Notification of a Notifiable Low Risk Dealing (NLRD)

Example No: 5

NLRD type:

PC2 (d)

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:

We seek approval to conduct all types of dealings with the GMOs 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.

The aim of this project is to generate infectious dengue virus clones bearing mutations in the prM and E proteins that modify fusion phenotype and the NS1 and NS3 proteins that modify replication, and assess their potential as attenuated vaccine candidates and in studies of viral replication. The remaining 6 viral proteins, C, NS2a, NS2b, NS4a, NS4b and NS5 will also be the target of selected mutagenesis.

Proposed methods:

- Exempt dealing relevant to this application: IBC/91E/SMMS/2008

  Description of NLRD dealings:

- Introduce selected mutations into the dengue viral genome of infectious dengue virus clones. Single and multiple mutations of interest will be introduced into the genomic length dengue 2 NGC cDNA clone (and others as available) using PCR mutagenesis followed by subgenomic fragment exchange.

- RNA transcripts will be prepared from the cDNA clones using T7 polymerase and electroporated into mammalian cell culture. Culture supernatants will be passaged in *Aedes albopictus* cells to produce virus stock to be used in further experiments.

- Growth characteristics, including virus titre production over time, plaque size and fusion phenotype will all be determined initially in cell culture. Neuro-virulence phenotype of both parent and mutant viruses will then be tested in mice.

- ~~This project will include indefinite storage of GMOs and/or GMO products~~.

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	Escherichia coli *K12 laboratory strains*	Standard non-conjugative cloning vectors. Full length dengue virus genome containing plasmid pDVWSK601 – by electroporation or chemical competence.	No	Antibiotic resistance gene (eg ampicillin) Full-length Dengue viral genome	-	Exempt
Animal cell lines	Animal cell lines eg BHK12	Transfection or electroporation of CMV promoter driven plasmid, pJWSAS and RNA transcripts of pDVWSK601.	Yes	C, prM, NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5 genes of dengue type 2 virus NGC strain.	-	Exempt
Dengue virus	Dengue virus NGC subtype 2	RNA transcripts (and mutated derivatives) generated from pDVWSK601 - via electroporation	No	Wild type and mutated derivatives of C, prM, NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5 genes of dengue type 2 virus NGC strain. The donor nucleic acid is characterised and is not known to alter the host range or mode of transmission, or increase the virulence, pathogenicity or transmissibility of the host or vector.	Cell lines and mice	PC2(d)