RETROVIRAL PROJECT CLASSIFICATIONS

As per Gene Technology Regulations 2019

INSTITUTIONAL BIOSAFETY COMMITTEE

University of South Australia

The purpose of this document is a guide to classify retroviral projects under the Gene Technology Regulations: 2019.
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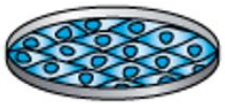
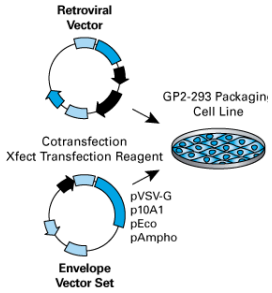
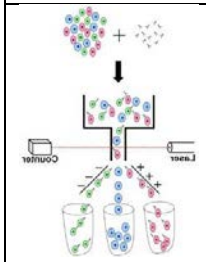
Reviewed by UniSA Institutional Biosafety Committee

This document refers to the IBC-2.6_GMO-Appl_Form.
All cultures in this example are under 25 litres in volume.
All host and target cells are less than Risk Group 3.
The classifications are for *invitro* studies only.

	Importing into UniSA or inheriting from a colleague, and storage of a packaging cell line which were previously genetically modified.	Exempt Item 4 Part 1 of Schedule 2 Item 9 of Part 2 of Schedule 2
	Importing into UniSA or inheriting from a colleague, and storage of retroviral vector plasmid and envelope vector plasmids.	Not gene technology Naked plasmid DNA that is not capable of giving rise to infectious agents when introduced to a host cell is not classified as gene technology.
	Culture of packaging cell line	Exempt Item 4 Part 1 of Schedule 2 Subject to Item 9 of Part 2 of Schedule 2

	Transfect packaging cell line with plasmids. And culturing less than 25 litres of transduced cell line.	Exempt Item 4 Part 1 of Schedule 2 Item 9 of Part 2 of Schedule 2 Tissue culture of human cells transduced with plasmids which contain a viral sequence which cannot give rise to infectious agents without additional non-host genes or gene products.
	Collect, concentrate, purify and titrate genetically modified replication defective retrovirus, able to transduce human cells.	NLRD PC2.1 (l)(i)(ii)(iii)(A) or (B) If the supernatant from the cell culture contains a replication defective retroviral vector able to transduce human cells. Or NLRD PC2.1 (m)(i)(ii)(iii)(iv)(A) or (B) If the supernatant from the cell culture contains a replication defective retroviral vector able to transduce human cells and a host not mentioned in Part 2 of Schedule 2.
	Transduction of target cells with replication defective retrovirus able to transduce human cells. Culture of GM target cells which still contain the GM retrovirus.	NLRD PC2.1 (l)(i)(ii)(iii)(A) or (B) If the supernatant from the cell culture contains a replication defective retroviral vector able to transduce human cells. Or NLRD PC2.1 (m)(i)(ii)(iii)(iv)(A) or (B) If the supernatant from the cell culture contains a replication defective retroviral vector able to transduce human cells and a host not mentioned in Part 2 of Schedule 2.
	GM target cells after 3-7 passages (no retrovirus present in culture) Enrichment of GM target cells, such as by FACS or antibody staining. Clonal expansion of the enriched cell line, less than 25 litres.	Exempt Item 4 Part 1 of Schedule 2 Item 9 of Part 2 of Schedule 2 After 3-7 passages the retrovirus has stopped replicating and has been removed through each change of media. Therefore, the genetically modified cell line/culture is the only GMO present in the culture and is classified as Exempt.

	Injecting GM target cells into mice	Exempt or PC1.1 (a) The cells are Exempt dealings, but the mouse may or may not be genetically modified. If the mouse is wild type, such as SCIDγ, nude or Balb/c, then the dealing is exempt. If the mouse was modified by SDN1 methods, they are not classified as genetically modified and the dealing is Exempt If the mouse was genetically modified then the dealing is classified as PC1.1(a)

Example of Table 4B Completion of GMO Application Form

4B	**Record of GMOs**				
NOTE: Please supply as many specific details as possible. This table is intended to be a concise, accurate record of all the GMOs to be generated or used. However, these details should not be so narrow as to preclude foreseeable and intended work which would then necessitate a new approval.					
COMMON NAME OF THE HOST ORGANISM (Organism that is/will be genetically modified)	**SCIENTIFIC NAME OF THE HOST ORGANISM**	**VECTOR(S) & METHOD OF TRANSFER**	**EXEMPT HOST/VECTOR SYSTEM (Yes/No)**	**GENETIC MODIFICATION IDENTITY, FUNCTION, ORGANISM OF ORIGIN**	**DEALING TYPE**
HEK-293T cells Human Embryonic Kidney Cells Packaging Cell Line	*Homo sapiens*	(1) plasmids; (2) replication defective viral vectors unable to transduce human cells;	Yes Yes	This is an intermediate step in viral production. Transgenes (list of transgenes) are introduced by plasmid transfection	Exempt Schedule 2 Part 2 Item 9 a) Tissue culture host cells and vectors 1) plasmids; 2) replication defective viral vectors unable to transduce human cells

Moloney Murine Leukemia Virus	*retroviridae (oncovirinae) Moloney Murine Leukemia Virus*	Transient transfection and transfer of packaging plasmids into the packaging cell line.	No	Replication defective ecotropic viral particles encoding the transgenes. Modified genes (list of modified genes) This virus has a pseudotyped with an amphotropic envelope of the gibbon ape leukaemia virus (GALV) or RD114.	PC2.1 (l)(i)(ii)(iii)(B)
Lymphocytes Target Cells	*Homo sapiens*	Replication defective amphotropic viral vector	No	Modified genes (list of modified genes)	PC2.1 (l)(i)(ii)(iii)(B) Transduced by virus
Lymphocytes	*Homo sapiens*	No transduction – lymphocytes previously modified by viral vector	Yes	Modified genes (list of modified genes)	Exempt Schedule 2 Part 2 Item 9 a) 1) FACS sorting 2) Clonal expansion
CDC Knockout Mouse *(Note – only list here animals which have not been modified by SDN1 methods)*	*Mus musculus*	No transduction – mouse previously modified	No	CDC $^{-/-}$ (List the modified gene/s)	PC1.1(a) Host for modified cells

Definitions

Amphotropic viral vector: Virus which can infect most mammalian cells. Can infect cells of both original and heterologous species.

Ecotropic viral particles: Can only infect cells of the original species.

Packaging cell line: packaging cell line means an animal or human cell line that contains a gene or genes that when expressed in trans are necessary and sufficient to complement packaging defects of a replication defective viral vector in order to produce packaged replication defective virions;

Replication defective: Mutant viruses which are defective for viral functions that are essential for viral genome replication and assembly of progeny virus particles. They can only replicate if expressed in complementing cell lines (packaging cell lines) that express the missing viral gene products.

Office of Gene Technology Regulator guidance on the classification of contained dealings with viral vectors

Australian Government
Department of Health
Office of the Gene Technology Regulator

Guidance on the classification of contained dealings with viral vectors*

Replication defective non-retroviral vectors			
Viral vector type	**Characteristics of donor nucleic acid, donor organism or modification**	***In vitro*[1]**	***In vivo***
Any	toxin or uncharacterised gene from toxin producing organism	DNIR, S3 p3.1 (a), (b) or (c)	
	genes whose expressed products are likely to increase the capacity of the viral vector to induce an autoimmune response	DNIR, S3 p3.1 (h)	
	creates novel replication competent virus with increased capacity to cause harm (e.g: new potential host species or mode of transmission; or increased virulence or transmissibility)	DNIR, S3 p3.1 (i)	
	can modify an organism so as to increase the likelihood of inheritance of particular nucleotide sequence(s) (i.e. create an engineered gene drive)	DNIR, S3 p3.1 (s)	
Risk Group 4 virus[2]	any	DNIR, S3 p3.1 (p)	
Risk Group 3 virus[2]	any	DNIR, S3 p3.1 (q) if not in an appropriate PC3 facility	
Unable to transduce human cells (and not Risk Group 3 [2])	unlikely to increase capacity to cause harm; cultures used are ≤ 25 L	Exempt, S2 p1 item 4	NLRD, S3 p2.1 (i)
	unlikely to increase capacity to cause harm; cultures used are > 25 L	NLRD, S3 p2.1 (f)	N/A
	may increase capacity to cause harm; uncharacterised nucleic acid from a pathogen	NLRD, S3 p2.1 (e)	NLRD, S3 p2.1 (i)
Able to transduce human cells: *Human adenovirus* or *Adeno associated virus*	does not confer an oncogenic modification or immunomodulatory effect in humans; not a toxin	NLRD, S3 p1.1 (c)	NLRD, S3 p2.1 (k)
	confers an oncogenic modification or immunomodulatory effect in humans; not a toxin	NLRD, S3 p2.1 (j)	DNIR, S3 p3.1 (d)
	would impair practical treatment of any disease or abnormality caused by the virus (e.g. drug resistance)	DNIR, S3 p3.1 (o)	
Able to transduce human cells: all other viruses	not a toxin	NLRD, S3 p2.1 (j)	NLRD, S3 p2.1 (k)
	oncogenic modification or immunomodulatory in humans	NLRD, S3 p2.1 (j)	DNIR, S3 p3.1 (d)
	would impair the practical treatment of any disease or abnormality caused by the virus (e.g. drug resistance)	DNIR, S3 p3.1 (o)	

DNIR = dealing not involving intentional release, exempt = exempt dealing, NLRD = notifiable low risk dealing; p = Part (of the Regulations); S = Schedule (of the Regulations)

[1] In cell or tissue culture, as packaged virions without a host, or naked vector nucleic acid (if the nucleic acid can produce infectious particles when introduced into a suitable host cell).

[2] Unmodified parent virus satisfies the criteria in AS/NZS 2243.3:2010 for classification in the indicated Risk Group.

*** Guidance only – refer to detail in the applicable clauses of the Gene Technology Regulations 2001, as current at the time. This guidance reflects the Commonwealth Regulations incorporating amendments from Schedule 1 of the Gene Technology Amendment (2019 Measures No. 1) Regulations 2019, which commence on 8 October 2019.**

Document last reviewed June 2021

Guidance on the classification of contained dealings with viral vectors*

Replication defective* retroviral *vectors			
Viral vector type	**Characteristics of donor nucleic acid, donor organism or modification**	***In vitro*[1]**	***In vivo***
Any	toxin or uncharacterised gene from toxin producing organism	DNIR, S3 p3.1 (a), (b) or (c)	
	genes whose expressed products are likely to increase the capacity of the virus/viral vector to induce an autoimmune response	DNIR, S3 p3.1 (h)	
	creates novel replication competent virus with increased capacity to cause harm (e.g: new potential host species or mode of transmission; or increased virulence or transmissibility)	DNIR, S3 p3.1 (i)	
	would impair practical treatment of any disease or abnormality caused by the viral vector (e.g. drug resistance)	DNIR, S3 p3.1 (o)	
	can modify an organism so as to increase the likelihood of inheritance of particular nucleotide sequence(s) (i.e. create an engineered gene drive)	DNIR, S3 p3.1 (s)	
Unable to transduce human cells	unlikely to increase capacity to cause harm; cultures used are ≤ 25 L	Exempt, S2 p1 item 4	NLRD, S3 p2.1 (i)
	unlikely to increase capacity to cause harm; cultures used are > 25 L	NLRD, S3 p2.1 (f)	N/A
	may increase capacity to cause harm (e.g. pathogenic determinant); not a toxin	NLRD, S3 2.1 (e)	NLRD, S3 p2.1 (i)
Able to transduce human cells[3]: Self inactivating **and/or** accessory genes **not** present	does not confer an oncogenic modification or immunomodulatory effect in humans; not a toxin	NLRD, S3 p2.1 (l)	NLRD, S3 p2.1 (m)
	confers an oncogenic modification or immunomodulatory effect in humans; not a toxin	NLRD, S3 p2.1 (l)	DNIR, S3 p3.1 (d) & (j)
Able to transduce human cells: not self inactivating **and** accessory genes **are** present	does not confer an oncogenic modification and not immunomodulatory effect in humans; not a toxin	DNIR, S3 p3.1 (j)	
	oncogenic modification or immunomodulatory in humans	DNIR, S3 p3.1 (d) & (j)	
Risk Group 4 virus[2]	any	DNIR, S3 p3.1 (p)	

[3] As well as including one of the indicated safety features to reduce the likelihood of recombination leading to replication competence being regained, additional requirements apply, including that all viral genes must be removed from the vector and only *gagpol*, *env rev* viral sequences may be present in the packaging system.

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Guidance on the classification of contained dealings with viral vectors*

Replication competent vectors			
Viral vector type	**Characteristics of donor nucleic acid or donor organism**	***In vitro*[1]**	***In vivo***
Any	can modify an organism so as to increase the likelihood of inheritance of particular nucleotide sequence(s) (i.e. create an engineered gene drive)	DNIR, S3 p3.1 (s)	
Non-pathogenic plant viral vector or Baculovirus (polyhedrin minus forms of *Autographa californica nuclear polyhedrosis virus*)	unlikely to increase capacity to cause harm; cultures used are ≤ 25 L	Exempt, S2 p1 item 4	NLRD, S3 p2.1 (c)
	unlikely to increase capacity to cause harm; cultures used are > 25 L	NLRD, S3 p2.1 (f)	N/A
	may increase capacity to cause harm	NLRD, S3 p2.1 (e)	DNIR, S3 p3.1 (f) & (g)
	toxin or uncharacterised gene from toxin producing organism	DNIR, S3 p3.1 (a), (b) or (c)	
	genes whose expressed products are likely to increase the capacity of the virus/viral vector to induce an autoimmune response	DNIR, S3 p3.1 (h)	
	creates novel replication competent virus with increased capacity to cause harm (e.g: new potential host species or mode of transmission; or increased virulence or transmissibility)	DNIR, S3 p3.1 (i)	
Risk Group 4 virus[2]	any	DNIR, S3 p3.1 (p)	
Risk Group 3 virus[2]	any	DNIR, S3 p3.1 (q) if not in an appropriate PC3 facility	
All other replication competent viruses	not a pathogenic determinant and not a toxin and not an oncogenic modification and not immunomodulatory in humans	NLRD, S3 p2.1 (c) or (d)	
	toxin or an uncharacterised gene from toxin producing organism	DNIR, S3 p3.1 (a), (b) or (c)	
	confers an oncogenic modification or immunomodulatory effect in humans	DNIR, S3 p3.1 (e)	
	pathogenic determinant or may otherwise increase capacity of virus to cause harm	DNIR, S3 p3.1 (f) or (g)	
	genes whose expressed products are likely to increase the capacity of the virus/viral vector to induce an autoimmune response	DNIR, S3 p3.1 (h)	
	creates novel replication competent virus with increased capacity to cause harm (e.g: new potential host species or mode of transmission; or increased virulence or transmissibility)	DNIR, S3 p3.1 (i)	
	would impair practical treatment of any disease or abnormality caused by the virus (e.g. drug resistance)	DNIR, S3 p3.1 (o)	

***Guidance only – refer to detail in the applicable clauses of the Gene Technology Regulations 2001, as current at the time. This guidance reflects the Commonwealth Regulations incorporating amendments from Schedule 1 of the Gene Technology Amendment (2019 Measures No. 1) Regulations 2019, which commence on 8 October 2019.**

References

Australian Commonwealth Government, *Gene Technology Regulations 2001* – Amended and in force 8 October 2020.
https://www.legislation.gov.au/Details/F2020C00957

Office of Gene Technology Regulator, 'Guidance on classification of contained dealings with viral vectors – effective October 2019',
http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/viral-vectors