Notification of a Notifiable Low Risk Dealing (NLRD)

EXAMPLE No: 4

NLRD Type:

PC1(a)

PC2(i)

PC2(j)

PC2(l)

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

Briefly describe the purpose of the Dealing (in no more than a few sentences) and proposed methods to be undertaken in the dealing (as dot points).

Purpose of the Dealing:

To define the signalling pathways used by the growth hormone receptor to control growth, metabolism, oncogensis and longevity, via:

- Creation of retroviral, lentiviral and adenoviral vectors to transduce mammalian cells with DNA to express genes of interest (see Part 2 table).
- Cross breading of transgenic mice (see Part 2 table). For the purpose of investigating growth hormone signalling and its role in metabolism, diabetes, lifespan, and oncogenesis.

We seek approval to conduct all types of dealing with the GMO's described here and in the following Table of this application. That is, we wish to create, culture, propagate, grow, import, transport, store, possess, conduct experiments with and dispose of the described GMOs.

Proposed methods:

Exempt dealings relevant to this Application:

- The following genes/gene classes: mammalian transmembrane receptor genes, growth factor genes, transcription factor genes, RNA metabolism genes, cell cycle regulatory genes, cell signaling genes, cellular trafficking genes, nuclear transport genes, gene fusions to a leucine zipper from c-Jun, fluorescent marker genes, antibiotic resistance genes, housekeeping genes, and RNA interference (RNAi) to knock-down gene expression (within the genes/gene classes listed above) will be cloned into E. coli using non-conjugative plasmid vectors (under [redacted]).
- These genes will be transformed into mammalian, avian or insect cell lines (see Part 2 table) via non-conjugative plasmid vectors (under [redacted]).

Description of NLRD dealings:

- The genes described above will be transduced into mammalian cell lines and or insect cell lines (see Part 2 table) via replication defective adenoviral vectors, replication defective ecotropic or pantropic retroviral vectors, ASLV-A RCAS retroviral vectors (RCAS vectors are a family of retroviral vectors derived from the SR-A strain of Rous sarcoma virus (RSV), a member of the avian sarcoma-leukosis virus (ASLV) family), ASLV-A psudeotyped lentiviral vectors (which are unable to transduce normal mammalian cells unless these cells express avian TVA by recombinant methods), and replication defective lentiviral vectors (which satisfy OGTR Regulations Schedule 3 Part 2, 2.1 (l)). No nucleic acid which is known to code for a vertebrate toxin will be used to transduce cells. No nucleic acid will be used which is known to be capable of correcting a defect in the vector leading to production of replication competent virions.
- Mice will be transformed (or imported) that carry a knock-out for any of the murine genes listed above (see Part 2 table for details).
- Mice will be transformed (or imported) that express full-length or mutant genes, marker genes, cre recombinase, or RNAi as described above (see Part 2 table). Transformation will be via non-conjugative plasmid vectors or naked DNA, via microinjection or pronuclear injection into mouse embryos. No GM animals will be produced under this dealing using retroviral vectors.
- This dealing will involve the importing of GMOs.

Table 2.1: 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'.

2A	2B	2C	2D	2E	2F	2G
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
Bacteria	E. coli (Exempt strains)	Non-conjugative plasmid vectors	Y	Mammalian cell surface receptor genes, transcription factor genes, RNA metabolism genes, cell signalling genes, growth factor genes, gene fusions to a leucine zipper from c-Jun, genes involved in development and function, housekeeping genes, antibiotic resistance genes, Cre recombinase, fluorescent protein genes (including GFP, YFP, DsRed and related genes) and RNAi.. No nucleic acid which is known to code for a vertebrate toxin will be used to transduce cells.	-	-
Mammalian cultured cells	Human cell lines (eg Plat-E, fibroblast cell line)	Non-conjugative plasmid vectors	Y	See above	-	-
Insect cell lines	Sf9 cells and other standard insect cell lines suitable for protein production	Baculovirus infection (*Autographa californica* polyhedron minus)	Y	See above	-	-
Avain cultured cells	Chicken cell line (DF-1)	Non-conjugative plasmid vectors	Y	See above	-	-
Mammalian cultured cells	Human cell lines (eg Plat-E, fibroblast cell line)	Replication defective ecotropic retroviral vectors (based on eg pMX, MSCV, or pBABE vectors)	N	See above No nucleic acid will be used which is known to be capable of correcting a defect in the vector leading to production of replication competent virions.	-	Exempt S2 p1 item 4

Mammalian cultured cells	Human cell lines (eg Plat-E, fibroblast cell line)	Replication defective pantropic retroviral vectors (based on pMX, MSCV, or pBABE the pMX vectors)	N	See above No nucleic acid will be used which is known to be capable of correcting a defect in the vector leading to production of replication competent virions.	-	PC2 NLRD, S3 p2.1 (j)
Mammalian cultured cells	Human cell lines (eg Plat-E, fibroblast cell line)	Replication defective lentiviral vectors , which satisfy OGTR Regulations Schedule 3 Part 2, 2.1 (l)	N	See above No nucleic acid will be used which is known to be capable of correcting a defect in the vector leading to production of replication competent virions.	-	PC2 (l)
Mammalian cultured cells	Human cell lines (eg Plat-E, fibroblast cell line)	Replication defective 3rd generation ASLV-A pseudotyped lentiviruses (unable to transduce mammalian cells unless expressing TVA with recombinant methods).	N	See above No nucleic acid will be used which is known to be capable of correcting a defect in the vector leading to production of replication competent virions.	-	PC2 (i)
Mammalian cultured cells	Human cell lines (eg HEK, fibroblast cell line)	Replication defective adenoviral vectors (based on the pAdEasy vectors)	N	See above No nucleic acid will be used which is known to be capable of correcting a defect in the vector leading to production of replication competent virions.	-	PC2 (j)
Avain cultured cells	Chicken cell line (DF-1)	retroviral vectors (ALSV-A RCAS - unable to transduce mammalian cells unless these cells express avian TVA).	N	See above No nucleic acid will be used which is known to be capable of correcting a defect in the vector leading to production of replication competent virions.	-	PC2 (i)
mouse	Mus musculus	ES cell transfection/injection into blastocyst	N	Mutations, nuclear localisation signal fusion, deletions and truncations of the Growth hormone receptor, and GHR gene fusions to a leucine zipper from c-Jun.	-	PC1a
mouse	Mus musculus	ES cell transfection/injection into blastocyst	N	Growth hormone receptor knock-out, murine cell surface receptor genes, transcription factor genes, RNA metabolism genes, cell signalling genes, growth factor genes, and genes involved in development and function.	-	PC1a,

mouse	Mus musculus	ES cell transfection/injection into blastocyst	N	floxed Stat5A/B, floxed cell signalling genes, floxed cell surface receptors, Cre recombinase, Growth hormone antagonist, chicken TVA gene, IGF-1.	-	PC1a