

# **The Ministerial Ordinance Providing Containment Measures to Be Taken in Type 2 Use of Living Modified Organisms for Research and Development**

**(Tentative Translation)**

(Purpose)

Article 1: This Ministerial Ordinance shall have the purpose of stipulating necessary matters concerning containment measures to be taken in Type 2 Use of living modified organisms for research and development (excluding the use toward commercialization or practical use of living modified organisms preferred to be examined in accordance with the ideas shown in the recommendations, dated July 16, 1986, of the Council of the Organization for Economic Cooperation and Development concerning Considerations on the Safety in Using Recombinants in Industry, Agriculture and Environment. Hereinafter the same applies) and concerning the confirmation of containment measures in the case where containment measures to be taken are not stipulated, and thereby to ensure proper implementation of Type 2 Use of living modified organisms for research and development.

(Definitions)

Article 2: In this Ministerial Ordinance, the terms mentioned in the following subparagraphs shall have the meanings stipulated respectively in these subparagraphs.

1. Genetic recombination experiment: Of Type 2 Use of living modified organisms for research and development, the use (excluding storage and transport excluding those in the process of experiment ) which involve living modified organisms having nucleic acid or its replicated product thereof (hereinafter “recombinant nucleic acid”) obtained by using technology provided in Article 2 Paragraph 2 Subparagraph 1 of the Law concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms (hereinafter “the Law”)
2. Experiments using microorganisms: Of genetic recombinant experiments, those which involve living modified organisms that are microorganisms of living modified organisms that are microorganisms (which mean living organisms belonging to Fungi (excluding mushroom), living organisms belonging to Protista, living organisms belonging to Monera, viruses and viroids. Hereinafter the same applies.) (excluding those which are set forth in the next subparagraph through Paragraph 5)
3. Large scale cultivation experiments: Of genetic recombination experiments, those which use living modified organisms that are microorganisms, and which use equipment for culture or fermentation (limited to those which have a total capacity

- of 20 or more liters. Hereinafter “Culture Equipment.”)
4. Experiments using animals: Of genetic recombination experiments, those which involve living modified organisms (excluding ones retaining living modified organisms) which are animals (meaning living organisms belonging to Animalia. Hereinafter the same applies) (hereinafter “animal development experiment”) and those which involve living modified organisms retained by animals (hereinafter “animal inoculation experiment”)
  5. Experiment using plants: Of genetic recombination experiments, those which involve living modified organisms (excluding ones retaining living modified organisms) which are plants (meaning organisms belonging to Plantae. Hereinafter the same applies) (hereinafter “plant development experiment”), those which involve living modified organisms that are mushroom (hereinafter “mushroom development experiment”) and those which involve living modified organisms retained in plants (hereinafter “plant inoculation experiment”)
  6. Cell fusion experiments: Of Type 2 Use of living modified organisms for research and development, the use (excluding storage and transportation excluding those in the process of experiment) which involves living modified organisms having nucleic acid or replicated products thereof obtained by the use of the technology stipulated in Article 2 Paragraph 2 Subparagraph 2 of the Law
  7. Recipient organism: An living organism into which recombinant nucleic acid is transferred.
  8. Vector: The recombinant nucleic acid that replicates the whole or a part of itself in a recipient organism into which it is transferred
  9. Donor nucleic acid: Recombinant nucleic acid excluding vectors.
  10. Donor organism: An living organism (including Homo) from which donor nucleic acid originates.
  11. Experiment classification: A classification made for recipient organisms or donor organisms, for use in deciding on containment measures to be taken in genetic recombination experiments in accordance with the degree of dispersal which possibly cause Adverse Effect on Biological Diversity.
  12. Identified nucleic acid: The donor nucleic acid set forth in any of A through C in the following.
    - A. Functions of the donor nucleic acid or protein or other products from the donor nucleic acid are assumed in the light of scientific knowledge on the basis of nucleotide sequences of genes.
    - B. Nucleic acid of a living organism belonging to the same taxonomical species as the recipient organism into which the nucleic acid is transferred, or nucleic acid of a living organism which is recognized to exchange nucleic acid with the taxonomical species to which the said recipient organism belongs under natural conditions

(except where the recipient organism is a virus or a viroid)

C. Nucleic acid of a virus or a viroid which exchanges nucleic acid with a recipient organism into which the said nucleic acid is transferred under natural conditions (only where the recipient organism is a virus or a viroid)

13. Authorized recipient organism/vector line: A combination of a recipient organism that is low in viability except under specific cultural conditions and a vector that is low in transmissibility to other living organisms than the said recipient organism, and is stipulated by the Minister of Education, Culture, Sports, Science and Technology.

(Experiment classification)

Article 3: The names of experiment classification and recipient organisms or donor organisms which belong to the experiment classifications shall be as stipulated respectively in the left columns and the right columns of the following table.

1. Class 1	Of microorganisms, mushroom and parasites, those which are not pathogenic to animals belonging to Mammalia and Aves (including Homo. Hereinafter "mammals") and are stipulated by the Minister of Education, Culture, Sports, Science and Technology, and animals (including Home and excluding parasites) and plants
2. Class 2	Of microorganisms, mushroom and parasites, those which are low in pathogenicity to mammals and are stipulated the Minister of Education, Culture, Sports, Science and Technology
3. Class 3	Of microorganisms and mushroom, those which are high in pathogenicity to mammals and low in propagation and are stipulated by the Minister of Education, Culture, Sports, Science and Technology
IV. Class 4	Of microorganisms, those which are high in pathogenicity to mammals and high in propagation and are stipulated by the Minister of Education, Culture, Sports, Science and Technology

(Categories and contents of containment measures concerning genetic recombination experiment)

Article 4: Categories and contents of containment measures concerning genetic recombination experiments (excluding the ones mentioned in Annexed Table 1. The same applies to the next Article) shall be as stipulated in the following subparagraphs for the types of genetic recombination experiments respectively

mentioned in these subparagraphs.

1. Experiments using microorganisms: The contents set forth in the right columns of Annexed Table 2 for the categories mentioned respectively in the left columns of the table.
2. Large scale culturing experiments: The contents set forth in the right columns of Annexed Table 3 for the categories mentioned in the left of the table.
3. Experiments using animals: The contents set forth in the right columns of Annexed Table 4 for the categories mentioned respectively in the left columns of the table.
4. Experiments using plants: The contents set forth in the right columns of Annexed Table 5 for the categories mentioned respectively in the left columns of the table.

(Containment measures to be taken for genetic recombination experiments)

Article 5: Containment measures to be taken for genetic recombination experiments shall be as stipulated in the following subparagraphs for the types of genetic recombination experiments mentioned respectively in these subparagraphs (excluding the cases set forth in Article 16 subparagraphs 1, 2 and 4 of the Regulations related to the Enforcement of the Law concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms (Ordinance No.1 of Ministries of Finance; Education, Culture, Sports, Science and Technology; Health, Welfare and Labour; Economy, Trade and Industry; and the Environment. Hereinafter “the Enforcement Regulations”) and a case where Type 2 Use is made without taking containment measures to be taken in Type 2 Use because of false information received).

1. Experiments using microorganisms: Containment measures shall be as stipulated in the following for the categories of living modified organisms mentioned therein.
  - A. Living modified organisms except those set forth in the B through D in the following: In case whichever not smaller of the numbers included in the names of experiment classification of the recipient organism and the donor organism is Class 1, Class 2 or Class 3, containment measures shall be respectively of the P1 Level, P2 Level or P3 Level mentioned in Annex Table No. 2.
  - B. Living modified organisms (excluding those set forth in C) using a special authorized recipient organism/vector line (which means a combination of a recipient organism that is extremely low in viability except under specific cultural conditions and a vector that is extremely low in transmissibility to other living organisms than the said recipient organism, and is stipulated by the Minister of Education, Culture, Sports, Science and Technology. Hereinafter the same applies): In case the experiment classification of donor organism is Class 1 and Class 2, containment measures shall be of the P1 Level mentioned in Annexed Table No. 2, and in case it is Class 2, containment measures shall be of the P2 Level mentioned

in Annexed Table No. 2.

C. Living modified organisms, of which the donor nucleic acid is an identified donor nucleic acid and is assumed, in the light of scientific knowledge, not to concern with pathogenicity and transmissibility to mammals: In case the experiment classification of recipient organism is Class 1 or Class 2, containment measures shall be respectively of the P1 Level or the P2 Level mentioned in Annexed Table No. 2.

D. Living modified organisms not using authorized recipient organism/vector line, of which the donor nucleic acid concerns with pathogenicity and transmissibility to mammals and is assumed, in the light of scientific knowledge, to significantly raise the pathogenicity of the recipient organism due to characteristics thereof: In case whichever not smaller of the numbers included in the names of experiment classes of recipient organism and donor organism is Class 1 or Class 2, containment measures shall be respectively of the P2 Level or P3 Level mentioned in Annexed Table No. 2.

2. Large scale culturing experiments: Containment measures shall be as stipulated in the following for the categories of living modified organisms mentioned therein.

A. Living modified organisms except those set forth in B through E in the following: In case whichever not smaller of the numbers included in the names of experiment classes of recipient organism and donor organism is Class 1 or Class 2, containment measures shall be respectively of LS1 Level or LS2 Level mentioned in Annexed Table No. 3.

B. Living modified organisms mentioned in Subparagraph 1 B (excluding those set forth in E): In case the experiment classification of donor organism is the Class 1 and Class 2, containment measures shall be of the LS1 Level mentioned in Annexed Table No. 3, and in case it is Class 3, containment measures shall be of the LS2 Level mentioned in Annexed Table No. 3.

C. Living modified organisms set forth in Subparagraph 1 C (excluding those set forth in E): In case the experiment classification of recipient organism is Class 1 or Class 2, containment measures shall be respectively of LS1 Level or LS2 level mentioned in Annexed Table No. 3.

D. Living modified organisms set forth in Subparagraphs 1 D: In case the experiment classification of both recipient organism and donor organism is the Class 1, containment measures shall be of LS2 Level mentioned in Annexed Table No. 3.

E. Living modified organisms set forth in the following (1) or (2): Containment measures shall be of LSC Level mentioned in Annexed Table No. 3.

(1) Of the living modified organisms using authorized recipient organism/vector lines, of which the experiment classification of donor organism is Class 1, those of which donor organism is identified nucleic acid and is assumed, in the light of

scientific knowledge, not to concern with pathogenicity and transmissibility to mammals.

(2) Living modified organisms stipulated by the Minister of Education, Culture and Sports, Science and Technology as being suitable for taking LSC level containment measures set forth in Annexed Table 3.

3. Experiments using animals: Containment measures shall be as stipulated in the following for the categories of living modified organisms respectively mentioned therein.

A. Living modified organisms except those set forth in B through E in the following:

In case living modified organisms are for animal development experiments and the experiment classification of recipient organism is Class 1 or Class 2, containment measures shall be respectively of P1A Level or P2A Level mentioned in Annexed Table No. 4, and in case living modified organisms (limited to those retained by animals) are for animal inoculation experiments and whichever not smaller of the numbers included in the names of experiment classification of the recipient organism and the donor organism is Class 1, Class 2 or Class 3, containment measures shall be respectively of P1A Level, P2A Level or P3A Level mentioned in Annexed Table No. 4.

B. Living modified organisms set forth in Subparagraph 1 B (excluding those set forth in E): In case the experiment classification of donor organism is Class 1 and Class 2, containment measures shall be of P1A Level mentioned in Annexed Table No. 4 and in case it is Class 3, containment measures shall be of the P2A Level mentioned in Annexed Table No. 4.

C. Living modified organisms set forth in Subparagraph 1 C (excluding those set forth in E): In case the experiment classification of recipient organism is Class 1 or Class 2, containment measures shall respectively be of the P1A Level or P2A Level.

D. Living modified organisms set forth in Subparagraph 1 D: In case living modified organisms (limited to those retained by animals) are for animal development experiment and the experiment classification is Class 1 or Class 2 and in case living modified organisms are for animal inoculation experiment and whichever not smaller of the numbers included in the names of experiment classification of the recipient organism and the donor organism is Class 1 or Class 2, containment measures shall be respectively of P2A Level or P3A Level mentioned in Annexed Table No. 4.

E. Living modified organisms which meet all the requirements set forth (1) through (4) in the following: Containment measures shall be of the special breeding section mentioned in Annexed Table No. 4.

(1) Donor nucleic acid is identified nucleic acid and is assumed, in the light of scientific knowledge, not to concern with pathogenicity and transmissibility to

mammals;

(2) Donor nucleic acid is integrated in the nucleic acid of chromosomes of recipient organisms and no transposable element is contained;

(3) It is assumed in the light of scientific knowledge that the kinetic ability related to escape does not increase in comparison with the recipient organism.

(4) Animals do not retain living modified organisms which are microorganisms.

4. Experiments using plants: Containment measures shall be as stipulated in the following for the categories of living modified organisms respectively mentioned therein.

A. Living modified organisms except those set forth in B through E in the following:

In case living modified organisms are for plant development experiment and the experiment classification of recipient organisms is Class 1, containment measures shall of P1P Level mentioned in Annexed Table No. 5, and in cases living modified organisms (limited to those retained in plants) are for plant inoculation experiment and for mushroom development experiment and whichever not smaller of the numbers included in the names of experiment classification of the recipient organism and the donor organism is Class 1, Class 2 or Class 3, containment measures shall be respectively of the P1P Level, P2P Level or P3P Level mentioned in Annexed Table No. 5.

B. Living modified organisms set forth in Subparagraph 1 B (excluding those set forth in E): In case the experiment classification of donor organism is Class 1 or Class 2, containment measures shall be of P1P Level mentioned in Annexed Table No. 5 and in case it is Class 3, containment measures shall be of P2P Level mentioned in Annexed Table No. 5.

C. Living modified organisms mentioned in Subparagraph 1 C (excluding those set forth in E): In case the experiment classification of recipient organism is Class 1 or Class 2, containment measures shall be respectively of P1P Level or P2P Level mentioned in Annexed Table 5.

D. Living modified organisms set forth in Subparagraph 1 D: In case living modified organisms are for plant development experiment and the experiment classification of recipient organism is Class 1, containment measures shall be of P2P Level mentioned in Annexed Table No. 5., and in case living modified organisms (limited to those retained by plants) are for plant inoculation experiment and for mushroom development experiment, whichever not smaller of the numbers included in the names of experiment classification of the recipient organism and the donor organism is Class 1 or Class 2, containment measures shall be respectively of P2P Level or P3P Level mentioned in Annexed Table No. 5.

E. Living modified organisms which meet all the requirements set forth (1) through (4) in the following: Containment measures shall be of the special screened

greenhouse mentioned in Annexed Table No. 5.

- (1) Donor nucleic acid is identified nucleic acid and is assumed, in the light of scientific knowledge, not to concern with pathogenity and transmissibility to mammals;
- (2) Donor nucleic acid is integrated in the nucleic acid of chromosomes of recipient organisms and no transposable element is contained;
- (3) It is assumed in the light of scientific knowledge that the dispersibility of pollen, spores and seeds (hereinafter to be referred to as “the pollen”) and crossability do not increase in comparison with recipient organisms.
- (4) Plants do not retain living modified organisms that are microorganisms.

(Containment measures to be taken in storage)

Article 6: Containment measures to be taken in storage(excluding storage in the process of a genetic recombination experiment or a cell fusion experiment) in Type 2 Use of living modified organisms for research and development shall be as stipulated in the following (excluding the cases set forth in Article 16 subparagraphs 1, 2 and 4 of the Enforcement Regulations, and in a case where Type 2 Use is made without taking containment measures to be taken in Type 2 Use because of false information received).

1. A living modified organism shall be put in a container of the structure that prevents the living modified organism from leaking , escaping or other dispersion and it shall be indicated in an easily visible spot on the outside of the said container that the living modified organism is contained.
2. The container of the preceding subparagraph containing the living modified organism shall be stored in a prescribed place. In case the place of storage is a refrigerator or other equipment for storage, it shall be indicated in an easily visible spot of the said equipment that the living modified organism is stored.

(Containment measures to be taken in conveyance)

Article 7: Containment measures to be taken in conveyance (excluding conveyance in the process of a genetic recombination experiment or a cell fusion experiment) in Type 2 Use of living modified organisms for research and development shall be as stipulated in the following (excluding the cases set forth in Article 16 subparagraphs 1, 2 and 4 of the Enforcement Regulations, and in a case where Type 2 Use is made without taking containment measures to be taken in Type 2 Use because of false information received).

1. A living modified organism shall be put in a container of the structure that prevents the living modified organism from leaking, escaping or other dispersion.



2. In case containment measures to be taken in a genetic recombination experiment or a cell fusion experiment of living modified organisms are other than those of P1 Level, P2 Level, LSC Level, LS1 Level, P1A Level, P2A Level, the special breeding section, P1P Level, P2P Level and the special screened greenhouse, in addition to the measures stipulated in the preceding subparagraph, the container stipulated in the preceding subparagraph shall be put in another container of the structure that prevents the living modified organism in the said container from leaking, escaping or other dispersion even if the said container is broken by an accident during ordinary conveyance.
3. It shall be indicated in an easily visible spot of the outermost container (should the container be packed, the packing) that careful handling is required.

(Matters mentioned in application)

Article 8: Matters to be stipulated by the ordinance of the competent ministries under Article 13 paragraph 2 subparagraph 4 of the Law shall be as follows.

1. The name of Type 2 Use.
2. The name and address of a place intended for making Type 2 Use.
3. The purpose and an outline of Type 2 Use.
4. Properties of an animal or a plant retaining a living modified organism (only in the cases of animal inoculation experiments and plant inoculation experiments).
5. Properties of cells (only those of other organisms than animals and plants. The same applies to this subparagraph.) retaining living modified organisms (only in the case of using a cell retaining living modified organisms which are microorganisms).

(Form of application)

Article 9: The form of the application stipulated in Article 13 paragraph 2 of the Law shall be as shown as an annex.

#### Supplemental Provision

This Ministerial Ordinance shall be enforced on the date of the enforcement of the Law (February 19, 2004.)

Annexed Table No.1 (Related to Article 4)

1. Of the experiments using microorganisms, those involving living modified organisms set forth in A through H in the following.
  - A. Living modified organisms for which either recipient organisms or donor organisms are other than those mentioned in the right columns of each subparagraph of the Table of Article 3 (excluding, of living modified organisms which use authorized recipient organism/vector lines and for which donor organisms are other living

- organisms (including Homo) than viruses and viroids, those for which donor nucleic acid is identified nucleic and is assumed, in the light of scientific knowledge, not to concern with pathogenicity and transmissibility to mammals)
- B. Living modified organisms for which the experiment classification of either recipient organisms or donor organisms is Class 4.
  - C. Living modified organisms for which the experiment classification of recipient organisms is Class 3.
  - D. Of the living modified organisms which do not use authorized recipient organism/vector lines and for which the experiment classification of donor nucleic acid is Class 3, those for which donor nucleic acid is not identified nucleic acid or is identified nucleic acid that concerns with pathogenicity and transmissibility to mammals and because of that characteristic, is assumed, in the light of scientific knowledge, to significantly increase the pathogenicity of recipient organisms to mammals
  - E. Living modified organisms (excluding those which are viruses and viroids) for which the experiment classification of recipient organisms is Class 2 and donor nucleic acid contains drug-resistant genes (limited to those which give the property of making a infectious disease attributable to the living modified organisms difficult to be treated in case mammals are infected with the living modified organisms)
  - F. Living modified organisms which are viruses or viroids having independent prolificity and infectivity (excluding those stipulated by the Minister of Education, Culture, Sports, Science and Technology) and proliferate through the use thereof.
  - G. Living modified organisms for which donor nucleic acid containing genes having connection with proteinaceous toxins of which the median lethal dose to mammals is 100 micrograms or less per 1kg of their body weights (excluding living modified organisms which use authorized recipient organism/vector lines of which the recipient organism is a *Escherichia coli*, and for which donor nucleic acid containing genes having connection with proteinaceous toxins of which the median lethal dose to mammals exceeds 100 nanograms per 1kg of their body weights)
  - H. Other than those mentioned in A through G, those stipulated by the Minister of Education, Culture, Sports, Science and Technology.
2. Of the large scale culturing experiments, those involving living modified organisms mentioned in A through E in the following
- A. Living modified organisms set forth in subparagraph 1 A through G.
  - B. Of the living modified organisms which do not use authorized recipient organism/vector lines and for which the experiment classification of recipient organisms or donor organisms is Class 2, those for which donor nucleic acid has connection with pathogenicity or transmissibility to mammals and because of that characteristic, is assumed, in the light of scientific knowledge, to significantly

increase the pathogenicity of recipient organisms to mammals.

- C. Living modified organisms which use no authorized recipient organism/vector lines and for which the experiment classification donor organisms is Class 3 (excluding those set forth in subparagraph 1 D).
  - D. Of the living modified organisms set forth in Article 5 subparagraphs 2 A through C, those which require to take the LSC Level containment measures set forth in Annexed Table 3 in the use thereof.
  - E. Other than those mentioned in A through D, living modified organisms stipulated by the Minister of Education, Culture, Sports, Science and Technology.
3. Of the experiments using animals, those involving living modified organisms set forth in A through D in the following
- A. Living modified organisms set forth in subparagraph 1 A through G
  - B. Living modified organisms for which recipient organisms are animals and donor nucleic acid contains genes that give to the recipient organisms a receptor (limited one that is not possessed by any organism belonging to the same taxonomical species as the recipient organisms) which causes infection of a microorganism that is pathogenic to mammals
  - C. Of the living modified organisms set forth in Article 5 subparagraphs 3 A through C, those which require to take the containment measures of the special breeding section mentioned in Annexed Table No. 4 in the use thereof
  - D. Other than those mentioned in A through C, living modified organisms stipulated by the Minister of Education, Culture, Sports, Science and Technology
4. Of the experiments using plants, those involving living modified organisms set forth in A through C in the following
- A. Living modified organisms set forth in subparagraph 1 A through G
  - B. Of the living modified organisms set forth Article 5 subparagraphs 4 A through C, those which require to take the containment measures of the special screened greenhouse mentioned in Annexed Table No. 4 in the use thereof
  - C. In addition to the living modified organisms set forth in A and B, those stipulated by the Minister of Education, Culture, Sports, Science and Technology
- Annexed Table No. 2 (Related to Article 4 subparagraph 1)

Category of Containment Measures	Contents of Containment Measures
1. P1 Level	<p>A. With regard to facilities, the laboratory shall have the structure and equipment as a laboratory for ordinary organisms.</p> <p>B. In carrying out genetic recombination experiments, the matters stipulated in the following shall be observed.</p> <p>(1) Before disposing waste products (including effluent.</p>

	<p>Hereinafter the same applies) containing living modified organisms, a measure for inactivating the living modified organisms shall be taken.</p> <p>(2) Before disposing or reusing (or washing when it is washed beforehand; hereinafter “the disposal.”) an equipment, an apparatus or an appliance stuck with living modified organisms, a measure for inactivating the living modified organisms shall be taken.</p> <p>(3) A testing bench shall be subjected to a measure for inactivating living modified organisms on each day on which an experiment is carried out upon end of the day’s experiment and immediately when a living modified organism sticks to it.</p> <p>(4) The door to the laboratory shall be kept closed (except when one gets in and out of it).</p> <p>(5) The windows of the laboratory shall be kept closed and other necessary measures shall be taken to prevent insects from entering.</p> <p>(6) In all operations, the production of aerosol shall be minimized.</p> <p>(7) When a living modified organism is taken out of the laboratory in process of an experiment, including the case when one intends to inactivate a living modified organism in any other place than the laboratory, the living modified organism shall be put in a container of the structure that prevents it from leaking or other dispersion.</p> <p>(8) To prevent a living modified organism from sticking to or infecting a person who handles it, necessary measures including hand washing after handling it shall be taken.</p> <p>(9) A measure shall be taken to prevent anyone having no knowledge of the contents of experiment from entering the laboratory without permission.</p>
2. P2 Level	<p>A. Facilities shall meet the following requirements.</p> <p>(1) The requirement set forth in A of the preceding subparagraph.</p> <p>(2) The laboratory shall be equipped with a biological safety cabinet for research (only when operation which tends to produce aerosol is carried out).</p> <p>(3) In case an autoclave is used to inactivate living modified</p>

	<p>organisms, the autoclave shall be provided in the building in which the laboratory is located.</p> <p>B. In carrying out genetic recombination experiments, the matters mentioned in the following shall be observed.</p> <p>(1) The matters set forth in B of the preceding subparagraph.</p> <p>(2) When operation which tends to produce aerosol is carried out, a biological safety cabinet for research shall be used. On a day on which an experiment is carried out, upon completion of the experiment and immediately when a living modified organism sticks to the biological safety cabinet for research, it shall be subjected to a measure for inactivating the living modified organism.</p> <p>(3) The note, "P2 Level experiment going on," shall be posted on the door to the laboratory and equipment for storing living modified organisms in the process of an experiment (hereinafter "storage equipment").</p> <p>(4) In case experiments for which containment measures of P1 Level, P1A Level or P1P Level are performed in the same laboratory at the same time, an area for each experiment shall be defined distinguishably, or containment measures of P2 Level, P2A Level or P2P Level shall be taken respectively.</p>
3. P3 Level	<p>A. Facilities shall meet the following requirements.</p> <p>(1) The matters mentioned in subparagraph 1 A</p> <p>(2) The doorway to the laboratory shall be equipped with an anteroom (that must have front and rear doors built to close automatically and must have an enough space for personnel to change clothes. Hereinafter the same applies).</p> <p>(3) The floor, wall and ceiling surfaces of the laboratory shall be of the structure that can be washed with water and fumigated easily.</p> <p>(4) The laboratory or the experiment section (which means a section composed of the laboratory and the anteroom. Hereinafter the same applies) shall be of the structure that prevents insects from entering and maintains a sealed condition for easy fumigation.</p> <p>(5) In the main doorway to the laboratory or to the anteroom, a hand-washing installation to be operated by a</p>

foot, an elbow or automatically shall be provided.

(6) An air feeder/exhauster to guide air from the doorway of the laboratory to its inside shall be provided.

(7) An exhauster shall prevent the exhaust from the laboratory (excluding the exhaust filtered through HEPA filter (including the exhaust from the biological safety cabinet for research)) from being re-circulated the laboratory and other rooms in the building in which the laboratory is located.

(8) A drainage equipment shall discharge effluent from the laboratory only after taking a measure to inactivate living modified organisms.

(9) In the laboratory, a biological safety cabinet for research shall be provided (only in the case where operation likely to produce aerosol is carried out).

(10) When a biological safety cabinet for research is provided, it shall be so arranged that examination, replacement of HEPA filters and fumigation can be performed without moving the cabinet.

(11) Autoclave shall be provided in the laboratory.

(12) In case a vacuum aspiration pump is used, it shall be for exclusive use for the laboratory and a catching device using disinfectant shall be provided.

B. In carrying out genetic recombination experiments, the following matters shall be observed.

(1) The matters set forth in subparagraph 1 B (1) through (4) and (6) through (9)

(2) In the laboratory, long-sleeved working clothes which do not open in front, protective footwear, a protective cap, goggles and protective gloves (here- in after "the working clothes") shall be put on.

(3) The working clothes shall be disposed of only after a measure to inactivate living modified organisms is taken.

(4) The doors provided in front and in rear of the anteroom shall not be opened at the same time.

(5) When operation likely to produce aerosol is carried out, the biological safety cabinet for research shall be used and no one shall get in and out of the laboratory. On each day on which an experiment is carried out, upon completion of

	<p>the experiment, and immediately when a living modified organism sticks to the biological safety for research, the cabinet shall be subjected to a measure for inactivating the living modified organism.</p> <p>(6) The note, “P3 Level experiment going on,” shall be posted on the door to the laboratory and the storage equipment.</p> <p>(7) In case experiments requiring to take containment measures of lower levels than P3 Level, P3A Level or a P3P Level are performed in the same laboratory at the same time, containment measures of P3 Level, P3A Level or P3P Level shall be taken respectively.</p>
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Annexed Table No.3 (related to Article 4 subparagraph 2)

Category of Containment Measures	Contents of Containment Measures
1.LSC Level	<p>A. With regard to facilities, an experiment area (an area in which genetic recombination experiments are carried out and which can be clearly distinguished from other areas. Hereinafter the same applies.) shall be provided.</p> <p>B. In carrying out genetic modification experiments, the following matters shall be observed.</p> <p>(1) The matters set forth in subparagraph 1 B (1), (2) and (6) through (9) of Annexed Table No. 2. In this case, these provisions shall read “the experiment area” for “the laboratory.”</p> <p>(2) The note, “LSC Level mass culture experiment going on,” shall be posted in the experiment area.</p>
2.LS1 Level	<p>A. Facilities shall meet the following requirements.</p> <p>(1) The matters set forth in A of the preceding subparagraph.</p> <p>(2) Culture Equipment shall prevent living modified organisms from flowing out of it.</p> <p>(3) The exhauster shall discharge the exhaust from Culture Equipment through a germicidal filter or an apparatus having equivalent germicidal effect to it.</p> <p>B. In carrying out genetic recombination experiments, the matters mentioned below shall be observed.</p> <p>(1) The matters mentioned in B (1) of the preceding subparagraph.</p>

	<p>(2) When living modified organisms are implanted in Culture Equipment, sampled from Culture Equipment, or transferred to another equipment or an apparatus, the living modified organisms shall be put in containers of the structure that prevents them from leaking or other dispersion or piping of a similar structure shall be used. In the event a living modified organism sticks to the outer wall of Culture Equipment, any other equipment or apparatus, the floor of the experiment area, or the ground, the living modified organism shall be inactivated immediately.</p> <p>(3) The note, "LS1 Level Large Scale Culturing experiment going on," shall be posted in the experiment area and the storage equipment.</p>
3.LS2 Level	<p>A. Facilities shall meet the following requirements.</p> <p>(1) The matters set forth in A of subparagraph 1.</p> <p>(2) Culture Equipment shall allow measures to be taken for preventing living modified organisms from flowing out of it and for inactivating living modified organisms therein without opening it, and a rotary seal, piping valves and other parts directly connected to the said Culture Equipment shall be capable of preventing living modified organisms from being discharged outside it.</p> <p>(3) An exhauster shall discharge the exhaust from Culture Equipment through the HEPA filter or an apparatus having equivalent germicidal effect to the HEPA filter.</p> <p>(4) In an experiment area, a biological safety cabinet for research or an installation having equivalent containment function to it (hereinafter "Biological Safety Cabinet) shall be provided (only in cases where operation which tends to produce aerosol is carried out).</p> <p>(5) When a Biological Safety Cabinet is installed, it shall be so arranged that examination, replacement of HEPA filters and fumigation can be performed without moving the Biological Safety Cabinet.</p> <p>(6) In case an autoclave is used to inactivate a living modified organisms, the autoclave shall be installed in the building in which the experiment area is located.</p> <p>(7) For Culture Equipment and apparatus directly</p>



	<p>connected to it, a device for monitoring the level of their airtightness while they are being used shall be provided.</p> <p>B. In carrying out genetic recombination experiments, the following matters shall be observed.</p> <p>(1) The matters set forth in subparagraph 1 B (1) and in B (2) of the preceding subparagraph.</p> <p>(2) In carrying out operation which tends to produce aerosol, a Biological Safety Cabinet shall be used. For the Biological Safety Cabinet, on each day on which an experiment is carried out, upon completion of experiment, and immediately when a living modified organism sticks to it, a measure to inactivate living modified organisms shall be taken.</p> <p>(3) While Culture Equipment and apparatus directly connected to it are used, the level of their airtightness shall be confirmed constantly by means of a monitoring device.</p> <p>(4) The note, "LS2 Level mass culture experiment going on," shall be posted in the experiment area and the storage equipment.</p>
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Annexed Table No.4 (related to Article 4 subparagraph 3)

Category of Containment Measures	Contents of Containment Measures
1.P1A Level	<p>A. Facilities shall meet the following requirements.</p> <p>(1) The laboratory shall have the structure and equipment as an ordinary animal breeding room.</p> <p>(2) In the doorway to the laboratory, windows and other places which will become escape routes of living modified organisms that are animals and animals retaining living modified organisms (hereinafter "recombinant animals"), equipment, apparatus or appliances for preventing the living modified organisms from escaping shall be provided suitably for their behaviors.</p> <p>(3) In case living modified organisms are contained in feces of modified animals, equipment, apparatus or appliances necessary to collect the feces shall be provided and the floor of the laboratory shall be of the structure that allows the feces to be collected.</p> <p>B. In carrying genetic recombination experiments, the</p>

	<p>following matters shall be observed.</p> <p>(1) The matters set forth in subparagraph 1 B (1) through (6), (8) and (9) of Annexed Table No.2.</p> <p>(2) or when living modified organisms are taken out of the laboratory in process of an experiment, including the case when one intends to inactivate a living modified organism in any other place than the laboratory, the living modified organisms shall be put in a container of the structure that prevents them from leaking or dispersing in any other way.</p> <p>(3) A measure that is capable of identifying modified animals by the types of recombinant nucleic acid transferred in them, or by the types of living modified organisms retained by them shall be taken.</p> <p>(4) The note, “Modified animal being bred,” shall be posted on the door to the laboratory.</p>
2.P2A Level	<p>A. Facilities shall meet the following requirements.</p> <p>(1) The requirements set forth in subparagraph 2 A (2) and (3) of Annexed Table 2.</p> <p>(2) The requirements set forth in A of the preceding paragraph.</p> <p>B. In carrying out genetic recombination experiments, the matters set forth in the following shall be observed.</p> <p>(1) The matters set forth in subparagraph 1 B (1) through (6), (8) and (9), and subparagraph 2 B (2) and (4) of Annexed Table No.2.</p> <p>(2) The matters set forth in B (2) and (3) of the preceding subparagraph.</p> <p>(3) The note, “Modified animal being bred (P2),” shall be posted on the door to the laboratory.</p>
2. P3A Level	<p>A. Facilities shall meet the following requirements.</p> <p>(1) The requirements set forth in subparagraph 3 A (2) through (12) of Annexed Table No.2.</p> <p>(2) The requirements set forth in subparagraph 1 A.</p> <p>B. In carrying out genetic recombination experiments, the matters set forth in the following shall be observed.</p> <p>(1) The matters set forth in subparagraph 1 B (1) through (4), (6), (8) and (9), and subparagraph 3 B (2) through (5) and (7) of Annexed Table No.2.</p> <p>(2) The matters set forth subparagraph 1 B (2) and (3).</p>

	(3) The note, “Modified animal being bred (P3),” shall be posted on the door to the laboratory.
4. Special breeding section	<p>A. With regard to facilities, a section for breeding modified animals (hereinafter “the breeding section”) shall be provided doubly with installations for preventing their escape suitably for their behaviors.</p> <p>B. In carrying out genetic recombination experiments, the matters set forth in the following shall be observed.</p> <p>(1) The matters set forth in subparagraph 1 B (1), (2), (4), (8) and (9) of Annexed Table No.2. In this case, these provisions shall read “the breeding section” for the “laboratory”.</p> <p>(2) The matters mentioned in subparagraph 1 B (2) through (4). In this case, these provisions shall read “the breeding section” for “the laboratory”.</p>

Annexed Table No.5 (related to Article 4 subparagraph 4)

Category of Containment Measures	Contents of Containment Measures
1. P1P Level	<p>A. Facilities shall meet the following requirements.</p> <p>(1) The laboratory shall have the structure and equipment as an ordinary plant culture room.</p> <p>(2) When operation which tends to scatter the pollen of living modified organisms that are plants or mushroom and plants retaining living modified organisms (hereinafter modified plants) is carried out, an exhaust system shall be capable of minimizing the pollen of the modified plants contained in the exhaust from the laboratory.</p> <p>B. In carrying out genetic modification experiments, the following matters shall be observed.</p> <p>(1) The matter set forth in subparagraph 1 B of Annexed Table No.2.</p> <p>(2) The note, “Modified plants being cultured,” shall be posted on the door to the laboratory.</p>
2. P2P Level	<p>A. Facilities shall meet the following requirements.</p> <p>(1) The requirements set forth in subparagraph 2 A (2) and (3) of Annexed Table No.2</p> <p>(2) The requirements set forth in A of the preceding subparagraph.</p> <p>B. In carrying out genetic recombination experiments, the</p>

	<p>following matters shall be observed.</p> <p>(1) The matter set forth in subparagraph 1 B and subparagraph 2 B (2) and (4) of Annexed Table No.2.</p> <p>(2) The note, “Modified plants being cultured (P2),” shall be posted on the door to the laboratory.</p>
3. P3P Level	<p>A. Facilities shall meet the following requirements.</p> <p>(1) The requirements set forth in subparagraph 3 A (2) through (12) of Annexed Table No.2.</p> <p>(2) The requirements set forth in subparagraph 1 A.</p> <p>B. In carrying out genetic recombination experiments, the following matters shall be observed.</p> <p>(1) The requirements set forth in subparagraph 1 B (1) through (4) and (6) through (9) and subparagraph 3 B (2) through (5) and (7) of Annexed Table No.2.</p> <p>(2) The note, “Modified plants being cultured (P3),” shall be posted on the door to the laboratory.</p>
4. Special screened greenhouse	<p>A. Facilities shall meet the following requirements.</p> <p>(1) Facilities for culturing modified plants (hereinafter “the screened greenhouse”) shall be equipped with a net and other equipment in positions opening to the air in order to reduce insects getting in from outside to the minimum.</p> <p>(2) In case direct access from the outdoors to the screened greenhouse is possible, an anteroom shall be provided to the doorway.</p> <p>(3) In the event the living modified organisms are contained in water discharged from the screened greenhouse, equipment, apparatus or appliances necessary to collect the discharged water shall be provided, or the floor or the ground of the screened greenhouse shall be of the structure that can collect the discharged water.</p> <p>B. In carrying out genetic recombination experiments, the following matters shall be observed.</p> <p>(1) The matters set forth in subparagraph 1 B (1), (2), (4) and (7) through (9) of Annexed Table No. 2. In this case, these provisions shall read “the screened greenhouse” for “the laboratory.”</p> <p>(2) Insects taking out the pollen of modified plants shall be eradicated.</p> <p>(3) The measures, including keeping windows closed in a</p>

	<p>season in which the pollen of modified plants scatters, for preventing the pollen of modified plants from scattering outside the screened greenhouse shall be taken (excluding the case where if the pollen of modified plants scatters outside the screened greenhouse, the said pollen neither crossbreeds nor sprouts.)</p> <p>(4) The note, "Modified plants being cultured," shall be posted on the door to the screened greenhouse.</p>
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Application Form (related to Article 9)

□ Serial No.

**Application for Confirmation of Containment Measures  
for Type 2 Use**

To: The Minister of Education, Culture, Sports, Science and Technology

Date:

Name

Seal

Applicant

Address

I wish to obtain your confirmation of containment measures to be taken in the Type 2 Use of living modified organisms and apply as follows under the provisions of Article 13 paragraph 1 of the Law concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms.

Name of Type 2 Use				
Place intended for Type 2 Use	Name			
	The site	Postal No. (         )		
		Phone No.		
Contact for administrative matters	Person in charge of managing experiments	Name of organization to which applicant belongs and official title		
		Name		
		Address	Postal No. (         )	
			Phone No.	
			Fax No.	
			e-mail Address	
		Name of organization to which the person belongs and official title		
		Name		
	Other contact	Address	Postal No. (         )	
			Phone No.	
Fax No.				
e-mail address				

Objective and outline of Type 2 Use	Type	<ol style="list-style-type: none"> <li>1. Experiments using microorganisms</li> <li>2. Large scale culturing experiments</li> <li>3. Experiments using animals               <ol style="list-style-type: none"> <li>(1) Animal development experiments</li> <li>(2) Animal inoculation experiments</li> </ol> </li> <li>4. Experiments using plants               <ol style="list-style-type: none"> <li>(1) Plant development experiments</li> <li>(2) Plant inoculation experiments</li> <li>(3) Mushroom development experiments</li> </ol> </li> <li>5. Cell fusion experiments</li> </ol>	
	Purpose		
	Outline		
	The Use for which confirmation is applied		
Character- istics of living modified organisms	Properties of donor organism		
	Properties of donor nucleic acid		
	Properties of vector		
	Properties of recipient organism		
	Properties of living modified organism (including difference from recipient organism)		
Properties of animal, plant or cell which retains living modified organism			
Containment measures	Category and reason for selection		
	Outline of facilities		
	Measure for in-activating living modified organism		
Others			

[Notes]

1. In case the applicant is a corporation, mention the name of the corporation and the name of its representative for “Name of applicant” and the address of the main office

for "Address of applicant."

2. Instead of mentioning the name (in the case of a corporation, the name of its representative) and sealing, the applicant (in the case of a corporation, the name of its representative) may sign his or her name.

3. For "Name of Type 2 Use," mention a name that expresses the objective and an outline of the Type 2 Use briefly.

4. For "Name and The site," the information shall be given on all of the laboratory, the experiment section, the experiment area, the breeding section and the screened greenhouse to be used for the Type 2 Use.

5. For "Person in charge of managing experiments," the information shall be given on a person who is directly manage the Type 2 Use in the place where the Type 2 Use is made.

6. For "Other contact," if there is any other contact for administrative matters than the person in charge of managing experiments, give the information on the contact.

7. For "Type," select all items under which the Type 2 Use falls.

8. For "Outline," all living modified organisms involved in the Type 2 Use and the categories of containment measures to be taken during the Type 2 Use shall be mentioned so as to show processes of the Type 2 Use. Besides, if the special breeding section or the special screened greenhouse is included in the categories of containment measures, the following shall also be mentioned:

(1) The number of lines or individuals of the modified animal or the modified plant for the Type 2 Use;

(2) The area of the breeding section or the screened greenhouse to be used in the Type 2 Use;

(3) The method of breeding the living modified animal for the Type 2 Use or culturing the living modified plant for the Type 2 Use.

9. For "The Use for which confirmation is applied," give the number of subparagraph of Annexed Table No.1 under which the Type 2 Use falls (only in the case of genetic recombination experiments).

10. For "Characteristics of donor organism," the following shall be mentioned about the donor organism for the living modified organism for the Type 2 Use (only in the case of genetic recombination experiments). Nevertheless, in the case of a donor organism from which donor nucleic acid that is drug-resistant genes and other marker genes and expression regulatory genes (excluding target genes) originates, mention on the following items may be omitted.

(1) Taxonomical position and experiment classification; and

(2) Pathogenicity, production of harmful substances and other characteristics;

11. For "Characteristics of donor nucleic acid," the following shall be mentioned about the donor nucleic acid of the living modified organism for the Type 2 Use. Nevertheless,



in the case of donor nucleic acid that is drug-resistant genes and other marker genes and expression regulatory genes (excluding target genes), mention on the following items may be omitted.

- (1) Type (such as genomic nucleic acid, complementary deoxyribonucleic acid or synthesized nucleic acid) and general name;
- (2) Functions, sizes and composition of component elements (target genes, expression regulatory genes, etc.); and
- (3) Nucleotide sequence information or an accession number to the nucleotide sequence database of, for example, the Japan DNA Databank (only in the case of donor nucleic acid that is identified nucleic acid).

12. For “Characteristics of vector,” mention the following about the vector of the living modified organism for the Type 2 Use (only in the case of genetic recombination experiments). Besides, characteristics of drug-resistant genes and other marker genes shall be mentioned.

- (1) Name, the taxonomic position of origin organism and experiment classification;
- (2) Composition; and
- (3) Transmissibility and recipient organism-specificity

13. “For “Characteristics of recipient organism,” mention the following about the recipient organism of the living modified organism for the Type 2 Use in the case of a genetic recombination experiment, and about the parent organism (which means a living organism from which nucleic acid, or its replicated product, obtained by using technology provided in Article 2 Paragraph 2 subparagraph 3 of the Law. Hereinafter the same applies.) of the living modified organism for the Type 2 Use in the case of a cell fusion experiment.

- (1) Taxonomical position and experiment classification;
- (2) State of distribution in natural environment and environment in which living or growth is possible;
- (3) The mode of reproduction or proliferation;
- (4) Pathogenicity, production of harmful substances and other properties
- (5) Auxotrophy, drug resistance and optimal conditions for growing (only in the case of Use of a living modified organism that is a microorganism (excluding a virus or a viroid));
- (6) The items set forth in 12 (only in the case where the recipient organism is a virus or viroid).

14. For “Characteristics of living modified organism (including difference from recipient organism),” in comparison with the recipient organism of the living modified organism for the Type 2 Use in the case of a genetic recombination experiments and in comparison with the parent organism of the living modified organism for the Type 2 Use in the case of a cell fusion experiment, properties expected to be newly given or

already given to the living modified organism for the Type 2 Use shall be mentioned. Besides, in case the special breeding section or the special screened greenhouse is included in the category of containment measures to be taken during the Type 2 Use, mention the following about the living modified organism for the Type 2 Use.

- (1) The method of transferring recombinant nucleic acid and the progress of rearing (including number of times of subculture);
- (2) The state of existence of donor nucleic acid and the stability of expression of character caused by the donor nucleic acid (only in the case of a genetic recombination experiment);
- (3) The mode of reproduction or proliferation;
- (4) Effect of climatic conditions in the place in which Type 2 Use on growth or existence; and
- (5) The survivability of the living modified organism that is a microorganism and the propagation of the living modified organism to other organisms (only in the case that a living modified organism that is a microorganism is used to develop a living modified organism that is a plant for the Type 2 Use).

15. For "Characteristics of animal, plant or cell which retains living modified organisms," in addition to pertinent items among those mentioned in (1) through (4) of 13, characters expected to be newly given or already given to an animal, a plant or a cell which retains the living modified organism for the Type 2 Use in comparison with animals, plants or cells which do not retain the living modified organisms for Type 2 Use shall be mentioned.

16. For "Category and reason for selection," basically, among the categories of containment measures shown in the left columns of Annexed Tables Nos. 2, 3, 4 or 5, mention all categories of containment measures to be taken during the Type 2 Use and give details of the reasons for selecting them respectively.

17. For "Outline of facilities," mention the following about the selected containment measures:

- (1) Names and positions of major facilities, equipment and apparatus;
- (2) A total capacity of the Culture Equipment (only in the case of a large scale culturing experiment);
- (3) The status of confirmation of the facilities;
- (4) In case an animal or a plant that has no relation with the Type 2 Use is bred or cultured in the laboratory, the experiment section, the experiment area, the breeding section or the screened greenhouse, the state of the breeding of the animal or the culture of the plant; and
- (5) In areas surrounding the place intended for the Type 2 Use, whether there is any plant that may hybridize with the modified plant, and a measure to prevent the hybridization (only in the case where the category of containment measures to be

taken during the Type 2 Use is the special screened greenhouse).

18. For “Measure for inactivating living modified organism,” about the containment measures to be taken during the Type 2 Use, mention a measure for inactivating waste products containing the living modified organism for the Type 2 Use and apparatus and appliances to which the living modified organism for the Type 2 Use sticks, and the effectiveness of the measure.

19. For “Others,” mention the following:

(1) A period scheduled for the Type 2 Use;

(2) The state of affairs with regard to the establishment of a committee or the like for safe handling of living modified organisms, and the title and the name of the chairman of such a committee;

(3) How the facilities for breeding animals is checked by a person in charge of facility management (only in the case of an experiment using animals); and

(4) Action to be taken against accidents and other emergencies (only in the case of large scale culturing experiments).

20. Leave the columns marked with □ blank.

21. Use paper of JIS 4A in size in a file form.

22. Should description is too long to be put in an appropriate column, give a note, “as per attached” and use a separate sheet of paper. Should there be any pertinent literature, give a note, “reference literature” and attach a copy of the literature.