University of Sydney Institutional Biosafety Committee

- This form is to be completed by the Principal Investigator/Project Supervisor and attached to the project proposal.

Name of Principal Investigator/Project Supervisor:
Dr Neville Firth

Project title:
DNA segregation in multiresistant *Staphylococcus aureus*

Type of project proposal:
☐ Exempt Dealing X NLRD ☐ DNIR

RIMS Project ID Code: (for projects that are part of a funded research grant)
100000815

- For completion by IBC.

IBC Reference Number	**Approval Date**

University of Sydney Institutional Biosafety Committee – Notification of a Notifiable Low Risk Dealing (NLRD) application form

Part 1: Project Supervisor

Project supervisor's name:	Dr Neville Firth
Position within the organisation:	Senior Lecturer
Relevant qualifications:	PhD (Monash University; 1994)
Relevant experience:	Over 20 years experience in recombinant DNA technology and handling both pathogenic and non-pathogenic bacterial strains

Contact details of the Project Supervisor

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Postal address:	School of Biological Sciences Macleay Building, A12 The University of Sydney NSW 2006, AUSTRALIA

Part 2: About the dealings with the GMO or GMOs

To answer the questions in this Part, please firstly remove any explanatory text (including this text) and then insert your answer directly under the question.

2.A What is the title of the project?

DNA segregation in multiresistant *Staphylococcus aureus*

2.B What is the proposed date of commencement of the project?

January 2009

2.C What is the proposed date of completion of the project?

Ongoing

2.D Who are the people or class of people who will be authorised to undertake dealings with the GMO(s)?

Dr N. Firth and Dr S. Jensen (Chief Investigators), Postdoctoral Fellows, Research and Technical Assistants, Visiting Scientists and PhD, MSc, BSc Honours and Grad Dip students.

2.E Describe the training and experience of personnel involved in the dealing.

Dr N. Firth and Dr S. Jensen have many years of experience in recombinant DNA technology and handling both pathogenic and non-pathogenic bacterial strains. All research personnel will be given a PC2 lab induction to be made aware of OGTR and PC2 containment guidelines; people working in the laboratory will additionally attend appropriate training courses, including radiation safety, hazardous substances and biosafety.

2.F Will the dealing involve the import of GMOs into Australia? If yes, please provide brief details.

The importation of GMOs into Australia is not anticipated.

2.G Briefly describe the project, including the purpose and aims of the proposed dealing. Please take no more than half a page for this answer.

The overall objective of the project is to understand DNA segregation mechanisms in staphylococci. Research focuses on partitioning of the *S. aureus* multiresistance plasmid pSK41 and staphylococcal chromosome segregation. Mutagenesis, plasmid segregational stabilty and transcriptional reporter gene assays will be used to determine the significance of the pSK41 centromere DNA repeats (*parC*) and key amino acids, as identified by structural studies, of ParM and ParR partitioning proteins (see Schumacher *et al*., 2007. Nature 450:1268-71). Protein-DNA interactions will be characterised using fluorescence polarisation assays, and DNA-affinity purification and electron microscopy will be used to study the relationship between loss of function and protein-protein (ParR-ParM and ParM-ParM) interactions. Cytological studies of plasmid and chromosome segregation will employ YFP fusions, IFM and FISH. Gene knockouts ("Targetron" system), DNA staining, segregational stability assays and growth experiments will be used to determine the contribution of ParB1 and ParB2 to chromosome segregation. Electrophoretic mobility shift assays, DNase I footprinting and fluorescence polarisation will be employed to characterise ParB binding to putative *parS* centromere DNA sites, and ParB binding partner/s will be identified using DNA-affinity purification and peptide mass fingerprinting.

Part 3: Description of the GMO

In this part, a description of the GMO(s) is required. This includes a description of all of the GMO(s) to be generated and/or used during the proposed dealings, for example, bacteria used for subcloning steps, tissue culture cell lines *etc*.

Use Table 1 at the end of the application form if using multiple GMOs.

Please replace any explanatory text with your answers.

3.A What are the common and scientific names of the parent organism(s)?

Staphylococcus aureus (attenuated lab strains such as RN4220) and *Escherichia coli* K-12 and B (standard laboratory strains such as DH5α and BL21).

3.B What vector(s) and methods are to be used for the transfer of genetic material?

Plasmids will be introduced into *S. aureus* by electroporation. Plasmids will be introduced into *E. coli* by electroporation, or transformation of chemically competent cells The vectors that will be used are non-conjugative *E. coli* plasmid vectors such as pUC18, pBLUESCRIPT, pTTQ18, pQE30 and other general *E. coli* cloning/expression vectors, and non-conjugative *E. coli-S. aureus* shuttle vectors derived from them by the incorporation of replication/maintainence functions and selectable antimicrobial resistance markers that operate in *S. aureus* (N.B. these shuttle plasmids are already covered by approved NLRDs 652/2003, 653/2003 & 598/2005 [IBC refs. 02N018, 02N019 & 04N023]).

The non-conjugative *E. coli-S. aureus* shuttle plasmid pNL9164 (Sigma-Aldrich), and "retargeted" derivatives thereof, will be used to deliver "targetrons" (N.B. the use of targetrons is already covered by approved NLRD 2288/2007, [IBC ref. 06N019]).

3.C Are any of the proposed host/vector systems to be used not listed as an exempt dealing in Part 2 of Schedule 2 of the Gene Technology Regulations 2001?

E. coli is listed in Part 2 of Schedule 2. However, we will also be using *S. aureus* as a host, which is capable of causing disease in animals. Since the donor DNA to be introduced into *S. aureus* is fully characterised (sequenced) and will only contain genes or parts of genes (e.g., transcriptional promoters) that will not increase virulence, this dealing falls under item d of NLRD types (see over).

3.D What are the identity and function of the gene(s) responsible for the modified trait?

Protein expression: *S. aureus rep* plasmid replication initiation genes and *par* plasmid and chromosome segregational stability genes.

Antibiotic resistance: various antimicrobial resistance genes from *E. coli* (e.g., *bla* – ampicillin resistance) and staphylococci (e.g., *tet, cat, ermC* – tetracycline, chloramphenicol and erythromycin resistance, respectively) cloned into *E. coli* and/or *S. aureus* as selectable markers (note that these will not compromise treatment options; see 6A below).

Reporter/marker gene expression: chloramphenicol resistance, *cat*; β-lactam resistance, *bla*; β-galactosidase activity, *lacZ*; *gfp* spectral variants (e.g. *cfp, yfp*), fluorescence.

Other: Carriage of transcriptional promoters of genes involved in pSK41/pSK1 replication (*rep*) or plasmid/chromosomal segregational stability (*par* genes). "Targetrons" are derived from the *Lactococcus lactis* LI.LtrB group II intron.

3.E From what organism were the gene(s) responsible for the modified trait(s) isolated?

Plasmid replication and segregation genes and antibiotic resistance genes: *S. aureus* (pSK1, pSK41); *E. coli* (pBR322, pUC18). Chromosomal segregation genes from *S. aureus* RN4220 *gfp* was originally isolated from *Aequorea victoria*. "Targetrons" are derived from the LI.LtrB group II intron of *Lactococcus lactis.*

3.F What are the organisms or tissues to be used in association with the GMO(s)?

None.

3.G Containment Facilities

Provide details of all facilities to be used for this NLRD. Add extra rows if required.

Facility No.	Room Number and Building Number	Facility Type (eg animal house, laboratory etc)	Physical Containment Level (eg PC1, PC2)	OGTR Certification Number
1	Rooms 216-218 (A12)	laboratory	PC2	Cert-418
2				
3				
4				

Part 4: Type of Notifiable Low Risk Dealing in relation to Schedule 3 (Parts 1 and 2) of the Gene Technology Regulations

Please place an X in the appropriate box

Mark item with X	Part 1 – item 1.1
	(a) A dealing involving a genetically modified laboratory mouse or a genetically modified laboratory rat, unless; i) an advantage is conferred on the animal by the genetic modification; or ii) because of the genetic modification, the animal is capable of secreting or producing an infectious agent
	(b) A dealing involving a host/vector system mentioned in Part 2 of Schedule 2, if the donor nucleic acid confers an oncogenic modification;
	(c) A dealing involving a defective viral vector able to transducer human cells in a host mention in item 4 of Part 2 of Schedule 2 (animal or human cell culture), unless: i) the vector is a retroviral vector; or ii) the donor nucleic acid confers an oncogenic modification.

Mark item with X	Part 2 – item 2.1
	(a) A dealing involving whole animals (including non-vertebrates) that: (i) involves genetic modification of the genome of the oocyte or zygote or early embryo by any means to produce a novel whole organism; and (ii)does not involve any of the following: (A)a genetically modified laboratory mouse; (B)a genetically modified laboratory rat; (C)a genetically modified *Caenorhabditis elegans*;
	(aa) A dealing involving a genetically modified laboratory mouse or a genetically modified laboratory rat, if: (i)the genetic modification confers an advantage on the animal; and (ii)the animal is not capable of secreting or producing an infectious agent as a result of the genetic modification;
	(ab)A dealing involving a genetically modified *Caenorhabditis elegans*, if: (i)the genetic modification confers an advantage on the animal; and (ii)the animal is not capable of secreting or producing an infectious agent as a result of the genetic modification;
	(b)a dealing involving a genetically modified plant (including a genetically modified flowering plant), if the dealing occurs in a facility that is designed to prevent the escape from the facility of: (i)pollen, seed, spores or other propagules which may be produced in the course of the dealing; and (ii)invertebrates that are capable of carrying the material mentioned in subparagraph (i);
	(ba) A dealing involving a genetically modified flowering plant, if, before flowering, all inflorescences are wholly enclosed in bags designed to prevent escape of viable pollen and seed;
	(c) A dealing involving a host and vector that are not mentioned as a host/vector system in Part 2 of Schedule 2, if: (i) the host has not been implicated in, or had a history of causing, disease in human beings, animals, plants or fungi; and (ii)the vector has not been implicated in, or had a history of causing, disease in human beings, animals, plants or fungi;
X	(d) A dealing involving a host and vector that are not mentioned as a host/vector system in Part 2 of Schedule 2, if: (i) either: (A) the host has been implicated in, or has a history of causing, disease in human beings, animals, plants or fungi; or (B) the vector has been implicated in, or has a history of causing, disease in human beings, animals, plants or fungi; and (ii) 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;
	(e) a dealing involving a host/vector system mentioned in Part 2 of Schedule 2, if the donor nucleic acid: (i) encodes a pathogenic determinant; or (ii) is uncharacterised nucleic acid from an organism that has been implicated in, or has a history of causing, disease in human beings, animals, plants or fungi;
	(f) A dealing involving a host/vector system mentioned in Part 2 of Schedule 2 and producing more than 10 litres of GMO culture in each vessel containing the resultant culture, if: (i)the dealing is undertaken in a facility that is certified by the Regulator: (A)as a large scale facility; and (B)to at least physical containment Level 2; and (ii) the donor nucleic acid satisfies the conditions set out in item 4 of Part 1 of Schedule 2;
	(g)A dealing involving complementation of knocked-out genes, if the complementation does 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 before the genes were knocked-out;
	(h)a dealing involving shot-gun cloning, or the preparation of a cDNA library, in a host/vector system mentioned in item 1 of Part 2 of Schedule 2, if the donor nucleic acid is derived from either: (i) a pathogen; or (ii) a toxin-producing organism;
	(i)a dealing involving the introduction of a replication defective viral vector able to transduce human cells into a host mentioned in Part 2 of Schedule 2 if: (i) the donor nucleic acid is incapable of correcting a defect in the vector leading to production of replication competent virions; and (ii) either: (A) the vector is a retroviral vector; or (B) the donor nucleic acid confers an oncogenic modification.

Part 5: Additional information for a GMO that is a whole plant or is to be used in conjunction with a whole plant

The following information is required if you propose to deal with a GMO that is a whole plant or is to be used in conjunction with a whole plant.

Applicable ☐ Not applicable x

5.A To what stage of development are the plants to be grown?

This relates to the potential spread of the GMO, for example, if the plant produces pollen or seed.

5.B What will be used as the growing medium for the plants?

Please indicate the type of medium (soil or soil substitute) to be used and how it will be subsequently sterilised or disposed of.

Part 6: Risk assessment and management

In this Part please briefly describe, in no more than half a page, the risks the proposed dealings pose to the health and safety of people and the environment

To answer the questions below, please firstly remove any explanatory text and then insert your answer directly under the question.

Health and safety of people

6.A What are the possible hazard(s) and the likelihood and consequence of the hazard(s) occurring (*ie* the risk) from the proposed genetic modification(s)?

All *E. coli* strains covered by this application are non-pathogenic and no known toxins have been introduced into them.

The *S. aureus* strains to be used as hosts in this project have been used for over 20 years, with no known infection issues, are laboratory strains with attenuated virulence. Insertion of the genes and gene fragments to be studied is highly unlikely to result in any increase in pathogenicity. These background laboratory strains are susceptible to all antibiotics commonly used to treat staphylococcal infections. In some cases one or at most two resistance genes will be introduced into these strains as selectable markers. However, all strains generated are completely susceptible to widely used anti-staphylococcal antibiotics, including methicillin and vancomycin.

6.B What are the possible hazard(s) and the likelihood and consequence of the hazard(s) occurring (*ie* the risk) from an unintentional release of the GMO(s) into the environment?

The *E. coli* strains are unlikely to survive outside of laboratory conditions, due to their nutritional requirements. The risk of these organisms causing a clinically significant infection is negligible; they represent no hazard to human health.

The *S. aureus* host strains have been used for over 20 years with no known infection issues. These are attenuated laboratory strains and therefore represent a minimal hazard. The gene and gene fragments to be introduced into these strains will not result in any increase in pathogenicity. These organisms therefore represent a minimal hazard to human health.

Environment

6.C What are the possible hazard(s) and the likelihood and consequence of the hazard(s) occurring (*ie* the risk) from an unintentional release of the GMO(s) into the environment?

The *E. coli* strains are unlikely to survive outside of the laboratory due to their nutritional requirements, and therefore pose little or no hazard.

The *S. aureus* strains are attenuated and therefore unlikely to survive in the environment, and therefore pose little or no hazard.

6.D Do you propose to transport the GMO(s) outside a certified facility?

If required, transport of the GMOs will be conducted in accordance with the conditions described in the Guidelines for the transport of GMOs. The organisms will be wholly contained within a primary sealed container, which in turn will be packaged in to a secondary sealed unbreakable container, labelled as described in the guidelines.

6.E How will the GMO(s) be disposed of?

All GMOs will be inactivated/killed by exposure to 1% hypochlorite solution or 80% (v/v) ethanol, or alternatively by autoclaving.

6.F What are the steps will you take in the event of an unintentional release of the GMO(s) outside the certified PC2 facility?

In the unlikely event that the GMOs are released to the environment we will isolate the contaminated material, contain it and decontaminate it immediately by use of 1% hypochlorite solution or 80% (v/v) ethanol, and/or autoclaving. The IBC will be promptly notified should unintentional release occur, via the IBC secretary (Risk Management Office, 1 4126).

6.G Are there any other actions and precautions you will take to minimise risks posed by the proposed dealing(s)?

All work performed in the laboratory is done in accordance with the PC2 containment guidelines.

Part 7: Signatures

I declare that to the best of my knowledge, having made reasonable inquiries, the information herein is true and correct. I understand that providing misleading information to the OGTR, deliberately or otherwise, is an offence under Commonwealth law.

Notifying Organisation's Representative

Signature: _____Date: _____

Printed Name: _____ Professor Merlin Crossley _____

Project Supervisor

Signature: _____Date: _____

Printed Name: _____Dr Neville Firth_____

IBC Chair

Signature: _____Date: _____

Printed Name: _____ Professor Anthony Weiss _____