FORM 2

Application for approval to

IMPORT INTO CONTAINMENT ANY NEW ORGANISM

under Section 40 of the Hazardous Substances and New Organisms Act 1996

Office use only ☐☐☐☐☐☐☐☐

Fees $_____ Date received ___/___ /___ Verified date ___/___ /___

_____Job manager

Application for approval to field test (including large scale fermentation) in containment any genetically modified organism under Section 40 of the Hazardous Substances and New Organisms Act 1996

IMPORTANT

Before you fill in this application form please talk to ERMA New Zealand. We can help you scope and prepare your application. The scale of information we need should match the potential significance of the application. For example, applications which may pose a significant risk to the environment or to human health need to be supported with more substantial information than applications which clearly pose a more minor risk.

We need all relevant information early on in the application process. Quality information up front will speed up the process.

Any extra material that does not fit in the application form must be clearly labelled and cross-referenced in the application form. Commercially sensitive information should be collated in a separate document.

All applicants must sign at the end of the form and enclose the correct application fee. The standard non-notified application fee is $750 (excl GST). We are unable to process applications that do not contain the correct fee.

All references to regulations in this form, unless otherwise noted, refer to the Hazardous Substances and New Organisms (New Organisms Forms and Information Requirements) Regulations 1998.

Copies of all our application forms will soon also be available on our website: www.ermanz.govt.nz, and also in electronic form (MS Word format).

If you have any suggestions for improvements to this form, please contact our operations staff at the address below.

You can get more information at any time by telephoning, writing to, or calling in at our Wellington office. One of our staff members will be able to help you.

List of application forms for new organisms:

These are all our application forms related to new organisms. Please check you have the right one.

Form 1 Application for approval under section 34 of the Act to import for release, or release from containment, any new organism -–including rapid assessment.

Form 2 application for approval under section (40)(1)(a) of the Act to import into containment any new organism **(this form)**.

Form 3 application for approval under section 40(1)(b) of the Act to develop in containment any genetically modified organism – including rapid assessment.

Form 4 application for approval under section 40(1)(c) to field test (including large scale fermentation) in containment any genetically modified organism.

Form 5 application for approval under section 47 to use a new organism in an emergency.

Form 6 application for approval under section 62 for grounds for reassessment of a new organism in containment.

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Application for approval to field test (including large scale fermentation) in containment any genetically modified organism under Section 40 of the Hazardous Substances and New Organisms Act 1996

Applicant details

1. Name and address in New Zealand of the applicant:

This should be the organisation or person formally responsible for this application.

Name: University of Otago
Address: c/o Professor G Petersen, Chair IBSC, Department of Biochemistry, PO Box 56, Dunedin
Phone: 03 479 7846

Name: University of Auckland
Address: c/o Dr N. Birch, Chair, University of Auckland, Biological Safety Committee, Molecular Neuroendocrinology Laboratory, School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland
Phone: 09 373 7599 ex 7279

Name: Massey University
Address: c/o Professor H. T. Blair, Chair IBSC, Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11222, Palmerston North
Phone: 06 356 9099

2. The applicant's address for service in New Zealand (if different from above):

Address: Professor Petersen at the University of Otago, as above.

3. Name of the contact person for the application (if different from applicant): This person should have sufficient knowledge to respond to queries and have the authority to make decisions on behalf of the applicant that relate to processing the application.

Name: Dr Iain L Lamont
Position: Member of University of Otago IBSC
Phone: 03 479 7869
Fax: 03 479 7866 **Email:** iain.lamont@stonebow.otago.ac.nz

4. Summary

Provide a summary of the information contained in this application relating to the identification of the organism.
The information should include summaries of:
- the identity of the organism;
- if it is a genetically modified organism, the source of the donor nucleic acid material and the purpose of the modification;
- what the organism will be used for and why it has been selected.

Provide a summary of the information contained in this application relating to the assessment of the effects of the organism.
The information should include summaries of:
- the risks, costs and benefits and the assessment of these;
- the containment system proposed.

This summary will be used to provide information to people and agencies who may request it. Applications to import any new organism into containment will not be publicly notified. However, as the information in this section may be released upon request, applicants should ensure that this summary does not contain any commercially sensitive information.

[Yes/No?] further information

contd/..

The organism is *Escherichia coli* strain K12 or strain B that has been genetically modified to contain DNA from other organisms. It is genetically crippled and has been shown to be unable to establish self-sustaining populations outside the laboratory environment. The donor material is from the vertebrates, invertebrates, plants, fungi and microorganisms listed in Appendix 1. It has been cloned into standard DNA cloning vectors that are not transmissible to other bacteria (listed in Appendix 2). The organism was selected because it cannot survive in the natural environment in the event of accidental release from containment and because it is the standard host for cloning and analysis of DNA. The organism will be used as a source of cloned DNA fragments for a variety of research purposes involving the analysis of gene structure, expression and function. The benefit of the organism is that it will advance scientific knowledge and understanding. The cost of not importing the organism would be a reduction in New Zealand's ability to carry out innovative research that has the long term aim of enhancing the country's economy and public well being. The proposed containment system is physical containment level 1 (PC1) as defined in the Australia New Zealand Standard for Microorganisms 2243.3:1995 and as registered by MAF to meet the MAF/ERMA Standard 154.03.02 *Containment Facilities for Microorganisms*.

Organism details

5. The identification of the organism:

This should include all information necessary to identify the organism and should include:

- the taxonomic classification and name of the organism;
- the essential characteristics that identify the organism and its behaviour in the environment;
- sufficient information to enable the Authority to uniquely identify the organism in the register as required by section 20(2)(b) of the Act.

(This section may also include the name by which the organism is generally known.)

The information in this section would include, for example, information on the habitat range and climatic sensitivity of the organism. References to the scientific literature supporting this information should be given here if appropriate.

In the separate box below the applicant should provide the name of the organism suitable for inclusion in the Authority's public register.

Information that is commercially sensitive should be clearly identified. If supplied separately, a cross-reference to it should be included.

Taxonomic Name: Genetically crippled derivatives of *Escherichia coli* K-12 and strain B

Characteristics: Non-pathogenic Gram negative rod-shaped bacterium; originally derived from non-pathogenic bacteria inhabiting the gut of mammals; carries mutations that would prevent it from establishing a self-sustaining population outside the laboratory; has been shown to be unable to colonise humans.

References:

1. Bachmann, B. (1996) Derivatives and genotypes of some mutant derivatives of *Escherichia coli* K-12. In: *Escherichia coli* and *Salmonella*: molecular and cellular biology. F. Neidhardt, Ed. ASM Press, Washington, USA.
2. Smith, H.W. (1975) Survival of orally administered *E. coli* K12 in alimentary tract of man. Nature 255:500-502.

[Yes/No?] further information [Yes/No?] commercially sensitive information

Name of the organism that may be used for the Authority's public register:

Escherichia coli K-12 or B derivatives as modified by DNA cloned from donor organisms into non-conjugative plasmid or phage vectors.

6. If the organism is a genetically modified organism, information on the details of the genetic modifications:

This information shall include full details of the genetic constructs and modifications and the source and characteristics of the foreign nucleic acid.

This information should clearly identify the source of the donor genetic material and the characteristics. The desired characteristic (eg, herbicide resistance) and any other significant characteristics that may be expressed by the donor genetic material in the organism should be described.

Information on the stability and homogeneity of the construct should be given, if known. If this information is not known then this should be explicitly stated. References to the scientific literature supporting this information should be given here if appropriate.

Information that is commercially sensitive should be clearly identified. If supplied separately a cross-reference to it should be included.

[Yes/No?] further information [Yes/No?] commercially sensitive information

The sources of the foreign nucleic acids are the organisms listed in Appendix 1. The DNA (genomic and copy DNA) is cloned into the vectors listed in Appendix 2. These vectors are approved for use with *Escherichia coli* K12 or *E. coli* B derivatives under Schedule 2 of the Hazardous Substances and New Organisms (Low Risk Genetic Modification) Regulations 1998. The desired characteristic is that the donor DNA is stably replicated in cells of *E. coli*. The donor DNA is well characterised stable homogeneous clones of DNA or libraries of DNA clones. This application is restricted to organisms that fall within Category A or B of Schedule 1 of the Hazardous Substances and New Organisms (Low Risk Genetic Modification) Regulations 1998; organisms that, due to the nature of the donor nucleic acid, fall within Category C of these Regulations are specifically excluded. Animals, fish and plants that are native to New Zealand are explicitly excluded from the list of donor organisms.

7. The reason why an application is necessary for the organism:

Refer to the definitions set out in Section 2 of the Act, to the prohibited organisms in the Second Schedule of the Act, and for genetically modified organisms, to the exemptions in the HSNO (Organisms Not Genetically Modified) Regulations 1998.

An application is necessary because this importation involves a genetically modified organism that falls into Category A and B of Schedule 1 of the Hazardous Substances and New Organisms (Low Risk Genetic Modifications) Regulations 1998. The imported organisms are not prohibited organisms listed in the Second Schedule to the Hazardous Substances and New Organisms Act (1996).

8. The purposes for which an approval is sought:

Reference should be made to the purposes specified in section 39(1) of the Act and the information should also provide sufficient details on the purpose of the application to enable the Authority to provide the information required in the register (under section 20(2)(c) of the Act).

The information in this section should be as expansive as possible. While the applicant may have only one potential use in mind, an approval would enable other uses as well. To enable the Authority to have access to all relevant information all the potential uses of the organism should be provided. The information on how well the organism performs these uses is necessary to enable the Authority to determine the performance characteristics of the organism.

Information that is commercially sensitive should be clearly identified. If it is supplied separately a cross-reference to it should be included.

[Yes/No?] further information [Yes/No?] commercially sensitive information

This application falls under section 39(1)(h) of the Hazardous Substances and New Organisms Act 1996, "importation of a new organism for such other purposes as the Authority thinks fit". The imported organism will be used for the following research purposes: as a source of DNA libraries to be screened for clones; as a source of cloned DNA for subcloning into other vectors; as a source of cloned DNA for sequencing, for mutagenesis experiments and as probes in hybridisation experiments; as a source of vectors for DNA cloning experiments; and for the expression of cloned genes to enable functional analysis of gene products.

Provide in this box a statement describing the purpose for making the application. This statement may be included in the Authority's public register (please use a maximum of 255 characters):

The purpose of this application is to permit importation of *Escherichia coli* bacteria that contains fragments of DNA that have been cloned from other species.

9. Information on any likely inseparable organisms:

Information should be provided on any organism which is unable to be separated from any new organism at the time of making the application. Examples may include foot and mouth and scrapie causing organisms in animals and viruses in plants.

[Yes/No?] further information

These will be pure cultures and will not contain any inseparable organisms.

Assessment of Effects

The information to be provided in these sections should cover the assessment of effects (both adverse and positive) of the organism. Where appropriate these sections may be combined in section 13 below.

Effects should be clearly assessed where relevant, including details as to how the risks will be controlled by the proposed containment system. **Where these adverse effects are identified, in the first instance by the applicant, as being minor then these do not require in-depth assessment.**

10. Information on all the possible adverse effects of the organism on the environment:

This should include information on the effects of the organism on ecosystems, public health, and Maori culture and taonga. It should also include information relevant to the matters in sections 4, 5, 6, 7, 8, and 37 of the Act and any regulations made under section 41 of the Act. The assessment should identify and assess risks, costs and benefits.

The information should give particular regard to:

Environmental and ecosystem effects (section 6(a) and (b) of the Act)

- assessment of the known and possible adverse effects throughout the life cycle of the organism on the sustainability of native and valued introduced flora and fauna and on the intrinsic value of ecosystems. *[Include an assessment of the ability of the organism to establish an undesirable self-sustaining population and the ease with which the organism could be eradicated if it was established.]*

Public health effects (section 6(c) of the Act)

- assessment of the known and possible adverse effects throughout the life cycle of the organism on public health. *[Assessment should take account of aspects of public health and safety including, where appropriate, effects from occupational exposure and effects from environmental exposure to the organism.]*

Relationship of Maori with taonga (section 6(d) of the Act)

- assessment of the known and possible adverse effects throughout the life cycle of the organism on the relationship of Maori and their culture and traditions with their ancestral lands, water, sites, wahi tapu, valued flora and fauna, and other taonga. [Include details of consultation (if any) carried out.]

The ability of the organism to escape from containment.

Environment and ecosystem effects. All the strains of bacteria to be used are derived from *E. coli* K-12 or the closely related *E. coli* strain B. Strain K-12 was originally isolated from the stool of a person in 1922. There is no evidence that the original isolate was associated with disease. By the 1950's, after 30 years of being subcultured, it was found that the strain had a greatly reduced ability to produce the surface antigens that are characteristic of wild-type strains. It was shown in 1975 that laboratory strains of *E. coli* K-12 are unable to colonise the alimentary tract of a human host and do not cause any symptoms of disease. Strain B is closely related to strain K-12 and has also been subcultured in research laboratories for over 30 years so that conclusions reached with strain K-12 can be extended to strain B. In addition to the above characteristics, strains of *E. coli* in current use have requirements for growth factors such as amino acids and vitamins further reducing the possibility of their survival outside the laboratory. These data show that the organism would not be able to establish a self-sustaining population even if it were to escape from containment.

References:

1. Bachmann, B. (1996) Derivatives and genotypes of some mutant derivatives of *Escherichia coli* K-12. In: *Escherichia coli* and *Salmonella*: molecular and cellular biology. F. Neidhardt, Ed. ASM Press, Washington, USA.

2. Smith, H.W. (1975) Survival of orally administered *E. coli* K12 in alimentary tract of man. Nature 255:500-502.

Public Health Effects. The organism will be held in approved containment facilities. For reasons described above the organism would not pose any threat to public health even if it were to escape from containment.

Relationship with Maori and taonga. This application does not involve native organisms and the organism will be held in containment so that no effect on the relationship of Maori with taonga is envisaged.

The ability of the organism to escape from containment. The organism will be unable to escape from containment unless accidental release occurs. Even if this were to occur, the organism would be unable to establish a self-sustaining population for reasons outlined above.

11. In the identification and assessment of risks, costs and benefits and other impacts, which may occur should the organism escape, include those matters set out below.

The information should comprise of the risks identified and include:

- the nature of the adverse effects of the organism.
- the probability of occurrence and the magnitude of each adverse effect.
- the risk assessed as a combination of the magnitude of the adverse effect and the probability of its occurrence.
- the options and proposals for managing the risks identified.
- the uncertainty bounds on the information contained in the assessment, expressed quantitatively where possible but otherwise through narrative statements.

The identification and assessment of costs and benefits required in each application must include.

- the nature of the costs and benefits associated with the proposed new organism and whether they are monetary or non-monetary;
- the magnitude or expected value of the costs and benefits and the uncertainty bounds on the expected value.

Relevant costs and benefits will be those which pertain to the New Zealand economy, society and environment and which would not arise if the application was not approved (ie the opportunity cost to New Zealand). They shall include the long term as well as short term, and consequential as well as direct costs and benefits.

The information on risks, costs and benefits shall include the distributional effects over time, space and groups in the community. It shall also include the uncertainty intervals associated with these estimates.

Costs and benefits of the organism. There is no foreseeable risk associated with importation of the organism. This is because it will be held in containment; even if it were to escape from containment, it would be unable to establish a self-sustaining population; and the organism has been very widely used in research laboratories world wide for over 20 years with no evidence of adverse effects. Importation of the organism is a non-monetary benefit that will advance scientific knowledge and understanding. The costs of not importing the organism include a major reduction in New Zealand's ability to carry out innovative research, reduced opportunities to train new researchers in molecular biology and biotechnology and a reduced ability for New Zealand researchers to be part of the international scientific research community. There are no costs associated with importation of the organism.

12. Information on the positive effects of the organism:

The organism will be essential in enabling New Zealand researchers to carry out a very large number of innovative research projects in the general areas of health and biotechnology, thereby enhancing scientific knowledge and understanding.

13. Assessment of effects

If the assessment of effects is combined into this section, applicants should clearly indicate how the information requirements in sections 10, 11 and 12 of this form are addressed.

[Yes/No?] further information [Yes/No?] commercially sensitive information

Response

Containment System

14. Information about proposed containment system:

Provide information on how it is proposed that the organism be adequately contained including how the proposed containment system conforms to the requirements of the Parts I and II of the Third Schedule of the Act as appropriate.

This may include reference to, and outlines of, appropriate standards and codes of practice.

[Yes/No?] further information

As the host organism and vectors listed under Schedule 2 of the Hazardous Substances and New Organisms (Low Risk Genetic Modification) Regulations 1998, this organism falls into Categories A and B of the Regulations. Therefore the containment level for the organism is PC1 of A/NZS 2243.3. The organism will be imported into transitional facilities approved by the Ministry of Agriculture and Fisheries pursuant to the Biosecurity Act 1993 and in accordance with MAF Regulatory Authority Standard 154.02.17, Transitional Facilities for Biological Products. Copies of Containment and Quarantine Manuals (University of Auckland and Massey University) are attached along with Certificates of Approval as Transitional Facilities for these Universities. ERMA NZ already has copies of the equivalent documentation from the University of Otago (Dunedin Campus and Malaghan Institute of Medical Research). Documentation for the University of Otago (Christchurch School of Medicine) will be sent to ERMA once this transitional facility has been approved by MAF.

International and related matters

15. Information on all occasions where the organism has been considered by the government of any prescribed State or country or by any prescribed organisation and the results of such consideration: Where no countries or organisations are prescribed by regulations made under section 140(1)9k) of the Act, this section can be omitted.

If the applicant is aware that the organism has previously been considered by, for example, any OECD or APEC country, information on the nature of that consideration, including the result, should be provided if known.

[Yes/No?] further information

The organism is exempt from the National Institute of Health (USA) Guidelines for Recombinant DNA Research because it does not present a significant risk to health or the environment.

Reference: National Institute of Health Guidelines for Research Involving Recombinant DNA Molecules. (http://www.nih.gov/od/orda/toc.htm).

This organism is exempt from the Genetic Manipulation Advisory Committee (GMAC) (Australia) guidelines if the foreign nucleic acids is not derived from a microorgansim able to cause disease in humans, plants or animals. If the foreign nucleic acid is a pathogenic determinant; uncharacterised DNA from microorganisms

able to cause disease in humans, animals or plants; or an oncogene then it requires approval by the Institutional Biosafety Committee and Notification to GMAC.

Reference: Genetic Manipulation Advisory Committee Guidelines for Small Scale Genetic Manipulation Work. April 1995. GMAC Secretariat, GPO Box 2183, Canberra, ACT 2601, Australia.

16. Information on New Zealand's international obligations that may be relevant to the application:

Where the applicant is aware that New Zealand's international obligations may be relevant to the application, indicate the nature of the obligation and the effect this may have on the application.

If the applicant is aware of obligations such as the WTO Agreements, the Convention on International Trade in Endangered Species (CITES), Trans Tasman Mutual Recognition Agreement and the like that may be relevant to the application, then information on these obligations should be provided, if known.

[Yes/No?] further information

Not applicable

Previous considerations

17. If the application relates to an organism that has been previously considered by the Advisory Committee on Novel Genetic Techniques or the Minister for the Environment on the recommendation of the Interim Assessment Group, details of the consideration and its results:

[Yes/No?] further information

The development of *E. coli* strains K12 and B modified by DNA from many of the donor organisms listed in Appendix 1 was approved by the Advisory Committee on Novel Genetic Techniques.

Reference: New Zealand Gazette Issue 101, pages 2346 - 2350 item 58.

Other relevant legislation

18. Information on other legislation relevant to the organism and its use throughout its life cycle.

If the organism is also subject to other legislation (eg. an Import Health Standard under the Biosecurity Act 1993, or resource consent under the Resource Management Act 1991), details should be provided.

[Yes/No?] further information

Importation of the organism requires a Ministry of Agriculture and Fisheries permit under the Biosecurity Act 1993.

Glossary

19. A glossary of scientific and technical terms used in the application.

This may be appended to the application on a separate form if desired.

[Yes/No?] further information

Copy DNA: double stranded DNA synthesised using RNA as a template.

DNA library: a set of clones each containing a different DNA fragment derived from a common source.

Genetically crippled: organisms that lack genes essential for their survival outside containment.

Hybridisation: the formation of double-stranded nucleic acid molecules where each strand is from a different source.

Mutagenesis: treatment of DNA in order to introduce heritable changes to the nucleotide sequence.

Non-conjugative: a plasmid that cannot transfer itself from one bacterial cell to another.

Other relevant information

20. Provide here any other information required by the Act or regulations not included under any other section of this form.

[Yes/No?] further information [Yes/No?] commercially sensitive information

Not applicable

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Summary of Application Contents

(Please check the application is complete and identify attachments)

[Yes] Fees enclosed

[No] Confidential information supplied

[Yes] Assessment of effects included

[Yes] Signed and dated

[Yes] Appendices attached and cross-referenced (list below)

Appendix 1 Organisms that are the source of donor nucleic acids.

Appendix 2 DNA cloning vectors

Appendix 3 Containment Manual of University of Auckland School of Biological Sciences

Appendix 4 Quarantine Manual of University of Auckland School of Biological Sciences

Appendix 5 Quarantine Manual for Massey University

Appendix 6 Certificate of Approval as a Transitional Facility: Massey University (Institute of Molecular Biosciences)

Appendix 7 Certificate of Approval as a Transitional Facility: University of Auckland (School of Biological Sciences)

Signature of applicant or person authorised on behalf of applicant _____

Date: _____

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