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

Proposed methods:

Exempt dealings relevant to this Application: IBC/142E/IMB/2009

- Cloning of DNA fragments encoding precursors for naturally occurring or engineered peptides, fragments encoding parts of plant viruses, fragments encoding standard reporter/marker genes, gene silencing suppressor genes, and fragments encoding genomic and cDNA sequences from various organisms that are not human pathogens into *Escherichia coli* K12 derivative strains. Genes will not encode gene products known to have an LD50 of 100µg/kg or less.
- Propagation of cloned fragments encoding precursors for naturally occurring or engineered peptides, fragments encoding parts of plant viruses, fragments encoding standard reporter/marker genes, gene silencing suppressor genes, and fragments encoding genomic and cDNA sequences from various organisms that are not human pathogens in *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes* strains. Genes will not encode gene products known to have an LD50 of 100µg/kg or less.
- Genetic modification of plant cell and tissue cultures carrying nucleic acid fragments from the above two dot points in: *Nicotiana tabacum, N. benthamiana, Arabidopsis thaliana*, *Viola* spp., Rubiaceae family members, Asteraceae family members, *Brassica* spp., *Solanum lycopersicum, Solanum tuberosum, Petunia hybrida, Petunia axillaris, Petunia integrifolia, Brachypodium distachyon, Sorghum bicolor*, and *Clitoria ternatea*.
- Genetic modification of insect cell cultures carrying nucleic acid fragments from the first two dot points above in: *Spodoptera frugiperda*, and *Trichoplusiani* spp.
- Genetic modification of *Pichia pastoris* with nucleic acid fragments from the first two dot points above.

Description of NLRD dealings:

Genetic modification and growth of full plants of the aforementioned plant species in a PC2 glasshouse or enclosed artificial environment (eg growth chamber) for the production of plant peptides and hypothesis testing the production efficiency of listed plant species expressing various constructs. Genes introduced to host plant cells will not encode gene products known to have an LD50 of 100µg/kg or less.

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, IF ANY, TO BE USED WITH THE GMO(S) & METHOD OF EXPOSURE***	**NLRD TYPE**
Bacteria	*Escherichia coli* K12 derivatives	Non-conjugative plasmids e.g. PCR2.1-TOPO (Invitrogen); *P.pastoris* expression vectors; bacteriophage lambda	Yes	Antibiotic resistance genes/ reporter/ marker genes carried on standard cloning vectors. cDNA and genomic DNA libraries of plant origin; plant viral suppressors of gene silencing; cyclotide genes and their variants; Genes encoding cysteine rich peptides from various sources	None	Exempt
Bacteria	Non-tumorigenic, disarmed *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes* strains	Standard binary T-DNA or direct DNA transfer vectors; Ri plasmid vectors	Yes	Antibiotic resistance genes/ reporter/ marker genes carried on standard cloning vectors. cDNA and genomic DNA of plant origin; plant viral suppressors of gene silencing; cyclotide genes and their variants	Plants (e.g. Solanaceae family members, *Arabidopsis thaliana*, *Viola* spp. Rubiaceae family members, Asteraceae family members, Fabaceae family members)	Exempt

Plant Tissue Culture **Tobacco** **Thale cress** **Violet** **Coffee family members** **Sunflower** **Canola** **Tomato** **Potato** **Petunia** **Purple false brome** **Sorghum** **Butterfly pea**	*Nicotiana tabacum* and *N. benthamiana*, *Arabidopsis thaliana* *Viola* spp Rubiaceae family members Asteraceae family members, esp *Helianthus* *Brassica ssp.* *Solanum lycoperiscum* *Solanum tuberosum* *Petunia hybrida*, including progenitor species *P. integrifolia ssp. inflata* and *P. axillaris* *Brachypodium distachyon* *Sorghum bicolor* *Clitoria ternatea*	Microprojectile bombardment or Agrobacterium *tumefaciens*-mediated transformation, or Agrobacterium *rhizogenes*- mediated transformation	Yes	Antibiotic resistance genes/ reporter/ marker genes, herbicide resistance genes carried on standard cloning vectors. Plant cDNAs and genomic fragments as follows: Native and mutated cyclotides and cyclotide-like peptides from various plant species the expression of which is likely to make the plants resistant to certain pests, diseases, or express putative therapeutic agents. Plant viral sequences encoding characterised suppressors of gene silencing Plant enzyme sequences involved with the isomerisation and cyclisation of cyclotides that are derived from cyclotide-producing plant species (e.g. *Oldenlandia sp.*, *Viola sp.*)	None	Exempt

Whole Plants **Tobacco** **Thale cress** **Violet** **Coffee family members** **Sunflower** **Canola** **Tomato** **Potato** **Petunia** **Purple false brome** **Sorghum** **Butterfly pea**	 *Nicotiana tabacum* and *N. benthamiana*, *Arabidopsis thaliana* *Viola* spp. Rubiaceae family members Asteraceae family members, esp *Helianthus* *Brassica spp.* *Solanum lycopersicum* *Solanum tuberosum* *Petunia hybrida*, including progenitor species *P. integrifolia ssp. inflata* and *P. axillaris* *Brachypodium distachyon* *Sorghum bicolor* (e.g. Tx430) *Clitoria ternatea*	Microprojectile bombardment or Agrobacterium-mediated transformation (*tumefaciens* or *rhizogenes*) TMV-Gate vector system for transient expression. Carries components of the tobacco mosaic virus genome but lacking gene sequences encoding the coat protein, thus rendering the virus avirulent. This system requires Agrobacterium for gene transfer. As detailed in (Kagale S *et al., 2012 Nature Scientific Reports 2: 874).* .	No	Antibiotic resistance genes/ reporter/ marker genes, herbicide resistance genes carried on standard cloning vectors. Plant cDNAs and genomic fragments as follows: Native and mutated cyclotides, cyclotide-like and cystine-rich peptides from various plant species the expression of which is likely to make the plants resistant to certain pests, diseases, or express putative therapeutic agents. Plant viral sequences encoding characterised suppressors of gene silencing Toxin sequences encoding insecticidal peptides derived from those of spiders (e.g. funnel web). These insecticidal distinct and structurally characterised toxins have no known vertebrate toxicity.	Plants may be challenged with insect pests or pathogens to determine activity of the expressed peptides. Insects to be tested are *Helicoverpa armigera, H. punctigera, Creontiades dilutes, Nezara viridula, Bemisia tabaci, Aphis gossypii,* and *Frankliniella occidentalis.*	PC2(b)

Yeast	*Pichia pastoris*	*P. pastoris* expression vectors, e.g. pPIC9K (Invitrogen)*	Yes	Genes encoding cysteine rich peptides from various sources; genes conferring expression of antibiotic resistance genes.	none	Exempt
Insect cells	*Spodoptera frugiperda* (e.g. SF9) or *Trichoplusiani* (e.g. Tn-368)	Commercial Baculovirus-based vectors (e.g. Bac-to-Bac system vectors from Invitrogen)	Yes	Genes encoding cysteine rich peptides from various sources; CAT and Gus are expressed from the vector DNA and are selective markers; resistance to gentimicin is encoded by the vector DNA	none	Exempt

*e.g. injection, co-culture, transplantation, infection