RISK ASSESSMENT REVIEW/REVISION RECORD

Risk Assessment Ref No:	CBE/BRA/018 CBE/BREGMO/018.	Version Number 1.0

This risk assessment should be reviewed **annually** or more frequently if there is any change in the work, or if new information becomes available that indicates the assessment may no longer be valid. **This form should be attached to the front of the current version of the risk assessment or to the new version of the risk assessment if one is issued**

The following review/revision has been carried out on the dates indicated and either the assessment remains valid or it has been amended as indicated.	
Name(s) of reviewer: Andrew Want	Date:17th February 2012
Signature:	

Amendments:

Minor Revision: Addition of Andrew Picken to the hESC culture biological risk assessment allowing him to undergo training in procedures such as feeding, passaging, cryopreservation, and flow cytometric analysis of hESCs. This will also cover ancillary processes such as preparing samples for karyotyping and aliquotting of Matrigel and preparation of Matrigel flasks.

There are no changes in the nature of the work or the hazards involved, so this risk assessment is still relevant to the work activity.

Centre for Biological Engineering

RISK ASSESSMENT REVIEW/REVISION RECORD

Risk Assessment Ref No:	CBE/BRA/018 CBE/GMO/018	Version Number 1

This risk assessment should be reviewed **annually** or more frequently if there is any change in the work, or if new information becomes available that indicates the assessment may no longer be valid. **This form should be attached to the front of the current version of the risk assessment or to the new version of the risk assessment if one is issued**

The following review/revision has been carried out on the dates indicated and either the assessment remains valid or it has been amended as indicated.	
Name(s) of reviewer: K. Brosnan	Date: 12/04/2010
Signature: K Brosnan	

Amendments:

The above risk assessment has been reviewed to add K. Brosnan to the project. The work will continue to take place solely in the class II laboratory, H25 and the volumes of culture and materials used will not exceed those stated within the risk assessment. As there are no changes to the biological or other (eg. chemical) hazards or the nature of the work, this risk assessment is still relevant to the work activity.

This review or revision must be approved by the person supervising the work and the CBE Quality Manager. Significant changes may require a revised version of the risk assessment to be issued for re-approval by the local BGMSA and/ or the BSO and/or GM Safety Committee, as appropriate.

Name of Approver: P Hourd	Date: 12/04/2010
Position: CBE QM	
Signature: P Hourd	
Name of Approver:	Date:
Position:	
Signature:	

Centre for Biological Engineering

RISK ASSESSMENT REVIEW/REVISION RECORD

Risk Assessment Ref No:	CBE/BRA/018 CBE/GMO/018	Version Number 1.0

This risk assessment should be reviewed **annually** or more frequently if there is any change in the work, or if new information becomes available that indicates the assessment may no longer be valid. **This form should be attached to the front of the current version of the risk assessment or to the new version of the risk assessment if one is issued**

The following review/revision has been carried out on the dates indicated and either the assessment remains valid or it has been amended as indicated.	
Name(s) of reviewer: Andrew Want	Date: 1st March 2010
Signature: AJW.	
Amendments: Revision Number 1 - Minor revision: The source of the MEF cells has changed, since writing of the risk assessment. These cells will now be obtained from R&D Systems. They are still classified as Hazard Group 1, and present minimal risk to the operator given the controls already in place.	

This review or revision must be approved by the person supervising the work and the CBE Quality Manager. Significant changes may require a revised version of the risk assessment to be issued for re-approval by the local BGMSA and/ or the BSO and/or GM Safety Committee, as appropriate.	
Name of Approver: Chris Hewitt	Date: 1/4/10.
Position: Supervisor	
Signature: CJ Hewitt.	
Name of Approver: Paul Hourd	Date: 03/03/10
Position: CBE Quality Manager	
Signature: P Hourd	
Name of Approver: C.J. Hewitt	Date: 1/4/10.
Position: Biological Safety Office	

CBE | BRA | 18

What hazard gp is the biological work. - See pg 5.
organism
- Should this be stated on the front cover

Loughborough University

For Health and Safety Unit Use only ASSESSMENT NO.

RISK ASSESSMENT OF WORK WITH BIOLOGICAL MATERIALS

Please note the following before completing this form:

1. University Health and Safety Policy requires that risk assessment of all work with biological materials 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 that may contain biological agents.
2. YOU SHOULD COMPLETE ALL OF PART A, THE APPROPRIATE SECTION(S) OF PART B, AND ALL OF PART C. THIS FORM SHOULD BE SUBMITTED TO THE HEALTH AND SAFETY UNIT FOR REVIEW (VIA YOUR DEPARTMENTAL SAFETY OFFICER).
3. It is the responsibility of the Principal Investigator 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:	4th December 2009	Date Approved:	10/1/10

PART A: Please provide the following general information:

School/Department

Chemical Engineering/CBE

The Project

Title of Project:
Developing scalable and standardised manufacturing methods for human pluripotent stem cells

Project Reference Number: BB/G010404/1

Person responsible for this work (*Principle Investigator*):

Name: Chris Hewitt	Position: Professor
Department: Chemical Engineering	University Faculty: Engineering

Person conducting this assessment

Name: Andrew Want		Position: Researcher	
Department:	Chemical Engineering	Date Risk Assessment Undertaken:	4th December 2009
Proposed Project Start Date:	December 15th 2009	Proposed Project End Date:	October 1st 2012

Assessment Review:
required at least once a year or immediately following any significant change to the project

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

A1 PROJECT SUMMARY

A1.1 Scientific Goals of the Project *Brief yet clear outline only*

Human embryonic stem cells (hESCs) and induced pluripotency stem cells (iPSCs) are a major emerging platform for a wide range of therapeutic cell-based products but their scale-up is a major barrier to commercial-scale production. This project aims to improve our understanding and the reliability of cell expansion for these cells by: 1) investigating the properties of several highly characterised cell lines that influence their processing and scale-up 2) optimise and validate protocols in stirred tank reactors and carry out further investigation of transfer of culture to automated cell culture platform (CompacT Select). Overall this work will identify and deliver a generic process that can be used to reproducibly produce human embryonic stem cells and iPSCs at a commercially relevant scale.

A1.2 Description of the Experimental Procedures

Describe laboratory procedures to be used and highlight any non-standard laboratory operations

1) Manual culture of hESC and iPSC lines – details pertaining to each cell line will be described in the relevant SOPs but briefly the standard protocol involves seeding cells in Matrigel-coated T-flasks in MEF (mouse-embryonic fibroblast) pre-conditioned media. Cells are cultured at 37°C, 5% CO_2 in a humidified, static incubator until confluent, with complete media changes taking place daily. Cells may be cultured in a variety of different media and passaged by a number of different ways including use of trypsin-EDTA, TrypLE Express and Accutase. It is the intention that during this project, alternative medium formulations will be used which do not entail MEF culture as detailed below. SOP087 & SOP091
2) Manual culture of MEFs and generation of conditioned media – details will be described in the relevant SOP but briefly MEFs are seeded into T-flasks and cultured, mitotically inactivated using mitomycin C and returned to the humidified, static incubator (37°C, 5% CO_2). Unconditioned medium is added daily, removed after 24 hours and stored at -80°C for a maximum of 3 months. SOP091
3) Cell counting – details described in SOP034 "Viable Cell Count Assessment Using Haemocytometer."
4) Cell Culture on Microcarriers -
    - In 24 well plates, static
    - In 24 well plates, shaken
    - In 100ml spinner flasks
5) Generation of embryoid bodies SOP092
6) Sample taking- samples will be taken from the various cultures at different stages for a number of different assays such as:
    - Spent media collected in universal bottles (maximum sample volume 5 mL) and run through Bioanalyser
    - Head space gas collected (to be run through SIFT-MS at Keele)
    - Cell-microcarriers to be collected for cell counts, direct microscopic observation
    - Samples for flow cytometry
    - Samples for fluorescent microscope

All procedures will be conducted in accordance with the laboratory Quality Management System requirements, Good Cell Culture Practice, Good Aseptic Technique, the local Code of Practice and the University Biological Safety Policy. All SOP's available (authorised access only) for review at:
https://internal.lboro.ac.uk/restricted/wolfson/CBE_SOP/5_SOPs/SOPs.html.htm

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 AND 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?
HUES1, 7, 8, 9 (Continuous)	Blastocyst	Human	University of Nottingham
H1, 9 (Continuous)	Blastocyst	Human	University of Nottingham
NOTT1, 2 (Continuous)	Blastocyst	human	University of Nottingham
NOTT-iPS1, 2, 3 (Continuous)	Skin	human	University of Nottingham
MEFs (Primary)	Skin	Mouse	University of Nottingham

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

Indicate in the adjacent box if Not Relevant (N/R)			
Material type and ID	Organ Source	Species	From where will it be obtained?
Foetal Bovine Serum	Blood	Cow	Established suppliers who source from accredited herds.

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 Details: hESC lines HUES7 and NOTT1 transduced with GFP using a lentiviral vector.	

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

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If Yes, provide details of the types of screening and agents screened for: Cells are obtained from the University of Nottingham solely for internal academic research purposes via reputable cell-line suppliers (UK National Stem Cell Bank/Cell-Line originators who have registered the lines with the NIH). The material is experimental in nature and may have hazardous properties since not all of its characteristics are known however, the donors are screened for HepB, HepC and HIV at the time of donation, and the lines are then subsequently tested for mycoplasma infection. The material has been grown in culture over a prolonged period prior to receipt at Loughborough, during which time the existence of any infectious agents would have been observed.	

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:
If yes, will a policy of rejection of samples from diseased patients be adopted? Explain
If yes, how will the information be disseminated in the course of the project?
If yes, will this information be anonomised?

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)	N/R
If Yes, summarise here:	

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 Embryonic Stem Cell H1, H9 HUES1, 7, 8, 9 NOTT1, 2 Human Induced Pluripotency Stem Cell NOTT-iPS1, 2, 3	low	All well authenticated continuous cell lines obtained from the University of Nottingham. The cells are not fully characterised but have been utilised extensively in peer-reviewed academic research. The cells are sourced from a leading academic laboratory that has successfully deposited cells to the UK Stem cell bank. Since these cell lines have been subjected to extensive sub-culture the risk of pathogenic agent contamination is very low. Categorised as Hazard Group 2 requiring baseline containment level 2.
Mouse Embryonic Fibroblast	low	Not fully characterised primary cell line of animal origin obtained from university of Nottingham, as described in section B2.1.4. Categorised as Hazard group 1 requiring baseline containment level 2.
		If low risk or 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 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

***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 routes of infection (in humans) for these adventitious agents (place a 'X' in the relevant box)

Percutaneous	Mucocutaneous	Inhalation	Ingestion	N/R
Details:				

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

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes
If Yes, describe: Human embryonic stem cells, due to their pluripotent nature, carry the risk of generating teratomas. However, this risk is negligible in individuals with a functioning immune system.	

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)	yes
If yes, Occupational Health must be consulted: See B2.2.4. Immunocompromised individuals will be forbidden from undertaking this work. All workers will be registered with Occupational Health for monitoring.	

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: All hESC and iPSC will be cultured manually as described in section A1.2 in T-flasks with liquid cell culture medium at 37°C, 5% CO_2 in a humidified, static incubator (SOP087). In addition, these will also be grown on microcarriers in multiwell plates (again in static incubator), 100 mL spinner flasks (SOP084). MEFs will only be cultured manually as described in section A1.2 in T-flasks with liquid cell culture medium at 37°C, 5% CO_2 in a humidified, static incubator.	

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 100 mL	Per experiment 1000 mL

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)	no
If yes, explain:	

B2.5 WORKING WITH MATERIAL DONATED BY YOURSELF OR COLLEAGUES - Persons MUST NOT work with their own cells.

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, cryogenic gases ionising radiation.

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	yes
If yes, identify these: 1) cryogenic processing with liquid nitrogen 2) generic and specific hazardous chemicals e.g. Mitomycin C 3) use of flow cytometer (non-ionising radiation source (laser))	
If yes, have these been risk assessed and any necessary approval obtained? 1) Liquid nitrogen – procedures will be carried out by trained personnel in accordance with SOPs 013, 031, 032. Risk Assessment Reference Number: CBE/007 2) All hazardous non-biological materials used in this project are subjected to COSHH assessment. 3) Use of the Altra Epics or Quanta flow cytometer – procedure will be carried out by trained personnel in accordance with SOPs 081 and 046.	

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:

All hESC and iPSC lines- no substitute is available. Use of these specific cells is critical to the value of the research. These cells are from reputable, and reliable, sources, University of Nottingham/UK Stem Cell Bank/Cell line originators.

MEFs- no substitute available. Use of these specific cells is critical to the growth and maintenance of the hES and iPS cultures. These cells are from a reputable, and reliable, laboratory at the University of Nottingham.
Matrigel – No substitute is available, this material is currently critical to the value of the research. Supplied by Becton Dickinson who test the material extensively for contamination and function, prior to lot release.

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:

Access to the Containment level 2 CBE lab unit is restricted to authorised workers with appropriate training in accordance with documented local Code of Practice and Quality Management System requirements for containment level 2 activities involving biological material.

Outside of normal working hours the laboratories are locked to ensure safe storage of biological agents and unauthorised entry. Keys are only issued to authorised users who have been granted out of hours access following risk assessment of their intended work.

There is no access to the laboratories by any cleaning or maintenance staff at any time unless a specific permit to work has been granted.

(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 restricted to those with documented training (training files held in CBE Office, H07) in accordance with the local Code of Practice and Quality Management System requirements.

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)	yes

If yes, list the sharps:

The sharps used that may cause damage to the skin include glass microscope slides and cover slips.

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

It is local practice in the CBE laboratory unit that the use of sharps is avoided wherever possible, with glass items replaced with plastic alternatives. However, the above sharps are essential for microscopy work (according to SOP033; "Use and Maintenance of Haemocytometer" and SOP080; "Use and Maintenance of Nikon TS100 Inverted Phase-Contrast Microscope.

If yes, describe there use and disposal:

Used sharps are placed directly into a sharps containers conforming to BS 7320. Sharps bins are removed when three quarters full and contents rendered safe by autoclaving prior to their removal from site.

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

Accident procedures for sharps and glass injuries are displayed in posters in all labs within the Unit

C1.2.2 Containment and Ventilation

(i) Is the use of BSC required for the protection of the worker ie 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:

A Class II Biological Safety Cabinet will be used for all manipulations that may produce aerosols or splashes but is primarily used to ensure protection of research materials as part of a quality assurance discipline. Procedures to be carried according to the following SOPs:

1) SOP009, "Use and Maintenance of HERASAFE KS Class II BSC"

(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)	no

If yes, specify:

C1.2.3 Transport and Storage within the laboratory

How and where are materials to be stored?

Material will be stored in a cryobank or temporary storage in designated cell culture incubators according to the following SOPs :

1) SOP005, "Storage and Transport of Biological Materials"
2) SOP008, "Receipt of Hazardous Biological Material"
3) SOP013, "Use and Maintenance of Liquid Nitrogen Stores"
4) SOP079, "Use and Maintenance of the Heracell Incubator"
5) SOP031, "Cryopreservation and Storage of Mammalian Cell Lines"

Storage units are located in Laboratories H22, H23 and H25 of the CBE Laboratory Unit

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.

Cells will always be transferred in closed containers. Appropriate spill response procedures are posted in the lab and documented in detail in the following SOPs:

1) SOP005, "Storage and Transport of Biological Material"
2) SOP038, "Biological Spill Response"

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

Transfer outside the CBE Laboratory Unit is not anticipated but any requirement is likely to be constrained within the University site. All transport will be subject to controlled procedures according to the local code of practise and SOP005 (see below). For example, if necessary, transfers will use double containment procedures Transport of research material between laboratories is done using sealed containers which are put into tube racks and trays and transported using trolleys according to the following SOPs. Waste potentially containing viable agents is not removed from the laboratories until it has been autoclaved.

1) SOP003, "Disposal of Biological Waste"
2) SOP005, "Storage and Transport of Biological Material"
3) SOP038, "Biological Spill Response"

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): Any Biological material to be shipped outside the CBE will be packaged and sent in accordance with SOP 005 "Storage and Transport of Biological Material"

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?

The material listed in B2.1.1 will be shipped from the University of Nottingham/UK Stem Cell Bank/Cell-line originators, according to their own procedures. The procedure for the safe receipt of packages containing potentially biohazardous material and their delivery to the appropriate recipient or other designated personnel is documented in SOP008; "Receipt of Hazardous Biological Material". This SOP is intended to minimize the consequences that could result from the failure of packaging methods and materials used to ship biohazardous materials.

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?

Sealed buckets will be opened within the Containment Level 2 (CL2) Laboratory Unit, unless there is evidence of a potential spillage, in which case the sealed buckets will be opened in the BSC (SOP009, "Use and Maintenance of HERASAFE KS Class II BSC"
The centrifuge is operated and maintained according to the following SOPs:

1) SOP088, "Use and maintenance of Eppendorf 5804 Centrifuge"
2) SOP038, "Biological Spill Response"
3) SOP089, "Use and Maintenance of the Sartorius-Stedim Centrisart A-14 Microcentrifuge"

(iii)Describe the procedures in place to deal with leaks and spillages in the centrifuge

Procedures to prevent, contain and respond to leakages and spillages in the centrifuge are detailed in the following SOPs:

1) SOP088, "Use and Maintenance of Eppendorf 5804 Centrifuge"
2) SOP038, "Biological Spill Response"
3) SOP089, "Use and Maintenance of the Sartorius-Stedim Centrisart A-14 Microcentrifuge"

Labelled Biological Spill kits are located in each laboratory within the CBE Laboratory Unit. Signs are posted throughout the Laboratory Unit to enable workers to locate the nearest biological (and chemical) spill kits. Posters are also displayed in each laboratory where a centrifuge is located to advise on spill response and reporting procedures.

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.

Static incubators are used. Procedures to prevent, contain and respond to spillages in the incubators are detailed in the following SOPs:

1) SOP079, "Use and Maintenance of the HeraCell Incubator"

C1.2.9 Disinfection

Specify the type and concentration of disinfectants to be used:

The disinfectants were carefully chosen for effectiveness in use. The number of disinfectants in use is strictly limited to avoid errors and ambiguities in use and accidental mixing of compounds that may give rise to hazardous reactions or the formation of toxic products. Unless there are compelling reasons to do otherwise, Virkon (1% w/v) is the sole disinfectant used in the laboratories other than 70% IMS which is used for general disinfection cleaning (SOP004) where Virkon cannot be used; for example stainless steel surfaces.

Virkon has a wide range of bactericidal, virucidal, fungicidal and sporocidal activities. Representative viruses from all the major virus families are inactivated by Virkon. Working solutions of 1% w/v have low toxicity and no irritancy. Selection and procedures detailed in the following SOPs:

1) SOP004, "General Laboratory Housekeeping"
2) SOP006, "Selection and Use of Virkon Disinfectant"
3) SOP039, "Storage, Handling and Disposal of Chemicals"

COSHH Risk Assessment reference for Virkon SAF/MM/1745

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

Is this sufficient

If yes, describe the procedure:

For Hazard Group 1 and 2 Biological agents it is normally be sufficient to rely on the manufacturers data, providing the recommended concentrations and contact times are used. Hence Virkon (1%) is used as per manufacturers instruction and according to standard procedures detailed in the COP and the following SOP:

1) SOP006, "Selection and Use of Virkon Disinfectant"

C1.2.10 Personal Protective Equipment (PPE)

(i) What type of lab coats will be worn and where will they be stored?

Side fastening Howie type lab coats are worn. They are stored outside the laboratory in purposely designed change rooms. Proper use of PPE is described in the following SOP: SOP037, "Use of Personal Protective Equipment (PPE)"

(ii) What type of gloves will be worn and where will they be stored?

1. Autoclave gloves, which will be stored in close proximity to the autoclave equipment in the Autoclave Room (H31) and the Automated Cell Culture Suite (H21/H22) and animal cell lab (H25)
2. Cryogenic gloves, which will stored in close proximity to the Liquid Nitrogen storage containers located in Gas Pod 3, Analytical Lab (H23)
3. Nitrile gloves for general use, which will be stored in the change rooms and point of entry to each laboratory within the CBE Laboratory Unit.

Correct use of PPE is described in SOP037, "Use of Personal Protective Equipment (PPE)"

(iii) Describe any other PPE to be used:

1. Laboratory safety glasses (including those for spectacle wearers)
2. Face Shields (primarily for handling liquid nitrogen)
3. Shoe covers, in case of a spillage
4. Aprons or disposable lab coats for extra protection over laboratory coat.

Correct use of the above PPE is described in SOP037, "Use of Personal Protective Equipment (PPE)"

C1.2.11 Hygiene Measures

Describe the hygiene facilities available and where they are located

1. Designated hand washing facilities are located in each laboratory change room and in the Analytical Laboratory (H23).
2. Eye Wash stations are located next to each 'hand washing only' sink in each laboratory change room and in the Analytical Laboratory (H23).

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?

	Treatment before disposal	Validation

how is this to be done?

Liquid waste	Virkon sterilise (SOP003 – Disposal of biological waste)	According to manufacturers instructions; see section C2.1.9
Solid waste	Autoclave sterilise (SOP003 – disposal and disinfection of biological waste)	Treatment cycle validated according to SOP024 & SOP025, "Use and Maintenance of the Systec VX95 Autoclave"; No CBE044 and No CBE045

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	*Autoclave cycle (temp, cycle time)*	*Treatment monitor*
Liquid waste	None		
Solid waste	Cell Culture consumables e.g pipette tips and flasks.	121°C for 15 minutes	Designated Autoclave tape monitors

Location of autoclave	Servicing details	Location of back-up autoclave	Designated area for storage of unsterilised waste
Autoclave Room H31	Annual	CBE Room H31 & H22	In secure cage within the Autoclave Room (H31)

C1.2.15 Liquid Waste Disposal

How will liquid waste be disposed of?
To the drain? With copious amounts of water in accordance with SOP003 – " Disposal of biological waste"?
As solid waste? Fill in this section.
Other?

C1.2.16 Solid Waste Disposal

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

European Waste Catalogue Code	Categorisation		Disposal Method
		Hatch relevant box(es)	
18 01 01	Sharps		Sharps bin>autoclave sterilisation if known or potentially infected >clinical waste disposal (incineration)
18 01 02 [human]	Human body parts, organs, including blood bags and blood preserves and excreta (unless identified as medium or high risk or known infected in Section 2.16 of this RA in which case they must be pre-treated before disposal and classified 18 01 04 [sealed bins])		Rigid one way sealed tissue bins>incineration only

18 01 02 [animal]	Animal body carcases or recognisable parts ((unless identified as medium or high risk or known infected in Section 2.16 of this RA in which case they must be pre-treated before disposal and classified 18 01 04 [sealed bins])		Rigid one way sealed tissue bins > incineration only
18 01 03	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 preference and is subject to special requirements
18 01 04 [bags]	Infected or potentially infected lab wastes that have been pre treated before leaving the site		Disinfection or sterilisation (as identified in C1.2.13) in the lab suite > placement in yellow clinical waste bags > clinical waste disposal (incineration)
18 01 04 [sealed bins]	Infected or potentially infected animal or human body parts, organs or excreta that have been pre treated before leaving site		Disinfection or sterilisation (as identified in C1.2.13) in the lab suite > placement in yellow one way sealed tissue bins > 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)	N/R
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 fermenter be used to culture a pathogen?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If yes, describe the size, and type of the 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:

Procedures for dealing with small and large spillages are detailed in the following SOPs:

1) SOP006, "Selection and use of Virkon Disinfectant"
2) SOP009, "Use and Maintenance of HERASAFE KS Class II BSC"
3) SOP038, "Biological Spill Response"
4) SOP052, "Use and Maintenance of Bioquell Advanced Microflow Biosafety Cabinet"

Labelled Biological Spill kits are located in each laboratory within the CBE Laboratory Unit. Signs are posted throughout the Laboratory Unit to enable workers to locate the nearest biological (and chemical) spill kits.Posters are also displayed in each laboratory within the Unit where a BSC is located to advise on spill response (inside the BSC) and reporting procedures.

Within the laboratory but outside the control measure e.g. BSC, spill tray

Procedures for dealing with small and large spillages are detailed in the following SOPs:

1) SOP006, "Selection and use of Virkon Disinfectant"
2) SOP038, "Biological Spill Response"

Labelled Biological Spill kits are located in each laboratory within the CBE Laboratory Unit. Signs are posted throughout the Laboratory Unit to enable workers to locate the nearest biological (and chemical) spill kits. A biological spill kit is available in Goods Inwards (Wolfson School). Posters are also displayed in each laboratory within the Unit to advise on spill response (outside the BSC) and reporting procedures.

Outside the laboratory e.g. during transport

Procedures for dealing with small and large spillages are detailed in the COP and the following SOPs:

1) SOP005, "Storage and Transport of Biological Material"
2) SOP006, "Selection and use of Virkon Disinfectant"
3) SOP038, "Biological Spill Response

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)

1. Procedures to respond to accidental exposure are detailed in SOP038, "Biological Spill Response" and the local COP. These are detailed in spill response posters located in each laboratory within the Unit. Accident procedures in the case of glass or sharps injury are described in the local COP and displayed in posters located in each laboratory within the Unit
2. Designated hand washing facilities are located in each laboratory change room and in the Analytical Laboratory (H23).
3. Eye Wash stations are located next to each 'hand washing only' sink in each laboratory change room and in the Analytical Laboratory (H23).
4. A First Aid Kit is located outside the Laboratory Unit. Signs are posted throughout the Laboratory Unit to enable workers to locate the nearest Medical Kit. Contact details for First Aiders are posted in each laboratory within the Unit
5. Essential and Emergency Contact details are posted in each laboratory within the Unit.

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 presence 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?

All procedures will be carried out under Containment level 2 (CL2) within the CL2 CBE Laboratory Unit. The work activities within this project involve biological agents (BAs) assessed as both Hazard Group 1 and Hazard Group 2. Work with the lower Hazard Group will be carried out under the management standards imposed by the higher level (Containment level 2). This applies under circumstances in which the project is divided into several elements that may be under way in the CBE Laboratory Unit simultaneously. This project, involving the use of Hazard Group 1 BAs that require Containment Level 1 are carried out at Containment Level 2 for reasons other than worker protection; this includes the need to ensure research material protection (e.g. the use of a class II safety cabinet) and to impose a quality assurance discipline

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

None

C3 FACILITIES

C3.1 Where will this work take place?

Room(s)	Building	Campus	Person in Control of area
CBE Laboratory Unit (*self contained suite of laboratories and ancillary rooms within the CBE*)	Centre for Biological Engineering	Holywell Park, Loughborough University	Carolyn Thomas Bob Temple Chris Hewitt

C4 PERSONNEL

C4.1 Names of Personnel involved in the Project

Surname	Initials	ID	Position
Want	AJ	5013324	Post-Doctoral Research Associate
Ramasamy	G	A911624	Doctoral Student

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.

Andrew Want will be performing the bulk of the work in the first instance, having received training in culture methods from collaborators at University of Nottingham.
Gaya Ramasamy will be supervised closely, at all times until competence is determined with the techniques, after which her training file will be updated accordingly.
Formal records of training are kept for all workers authorised to work at Containment Level 2 (CL2) within the CBE CL2 Laboratory Unit. Instruction against local Code of Practice and QMS ie SOPs is provided.
Each individual will record that they have read and understood this risk assessment and the associated GMO risk assessment.

C4.3 Relevant Experience/Training:

Surname	Experience/Training
Want	Documented in Personal Training File
Ramasamy	Documented in Personal Training File

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

Details:

NONE: Cleaners and Maintenance workers are not authorised to enter the laboratory. All laboratory cleaning is undertaken by authorised personnel (ie CBE staff). Access for non-laboratory workers is subject to a local permit-to-work procedures. If access is needed for essential maintenance of equipment for example a clean down and decontamination of the laboratories will be performed. This will be documented with decontamination certificates and the maintenance worker fully supervised according to SOP004 " General Laboratory Houskeeping" and the local Code of Practice Two laboratory shut downs occur every year for a week for maintenance work to be done in the CBE Laboratory Unit. Prior to these shut down weeks a full deep clean decontamination will be performed in the all laboratory areas.

All other workers in the CBE Laboratory Unit are authorised personnel.

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 if required. All workers involved with handling unscreened blood, blood products and other tissues are recommended to have Hepatitis B immunization

Certificate for Hepatitis B immunization documented in personal training file.

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).

None required

C6. NOTIFICATIONS- Human Tissue Act

C6.1.1 Relevant material covered by the Human Tissue Act

Are any of the cell, 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

Why ?

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)	Yes (attached)
Approval number: SCSC09-43	
Date obtained: 21/09/2009	Ethics committee name: MRC Steering committee for the UK Stem Cell Bank

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)	Yes
If Yes, give details: The HSE has been notified of the intention to use a Class I Genetically Modified Organism at the CBE.	

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
NOTE: The regulations covering the import of animal products (including tissue cultures, tissues, body fluids or fractions thereof) are in a state of flux. See the DEFRA website for details. UNLESS THIS SECTION IS NOT RELEVANT (N/R) ie THE INTENDED WORK DOES NOT USE ANIMAL PRODUCTS - CONSULT THE LU H&S OFFICE TO REVIEW APPLICATION REQUIREMENTS BEFORE ANY SUBMISSIONS	

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

- accept that for some Containment Level 2 and all CL3 activities **a statutory notification period of 20 days** may be required before work can commence
- 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**
Andrew Want		4/12/09.
Name: Other signature (s) (if required – please state position)		
PAUL HOURD (CBC QUALITY MANAGER)		4/12/09
Name: Principal Investigator	**Signature**	**Date**
C.J. Aewtt		4/12/09

9.APPROVAL		
Name: Departmental Safety Officer	**Signature**	**Date**
C. J. Aewtt		4/12/09.
Name: University Biological Safety Officer	**Signature**	**Date**
BSO	G. M. Moore.	2/2/10.

STEERING COMMITTEE FOR THE UK STEM CELL BANK AND FOR THE USE OF STEM CELL LINES

APPLICATION FORM TO:

Use stem cell lines from sources within the UK other than the UK Stem Cell Bank

---

Name and title of Principal Investigator:	**Professor Christopher Hewitt**
Title of project	**Developing scalable and standardised manufacturing methods for human pluripotent stem cells**
Provider of cell line(s)	**Nottingham University**
Name/number of cell line(s) designated by originator	**Hues-1,7,8,9; Nott-1,2; H1; H9**
Is the cell line Clinical or Research grade?	**Research**
Is the cell line listed on the Register of Steering Committee approved Stem Cell Lines? (http://www.mrc.ac.uk/index/strategy-strategy/strategy-science_strategy/strategy-strategy_implementation/strategy-government_spending_review_initiatives/strategy-stem_cells/strategy-stem_cell_governance/strategy-registry_of_stem_cell_lines.htm)	**Yes**
Is the cell line listed on the NIH Registry? (http://stemcells.nih.gov/research/registry/)	**H1 and H9 - Yes. Others No.**

INSTRUCTIONS:

- **It is important that this application is understandable by lay members and abbreviations explained.**
- **Please submit this application form and a <u>one page</u> C.V of the Principal Investigator by email to the Stem Cell Steering Committee: stemcellsecretary@headoffice.mrc.ac.uk**
- **For further information please contact the secretary for the Steering Committee at: stemcellsecretary@headoffice.mrc.ac.uk**

  Or for scientific queries please contact Dr Matthew Wakelin at: Matthew.Wakelin@headoffice.mrc.ac.uk

Key to abbreviations:

hES = Human Embryonic Stem (cell line)
MHRA = Medicines and Healthcare products Regulatory Agency
HFEA = Human Fertilisation and Embryology Authority
NIH = National Institutes of Health

SECTION 1 – CONTACT DETAILS

1.1 Principal investigator (*attach a one page C.V.*)

Name and title: Professor Christopher Hewitt

Post held: Professor of Pharmaceutical Engineering

Address: Department of Chemical Engineering
Loughborough University,
Leicestershire,
LE11 3TU

Tel: 01509 222506
Fax:
Email: C.J.Hewitt@lboro.ac.uk

1.2 Contact person if different from person listed at 1.1

Name and title:

Post held:

Address:

Tel:
Fax:
Email:

1.3 Collaborators (Name and institution of Principal Investigators who wish to have access to hES cell lines as part of this application)
(You must inform the Stem Cell Steering Committee if collaborators join the project subsequent to this application)

SECTION 2 – DETAILS OF CELL LINE(S)

2.1 Provider of cell line(s) and contact details

Name and title: Prof. Lorraine Young

Post held: Professor in Molecular Embryology & Director of STEM

Address: Wolfson Centre for Stem cells, Tissue Engineering and Modelling (STEM)
The University of Nottingham
Centre for Biomolecular Sciences
University Park
Nottingham, NG7 2RD

Tel: 0115 (82) 31238
Fax: 0115 (82) 31230
Email: Lorraine.Young@nottingham.ac.uk

2.2 For hES cell lines derived in the UK, please provide the HFEA Licence number

R0141

2.3 For hES cell lines derived abroad that are not listed on the Register of Steering Committee approved Stem Cell Lines or the NIH Registry, please complete the following:

a) Was the study to derive the cell line(s) approved by an ethics committee (or its equivalent if the application is from overseas)?

Yes ☐ No ☐

b) Have you clarified with the consenting clinician that informed consent in line with UK guidelines has been given?
(The following criteria constitute best practice in the UK. Please tick as appropriate)

At the time of consenting, the Donor was informed*:

i. about the specific research project, including any tests may be performed as part of the licensed research project on embryos or cells derived from the embryos

Yes ☐ No ☐

ii. that any stem cells lines created may continue indefinitely and be used in many different research projects

Yes ☐ No ☐

iii that the decision whether to donate will not affect their treatment in any way;

Yes ☐ No ☐

iv. about whether the embryos will be reversibly or irreversibly anonymised, and the implications of this

Yes ☐ No ☐

v. whether any information will be fed back to the donors

Yes ☐ No ☐

vi. that the donors can vary or withdraw the terms of their consent until the point the embryos are used in the project of research

Yes ☐ No ☐

vii. that once an embryo has been used in the project of research the donors have no control over any future use of the embryonic cells and any stem cell lines derived

Yes ☐ No ☐

viii. that stem cell lines derived in this project will be deposited in the UK Stem Cell Bank and the implications of this including they may be used for other projects

Yes ☐ No ☐

ix. that stem cell lines must not be generated from donated embryos where the consent from the relevant donors, or one of them, places a constraint on future use

Yes ☐ No ☐

x. that cell lines may be used for commercial purposes, but that the donor will not benefit financially from this

Yes ☐ No ☐

xi. that any cell lines derived, or discoveries made using them, could be patented, but that the donor will not benefit financially from this

Yes ☐ No ☐

xii. about how the research is funded, including any benefit which will accrue to researchers and/or their departments

Yes ☐ No ☐

*The Steering Committee considers all applications on a case by case basis and appreciates that there may be occasions where not all criteria are fulfilled. The Steering Committee reserves the right to ask for original documentation if considered necessary.

SECTION 3 - DETAILS OF THE RESEARCH PROJECT FOR WHICH THE CELL LINE(S) IS BEING USED

3.1 Title of the project:

Developing scalable and standardised manufacturing methods for human pluripotent stem cells

3.2 Has a study previously approved by the Stem Cell Steering Committee?

Yes [X] **No** [ ]

3.3 Abstract of research project including aims and objectives *(Approx. 300 words):*
(The Stem Cell Steering Committee needs to satisfy itself that hES cell lines are not used for trivial purposes and their uses are within the remit of HFEA regulations. The Stem Cell Steering Committee will not conduct a scientific review of experimental detail or repeat the peer review.)

Pluripotent hESCs are a major emerging platform for a wide range of therapeutic cell based products and pharmaceutical assays, however there are major barriers to their commercial-scale production. Our multidisciplinary collaboration will improve the understanding and reliability of cell expansion for pluripotent human embryonic stem (hESC) and induced pluripotency (hIPSC) cells by:

1) investigating properties of pluripotent cells that influence their processing and scale-up using our experience of multiple cell lines and culture conditions to scope generic process conditions

2) optimising and validating automated bioprocess protocols to enable robust and reproducible manufacture of hESC-based products at commercial scales.

To maximise the range of manufacturing scales that are likely to be required for e.g. pharmaceutical screening processes or regenerative medicine applications, we will develop medium scale (entirely automated 90 X T175 flask T-flask culture in the CompacT SelecT) and larger scale (for potentially up to 1000L bioreactor) systems in parallel, using the same source of highly characterised cells. The processes that we deliver will have improved cost-effectiveness over current systems and will allow standardised culture protocols to be applied to multiple human pluripotent cell lines. Statistically-designed factorial experiments, underpinned by systematic process improvement, will identify the variables in manual culture methods that affect the practicality of scaled hESC manufacture. Factorial experimentation & quality optimisation (biological function, variation & cost) of the bioprocessed cell product will be achieved through gaining an understanding of all relevant variables through a unique collaboration between stem cell biologists and bioprocess/biomanufacturing engineers.

3.4 Has the research project been subjected to peer review?

Yes [Y] **No** [ ]

If yes, please provide details (Funding Body, etc)

BBSRC – Bioprocessing Research Industry Club (BRIC); BB/G010404/1

If no, please explain why not (e.g generation of preliminary data) and state how the research will be supported

3.5 Does the research project include experiments in animals?

Yes [ ] **No** [N]

If yes, please provide details

3.6 Do you intend to perform experiments creating hES cell/animal embryo aggregation chimaeras?

Yes ☐ No N

If yes, please provide details

3.7 Are all experiments involving animals covered by appropriate Home Office Animal Procedures Licences? N/A

Yes ☐ No ☐

3.8 Was the stem cell line derived in clinical grade facilities accredited by the UK MHRA?

Yes ☐ No N

If yes, do you have access to clinical grade facilities accredited by the UK MHRA (?

Yes ☐ No ☐

SECTION 4 - DECLARATION TO USE HUMAN EMBRYONIC STEM CELL LINES FROM SOURCES OTHER THAN THE UK STEM CELL BANK

4.1 By submitting this application form, the person responsible (e.g. Head of Department at the host institution) of the recipient of the exported stem cell line(s) confirms that;

the cell line(s) (insert name/no) **Hues-1,7,8,9; Nott-1,2; H1; H9** will only be used for the following purposes:

1. The information in this form is accurate to the best of my knowledge and belief and I take full responsibility for it.
2. Research that is consistent with UK legislation (as specified in the Code of Practice for the Use of Stem Cell Lines* and the recipient hereby agrees to abide by this Code.
   (a) research which has the long term goal of helping to increase knowledge about serious diseases and their treatment
   (b) basic cell research which underpins these aims
   (c) development of cell based therapies for clinical trials in respect of serious human diseases
3. Research that does not contravene UK legislation such as human reproductive cloning.

On behalf of Host Institution
(Person Responsible [e.g. Head of Department/Dean])
Signature: C D Rielly

Date 3/9/09

Principal Investigator

Signature:

Date 25/8/09.

Name and title of host Institution Signatory: PROFESSOR CHRIS RIELLY

Post held: PROFESSOR OF CHEMICAL ENGINEERING/HEAD OF DEPARTMENT

Institution: LOUGHBOROUGH UNIVERSITY

Postal Address: LOUGHBOROUGH UNIVERSITY
LOUGHBOROUGH
LEICESTERSHIRE, LE11 3TU

Country: UK
Tel: 01509 222504
Fax:
Email: C.D.RIELLY@LBORO.AC.UK

PROTECT: RESEARCH

Professor Christopher Hewitt
Department of Chemical Engineering
Loughborough University
Leicestershire
LE11 3TU

21 September 2009

Our ref: SCSC09-43

Dear Professor Hewitt,

Re: Application to Use stem cell lines from sources within the UK other than the UK Stem Cell Bank

Thank you for your application to use stem cell lines for the following project *"Developing scalable and standardised manufacturing methods for human pluripotent stem cells."*

I am pleased to inform you that Steering Committee for the UK Stem Cell Bank and for the Use of Stem Cell Lines (herein after referred to as the 'Steering Committee') has approved your request for the following lines:

Hues-1, Hues-7, Hues-8, Hues-9, Nott 1, Nott 2, H1 and H9

Please note we are only sending this notification to the principal applicant and would request that co-applicants be informed of the Committee's decision. Any major new uses of these cell lines will need to be approved by the Steering Committee.

The Steering Committee would like to remind you that the proposed research must be consistent with the UK Code of Practice and the current regulations in the country where the hES cells will be used.

An increasing number of lines are being made available through the UK Stem Cell Bank; please refer to the Bank's website at www.ukstemcellbank.org.uk. It is best practice to use the Bank where possible, in order to ensure that you are working with standardised high quality lines. This will also save the originator labs from having to process a number of potential requests.

For future applications; the Steering Committee requests that you first determine whether or not your required cell lines are available via the UK Stem Cell Bank, and if so source these from the Bank accordingly. Should there be circumstances where the requested lines are banked but you wish to have them supplied from elsewhere, please could you write to me briefly specifying why this is the case.

Please do not hesitate to contact me if you have any questions.

With best wishes,

Dr Catriona Crombie
Programme Manager: Stem Cells, Regenerative Medicine and Developmental Biology
Molecular and Cellular Medicine Board (MCMB)
Telephone: +44 (0) 20 7670 5445
Fax: +44 (0) 20 7636 6289
Email: catriona.crombie@headoffice.mrc.ac.uk

Cc Dr Charles Hunt, UK Stem Cell Bank

Loughborough University

For Health and Safety Unit Use only ASSESSMENT NO.
CBE/GMO/018

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	4th December 2009	**Date approved**	

Please provide the following general information:

School/Department	Chemical Engineering/Centre for Biological Engineering (CBE)

Principal investigator	Christopher Hewitt	**Position**	Professor of Pharmaceutical Engineering
E-mail address	C.J.Hewitt@lboro.ac.uk	**Phone no.**	222506

Please give a brief and descriptive title for this risk assessment

Title	Developing scalable and standardised manufacturing methods for human pluripotent stem 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.

No GMMs are produced at Loughborough in this project. Human embryonic stem cell lines, virally transduced with DNA encoding green fluorescent protein will be obtained from the University of Nottingham. Human fibroblasts will be virally transduced with transcription factors, associated with pluripotency, to form induced pluripotency stem cells. In both cases, the cells will have been cultured at Nottingham for sufficient time to remove the virus from the culture, such that the only GMO present is the cell of interest. We have classified these cells as HG2. The biological risk assessment for these cells is attached and should be referred to for the 'characteristics' sections of this GM risk assessment. This GM risk Assessment is therefore only assessing the risks involved in handling the GM organism not the GM process or the vectors

Donor	All sequences obtained from Addgene (MA, USA)
Name of gene/nucleic acid sequences	GFP/Oct4; Sox2; Nanog; Lin28
Vector	PLVTHM/pSIN based cloning vectors.
Host	Human embryonic stem cell/human fibroblast
ACDP category* of host (where appropriate)	2

*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)**

All plasmids obtained from Addgene (MA, USA)

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")

Green fluorescent protein is a colour-based reporter gene which fluoresces following illumination with light of a specific wavelength. It is used in this case to render the cells more easily visible. Oct4; Sox2; Nanog and Lin28 are transcription factors implicated in maintenance of pluripotency.

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"

The plasmid vectors are based on a pSIN/PLVTHM backbone and are integrated into the chromosomes of host cells. These plasmids will be delivered (in work performed at the University of Nottingham) by lentiviral 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"

The host cells are either human embryonic stem cell lines, received from an established laboratory, having undergone a number of passages at the University of Nottingham. Prior to this, the lines have been obtained from cell banks or other reputable sources. All of the original donors are routinely screened for HepB, HepC and HIV and the cell cultures tested for Mycoplasma.

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?

Yes. All sequences encode proteins which will be constitutively expressed in the target cell under control of the EF2 and CMV promoters for GFP and Oct4/Sox2/Nanog/Lin28 respectively.

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 expected
Humans at increased risk of the above effects (e.g. immunocompromised, pregnant or breastfeeding women)
None Expected

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

Maximum single culture vessel size: 100 mL liquid volume containing a maximum of 1.76×10^{10} cells. At any one time, there may be up to 10 of these such vessels in operation at any one time.

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)

The cells are categorised as Class 1 GMOs and Hazard Group 2 biological agents (see attached Risk Assessment). The cells are no more hazardous when modified. All procedures will be carried out under Containment level 2 (CL2) within the CL2 CBE Laboratory Unit ie under management standards imposed by a higher level of Containment. This is for reasons other than worker or environmental protection and includes the need to ensure research material protection (e.g. the use of a class II safety cabinet); to impose a quality assurance discipline and because other projects using HG2 BAs may be under way in the CBE Laboratory Unit simultaneously.

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?

Aerosols may be generated when pipetting or manipulating solutions. All such manipulations and vial openings will be conducted in a Biological Safety Cabinet as detailed in the attached biological Risk Assessment

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

Sharps used that may cause damage to the skin include glass microscope slides and cover slips. Control measures are specified in the attached Biological Risk Assessment

Protective equipment and clothing to be used

Standard laboratory safety equipment will be used. Nitrile gloves and side fastening Howie type lab coats will be worn. See details in attached biological Risk Assessment.

Transport and storage arrangements

No special arrangements required. Details in attached Biological Risk Assessment.

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

Disinfection

1% Virkon for liquids as specified in SOP 003 Disposal and Disinfection of Biological waste. Solid waste will be autoclaved and sent for incineration. Validated procedures used as detailed in attached Biological Risk Assessment.

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

1% Virkon for liquids as specified in and SOP 003 Disposal and Disinfection of Biological waste. Solid waste will be autoclaved and sent for incineration. Waste inactivated by validated methods as detailed in the attached Biological RA.

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.

This is not made clear in the associated RA. (See pg 12).

Monitoring of Containment and Control Methods

Monitoring of containment at point of use

Not required as these cells will not survive outside a highly specialised environment

Monitoring of waste inactivation methods

As in SOP 003 – periodic validation of autoclave. Detailed in Biological Risk Assessment — See Comment on last Valid^n pg12

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

An emergency plan is not required because the small scale activities that may result in significant and unintended release of the Class 1 low risk GMO ie as a result of any foreseeable accident would not affect the health and safety of people outside the premises or present a serious risk to the environment. Nevertheless the following procedures will be implemented all staff are trained in emergency procedures, appropriate safety equipment and personal protective equipment is to be used; first aid arrangements are in place, procedures for cleaning up and disposal of waste are in place; procedures for reporting accidents/incidents are in place. Further details can be found in the attached Biological Risk Assessment

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

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)	**N/R**

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 – see attached Biological Risk Assessment

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.

Negligible – see attached Biological Risk Assessment

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.

Effectively Zero – See Biological Risk Assessment

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 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. No

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.**

N/R

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**
CBE Laboratory Unit; H25: Cell culture Laboratory	2
CBE Laboratory Unit; H23: Analytical laboratory	2

Workers initially involved in work:	**Post/experience/training:**
Andrew Want	Post-doctoral Research Associate 5 years experience working with Class I GMOs.
Gayatri Ramasamy	PhD student; Limited previous practical experience with GMO.
Training and assessment of competence for existing and future personnel ***Specify arrangements for provision for existing and future personnel***	
Standard laboratory training SOP - See attached Biological Risk Assessment. Full training will be provided for GR; with increasing workload in line with increasing competence, as determined by experienced personnel (AW). GR's training file will be updated on an ongoing basis, starting with 100 % supervision, moving towards independent working over 3-6 months.	

Authorisation and Notification

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

Signature of proposer ..... **Date** 4th December 2009..

Please print name ...Andrew Want.....

Other Signature (s) (if required – please state position) ..... (CBE QUALITY MANAGER) **Date** 4/12/09

Please print name ....PAUL HOWARD.....

Signature of Biological Safety Officer or authorised Deputy ..... **Date** 4/12/09

Please print name .....C.J. Hewitt.....

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 ..... **Approval Date** 11/1/00

This page is intentionally blank

TABLE 1A: LABORATORY ACTIVITIES

Containment measures	Containment level 1		Containment level 2		Containment level 3	
Laboratory suite - isolation	Not required		Not required	X	Required	
Laboratory - sealable for fumigation	Not required		Not required	X	Required	
Equipment						
Impervious/easy to clean surfaces	Required for bench		Required for bench	X	Required for bench and floor	
Entry to lab via air lock	Not required		Not required	X	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		Required where and to the extent the risk assessment shows it is required	X	Required	
Extract and input air in laboratory should be HEPA filtered	Not required		Not required	X	HEPA filters required for extract air	
Use of microbiological safety cabinet/enclosure	Not required		Required where and to the extent the risk assessment shows it is required	X	Required and all procedures with infective materials required to be contained within cabinet/enclosure	
Autoclave	Required on site		Required in the building	X	Required in the laboratory suite	
System of work						
Access restricted to authorised personnel only	Not required		Required	X	Required	
Specific measures to control aerosol dissemination	Not required		Required so as to minimise	X	Required so as to prevent	
Shower	Not required		Not required		Required where and to the extent the risk assessment shows it is required	
Protective clothing	Suitable protective clothing required		Suitable protective clothing required	X	Suitable protective clothing required; Footwear required where and to the extent the risk assessment shows it is required	
Gloves	Not required		Required where and to the extent the risk assessment shows it is required	X	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		Required	X	Required	
Specified disinfection procedures in place	Required where and to the extent the risk assessment shows it is required		Required	X	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		Not required	X	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		Required by validated means	X	Required by validated means with waste inactivated in lab. suite	
Other measures						
Laboratory to contain own equipment	Not required		Not required	X	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		Required where and to the extent the risk assessment shows it is required	X	Required	
Safe storage/transport of GMMs	Required where and to the extent the risk assessment shows it is required		Required	X	Required	
Written records of staff training	Not required		Required where and to the extent the risk assessment shows it is required	X	Required	

HIGHEST LEVEL OF CONTAINMENT SELECTED ABOVE: CL2

CORRESPONDING CLASS OF GMM: Class 1

MRC Medical Research Council

Dr David Williams
Wolfson School of Manufacturing and Mechanical Engineering,
Loughborough University,
Leicestershire,
LE11 3TU

8 August 2007

Dear Dr Williams,

Re: Application to use human embryonic stem cell lines HUES-1, HUES-7, HUES-8, HUES-9, NOTT-1, NOTT-2, BG01, H1 and H9

I am pleased to inform you that the Stem Cell Steering Committee has considered and approved your application to use the human embryonic stem cell lines HUES-1, HUES-7, HUES-8, HUES-9, NOTT-1, NOTT-2, BG01, H1 and H9 for the proposed work: *Automated culture of human embryonic stem cells.*

Please note that any major new uses for these cell lines need to be approved by the Steering Committee.

The Steering Committee stressed that the proposed research must be consistent with the UK Code of Practice and the current regulations in the country where the hES cells will be used.

Please also note that an increasing number of lines are being made available through the UK Stem Cell Bank (www.ukstemcellbank.org.uk). Best practice is to use the Bank where possible, in order to ensure that different labs are using standardised high quality lines as well as to save the originator labs from having to process a number of potential requests. Accordingly the Steering Committee has asked that you check whether any of your requested lines are now stocked by the Bank, and if so to source these from the Bank. Please contact either Dr Glyn Stacey (gstacey@nibsc.ac.uk) or Dr Charles Hunt (chunt@nibsc.ac.uk) to find out the latest situation. Should there be circumstances where the requested lines are banked but you wish to have them supplied from elsewhere, please could you write to me briefly specifying why this is the case.

Please do not hesitate to contact me if you have any further questions.

With best wishes,

Rob Buckle

Dr Robin Buckle
MRC Molecular and Cellular Medicine Board
and Secretary to the Stem Cell Steering Committee

Cc. Dr C Hunt, UK Stem Cell Bank

C.L.Kavanagh@lboro.ac.uk

From: A.J.Want@lboro.ac.uk
Sent: 03 February 2010 09:10
To: C.L.Kavanagh@lboro.ac.uk
Subject: FW: Biological Risk Assessments for approval

Hi Carolyn,

Copied below is the email from Cathy Moore, via Chris Hewitt confirming acceptance of the Bio and GMO risk assessments. I've also attached a copy of the CBE general risk assessment for the hESC culture, as requested.

Andrew

Post-Doctoral Research Associate
Cell Technologies Group
Centre for Biological Engineering
Loughborough University
LE11 3TU
t: 01509 564891
w: www.lboro.ac.uk/lcbe

From: C.J.Hewitt@lboro.ac.uk [mailto:C.J.Hewitt@lboro.ac.uk]
Sent: 18 January 2010 09:43
To: A.J.Want@lboro.ac.uk
Subject: FW: Biological Risk Assessments for approval

Andrew,

Can you respond to the queries below. Please note that the risk assessment has been accepted and work can now continue.

Cheers

C

Chris J. Hewitt CBiol CEng CSci FSB FIChemE
Professor of Biological Engineering
Director of the DTC in Regenerative Medicine

PA Elizabeth Attenborough
E.A.Attenborough@lboro.ac.uk
01509 222511

Centre for Biological Engineering
Dept of Chemical Engineering
Loughborough University
Leicestershire
LE11 3TU
United Kingdom

www.dtcregen-med.com

www.lboro.ac.uk/lcbe

From: C.M.Moore@lboro.ac.uk [mailto:C.M.Moore@lboro.ac.uk]
Sent: 15 January 2010 08:34
To: R.J.Thomas; C.J.Hewitt
Cc: R.I.Temple@lboro.ac.uk
Subject: FW: Biological Risk Assessments for approval

The biological risk assessment BB/G010404/1 and project A8180651 have been checked by Karen Coopman and David Williams. David had no concerns and Karen has made the following comments.

Both reviewers are happy for the projects to proceed.

Regards
Cathy

Catherine Moore
Health, Safety and Environment Manager

Tel 01509 222180
Fax 01509 223904

From: Karen Coopman [mailto:K.Coopman@lboro.ac.uk]
Sent: 12 January 2010 15:41
To: C.M.Moore@lboro.ac.uk
Subject: RE: Biological Risk Assessment for approval

Dear Cathy,

Risk Assessment- Investigating the potential of Mesenchymal stem cells from umbilical cord

I am happy for this project to proceed and have only a few queries :
1) under sharps (section C1.2.1) they have not mentioned the use of coverslips. These are commonly used in conjunction with a haemocytometer to perform cells counts. Is this an ommision or are they performing all of the counting on the Cedex associated with the CompacT SelecT?
2) under centrifugation (section C1.2.7) they mention using centrifugation only for cell extraction. Are there no plans to remove cells from tissue culture plates and centrifuge them before analysis or for any passaging?
3) Section C4.2 - it reads as if Andreea is and will only ever be granted conditional access (requiring full time supervision for her work)- did they mean this or is there a way for unconditional access to be granted following further training?

Other minor comments which you may wish to forward to Rob and Andreea are as follows:

- some mention of where the work (ie CBE, class II labs) in section A1.2 would help put this initial section into context.
- although implied, it could be useful to state more obviously that the MSCs isolated and cultured are adherent cells.

Risk Assessment- Developing scalable and standardised manufacturing methods for human pluripotent stem cells.

I am happy for this project to proceed and have only a few queries:
1) Section B2.1.3- if listing which cells are GMOs, should include the IPS lines too.
2) Section B2.4.3 - only a volume indicated, no mention of cell densities.
3) GMO risk assessment page 3 - "host" section - incomplete as not mention the fibroblasts which are used to generate the induced pluripotent stem cell lines mentioned on page 1

Other minor comments which you may wish to forward to Andrew are as follows:

- some mention of where the work (ie CBE, class II labs) in section A1.2 would help put this initial section into context.

Best wishes,

Karen

03/02/2010

P.Hourd@lboro.ac.uk

From: Andrew Want [A.J.Want@lboro.ac.uk]
Sent: 25 March 2010 09:23
To: Paul Hourd
Cc: Kathryn Brosnan
Subject: RE: Bio Assessment

Paul,

In response to the comments on the approved assessment:

"What hazard group is the organism? Should this be stated on the front cover?"
The hazard group of the organism is listed on the relevant section of the form.

"?" page 6.
This was an omission on my part, and should read N/R, however, makes no material difference, as the content is dependent on a previous answer.

"Is this sufficient?" (referring to the use of Virkon for waste inactivation) And the associated query regarding method of validation.
As far as I understand, the manufacturer's instructions for concentration and contact time that are suggested are more than adequate for the destruction of less condition-sensitive organisms than the ones in use here.

"Fill in this section" page 13.
Liquid waste will not be disposed of as solid waste, hence this part of the form was left blank.

C6.1.1 page 18 "Why?" in reference to classification of these organisms as not covered by the human tissue act.
They are not human tissues.

Hope this clarifies.

Andrew

Post-Doctoral Research Associate
Cell Technologies Group
Centre for Biological Engineering
Loughborough University
LE11 3TU
T: 01509 564891
W: www.lboro.ac.uk/lcbe

-----Original Message-----
From: P.Hourd@lboro.ac.uk [mailto:P.Hourd@lboro.ac.uk]
Sent: 25 March 2010 08:58
To: Andrew Want
Cc: Kathryn Brosnan
Subject: FW: Bio Assessment

Andrew

What is the status on this?

Paul
-----Original Message-----
From: P.Hourd@lboro.ac.uk [mailto:P.Hourd@lboro.ac.uk]
Sent: 18 March 2010 12:13
To: Andrew Want
Subject: RE: Bio Assessment

Andrew

Couple of points