Details

Application Reference : IBC/1397/MRI/TRI/2022
Title : Macrophage regulation of homeostasis and disease.
Application Status : Approved
Expiry Date : 30/3/2027
Leader : Allison Robyn Pettit
Applicant : Katharine Margaret Irvine
Contact : James Joseph Lynch
Business Unit : Translational Research Institute
Activity Type : Biological activities
Activity Category : Dealings with genetically modified organisms
Type of Dealing : NLRD

Application Form

Project description :

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The overall goal of the research program is to understand how different populations of macrophages, and their activation phenotype, influences healthy development, wound healing, tissue regeneration and disease. A powerful tool in testing the scientific hypothesis is to be able to delete or manipulate the phenotype of macrophages in vitro and in vivo using different genetically modified mouse strains.
We seek approval to conduct all types of dealings with the GMO’s described here and in the following Table of this application. That is, we wish to create, culture, propagate, grow, transport, store, possess, conduct experiments with and dispose of the proposed GMOs.

Please note the IBC identifier of any associated exempt dealing.
Associated exempt dealing :
IBC/576E/MRI/TRI/2022

Please note the IBC identifier of any previous dealing/s.
Previous dealing number :
IBC/1182/TRI/MRI/2018

IBC/1048/TRI/MRI/2016

Is this a storage only application, i.e. the only dealing that will take place is storage of an NLRD GMO(s)?
GMO storage (Y/N)? : No

Will this project involve the use of animals?
Animal use (Y/N)? : Yes

If yes, please provide the title(s) and clearance number(s) of your Animal Ethics Committee (AEC) approvals or applications if approval has not yet been given. Please note, work with animals cannot be undertaken without AEC approval.
Animal ethics : Approved
2021_AE000153 Res - Characterisation of mice expressing a fluorescent calcium indicator in macrophage lineage cells
2021/AE000181 Res - Mechanisms of macrophage-mediated metabolic regulation
2021/AE000327 Res - The role of macrophages in multiple sclerosis
2021/AE000337 Breed • Breeding of IL34 knockout rats
2021/AE000495 Res • Utilisation of CSF1R transgenic mouse strains to understand macrophage biology
2021/AE000760 Res - Investigating the functional consequences of CSF1R mutations
2021/AE000769 Breed/Res • Analysis of the phenotype of CSF1R transgenic mouse lines
2021/AE000795 Breed - Breeding of CSF1R mutant and reporter transgenic lines
2017/AE000318 The role of Csf1r signaling in homeostasis and disease
2016/AE000503 Breeding of CSF1R transgenic rat colony
2020/AE000241 The use of bone marrow transplantation to study the turnover of tissue macrophages in mice
2020/AE000355 Breeding of mice expressing a fluorescent calcium indicator in macrophage lineage cells
2019/AE000362 Analysis of the phenotype of CSF1R transgenic mouse lines
2019/AE000407 Macrophage-targeted therapy for chronic liver disease
2018/AE000424 The use of bone marrow transplantation to study the origins of tissue macrophages
2017/AE000457 Breeding of MacApple transgenic rats
2018/AE000533 Breeding of Csf1r mutant and reporter mouse lines
2019/AE000538 Breeding of mouse models of diabetes and obesity
2017/AE000566 The Impact of CSF1 Treatment on metabolism
2018/AE000157 Breed CD5L Null Mouse Breeding Colony

2019/AE000311 Breed CD169-Cre mice breeding colony
2020/AE000452 Res Enhancing macrophage resilience to improve haematopoietic stem cell transplantation outcomes
2017/000378 Breed CD169-DTR mice breeding colony
2018/AE000599 Res The role of bone marrow macrophages in clinically relevant models of stem cell transplantation

Applications under consideration
2021/AE000958 Res - Macrophage-targeted therapy in chronic liver disease
2021/AE001101 Breed - Breeding Foz/Foz Mice

Will this project involve the use of human samples?
Use of human samples (Y/N) : Yes

If yes, please provide the title(s) and clearance number(s) of your Human Research Ethics Committee (HREC) approvals or applications if approval has not yet been given. Please note, work with human samples cannot be undertaken without HREC approval.
Human ethics : HREC/99/QPAH/076 (1999/090); Q2003000092: Factors affecting the progression of liver disease and response to treatment.
HREC/MML/70236 Increasing haematopoietic stem cell niches post transplantation through enhancing bone marrow macrophage resilience and regeneration mechanisms

Type of Notifiable low risk dealing :

Dealing with a GM animal
[X] PC1(a)
[ ] PC2(a)
[ ] PC2(aa)

Dealing with a GM plant
[ ] PC2(b)

Dealing involving a lentiviral vector
[ ] PC2(i)
[ ] PC2(j)

[X] PC2(l)
[X] PC2(m)

Dealing involving an adeno or adeno associated viral vector
[ ] PC1(c)
[ ] PC2(i)
[ ] PC2(j)
[ ] PC2(k)

Dealing involving pathogenic host/vector or donor nucleic acid
[ ] PC2(c)
[X] PC2(d)
[X] PC2(e)

Dealing involving large scale culture (greater than 25L)
[ ] PC2(f)

Dealing involving complementation
[ ] PC2(g)

Dealing involving shot-gun cloning
[ ] PC2(h)

Project details :

Enter details of the proposed NLRD dealings
Proposed dealings :
Exempt Dealings: IBC/576E/MRI/TRI/2022

- Cloning - This project will use standard molecular cloning approaches as well as Gibson assembly and related methods.
-

Non-conjugative expression vectors are already introduced or will be introduced via techniques such as electroporation and heat shock in exempt strains. These include: Escherichia coli K12, B, C, Nissle 1917

Plasmids typically contain an antibiotic resistance cassette for the selection of transformants and fluorescent markers or characterised non-virulent genes such as ovulbumin.

- Tissues collected from GM Mice and Rats

Description of NLRD dealings:
PC1a work:

- Breeding of transgenic, knock-in and knockout GMO mouse and rat strains, to generate stock for inter-breeding and experimental purposes, which will be valuable experimental tools for dissecting the role of macrophages in the regulation of homeostasis and disease. Breeding will require tissue collection for DNA extraction and subsequent genotyping.

Mice types include but are not limited to:

1. Mafia (macrophage Fas-induced apoptosis) transgenic mice: express a green fluorescent protein (GFP, from Aequorea victoria) and a genetically engineered plasma membrane receptor consisting of the extracellular and transmembrane domain of the human low-affinity nerve growth factor receptor, a cytoplasmic FK506 binding protein (that has a single amino acid substitution) and the cytoplasmic Fas death domain. Transgenic construct expression is driven by mouse c-fms gene promoter elements.
2. Interleukin-4 receptor (IL-4R) alpha floxed mice: generated by gene targeting in BALB/c embryonic stem (ES) cells and Cre/loxP-specific site-specific recombination to generate IL-4Ralpha flox/flox mice
3. Lysozyme M (LysM) Cre mice: generated using a targeting vector constructed such that the cDNA for Cre was introduced into the endogenous ATG-start site within the first exon of the lysozyme M gene and electroporated into Sv129/C57B1/6/CB.20 ES cells with subsequent injection into blastocysts.
4. IL-4R alpha knockout mice: IL-4R alpha-deficient mice were generated using gene targeting with an isogenic target vector (gene derived from BALB/c) and Cre- mediated site-specific recombination in BALB/c ES cells.
5. MacGreen mice: macrophage/myeloid cells express the green fluorescent protein (GFP). Transgenic vector contained the 3.5 kb sequence flanking mouse c-fms exon 2 plus the whole intron 2 sequences driving the EGFP gene (p7.2fms-EGFP).
6. MacApple mice: macrophage/myeloid cells express the red fluorescent protein mApple. Transgenic vector contained the 3.5 kb sequence flanking mouse c-fms exon 2 plus the whole intron 2 sequences driving the mApple gene (p7.2fms-mApple).
7. CD169-Diphtheria Toxin Receptor (DTR) knock-in mice: To generate CD169-DTR mice, ES cells were transfected with the linearized targeting vector containing human DTR cDNA replacing a 28-bp fragment including the endogenous ATG start site in exon 1 of the CD169 sialoadhesion gene was replaced with human DTR cDNA with a polyA tail by a recombinant PCR technique. To

allow selection for homologous recombinants, a loxP-flanked Neo cassette was cloned downstream of the human DTR gene. The thymidine kinase gene was inserted downstream of the 3′ arm to select against random integrants.

8. CD11b-DTR mice – These transgenic mice have a transgene insert that contains a fusion product involving simian DTR and GFP under the control of the human ITGAM (integrin alpha M) promoter (CD11b) that was introduced into fertilized FVB/N donor eggs.

9. Ubiquitin C promoter (UBC-GFP) – A transgenic construct containing an enhanced GFP open reading frame under the control of the human ubiqutin C promoter was microinjected into fertilized C57BL/6 oocytes.

Rat types include but are not limited to:

- CSF1R knockout rats – CSF1R-deficient rats were generated using ES cell targeting: ES cells were transfected with a targeting vector containing the open reading frame for eGFP and a loxP-flanked Neo cassette, which replaced exon 1 of one allele of the rat Csf1r gene, preventing expression of the gene.

Controls:

- Non-GM, in vivo animal models, will be utilized in conjunction with the above mouse strains, including but not limited to: Tibial injury model, Calvarial defect model, MouseFix fracture models, Osteoporosis models (OVX), Ectopic ossification models, Haematopoietic stem cell mobilization, total body irradiation and bone marrow transplantation (BMT), liver, gut and muscle injury models.

PC2d work:
Bacteria:
Recombinant protein expression in E.coli: Standard non-conjugative cloning and expression vectors (eg Invitrogen Champion pET300/NT-DEST) transferred by chemical transformation or electroporation for recombinant protein expression. Rat and mouse genes (e.g. CSF1, CD5L, IL34, ADGRE1) will be cloned into expression vectors for the production of recombinant proteins for the following purposes:

- Immunisation of mice for the production of monoclonal antibodies
- Generation of recombinant proteins to test therapeutic potential in in vivo models.

Mutation of bacterial genes in E.coli and Klebsiella: Standard non-conjugative cloning and expression vectors transferred by chemical transformation or electroporation. Genes to be be mutated include those that encode factors associated with bacterial survival and pathogenesis (eg adherence). The impact of these modifications will be investigated by immune responses to the modified bacteria in vitro (human fluids, human cells). All work will be conducted in a BSCII with appropriate risk assessments and bacteria will be killed and disposed of with human material in

clinical waste streams at the end of the experiment. In the event the modifications confer an advantage all work will cease immediately and the IBC will be informed and work reassessed before proceeding.
Genes/sequences to be introduced and/or modified include:
1) Introduction of reporter genes (e.g. GFP and its variants derived from Aequorea victoria).
Function: confirm the transfer of genetic material to host.
2) Introduction of genes encoding proteins for in vitro recombinant protein production for immunisation of mice to produce monoclonal antibodies against the protein of interest (eg soluble and cell surface immune regulators such as CSF1, IL34, ADGRE1)
Function: protein expression for monoclonal antibody production
3) Modification of bacterial genes implicated in bacterial survival or pathogenesis (e.g. cell cycle or structural genes involved in adherence)
Function: confirm role in bacterial survival or pathogenesis.
DNA vectors will also contain characterised antibiotic resistance gene(s) such as ampicillin (Amp), tetracycline (Tet), blasticydin (Blast), and puromycin (puro)

PC2L and PC2m work
Lentivirus: Standard replication-defective viral vectors utilised for genome editing in vitro and in vivo (e.g. Addgene Lenti Cas9 2A Blast, pX330A-1x2, pX330S-2, lentiGuide-Puro). These vectors will introduce new genetic material (e.g. fluorescent reporter genes) and/or mutate existing genes and regulatory elements (eg gene knockout, protein sequence mutation, regulatory sequence mutation).
These vectors will be used to transduce cultured mammalian (human, mouse, rat) cell lines or primary cells (e.g. macrophages) for in vitro experiments.
Viral vectors will also be directly injected into wild type murine embryos. Animals will then be sacrificed at specified times before or after birth. These vectors will also be used to genetically modify somatic tissue of laboratory rodents with genes involved in immune function. Animals will be sacrificed and tissues used for biochemical studies, in vitro cultures, or will be used in behavioural studies involving live animals which will be sacrificed upon completion of the study. All work with lentivirus will be conducted in a BSCII cabinet. No genes encoding oncogenes or genes that lead to sustained cell proliferation will be used. The donor nucleic acid is incapable of correcting a defect in the vector leading to the production of replication-competent virions.
Genes/sequences to be introduced and/or modified include:
1) Reporter genes (e.g. GFP and its variants derived from Aequorea victoria).
Function: confirm the transfer of genetic material to host.
2) Site-directed recombinases (e.g. Cre and FLP recombinase)
Function: rearrange DNA segments at specific DNA sites (sequences)
3) Regulators of gene transcription (e.g. microRNA, lncRNA, transcription factors)

Function: control of gene expression
4) Cytokines (e.g. macrophage growth factors CSF1 and IL34)
Function: immune system development and function
5) Gene regulatory elements (e.g. promoter and enhancer sequences)
Function: regulation of gene expression
6) CRISPR-Cas9 elements (e.g. gRNA, Cas9)
Function: genome editing

Genes or gene fragments of interest are from mammals such as humans, mice and rats, and fruit fly, round worms or zebrafish.
Standard reporter genes are from algae or jellyfish.
Cre recombinase is derived from P1 bacteriophage
FLP recombinase is derived from yesat Saccharomyces cerevisiae
CRISPR-Cas9 is derived from bacteriophage
The donor nucleic acid is incapable of correcting a defect in the vector leading to the production of replication-competent virions.

DNA vectors will also contain characterised antibiotic resistance gene(s) such as ampicillin (Amp), tetracycline (Tet), blasticydin (Blast), and puromycin (puro)

Should we become aware that any of the genes are oncogenic, then all work is to be ceased and confirmation that the applicant has a DNIR to cover this product is confirmed. We contact Biosafety if this can not be determined and do not continue this particular modification.

Risk assessments attached
-Animal work is at TRI and PACE BRF with TRI/PACE processes. - RA 14903, RA 6462 and RA 17794
Post-exposure is also attached.
If viral material has come into contact with the mucous membranes or broken skin of a researcher, it should be treated according to the first aid protocol below.

1. Cease procedure immediately and notify supervisor.

2.Do not continue to use the sharp involved
3.Apply first aid and dispose or secure the sharp safely
4.Seek medical practitioner advice from the nearest hospital infection control clinic or Contact Clinical Unit Team Leader; OHS Manager or Health Nurse
5.Take pathogen safety data sheet or information regarding genetic modifications made to virus,

with you when you go to the consultation with a practitioner
6. Baseline testing (for all exposed or potentially exposed persons as well as source persons).
7. Submit Online Incident Report select Sharps –Contaminated as Agency of Injury.
8.Counselling and ongoing treatment, including 3- and 6- monthly follow up blood tests (if required)

Access to the lab is via security swipe after training has been completed.
Staff working on viruses will be trained to recognise virus infection and the importance of reporting any incident that may lead to an accidental infection.

Please provide details of disposal methods for GMOs, including specific details on what method or chemical will be used for disposal of different wastes e.g. liquid waste, solid waste, animal carcasses. Refer to the OGTR Transport, storage and disposal of GMO guidelines for more information.
GMO disposal :
TRI

- All waste generated will be disposed of via the documented TRI waste management guideline for waste. This involves placing in double-bagged waste bin, keeping sealed before autoclaving. All items are either decontaminated or sealed for disposal or kept for storage.
- Any liquid waste will be chemically decontaminated with an appropriately active disinfectant (i.e. Farmdyne 2% = 60mL per litre dilution– idophore disinfectant) for 30mins and then subject to autoclaving in an approved container.
- Any re-usable items will be chemically decontaminated with an appropriately active disinfectant (i.e. Farmdyne) over an adequate timeframe and then subject to autoclaving.
- Any pipette tips, serological pipettes or solid waste will be autoclaved in an approved bag or containers.
- Ensure that when using disinfectants, an appropriate contact time is allowed before the disposal of waste
- Surfaces will be wiped down with farmdyne (2% = 60mL per litre dilution) with 10 mins contact time followed by wiping with water to remove detergent followed by wiping with 80% ethanol.
- Select appropriate disinfectants (AS 2243.3).
-
- Farmdyne is not suitable as a surface disinfectant.
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- 80% (v/v) ethanol will be used for general (non-spill) disinfection
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- Work areas and contaminated metals will be disinfected with ethanol (80% v/v) after the

completion of the task, followed by a minimum of 20 minutes exposure to UV light.

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- For clean-up of spills, a daily-prepared dilution of bleach is adequate (AS 2243.3)

Animal House Waste:

- Waste within the animal facility will be disposed of in accordance with the policies/procedures in place in the animal facility. Animal infectious waste will be autoclaved before being disposed of via the animal waste stream. Mice will be frozen prior to incineration.
- Decontamination of the workspace by 80% v/v ethanol with 10 minutes contact time as per the pathogen safety data sheet.
- All liquid waste generated will be autoclaved at the end of each day prior to disposal.
- Mice will be euthanized and mice disposed of according to animal house procedures.
- Sharps waste (e.g. syringe needles) will be placed into dedicated sharps containers.
- Appropriate PPE (back- or side-fastening lab gown, gloves, covered footwear and safety glasses) will be worn at all times during work and waste disposal.
- Tissues that are harvested and analysed will be disposed of as clinical waste.

Decontamination:

- Work areas and contaminated metals will be disinfected with ethanol (80% v/v) after the completion of the task, followed by a minimum of 20 minutes exposure to UV light. Spent culture media, plastic pipette tips, cell culture items (e.g. cell scrapers) and other contaminated items will be decontaminated using bleach (1% sodium hypochlorite concentration, as this is the active ingredient) or farmdyne (4% final concentration) for a minimum of 12 hours contact time. The bleach or farmdyne will then be decanted and pipette tips/cell culture items or other contaminated materials will be discarded via the clinical waste stream.
- Be sure to note the expiry date of the bleach or farmdyne prior to use, information regarding this can be found on the bottle. Contaminated liquids containing phenol should never be mixed with bleach and liquids containing bleach must never be autoclaved.
- Appropriate PPE (back- or side-fastening lab gown, gloves, covered footwear and safety glasses) will be worn at all times during work and waste disposal/decontamination.

<u>Procedure for spills:</u>

- Minor spills in the biosafety cabinet will be decontaminated immediately. Any spills outside the BSC will be dealt with promptly following the Biological Spills Procedure and use of the PC2 Biological Spill kit available in the laboratory. All spills will be reported to the Safety Manager.
- Note: Mice will be kept in PC2-certified animal holding facilities, or for short-term

experimentation in PC2-certified laboratories (less than 1 day).

Are the GMOs being transported between facilities (either inside the same building or between buildings) or off campus?
GMO transport (Y/N)? : Yes

If yes, please indicate why it will be required and what arrangements will be made. Transport includes: between facilities listed previously, from a laboratory to an autoclave or animal house, across corridors or via lifts which are not part of a certified facility, to storage facilities external to the facility where activities are being undertaken etc.
Samples are double-contained for transport outside of laboratory environment. Primary storage of samples will be within its own container. These will be placed in a storage box. Storage boxes will be transported within designated sealed transport containers labelled with appropriate biosafety warnings. The sealed transport container will only be opened within PC2 laboratory. Dedicated animal containers are used clearly labelled and double contained between laboratories and animals are not kept overnight in PC2 facilities.

Will the dealing involve a direct import of GMOs into Australia?
Importing material : Yes

If Yes – please provide details of the import, including the Import Permit Number and whether it needs to be directed to and/or held within and Approved Arrangement facility. Please provide a copy of the import permit in the attachment section of this applications.
If the dealing involves in vivo work on non-laboratory animals (defined as all species excluding guinea pigs, hamsters, mice, rats, rabbits and microorganisms) AND you don't have specific permission in your import permit conditions, then you will need to apply to Department of Agriculture for an IVA (In vivo approval).
Does it need Approved Arrangement facility = no

People

Allison Robyn Pettit : (Project leader)
Comments :

Katharine Margaret Irvine : (Post-doctoral researcher)
Comments :

Deon Paul Knight : (Biosafety contact)
Comments :

Kim Summers : (Research academic)
Comments :

David Arthur Hume : (Research academic)
Comments :

Sahar Keshvari : (Post-doctoral researcher)
Comments :

Yajun Liu : (PhD student)
Comments :

Simranpreet Kaur : (PhD student)
Comments :

Michelle Cestari : (PhD student)
Comments :

Omkar Patkar : (Post-doctoral researcher)
Comments :

Jessica Anne Ineson : (Biosafety contact)
Comments :

Jesse Masson : (PhD student)
Comments :

Jennifer Stables : (PhD student)
Comments :

Stephen Huang : (Post-doctoral researcher)
Comments :

Ngari Joan Teakle : (Research assistant)

Comments :

Dylan Carter-Cusack : (PhD student)
Comments :

Emma Claire Maxwell : (Research assistant)
Comments :

Leanne Addison : (Biosafety contact)
Comments : Mater safety Leader

Sebastien Jacquelin : (Post-doctoral researcher)
Comments :

Location & Facilities

TRI - 1008-1077 : (Main dealing location)
Type : Suite
Comments :
PC2 Facility certification : Not currently held
PC2 Animal Facility : (Licence Number: 3592 (AN)) Valid to 20/11/2022
PC1 Facility Certification : Not currently held
PC2 Insectary : Not currently held
PC2 Plant facility certification : Not currently held
PC3 Facility Certification : Not currently held

TRI - 1000-1079A : (Main dealing location)
Type : Suite
Comments :
PC2 Facility certification : Not currently held
PC2 Animal Facility : (Licence Number: 3593 (AN)) Valid to 20/11/2022
PC1 Facility Certification : Not currently held
PC2 Insectary : Not currently held
PC2 Plant facility certification : Not currently held
PC3 Facility Certification : Not currently held

PACE - 3033-3123 : (Main dealing location)
Type : Suite
Comments :
PC2 Facility certification : Not currently held
PC1 Facility Certification : Not currently held
PC2 Insectary : Not currently held
PC2 Plant facility certification : Not currently held
PC3 Facility Certification : Not currently held
PC2 Animal Facility : (Licence Number: 3014) Valid to 22/12/2024

TRI - 4018-4104 : (Main dealing location)
Type : Suite
Comments :
PC2 Facility certification : (Licence Number: 3602) Valid to 20/11/2022
PC2 Animal Facility : Not currently held
PC1 Facility Certification : Not currently held
PC2 Insectary : Not currently held
PC2 Plant facility certification : Not currently held
PC3 Facility Certification : Not currently held

TRI - 4045-4113 : (Main dealing location)
Type : Suite
Comments :
PC2 Facility certification : (Licence Number: 3603) Valid to 20/11/2022
PC2 Animal Facility : Not currently held
PC1 Facility Certification : Not currently held
PC2 Insectary : Not currently held
PC2 Plant facility certification : Not currently held
PC3 Facility Certification : Not currently held

RBWH building 59 - 101-235 : (Main dealing location)
Type : Suite
Comments :
PC2 Facility certification : Not currently held
PC2 Animal Facility : (Licence Number: 1272 (AN)) Valid to 25/05/2023
PC1 Facility Certification : Not currently held

PC2 Insectary : Not currently held
PC2 Plant facility certification : Not currently held
PC3 Facility Certification : Not currently held

Materials

Material Type : NLRD Material May 2019
Common Name : 2. Mouse and Rodents
Scientific Name : Mus musculus, Rattus norvegicus
Organism/sample type : Animal
Risk group : Risk group 1
Strain/species details : All mouse strains described below are C57BL/6 Wild type background (Source: Jackson Lab,https://www.jax.org/strain/000664).
Rat strains are Sprague Dawley or Dark Agouti wild type background
Vector and method of transfer of genetic material : Mus musculus -Transgene injected into pronuclei of fertilized eggs.

Transgene electroporated into ES cells with subsequent injection into blastocysts.
Gene knockout via isogenic target vector and Cre- mediated site-specific recombination with subsequent injection into blastocysts.

Rattus Norvegicus -Transgene injected into pronuclei of fertilized eggs.

Transgene electroporated into ES cells with subsequent injection into blastocysts.
Gene knockout via isogenic target vector and Cre- mediated site-specific recombination with subsequent injection into blastocysts.

Mafia (macrophage Fas-induced apoptosis) transgenic mice: express a green fluorescent protein (GFP, from Aequorea victoria) and a genetically engineered plasma membrane receptor consisting of the extracellular and transmembrane domain of the human low-affinity nerve growth factor receptor, a cytoplasmic FK506 binding protein (that has a single amino acid substitution) and the cytoplasmic Fas death domain. Transgenic construct expression is driven by mouse c-fms gene promoter elements.
Interleukin-4 receptor (IL-4R) alpha floxed mice: generated by gene targeting in BALB/c embryonic stem (ES) cells and Cre/loxP-specific site-specific recombination to generate IL- 4Ralpha flox/flox mice

Lysozyme M (LysM) Cre mice: generated using a targeting vector constructed such that the cDNA for Cre was introduced into the endogenous ATG-start site within the first exon of the lysozyme M gene and electroporated into Sv129/C57B1/6/CB.20 ES cells with subsequent injection into blastocysts.
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MacApple mice: macrophage/myeloid cells express the red fluorescent protein mApple. Transgenic vector contained the 3.5 kb sequence flanking mouse c-fms exon 2 plus the whole intron 2 sequence driving the mApple gene (p7.2fms-mApple).
CD169-Diphtheria Toxin Receptor (DTR) knock in mice: To generate CD169-DTR mice, ES cells were transfected with the linearized targeting vector containing human DTR cDNA replacing a 28-bp fragment including the endogenous ATG start site in exon 1 of the CD169 sialoadhesion gene was replaced with human DTR cDNA with a polyA tail by a recombinant PCR technique. To allow selection for homologous recombinants, a loxP-flanked Neo cassette was cloned downstream of the human DTR gene. The thymidine kinase gene was inserted downstream of the 3′ arm to select against random integrants.
CD11b-DTR mice – These transgenic mice have a transgene insert that contains a fusion product involving simian DTR and GFP under the control of the human ITGAM (integrin alpha M) promoter (CD11b) that was introduced into fertilized FVB/N donor eggs.
Ubiquitin C promoter (UBC-GFP) – A transgenic construct containing an enhanced GFP open reading frame under the control of the human ubiqutin C promoter was microinjected into fertilized C57BL/6 oocytes.
Actin-RFP mice – A transgenic construct containing red fluorescent protein (RFP) open reading frame under the control of the xx actin promoter
Cg-Csf1rtm1Jwp/J (commonly known as Csf1rfl/fl): Csf1rfl/fl floxed mutant mice possess loxP sites flanking exon 5 of the colony-stimulating factor 1 receptor (Csf1r) gene, which do not interrupt gene transcription.
Rats

CSF1R knockout rats – CSF1R-deficient rats were generated using ES cell targeting: ES cells were transfected with a targeting vector containing the open reading frame for eGFP and a loxP-flanked Neo cassette, which replaced exon 1 of one allele of the rat Csf1r gene, preventing expression of the gene.

OGTR exempt host/vector system (Y/N)? : No

Identity, function and origin of transferred/mutated gene/s : Standard laboratory antibiotic resistance genes
Reporter and marker genes eg GFP
Whole, partial and truncated mammalian genes involved in macrophage regulation of homeostasis and disease.
Knockouts of the above murine genes
Promoter sequences associated with the above genes
Cre/Lox recombinase system

Mafia (macrophage Fas-induced apoptosis) transgenic mice: express a green fluorescent protein (GFP, from Aequorea victoria) and a genetically engineered plasma membrane receptor consisting of the extracellular and transmembrane domain of the human low-affinity nerve growth factor receptor, a cytoplasmic FK506 binding protein (that has a single amino acid substitution) and the cytoplasmic Fas death domain. Transgenic construct expression is driven by mouse c-fms gene promoter elements.
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Rats

CSF1R knockout rats – CSF1R-deficient rats were generated using ES cell targeting: ES cells were transfected with a targeting vector containing the open reading frame for eGFP and a loxP-flanked Neo cassette, which replaced exon 1 of one allele of the rat Csf1r gene, preventing expression of the gene.
Cg-Csf1rtm1Jwp/J (commonly known as Csf1rfl/fl): Csf1rfl/fl floxed mutant mice possess loxP sites flanking exon 5 of the colony-stimulating factor 1 receptor (Csf1r) gene, which do not interrupt gene transcription.

Organism or tissue to be used with the GMO : None
NLRD category : PC1(a)
Biosecurity (quarantine) material (Y/N)? : No

Material Type : NLRD Material May 2019
Common Name : 1. Escherichia Coli
Scientific Name : Escherichia coli K12, exempt strains
Organism/sample type : Bacterial
Risk group : Risk group 1
Strain/species details : Exempt strains resistant to standard laboratory antibiotics. Antibiotics used are not clinically relevant to the organism in question or are obsolete and no longer in clinical use.
Vector and method of transfer of genetic material : (i) Non-conjugative plasmid vectors (pBluescript, pEF/myc/cyto, pCDM8, pCDNA, pIRES).
(ii) Heat shock or electroporation of cDNAs into E. coli.
OGTR exempt host/vector system (Y/N)? : No
Identity, function and origin of transferred/mutated gene/s : Genes/sequences to be introduced and/or modified include:

1) Introduction of reporter genes (e.g.GFP and its variants derived from Aequorea victoria).
Function: confirm the transfer of genetic material to host.
2) Introduction of genes encoding proteins for in vitro recombinant protein production for immunisation of mice to produce monoclonal antibodies against the protein of interest (eg soluble and cell surface immune regulators such as CSF1, IL34, ADGRE1)
Function: protein expression for monoclonal antibody production
3) Modification of bacterial genes implicated in bacterial survival or pathogenesis (e.g. cell cycle or structural genes involved in adherence)
Function: confirm role in bacterial survival or pathogenesis.
DNA vectors will also contain characterised antibiotic resistance gene(s) such as ampicillin (Amp), tetracycline (Tet), blasticydin (Blast), and puromycin (puro)

Organism or tissue to be used with the GMO : Mice, mammalian cell lines

NLRD category : PC(e)

Biosecurity (quarantine) material (Y/N)? : No

Material Type : NLRD Material May 2019

Common Name : 3. Klebsiella pneumoniae

Scientific Name : Klebsiella pneumoniae

Organism/sample type : Bacterial

Risk group : Risk group 1

Strain/species details : Klebsiella pneumoniae

Vector and method of transfer of genetic material : Standard non-conjugative cloning vectors and standard expression plasmids (eg pQF50-Cm), transferred by chemical transformation or electroporation

OGTR exempt host/vector system (Y/N)? : No

Identity, function and origin of transferred/mutated gene/s : Genes to be modified:bacterial genes implicated in bacterial survival or pathogenesis (e.g. cell cycle or structural genes involved in adherence)
Function: confirm role in bacterial survival or pathogenesis.
DNA vectors will also contain characterised antibiotic resistance gene(s) such as ampicillin (Amp), tetracycline (Tet), blasticydin (Blast), and puromycin (puro)

In the event that modifications confer an advantage, all work will cease immediately, the IBC/Biosafety will be informed, and work reassessed before proceeding

Organism or tissue to be used with the GMO : Human fluids (ascites fluid, blood, urine) and interaction with human cells (macrophages and epithelial cells).

Bacteria will be killed and disposed of with human material in clinical waste streams at the end of the experiment.

NLRD category : PC2(d)

Biosecurity (quarantine) material (Y/N)? : No

Material Type : NLRD Material May 2019

Common Name : 5. Lentivirus

Scientific Name : Lentivirus

Organism/sample type : Virus

Risk group : Not applicable

Strain/species details : Replication-defective lentiviral vectors (eg.pLV-mCherry)(as defined according to OGTR Regulations Schedule 3 Part 2, 2.1 (l)).
Created in the laboratory from plasmids or purchased high titre Lentivirus particles (amphotropic) sourced from Addgene or similar commercial supplier.

Vector and method of transfer of genetic material : (i)Lentiviral plasmid vectors (pMD2G, psPAX2, pL-CRISPR.EFS, pLX_TRC311/NLS-Cas13d-P2A-Blast, lenticas9-blast, pLentiRNAGuide, pLentiguide-puro)
(ii) Virus transduction following virus production, plasmid transfection, plasmid electroporation.
The procedure is as follows:
Live virus is added to the cells in vitro.After a specific time course, the cells can be assessed for transduction efficiency (the viruses express a fluorescent protein). The cells are collected, spun down in a centrifuge, and the supernatant (media containing the virus) is discarded. The cells are washed three times in PBS and re-suspended in virus-free media.
No live virus remains.

OGTR exempt host/vector system (Y/N)? : No

Identity, function and origin of transferred/mutated gene/s : These Genome editing related vectors use CRISPR CAS technology to target (knock out, inhibit or activate gene/RNA expression).
These DNA vectors will typically contain:

characterised antibiotic resistance gene(s) such as ampicillin (Amp), tetracycline (Tet), blasticydin (Blast), and puromycin (puro)
CRISPR cas enzyme (cas9 and cas13d)
GUIDE RNA (SgRNA) targeting mouse, rodents and human gene or transcript
Reporter gene such as fluorescent proteins (GFP, YFP, RFP, CFP, ZSgreen, mcherry)
Envelop and packaging gene necessary for virus particle production In the case of PMD2G AND PSPAX2 vectors.

Site-directed recombinases (e.g. Cre and FLP recombinase)

Genome sequences to be targeted include protein coding and gene regulatory sequences, e.g.:
1) Regulators of gene transcription (e.g. microRNA, lncRNA, transcription factors)
Function: control of gene expression
2) Cytokines (e.g. macrophage growth factors CSF1 and IL34)
Function: immune system development and function
3) Gene regulatory elements (e.g. promoter and enhancer sequences)
Function: regulation of gene expression

Organism or tissue to be used with the GMO : mouse cell lines and primary cells for example:
Mouse cell lines: P19, C2C12,AIP WT-fibroblasts, 17F4.F4, D12,NIH3T3
Rat cell lines: PC12, H9c2 cells
Hamster cell lines: CHO cells
Monkey cell lines: COS cells
Human cell lines: HEK 293T, IMR32, HUVEC, SH-SY5Y, HASMC, THLE-3, HK-2, WT 9-12, hTERT.

NLRD category : PC2(I)

Biosecurity (quarantine) material (Y/N)? : No

Material Type : NLRD Material May 2019

Common Name : 4. Mammalian Cells (lentivirus packaging)

Scientific Name : Mammalian Cell lines

Organism/sample type : Cell line

Risk group : Risk group 1

Strain/species details : Mammalian cell lines such as HEK293, HEK293T and similar HEK cell line variants will be used to package lentivirus.

Vector and method of transfer of genetic material : Retroviral (lentiviral) vectors will be constructed in vitro using packaging vectors (e.g. psPAX2, pMDLg/pRRE, pRSV-Rev, pMD2.G) purchased from commercial suppliers and transfer vectors containing the genes of interest (eg.pLV-mCherry). For all replication-defective retroviral (lentiviral) vectors to be used in this dealing, the following will apply (see Schedule 3 Part 2(I) OGTR Regulations 2001):
(i) viral genes have been removed from the retroviral vector so that it cannot replicate or assemble into a virion without these functions being supplied in trans; and
(ii) viral genes needed for virion production in the packaging cell line are expressed from

independent, unlinked loci with minimal sequence overlap with the vector to limit or prevent recombination; and
(iii) either:
(A) the retroviral vector includes a deletion in the Long Terminal Repeat sequence of DNA that prevents transcription of genomic RNA following integration into the host cell DNA; or
(B) the packaging cell line and packaging plasmids express only viral genes gagpol, rev, and an envelope protein gene, or a subset of these. NB. If the second generation packaging plasmid psPAX2 is used (expresses viral genes gagpol, rev, and tat), the system will be neither second nor third generation, however the transfer vector used will include a deletion in the Long Terminal Repeat sequence of DNA that prevents transcription of genomic RNA following integration into the host cell DNA in order to comply with the OGTR classification (Schedule 3 Part 2(l) OGTR Regulations 2001).

OGTR exempt host/vector system (Y/N)? : No

Identity, function and origin of transferred/mutated gene/s : 1) Reporter genes (e.g.GFP and its variants derived from Aequorea victoria).
Function: confirm the transfer of genetic material to host.
2) Site-directed recombinases (e.g. Cre and FLP recombinase)
Function: rearrange DNA segments at specific DNA sites (sequences)
3) Regulators of gene transcription (e.g. microRNA, lncRNA, transcription factors)
Function: control of gene expression
4) Cell cycle regulators (e.g. p53)
Function: control of cell cycle
5) Cytoskeletal proteins (e.g. WDR62)
Function: cellular structure, motility, organelle movement
6) CRISPR-Cas9 elements (e.g. gRNA, Cas9)
Function: genome editing
7) Reprogramming genes (e.g. OCT4, SOX2, KLF4, MYC)
Function: Reprogram somatic cells to become pluripotent stem cells
Genes or gene fragments of interest are from mammals such as humans, mice and rats, and fruit fly, round worms or zebrafish.
Standard reporter genes are from algae or jellyfish.
Cre recombinase is derived from P1 bacteriophage
FLP recombinase is derived from yesat Saccharomyces cerevisiae
CRISPR-Cas9 is derived from bacteriophage
The donor nucleic acid is incapable of correcting a defect in the vector leading to the production of replication-competent virions.
No genes encoding oncogenes, immuno-modulatory genes, or genes that lead to sustained cell proliferation will be used. Should we become aware that any of the genes are oncogenic

orimmunomodulatory, then all work is to be ceased and confirmation that the applicant has a DNIR to cover this product is confirmed. We contact Biosafety if this can not be determined and do not continue this particular modification.

Organism or tissue to be used with the GMO : None

NLRD category : PC2(I)

Biosecurity (quarantine) material (Y/N)? : No

Material Type : NLRD Material May 2019

Common Name : 6. Lentivirus (Lentivirus injection into rodents)

Scientific Name : Lentivirus

Organism/sample type : Virus

Risk group : Risk group 2

Strain/species details : Replication-defective retroviral vectors and replication-defective lentiviral vectors (as defined according to OGTR Regulations Schedule 3 Part 2, 2.1 (l)).
Created in the laboratory from plasmids or purchased high titre Lentivirus particles (amphotropic) sourced from Addgene.

Vector and method of transfer of genetic material : Replication-defective retroviral vectors (e.g. pLV-mCherry,pHIV-Luc-ZsGreen, FUGW) synthesised by us or by a commercial company such as Addgene.
Vectors are able to transduce human cells, but are self inactivating and/or accessory genes are not present.
CRISPR genome editing will be performed by the UQ Transgenic Animal Service (TASQ) under SOPs. Genetically modified progeny will be transferred to TRI for breeding and use in approved experiments.
Should we become aware that any of the genes are oncogenic or immunomodulatory, then all work is to be ceased immediately and Biosafety notified to allow for DNIR assessment before continuing.

OGTR exempt host/vector system (Y/N)? : No

Identity, function and origin of transferred/mutated gene/s : These Genome editing related vectors use CRISPR CAS technology to target (knock out, inhibit or activate gene/RNA expression).
These DNA vectors will typically contain:

characterised antibiotic resistance gene(s) such as ampicillin (Amp), tetracycline (Tet), blasticydin (Blast), and puromycin (puro)
CRISPR cas enzyme (cas9 and cas13d)
GUIDE RNA (SgRNA) targeting mouse, rodents and human gene or transcript

Reporter gene such as fluorescent proteins (GFP, YFP, RFP, CFP, ZSgreen, mcherry)
Envelop and packaging gene necessary for virus particle production In the case of PMD2G AND PSPAX2 vectors.
Site-directed recombinases (e.g. Cre and FLP recombinase)

Genome sequences to be targeted include protein coding and gene regulatory sequences, e.g.:
1) Regulators of gene transcription (e.g. microRNA, lncRNA, transcription factors)
Function: control of gene expression
2) Cytokines (e.g. macrophage growth factors CSF1 and IL34)
Function: immune system development and function
3) Gene regulatory elements (e.g. promoter and enhancer sequences)
Function: regulation of gene expression
NB: Immunomodulatory genes or oncogenes will notbe used with replication-defective lentiviral vectors.

Organism or tissue to be used with the GMO : Rodent - Mus musculus Mice: Wildtype

NLRD category : PC2(m)

Biosecurity (quarantine) material (Y/N)? : No

Risk Assessments

Risk Number : 14903
Title : UQDI Slape - Animal Handling

Risk Number : 6462
Title : UQDI Slape - PC2 Facility Laboratory work (general microwave, centrifuge, water bath, heatblock use)

Risk Number : 17794
Title : UQDI - Slape Lab - Working with GMO mice

Attachments

Name : Post Exposure protocol - animal_mice.docx
Comments :

Name : BIOGRAM_English.pdf

Comments :

Name : FARMDYNE_English_SDS.pdf
Comments :

Name : TRI facilities for NLRD forms - v4.0 - May2021.docx
Comments :

Name : Approval Letter UQ Safe 1397 ref 1326 Mar3 2022.pdf
Comments :

Declarations

Person : David Arthur Hume
Type : Approved
Declared By : Sophie O'Neill
Date of Declaration : 31/03/2022
Status : Accept

Person : Michelle Cestari
Type : Approved
Declared By : Sophie O'Neill
Date of Declaration : 31/03/2022
Status : Accept

Person : Leanne Addison
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Status : Accept