ENVIRONMENTAL RISK MANAGEMENT AUTHORITY DECISION

18 March 2010

Application code:	ERMA200332
Application category:	To develop in containment genetically modified organisms under sections 40(1)(b) and 42A of the Hazardous Substances and New Organisms (HSNO) Act 1996.
Applicant:	AgResearch Limited
Purpose:	To genetically modify *Escherichia coli* to understand the evolutionary processes involved in copying and repairing genetic material, and adapting to living, with or without oxygen.
Date application received:	15 March 2010
Consideration date:	18 March 2010
Considered by:	Chief Executive, ERMA New Zealand

1 Summary of Decision

1.1 Application ERMA200332 to develop, as a project, genetically modified organisms (as described in Table 1 of this decision) in containment is **approved, with controls** (see Appendix 1 of this decision), having been considered in accordance with section 42A of the Hazardous Substances and New Organisms (HSNO) Act 1996 (the Act), the HSNO (Low-Risk Genetic Modification) Regulations 2003 (the Regulations), and the HSNO (Methodology) Order 1998 (the Methodology).

The organisms approved are:

1.2 The organisms approved for development are the genetically modified organisms described in Table 1:

Table 1: Organisms as recorded on ERMA New Zealand Register

Host organism	Category of host organism	Category of modification/ containment level	Nature and range of the proposed genetic modification:
Escherichia coli (Migula 1895) Castellani and Chalmers 1919 Non-pathogenic laboratory strains	1	A/PC1	Standard non conjugative and conjugative (mobilisable[1]) *E. coli* cloning and expression plasmid vectors containing donor DNA from *E. coli*. The genetic modifications will be: (1) Site-directed mutagenesis of *E. coli* genes and genomic loci involved in maintaining genome fidelity. Genes may encode products involved in DNA replication and repair, transcription and translation. (2) Disrupting genes (eg, gene knockouts) involved in maintaining genome stability and enhancing fitness to survive under different oxygen conditions. These genes are known or are predicted to be involved in DNA replication and repair, stress response pathways, and the processing of reactive oxygen species and oxidatively damaged cellular components. (3) Expressing gene products involved in maintaining genome fidelity, genome stability and enhancing fitness for complementation studies with genetically modified *E. coli* strains developed as listed above in (1) and (2). Vectors will include promoters and other gene regulatory elements, plasmid mobilisation regions, selectable marker genes, reporter genes, secretory and

[1] Plasmids vectors carrying *mob* (mobilisation) but not *tra* (transfer) genes. These can be mobilised from host to recipient *E. coli* strains by conjugation, in the presence of transfer proteins.

			targeting signals, *Cre* recombinase gene, recombination sites and flanking sequences (CRE/Lox system), protein purification tags and origins of replication. The genetic modifications will exclude: • genes encoding known or predicted vertebrate toxins. • uncharacterised sequences from pathogenic microorganisms. The genetic modifications will not: • increase the pathogenicity, virulence or infectivity of the host organism. • result in the genetically modified organism having a greater ability to escape from containment than the unmodified host organism.

2 Consideration

Sequence of the consideration

2.1 The application was formally received and verified as containing sufficient information on 15 March 2010.

2.2 The decision was based on the information supplied by the applicant in the application form: *Develop in containment a project of low risk genetically modified organisms by rapid assessment* (NO3P).

2.3 The application was considered by Rob Forlong, the Chief Executive of ERMA New Zealand. Relevant staff within ERMA New Zealand, including the General Manager, Māori, were involved in providing advice on the consideration of the application.

2.4 In reaching my decision I considered that the organism description and purpose described in this application fall within the bounds of a project. This project represents a particular line of scientific inquiry and has clearly defined objectives ie, to investigate genes in *E. coli* that are involved in copying and repairing genetic material, and dealing with oxidative stresses so that there is a better understanding of the evolutionary processes involved in adapting to living with and without oxygen. The description of the organisms includes the taxonomy of the host, the types of vectors to be used, and a detailed description of the source and function, and the nature and range of proposed genetic modifications, including the range of regulatory

sequences and selectable markers (see Table 1). The application provides a sufficient description of the genetically modified organisms which will be produced to confirm that they conform to the Regulations and the controls imposed by this approval.

2.5 In reaching my decision I have considered matters relevant to the purpose of the Act, as specified in Part II, and followed the relevant provisions of the Methodology.

2.6 In accordance with section 42A of the Act for rapid assessment, the approach adopted was to identify the host organisms and the nature and range of the genetic modification, to evaluate these against the criteria specified in the Regulations established under section 41 of the Act, and to consider whether there are any residual risks that require further consideration. This approach covered the following issues:

- purpose of the application (section 39 of the Act);
- assessment against the criteria of the Regulations;
- identification and assessment of the risks and other impacts of the organism;
- precedents;
- assessment of whether the applicant needs to provide progress reports on the research; and
- identification and assessment of any controls necessary to provide for each of the matters specified in Schedule 3 of the Act.

Purpose of the application

2.7 The purpose of this application is to identify and describe the function of genes in *E. coli* that are involved in adapting to living in environments with and without oxygen. The aims of this project are to understand the effects of varying oxygen concentrations on evolutionary processes in bacteria and the mechanisms that are involved.

2.8 The applicant proposes to grow laboratory strains of *E. coli* under different oxygen conditions to identify individuals that survive better in different concentrations of oxygen. To increase the chances of discovering such individuals, these bacteria may be genetically modified so that their ability to faithfully reproduce genetic material is compromised and their ability to deal with external stress and internal stresses such as damaging types of oxygen, and effects of these damages within the cell is altered. In addition, *E. coli* may be genetically modified to express the products of these genes for complementation studies to determine how these genes contribute to the ability of *E. coli* to survive better in different oxygen concentrations.

2.9 I have determined that this application is for a valid purpose being the development of any new organism as provided for in section 39(1)(a) of the Act.

Assessment against the criteria for low-risk genetic modification

Category of host organism

2.10 The non-pathogenic laboratory strains of *E. coli* to be used by the applicant are not capable of causing disease in humans, animals, plants or fungi, do not normally infect, colonise, or establish in humans, nor do they produce desiccation-resistant structures, such as spores or cysts. As such, non-pathogenic laboratory strains of *E. coli* are considered Category 1 host organisms as defined in clause 7(1) of the Regulations.

Category of genetic modification

2.11 The genetic modifications to non-pathogenic laboratory strains of *E. coli* (described in Table 1) are not expected to increase the pathogenicity, virulence or infectivity of the organism to laboratory personnel, the community, or the environment. In addition, the developments will not result in the organism having a greater ability to escape from containment than the unmodified organism. Therefore, the genetic modifications to non-pathogenic laboratory strains of *E. coli* as described in Table 1 of this decision are Category A genetic modifications as defined in clause 5(1) of the Regulations and shall be contained at a minimum of Physical Containment level 1 (PC1).

2.12 The applicant has proposed to use conjugative plasmid vectors to facilitate the transfer of these vectors from host to recipient *E. coli* non-pathogenic laboratory strains. The conjugative plasmid vector cannot move itself (not self-transmissible) because it does not carry gene(s) which code for transfer proteins. Transfer proteins are required for moving these plasmid vectors during conjugation and may be supplied by the recipient *E. coli* strain. After considering whether these developments might affect the category of genetic modifications for *E. coli* non-pathogenic laboratory strains, I determined that the use of conjugative plasmids does not change the category of genetic modification as described in paragraphs 2.11.

2.13 I am satisfied that the developments meet the criteria for low-risk genetic modification specified in the Regulations. The developments involving non-pathogenic laboratory strains of *E. coli* meet the requirements of Category A modifications as defined in clause 5(1) of the Regulations.

Identification and assessment of the risks, costs and other impacts of the organism

2.14 I consider that the information provided by the applicant is relevant and appropriate to the scale and significance of the risks, costs, and benefits associated with the application (as required by clause 8 of the Methodology). In accordance with clauses 9, 10 and 12 of the Methodology (which incorporate sections 5, 6, and 8 of the Act) the information supplied by the applicant has been evaluated as follows:

2.15 I consider that, given the biological characteristics of the organisms, the containment system and the controls attached to this approval (see Appendix 1 of this decision), there is no evidence for, nor any reason to expect, any non-negligible adverse effects of the proposed genetically modified organisms on humans, animals, plants, other organisms or the environment.

2.16 I have considered the potential Māori cultural effects in accordance with sections 6(d) and 8 of the Act and clauses 9(b)(i), 9(c)(iv) of the Methodology, in consultation with the General Manager, Māori. This application is for development in containment and does not involve the use of genetic material from native or valued flora and fauna or from Māori. The applicant did consult with Māori to keep the local iwi informed of the scientific research plan, as a means of relationship building and to ensure that there were no issues with this containment application. In consultation, it was noted that the proposed research did not impinge on their culture or traditions and no other issues were raised.

2.17 Although recognising that iwi/Māori maintain an ongoing interest and concern in the potential long term cultural implications of genetic modification generally, I consider that this application poses negligible risk of adverse effects to the relationship of Māori culture and traditions with their ancestral lands, water, sites, waahi tapu, valued flora and fauna, and other taonga.

2.18 This assessment is made with the understanding that all associated containment regulations, controls and conditions are met by the applicant.

Precedents

2.19 I must consider each application on its merits, and am therefore not bound by the stance taken in previous decisions. However, in reflecting on previous decisions that involved similar genetic modifications to those proposed by this application, I note that genetic modifications of non-pathogenic laboratory strains of *E. coli*, conforming to the Regulations, have been considered and approved on several occasions by both Institutional Biological Safety Committees (IBSCs) and the Chief Executive of ERMA New Zealand, under delegated authority. For example, in application GMD9009, a proposal to generate a series of random mutation libraries of both non-

pathogenic and pathogenic *E. coli* relevant to the food industry to look at survival and persistence mechanisms, was approved by the Chief Executive.

2.20 I consider that this current application does not raise any novel issues and as such do not require progress reports on this application.

Containment

2.21 The experiments proposed in this application to develop genetically modified non-pathogenic laboratory strains of *E. coli* meet the requirements of Category A genetic modifications as defined in clause 5(1) of the Regulations. Category A experiments are required to be contained within a Physical Containment level 1 facility (PC1).

2.22 The facility to be used shall be approved as a containment facility under section 39 of the Biosecurity Act, in accordance with the MAF/ERMA New Zealand Standard: *Facilities for Microorganisms and Cell Cultures: 2007a*. This containment regime contains clear guidelines for the safe handling and disposal of cultures.

3 Decision

3.1 I am satisfied that this application is for one of the purposes specified in section 39(1) of the Act, being section 39(1)(a): *the development of any new organism*.

3.2 Based on consideration and analysis of the information provided, and having considered the characteristics of the organisms that are the subject of this approval, the modifications and the criteria for low-risk genetic modification detailed in the Regulations, I am of the view that the organisms meet the criteria for rapid assessment under section 42A of the Act.

3.3 I have considered all the matters to be addressed by the containment controls for Importing, Developing or Field testing of Genetically Modified Organisms detailed in the Third Schedule Part I, of the Act, and in accordance with section 42A(3)(b) of the Act, this approval is subject to the controls specified in Appendix 1.

3.4 I consider that this current application does not raise any novel issues and there are no residual issues that require further consideration.

3.5 Pursuant to section 42A(3)(a) of the Act, and acting under delegation from the Authority provided for in section 19 of the Act, I have **approved** this project application for genetically modified non-pathogenic laboratory strains of *E. coli* described in Table 1 of this decision, subject to the controls specified in Appendix 1 of this decision.

_____ 18 March 2010

Mr Rob Forlong Date

Chief Executive, ERMA New Zealand

Approval code: GMD100267

Appendix 1: Controls required by this approval

In order to provide for the matters detailed in Part I of Schedule 3 of the Act[2], *Containment Controls for Importation, Development and Field Testing of Genetically Modified Organisms,* and other matters in order to give effect to the purpose of the Act, the approved organisms are subject to the following controls:

1 To limit the likelihood of any accidental release of any organism or any viable genetic material[3].

1.1 The approved organisms shall be developed and maintained within a containment facility which complies with these controls.

1.2 The Operator must ensure all personnel involved in the handling of the organisms are made aware of and understand these controls.

1.3 The facility shall be approved by MAF as a containment facility under section 39 of the Biosecurity Act, in accordance with the MAF/ERMA New Zealand Standard (below).

1.4 The construction, operation and management of the containment facility shall be in accordance with the:

1.4.1 MAF/ERMA New Zealand Standard: *Facilities for Microorganisms and Cell Cultures: 2007a*[4];

1.4.2 Australian/New Zealand Standard AS/NZS 2243.3:2002[4]: *Safety in laboratories: Part 3: Microbiological aspects and containment facilities*; and

1.4.3 Physical Containment level 1 (PC1) requirements of the above Standards.

[2] Bold headings in the following text refer to Matters to be Addressed by Containment Controls for Import, Development and Field Testing of Genetically Modified Organisms, specified in the Third Schedule of the Act.
[3] Viable Genetic Material is biological material that can be resuscitated to grow into tissues or organisms. It can be defined to mean biological material capable of growth even though resuscitation procedures may be required, e.g. when organisms or parts thereof are sub-lethally damaged by being frozen, dried, heated, or affected by chemical.
[4] Any reference to this standard in these controls refers to any subsequent version approved or endorsed by ERMA New Zealand.

2 To exclude unauthorised people from the facility.

2.1 Construction and operation of the containment facility shall comply with the requirements of the standards listed in control 1.4 relating to the identification of entrances, numbers of and access to entrances and security requirements for the entrances and the facility.

3 To exclude other organisms from the facility and to control undesirable and unwanted organisms within the facility.

3.1 Construction and operation of the containment facility shall comply with the requirements of the standards listed in control 1.4 relating to the exclusion of other organisms from the facility and the control of undesirable and unwanted organisms within the facility.

4 To prevent unintended release of the organism by experimenters working with the organism.

4.1 Construction and operation of the containment facility shall comply with the requirements of the standards listed in control 1.4 relating to the prevention of unintended release of the organism by experimenters working with the organism.

5 To control the effects of any accidental release or escape of an organism.

5.1 Construction and operation of the containment facility shall comply with the requirements of the standards listed in control 1.4 relating to controlling the effects of any accidental release or escape of an organism.

5.2 If a breach of containment [5]occurs, the Operator must ensure that the MAF Inspector responsible for supervision of the facility has received notification of the breach within 24 hours.

5.3 In the event of any breach of containment of the organism, the contingency plan for the attempted retrieval or destruction of any viable material of the organism that has escaped shall be implemented immediately. The contingency plan shall be included in the containment manual in accordance with the requirements of standards listed in control 1.4.

[5] Breach of containment includes: escape of organism(s), unauthorised entry to facility, and/or structural integrity of facility compromised.

6 Inspection and monitoring requirements for containment facilities.

6.1 The operation of the containment facilities shall comply with the requirements contained in the standards listed in control 1.4 relating to the inspection and monitoring requirements for containment facilities.

6.2 The containment manual shall be updated, as necessary, to address the implementation of the controls imposed by this approval, in accordance with the standards listed in control 1.4.

7 Qualifications required of the persons responsible for implementing those controls.

7.1 The training of personnel working in the facility shall be in compliance with the standards listed in control 1.4.