Notification of a Notifiable Low Risk Dealing (NLRD)

Example No: 6

NLRD type:

PC2 (e)

PC2(d)

PC2(g)

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, import, transport, store, possess, conduct experiments with and dispose of the described GMOs.

The aim of this work is to understand the mechanisms employed by bacteria to colonize the human urinary tract and cause disease. We will study a range of processes important to adherence, colonisation, survival and pathogenesis by selected Gram-negative and Gram-positive bacteria that cause urinary tract infection. These processes include expression and function of membrane proteins/organelles/lipopolysaccharides/glycans, mechanisms of nutrient acquisition, mechanisms of resistance, metabolic process, stress responses and global gene regulation of these processes.

Proposed methods:

- This dealing is to be undertaken association with Exempt dealing **IBC/126E/[redacted]/2008**.
- Cloning of genes from bacterial pathogens that cause urinary tract infection into non-conjugative plasmids and expression in *E. coli* K-12, B or C strains.
- Creation of mutations in the gene/s of a particular pathway to assess its role in a particular cell process. Mutants will be constructed by random transposon mutagenesis or site-specific methods. Antibiotics used are not clinically relevant to the organism in question, or are obsolete and no longer in clinical use.
- Creation of gene fusions to report the expression of a particular gene and introduction of these constructs into wild-type strains.
- Creation of tagged gene constructs (e.g. 6xHisTag) and purification of respective proteins for antibody production
- Transcriptomic and proteomic approaches to study bacterial growth (wild-type strains and GMOs) in different environments.
- Complementation of mutants using low copy number plasmids. Complementation will not alter the host range or mode of transmission, or increase the virulence, pathogenicity, or transmissibility of the host above that of the parent organism. Antibiotics used are not clinically relevant to the organism in question, or are obsolete and no longer in clinical use.
- Analysis of wild-type strains and GMOs in biofilms, tissue culture assays and mouse infection models.
- ~~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* strains K12, B & C	Standard non-conjugative cloning vectors and standard expression plasmids, transferred by chemical transformation or electroporation	Yes	Bacterial genes that encode factors associated adherence, colonisation, survival and pathogenesis as well as characterised/fully sequenced genes. These genes **do not** encode for toxins or oncogenes. Genes will be cloned from *Escherichia coli*, *Salmonella* species, *Shigella* species, *Pseudomonas aeruginosa*, *Klebsiella* species, *Proteus* species, *Serratia* species, *Enterobacter* species, *Acinetobacter* species, *Citrobacter* species, *Morganella* species, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Streptococcus* species and *Enterococcus* species.	Human tissue culture cell lines (e.g. HeLa, T24 bladder, A498 kidney cells) will be infected with modified bacteria.	Exempt PC2(e)

Bacteria	*Escherichia coli*, *Salmonella* species, *Shigella* species, *Pseudomonas aeruginosa*, *Klebsiella* species, *Proteus* species, *Serratia* species, *Enterobacter* species, *Acinetobacter* species, *Citrobacter* species, *Morganella* species, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Streptococcus* species and *Enterococcus* species	Temperature sensitive plasmid systems for generation of targeted gene mutations; Transposons such as miniTn5 for random mutagenesis	No	Resistance to standard laboratory antibiotics. Antibiotics used are not clinically relevant to the organism in question, or are obsolete and no longer in clinical use. Reporter gene expression (eg Gfp)	Human tissue culture cell lines (e.g. HeLa, T24 bladder, A498 kidney cells) will be infected with modified bacteria. *Mus musculus* will be injected with modified bacteria	PC2(d)
Bacteria	*Escherichia coli*, *Salmonella* species, *Shigella* species, *Pseudomonas aeruginosa*, *Klebsiella* species, *Proteus* species, *Serratia* species, *Enterobacter* species, *Acinetobacter* species, *Citrobacter* species, *Morganella* species, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Streptococcus* species and *Enterococcus* species	Standard non-conjugative cloning vectors and standard expression plasmids, transferred by chemical transformation or electroporation	No	Complementation of mutants generated above. Complementation will not alter the host range or mode of transmission, or increase the virulence, pathogenicity, or transmissibility of the host above that of the parent organism. Antibiotics used are not clinically relevant to the organism in question, or are obsolete and no longer in clinical use.	Human tissue culture cell lines (e.g. HeLa, T24 bladder, A498 kidney cells) will be infected with modified bacteria. *Mus musculus* will be injected with modified bacteria	PC2(g)