Loughborough University

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Ref No:	
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Ref No:	CBE/GMO/0XX

RISK ASSESSMENT of WORK with

GENETICALLY MODIFIED ORGANISMS

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

Date submitted	January 2015	**Date approved**	

Please provide the following general information:

School/Department	Centre for Biological Engineering, Wolfson School of Mechanical and Manufacturing Engineering

Principal investigator	Elizabeth Ratclliffe	**Position**	Enterprise Fellow
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Please give a brief and descriptive title for this risk assessment

Title	Enterprise Fellowship to Develop a suite of Synthetic Biology Strengthened Tools for Gene Therapy

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

This risk assessment accompanies Biological Risk Assessment CBE/BRA/0XX. The human embryonic kidney (HEK293T/17) cell line to be used is classed as GMO1 Hazard Group 2. This base cell line has been historically genetically modified and has been previously assessed and this information is captured in GMO Risk Assessment CBE/GMO/0XX.

The main scientific goals of this project are to modify collaborator supplied adeno-associated virus (AAV) with a synthetic CMV promoter (supplied by a 2nd collaborator). The modified vector will be assessed for functionality in a clinically relevant manufacturing process where cells are expanded and transfected on the automated CompacT SelecT culture platform. Techniques involving the HEK cells include manual and automated cell culture, passaging of cells, cryopreservation and thawing of cells, cell observation, imaging and counting. Techniques involving rAAV are standard molecular cloning for plasmid construction and harvest, as well as cell transfection using the PEIPro transient transfection method, viral genome particle harvesting from cell supernatant, purification and quantification techniques.

The project seeks to improve yields of viral genome particles using scalable processes and synthetic biology approaches.

Donor	Cytomegalovirus (promoter)
Name of gene/nucleic acid sequences	Cytomegalovirus (CMV) promoter (supplied by Synpromics Ltd)
Vector	Adeno-Associated Virus vector; pRK-AILP1 plasmid (supplied by UCL)

Host	1. *E. coli* XL2 Blue Ultracompetent cells (for genetic modification of vector) 2. Human embryonic kidney cell line HEK293T (for production of modified vector)
ACDP category* of host (where appropriate)	The biological properties of the expressed gene products represent a low risk to human health. 1. *Escherichia coli* XL2 Blue, XL1 Blue derivative, K-12 derivative. ADCP Category 1, refer to SAGM compendium of guidance, Part 2. Supplied from a commercial source; Stratagene Ltd with certificate of analysis for safety screening (attached to accompanying Biological risk Assessment) *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*q*ZΔM15* 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*q*ZΔM15* gene on the F′ episome allows blue-white screening for recombinant plasmids. 2. AAVs are classified as Dependoviruses (SACGM), they are relatively defective requiring co-infection with a helper virus in order to replicate. The AAV vectors are non-pathogenic, low hazard and can be used safely at Containment Level 1, however all procedures will be carried out at Containment Level 2. The host cell line 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. The cells will be treated as Biosafety Level 2 agents and handled at Containment Level 2. The AAV-HEK293T system is commercially available and is also currently used by the project partner; UCL Wolfson Gene Therapy Unit for gene therapy development and manufacture under GM Class 1 conditions (Attachment 1). The plasmid pRK-AILP1 is supplied by UCL and produced under GMP conditions, a certificate of analysis is attached (Attachment 2)

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

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

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

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

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

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

Characteristics of the Donor, Insert, Vector and Host

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

Cytomegalovirus (CMV) promoters are commonly used in mammalian expression vectors to drive protein expression. The promoter initiates transcription and is the point of control for the expression of genes. The donor, Cytomegalovirus is a viral genus of the viral family known as Herpesviridae or herpesviruses. CMV promoter sequences are non-pathogenic.

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

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

Synthetic CMV; A synthetic promoter is a sequence of DNA that does not exist in nature and which has been designed to control gene expression of a target gene. *Cis*-regulatory sequences derived from naturally-occurring promoter elements are used to construct these synthetic promoters using a building block approach. The result is a sequence of DNA composed of several distinct *cis*-regulatory elements in a completely novel orientation that can act as a promoter enhancer. The Synthetic CMV promoter will be provided by Synpromics Ltd and the methods to contstruct the promoter and the promoter sequence is proprietary.

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

Name and characteristics of the "vector"

Adeno-Associated Virus vector; pRK-AILP1 plasmid (supplied by UCL)

The AIPL1 (aryl hydrocarbon receptor interacting protein-like 1) gene is a protein coding gene that encodes the photoreceptor specific hydrocarbon receptor-interacting protein-like 1.

Mutations in this gene are known to cause a heterogeneous set of clinical conditions depending on the severity of the mutation. Diseases range from milder forms of retinitis pigmentosa and cone-rod dystrophy to Leber Congenital Amaurosis (LCA), the most severe inherited retinal degenerative disease with the earliest age of onset. Individuals affected with LCA are diagnosed at birth or in the first few months of life with severely impaired vision or blindness. There is currently no effective treatment for LCA and inherited retinal dystrophies, which are the commonest cause of childhood blindness. AIPL1 is expressed in retinal photoreceptors (rod and cone cells) and is required for the biosynthesis of photoreceptor phosphodiesterase (involved in visual excitation).

The pRK-AIPL1 plasmid contains a normal human AIPL1 gene sequence, the efficacy of AILP1 gene replacement in pre-clinical testing using this AAV vector has been shown in varying rates of degeneration that reflects the clinical spectrum of the disease, it is under investigation as a potential gene therapy against the above incurable inherited genetic diseases.

This gene sequence is already part of the vector and will not undergo modification, only the promoter region of the vector will be modified using site-directed cloning.

The SAGM states that Adeno-associated viruses (AAV) belong to the family *Parvoviridae,* non-autonomously replicating and non-pathogenic virus present in human population and there is no known link to any human illnesses. AAVs are relatively replication defective, requiring co-infection with a helper virus (for example Adenovirus or Herpes simplex virus) in order to replicate and this has led to their classification as *Dependoviruses*, a discrete genus within this family. Replication can also be induced during cellular stress (for example in the presence of genotoxic agents or following UV irradiation), suggesting that AAVs are not fully defective but are rather reliant upon certain cellular conditions for replication. There are six known human AAV serotypes that appear to be highly prevalent. For example, over 80% of individuals are seropositive for AAV serotype 2 (AAV-2) and this immunity appears to be long-lasting. The SAGM states that since AAVs are defective in nature and not associated with human illnesses, the hazards posed to human health can be expected to be low. The main hazards arising from AAV vectors are likely to arise from the

properties of any inserted genetic material.

The ACDP approved list of biological agents lists some Parvoviridae (human bocavirus, human parvovirus B19, 4, 5) and they are all assigned Hazard Group 2. Adeno-associated virus is not listed by the ACDP. The ACDP states that all viruses which have been isolated from humans, but which do not have an approved classification, should be classified in Hazard Group 2 as a minimum, unless and until there is evidence that they are unlikely to cause disease in humans. The vectors are extensively used for gene therapy research, they are well established and authenticated.

Cultures of replication defective AAV vectors are non-infectious and are not hazardous materials as defined by OSHA 1919.1200 (US Occupational Safety & Health Administration; aligned with the UN Globally Harmonized System of Classification and Labelling of Chemicals, http://genetherapy.unc.edu/forms/JVL%20MSDS%20AAV.pdf). However, these materials are produced in cells where there is the possibility of recombination to form wild type virus. As such, they should be handled as potentially infectious material. Handle as biohazardous material under Biosafety Level 2 containment.

The AAV-HEK293T system is commercially available and is also currently used by the project partner; UCL Wolfson Gene Therapy Unit for gene therapy development and manufacture under GM Class 1 conditions (Attachment 1).
The plasmid pRK-AILP1 is supplied by UCL and produced under GMP conditions, a certificate of analysis is attached (Attachment 2)

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

Name and characteristics of the "host"

Refer to CBE/BRA/075 and CBE/GMO/075 for further information regarding HEK293T cells

The HEK293T/17 cell line used in this project is a derivative of the 293T (293tsA1609neo) cell line. 293T is a highly transfectable derivative of the 293 cell line into which the temperature sensitive gene for SV40 T-antigen was inserted.
The human embryonic kidney (HEK) 293 cell line has been very widely distributed since its isolation more than 35 years ago. The cells are extensively used for the production of E1-deleted Ad vectors and in a variety of transfection studies. This cell line is well established and authenticated.

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

Characteristics of the Genetically Modified (Micro)Organism

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

Promoter expression and function will occur in the genetically modified organism, as the study is based on optimizing promoter efficiency the level of expression could vary. During production of the virus the 293T cells may express the AILP1 gene, as gene expression is dependent on promoter expression and functionality, the levels of gene expression will also vary accordingly.

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

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

Effects on human health (include colonisation, infection, allergy, toxin-mediated disease)
There are two aspects that should be considered with respects to effects of these experiments on human health and these are (1) the potential for virus produced to infect the user and (2) the potential for the virus to replicate after infecting the target cells. 1. AAV is non-autonomously replicating and non-pathogenic, low hazard virus present in the human population which can be used safely at Containment Level 1. Due to the low pathogenicity of the recipient virus, the major hazards that will be posed by recombinant AAV vectors will depend upon the properties of the inserted genetic material and any products that it may encode. The biological properties of the expressed gene products represent a low risk to human health. The sequences in the plasmids cannot be transferred accidentally in humans. 2. All viral coding sequences (REP and CAP genes; viral replication and packaging) are deleted from the recombinant AAV genome leaving only 145 nucleotide of the original viral genome, which represent regulatory and cis-acting sequences (ITRs). The final viral product will be assayed for Replication Competence as appropriate.
Humans at increased risk of the above effects (e.g. immunocompromised, pregnant or breastfeeding women)
There is no increased risk to these individuals.

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

Does this project involve work with animals? Provide details

No

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

Quantity of organisms to be used

Growth of virus may involve maximal automated handling of up to 90 T175 flasks per experiment, though most experiments are anticipated at ≤10 flasks. Harvested cell supernatant containing virus may be up to 1L at maximum automated handling, though most experiments are anticipated at <500ml. Concentrated viral stocks may be up to 10ml (e.g. up to 10^{12} particles per ml). Thus the volume of liquid spills can be readily contained by standard laboratory procedures.

Specify volumes and concentrations/culture density

Interim Assignment of Containment Conditions to Protect Human Health

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

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

The ACDP approved list of biological agents lists some Parvoviridae (human bocavirus, human parvovirus B19, 4, 5) and they are all assigned Hazard Group 2. Adeno-associated virus is not listed by the ACDP. The ACDP states that all viruses which have been isolated from humans, but which do not have an approved classification, should be classified in Hazard Group 2 as a minimum, unless and until there is evidence that they are unlikely to cause disease in humans.

The SAGM states that since AAVs are defective in nature and not associated with human illnesses, the hazards posed to human health can be expected to be low. Therefore AAVs can be classified as Hazard Group 1. The main hazards arising from AAV vectors are likely to arise from the properties of any inserted genetic material.
The CMV promoter and the AIPL1 gene do not increase the potential hazard, thus the rAAV with the transgene and / or promoter can also be classified as Hazard Group 1.

HEK cell line has been transformed by HadV-5 and SV40 viruses, both viruses are classified as biological Hazard Group 2 by the ACDP and the resultant clone HEK293T/17 is also therefore classified as biological Hazard Group 2 (Refer to CBE/BRA/075, CBE/GMO/075).

The required containment level for the transfection procedure is Containment Level 2 and all work will be carried out under CL2 conditions in a CL2 facility.

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

Please provide the following information for the Committee:

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

Some aerosols may be generated during culture, manipulation and pipetting of the cells, risk is minimised by careful handling.
A Class II Biological Safety Cabinet or laminar flow protected automated processing platform (CompacT SelecT, TAP Biosystems Ltd.) will be used for all cell culture and viral transfection work to protect against aerosols or splashes. Post-transfection virus stocks will also be handled within the BSC.
All work will be carried out using aseptic techniques, maintaining a sterile environment and also protecting the operator and other users of the laboratory from biological agents using a class 2 biological safety cabinet / equivalent automated processing cabinet.
Procedures to be carried according to the following SOPs:

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

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

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

None

Protective equipment and clothing to be used

Personal protective equipment will be used at all times and in particular, latex gloves, howie style lab coat and shoe covers will be worn and safety goggles when required.
Proper use of PPE is described in the following SOP: SOP037, "Use of Personal Protective Equipment (PPE)"

Transport and storage arrangements

Cloning and associated 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. Project specific dedicated incubation equipment will be used to mitigate the risk of cross-contamination. The H29 BSC will be used for cloning and associated 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 used for cloning (refer to attached biological risk assessment) will be stored at -80°C (H34) in segregated secondary containment. All plasmids and DNA will be stored at -20°C (H29) in a project specific freezer in segregated secondary containment.
HEK293T Cells will be stored in closed flasks within a designated incubator (H29), automated platform incubator (H21) or in sealed vials in liquid nitrogen storage.
Virus and cell stocks will be transported within the laboratory in sealed tubes or flasks in secondary containment.
There will be no transport of cell stocks outside of the CBE laboratory or shipping to other sites. Non-infectious DNA samples may be shipped for sequencing or quality analysis by project partner.

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

Disinfection

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) equipment manufacturer's recommend minimal use of Virkon; for example low grade stainless steel surfaces.

70% IMS is not suitable for use with rAAV therefore Virkon (1% w/v) will be used for all disinfection and cleaning requirements. A timer will be used for surface disinfection purposes to ensure the manufacturer's 10 minute contact time for effective disinfection does not over-run and cause surface damage. Surfaces (e.g. low grade stainless steel) will be inspected on a monthly basis to note any changes caused by the disinfection regimen. Surface replacement will be considered on an annual basis (or more or less frequently as determined by the monitoring).

Virkon has a wide range of bactericidal, virucidal, fungicidal and sporocidal activities. Representative viruses from all the major virus families are inactivated by Virkon, including the parvoviridae family of which adeno-associated viruses belong (Attachment 3) and commercial suppliers of rAAV materials recommend Virkon for disinfection and disposal (Attachment 4). 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

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 Virkon is used per manufacturer's instructions and according to the local Code of Practice and SOP006-"Preparation of disinfectants for use in the CBE laboratories"

Independent studies have reported that 1% Virkon completely destroys a wide spectrum of organisms within a

contact time of 10mins.

Virkon will be used per the manufacturer's instructions as follows:

- Laboratory surface disinfection (benches, equipment, BSC); wipe surface with 1% concentration with a paper towel to ensure all the surface is covered and dry with a paper towel, ensure contact time is no longer than 10minutes for susceptible surfaces, follow by wiping surfaces with 70% IMS and drying with a paper towel.
- Aspirated or automated platform (CompacT SelecT, TAP Biosystems) liquid waste disinfection: Cell culture liquid waste will be disinfected for 24hrs with 10% Virkon (as recommended by commercial AAV suppliers, Attachment 4) then waste is poured down the sink with copious amounts of water. These disinfectants are well known to be effective against a wide range of viruses, fungi and bacteria. For Hazard Group1 and 2, it is sufficient to rely on data from the manufacturer, providing the recommended concentrations and contact times are used.

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

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

Inactivation of GMMs in waste, and subsequent disposal

Decontamination will be performed in accordance with procedures outlined in SOP003 "Disposal of Biological Waste". The procedures outlined are expected to generate 100% degree of kill for autoclaved waste and 99.999% degree of kill (5 log10 reduction) for chemically disinfected waste.

Cell culture liquid waste will be disinfected for 24hrs with 10% Virkon (Attachment 4) then waste is poured down the sink with copious amounts of water (>10-fold dilution). These disinfectants are well known to be effective against a wide range of viruses, fungi and bacteria. For Hazard Group1 and 2, it is sufficient to rely on data from the manufacturer, providing the recommended concentrations and contact times are used.

For solid waste, such as tissue culture plastic and other consumables, decontamination by autoclaving will be carried out following the appropriate SOPs. The autoclave is a validated method of decontamination of biological waste, using cycle 4 for solid waste, Minimum 121°C for 15 minutes. Treatment Cycle (4) is validated according to SOP024 "Maintenance of Systec VX-95 Autoclave CBE044". Annual validation is conducted by an external contractor, validation of individual cycles is performed using autoclave tape monitors. Disposable solid waste, which is or may be contaminated with GMOs is inactivated by autoclaving at 121°C for 15 minutes, before removal as "clinical waste" by specialist contractors, with final disposal by incineration.

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

Monitoring of Containment and Control Methods

Monitoring of containment at point of use

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

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

Monitoring of waste inactivation methods

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

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

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

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

Occupational Health issues

The HEK293T cell line is biological Hazard Group 2, and therefore has the potential to cause disease in humans and be hazardous to those in contact with the biological agent. However, because the cells contain attenuated sections of Adenovirus and Simian Virus 40 T-antigen genetic material they represent a much reduced risk of harm and the risk of generation of these viruses is extremely low. Additionally, the cells will not survive outside a highly specialised environment.

The SAGM states that Adeno-associated viruses (AAV) belong to the family *Parvoviridae* and there is no known link to any human illnesses. The promoter / transgenes do not increase the potential hazard.

No specific requirements for immunisation or health monitoring. The cells, virus and GMOs will be handled in CL2 laboratories at all times and will be used within a Class 2 BSC / equivalent automated platform and personnel involved on the project will wear the correct personal protective equipment and follow the local COP and SOPs to mitigate risks.

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

Environmental Considerations

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

Risk to animals, fish, plants etc

If the recipient microorganism is controlled by DEFRA, do you have a DEFRA licence? (delete as appropriate)	**Not controlled by DEFRA**

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

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

The cells will not survive outside a highly specialised environment. The sequences in the plasmids cannot be transferred accidentally in humans. The modification (insertion of CMV promoter) is not expected to alter the recipient organism's ability to survive in the environment or pose any potential threat. The AIPL1 transgene encoded by the virus may be able to modify the behaviour of cells they infect, but this would only occur if the virus came in direct contact with mammalian cells and helper/packaging elements, these risks are controlled by use of containment level 2 measures. AAV vectors are derived from human viruses, which are not thought to be able to replicate or cause disease in any animal species. All viral coding sequences (REP and CAP genes; viral replication and packaging) are deleted from the recombinant AAV genome leaving only 145 nucleotide of the original viral genome, which represent regulatory and cis-acting sequences (ITRs).The sequences in the viral vectors are unlikely to cause harm if transferred accidentally in humans or in species in the environment. Therefore it is unlikely that accidental release will represent any significant risk to the environment.

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

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

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

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

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

Effectively Zero

Additional Containment

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

Additional containment provisions for environmental protection

None

Assign your final containment level.

Work with virus: CL2 is sufficient to control all hazards. All work with live virus will be done in a Class II BSC or equivalent automated platform and incubators designated for viral work.	
Are all hazards now controlled by this proposed level of containment?	**Yes**

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

Class 1 activity, not notifiable.

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

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

Yes

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

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

N/R

Workers Involved in the Project and Facilities Used for the Work

Please indicate the areas where work will be carried out (including Room No. and Designation):	
Room No. and designation	**ACGM Categorisation**
Laboratories H29 & H21, Centre for Biological Engineering, Holywell Park, Loughborough University	CL2 Facilities

Workers initially involved in work:	**Post/experience/training:**
E. Ratcliffe	Documented in personal training file. Manual and automated human cell culture expertise, 6 years of experience gained within the Centre for Biological Engineering with a variety of cell types, automated culture platforms and experimental techniques. Experience of setting up new Containment Level 2 laboratories including a Class 1 Genetic Modification (GM) activity and associated facilities within a hospital-based setting. Experience as a departmental GM safety representative; procedural and laboratory risk assessment activities, advising other researchers on their GM work and supervising students performing GM work.
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	.....	**Date**	.....
Please print name	ELIZABETH RATCLIFFE		
Other Signature (s) (if required – please state position)	.....	**Date**	.....
Please print name	ANDREW PICKEN (Post-doc reviewer)		
Other Signature (s) (if required – please state position)	.....	**Date**	.....
Please print name	PAUL HOURD (Quality Manager)		
Signature of Biological Safety Officer or authorised Deputy	R J Temple	**Date**	08/04/2015 .....
Please print name	ROBERT TEMPLE (Department Biological Safety Officer)		
Signature of Biological Safety Officer or authorised Deputy	C. M. Moore	**Date**	8/4/15 .....
Please print name	CATHY MOORE (University Biological Safety Officer)		

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

APPROVAL of the RELEVANT SAFETY COMMITTEE

On behalf of SC ..... **Approval Date** .....

ANNEX 1

TABLES OF CONTROL MEASURES AND CONTAINMENT LEVELS

The basic principles of classification are that you:

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

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

***

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

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

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

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

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

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

TABLES OF CONTAINMENT MEASURES

TABLE 1A: LABORATORY ACTIVITIES

TABLE 1B: PLANT GROWTH FACILITIES

TABLE 1C: ANIMAL FACILITIES

TABLE 2: OTHER ACTIVITIES (LARGE SCALE)

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TABLE 1A: LABORATORY ACTIVITIES

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

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

HIGHEST LEVEL OF CONTAINMENT SELECTED ABOVE: 2

CORRESPONDING CLASS OF GMM: 1 (In this case Containment Level does not equate to GM Class)

amended 20.3.08