Insert BA Categorisation (Hazard Group 1 or 2/ or GMO Class 1):
HG2

Loughborough University

Health & Safety Unit Use Only	
Ref No:	
Department Use Only	
Ref No:	

RISK ASSESSMENT OF WORK WITH BIOLOGICAL AGENTS

Please note the following before completing this form:

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

Date Submitted:	28th January 2015	Date Approved:	
Version Number:	1.0	Supersedes *(insert version number if applicable)*	N/A

PART A: Please provide the following general information:

School/Department			
Centre for Biological Engineering, Wolfson School of Mechanical and Manufacturing Engineering			
Title of Project			
Enterprise Fellowship to Develop a suite of Synthetic Biology Strengthened Tools for Gene Therapy			
Project Reference Number:	EPG64-P4		
Person responsible for this work (*Principle Investigator*)			
Name:	Elizabeth Ratcliffe	Position:	Enterprise Fellow
Department:	Healthcare Engineering	University School:	Wolfson School
Person conducting this assessment			
Name:	Elizabeth Ratcliffe	Position:	Enterprise Fellow
Department:	Healthcare Engineering	Date Risk Assessment Undertaken:	January 2015
Proposed Project Start Date:	01/03/2015	Proposed Project End Date:	31/07/2016

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

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

A1 PROJECT SUMMARY

A1.1 Scientific Goals of the Project.

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

This risk assessment is for a research project on the improvement of Gene Therapy manufacturing processes, specifically the modification of Adeno-Associated viral (AAV) vector with a strong Synthetic Biology designed promoter region and the transfer and scaling of manual cell culture and expansion of human embryonic kidney cells (HEK293T) to CompacT SelecT (The Automation Partnership) automation.

A1.2 Description of the Experimental Procedures

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

Microbiological and Molecular Cloning Procedures

Standard Containment Level 2 microbiological culture and molecular cloning techniques with *E. coli* will be used. Reference for this section; *Gray, J and Zolotukhin S. 2011. Design and construction of functional AAV vectors, Methods in Molecular Biology, 807: 25-46.*

A standard PCR cloning kit, provided by commercial vendor (e.g. pGEM-T-Easy, Promega, Topo, Invitrogen) will be used. Restriction endonucleases, high fidelity PCR enzymes and kits (PfU Turbo, Stratagene or Phusion, New England Biolabs), *E. coli* XL1 Blue Ultracompetent cells and standard molecular cloning equipment will also be used.

Plasmid construction and transformation- CMV Promoter cDNA will be ligated into the backbone AAV-AILP1 plasmid via site specific restriction endonuclease digestion. Individual components will be amplified via PCR using site specific primers containing the necessary restriction enzyme sites, ligation into a PCR cloning plasmid and high fidelity polymerase. Component fragment plasmids will be fully sequenced and stocks saved. Fragments will be purified from parental plasmids after restriction digestion, ligated together and used to transform *E. coli* (standard heat pulse method according to *E. coli* manufacturer's instructions), which will then be cultured overnight at 37°C.

Microbial culture will take place in a designated laboratory (H29) and will be segregated from the other laboratories of CBE, only authorised users are permitted to work in lab H29. Microbial culture will be performed in project specific dedicated incubation equipment to mitigate the risk of cross-contamination. The H29 BSC will be used for microbial culture maintenance and manipulation, after each operation the BSC will be cleaned with 1% Virkon followed by 70% IMS before any work with animal or human cultures takes place to mitigate the risk of cross-contamination. *E. coli* cells will be stored at -80°C (H34) in segregated secondary containment. Plasmids will be stored at -20°C (H29) in a project specific freezer in segregated secondary containment.

Small scale culture and plasmid validation- Small scale plasmid preparations will be screened for structural integrity. After small scale culture of *E. coli*, a small glycerol stock of the transformed cells will be prepared and frozen. The remaining culture will be used to purify the plasmid DNA, plasmid preparations will be screened via restriction endonuclease digestion to confirm proper assembly of the fragments and to monitor ITR deletion.

Large scale culture and plasmid harvest- Once all screening criteria have been met, a large scale plasmid preparation will be prepared by re-streaking the frozen cells to obtain fresh, individual colonies and inoculating a single colony into a larger culture. Large-scale culture will be grown to late log phase, aliquots of the cell culture will be prepared for long term frozen storage and the remaining culture cells will be lysed to purify the DNA according to standard methods and kits (e.g. Qiagen DNA purification).

Human Cell Culture Procedures

Standard Containment Level 2 aseptic cell culture and passive transfection techniques with HEK293T cells will be used.

Thawing vials- Vials will be thawed in accordance to standard procedures as detailed in SOP032 "*Resuscitation*

of Cryo-Preserved Mammalian Cell Lines". Vials will be removed from liquid nitrogen storage, defrosted by rapid water bath thaw and transferred to the BSC, followed by slow addition of warmed culture media. In some instances a modified version of SOP032 will be used where the cells are thawed on dry ice for 30 minutes in accordance with the collaborator GMP thawing process. Cell suspension will be centrifuged at 1500rpm for 5mins before being re-suspended in fresh media and placed in the Sanyo MCO-18AIC CO2 incubator in accordance with standard procedures outlined in SOP053 "*Use and Maintenance of the Sanyo MCO-18AIC CO2 Incubator*".

Feeding Cells- Flasks will be transferred to BSC and media will be removed from culture flasks and replaced with fresh media. Flasks will be returned to the incubator immediately.

Passaging Cells- Within a BSC, this will involve aspirating the media off the cells, washing them gently in PBS and detaching them from the culture flask using trypsin at room temperature for up to 5 minutes. Culture media will be added to quench the trypsin reaction and the cells will be transferred to a sterile centrifuge tube. The cell suspension will be centrifuged e.g. at 1500rpm for 5 minutes. The supernatant will be removed to waste and the cell pellet will be re-suspended in fresh culture media. An aliquot of cell suspension (up to 1mL) will be removed for cell counting.

Counting Cells- Cell counting will be performed using the automated Nucleocounter system and Via-1 cassettes in accordance with SOP121 "*Use and maintenance of the Chemometec NC100 nucleocounter*" or using the automated Cedex cell counter in accordance with SOP035 "*Use and maintenance of the CompacT SelecT*". Following calculation of viable cell number, cells will be seeded into new culture flasks.

Transfection- the passive triple plasmid or dual plasmid transfection method will be used (dependent on which helper / packager plasmids are in use at the time), within a BSC the vector of interest plasmid along with a combined helper / packaging plasmid or singular helper and packaging plasmids will be mixed within a PEI pro transfection reagent according to manufacturer's instructions (Polypus Transfection Ltd). Flasks to be transfected will have spent medium aspirated and the transfection mixture aseptically added, flasks will then be returned to the incubator for transfection to occur.

Transfection analysis- transfection efficiency will be analysed using the Transfection Efficiency assay on the automated Nucleocounter system, plaque assay techniques, DNA quantitation techniques including the use of the Nanodrop spectrophotometer in accordance with SOP145 *"use and maintenance of the Nanodrop spectrophotometer"* and GFP fluorescence microscopy.

Automated Cell Culture
CompacT SelecT will be used for the automated cell culture and will mimic manual cell culture, cell counting and transfection steps, with the exception that the integral robot arm performs cell culture manipulations under a sterile air curtain environment and the operator interacts with the robot for programming and to ensure stocks of reagents and consumables are maintained. The protocols are described in SOP035 "*Use and maintenance of the CompacT SelecT*".

Freezing Cells- Working cell banks will be prepared in accordance with standard procedures as detailed in SOP031 "Cryopreservation and Storage of Mammalian Cell Lines". Freeze media containing ~10% DMSO will be prepared and 1ml cell suspensions will be added to labelled cryovials, before placing at -80°C for 24h. Cells will then be transferred to vapour phase liquid nitrogen for longer term storage.

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 1: MICRO-ORGANISMS

B1.1 HAZARD AND RISK IDENTIFICATION: NATURE OF MICRO-ORGANISMS

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

B1.1.1 List all micro-organisms to be used

Name	Strain	ADCP cat*	Source
E. coli XL2 Blue Ultracompetent cells	*Escherichia coli XL2 Blue, XL1 Blue derivative, K-12 derivative*	1, refer to SAGM compendium of guidance, Part 2	Stratagene, Agilent Technologies UK Ltd

see The Approved List of Biological Agents – available on the Health & Safety website

B1.1.2 Has any strain 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	

B1.2 DESCRIPTION OF RISK TO HUMANS

B1.2.1 The disease(s) caused to humans

Describe the type and severity of effects or disease(s) on human health (including colonisation, infection, allergy, toxin-mediated disease) by each of the agents or strains to be used

Indicate in the adjacent box if Not Relevant (N/R)		
Name	Type	Severity
E. coli XL2 Blue	Temporary colonisation	Low, disabled strain, minimal risk of colonisation and unable to survive outside specialised environment

B1.2.2 What is the likelihood of infection of this material? Indicate as None, Low Risk, Medium Risk, High Risk, Known Infected

Name of agent	Risk Category	Justification for Selection
E. coli XL2 Blue	Low risk	Disabled strain, screened with certificate of analysis from commercial supplier (attached to this risk assessment)
		If none proceed to section B1.3

B1.2.3 Infectivity to humans

Describe ALL the route(s)of infection (relevant to the laboratory setting) and the minimum infectious dose(s) if known (e.g. percutaneous, mucocutaneous, inhalation, ingestion)

Name of agent(s)	**Route(s) of infection**	**Minimum infectious dose**

E. coli XL2 Blue	Percutaneous, mucocutaneous, inhalation, ingestion	unknown

B1.2.4 Drug resistance
Is there any known or suspected drug resistance amongst the strains to be used? Identify & describe.

The strain has been genetically modified to incorporate antibiotic resistance genes to allow for clone selection during cloning procedures; XL2 Blue cells are tetracycline and chloramphenicol resistant.

B1.2.5 Attenuation or increased virulence
Are the strains attenuated or do they have an increased virulence in any way?

Identify and describe: *E. coli* XL2 Blue is an attenuated strain derived from *E. coli* XL1 Blue which was originally a K-12 derivative. It is a non-pathogenic / disabled *E. coli* K-12 derivative strain with a long history of safe use, described as non-pathogenic to humans by the SACGM compendium, it has a known genotype and will not grow outside a specially supplemented environment. The strain is from a commercial source and it's genotype and background are as follows:
endA1 supE44 thi-1 hsdR17 recA1 gyrA96 relA1 lac [F′ *proAB lacI*qZΔ*M15* Tn*10* (Tetr) Amy Camr]. (Genes listed signify mutant alleles. Genes on the F′ episome, however, are wild-type unless indicated otherwise.)
XL2-Blue cells are endonuclease (*endA*), and recombination (*recA*) deficient. The *hsdR* mutation prevents cleavage of cloned DNA by the *Eco*K endonuclease system. The *lacI*qZΔ*M15* gene on the F′ episome allows blue-white screening for recombinant plasmids.

B1.2.6 Ability to survive
In what form is the agent present e.g. spores or vegetative bacteria, and are there any issues about the agents' robustness, including any resistance to chemical disinfectants?

Identify and describe: the agent is present as vegetative bacteria that will grow in specially supplemented medium, there are no issues regarding the agents robustness or resistance to chemical disinfectants, the agent is susceptible to the type and concentration of disinfectants used in CBE.

B1.2.7 Most hazardous procedure?
Identify and describe the most hazardous procedure(s) to be used.

Large scale culture due to the increased volumes of bacteria, however all manipulations with the bacteria will be under Containment Level 2 conditions within a Biological Safety Cabinet, minimising the risk.

B1.3 HUMANS AT INCREASED RISK OF INFECTION

B1.3.1 Are there any pre-existing medical conditions that increase the risk associated with this agents listed in section 1.1 (including immunocompromised workers, pregnant workers, breast feeding mothers, diabetic workers)?

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

B1.4. PROPAGATION OR CONCENTRATION OF ADVENTITIOUS AGENTS

B1.4.1 Give details of the volumes and concentrations of organisms to be used

Name & Strain	Volume	Concentration
E. coli XL2 Blue	1L	$OD_{600} \geq 1.5$

B1.5 ENVIRONMENTAL CONSIDERATIONS:

B1.5.1 Are any of the agents capable of causing disease or other harm in animals, fish or plants?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If yes, describe briefly here (A separate risk assessment may be required if the agent to be used poses a significant risk to the environment):	

B1.5.2 Will there be any other environmental risks?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If yes, describe briefly here (NOTE: A separate risk assessment may be required if the agent to be used poses a significant risk to the environment):	

B1.6 OTHER HAZARDS

B1.6.1 Are there any other hazards associated with this work? For example, hazardous chemicals, cryogenic gases ionising radiation.

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If yes, identify these:	
If yes, have these been risk assessed and any necessary approval obtained?	

B1.6.2 Are there any conditions associated with the hazards described in B1.6.1 that require special attention in Section C of this risk assessment? For example, material incompatibilities with disinfectants such as Virkon or hazardous product decomposition associated with high temperatures (ie autoclaving).

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, provide details and ensure that appropriate control measures are addressed in Section C:	

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

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

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

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

Indicate in the adjacent box if Not Relevant (N/R)			
Cell or tissue type and ID	Organ Source	Species	From where will it be obtained?
Human Embryonic Kidney Cell Line (HEK293T) Continuous ATCC CRL-11268	**Kidney**	**Human**	**ATCC**

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

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

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

The 293T cell line, is a highly transfectable derivative of human embryonic kidney 293 cells and contains the SV40 T-antigen, making it an ideal host for genetic modification research. ATCC CRL-11268 HEK293T/17 cells constitutively express the simian virus 40 (SV40) large T antigen, and clone 17 was selected specifically for its high transfectability.
HEK293T cells are classified as Biosafety Level 2 because they contain Adenovirus and Simian Virus 40 T-antigen genetic material. However, because these cells do not contain the complete viral genome of the respective viruses the risk of generation of these viruses by these cells is extremely low. Proper aseptic microbiological techniques will be used so as not to contaminate these cells with virus that might recombine and mobilize these viral genes into infective particles, as such they will be treated as Biosafety Level 2 agents and handled at Containment Level 2.

Refer to attached GMO Risk Assessment Form.

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

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

If Yes, provide details of the types of screening and agents screened for: Safety screening information has been provided by the commercial cell supplier, ATCC and is attached to this risk assessment. All tests for infectious agents were negative.

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)	Yes
If Yes, summarise here: tests include total cell count, viability, sterility by direct inoculation method, mycoplasma detection, DNA fingerprinting for HEK293 origin, extended in vitro assay for virus detection, in vitro assay for porcine virus detection, test for in-apparent viruses, FPERT assay for absence of retroviral reverse transcriptase activity, transmission electron microscopy for contaminants and PCR for a range of specified viruses. Passed all tests and safety criteria.	

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

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes
If Yes, provide details of the route of provenance back to the originator of the cell line, together with a Certificate of Analysis; identifying the methods used to qualify the cell type. HEK cells appear on the list of cross-contaminated / misidentified cell lines, HEK293T cells are not specifically listed but the cells have been tested for their origin by DNA fingerprint / STR analysis confirming they are not cross-contaminated or misidentified.	

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 Kidney Cell Line HEK293T	Low	Well authenticated continuous cell line deposited with the ATCC (CRL-11268). The cells are not fully characterised but have been utilised extensively in peer reviewed academic research. Since the cell line has been subject to extensive sub-culture the risk of pathogenic agent contamination is very low. Hazard group 2 requiring baseline containment level CL2

		HEK293T cell lines are classified biosafety level 2 because they contain Adenovirus and Simian Virus 40 genetic material. This cell line has been well characterised and authenticated with low risk of endogenous infection, and low risk of exposure to further wild type viral genetic material (as explained in section B.2.2.3). HEK293T cells contain an attenuated section of wild type adenovirus genome and therefore represent a much reduced risk of harm compared to wild-type virus, furthermore the cell stock is demonstrably free of any replicative virus or any further viral particles or reversion events. Cell line presents low risk to operator under Containment Level 2 conditions / microbiological asepsis and has been tested for pathogen contamination (safety screening information is attached to this risk assessment). As part of the CBE quality system, samples are routinely sent for mycoplasma testing.
		If none proceed to section B2.2.4

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

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

Name of Agent	Classification
Adenovirus (other viral particles)	2
Simian vacuolating virus 40	2

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

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

Percutaneous	Mucocutaneous	Inhalation	Ingestion	N/R
	X	X	X	

Details:
Referring to Section B2.2.2., the material has been safety screened for bacterial, fungal and viral contaminants and passed several screening tests (as outlined in the attached safety screening report) indicating that it is not contaminated / infected with any Biological Agents.

There is no risk of exposure to further wild type Simian vacuolating virus genetic material as this material is not handled or stored within the CBE facility and none of the researchers working on this project (or any other CBE project) have access to Simian cells or DNA. SV40 is found in humans but exposure is extremely rare, e.g. exposure via contaminated polio vaccine produced between 1955 and 1961 (all researchers working on the project were vaccinated after this period with safe vaccine).

The tick boxes above for Section B2.2.3 and the paragraph below describes the potential route of infection of wild type Adenovirus infection in humans. In order to help explain the low risk of the HEK293T cells being used in the research becoming exposed to wild type Adenovirus genetic material.

Wild type Adenovirus: Adenoviruses are ubiquitous pathogens of both mammals and birds. Over 100 serotypes are known, 51 of which infect humans. The following guidance will focus on the use of human adenoviruses. However, many of the principles will also apply to work involving the adenoviruses that infect animals. The severity of these infections varies from acute respiratory disease (ARD) in adults (Ad4; Ad7) to mild respiratory symptoms in children (Ad2; Ad5), gastroenteritis (Ad40; Ad41), conjunctivitis (Ad8; Ad19; Ad37), cystitis or subclinical infection (Ad12). Certain serotypes have also been shown to be tumourigenic in neonatal rats (Ad12; Ad7), although this has never been observed in humans. Primary infection generally occurs in childhood via the airborne or faecal-oral routes and can be persistent with viral shedding continuing for months. Latent infection of lymphoid tissue can also occur and reactivation in the immunocompromised can lead to serious complications. However, the precise mechanism of latency remains unknown. Immunity is thought to be lifelong and over 90% of individuals are seropositive for Ad2 and Ad5 (SAGM compendium of guidance).

Wild-type Human Adenoviruses are ACDP Hazard Group 2. Therefore, Containment Level 2 should be adopted as a minimum requirement when handling wild-type virus. Adenovirus vector strains that can be shown to pose a much-reduced risk of harm compared to the wild-type virus might be handled at Containment Level 1 (SAGM compendium of guidance).

HEK293T cells contain an attenuated section of wild type adenovirus genome and therefore represent a much reduced risk of harm compared to wild-type virus, furthermore the cell stock is demonstrably free of any replicative virus or any further viral particles or reversion events.

Under the Containment Level 2 microbiological asepsis handling conditions described in Section A1.2. the risk of the HEK293T cells being exposed to wild-type adenovirus is mitigated. Additionally, if for example there was a serious breach of containment / microbiological asepsis that was unnoticed by the highly experienced lab users, the risk of wild type adenovirus generation is infinitely rare as a series of extremely rare recombination events would need to occur.
If there was a serious breach of containment / microbiological asepsis, all potentially exposed cells would be immediately destroyed (in accordance with SOP038 "Biological spill response" and SOP003 "Disposal of biological waste") in order to mitigate the risk of a recombination event occurring and any tangible outcome from such an event.

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

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

B2.3 HUMANS AT INCREASED RISK OF INFECTION

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

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

B2.4. PROPAGATION OR CONCENTRATION OF ADVENTITIOUS AGENTS

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

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes
If yes, identify the cells and the conditions these will grow: All cells will be cultured in closed T-flasks in cell culture medium in incubators (37°C humidified system). All manipulations of cultured cells will occur under user & product protected laminar flow in BSC's or the CompacT SelecT.	

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 T175 flasks: max $80x10^6$cells T500 flasks: max $250x10^6$cells	Per experiment Maximum of up to 90 flasks per experiment, most experiments at ≤10 flasks. T175 Flask working volume, max 50ml of medium per flask, max $80x10^6$ cells per flask. Max 90 T175 flasks gives maximum volume of 4.5L and $7.2x10^9$ cells T500 Flask working volume, max 250ml of medium per flask, max $250x10^6$ cells per flask. Max 90 T500 flasks gives maximum volume of 22.5L and $2.25x10^{10}$ cell

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:

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

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

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, explain what precautions are to be taken to prevent that person being exposed to the cells:	

If yes, where will this material be collected:
If yes, provide justification for not using a safer source:
If yes, how will confidentiality be assured:
If yes, has Ethics Committee approval been obtained:

B2.6 ENVIRONMENTAL CONSIDERATIONS:

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

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

B2.6.2 Will there be any other environmental risks?

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

B2.7 OTHER HAZARDS

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

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes
If yes, identify these: Trypan Blue – essential for cell counting; will be used and disposed in accordance with CBE COP, COSHH RA CBE020 and SOP029 "*Safe Handling and Disposal of Trypan Blue*" Liquid Nitrogen – essential for maintaining cryostores containing cell banks; will be used in accordance with CBE COP, CBE/SAF/7, COSHH RA CBE033 and SOP013 "*Use and Maintenance of Liquid Nitrogen Stores*" DMSO - Cryoprotectant added to media to inhibit cell death during freezing, COSHH RA CBE 035 Potentially other chemical hazards will arise as the work progresses, for example chemicals used in cloning procedures, before use all chemicals will be COSHH assessed	
If yes, have these been risk assessed and any necessary approval obtained? COSHH RA CBE020 COSHH RA CBE033 COSHH RA CBE035 Further COSHH assessments will be performed as the work progresses as required.	

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

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes
If yes, provide details and ensure that appropriate control measures are addressed in Section C: Trypan Blue will be disposed of via the cytotoxic material disposal route, addressed in Section C	

PART C: CONTROL MEASURES

C1. CONTROL MEASURES

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

C1.1 Preventing Exposure

C1.1.1 Substitution with a Safer Alternative

Is substitution with a safer alternative practical, by for example, replacement of a clinical strain or pathogen with one that is lab adapted? Provide reasons for your answer:
No, the cell line is well authenticated and used routinely in the gene therapy field, it is the base cell line used for the majority of gene therapies and is used by our collaborator site. Substitution is not practical, in order for the research to be fit for purpose the work must use the identical cell line. The cells are commercially classified as biosafety level 2 and will be handled appropriately in a CL2 laboratory suite as per the CBE quality system.

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:
The Containment Level 2 CBE Laboratory Unit is restricted to authorised laboratory workers with appropriate training in accordance with documented local Code of Practice (COP) and Quality Management System (OMS) requirements for Containment Level 2 work activities involving biological materials.
A designated externally ducted BSC will be used for all work involving this project (it is anticipated that work will be performed in the BSC in laboratory H29). Where appropriate, after each culture shared equipment will be cleaned and decontaminated according to procedures detailed in CBE equipment SOPs. Cultures will be manipulated under laminar flow within a BSC or the semi-closed automated platform and incubated in closed flasks. Risk of cross contamination is extremely low.

(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 to the Containment Level 2 CBE Laboratory Unit is restricted to authorised laboratory workers with appropriate training in accordance with documented local Code of Practice (COP) and Quality Management System (OMS) requirements for Containment Level 2 work activities involving biological materials (CL1 & 2).

The laboratories are locked at all times outside of normal working hours to ensure safe storage of biological agents and unauthorised entry. Keys to the laboratories are only issued to authorised users. Access is also restricted to the building and CBE (swipe card entries) during normal working hours. Out of Hours/Lone working is logged and permitted subject to risk assessment.

No cleaning personnel are permitted in the CBE Laboratory Unit. Access by other Non-Laboratory or maintenance personnel is subject to risk assessment and Permit-to-Work system documented in the local COP.

C1.2 Controlling Exposure

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

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If yes, list the sharps:	
If yes, justify there use – is there an alternative?	
If yes, describe there use and disposal:	
If yes, describe any additional precautions employed to reduce risk:	

C1.2.2 Containment and Ventilation

(i) Is the use of BSC required for the protection of the worker i.e. do the work procedures generate aerosols or splashes that pose a risk to workers?

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

If yes, specify the type(s) and when they will be used:

A Class II Biological Safety Cabinet or laminar flow protected automated processing platform 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 out according to the following SOPs:

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

For vial defrosts that have an incubation step using a small volume of dry ice, only ducted BSCs will be used.

(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 listed in B2.1.1 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) SOP031, "Cryopreservation and Storage of Mammalian Cell Lines"
5) SOP114, "Use and Maintenance of HERACell 150i C02 Incubators
6) SOP035 "Use and Maintenance of the CompacT SelecT"

Storage units are located in Laboratory H21, H26 and H29 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.
Vial removal from the LN_2 stores will only be performed by authorised users, the sealed vial will be placed on a small volume of dry ice in secondary containment for transport to the ducted BSC.
Cells will always be transferred in closed secondary containers large enough to carry the designated material.
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 COP 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): Shipping non-infectious DNA samples for sequencing or quality analysis by project partner.						
Description of material to be shipped (*indicate in available boxes*). Is this:						
Category A		UN2814		UN2900		*Packaging instruction 602 or 620 must be followed*
Or?						
Category B		UN3373		*Packaging instruction 650 must be followed*		
Or?						
Non-hazardous	X	*Should be packaged to protect sample*				

C1.2.6 Receipt of material

If material will be received from other sites or organisations, what precautions are being taken to ensure that the material is shipped correctly?

The materials listed in B1.1.1, B2.1.1 and non-infectious DNA sequences will be shipped from commercial sources (Stratagene, ATCC, Synpromics) according to their own Quality Management 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 centrifuges are operated and maintained according to the following SOPs:

1) SOP153, "Use and Maintenance of the H29 Centrifuge"
2) SOP038, "Biological Spill Response"
3) SOP111, "Use and Maintenance of the Sigma 1-14 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) SOP153, "Use and Maintenance of the H29 Centrifuge"
2) SOP038, "Biological Spill Response"
3) SOP111, "Use and Maintenance of the Sigma 1-14 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 and shaker incubators are used. The shaker incubators are used for culture of *E. coli* bacteria. The static incubators are used for human cell culture. Procedures to prevent, contain and respond to spillages in the incubators are detailed in the following SOPs:

1. SOP114, "Use and Maintenance of HERACell 150i C02 Incubators
2. SOP038, "Biological Spill Response
3. SOP035 "Use and Maintenance of the CompacT SelecT"
4. SOP120 "Use and Maintenance of MaxQ Mini 4450 Incubator-Shaker

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 used 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 CBE/39

The use of Virkon for the GMO's produced form this project have been assessed and are documented in the attached GMO risk assessment

Have these disinfectants been validated for use with the recipient biological material?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes

If yes, describe the procedure:
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 the local Code of Practice and SOP006-*"Selection and Use of Virkon Disinfectant"*

Independent studies have reported that 1% Virkon completely destroys a wide spectrum of organisms within a contact time of 10mins.

The use of Virkon for the GMO's produced form this project have been assessed and are documented in the attached GMO risk assessment

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 laboratories in a purposely designed change room. 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).
2. Cryogenic gloves; which will stored in close proximity to the Liquid Nitrogen storage containers located in H31 and H26
3. Latex powder free gloves for general use, which will be stored in the change room 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 when necessary (including those for spectacle wearers)
2. Face Shields (primarily for handling liquid nitrogen)
3. Shoe covers
4. Aprons or disposable lab coats for extra protection over Howie type laboratory coat when necessary.

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

Use of PPE for specific procedures is also described in SOP013 "*Use and Maintenance of Liquid Nitrogen Stores*" and SOP025 "*Use and Maintenance of Systec VX-95 Autoclave CBE045*"

C1.2.11 Hygiene Measures

Describe the hygiene facilities available and where they are located

Designated hand washing and eye wash facilities are located in the laboratory change room and at the point of entry to most laboratories, the work will be primarily carried out in H29 to which the nearest hand wash station is at the point of entry to H27, as well as in H21 which has its own hand wash station at point of entry. Both H29 and H21 have eye wash facilities.

C1.2.12 Vaccination

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

C1.2.13 Waste Treatment before Disposal

How must waste to be treated before disposal and how has it been validated as being effective?

Type of Waste	Treatment before disposal	Validation of this treatment
Liquid waste	Virkon Decontamination according to SOP003 "*Disposal of Biological Waste*" All waste will be labelled appropriately and only processed by those persons involved in the project to ensure correct processing occurs	According to manufacturer's instructions, see section C2.1.9
Solid waste	Autoclave Decontamination according to SOP003 *"Disposal of Biological Waste*" All waste will be labelled appropriately and only processed by those persons involved in the project to ensure correct processing occurs	Treatment Cycle (4) is validated according to SOP024 "Maintenance of Systec VX-95 Autoclave CBE044". Annual validation is conducted by an external contractor

C1.2.14 Autoclave sterilisation

If waste is treated by autoclave sterilisation then this section must be completed. If this section is not relevant then hatch the box

Type of Waste	Composition of waste	Autoclave cycle (temp, cycle time)	Treatment monitor

Liquid waste	None	N/R	N/R
Solid waste	Cell Culture Consumables e.g. pipette tips and flasks	Minimum 121°C for 15 mins (under clinical vacuum) CYCLE#4	Designated Autoclave tape monitors
Location of autoclave	Servicing details	Location of back-up autoclave	Designated area for storage of unsterilised waste
CBE- Autoclave Room H31	Annual	CBE/045- In autoclave room H31. Refer to SOP152 for emergency autoclave procedure should back up autoclave also be unavailable	Temporary storage in secondary containment in laboratory H29.

C1.2.15 Liquid Waste Disposal

How will liquid waste be disposed of?
To the drain? Non-GMO liquid waste: after 1% Virkon decontamination for 24 hours, waste is poured down the drain with copious amounts of water. Refer to SOP003 "*Disposal of Biological Waste*" The use of Virkon for the GMO's produced form this project have been assessed and are documented in the attached GMO risk assessment
As solid waste? No
Other? N/A

C1.2.16 Solid Waste Disposal

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

Colour Code	Categorisation	*Hatch relevant box(es)*	Disposal Method
Yellow	Sharps (not contaminated with cytotoxic/cytostatic material)		Yellow Sharps bin>autoclave sterilisation if known or potentially infected >clinical waste disposal (incineration)
Purple/Yellow Special case, contact DSO	Sharps (contaminated with cytotoxic/cytostatic material)		Purple/Yellow lidded Sharps bin>clinical waste disposal (incineration @ 1000C)
Yellow	Human body parts, organs, including blood bags and blood preserves and excreta (unless identified as medium or high risk or known infected in Section 2.2.1 of this RA in which case they must be pre-treated before disposal)		Yellow rigid one way sealed tissue bins>clinical waste disposal (incineration)
Yellow	Animal body carcasses or recognisable parts ((unless identified as medium or high risk or known infected in Section 2.2.1 of this RA in which case they must be pre-treated before disposal		Yellow rigid one way sealed tissue bins > clinical waste disposal (incineration)

Special Case – Contact DSO	Potentially or known infected lab wastes (including sharps) of HG2, GM Class 2, DEFRA Cat 2 or higher, that have not been pre-treated before leaving the site.		This is not a route of preference and is subject to special requirements
Orange	Infected or potentially infected lab wastes that have been pre treated before leaving the site		Disinfection or sterilisation (as identified in C1.2.14) in the laboratory suite > orange clinical waste bags > clinical waste disposal (incineration)
Yellow	Infected or potentially infected animal or human body parts, organs or excreta that have been pre treated before leaving site		Disinfection or sterilisation (as identified in C1.2.14) in the laboratory suite > yellow one way sealed tissue bins > clinical waste disposal (incineration)

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

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

C1.2.19 Other Control Measures Required?

None

C1.3 Emergency Procedures

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

Within the BSC:

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

1) SOP038, "Biological Spill Response"
2) SOP009, "Use and Maintenance of HERASAFE KS Class II BSC"
3) SOP006, "Preparation of Disinfectants for use within the CBE Laboratories"
4) SOP104, "Use and Maintenance of HERASAFE KS Class **II** re-circulating BSCs"

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) SOP038, "Biological Spill Response"
2) SOP006, "Preparation of Disinfectants for use within the CBE Laboratories"

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 to advise on spill response (ouside the BSC) and reporting procedures.

Outside the laboratory e.g. during transport

Cells will not be transported from the CBE unit. If they are, any movement is likely to be constrained within the University campus using local procedures. 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) SOP038, "Biological Spill Response
3) SOP006, "Preparation of Disinfectants for use within the CBE Laboratories"

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 Bioreactor Laboratory (H23).
3. Eye Wash stations are located next to each 'hand washing only' sink in each laboratory change room and in the Bioreactor Laboratory (H23). There are also eye wash facilities at the point of use in H29.
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 the CBE laboratories.

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? (see COSHH Schedule 3, Part II for a list of criteria)

All work activities within this project involve biological agents (BAs) assessed as Hazard Group 2. All procedures shall be carried out under the management standards imposed by Containment Level 2 (CL2) within the CL2 certified CBE Laboratories. The procedures and standards are appropriate for worker protection, research material protection and the required quality assurance disciplines as described under the CBE Code of Practice.

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

:

E. coli cells will be stored at -80°C in labelled segregated secondary containment. Only authorised users have access to the -80°C freezer and all users will be notified when and where the cells will be stored.
Where possible, all materials will be stored in the project designated laboratory (H29), this includes plasmid storage at -20°C in a project specific freezer within lab H29.
Microbial culture will take place in a designated laboratory (H29), work and materials will be segregated from the other laboratories of CBE, only authorised users are permitted to work in lab H29. All CBE users will be notified when work begins and all H29 users will be trained in the extra controls.
Microbial culture will be performed in project specific dedicated incubation equipment to mitigate the risk of cross-contamination. The H29 BSC will be used for microbial culture maintenance and manipulation, after each operation the BSC will be cleaned with 1% Virkon followed by 70% IMS before any work with animal or human cultures takes place to mitigate the risk of cross-contamination or accidental release. The BSC in H29 has been supplied with designated sets of pipettes, serological pipette aids and aspirators for microbial and mammalian cell culture to enable effective work segregation and mitigate risk of cross-contamination.

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),* primarily within the Automated cell culture suite (H21) and Dark room laboratory (H29) .	Centre for Biological Engineering	Holywell Park, Loughborough University	R. Temple (Department Safety Officer) P. Hourd (Quality Manager) K. Sikand / C. Kavanagh (Laboratory Manager)

C4 PERSONNEL

C4.1 Names of Personnel involved in the Project

Surname	Initials	University ID	Position
Ratcliffe	E	5012183	Post Doc

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.

Dr Ratcliffe is trained in all procedures and equipment required for the project. 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.

C4.3 Relevant Experience/Training:

Surname	Experience/Training
Ratcliffe	Documented in personal training file. Manual and automated cell culture expertise, worked in the CBE for 5 years.

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

Certificate and status of Hepatitis B immunisation documented in personal training file of all named personnel.

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. Self-monitoring of health is sufficient.

C6. NOTIFICATIONS: Human Tissue Act

C6.1.1 Relevant material covered by the Human Tissue Act

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

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

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

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

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

7. LICENSING REQUIREMENTS FOR ANIMAL PRODUCTS

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

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

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

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

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

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

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

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

8. DECLARATION

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

I, the undersigned:

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

Name: Person conducting assessment & Principle Investigator	**Signature:**	**Date:**
Elizabeth Ratcliffe	E. Ratcliffe	**28/01/2015**
Name: Supervisor/Line Manager	**Signature:**	**Date:**
David Williams		

9.APPROVAL

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

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

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

Name: Authorised CBE Personnel (please indicate position)	**Signature**	**Date**
Andrew Picken (Post Doc, Reviewer)		
Name: Authorised CBE Personnel (please indicate position)	**Signature**	**Date**
Paul Hourd (Quality Manager)		
Name: Departmental Biological Safety Advisor	**Signature**	**Date**
Robert Temple	R J Temple.	08/04/2015
Name: University Biological Safety Officer (or Deputy)	**Signature**	**Date**
Catherine Moore	C. M. Moore.	8/4/15