Loughborough University **Biological Risk Assessment**	Safety Department use only	Material(s) Classification
	Reference Number:	Hazard Group 1 ☑
		Hazard Group 2 ☐
	CBE Use only	GMO ☑
	Reference Number:	HTA Licensable ☐

FORM CBE-RA-Form/002 Version 1.2

RISK ASSESSMENT AND PROJECT REGISTRATION FOR WORK INVOLVING BIOLOGICAL MATERIAL

PLEASE READ CAREFULLY

This form acts to register projects involving the use of Biological Agents and / or Genetically Modified Micro-Organisms, or of materials that may be contaminated with these agents. It assesses the hazards and risks associated with the project as well as identifying those at risk and the measures necessary for preventing, or controlling these risks. Please ensure that sufficient detail is provided when completing this form and that the relevant written SOPs are referenced where required. Once completed and approved, all risk assessments must be supplied to all those working within this project. The work described within this form must not commence until this risk assessment has been completed and approved and that all necessary control measures are in place.

Any changes to the work, or the persons involved, must be notified to the authorised person. All changes requested must be recorded within the risk assessment change control form and may also need to be incorporated within an amended version of this form.

A separate risk assessment will be required for assessing risks associated with GMO activities.

The following declaration must be completed and undersigned by the Principal Investigator or Person Responsible for the project

- All information contained in this form is accurate and comprehensive.
- All workers involved will be instructed that their work must remain within the boundaries of this project registration & assessment.
- All workers have been given, or will be given before they become involved, adequate training and where necessary their competency assessed.
- All workers have, or will be before their involvement begins, enrolled with Occupational Health for health clearance where necessary.
- It is understood that this risk assessment shall not be transferred to a third party without the PI/Supervisor/Line Manager named in this form either taking responsibility for the new activities, or ensuring that a new proposal is submitted.
- All changes to the work covered by this form will be reassessed & the changes submitted to the authorised person before those changes are made to the work.

Principal Investigator		Person conducting this risk assessment	
Name	Robert Thomas	Name	Preeti Holland
Position	Professor of Manufacturing for Cell and Gene Therap	Position	Research Associate
Department	Centre of Biological Engineering	Department	Centre of Biological Engineering
School	Wolfson of MEME	School	Wolfson of MEME

The Project Activity			
Title	Technology transfer and scale up of the differentiation of a human ESC line to megakaryocytes using the modified iMS10.8A cell line from University of Cambridge.		
Reference Number			
Start Date	21 Feb 2022	End Date	31 May 2023

Names of others involved in the work	+
Katie Glen	x
Jon Harriman	x
Catherine Beltran-Rendon	x
Robert Thomas	x

Name	Signature	Date
Preeti Holland	Preeti Holland Digitally signed by Preeti Holland Date: 2022.02.22 15:43:19 Z	22 Feb 2022

1. INTRODUCTION

1.1 Background & aim of project	The aim of this project is the transfer of the current protocol used at the University of Cambridge for the forward programming of ESCs to megakaryocytes using the iMS10.8A cell line and testing various platforms for scale up optimisation of the process.
1.2 Description of experimental procedures	The following standard laboratory procedures will be used: 1. Sterile medium and medium supplements will be prepared as per manufacturer's instructions within a Class II biological safety cabinet and using sterile lab-ware. 2. The use of the autoclave to sterilise lab-ware and to decontaminate biological waste. 3. Frozen cells will be defrosted and seeded into appropriate vessels (cell culture plates, T-flasks or AMBR 15ml cartridges) in a Class II biological safety cabinet. 4. The use of the microscope to visually inspect T-flask and well-plate cultures and perform haemocytometer cell counts 5. Flow cytometry analysis of cells harvested from T-flasks, well plates or AMBR cartridges. 6. Use of the NC-3000 for cell counting Thawing vials- Vials will be thawed in accordance to standard procedures as detailed in SOP032 "Revival of Cryo-Preserved Mammalian Cell Lines". Vials will be removed from liquid nitrogen storage and placed in 37˚C water bath before being transferred to the BSC and added to 9ml of warmed culture medium. Cell suspension will be centrifuged at 300g for 5mins before being suspended in fresh medium and placed in the Sanyo MCO-18AIC CO2 incubator in accordance with standard procedures outlined in SOP110 "Use and Maintenance of the Sanyo and Panasonic multigas incubators". Feeding Cells- Flasks / AMBR vessels will be transferred to BSC. Cells and media will be transferred into sterile centrifuge tubes. These tubes will be centrifuged at 300g for 5 mins, and a proportion of the supernatant will be removed. Cells will then be re-suspended in fresh medium and media will be removed from culture flasks and replaced with fresh media. Flasks / AMBR vessels will be returned to the incubator / AMBR bioreactor immediately. Cell Counting- Refer to SOP095 "Use and Maintenance of AMBR System v5" and SOP029 "Safe Handling and Disposal of Trypan Blue" Cell Analysis - The BD FACS CANTO II Flow Cytometer will be used on cells at various stages of the differentiation process for phenotype analysis of cells. Freezing Cells- A working cell bank will be prepared in accordance to standard procedures as detailed in SOP031 "Cryopreservation and Storage of Mammalian Cell Lines". Freeze medium containing ~10% DMSO will be prepared and 1ml cell suspensions will be added to labelled cryovials, before passive cooling in a -80˚C freezer. Cells will then be transferred to vapour phase liquid nitrogen. Automated cell culture – The AMBR Workstation will be used to culture cells as described in SOP095 "Use and Maintenance of AMBR Systems v5" As the project progresses, further scale up platforms will be used to assess the efficiency and efficacy of megakaryocyte production from the iMS10.8A hES cell line and therefore the suitability of the platform to the process. Any future experimental plans and changes to the quantity of cells to be cultured will be risk assessed according to this risk assessment. The work will be reviewed regularly through a risk assessment review form. However, if the associated risks with any work plan change significantly to what is stated within this risk assessment, a new risk assessment will be completed.
1.3 Where will this work be carried out?	Rooms/areas: H21, H34
	Building(s): CBE, Door H, Charnwood Building, Holywell Park

☑ **2.1 Human or animal tissues, cells, body fluids or excreta will be used in this project**

2. TISSUES, CELLS, BODY FLUIDS OR EXCRETA

2.2 List all cells, tissues, body fluids and excreta to be used. For cells, indicate primary, continuous or finite.

Material type	Organ source	Species	Where it will be obtained from (Include country of origin)
modified embryonic stem cell line	Embryo	Human	University of Cambridge, originally a Mastershef cell line MS10.

☐ **2.3 Material(s) listed in section 2.2 above are considered to be 'relevant material' under the Human Tissue Act 2004.**

☑ **2.11 Biological agents will be used in this project**

2. BIOLOGICAL AGENTS (i.e. micro-organisms such as bacteria, fungi, microscopic endoparasites)

2.12 List the biological agents to be used	Name of Agent	Strain(s)	ACDP / Defra Classification
	hiESC	iMS10.8A	Hazard Group 1
2.13 Describe the type and severity of the disease that can be caused to humans, animals or plants by each of the agents and if relevant, the particular strains in use	This risk assessment assumes that the donor tissue used to generate the cell lines were screened for blood-borne viruses (BBV) including HIV, HBV and HCV.		
2.14 Has any strain listed in Section 2.12 been genetically modified in any way?	☑ Yes ○ No	Ref	

3. CLASSIFICATION OF HAZARD GROUP

3.1. Are you confident that any non-GM organism, tissue, cell, body fluid, excreta or any component thereof covered by this assessment cannot potentially pose a threat to humans or cause human diseases?	☑ Yes - Classify as HG1
3.1.1. Can any non-GM organism, tissue, cell, body fluid, excreta or any component thereof cause human disease and potentially be a hazard to humans but is unlikely to spread to the community and for which there is usually effective prophylaxis or treatment available?	○ Yes - Classify as HG2
3.1.2. Can any non-GM organism, tissue, cell, body fluid, excreta or any component thereof cause severe human disease and potentially be a serious hazard to humans and that may spread to the community, where effective prophylaxis or treatment may or may not be available?	○ Yes
3.2. Do any of the materials contain pathogens or toxins covered by the Anti-Terrorism Crime and Security Act?	○ Yes **ATCSA Schedule 5**
ASSIGNMENT OF CONTAINMENT LEVEL	HG1

4. TISSUES, CELLS, BODY FLUIDS OR EXCRETA

4.2. Will any culturing of the material described in section 2 take place? *If Yes, describe which cell(s) will be cultured and under what conditions.*	☑ Yes ○ No	The iMS10.8A cell line will be cultured. Sterile medium and medium supplements will be prepared as per manufacturer's instructions within a Class II biological safety cabinet and using sterile lab-ware. Feeding Cells- Flasks / AMBR vessels will be transferred to BSC. Cells and media will be transferred into sterile centrifuge tubes. These tubes will be centrifuged at 300g for 5 mins, and a proportion of the supernatant will be removed. Cells will then be re-suspended in fresh medium and media will be removed from culture flasks and replaced with fresh media. Flasks / AMBR vessels will be returned to the incubator / AMBR bioreactor immediately.
4.3. Could HIV permissive cells be present*? *If Yes, describe the cells and for how long these cultures will be allowed to grow.* *If unsure seek advice. Refer to CBE Code of Practice for details on additional precautions.*	○ Yes ☑ No	
4.4. What is the maximum volume of culture grown?	Per Vessel	500
	Number of vessels	4
4.5. Will the tissues, cells, body fluids or excreta be manipulated in any way that could result in the concentration of adventitious biological agent present? *If Yes, explain.*	○ Yes ☑ No	
4.6. Will any of the tissues, cells or fluids be donated by you or your colleagues working in or with access to the labs?	○ Yes ☑ No	

4. BIOLOGICAL AGENTS (ie micro-organisms such as bacteria, viruses, fungi, microscopic endoparasites)

4.8. Describe ALL route(s) of infection (relevant to the laboratory setting) and the minimum infectious dose(s), if known	Name of agent	Route(s)	Minimum infectious dose
	hESC line	Ingestion, inhalation, broke	Not known

4.9. What is the highest concentration and volume of agent(s) to be worked with?	Per experiment	Total stored
	10,000,000	100,000,000

4.10. Are there any known drug resistances amongst the strains to be used? If Yes, explain what these are and the consequences	○ Yes ☑ No

4.11. What forms of agent will be used e.g. spores, vegetative forms and are there any issues over the robustness of these particular forms e.g. resistance to disinfectants or increased stability on dry surfaces?	Human iPSCs are mammalian cells and as such will only survive in specialist conditions (i.e. in ste

4.12. What will be the most hazardous procedure involving the use of this material?	Use of nucelocounter slides as glass shards may pierce gloves

5. RISKS AND CONTROL MEASURES

Risk		How will this be controlled?	Reference to SOP's / Other documentation
5.1. Might infectious droplets, aerosols or splashes be created, either deliberately or by accident?	☑ Yes ○ No	Aerosols may be generated when manually pipetting or manipulating solutions. A class 2 BSC will be used for all open manipulations to protect cell line from contamination and to ensure any aerosols generated are contained. BSCs will be operated in accordance to SOP009 "Use and Maintenance of the Herasafe Biological Safety Cabinets Ducted and Non Ducted"	SOP009 "Use and Maintenance of the Herasafe Biological Safety Cabinets Ducted and Non Ducted"
5.2. Will this material be transported within the laboratory e.g. between BSC & incubator?	☑ Yes ○ No	The cells will be cultured in sealed tissue culture flasks and transported within the laboratory in sealed tissue culture flasks. In the event of an accidental breakage, resulting in a biological spill, this will be cleaned up immediately according to SOP038 "Biological Spill Response"	SOP038 "Biological Spill Response"
5.3. Will this material (including waste) be transported locally between sites on campus but outside the laboratory?	☑ Yes ○ No	Transport outside CBE lab unit is highly unlikely, any movement is likely to be constrained within the University campus in sealed flasks and sealed secondary containers with outer packaging and using local procedures: SOP038 "Biological Spill Response"	SOP038 "Biological Spill Response" SOP005 - Storage & Transport of Biological Agents
5.4. Will material(s) listed in section 2.2 or section 2.3 be shipped to organisations elsewhere in the UK or abroad?	☑ Yes ○ No	Transportation is unlikely, but if required, cells will be packaged in sealed containers within secondary containment vessels with the appropriate hazard labels used. Approved couriers will be used when required.	SOP005 - Storage & Transport of Biological Agents
5.5. Will this material be received from organisations elsewhere in the UK or abroad?	☑ Yes ○ No	A frozen vial(s) of the iMS10.8A cell line will be received from the University of Cambridge. They will be shipped frozen in a dry shipper or double packed by courier. They will be shipped from the Department of Haematology, University of Cambridge. 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 "Management and Control of Incoming Biological Material". This SOP is intended to minimise the consequences that could result from failure of packaging methods and materials used to ship biohazardous materials.	SOP005 - Storage & Transport of Biological Agents SOP008 ""Management and Control of Incoming Biological Material"
5.6. Will this material be stored?	☑ Yes ○ No	Any vial will be removed from the liquid nitrogen stores by an authorised user according to SOP013 "Safe Use and Maintenance of Liquid Nitrogen Stores". Any further cell stocks will be stored within the liquid nitrogen cryostores in designated storage racks in accordance with SOP031 "Cryopreservation and Storage of Mammalian Cells".	SOP013 "Safe Use and Maintenance of Liquid Nitrogen Stores" SOP031 "Cryopreservation and Storage of Mammalian Cells" SOP032 "Revival of Cryopreserved Mammalian Cells"

Risk		How will this be controlled?	Reference to SOP's / Other documentation
5.7. Will this material be centrifuged?	☑ Yes ☐ No	Sealed rotors and buckets will always be used. Sealed Buckets will be opened upon bench top, unless a spillage within a bucket is suspected, in which case the buckets will be transferred to a BSC and opened within a controlled environment. The following SOPs will be strictly adhered to: SOP088- "Use and Maintenance of the centrifuges" SOP089- "Use and Maintenance of the micro centrifuges" SOP308- "Biological Spill Response" Biological spill kits are readily available in each laboratory change room or directly inside laboratories that do not have change rooms.	SOP088- "Use and Maintenance of the centrifuges" SOP089- "Use and Maintenance of the micro centrifuges" SOP308- "Biological Spill Response"
5.8. Are biological samples to be cultured in an incubator?	☑ Yes ☐ No	Yes all biological samples will be cultured in a static incubator at 5% CO2 37˚C. Leaks and/or spillages will be dealt with according to SOP038 "Biological Spill Response" which specifically detail methods to prevent, contain and respond to leakages and spillages in an incubator, such procedures are detailed in.	SOP038 "Biological Spill Response"
5.9. Are sharps to be used at any stage during this activity?	☑ Yes ☐ No	Glass NC-3000 slides will occasionally be used. Only trained users will operate the NC-3000 and will be made aware of the risks of glass shards. Occasionally needles will need to be used to remove reagents (such as DMSO) from sealed bottles. In these cases users will never re-sheath needles. Sharps will be disposed of in either cytotoxic sharps bins (when using chemicals) or autoclavable bins for biological materials only.	
5.10. Are animals to be used in this project?	☐ Yes ☑ No		
5.11. Will a fermenter / bioreactor be used to culture a biological agent or material?	☑ Yes ☐ No	The aim of this project is to carry out scale up work and the plan is to test several scale up platforms to identify an optimum bioreactor for the successful scale up of the megakaryocytes. Initially, the AMBR bioreactor will be used in accordance with SOP095 "Use and Maintenance of AMBR systems v5". Other platforms have not been decided on but this risk assessment will be continually reviewed and the risk assessed. If changes need to be made, a review form will be completed or if the risks change significantly, a new risk assessment will be completed.	SOP095 "Use and Maintenance of AMBR systems v5".
5.12. Is there any stage within the experimental procedures when an infectious material is inactivated (other than for disposal)?	☐ Yes ☑ No		
5.13 Are any of the following to be used in conjunction with the project? You must complete a cryogen risk assessment before work begins and add the reference here.	☐ Carcinogens or Mutagens ☐ Toxins		
	☑ Liquid Nitrogen	Liquid nitrogen cryostores are used to store cyrovials of cells. Handling the cryostores and cryovials will be done in accordance with the relevant SOPs and risk assessments stated.	SOP013 "Safe Use and Maintenance of Liquid Nitrogen Stores" SOP031 "Cryopreservation and Storage of Mammalian Cells" SOP032 "Revival of Cryopreserved Mammalian Cells" CBE 188 SAFMEME 6720 "Cell Cryopreservation" SAFMM6405 Risk Assessment :"Liquid Nitrogen: delivery and manual refill"
	☐ Ionising radiation		

You must complete a lone working risk assessment before work begins and add the reference here.	☑ Lone working	Occasionally, the nature of the experiment may require lone working during the week or on weekends. A lone working risk assessment has been completed along with local emergency contacts if needed during lone working.	
5.14. Are there any conditions associated with the hazards described in section 5.13 that require additional control measures?	○ Yes ☑ No		

6. PPE AND HYGENE

Control Measure	Details		Reference to SOPs / other documentation
6.1 When will gloves be worn?	Gloves will be worn at all times within the CBE		SOP037 "Use of Personal Protective Equipment"
6.2 What type and where will they be stored?	Nitrile	In Lab and in Changing Area	SOP037 "Use of Personal Protective Equipment"
6.3 When will laboratory coats be worn and what type are these?	On entering the CBE lab space	White Howie	SOP037 "Use of Personal Protective Equipment"
6.4 Where will lab coats be stored and what are the arrangements for cleaning or disposal?	Lab coats are stored outside the laboratory in the dedicated change area or first change	Lab coats are sent for cleaning externally once a month.	SOP037 "Use of Personal Protective Equipment"
6.5 Provide details of any other types of PPE to be used?	Laboratory safety glasses will be worn as directed by relevant SOPs when working within the CBE. Face shield (primarily for handling liquid nitrogen) will be worn when retrieving cell vial from storage in the CBE as directed by SOP013 "Use and Maintenance of Liquid Nitrogen Stores" Full length aprons will be worn when retrieving cell vial from liquid nitrogen stores in the CBE facility. When autoclaving, laboratory glasses, apron and autoclave gloves will be worn when handling autoclaved waste/equipment.		SOP013 "Safe Use and Maintenance of Liquid Nitrogen Stores" and when operating the autoclave as directed by SOP025 "Use and Maintenance of Systec VX-95 Autoclaves"
6.6 Describe the lab hygiene facilities available and where they are located	Designated eye wash stations and hand washing facilities are available in the change room of each laboratory; other hand basins are situated directly inside the analytical laboratory and in the main change area as entering and exiting the facility.		SOP038 "Biological Spill Response"
6.7 Where are the first aid boxes and emergency spill kits located?	All biological spill kits are in the changes rooms and autoclave rooms. Chemical spill kits are located in H34 and the first change. First aid kit is located in CBE office.		

7. WASTE

7.1 How will waste be treated prior to disposal

(Note that all differently treated wastes must be included e.g. if some liquid is autoclaved, but others not, then describe both)	Treatment prior to disposal	Is the treatment validated?	Reference to SOPs / other documentation
☑ Liquid waste	Virkon Decontamination according to SOP003 "Decontamination and disposal of Biological (Healthcare) Waste"	☑ Yes ○ No	SOP003 "Decontamination and disposal of Biological (Healthcare) Waste"

7. WASTE

☑ Solid waste	Autoclave Decontamination according to SOP003 "Decontamination and disposal of Biological (Healthcare) Waste" and operated autoclaves according to operating the autoclave as directed by SOP025 "Use and Maintenance of Systec VX-95 Autoclaves"	☑ Yes ○ No	SOP003 "Decontamination and disposal of Biological (Healthcare) Waste" SOP025 "Use and Maintenance of Systec VX-95 Autoclaves"
☐ Other (Specify)			

7.2 Is any waste being autoclaved?	☑ Yes ○ No	SOP025 "Use and Maintenance of Systec VX-95 Autoclaves"
All cycles have been validated for the actual load types used? *(If Yes, documentary evidence of the validation must be available)*	☑ Yes ○ No	There are specific validated cycles that are used for the load type such as waste and liquid cycles. Cycles 4, 5 and 6 are validated cycles. See CBE SOP025 "Use and Maintenance of Systec VX-95 Autoclaves" for more information.
The successful completion of every load is checked prior to disposal?	☑ Yes ○ No	A printout receipt of each cycle is checked once the cycle is complete to ensure that the cycle passed. The receipts are kept as a record of each cycle. This is then logged on a record sheet for autoclave usage as well as the cycle number, date and user. The autoclave tape used on waste bags is also an indicator that the autoclave cycle reached the correct temperature and changes colour from green stripes to black. This is also checked before disposal of the autoclaved waste.

7.3 How will liquid waste be disposed of?

☑ To drain?	After treatment with virkon	☑ Yes ○ No	After 1% Virkon decontamination for 24 hours, waste is poured down the drain followed by copious amounts of water. Refer to SOP003 "Decontamination and Disposal of Biological (Healthcare) Waste" In the occurrence of a contamination, flask will be treated with 3% Virkon overnight before being disposed of, refer to "Decontamination and Disposal of Biological (Healthcare) Waste"
☐ As solid waste?			
☐ Other (Specify)			

7.4 How will solid waste be disposed of?

Categorisation	Waste stream colour code	Disposal method (Edit as required)

Categorisation	Waste stream colour code	Disposal method (Edit as required)
☑ Sharps	**Orange**	Yellow/Orange lidded sharps bin > autoclave sterilisation if known or potentially infected > clinical waste disposal (incineration)
☑ Sharps contaminated with cytotoxic or cytostatic material	**Purple**	Yellow/Purple lidded Sharps bin >clinical waste disposal (incineration @ 1000C)
☑ Human body parts, organs, including blood bags and blood preserves and excreta that have been pretreated before leaving the site	**Orange**	Disinfection or sterilisation in the lab site > Yellow/Orange lidded rigid one way sealed tissue bins > clinical waste disposal (incineration) *Human tissue waste must be placed in separate containers from non-human waste and labelled 'HTA waste'
☐ Animal body carcasses or recognisable parts that have been pretreated before leaving the site		
☑ Potentially or known infected lab wastes contaminated or potentially contaminated with cytotoxic or cytostatic material that have **NOT** been pretreated before leaving the site	**Purple**	Yellow/Purple clinical waste bags > clinical waste disposal (incineration)
☑ Potentially or known infected lab wastes that have **NOT** been pretreated before leaving the site	**Yellow**	Yellow clinical waste bags > clinical waste disposal (incineration)
☑ Infected or potentially infected lab wastes that **HAVE** been pretreated before leaving site	**Orange**	Disinfection or sterilisation in the lab site > orange clinical waste bags > clinical waste disposal (incineration)

8. MAINTENANCE

8.1 Are preventative maintenance and monitoring regimes in place for the following laboratory equipment?

	Inspection / Servicing Frequency	Cleaning / Disinfection Frequency	Monitoring / Alarms Frequency	Reference to SOPs
☑ Centrifuges	Weekly inspections carried out during lab clean. Serviced every 2 years	Performed according to relevant SOP		SOP004 – General laboratory housekeeping SOP088- "Use and Maintenance of the centrifuges" SOP089- "Use and Maintenance of the micro centrifuges"
☑ BSCs	Weekly inspections carried out during lab clean. Serviced every 12 months.	Before and after every use the BSC is wiped down with 1:50 chemegene, which is left to dry then followed by 70% IMS. There is a thorough weekly clean with 1:20 Chemgene which is left to dry then followed by 70% IMS.	Alarms are present on the BSCs to inform if the sash is not correctly positioned. The display in the BSC also detailed the level of air flow which is monitored and recorded on every use.	SOP004 – General laboratory housekeeping SOP009 "Use and Maintenance of the Herasafe Biological Safety Cabinets Ducted and Non Ducted"
☐ Fume Hoods				
☑ Autoclaves	Serviced every 6 months	Autoclaves have weekly and monthly cleaning as detailed in SOP. The usage is recorded each time it is used and whether issues occurred.	The autoclave alarms when a cycle fails	SOP025 "Use and Maintenance of Systec VX-95 Autoclaves"
☑ Incubators	Inspection during weekly lab duties. Annual servicing.	Decontamination is accordance with SOP.	Alarms triggered for incorrect temperature and CO2 concentration	SOP110 "Use and Maintenance of the Sanyo and Panasonic multigas incubators".
☑ Liquid N_2 Stores	LN2 stores are checked and topped up twice weekly		O2 alarms are in place any time that LN2 stores are being refilled. LN2 stores are connected to temperature probes to monitor storage temperatures.	SOP013 "Safe Use and Maintenance of Liquid Nitrogen Stores"

8. MAINTENANCE

Failure contingency plan	Working banks will be generated from the original stock vials and will be spread across the cryostores in case one or more banks fail and therefore not all cryovials are lost.			
☑ Freezers	Inspected / defrosted and cleaned every 6 – 12 months		On board alarms and thermocouples linked to monitoring system.	SOP016 "Use and maintenance of Fridges and Freezers"
Failure contingency plan	Temperature monitoring system alerts key laboratory personnel and lab managers if there is a problem with a fridge or freezer. In this case, consumables/reagents will be moved to a different fridge/freezer temporarily.			
☑ Fridges	Inspected / defrosted and cleaned every 6 – 12 months		On board alarms and thermocouples linked to monitoring system.	SOP016 "Use and maintenance of Fridges and Freezers"
Failure contingency plan	Temperature monitoring system alerts key laboratory personnel and lab managers if there is a problem with a fridge or freezer. In this case, consumables/reagents will be moved to a different fridge/freezer temporarily.			
☑ Others	Nucleocounter NC-3000 BD FACS CANTO II	The BD FACS CANTO II is cleaned thoroughly after use. A long clean is run monthly and preventative maintenance is performed by a BD engineer every 6 months.		SOP121 "Use and Maintenance of NC-3000 Nucleocounter v3"

9. TRAINING

9.1. Have all project research workers undertaken safety training for working with hazardous or potentially hazardous biological materials and agents at CL2?

Name of researcher	Had Training	Date training completed (or will be completed)	If no, state why
Preeti Holland	◉ Yes ○ No	25 Jan 2013	
Katie Glen	◉ Yes ○ No	1 May 2011	
Jon Harriman	◉ Yes ○ No	30 June 2014	
Catherine Beltran-Rendon	◉ Yes ○ No	Jan 2018	

☐ 9.2. This work involves HTA 'Relevant Material', confirm that all project research workers have undertaken HTA training

10. EMERGENCY PROCEDURES

10.1 Are procedures in place for dealing with spillage of infectious or potentially infectious material

Equipment	Reference to SOPs
☑ Within the BSC	SOP006- Selection and Use of Disinfectants, SOP009- Use and Maintenance of Herasafe Biological Safety Cabinets Ducted and Non Ducted , SOP038- Biological Spill Response
☑ Within the centrifuge	SOP088- "Use and Maintenance of the centrifuges", SOP089- "Use and Maintenance of the micro centrifuges", SOP038- Biological Spill Response
☑ Within the laboratory, but outside any primary control measures (e.g. BSC)	SOP006- Selection and Use of Disinfectants, SOP038- Biological Spill Response
☑ Outside the laboratory	SOP038- Biological Spill Response, Spill responses are detailed in SOP005 - Storage & Transport of Biological Agents.

10. EMERGENCY PROCEDURES

Are procedures in place for the security of these HTA Relevant samples?

☐ Loss or theft of samples (including whilst in transit)	
☐ Loss of traceability of samples	
☐ Incorrect disposal of samples	

10.2 Describe the procedures in place for an accidental exposure

Immediate action	Procedures to respond to accidental exposure are detailed in CBE SOP038 "Biological Spill Response" and the CBE COP. These are detailed in spill response posters located in the CBE laboratories. Designated hand washing facilities are located in laboratory change areas and immediately inside the analytical lab where the cryostorage unit is located in the CBE facility. Eye wash stations are readily available in each laboratory change area and within laboratories that do not have a change area. 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 laboratories. Any sharps injury is to be reported and treated by local first aider immediately.	Ref to SOP's	SOP038 "Biological Spill Response"
When and whom to report the incident	Immediately to laboratory management, first aiders and lab users in t	Ref to SOPs	SOP038 "Biological Spill Response"
Immediate action	To report the incident on the University reporting system	Ref to SOP's	
When and whom to report the incident	Go onto the university health and safety web page and report incider	Ref to SOPs	https://www.lboro.ac.uk/services/health-safety/

11. ACCESS

		Explanation	References
11. Is/are the lab(s) adequately separated from other areas (e.g. offices)?	☑ Yes ○ No		
11.2. Is/are the lab(s) or other work areas shared with other users not involved in the project?	☑ Yes ○ No	Access to CBE laboratories is restricted to authorised users. In order to obtain authorised user status, operators must satisfy minimum training requirements set by CBE management and Health and Safety Committee. Basic training modules include a detailed review of the current Code of Practice (CoP), this document details specific aspects of class 2 working in relation to handling biological agents, waste management, training requirements of lab equipment and emergency procedures including spill responses. All training is documented in a personal training file, which is held in the CBE office at all times. Prior to being granted access to CBE labs, each training file must be reviewed and signed off by both lab management and the departmental safety officer (DSO). Once authorised access has been granted, it is the responsibility of the operator to identify specific training needs prior to the start of new projects. SOPs and risk assessments relevant to project equipment and/or procedures can be used as training aids. Training files are live documents and must be continually updated to record all training acquired. For this project P Holland will partake in practical aspects of the work. J. Harriman, K. E. Glen and C. Beltran-Rendon may help with some of the practical work. R. Thomas will also undertake a supervisory role.	

11. ACCESS

11.3. Describe the measures in place to ensure that hazardous biological agents or **HTA relevant** material is secure	☑ Yes ☐ No	N/A	

12. OCCUPATIONAL

12.1. All workers involved with handling unscreened blood, blood products and other tissues are recommended to have Hepatitis B immunisation. Have all workers involved in this project been immunized?	☑ Yes ☐ No
12.2. Is health surveillance required?	☐ Yes ☑ No

13. NOTIFICATIONS

☐ 13.1. Are any of the cells, tissues or fluids covered by the Human Tissue Act (HTA) under the University HTA Licence?	
☐ 13.2. Are any of the cells, tissues or fluids obtained from a HTA licensed biobank with REC approval for generic research use?	
☐ 13.3. Does this work have ethical approval from a recognised NHS Research Ethics Committee?	
☐ 13.4. Does any of the work require approval from the University Ethical Committee?	
☐ 13.5. Do any of the materials require approval for use from the UK Stem Cell Bank Steering Committee (MRC)?	
☐ 13.6. Do any of the materials or biological agents listed require any other licenses?	

14. APPROVALS

Authorised Person	Robert Thomas Digitally signed by Robert Thomas Date: 2022.03.01 21:40:44 Z
Departmental Biological Safety Advisor	Julie Turner Digitally signed by Julie Turner Date: 2022.04.14 12:23:11 +01'00'

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.*

Please provide the following general information:

Date submitted		Date approved	
Title	Technology transfer and scale up of the differentiation of human induced pluripotent stem cells (hiPSCs) to megakaryocytes using the iMS10.8A cell line from University of Cambridge.		
Donor	No data available	Name of gene / nucleic acid sequences	GATA1, FLI1 and TAL1
Vector	Zinc-finger nuclease vectors with integrated cassette	Host	MS10 hES cell line
ACDP category of host (where applicable)			

Characteristics of the Donor, Insert and Host

Name (species/strain if appropriate) and characteristics of the source of the nucleic acid sequences ("the donor")	No data available
Name, description and function of the gene/nucleic acid sequences involved ("the insert")	GATA1, FLI1 and TAL1
Name and characteristics of the "vector"	Zinc-finger nuclease (ZFN) vectors targeted to the AAVS1 locus with integrated cassette
Name and characteristics of the "host"	MS10 hES cell line

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. The iMS10.8A cell line is a human ES cell line and so did not need reprogramming to become an induced pluripotent cell. However, forward programming of the iMS10.8A cells has been carried out using ZFN's with integrated cassette containing the three transcription factors, GATA1, FLI1 and TAL1, so that the cells can be induced towards megakaryocytes by chemically controlling the expression of the three transcription factors. The cells will not excrete proteins or other functional products.

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)	Ill effects are highly unlikely. If cells entered the blood stream through pierced skin, it is highly unlikely they would survive and proliferate. All genetic modification has been carried out prior to arriving at the CBE as the cells will be recieved from the Department of Haematology at the University of Cambridge.
Humans at increased risk of the above effects (e.g. immunocompromised, pregnant or breastfeeding women)	This cell line will only be used for the work outlined in this risk assessment and the biological risk assessment and will not be used for any other study or by any other personnel other than those listed in the risk assessments, whom are immune-competent.
Does this project involve work with animals? Either use of transgenic animals or work with GMMs in animal models	N/A
Quantity of organisms to be used	500

Interim Assignment of Containment Conditions to Protect Human Health

Interim containment level and corresponding Class (classes) of GMO(s) involved in the work (& explanation)	For operational purposes, all procedures will be carried out under Containment Level 2 within the CBE labs. No hazards are presented by these cells since they have been screened for infectious agents, therefore they should be classified as HG1.

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 handling cultures or when pipetting/manipulating cell solutions. Therefore the work will be undertaken in a class 2 BSC for all open manipulations to protect the cell line from contamination and to ensure any aerosols generated are contained. BSCs will be operated following SOP009 "Use and Maintenance of the Herasafe Biological Safety Cabinets Ducted and Non Ducted"
Identify any use of sharps in the work; justify their use and specify control measures	Glass NC-3000 slides will occasionally be used. Only trained users will operate the NC-3000 and will be made aware of the risks of glass shards. Occasionally needles will need to be used to remove reagents (such as DMSO) from sealed bottles. In these cases users will never re-sheath needles. Sharps will be disposed of in either cytotoxic sharps bins (when using chemicals) or autoclavable sharps bins for biological materials only.
Protective equipment and clothing to be used	Lab coats and nitrile gloves will be worn at all times within the CBE. Safety glasses will be worn when required according to relevant SOPs. SOP037 "Use of Personal Protective Equipment" will be adhered to.
Transport and storage arrangements	It is highly unlikely that material will be transported, however 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 "Management and Control of Incoming Biological Material". This SOP is intended to minimise the consequences that could result from failure of packaging methods and materials used to ship biohazardous materials.
Disinfection	70% IMS, 1:50 Chemgene and 1% Virkon will be used. For hazard group 1 and 2, biological agents it is normally sufficient to rely on the manufacturer's data providing the recommended concentrations and contact times are used. Hence 1% Virkon is used per manufacturer's instructions and according to local Code of Practice and SOP006- "Selection and Use of Disinfectants" Independent studies have reported that 1% Virkon completely destroys a wide spectrum of organisms within a contact time of 10mins
Inactivation of GMMs in waste, and subsequent disposal	There will be no GMMs present in cell culture, however all waste will be treated to inactivate the cells cultures and any adventitious organisms. Cell Culture liquid waste will be disinfected with 1% Virkon for 24 hours then waste is poured down the drain followed by copious amounts of water. Refer to SOP003 "Decontamination & Disposal of Biological (Healthcare) Waste". These disinfectants are well known to be effective against a wide range of viruses, fungi and bacteria. For hazard group 1 or 2, it is sufficient to rely on data from the manufacturer, providing the recommended concentrations and contact times are used. Solid waste, such as tissue culture plastic and other consumables, will be decontaminated using an autoclave as directed by SOP025 "Use and Maintenance of Systec VX-95 autoclave". The autoclave is a validated method of decontamination for biological waste, using cycle 4 for solid waste, minimum 121˚C for 15 minutes.

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	According to procedures detailed in attached biological risk assessment
Emergency procedures - Is an emergency plan required? Provide details (or attach)	N/A
Occupational Health issues	No specific requirements for health monitoring. The cells will be handled in CL2 laboratories at all times and will be used within a class 2 BSC and personnel involved on the project will wear the correct PPE and follow local SOPs to reduce risk.

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?	N/A	
Identify any identifiable potential hazards to the environment, which might occur if the genetically modified organism were to be accidentally released.	Negligible	

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.	Negligible
Grade the overall Risk to the environment (= Potential harm x Likelihood)	Negligible

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	The cells are not viable outside specific culture conditions (e.g. 37°C in a humidified incubator), and therefore would not proliferate in an event of loss of containment.
Assign your final containment level.	CL1
Are all hazards now controlled by this proposed level of containment?	Yes
Final classification of the activity, i.e.Class 1/2/3/4.	Class 1
Is the activity notifiable to HSE?	No
Do you intend to apply all control measures from your highest selected level of containment? If not, please justify the exclusion of any control measures not used.	Yes
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 whether they will be subject to transboundary movements.	N/A

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	
H21 and H34, Centre for Biological Engineering, Holywell Park, Loughborough University	CL2 Facilities	X

Workers initially involved in work:	Post/experience/training:	
Preeti Holland	Research Associate with 8 years cell culture experience in the CBE	X
Katie Glen	Research Associate with 10 years cell culture experience in the CBE	X
Jon Harriman	Technician with 8 years cell culture experience in the CBE	X
Catherine Beltran - Rendon	PhD student with 4 years cell culture experience in the CBE	X
Robert Thomas	Professor with 16 years cell culture experience	X

Training and assessment of competence for existing and future personnel

Specify arrangements for provision for existing and future personnel

Authorisation and Notification

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

Signature of proposer	Preeti Holland Digitally signed by Preeti Holland Date: 2022.02.22 15:44:06 Z	Date	22 Feb 2022
Name	Preeti Holland		
Other Signature	Robert Thomas Digitally signed by Robert Thomas Date: 2022.03.01 21:36:41 Z	Date	1 Mar 2022
Name	Robert Thomas		
Signature of Biological Safety Officer	Julie Turner Digitally signed by Julie Turner Date: 2022.04.14 12:24:22 +01'00'	Date	
Name			

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

Approval of the relevant Safety Committee

On behalf of the SC	Julie Turner Digitally signed by Julie Turner Date: 2022.04.14 12:25:10 +01'00'	Date	