Notification of a Notifiable Low Risk Dealing (NLRD)

EXAMPLE No: 1

NLRD Type:

PC1(a)

PC1(c)

PC2(a)

PC2(l)

PC2(m)

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:

This project aims to identify and characterize genes involved in the development of the nervous system. 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, import, transport, store, posses, conduct experiments with, and dispose of the described GMOs.

- We will investigate the role of these genes in the development of a functional nervous system and in the repair processes occurring after brain and disease. We will also investigate the role of these genes in non-neural developmental processes in the embryo as these functions will help shed light on the neural function of these genes.
- We will investigate the effect of the gene products on cell growth, differentiation, migration, cell death, and repair, by expressing full-length or mutated cDNAs in vertebrate cell lines, yeast, and zebrafish and mice. In some instance we will also study loss-of-function mutants in these organisms by introducing anti-sense oligonucleotides or small interfering double-stranded RNAs (shRNAs). In some instances, transfected vertebrate cells will be transplanted into embryonic and adult mice to study their activity in a normal *in vivo* environment.
- Transgenic and "knockout" mice and zebrafish will be created to study the function of the neural genes in the context of the whole animal.
- The 3rd generation replication-defective lentiviral vectors carry neural genes or shRNAs will be directly injected into embryonic or adult mouse brains. Animals will then be sacrificed at specified time before or after birth. NOTE: these animals will **not** be used for breeding.

Proposed methods:

- *Exempt dealings relevant to this Application:*

- Clone a series of full-length and truncated neural cDNAs, or neural cDNAs carrying point mutations into E.Coli. These cDNAs will harbour deletions, point mutations, or insertions designed to increase or decrease the activity of the gene product. cDNAs will be cloned into non-conjugative plasmid vectors such as pBluescript, pEF/myc/cyto, pCDM8, pCDNA, pIRES, or other similar non-conjugative plasmid vectors.
- cDNAs encoding antibiotic resistance genes, marker genes (eg. GFP, LacZ, Tau-LacZ), and/or Cre Recombinase will also be cloned into E.Coli using non-conjugative plasmid vectors such as pBluescript, pEF/myc/cyto, pCDM8, pCDNA, pIRES, or other similar non-conjugative plasmid vectors.
- cDNAs encoding shRNAs will also be cloned into E.Coli using non-conjugative plasmid vectors as described above.
- Non-conjugative BAC vector libraries in E. Coli will also be used to screen for candidate developmental genes.

Description of NLRD dealings:

- Produce a series of non-conjugative plasmid vectors harbouring the wildtype and mutant neural cDNAs and shRNAs for expression in mammalian cell lines.
- Produce a series of replication-defective adenovirus vectors harbouring the wildtype and mutant neural cDNAs and shRNAs.
- Produce a series of 3rd generation, replication-defective lentiviruses, harbouring wildtype and mutant neural cDNAs and shRNAs.
- BAC libraries carrying candidate neural cDNAs
- Produce mammalian cell lines expressing wildtype and mutant neural cDNAs and shRNAs generated from non-conjugative expression plasmid vectors, adenovirus or lentivirus.
- Produce a series of yeast strains carrying the full-length or mutant cDNAs described above.
- Produce or import transgenic mice harbouring the full-length or mutant cDNAs described above, and also harbouring antibiotic resistance genes and marker genes.
- Produce or import knock-out mice lacking alleles of the neural genes and harbouring antibiotic resistance genes and marker genes, including Cre/lox loci.
- Produce or import transgenic zebrafish harbouring the full-length or mutant cDNAs described above, and also harbouring marker genes.
- Produce mouse embryos in which wildtype or mutant neural cDNAs and shRNAs are delivered by injection of non-conjugative plasmids or lentivirus into the embryonic brain while in utero. Embryos will then be placed back into the mother and development allowed to proceed for a given length of time, after which the animals will be sacrificed. Depending on the experiment, embryos may be sacrificed prior to birth or allowed to proceed to birth, after which they will be sacrificed before or at 8 weeks of age. NOTE: these animals will **not** be used for breeding.
- Produce mouse neonates or adults in which lentivirus carrying wildtype or mutant neural cDNAs and shRNAs are injected into the brain. Depending on the experiment, animals may be sacrificed before or at 8 weeks of age. NOTE: these animals will **not** be used for breeding
- ~~This project will include indefinite storage of GMOs and/or GMO products~~.
- In some instances GMO cell lines, mouse or zebrafish strains may be exported to or imported from both overseas and interstate colloborating labs.

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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**
Bacteria	E.Coli	(i) Standard non-conjugative plasmid vectors, including BAC vectors (ii) Heat shock or electroporation of cDNAs and BACs into E. coli.	yes	antibiotic resistance genes: such as chloramphenicol, tetracycline, kanamycin, ampicillin cDNAs and siRNAs as described under "Cell Lines" below	none	exempt
yeast	Saccharomyces cerevisiae	(i) Non-conjugative plasmid vectors (ii) Heat shock or electroporation of cDNAs into yeast	yes	Auxotrophic markers Mutations in genes encoding essential amino acids cDNAs and siRNAs as described under "Cell Lines" below	none	exempt
cell lines	Mouse cell lines: P19 cells, neurospheres, embryonic stem cells, primary neural stem cells, primary fibroblasts, primary neurons, other primary cells Rat cell lines: PC12 cells Hamster cell lines: CHO cells Monkey cell lines: COS cells Human cell lines: HEK 293T, IMR32, MCF7, Caco2 cells Other mammalian cell lines	Electroporation, nuclear injection, or transfection with commercial reagents such as Fugene and Lipofectamine, or, calcium phosphate, LDH nanoparticle, or dextran solutions prepared in the laboratory	yes	Genes involved in axon guidance, cell adhesion, migration, synapses, cell death, intracellular signalling, growth and differentiation, neural development in mouse, human and zebrafish. Genes encoding cell surface receptors, membrane channels and transcription factors NB: some of these genes may have oncogenic modifications Marker genes: LacZ: E.Coli, Tau: Bovine, GFP or similar fluorescent proteins from a range of species, eg. jellyfish Cre recombinase: P1 bacteriophage Antibiotic resistance genes, including hygromycin: bacteria neomycin: TN5 transposon siRNAs, shRNA plasmids from mouse, human, zebrafish	none	exempt

cell lines	Cell lines as above (row 3)	Electroporation, nuclear injection, or transfection with commercial reagents such as Fugene and Lipofectamine, or, calcium phosphate, LDH nanoparticle, or dextran solutions prepared in the laboratory	yes	cDNAs and siRNAs as described under "Cell Lines" above (row 3)	Cell lines will be transplanted into somatic tissues of mice	exempt
cell lines	Cell lines as above (row 3)	Transduction with replication-defective adenovirus	no	cDNAs and siRNAs as described under "Cell Lines" above (row 3)	none	PC1(c)
cell lines	Cell lines as above (row 3)	Transduction with third generation replication-defective lentiviral vectors	no	cDNAs and siRNAs as described under "Cell Lines" above (row 3)	none	PC2(l)
mouse	Mus musculus	Pronuclear injections and breeding out of transgenic mouse lines	no	cDNAs and siRNAs as described under "Cell Lines" above (row 3)	none	PC1(a)
mouse	Mus musculus	Injection of targeted embryonic stem cells into blastocysts and breeding out of knock-out mouse lines	no	cDNAs and siRNAs as described under "Cell Lines" above (row 3)	none	PC1(a)
mouse	Mus musculus	Third generation replication-defective lentiviral vectors into somatic tissues	no	cDNAs and siRNAs as described under "Cell Lines" above (row 3) NB: Immunomodulatory genes or oncogenes **will not** be used with replication defective lentiviral vectors.	none	PC2(m)
zebrafish	*Danio rerio*	standard non-conjugative plasmids microinjected into embryos	no	(i) Transcriptional activators (eg. GAL4 from Saccharomyces cerevisiae (ii) shRNAs and full-length or mutated forms of genes described under "cell lines" -see above (iii) standard marker genes (eg. GFP from *Aequorea victoria* and Kaede from *Trachyphylla geoffroyi*	none	PC2(a)