approval of the University's relevant Safety Committee is required before work starts.

APPROVAL of the RELEVANT SAFETY COMMITTEE

On behalf of SC C. M. Moore **Approval Date** 2.4.15

ANNEX 1

TABLES OF CONTROL MEASURES AND CONTAINMENT LEVELS

The basic principles of classification are that you:

1. Determine the containment and control measures required by the risk assessment to control the risk of the activity;
2. Where this corresponds to a single containment level this will read across directly to give you the activity class, i.e. level 1 = class 1, level 2 = class 2, etc;
3. Where the measures identified correspond to measures from two different levels of containment the class corresponds to the higher of the two levels.

Further information can be found in the guide to the Contained Use Regulations and in the ACGM Compendium of guidance

***

Please consider the table(s) overleaf. Select the appropriate table for the work you are involved in. In most cases this will be **Table 1A (Laboratory Activities). Where your project involves the use of GMMs in plant growth facilities or animal facilities, you should consider Table 1B or 1C in conjunction with table 1A.** (In the final column of Tables 1B and 1C "additional" specifies use of that control measure in addition to the measures in Table 1A, while "modification" specifies that this measure shall be substituted for the relevant measure in Table 1A).

Large scale activities should be classified using **Table 2.**

Select your control measures. You should place a **X** in the appropriate box on each row to indicate whether that containment measure is required or not.

Determine the corresponding level of containment and hence the class of GMO. Where controls are selected from more than one containment level the Class corresponds to the higher of the containment levels.

FOR FURTHER INFORMATION PLEASE REFER TO ACGM NEWSLETTER 27 OR THE ACGM COMPENDIUM OF GUIDANCE

Please delete tables not relevant to your risk assessment. You may also delete this explanatory page from your final risk assessment

TABLES OF CONTAINMENT MEASURES

TABLE 1A: LABORATORY ACTIVITIES

TABLE 1B: PLANT GROWTH FACILITIES

TABLE 1C: ANIMAL FACILITIES

TABLE 2: OTHER ACTIVITIES (LARGE SCALE)

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TABLE 1A: LABORATORY ACTIVITIES

Containment measures	Containment level 1		Containment level 2		Containment level 3	
Laboratory suite - isolation	Not required	X	Not required		Required	
Laboratory - sealable for fumigation	Not required	X	Not required		Required	
Equipment						
Impervious/easy to clean surfaces	Required for bench	X	Required for bench		Required for bench and floor	
Entry to lab via air lock	Not required	X	Not required		Required where and to the extent the risk assessment shows it is required	
Negative pressure relative to the pressure of the immediate surroundings	Not required	X	Required where and to the extent the risk assessment shows it is required		Required	
Extract and input air in laboratory should be HEPA filtered	Not required	X	Not required		HEPA filters required for extract air	
Use of microbiological safety cabinet/enclosure	Not required	X	Required where and to the extent the risk assessment shows it is required		Required and all procedures with infective materials required to be contained within cabinet/enclosure	
Autoclave	Required on site	X	Required in the building		Required in the laboratory suite	
System of work						
Access restricted to authorised personnel only	Not required	X	Required		Required	
Specific measures to control aerosol dissemination	Not required	X	Required so as to minimise		Required so as to prevent	
Shower	Not required	X	Not required		Required where and to the extent the risk assessment shows it is required	
Protective clothing	Suitable protective clothing required	X	Suitable protective clothing required		Suitable protective clothing required; Footwear required where and to the extent the risk assessment shows it is required	
Gloves	Not required	X	Required where and to the extent the risk assessment shows it is required		Required	
Efficient control of disease vectors (eg for rodents and insects) which could disseminate GMMs	Required where and to the extent the risk assessment shows it is required	X	Required		Required	
Specified disinfection procedures in place	Required where and to the extent the risk assessment shows it is required	X	Required		Required	

	Containment level 1		Containment level 2		Containment level 3	
Waste						
Inactivation of GMMs in effluent from handwash sinks and showers and similar effluents	Not required	X	Not required		Required where and to the extent the risk assessment shows it is required	
Inactivation of GMMs in contaminated material and waste	Required by validated means	X	Required by validated means		Required by validated means with waste inactivated in lab. suite	
Other measures						
Laboratory to contain own equipment	Not required	X	Not required		Required, so far as is reasonably practicable	
An observation window or alternative to be present so that occupants of lab can be seen	Required where and to the extent the risk assessment shows it is required	X	Required where and to the extent the risk assessment shows it is required		Required	
Safe storage/transport of GMMs	Required where and to the extent the risk assessment shows it is required	X	Required		Required	
Written records of staff training	Not required	X	Required where and to the extent the risk assessment shows it is required		Required	

HIGHEST LEVEL OF CONTAINMENT SELECTED ABOVE: 1

CORRESPONDING CLASS OF GMM: 1

TABLE 1B: PLANT GROWTH FACILITIES

Containment measures	Containment level 1	Containment level 2	Containment level 3	Additional/Modification
Building				
Permanent structure*	Required where and to the extent the risk assessment shows it is required	Required	Required	Modification
Equipment				
Entry via a separated room with two interlocking doors	Not required	Required where and to the extent the risk assessment shows it is required	Required where and to the extent the risk assessment shows it is required	Additional
Control of contaminated run off water	Required where and to the extent the risk assessment shows it is required	Required so as to minimise run off	Required so as to prevent run off	Additional
System of Work				
Effective control of disease vectors such as insects, rodents, arthropods which could disseminate GMMs	Required	Required	Required	Additional
Effective control of pollen, seeds and other plant material which could disseminate GMMs	Required where and to the extent the risk assessment shows it is required	Required so as to minimise dissemination	Required so as to prevent dissemination	Additional
Procedures for the transfer of living material between plant growth facilities, protective structure and laboratory shall control dissemination of GMMs	Required so as to minimise dissemination	Required so as to prevent dissemination	Required so as to prevent dissemination	Additional

*A permanent structure refers to a fixed structure with walls, roof and floor. Where the structure is a greenhouse, that structure shall also have a continuous waterproof covering and self closing, lockable doors, and be located on a site designed to prevent the entry of surface run off water.

TABLE 1C: CONTAINMENT MEASURES FOR ACTIVITIES IN ANIMALS UNITS

Containment measures	Containment level 1	Containment level 2	Containment level 3	Additional/Modification
Facilities				
Isolation of animal unit (Note 1)	Required where and to the extent the risk assessment shows it is required	Required	Required	Modification
Animal facilities(Note 2) separated by lockable doors	Required where and to the extent the risk assessment shows it is required	Required	Required	Additional
Animal facilities (cages etc) designed to facilitate decontamination (waterproof and easily washable material)	Required where and to the extent the risk assessment shows it is required	Required where and to the extent the risk assessment shows it is required	Required	Additional
Floor and/or walls and ceiling easily washable	Required where and to the extent the risk assessment shows it is required	Required for floor	Required for floor and walls	Modification
Appropriate filters on isolators or isolated rooms (Note 3)	Not required	Required where and to the extent the risk assessment shows it is required	Required	Additional
Incinerator for disposal of animal carcasses	Required to be accessible	Required to be accessible	Required to be accessible	Additional
Appropriate barriers at the room exit, and at drains or ventilation duct work	Required	Required	Required	Additional
Animals kept in appropriate containment facilities such as cages, pens or tanks but not isolators	Required where and to the extent the risk assessment shows it is required	Required where and to the extent the risk assessment shows it is required	Required where and to the extent the risk assessment shows it is required	Additional
Animals kept in isolators	Required where and to the extent the risk assessment shows it is required	Required where and to the extent the risk assessment shows it is required	Required	Additional

Note 1: In the table, "Animal Unit" means a building or separate area within a building, containing an animals facility and other areas such as changing rooms, showers, autoclaves, food storage areas etc.
Note 2: In the table, "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.
Note 3: "Isolators" means transparent boxes where small animals are contained within or outside a cage; for large animals, isolated rooms may be appropriate.

TABLE 2: CONTAINMENT MEASURES FOR ACTIVITIES INVOLVING LARGE SCALE WORK

Containment measures	Containment level 1	Containment level 2	Containment level 3
General			
Visible micro-organisms should be contained in a system which separates the process from the workplace and wider environment (closed system)	Required where and to the extent the risk assessment shows it is required	Required	Required
Closed systems located within a controlled area	Not required	Required where and to the extent the risk assessment shows it is required	Required
Control of exhaust gases from the closed system	Not required	Required so as to minimise release	Required so as to prevent release
Control of aerosols during sample collection, addition of material to a closed system or transfer of material to another closed system	Required where and to the extent the risk assessment shows it is required	Required so as to minimise release	Required so as to prevent release
Inactivation of bulk culture fluids before removal from the closed system	Required where and to the extent the risk assessment shows it is required	Required by validated means	Required by validated means
Seals should be designed so as to minimise or prevent release	Not required	Required so as to minimise release	Required so as to prevent release
Controlled area designed to contain spillage of the entire contents of the closed system	Required where and to the extent the risk assessment shows it is required	Required where and to the extent the risk assessment shows it is required	Required
Controlled area sealable to permit fumigation	Not required	Required where and to the extent the risk assessment shows it is required	Required where and to the extent the risk assessment shows it is required
Biohazard signs posted	Required where and to the extent the risk assessment shows it is required	Required	Required
Equipment			
Entry via airlock	Not required	Not required	Required where and to the extent the risk assessment shows it is required
Surfaces resistant to water, acids, alkalis, solvents, disinfectants, decontamination agents and easy to clean	Required for any bench	Required for any bench	Required for bench and floor
Specific measures to adequately ventilate the controlled areas in order to minimise air contamination	Required where and to the extent the risk assessment shows it is required	Required where and to the extent the risk assessment shows it is required	Required where and to the extent the risk assessment shows it is required

Containment measures	Containment level 1	Containment level 2	Containment level 3
Equipment (continued)			
Controlled area maintained at an air pressure negative to the immediate surroundings	Not required	Not required	Required where and to the extent the risk assessment shows it is required
Extract and input air from the controlled area should be HEPA filtered	Not required	Not required	Required where and to the extent the risk assessment shows it is required
System of work			
Access restricted to nominated personnel only	Not required	Required	Required
Decontamination and washing facilities provided for personnel	Required	Required	Required
Personnel should shower before leaving the controlled area	Not required	Not required	Required where and to the extent the risk assessment shows it is required
Personnel should wear protective clothing	Work clothing required	Work clothing required	Required
Written procedures and records of staff training	Not required	Not required	Required
Waste			
Inactivation of GMMs in contaminated material and waste including those in process effluent before final discharge	Required by validated means	Required by validated means	Required by validated means
Inactivation of GMMs in effluent from handwashing sinks and showers or similar effluents	Not required	Not required	Required where and to the extent the risk assessment shows it is required

HIGHEST LEVEL OF CONTAINMENT SELECTED:

CLASS OF GMM: