Notification of a Notifiable Low Risk Dealing (NLRD)

EXAMPLE No: 3

NLRD Type:

PC1(a)

PC2(e)

PC2(j)

PC2(l)

Part 2: Description of the Dealings and GMO(s)

Purpose of the Dealing:

We seek approval to conduct all types of dealings with the GMOs described here and in Table 2 of this application. That is, we wish to create, breed, import, transport, store, possess, culture, propagate, grow, conduct experiments with and dispose of the described GMOs. We also seek approval for indefinite storage or the GMOs described in this application. The aim of the project is to understand the mechanism by which cancer is formed, maintained and spread. These processes are the result of changes in cellular gene expression either through mutation, deletion, amplification or over expression. Furthermore, each cancer is the result of changes in a unique gene or set of genes. In order to investigate this we wish to undertake a range of experiments that will require genetic manipulation of primary and cancerous cells of mammalian or insect origin which will result in, among other things, altered growth characteristics of the cells. We are investigating a range of biological processes including, but not limited to, the cell cycle, cell survival, transcription, signal transduction, differentiation, and hematopoesis. We are also investigating the formation of cancers including but not limited to leukaemia and breast cancer, using cell and laboratory animal models. The methods require altering the gene expression of cells either by gene knockdown (eg siRNA, shRNA, miRNA etc) or over expression (eg cDNA constructs in plasmids or viral vectors).

More explicitly we propose to:

- Clone a series of full length mammalian cDNAs of the gene classes listed in Table 2, in non-conjugative plasmid vectors in *E.coli*. Individual cDNAs will harbour random or specific truncations, insertions or point mutations. In addition, cDNAs encoding antibiotic resistance genes, marker genes (eg GFP, YFP CFP, Lacz, Lucifierase), Cre-recombinase and/or FLP- recombinase will also be cloned in *E.coli*. cDNAs will be cloned in a variety of non-conjugative plasmid vectors including, but not limited to, pBluescript (cloning vector), pIRES (mammalian expression vector) pCAL or pET (bacterial expression vectors) for protein purification or pCMV-Myc (mammalian expression vector).
- Clone corresponding genomic DNA of the gene classes listed in Table 2. Genomic DNA clones will be harboured in non-conjugative plasmid vectors, lambda bacteriophage vectors of bacterial artificial chromosome vectors (BACS).
- Clone individual genes from mammalian viruses including those from Herpes viruses, vaccinia virus, adenovirus (various types), Human Immunodeficency virus, Papillomavirus (various types), Simian virus type 40, Epstein Barr virus. Genes will be cloned in a variety of non-conjugative plasmid vectors. Individual genes may harbour random or specific truncations, insertions or point mutations.
- Clone and express a series of short-haripin RNAs targeted against the genes listed in Table 2. ShRNAs will be harboured in non-conjugative plasmid vectors.
- Replication-defective adenoviral, AAV, and retroviral (including lentiviral) vectors will be produced containing the genes of interest or shRNAs against them. The donor nucleic acid is incapable of correcting a defect in the vector leading to the production of replication competent virions.
- Each gene or shRNAs will transferred into a variety of mammalian and insect cell lines in vitro using either liposome transfection, electroporation of plasmid DNA, or viral transfection. These GM cells may be introduced into somatic tissue of whole animals. Virus will not be injected into whole animals.
- Each gene will transferred into yeast using either liposome transfection, or electroporation of plasmid DNA.
- We will make and use transgenic and knockout mice with genes of the classes described in Table 2, *excluding* viral genes. Animals will not be transformed using viral vectors.

Note:

- This work will be performed in association with UQ Exempt dealings including [illegible].
- [illegible].

Part 2 Table: is intended to generate a concise, accurate record of all the GMOs to be generated or used and the purpose of the proposed dealings. Attachment 1 provides example reference responses to the description of the GMOs. Attachment 2 provides information relating to the completion of the column headed 'NLRD Type'.

4A	4B	4C	4D	4E	4F	4G
COMMON NAME OF PARENT ORGANISM	SCIENTIFIC NAME OF PARENT ORGANISM	VECTOR(S) & METHOD OF TRANSFER	EXEMPT HOST/ VECTOR SYSTEM ?	IDENTITY & FUNCTION OF NUCLEIC ACID & ORGANISM OF ORIGIN	ORGANISMS OR TISSUES TO BE USED WITH THE GMO(S)	NLRD TYPE
Escherichia	E.coli K12 and B and C strains	Non-conjugative plasmids and BACs Bacteriophage lambda	Yes	Antibiotic resistance genes/ reporter/ marker genes carried on standard cloning vectors. **Mammalian cDNAs** (eg human and mouse cell lines and primary tissues) and genomic fragments from the following gene classes : MDR (multidrug resistance), ABC reporters, ion channel, transcription factor, RNA metabolism, cell signaling, cell cycle, growth control, tumour suppressors, cytoskeletal structure and maintenance, growth factors, development, proliferation, differentiation, reactive oxygen species metabolism, signal transduction, apoptosis housekeeping genes. **Viral Genes** (eg genes from Herpes viruses, vaccinia virus, orf virus, adenovirus (various types), Human Immunodeficency virus, Papillomavirus (various types), Simian virus type 40; and Epstein Barr virus) siRNA, shRNA and miRNA directed against the genes listed above		Exempt PC2(e)

Cell lines	Mammalian cell lines and primary cells. (Eg HeLa, SiHa, Caski, TC-1 B16 etc)	Non-conjugative plasmid DNA introduced by either liposome mediated transfection or electroporation	Yes	As listed for bacteria above.	Somatic tissue of whole animals	Exempt PC2(e)
Cell lines	Mammalian cell lines and primary cells.	Replication defective viral vectors eg adenoviral vectors	Yes	As listed for bacteria above. The donor nucleic acid is incapable of correcting a defect in the vector leading to the production of replication competent virions	Somatic tissue of whole animals	PC2(j)
Cell lines	Mammalian cell lines and primary cells.	Replication defective retroviral vectors eg 3rd generation lentivirus	Yes	As listed for bacteria above. The donor nucleic acid is incapable of correcting a defect in the vector leading to the production of replication competent virions	Somatic tissue of whole animals	PC2(l)
Cell lines	Insect cell lines and primary cells	Non-conjugative plasmid DNA introduced by either liposome mediated transfection or electroporation	Yes	As listed for bacteria above.		Exempt PC2(e)
Cell lines	Insect cell lines and primary cells.	Replication defective viral vectors eg adenoviral vectors	Yes	As listed for bacteria above. The donor nucleic acid is incapable of correcting a defect in the vector leading to the production of replication competent virions		PC2(j)
Cell lines	Insect cell lines and primary cells.	Replication defective retroviral vectors eg 3rd generation lentivirus	Yes	As listed for bacteria above. The donor nucleic acid is incapable of correcting a defect in the vector leading to the production of replication competent virions		PC2(l)
Yeast	*Saccharomyces cerevisiae*	Standard yeast expression vectors	Yes	As listed for bacteria above.		Exempt PC2(e)

Mouse	*Mus Musculus*	Pronuclear microinjections and breeding out of transgenic/knockout lines Once established knockout lines may be bred onto other transgenic and knockout lines	No	As listed for bacteria (excluding viral genes) as well as antibiotic resistance genes and reporter/marker genes No advantage is conferred on the animal by the genetic modification, and the animal is incapable of secreting or producing an infectious agent		PC1(a)