LA TROBE UNIVERSITY

Research and Graduate Studies Committee
La Trobe Institutional Biosafety Committee

Research Office

APPLICATION NUMBER: <<Office Use Only>>

Application and Risk Assessment for Gene Technology[1,2]
NLRDs and Exempt Dealings

[1]Conducting of dealings with Genetically Modified Organisms under the Gene Technology Act 2000 and Gene Technology Regulations 2001. Regulation 13B(a) requires an IBC that has assessed a proposal as being an NLRD to make a record of its assessment, in a form approved by the Regulator, and specifies the information that this record must contain. The information requested within this application to be assessed by the LTIBC are in accordance with Regulation 13B(a)(i)-(x).
[2]Notifiable Low Risk Dealings (NLRD's) are listed in Schedule 3 of the Gene Technology Regulations 2001. It is a requirement under the Gene Technology Act 2000 that applications for NLRD's be submitted to the LTIBC for consideration prior to commencement of GM dealings. In addition, La Trobe University requires submission of applications for any dealings that may be Exempt as listed in Schedule 2 of the Regulations.

Complete this form and associated Schedules then submit for assessment to: biosafety@latrobe.edu.au

Section 1. Project Details

Project Title: Cross-presentation of antigens by dendritic cells

Does this application replace an existing approval?	Is the project funded by an external agency?	Are any other approvals required for this project?
☐ YES ☒ NO	☒ YES ☐ NO	☒ YES ☐ NO
If YES, provide all relevant reference numbers	*If YES, provide all relevant reference numbers* NHMRC (GNTxxxxxxx).	*If YES, provide all relevant details* Pending AEC approval.

Principle/Chief Investigator *person who will have overall responsibility for this program*

Title/Full Name	Dr X		
Position	Senior research fellow / Lab head	**Staff ID**	X
Research Group and Department	Department of Biochemistry and Genetics	**Phone**	X
Email	X@latrobe.edu.au		
Role/Qualifications	Chief Investigator/PhD		

Primary Contact *If someone other than the project Chief Investigator listed above*

Title/Full Name	Dr X		
Position	Postdoctoral fellow	**Staff ID**	X
Research Group and Department	Department of Biochemistry and Genetics	**Phone**	X
Email	X@latrobe.edu.au		
Role/Qualifications	Researcher/PhD		

Duration of Project Approval *(For Research Office Use Only)*

Note that the LTIBC can only approve an application for a maximum of 5 years

Project Commencement Date:	20/05/2019	**Project Conclusion Date:**	20/05/2024

Project Summary

Provide a simple overview of the program of activity that involves Gene Technology (i.e. aim of the project, background, intended use/purpose of the GMO etc.)

Initiation of the CD8 T cell response, or priming, involves recognition of pMHC I complexes (Peptide- major histocompatibility complex I complexes) presented by professional antigen presenting cells (pAPCs). CD8 T cell priming can be further classified into direct- or cross-priming based on the initial pAPC antigen source. MHC I presentation of endogenously synthesized antigens in the pAPC leads to direct-priming while presentation of peptide derived from exogenous antigens leads to cross-priming of the naïve CD8 T cell. Dendritic cells (DCs) are known as the main cross-presenting pAPCs *in vivo*. DCs uptake exogenous antigens and then migrate to the draining lymph node (DLN). During this process they mature, as marked by the up-regulation of co-stimulatory molecules and become available for T cell priming. The cross-priming pathway is crucial in eliciting CD8 T cell responses to viruses that do not directly infect pAPCs or inhibit direct-priming of pAPCs upon infection and in the shaping of anti-tumour immunity against non-lymphoid tumours.

The aim of our project is to define the mechanism(s) and function(s) of cross-presenting DCs using *in vitro* and *in vivo* experimental models. Thus, the intended use of the GMO is to target molecular machinery of cross-presentation.

Section 2. Classification of Genetically Modified Organisms

Project Classification Summary *Use the flowchart below and for viral vectors also use Appendix 1 to complete. Note: more than one box can be checked*

		*List the classification(s) as determined from **the checkboxes below** (e.g. Schedule 2, Part 2, Item 1 and Item 4; Schedule 3, Part 2.1 (e), (h)).*
☒	Exempt Dealing	
☐	NLRD Suitable for PC1	Schedule 2, part 1, item 4 Schedule 2, part 2, item 1 and 4 Schedule 3, part 1, item 1.1 (a) Schedule 3, part 2, item 2.1 (a), (g), (l), (m)
☐	NLRD Suitable for PC2	
☐	NLRD Suitable for PC3	
☒	NLRD Suitable for PC1 and PC2	

Check the box(es) that are applicable to each class of Dealings. List those that have been checked in Section 2 of the Application Form. If you need assistance in determining which are applicable, please contact the LTIBC biosafety@latrobe.edu.au

☒ **Schedule 2, Part 1, Exempt Dealings (PC1)**

- ☐ **Item 2**, a dealing with a genetically modified *Caenorhabditis elegans*
- ☐ **Item 3**, a dealing with an animal into which genetically modified somatic cells have been introduced
- ☐ **Item 3A**, a dealing with an animal whose somatic cells have been genetically modified in vivo by a replication defective viral vector
- ☒ **Item 4**, (1) Subject to sub-item (2), a dealing involving a host/vector system mentioned in Part 2 of this Schedule and producing no more than 25 litres of GMO culture in each vessel containing the resultant culture
- ☐ **Item 5**, a dealing involving shot gun cloning, or the preparation of a cDNA library, in a host/vector system mentioned in item 1 of Part 2 of this Schedule

☒ **Schedule 2, Part 2, Host/vector systems for exempt dealings (PC1)**

- ☒ **Item 1**, Bacteria
- ☐ **Item 2**, Fungi
- ☐ **Item 3**, Slime Moulds

Commented [Sara Oveissi]: For more detail, please refer to: http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/exemptdealings-1Sept2011-htm

X **Item 4**, Tissue Culture

☒ **Schedule 3, Part 1, Notifiable low risk dealings suitable for at least physical containment level 1 (PC1)**

☐ **1.1 Kinds of dealing suitable for at least PC1**

☒ **(a)** a GM laboratory guinea pig, mouse, rabbit or rat

☐ **(c)** a replication defective vector derived from Human adenovirus or Adeno associated virus in host tissue culture mentioned in item 4 of Part 2 Schedule 2

☒ **Schedule 3, Part 2, Notifiable low risk dealings suitable for at least physical containment level 2 (PC2)**

☒ **2.1 Kinds of dealing suitable for at least PC2**

☒ **(a) Whole animals (including non-vertebrates)** THAT (involves genetic modification of the genome of the oocyte or zygote or early embryo to produce a novel whole organism) AND (does not involve a GM laboratory guinea pig, mouse, rabbit, rat or Caenorhabditis elegans).

☐ **(aa) A GM laboratory guinea pig, mouse, rabbit, rat or Caenorhabditis elegans** IF (an advantage is conferred) AND (it does not result in the capacity to secrete or produce infections).

☐ **(b) A genetically modified plant**

☐ **(c) A host/vector system not mentioned in NLRD PC1 1.1(c) or Part 2 of Schedule 2** IF (neither is implicated in, or have a history of causing, disease).

☐ **(d) A host AND vector not mentioned as a host/vector system in Part 2 of Schedule 2** if (either HAS been implicated in, or has a history of causing, disease) AND (donor nucleic acid is characterised) AND (host or vector unlikely to gain increased capacity to cause harm).

☐ **(e) A host/vector system mentioned in Part 2 of Schedule 2** IF donor nucleic acid (encodes a pathogenic determinant) OR (is uncharacterised, from organism implicated in, or with a history of causing, disease).

☐ **(f) A host/vector system mentioned in Part 2 of Schedule 2** and producing more than 25 litres of GMO culture in each vessel containing the resultant culture IF (it is undertaken in a certified large scale facility) AND (the donor nucleic acid satisfied specified conditions in subitem 4(2) of Part 1, Schedule 2.

☒ **(g) Complementation of knocked-out genes** IF (unlikely to increase GMO's capacity to cause harm compared to parent capacity)

☐ **(h) Shot-gun cloning or preparation of a cDNA library, in a host/vector system mentioned in item 1 of Part 2, Schedule 2** IF the donor nucleic acid is derived from a pathogen OR toxin-producing organism

☒ **(i)** The introduction of a **replication defective VIRAL vector that CANNOT enter intact human cells into a host NOT mentioned in Part 2 of Schedule 2** IF the donor nucleic acid cannot restore replication competence

☐ **(j)** The introduction of a **replication defective NON-RETROVIRAL vector that CAN enter intact human cells, other than NLRD PC1 1.1 (c), into a host mentioned in Part 2 of Schedule 2** IF the donor nucleic acid cannot restore replication competence

☐ **(k)** The introduction of a **replication defective NON-RETROVIRAL vector that CAN enter intact human cells into a host NOT mentioned in Part 2 of Schedule 2** IF the donor nucleic acid cannot restore replication competence

AND DOES NOT confer an oncogenic modification in humans OR encode a protein with immunomodulatory activity in humans

☒ **(l)** The introduction of a **replication defective RETROVIRAL vector that CAN enter intact human cells into a host mentioned in Part 2 of Schedule 2 IF** (all viral genes have been removed) AND (viral genes needed for virion production meet specified conditions, to limit or prevent recombination) AND (the retroviral vector includes a specified deletion to prevent transcription after integration OR the packaging cell line and packaging plasmids meet specified conditions)

Commented [Sara Oveissi]: For more information please refer to: http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/2011-excerpt-htm

☒ **(m)** The introduction of a **replication defective RETROVIRAL vector that CAN enter intact human cells into a host NOT mentioned in Part 2 of Schedule 2** IF the donor nucleic acid DOES NOT confer an oncogenic modification in humans OR encode a protein with immunomodulatory activity in humans AND (all viral genes have been removed) AND (viral genes needed for virion production meet specified conditions, to limit or prevent recombination) AND (the retroviral vector includes a specified deletion to prevent transcription after integration OR the packaging cell line and packaging plasmids meet specified conditions)

☐ **2.2 Kinds of dealing suitable for at least PC3**

☐ **Schedule 3, Part 3, Notifiable low risk dealings suitable for at least physical containment level 3 (PC3)**

Section 3. Types of GMOs

List all the GMOs to be generated and or used

Scientific name of the unmodified organism	Vectors and method of transfer	Gene identity and species of origin
E.coli	PLASMIDS - non conjugative Examples include but not limited to: pCDNA3.1, PMIG, SpCas9 and SpCas9-2A-EGFP series. E. coli strains (examples include but not limited to): TOP10, Stable2 and DH5-*α*.	One or multiple genes (wildtype or mutant) for class of genes including viral genes (including but not limited to HSV and IAV) and cellular genes (including but not limited to mOVA antigens), marker/reporter genes (including but not limited to GFP, mCherry and DsRed) and other genes including but not limited to IRES and 2A (self-cleaving 2a sequence). The immune response to these genes (wildtype or mutant) will be assessed *in vivo*, *ex vivo* and *in vitro*.
Human cell culture	Lentiviral and retroviral Transduction;	As Above.
Human cell culture (Exempt dealing)	Mammalian cell lines will be transfected by standard chemical-based or electrochemical based methods with mammalian expression vectors (e.g. pcDNA3.1-derived plasmid). Vectors expressing synthetic proteins regulatory genes (wildtype or modified) and/or reporter genes (e.g. GFP, DsRed, mCherry).	As Above.
Murine cell culture	Same as human cell culture as described above.	As Above.
Mus musculus	1. non-GM and GM mice strains (transgenic and knockout) will be utilized.	- GM mice (transgenic and KO) for class of genes (wildtype or mutant).

	2. Interbreeding of GM mice strains (transgenic and knockout).	- Viral genes (including but not limited to HSV glycoprotein B and IAV nucleoprotein). - Cellular genes (including but not limited to membrane bound chicken ovalbumin [mOVA] antigens). - Marker/reporter genes (including but not limited to GFP, mCherry and DsRed). - Other genes including but not limited to IRES and 2A (self-cleaving 2a sequence).

Section 4. Modified Trait(s) and Gene(s)

List all the GMOs to be generated and or used

Class of modified trait	Details
Bacterial cultures: Plasmid propagation	Propagation of class of genes (wildtype or mutant): - **Viral genes:** 1. IAV: I. Nucleoprotein (NP) encapsulates the negative strand IAV viral RNAs, protecting it from nucleases. Human and murine IAV infected cells will be utilized as a source of viral nucleoprotein $NP_{366-374}$ epitope to evaluate T_{CD8+} responses. 2. HSV: I. $GB_{498-505}$ viral glycoprotein B epitope will be used as an antigen source to evaluate T_{CD8+} responses. - **Cellular genes**: 1. Membrane bound chicken ovalbumin (mOVA): cell lines expressing mOVA as self-antigen under the control of the mammalian promoter. These GM cell lines will be used as source of self-antigen while T_{CD8+} responses to the $OVA_{257-264}$ (SIINFEKL peptide) will be assessed using OT-I transgenic cytotoxic T cells (OT-I: TCR transgenic line which produces MHC class I-restricted, ovalbumin-specific T_{CD8+}). - **Marker/reporter genes** (including but not limited to GFP, mCherry and DsRed). - **Other genes** including but not limited to IRES and 2A (self-cleaving 2a sequence).
Mammalian Cell lines: Protein expression	Expression of class of genes (wildtype or mutant) with or without reporter genes (e.g. GFP, DsRed, mCherry).

Mammalian Cell lines: Transduction resulting in altered protein expression (Replication incompetent)	Gene-editing technology (including but not limited to CRISPR/Cas9) with or without reporter genes (e.g. GFP, DsRed, mCherry).
Mus musculus Cell lines: Protein expression	Expression of class of genes (wildtype or mutant) with or without reporter genes (e.g. GFP, DsRed, mCherry).
Mus musculus Cell lines: Transduction resulting in altered protein expression (Replication incompetent)	Gene-editing technology (including but not limited to CRISPR/Cas9) with or without reporter genes (e.g. GFP, DsRed, mCherry).
Mice: change in cell clearance and immunity, or fluorescent macrophages (including but not limited to these trait).	These GM strains (transgenic and KO) may have altered immune cell cohorts (e.g. Batf3$^{-/-}$ line which lacks CD103^{+} DCs and CD8α^{+} DCs), leading to a change in immune responses. Some of GM mice strains contain fluorescent markers (e.g. Lang-EGFP strain where EGFP protein is expressed downstream of the internal stop codon of CD207 gene leading to EGFP^{+} Langerhans cells), which enables specific immune cell tracking.
Fluorophores	Fluorescently labelled cells
Gene-editing technology	To inactivate or functionally modify the target gene. The target genes are those that affect (including but not limited to cellular immunity, antigen processing and presentation).

Section 5. Types of GMO Dealings

Identify all relevant dealings and provide the specific procedures for each relevant category. Indicate which classification of GMO (from Section 2 of this form) the dealing is applicable to. Aim to keep descriptions broad to avoid the need to submit new applications for future work.

☒ Conduct experiments with the GMO	1. Use bacteria to prepare non-conjugative plasmids. 2. Use cell lines including transformed cell lines or primary cells derived from either GM and non-GM mice. 3. Use GM mice (transgenic and KO) for class of genes (wildtype or mutant) including viral genes (including but not limited to HSV and IAV) and cellular genes (including but not limited to mOVA antigens), marker/reporter genes (including but not limited to GFP, mCherry and DsRed) and other genes including but not limited to IRES and 2A (self-cleaving 2a sequence). 4. Transduce cells with GM retroviral and lentiviral vectors (including but not limited to PMIG and CRISPR/Cas9). Various marker genes (e.g. fluorescent, chemiluminescent, antibiotic etc.) and other genes including, but not limited to IRES, 2A self-cleaving peptide. 5. Non-GM or GM mice will be immunised with virus (including IAV strains) to study the role of dendritic cells during cross-priming.

☒ Make, develop, produce or manufacture the GMO	1. GMOs derived from either cell lines or primary cells modified by retroviral or lentiviral transduction and/or genome editing techniques (e.g. CRISPR/Cas9) for various class of genes as mentioned in *section 4*. 2. GM and non-GM mouse strains will be bred. GM and non-GM mice will be immunised with IAV and HSV to study the cross-presentation during $CD8^+$ T cell immune responses.
☒ Breed the GMO	1. GM mouse strains suitable for studying cross-presentation.
☒ Propagate the GMO	1. Cell lines including transformed cell lines or primary cells derived from either GM and non-GM mice will be propagated. 2. Generated cell lines by either transfection (non-retroviral/lentiviral vectors) and transduction (via replication defective lentiviral/retroviral vectors) of cell lines or primary cells will be propagated.
☒ Use the GMO to manufacture something that is not the GMO	GM mouse strains can generate progeny that are not GMO (e.g. from breeding heterozygotes to generate wild type littermates).
☒ Grow, raise or culture the GMO	1. GMOs derived from either cell lines or primary cells modified by retroviral or lentiviral transduction technique or transfected by standard chemical-based or electrochemical based methods with mammalian expression vectors (various fluorescent and/or antibiotic marker genes will be utilized). Bicistronic vector (containing IRES and 2A) will be utilized. 2. Production of virus including retrovirus, lentivirus, non-retrovirus including IAV and HSV.
☒ Import the GMO	GMOs will be imported in accordance with the OGTR Guidelines for Transport, Storage and Disposal of GMOs 2011 and as approved by the La Trobe IBC.
☒ Transport the GMO	GMOs will be transported in accordance with the OGTR Guidelines for Transport, Storage and Disposal of GMOs 2011 and as approved by the La Trobe IBC.
☒ Dispose of the GMO	GMOs will be disposed of in accordance with the OGTR Guidelines for Transport, Storage and Disposal of GMOs 2011 and as approved by the La Trobe IBC.
☒ Possession, supply or use of the GMO for the purposes of, or in the course of, a dealing mentioned in any of the above categories	This box is checked if any category of dealing above is also checked

Section 6. Classes of Facilities

Facilities in which the work will be conducted

Notes: Must also include details of any non-LTU facilities to be considered by the LTIBC

Building Name	Room Number(s)	Containment Level	Facility Type	OGTR Certification Number	Certification Expiry Date
LARTF	XXXX	PC2	Animal facility	Cert-XXXX	DD.MM.YYYY
LARTF	XXXX	PC1	Animal facility	Cert-XXXX	DD.MM.YYYY
LIMS1	Level x- room x	PC2	Laboratory	Cert-XXXX	DD.MM.YYYY
LIMS2	Level x- room x	PC1	Laboratory	Cert-XXXX	DD.MM.YYYY
LIMS2	Level x- room x	PC1	Instrument room	N. A	N. A

Storage outside of Physical Containment	☒	**Required**	Discuss with the LTIBC. Storage will only be approved if conditions of storage are in accordance with the *OGTR Guidelines for Transport, Storage and Disposal of GMOs*

Class(es) of Facility Approved *(For Research Office Use Only)*

This approval allows dealings in at least Physical Containment Level X (PCX) facilities as certified by the Office of the Gene Technology Regulator and approved by the LTIBC. The CI is to notify the Research Office of any changes/amendments to the list provided in this original application.

Section 7. Class(es) of Personnel

Class(es) of Personnel who may work with the GMOs

Notes: All personnel who intend to work with GMOs must complete the LTU Biosafety Training.

☒	Researchers (e.g. Post-Docs/Technicians)	*If OTHER, specifically indicate who:*
☒	LTU Students (e.g. Undergrad and Postgrad)	1. BioResources Facility (BRF) at Austin health (technicians and animal veterinarians). 2. x
☒	LARTF Personnel	
☒	Visitors (only those that will deal with a GMO)	
☒	Contractors (e.g. for waste disposal or transport)	
☒	Other	

Do Personnel have the Appropriate Training?

☒ YES ☐ NO

Notes: Records of training and trained personnel applicable to this application should be kept within the Certified Facility
If NO, then please provide an explanation/justification for LTIBC assessment

All classes of persons that will deal with GMOs under this program will be appropriately trained in accordance with La Trobe University Biosafety training.

Class(es) of Personnel Approved *(For Research Office Use Only)*

This approval allows dealings by authorised and trained persons in the classes listed above. The CI is to provide the Research Office with a list of personnel associated with this approval. The CI is to maintain a current list of personnel and notify the Research Office of any changes/amendments to the list provided in this original application.

Details of Additional Personnel

Please complete this section for each person involved in procedures on this application. All personnel must be listed and approved by the La Trobe Institutional Biosafety Committee. Please notify the LTIBC via email of any changes in personnel associated with this application following LTIBC approval.

Title/Full Name	Dr. x		
Position	Post-doctoral research fellow	**Staff ID**	X
Research Group and Department	Department of Biochemistry and Genetics	**Phone**	X
Email	X@latrobe.edu.au		
Role/Qualifications	Researcher/PhD		

Title/Full Name	Mr. X		
Position	Laboratory assistant	**Staff ID**	X
Research Group and Department	Department of Biochemistry and Genetics	**Phone**	X
Email	X@latrobe.edu.au		
Role/Qualifications	Researcher/BSc		

Title/Full Name	Mrs. X		
Position	PhD Student	**Staff ID**	X
Research Group and Department	Department of Biochemistry and Genetics	**Phone**	X
Email	X@latrobe.edu.au		
Role/Qualifications	Student		

Title/Full Name	Mrs. X		
Position	Visitor scientist	**Staff ID**	X
Research Group and Department	Department of Biochemistry and Genetics	**Phone**	X
Email	X@latrobe.edu.au		
Role/Qualifications	Research/ Visitor scientist		

If required, please provide the details of additional personnel.

Section 8. Risk Assessment and Risk Management

Note, depending on the donor DNA, a DNIR licence may be required from the OGTR

Are any of the proposed organisms or classes of GMOs potentially harmful to, or have a history of causing disease in otherwise healthy organisms?

Identify which of the organisms or GMOs are potentially harmful

☒ Humans?	**Comments:** 1. Influenza viruses to be used (H1N1 and H3N2) have either slightly or significantly impaired replication ability. 2. HSV virus to be used has a reduced infectious capacity. 3. Proposed retroviruses and lentiviruses are replication defective. 4. The GM mice do not pose any known hazard or risk to staff as the transgenic or gene knockout animals are not infectious. 5. Virally infected GM and non-GM will be treated as if it produces the virus.
☒ Animals?	**Comments:** 1. Influenza viruses to be used (H1N1 and H3N2) have either slightly or significantly impaired replication ability. 2. HSV virus to be used has a reduced infectious capacity.
☐ Plants?	**Comments:**
☒ Environment?	**Comments:** 1. Every effort will be made to prevent any unintentional release of the GMOs into the environment, by adhering strictly to the PC2 practice. 2. None of the GMOs are likely to persist outside of a laboratory setting.
☐ NO, none of the organisms or classes of GMOs are considered harmful to any of the above.	

Will any viral vectors be used in the project?

If, YES, use the OGTR tables to assist with appropriate classification

☒ YES ☐ NO	**Comments:** 1- 3rd generation lentiviral and retroviral mammalian expression vectors. 2- Herpes Simplex virus-1 (HSV-1) with reduced infectious capacity when compared with its wild type parental virus. 3- Replication-impaired IAVs with reduced infectivity.

Are the viral vectors replication defective?

☒ YES ☐ NO	**Comments:** 1. 3rd generation lentiviral and retroviral vectors: replication defective. NOTE: 1. IAVs to be used are H1N1 and H3N2 which either slightly or significantly have replication impairment. 2. HSV-1 to be used has reduced infectious capacity.

Can the vectors transduce/infect human cells?

☒ YES ☐ NO	**Comments:** 1. All researchers will be instructed to adhere to strict PC2 procedures to minimize any chance of exposure to the virus. 2. Suitable PPEs will be used.

Are any of the proposed donor DNA considered harmful to humans?

If yes, add comments.

☒ The project will deal with donor DNA considered to be a **Pathogenic Determinant**	GMO mice do not pose any known hazard or risk to staff as the transgenic or gene knockout animals are not infectious. However, when any of these mice are infected with any proposed viruses, virally infected mice will be treated as if they produce the virus.
☐ The project will deal with donor DNA considered to be **Oncogenic** in humans	
☐ The project will deal with donor DNA considered to be **Immunomodulatory** in humans	

☐ There an advantage conferred on the organism by the genetic modification	
☐ The modification increase virulence, pathogenicity or transmissibility	
☐ The GMO secretes or produces an infectious agent	
☐ The donor DNA is not characterised	
☐ The donor DNA is from a toxin producing organism(s)	
☐ NO, the donor DNA has been characterised, does not confer an advantage and is not considered harmful	

Identify Potential Hazards or Risks to People

Note, list those applicable to the proposed organisms and GMOs (e.g. exposure to allergens, infectious zoonotics, experimental agents, bites/scratches from animals).

Risk or Hazard	Proposed Controls
Working with virus	1- Herpes Simplex virus-1 (HSV-1) has a reduced infectious capacity compering with its wild type parental virus and can rarely cause extensive debilitating blisters in some patients with skin conditions, eye disease (herpetic keratitis) leading to blindness and even herpetic encephalitis. 2- Proposed replication-impaired IAVs have reduced infectivity in mice and, likely also true, in humans although no such incidence has been reported. 3- Proposed retroviruses and lentiviruses are replication defective. Viral vector systems separately provide the genes required for packaging (gag), replication and integration (pol) and infection (env) which minimises the chances of producing replication-competent virus due to recombination in the packaging cells. 4- Virally infected GM and non-GM mice will be treated as if they produce the virus. The infected mice will be identified by biohazard signs and the cage and bedding are not routinely changed for two weeks to minimize potential personnel exposure. All work will be undertaken by appropriately trained personnel within PC2 facilities (Viral work will be undertaken in BSC Class 2). The risk of accidental infection will also be reduced by ensuring that all staff wear personal protective equipment suitable to the task and specific to the laboratory/facility: 1. Laboratories: o Flu virus: gloves and lab coat (white lab coat- disposable) will be used; o Retroviral and lentiviral: gloves and lab coat (white lab coat- disposable) will be used; o HSV: long cuff gloves, safety glasses and lab coat (white lab coat- disposable) will be used; 2. Animal facility: o PPE appropriate to the task and specific to the animal facility requirement will be used as directed by the Facility (e.g. Long cuff gloves and gowns).
Aerosols from viral work	Procedures with viruses such as centrifuging and vortexing in sealed tubes can be performed outside a biological safety cabinet but the tubes will only be opened in a biological safety cabinet. Safety buckets will be used to contain potential aerosols for centrifugation outside of a biological safety cabinet. *NOTE: All work undertaken by appropriately trained personnel within PC2 facilities. Viral work undertaken in BSC Class 2. Personnel will wear PPEs suitable to the task as previously described.*
Exposure to biological agents	All work will be undertaken by appropriately trained personnel within PC2 facilities. Personnel will wear PPEs suitable to the task and facility.
Bites/Scratches from mice	• All work undertaken by appropriately trained personnel (including Animal Facility personnel). • All activities with mice will be undertaken by appropriately trained personnel and in accordance with Animal Facility procedures. • PPEs appropriate to the work and Animal Facility requirement will be used.
Needle stick from injecting mice	All work undertaken by appropriately trained personnel (including Animal Facility staff). Personal protective equipment is accordance with animal facility procedures. NOTE: Needles will be disposed using safe needle and syringe handling techniques immediately after use.
Allergic to animals	At risk researchers will wear appropriate PPEs such as a specialist face mask to minimise exposure to allergenic materials.

	If allergy happens, the researcher should stop working with animals, seek medical attention and report to LARTF staff and LTU incident and hazard reporting system.

Additional Comments:

If exposure to biological agents occur;

1. Necessarily medical attention will be undertaken and incident will be reported to the facility management and LTIBC (*via biosafety@latrobe.edu.au*).
2. Incident and hazard reporting will be followed in accordance with LTU's incident reporting system (https://www.latrobe.edu.au/incident-reporting/login.php or via direct contact to LTU Health and Safety at ohs@latrobe.edu.au or 9479 2462).

Identify Potential Hazards or Risks to the Environment

Note, list those applicable to the proposed organisms and GMOs if unintentionally released into the environment (e.g. potential to survive or persist in the environment).

Risk or Hazard	Proposed Controls
Escape of GM mice	1. All animal work will be undertaken in licenced and certified animal facilities as per LTU AEC and LTIBC approvals, respectively. Facilities which will be used for conducting proposed project have procedures in place for the management of unintentional escape. Moreover, none of GM mice used in this project would pose an advantage or expected to have Trojan effect and therefore possibility of survival or establishment in the environment is minimal. Nonetheless, risk assessment and management will be conducted to assess potential harms posed to the environment in unlikely event of GM and non-GM mice escape. 2. Mice infected with virus will be treated as if they produce the virus. Infected mice will be identified by biohazard signs and the cage and bedding are not routinely changed to minimize potential exposure. 3. In the event of GM or non-GM mouse escape from the physical-containment-certified facility, the incident cage will be identified and its lid will be properly placed to prevent further unintentional release. It should be noted that: (i) effort will be made to catch the Mouse, (ii) Preventative measures will be updated to prevent such incidents in future, and (iii) captured mouse will be either contained or euthanised (*according to the LTU AEC approvals for the project*) as deemed appropriate.
Spill of GM preparations/cultures during transport outside of containment	1. Transport to be undertaken in accordance with OGTR Transport, Storage and Disposal Guidelines. 2. GM bacterial and viral preparations/cultures cannot survive in the environment without intervention. Spills can be decontaminated with appropriate disinfectants/decontamination agents (e.g. 1% Virkon, 80% ethanol, 2% sodium hypochlorite solutions). 3. Plates and flasks containing retrovirus, lentivirus, HSV-Cre and IAVs will be kept in the glass trays when placed in the incubator (to contain incidents of spills). Incubator and biological safety cabinet(s) will also be labelled with signs indicating what type of viruses are being used.
Accidental exposure	1. Unbroken affected skin area will be washed with 80% Ethanol solution. 2. First aid guidance will be sought from the Laboratory Manager where broken skin is involved. 3. All occupational exposures to virus will be reported LTU Health and Safety, laboratory or facility managements where the incident occurred and where deemed necessary by the laboratory and facility managements to the Research Office via biosafety@latrobe.edu.au.

Additional Comments:

Incident and hazard reporting will be followed in accordance with LTU's incident reporting system (https://www.latrobe.edu.au/incident-reporting/login.php or via direct contact to LTU Health and Safety at ohs@latrobe.edu.au or 9479 2462).

Provide Details of the Action Management Plan Should an Unintended Release Occur

Provide details below. As a minimum, the plan must address the following:

1. *Relevant notifications (eg to IBC).*
2. *Noting the location(s) and extent of presence or escape.*
3. *Containment and recovery strategies (if applicable).*
4. *Methods for rendering the GMO(s) non-viable (if applicable).*
5. *Following instructions provided by representatives of the LTIBC and/or OGTR.*

Note, that in the event of a suspected unintentional release, the LTIBC must be notified immediately.

In the unlikely event that a GM organism (animal, cell line, or viral material) were to unintentionally escape into the environment, the following actions would be undertaken:

1. The LTIBC would be immediately notified, noting the nature of the unintentional release, location and extent.
2. In the event of GM or non-GM mouse escape from the physical-containment-certified facility, the incident cage will be identified and its lid will be properly placed to prevent further unintentional release. It should be noted that: (i) effort will be made to catch the Mouse, (ii) Preventative measures will be updated to prevent such incidents in future, and (iii) captured mouse will be either contained or euthanised (*according to the LTU AEC approvals for the project*) as deemed appropriate.
3. For GM cell lines, bacterial or lentiviral materials, spills can be decontaminated using appropriate disinfectants/decontamination agents (e.g. 1% Virkon, 80% ethanol, 2% sodium hypochlorite solutions).
4. If instructed, any other actions as directed by either the LTIBC and/or the OGTR.

Section 9. Transport

The transport, storage or disposal of GMOs must be undertaken in accordance with the *OGTR Guidelines for Transport, Storage and Disposal of GMOs*. This includes transport between approved facilities and institutions and the import and export of GMOs. Please keep in mind any future work you may want to undertake.

Do you intend to import GMOs into Australia? ☒ YES ☐ NO

If *Yes* – you need to ensure that you have the necessary import approvals (e.g. import permit) and that the conditions of use in that permit allow for GMOs and the activities/dealings that you require (e.g. the permit allows for *in vivo* activity if required not just *in vitro* work). Contact biosafety@latrobe.edu.au for assistance.

Will any of the GMOs be transported between facilities and/or institutions?

If, YES, refer to the OGTR Guidelines for the Transport Storage and Disposal of GMOs and complete the acknowledgement below. Include in the comments any commercial transportation companies that might be used

☒ YES ☐ NO

Comments:

Cell lines, animal tissues and viruses may be transported to between mentioned facilities (*section 6*). Other GM materials may be required to be transported to other PC facilities for collaborative purposes or to use specialist equipment.

Transport of GMOs declaration

When transporting GMOs, I, acknowledge that this must be undertaken in accordance with the *OGTR Guidelines for the Transport, Storage and Disposal of GMOs*. I understand and agree to be bound by these guidelines. I confirm that GMOs will not be transported unless:

☒ personnel transporting GMOs are appropriately trained and have read the *OGTR Guidelines for the Transport, Storage and Disposal of GMOs* and they agree to comply with and be bound by all the requirements for transport. This includes any contractors used for transport.

☒ the GMOs are appropriately contained (i.e. double contained). GMOs (e.g. micro-organisms and plants) to be transported must be wholly-contained within a sealed, unbreakable primary container. The Primary container must be packaged in a sealed, unbreakable secondary container.

☒ the outermost container is appropriately labelled to include:
1. Name, Address and Contact Details of the person responsible for the dealings.
2. A description to state that the container contains a GMO
3. A Biohazard label must be attached to any containers holding GMOs.

☒ an Emergency Response Procedure is included in the transportation documentation

☒ a documented and traceable accounting procedure is implemented ensuring that all GMOs are accounted for during and following transportation

☒ access to all GMO material will remain restricted to authorised and trained persons only. This means persons that have completed the required biosafety training or approved reputable transportation companies

☒ the external surfaces of the GMO transport containers will be decontaminated prior to transport, and the external and internal surfaces will be decontaminated (via thorough wiping of all surfaces) upon completion of transport. Decontamination will be undertaken with disinfectants appropriate to the GMO.

☒ all packaging will be disposed of through the appropriate biological waste stream or decontaminated prior to disposal

☒ Where relevant, other packaging and transport regulations will be complied with for the transport of GMOs

Section 10. Storage (if Applicable)

Is this an application for storage of GMOs outside of an authorised physical containment facility?

If Yes – this application must include full details of the GMOs to be stored, where and how the GMOs will be stored and how access will be restricted to authorised personnel only. Complete the acknowledgement below.

☒ YES ☐ NO	**Comments:** Storage of GMOs will be in accordance with the OGTR Guidelines for the Transport Storage and Disposal of GMOs. X building, Level X, Cryostore room X: Liquid Nitrogen Tanks are used to store cell lines. A list of master stock cell lines are kept ONLY by the chief investigator of this application and the primary contact person in charge of record keeping and maintenance. Access is restricted to staff only and access requires swipe cards.

Storage of GMOs declaration (if applicable)

When storing GMOs, I, acknowledge that this must be undertaken in accordance with the *OGTR Guidelines for the Transport, Storage and Disposal of GMOs*. I understand and agree to be bound by these guidelines. I confirm that an approval to store GMOs outside of authorised containment will be in accordance with the following conditions:

- ☒ Whole, viable GM animals must not be stored outside of an authorised physical containment facility without permission, in writing, from the Regulator. This restriction does not apply to the sperm, fertilised eggs or embryos of GM animals.
- ☒ Whole, viable GM plants must not be stored outside of an authorised physical containment facility without permission, in writing, from the Regulator. This restriction does not apply to the pollen, seeds, tubers, bulbs, corms or dormant stems of GM plants.
- ☒ GMOs must not be stored in a site that is prone to flooding, storm surges or other natural disasters
- ☒ GMOs, including organisms containing GMOs, being stored must be wholly contained inside a sealed, unbreakable primary container.
- ☒ GMOs for which the minimum permitted physical containment level is PC2, must be packed inside a sealed, unbreakable secondary container. In the case of a small storage unit, such as a refrigerator, freezer, or cryogenic storage container, the storage unit is permitted to be the secondary container.
- ☒ In the event of the escape, unintentional release, spill, leak or loss of GMOs from storage:
  - efforts must be implemented as soon as reasonably practicable to locate and/or retrieve the GMOs and return the GMOs to containment or render them non-viable; and
  - the incident must be reported to the Regulator as soon as reasonably practicable.
- ☒ GMOs must not be stored unless a supply of decontamination agents effective against the GMOs being stored is readily available for decontamination purposes. All containers of decontamination agents, including any solutions for decontaminating hands, must be labelled with the contents and, where necessary, the expiry date. Decontamination agents must not be used after their expiry date.
- ☒ A person supplying the GMO for storage must label the material to be stored in a manner capable of notifying any other handler of the material that the item to be stored is, or contains a GMO.
- ☒ The primary container must be labelled to clearly show the name or other identifier of the GMO being stored.
- ☒ The storage unit, or any other secondary container, must be labelled to clearly show the name and contact details of the person responsible for the dealings, so that the person can be contacted should any GMOs be spilled or lost.
- ☒ A biohazard label must be attached to the storage unit when storing any GM micro-organisms that satisfy the criteria for classification as a Risk Group 2 organism as defined in AS/NZS 2243.3.
- ☒ Procedures must be in place to ensure that all GMOs stored can be accounted for.
- ☒ A record(s) of GMOs being stored must be maintained and made available to the Regulator upon request.
- ☒ The record(s) of GMOs being stored must allow the person storing the GMOs to find the exact location of where the GMO is being stored.
- ☒ During the storage of GMOs outside of an authorised physical containment facility, access to the GMOs must be restricted, by any means that is effective, to only a person or class of persons mentioned in the LTIBC's record of assessment as having the appropriate training and experience to deal with the GMOs

Section 11. Disposal

Will GMOs be made non-viable before disposal?

List all methods (e.g. autoclaving, disinfection etc.)

Bleach treatment, autoclaving

1. GMOs will be made non-viable by treatments in accordance with appendix F of AS2243 (*e.g. for bacterial cultures, an appropriate sodium hypochlorite solution with a suitable contact time will be used. Also, cell cultures will be autoclaved prior to disposal through LIMS biological waste stream*).

2. All GM mice will be humanely euthanised at the end of the experimentation in accordance with the AEC approved humane euthanasia procedures. It should be noted that following euthanasia, GM mice remains will be disposed via the LARTF disposal procedures (or other relevant animal facilities as outlined under the AEC approved project). waste arising from licensed dealings is first autoclaved then disposed of via high temperature incineration through approved contractor. All the dead animal bodies are placed in a clinical waste bag in the waste freezer prior to disposal by high temperature incineration by an approved contractor.

What method(s) will be used for disposal?

Include the details of all methods (e.g. municipal landfill, incineration.)

Municipal landfill and incineration (in accordance with AS/NZS 2243.3: 2010 and Gene Technology Act 2000)

1. In the laboratory settings, Biological and chemical waste will be disposed of via waste management stream outlined by the laboratory and department. For example at LIMS, biological liquid waste will be decontaminated using 5000-10000 p.p.m. (0.5-1%) sodium hypochlorite which will then be disposed of via sink and flushed with water. Biological solid waste will be decontaminated by autoclaving followed by steam sterilisation through authorised contractors (Veolia).

2. LARTF: Disposal of animals follows the following approved process. Once the animal is confirmed as dead all waste is placed in a clinical waste bag and placed in the waste freezer prior to disposal by incineration by an approved contractor, currently SteriHealth. A receipt of collection is obtained and filed for records. Only LARTF staff or persons listed on the NLRD will be involved in the disposal process. All have completed the required compulsory training.

3. Tissues and organs derived from GMO mice are not viable and cannot replicate and pose no risk to staff or the environment. All tissues harvested from GMO mice will ultimately be disposed of by placing them in a biohazard bin for subsequent incineration (as above).

**These procedures comply with the Gene Technology Regulations 2001, the Gene Technology Amendment regulations 2011 and the OGTR Guidelines for the Transport, Storage and Disposal of GMOs.

** All students and staff are adequately trained and competent for handling waste and dealing with spills.

Who will be involved in the disposal of GMOs?

Also, if applicable, include the details of any centralised waste disposal within the School.

Only authorised researchers and appropriately trained personnel.

**These procedures comply with the Gene Technology Regulations 2001, the Gene Technology Amendment regulations 2011 and the OGTR Guidelines for the Transport, Storage and Disposal of GMOs

Will any Contractors be used for waste disposal?

Provide the details of any contractors, including whether they have appropriate training for GMO disposal

1. LIMS waste will be decontaminated by autoclave on site. Decontaminated waste will then be picked up by waste contractor Veolia (or other appropriate contractors authorised by LIMS) for incineration.
2. LARTF: SteriHealth or other appropriate contractors authorised by the animal facility (for disposal of non-viable biological waste)

Will persons involved in disposal be appropriately trained?

If Yes – indicate how they have been trained. If No, provide justification

☒ YES ☐ NO

Comments: Staff and student would have completed (gene technology training module) and School appointed contractors for waste disposal should have the appropriate training.

Disposal of GMOs declaration

When disposing of GMOs, I, acknowledge that this must be undertaken in accordance with the *OGTR Guidelines for the Transport, Storage and Disposal of GMOs*. I understand and agree to be bound by these guidelines. I confirm that GMOs will not be disposed of unless:

☒ personnel disposing of GMOs are appropriately trained and have read the *OGTR Guidelines for the Transport, Storage and Disposal of GMOs* and they agree to comply with and be bound by all the requirements for disposal. This includes any contractors used for disposal.

☒ GMOs, or non-GM organisms containing GMOs, are rendered non-viable prior to disposal if the method of disposal is not also the method of decontamination (e.g. incineration).

☒ Any wastes containing GMOs must be decontaminated prior to disposal if the method of disposal is not also the method of decontamination.

☒ A person supplying the GMO for disposal must label the material in a manner capable of notifying any other handler of the material that the item to be disposed of is, or contains a GMO

☒ Decontamination of GMOs must not be performed using defective equipment, expired chemical agents or any method that has not been validated as effective for the decontamination of the GMOs

☒ access to all GMO waste prior to disposal, that has not been decontaminated will remain restricted to authorised and trained persons only. This means persons that have completed the required biosafety training or approved reputable waste contractors

Section 12. Principal Investigator Declaration

By submitting this application, we, the Principle Investigator and all Project Personnel, declare that we

- ☒ have read the *Gene Technology Act 2000* and the *Gene Technology Regulations 2001* (and subsequent amendments) and agree to comply with and be bound by all the requirements of the legislation regulating the conduct of gene technology research. I have considered the ethical principles in relation to this dealing and will act accordingly;
- ☒ are aware of and abide by the Statement of Ethical Principles for Biotechnology in Victoria and the National Framework for the Development of Ethical Principals of Gene Technology;
- ☒ abide by the terms and conditions set by the LTIBC;
- ☒ will ensure that the qualifications and/or experience of all personnel involved with the project are appropriate to the procedures performed;
- ☒ will ensure that appropriate permits from relevant State or Federal agencies will be obtained and that any imposed conditions will be observed;
- ☒ will seek animal ethics and/or human ethics approval, if required;
- ☒ have successfully completed the appropriate training prior to conducting any research and will ensure that all personnel that may work with the GMOs have or will be appropriately trained;
- ☒ certify that the information contained in this application is true and accurate;
- ☒ understand that the information contained in this application is given on the basis that it remains confidential in accordance with relevant La Trobe University policies;
- ☒ will seek approval from the LTIBC for any modifications or amendments to the research prior to their implementation and understand that any amendment that varies the scope of the original proposal assessed and approved by the LTIBC may require the submission of a new application

By submitting this application, **I, the Principle Investigator**, declare that, I:

- ☒ Have obtained agreement from all personnel and will retain evidence of this agreement. This evidence may consist of a hard-copy signed document or email from personnel agreeing to participate in and abide by the conditions described in this application.

Dr. X

Name of Chief Investigator

Signed by Chief Investigator

Date: 20/05/2019

Commented [Sara Oveissi: Please sign the application following completion

Section 13. Approvals–Record of Assessment *(For Office Use Only)*

Regulation 13B(a) requires an IBC that has assessed a proposal as being a NLRD to make a record of its assessment, in a form approved by the Regulator, and specifies the information that this record must contain.

The information contained within this application and assessed by the LTIBC are in accordance with Regulation 13B(a)(i)-(x).

LTIBC Conditions/Comments

- ☐ APPROVED
- ☐ APPROVED WITH CONDITION(S)
- ☐ SUBJECT TO CHANGES TO THE SATISFACTION OF THE IBC
- ☐ DEFERRED
- ☐ NOR APPROVED, MAYBE RE SUBMITTED TO A QUORATE MEETING OF THE IBC
- ☐ NOT APPROVED

LTIBC Approval Number:		Expiry Date:	

Date:

Signed by LTIBC Chair

IBC Name:	La Trobe University	IBC Number:	
Accredited Organisation:	La Trobe University	Accreditation Number:	
OGTR Submission Number:		Submission Date:	

Section 12. OGTR Submission Template *(For Office Use Only)*

IBC_Assessed	IBC_NLRD_Identifier	Assessment_Date	IBC_Name	Notifying_Organisation*	Proposing_Organisation	Project_Title*	GMO_Details	Dealing_Type*
*Only submit NLRDs to the Gene Technology Regulator that have actually been assessed to be a NLRD. (The answer to this should be "**True**".)*	*Enter a unique identifier (this can include letters and numerals and other characters), eg. "COL 2011/43" Please do not enter extraneous information in this field such as project titles/purpose.*	*This must be a date only (no text) between 21 June 2001 and 30th June of the reporting period (future dates not permitted). Use any date format you wish - the cell will format the date automatically.*	*This is the IBC used by the organisation. e.g. ABCD Institutional Biosafety Committee.*	*Name of the organisation that submitted the NLRD proposal to the IBC. This should also be the organisation that notifies the Regulator.*	*Name of the organisation(s) proposing to undertake the NLRD. This can be a number of organisations involved in undertaking the dealing.* *DETAILS ONLY REQUIRED FOR DEALINGS ASSESSED DURING AND AFTER THE 2018-2019 REPORTING PERIOD.*	*Brief project title, for listing on OGTR website (exclude name of the supervisor). DO NOT include any CCI information in the title.*	*Genus and species (where known) and for viruses, the family. If not known, describe the GMO as best you can. For multiple GMOs, please separate by a comma, or semi colon.*	*"[1 Sep 2011]" followed by the paragraph number(s) relating to the kinds of dealing relevant for each containment level as detailed in Schedule 3 of the Gene Technology Regulations e.g. [1 Sep 2011] PC1 - (a), PC2 - (a), (m) and (l), PC3*

APPLICATION NUMBER: <<Office Use Only>>

IBC_Assessed	IBC_NLRD_Identifier	Assessment_Date	IBC_Name	Notifying_Organisation*	Proposing_Organisation	Project_Title*	GMO_Details	Dealing_Type*