

April 1, 2008

Notes on Experiments involving Recombinant DNA

(For *Chikyu*)

Safety and Environment Management Office
Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

This document outlines the necessary procedures and precautions pertaining to experiments involving recombinant DNA. Investigators who do not comply with these procedures and precautions shall not be permitted to conduct onboard recombinant DNA experiments.

1. Securing space for experiments

Appropriate space must be secured to conduct experiments involving recombinant DNA. Availability of such space depends on operational and lab conditions of *Chikyu*. Please contact the Expedition Project Manager at CDEX/JAMSTEC (EPM) well in advance of the expedition.

2. Equipment and materials to be prepared

Inform the EPM of equipment, materials, and chemicals to be used in your experiments well in advance of the expedition. Although basic supplies are available or provided onboard, each scientist is responsible to confirm all necessary items are available onboard. Contact the EPM to see the list of equipment, materials, and chemicals available onboard. If any extra equipment, materials, or chemicals are needed on board for the experiments, get permission from the EPM in advance.

3. Selection of the Experiment Leader

The Primary Investigator, or an assigned scientist in charge of the onboard experiments involving recombinant DNA, shall be responsible for the following procedures as the Experiment Leader. When multiple investigators work on the experiments involving microorganisms, please designate one person as the Leader with overall responsibility for said experiments. For details, consult with the EPM.

- **Application Forms**

The Leader shall submit the “Application Form for Approval of Recombinant DNA Experiments” and “Report on Completion of Recombinant DNA Experiments” after compiling information on all relevant experiments to be conducted.

- **Selection of the Research Party**

The Leader must provide a list of names and other relevant information of persons who will be conducting the above mentioned recombinant DNA experiments as “Research Party” on the “Application Form for Approval of Recombinant DNA Experiments.” Persons not on the list will not be allowed to conduct recombinant DNA experiments. The Leader designates *Chikyu’s* research support specialists who perform their duties, including facilities management and experiment assistance as “Research Party.” Check with the EPM for details.

- **Education and training**

The Leader is responsible to provide education and training covering all of the following items with regard to planned recombinant DNA experiments to the Research Parties prior to boarding:

- 1) Technologies for the safe handling of microorganisms, in accordance with risk level
- 2) Physical containment
- 3) Biological containment
- 4) Accident prevention and corrective action
- 5) Other safety-related items necessary to prevent experiment-related accidents

However, education and training are not required if it is determined that, based on his or her past record and experience, the Research Party has sufficient knowledge and skill to conduct recombinant DNA experiments.

- **Pledge**

The Leader must pledge to comply with JAMSTEC’s Safety Regulations for Recombinant DNA Experiments and to guide and supervise the Research Party.

- **Identification of regulations**

The Leader needs to identify local national regulations concerning the movement (import/export) of recombinant DNA material when scientist embarks/disembarks outside Japan. The Leader must inform the EPM of such regulations.

4. Submission of the Application Form for Approval of Recombinant DNA Experiments

The Leader must fill in the required items on the “Application Form for Approval of Recombinant DNA Experiments” (Form 2) and submit it to the EPM at least three months prior to the date of boarding.

Experiments involving recombinant DNA require prior approval from the President of JAMSTEC. Approval shall be based on discussion within the Safety Control Committee for Recombinant DNA.

5. Report on Completion of Recombinant DNA Experiments

When recombinant DNA experiments have been completed, the Leader must submit a

Report on Completion of Recombinant DNA Experiment (Form 3) within one month of disembarking from *Chikyu*. This report shall be reviewed by the Safety Control Committee for Recombinant DNA.

6. Health examination

Both the Leader and Research Party must have health examination provided by their organizations. All personnel must have a current health examination no older than 1 year prior to boarding *Chikyu*. Persons without a current health examination must receive one before being allowed aboard *Chikyu*.

7. Securing the laboratory doors and entry restrictions

Make sure that the laboratory's door and portholes are always closed when conducting experiments involving recombinant DNA; never leave doors or portholes open. Prohibit unauthorized persons from entering the laboratory. The Leader must comply with these regulations at all times.

8. Removal of contamination and disposal of samples

After experiments, the Leader is required to ensure that all equipment, materials, and supplies are properly disposed of and cleaned. These procedures must reflect the proper disposal or cleaning techniques appropriate for the equipment and materials used. Waste materials must be disposed of in accordance with the rules and regulations in force aboard *Chikyu*. All genetic or biological samples must be rendered inactive, inert or lifeless before disposal.

9. Containment measures for transport and storage of recombinant DNA

When transporting or storing samples that include recombinant DNA onto *Chikyu*, the Leader shall follow Article 6 and Article 7 of "The Ministerial Ordinance providing Containment Measures to be Taken in Type 2 Use of Living Modified Organisms for Research and Development" (Ministerial Ordinance No. 1 of 2004 of the Ministry of Education, Culture, Sports, Science and Technology and Ministry of the Environment) to ensure that said samples do not escape.

While at sea, the vessel may heel violently in a stormy weather. Take measures to prevent damage to containers caused by stormy weather.

10. Importing and exporting

The Leader agrees to conform with home country and local national laws concerning the movement of recombinant DNA material. When such events are occurred, the Leader or scientist has taken to comply with them. The Leader must also comply with the stipulations of the Law Concerning the Conservation and Sustainable Use of Biological

Diversity through Regulations on the Use of Living Modified Organisms (Law No. 97 of 2003).

Although JAMSTEC will bear responsibility for shipping samples that are collected by *Chikyu*, note that procedures associated with import or export of samples shall be the responsibility of each relevant researcher.

11. Response to accidents

In the event that an accident or problem (infection by recombinant DNA, loss of sample, etc.) occurs on board *Chikyu*, the Leader and the Researching Party must take appropriate and effective measures (notification of people in the vicinity, etc.), and immediately notify the EPM, the onboard CDEX representative (Operation Super Intendant; OSI), and/or the captain of *Chikyu*.

If an accident or problem (infection by microorganisms, loss of sample, etc.) occurs after disembarking from *Chikyu*, immediately contact IODP Department, Center for Deep Earth Exploration, JAMSTEC (045-778-5811).

12. Other items

Other agreements must be based on JAMSTEC's Safety Regulations for Recombinant DNA Experiments as well as relevant laws and ordinances. Contact JAMSTEC if any questions appear.

Explanation of items in the Application Form for Approval of Recombinant DNA Experiments

Terminology

“Experiment using microorganisms” refers to experiments involving living modified organisms that belong to the kingdom Fungi [excluding mushrooms], kingdom Protista, or kingdom Procaryotae (specifically, viruses and viroids). However, the organisms mentioned in Article 2 paragraphs 3 to 5 of “The Ministerial Ordinance providing Containment Measures to be Taken in Type 2 Use of Living Modified Organisms for Research and Development” (Ministerial Ordinance No. 1 of 2004 of the Ministry of Education, Culture, Sports, Science and Technology and Ministry of the Environment) are excepted.

“Nucleic acid donor” refers to an “organism (including a human being) from which donated nucleic acid (a recombinant nucleic acid other than a vector) is derived.”

Explanation of required items

1. Please do not write in spaces marked with asterisks.
2. The experiment applicant shall assign the Experiment Leader.
For the contact information, provide name, affiliation, telephone number and email address.
3. For the “Research Party” space in “official contact information,” if more than one Research Party exists, please write “same as in List of Research Party” and then fill in the list of Research Party. For the contact information, provide name, affiliation, telephone number and email address.
4. In the “type” space of “purpose and general outline of type 2 use,” enter “experiment using microorganisms.”
5. In the “purpose” space of “purpose and general outline of type 2 use,” describe concisely the reason for and method to be applied in the planned type 2 use.
6. In the “general outline” space of “purpose and general outline of type 2 use,” provide a simple flowchart that shows the process to be used in the experiment. This flowchart may be attached to the form on a separate paper.

Example: Genetic segregation from samples obtained in the Marianas

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Extraction in the laboratory

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PCR amplification

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(All steps below in the P1 laboratory)

Vector preparation

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Introduction

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Storage

7. In the “characteristics of nucleic acid donor” space of “characteristics of living modified organism,” provide the following information for all nucleic acid donors to be involved in the type 2 use:
 - 1) Taxonomic affiliation
 - 2) P1, P2 containment measures
 - 3) Characteristics in terms of pathogenicity and reproductivity of harmful substances
8. In the “characteristics of donated nucleic acid” space of “characteristics of living modified organism,” provide the following information for all donated nucleic acid to be involved in the type 2 use:
 - 1) Name
 - 2) Type (including genomic DNA, cDNA, synthetic nucleic acid, etc.)
 - 3) Function of components (target gene, expression regulatory gene, etc.), size, and structure
 - 4) When a donated nucleic acid is an identified nucleic acid, its base sequence information or accession number in the DNA Data Bank of Japan or other base sequence database (please specify the base number in the sequence)
9. In the “characteristics of vector” space of “characteristics of living modified organism,” provide the following information for all vectors to be involved in the type 2 use:
 - 1) Vector name
 - 2) Taxonomic affiliation of organism from which vector is derived
 - 3) Experiment classification (class 1, class 2) for organism from which vector is derived
 - 4) Transmissibility and host specificityIn addition, note the characteristics of marker genes (drug resistance genes, etc.)
10. In the “characteristics of host” space of “characteristics of living modified organism,” provide the following information pertaining to the host:
 - 1) Taxonomic affiliation
 - 2) Experiment classification (class 1, class 2)
 - 3) Distribution in the natural environment, and environments in which survival, growth, and development are possible
 - 4) Propagation and breeding patterns
 - 5) Characteristics in terms of pathogenicity and reproductivity of harmful substances
 - 6) If the host is a microorganism (excepting cases in which the host is a virus or viroid), note auxotrophy, drug resistance, and optimal life conditions. If the host is a virus or viroid, please also provide the following information following 1) to 6) above:
 - 7) Vector name
 - 8) Taxonomic affiliation of organism from which vector is derived

- 9) Experiment classification (class 1, class 2) for organism from which vector is derived
- 10) Transmissibility and host specificity
11. In the “characteristics of living modified organism (including differences with the host)” space of “characteristics of living modified organism,” provide characteristics whose new creation is anticipated or that have been created.
12. In the “characteristics of animal, plant, or cell having living modified organism” space, provide the following information if mixing is to be performed through infection/symbiosis, transplantation, etc.
 - 1) Taxonomic affiliation
 - 2) Experiment classification (class 1, class 2)
 - 3) Distribution in the natural environment, and environments in which survival, growth, and development are possible
 - 4) Propagation and breeding patterns
 - 5) Characteristics in terms of pathogenicity and reproductivity of harmful substances
13. In the “classification and reason for selection” space of “containment measures,” enter “P1” as the measure and provide the reason.
14. In the “room name” space of “containment measures,” enter both the research vessel name and laboratory name in which type 2 use will take place (be precise).
15. In the “general description of equipment” space of “containment measures” provide the following information pertaining to the selected containment measure:
 - 1) Location and name of the facility, equipment, or device, as well pertinent maintenance information
 - 2) Status of confirmation of equipment, etc., by JAMSTEC’s Safety Control Committee for Recombinant DNA (year of approval, etc.)
 - 3) If animals or plants not related to type 2 use will be bred or cultivated, a description of this activity

If making a new application, attach a separate sheet showing a general layout of the research vessel and detailed diagram of the experiment area (be as detailed as possible when showing equipment, etc.).

As required, you may be asked to attach separate sheets showing a structural diagram of the laboratory or equipment related to the containment measure, flowchart showing systems related to air conditioning or culture, or other diagrams that are in addition to the abovementioned diagrams.

16. In the “measures to inactivate living modified organisms” space of “containment measures,” describe methods for inactivating living modified organisms used in the experiment as well as devices and equipment on which such organisms may be attached. Make your notations so that treatment circumstances (items and conditions of treatment, etc.) can be identified. If inactivation is to occur under special conditions due to unique characteristics of the host or in order to recover living modified organisms, you must

demonstrate the effectiveness of inactivation by providing concrete data.

17. In the “other” space, note the scheduled time period for type 2 use.

18. List of Research Party

Enter the name, affiliated organization, position, telephone number and e-mail address of each Research Party.

19. Education and training

If education/training has been completed, circle “completed.” If, based on past experience and activities, it is judged that the Research Party does not require education/training, circle “not required.” If education/training has not been completed, circle “not completed.” If education/training has not been completed, see that it is completed prior to the Research Party’s boarding of *Chikyu*. Also, prepare and submit a separate “report on education and training” (any format accepted) that provides the “dates and content of education/training, the person to receive education/training, and the person that will provide education/training.”

20. Date of health examination

If the Research Party has received health examination within one year prior to boarding *Chikyu*, write “completed.” If the Research Party has not received health examination within one year prior to boarding *Chikyu*, write “not completed”; The Experiment Leader must ensure that all members of the Research Party have completed and submitted a copy of a recent health examination.

Signature and seal of the Experiment Leader

The Experiment Leader is responsible to sign and seal the “Application Form for Approval of Recombinant DNA Experiments” to certify that there are no inaccuracies in the information provided in the form.

Explanation of items in the Report on Completion of Recombinant DNA Experiment

1. Do not write in spaces marked with asterisks.
2. The experiment reporter shall note the Experiment Leader.
3. For the “Research Party” space in “official contact information,” if more than one Research Party exists, write “same as in List of Research Party” and then fill out the list of Research Party.
4. In the “biological containment level” space, indicate the appropriate level.
5. In the “physical containment level” space, enter “P1.”
6. For “No. of times/days insects, mice, etc., were destroyed in the laboratory” space, enter the number of times insects, etc., were destroyed during the voyage.

Form 2

Date: _____

Application Form for Approval of Recombinant DNA Experiments

Receipt No*	
DNA safety 験 inspector*	(signature)

To the President of the Japan Agency for Marine·Earth Science and Technology:

Applicant

Name: _____ (signature)

Title: _____

Organization: _____

I hereby submit this application form to receive confirmation of containment measures of living modified organisms that will be implemented during type 2 use.

Research topic (research voyage No.)		(—)
Official contact information	Experiment Leader	Name Title and organization Telephone (extension No.): E-mail:
	Research Party	Name Title and organization Telephone (extension No.): E-mail:
Purpose and general outline of type 2 use	Type	Experiment using microorganisms
	Purpose	
	General outline	
Characteristics of living modified organism	Characteristics of nucleic acid donor	
	Characteristics of donated nucleic acid	
	Characteristics of vector	
	Characteristics of host	
	Characteristics of living modified organism	

	(differences)	
Characteristics of animal, plant, or cell having living modified organism		
Containment measures	Classification and reason for selection	Experiment using microorganisms P1
	Room name	
	General description of equipment	
	Measures to inactivate living modified organisms	
Other		Scheduled time period for type 2 use (month, day, year) From _____ to _____

Application Form for Approval of Recombinant DNA Experiments (continued)

Pledge, List of Research Party, and Report

As the Experiment Leader, I hereby pledge to comply with the provisions of the Safety Regulations for Recombinant DNA Experiments established by the Japan Agency for Marine-Earth Science and Technology when conducting experiments involving recombinant DNA on board *Chikyu*. I also hereby provide notification that the persons mentioned below shall be the Research Party, and agree that I shall provide guidance and supervision to said Research Party. I further submit the following report on conclusion of education and training for experiments involving recombinant DNA, and proof of recent health examination by the Research Party.

List of Research Party

Name	Affiliation, position, and contact information (telephone number and e-mail address)	Education and training	Health exam
(Example) Taro Kaiyo	Assistant Professor Department of Marine Sciences Ocean Research Institute 012-345-6789 k-taroh@kaiyoh.or.jp	Completed Not required Not completed	Completed
(Example) Hanako Kaiyo	Student Marine Sciences Course, Dept. of Marine Sciences Ocean Research Institute 09-8765-4321 hanako@umi-net.ne.jp	Completed Not required Not completed	Not completed
		Completed Not required Not completed	
		Completed Not required Not completed	
		Completed Not required Not completed	
		Completed Not required Not completed	
		Completed Not required	

		Not completed	
		Completed Not required Not completed	
		Completed Not required Not completed	
		Completed Not required Not completed	
		Completed Not required Not completed	

Signature and seal of the Experiment Leader _____ (signature)

(All personal information supplied on this form shall be used solely for the purpose of managing the safety of experiments involving recombinant DNA and for no other purpose. Moreover, said information shall be handled in strict compliance with the Regulations on Protection and Management of Personal Information of the Japan Agency for Marine-Earth Science and Technology.)

Form 3

Date: _____

Report on Completion of Recombinant DNA Experiment

Receipt No*	
DNA safety inspector*	(signature)

To the President of the Japan Agency for Marine·Earth Science and Technology:

Applicant

Name: _____ (signature)

Title: _____

Organization: _____

I hereby submit this report in accordance with Article 18 of the Safety Regulations for Recombinant DNA Experiments.

Experiment authorization No.: —

Research topic		
Official contact information	Experiment Leader	Name Title and organization Telephone (extension No.): E-mail:
	Research Party	Name Title and organization (If same as in List of Research Party, state so here.) Telephone (extension No.): E-mail:
Biological containment level		
Physical containment level		
Place experiment took place (Building. name, room No.)		
Actual period experiment was		From _____ to _____

conducted (month, day, year)				
Frequency with which experiment was conducted	Nearly every day ___ times during period Not conducted			
Carrying in/out of materials that include recombinants	Were materials carried in/out?	Yes No	Is there a record of this?	Yes No
Storage of materials that include recombinants	Are materials stored	Yes No	Is there a record of this?	Yes No
	Place of storage			
Safety cabinet (fill in if P2)	Date of most recent regular inspection			
	Date of most recent filter replacement			
	Date of most recent fumigation			
No. of times/days insects, mice, etc., were destroyed in the laboratory	No. of times		Date	
Status of experiment (in progress/completed)				

Do not write in spaces marked with asterisks.

Send forms and direct inquiries to:

IODP Department

Center for Deep Earth Exploration (CDEX)

JAMSTEC

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Website: <http://www.jamstec.go.jp/>