Form20-1 JASRI Safety Office←Person in charge of managing experiments←Project Leader

Safety Supervisor		Confirmation by Safety Office		Receipt by the Safety Office	20YY, MM, DD

Application Form for Genetic Modification Experiment

Date of submission: June 1, 2016

To: The Director General, Japan Synchrotron Radiation Research Institute

(Person in charge of experiment)[1)]
Name of the organization
XX University, Graduate School
Department and title
XX Department XX Assistant
Name (print and signature)
Koukido Hanako

(Manager)[2)]
Name (Leave it blank if User is applying) Seal

To carry out the following genetic modification experiment, I hereby apply for approval of the genetic recombination committee.

Receipt number[3)]	(Do not write when submitting)
Type of application[4)]	■ New ☐ Renewal (Previous receipt number ☐ Amendment (Previous receipt number
Title of experiment[5)]	Crystallization and crystal diffraction experiments of lysozyme expressed in insect cells
Type of experiment[6)]	■ Microbiology experiment ☐ Large-scale cultivation experiment ☐ Animal experiment (☐ Animal inoculation・☐ Animal modification) ☐ Plant experiment (☐ Plant inoculation・☐ Plant modification・☐ Fungus modification)
The purpose	Structural analysis of lysozyme by crystallization and crystal diffraction experiments using solution containing proteins expressed in insect cells and baculovirus.
Summary[7)]	To efficiently obtain purified crystals for the analysis of the structure and functions of egg-white lysozyme, an expression system of insect cells will be developed. Then, the protein solution will be crystallized for use in the X-ray diffraction experiment. The protein solution will be purified by column chromatography and ultracentrifugation. We apply for the approval of genetic modification experiment for this research proposal because the existence of infectious baculovirus particles cannot be denied. The target protein is egg-white lysozyme. The sequence and functions of the base have been identified. The protein to be obtained is a noninfectious enzyme.
Expected duration of experiment[8)]	October **, 2016 to March 31, 2019
Contact information of the person in charge of the experiment	Address (Postal code) 1-1-1 Koto, Mikazuki-cho, Sayo-gun, Hyogo Phone (ext./PHS) 07**—**—****(2***) Fax: 07**-**-**** E-mail: hanako@********.**.jp

Prolongation of duration due to the completion of the valid period of the approved experiment → "Renewal"
Change in experimental material or place → "Amendment"
Check appropriate box and write the previous receipt number.

Other contacts[9)]	Organization and department of the person in charge of communications XX University, Graduate School XX Faculty XX Lab Office Name of the person in charge of communications Last name First name Address (Postal code) 1-1-1 Koto, Mikazuki-cho, Sayo-gun, Hyogo Phone (ext./PHS) 07**—**—****(3***) Fax: 07**-**-**** E-mail: shikaku@********.**.jp

Place of the experiment (place for animal keeping and raising)[10)] and place for storage of genetically modified organisms[11)]

Building	Room	Containment measures						
		P1	P1A	P1P	P2	P2A	P2P	Storage
Experiment Hall	☐BL20B2 experimental hutch							
	☐BL28B2 optics hutch							
	☐BL40XU experimental hutch							
	☐BL20B2 animal operation room							
	☐Mobile operation room							
	☐BL38B1 experimental hutch							
	■ PXBL* experimental hutch	○						
Experimental Animal Facility	☐Mouse room							
	☐Genetic experiment room							
	☐Treatment room							
Medium-length Beamline Facility (Experiment building)	☐BL20B2 experimental hutch							
	☐BL20XU experimental hutch							
	☐Animal operation room							
	☐							
Medium-length Beamline Facility (Research building)	☐Room 101							
	☐Room 201							
	☐Room 202							
	■Room 204	○						○
	■Room 212	○						○
	☐Room 213							
	■Biochemistry lab 1 Room 208							
	■Biochemistry lab 2 Room 209							
	☐Biochemistry lab 3 Room 210							
SACLA	☐Experimental hutch (EH3)							
	☐Biological sample prep. room							
	☐							
Others (Write the name of the building and room)	■***** building …… room	○						○

Attach documents explaining the followings.
(1) Place of major facility, equipment, and instrument (a detailed drawing of the experimental area)
(2) The state of animals or plants that are kept in the room of area, but are not related to the experiment.

Nucleic acid donor/Donor nucleic acid[12)]

Nucleic acid donor	Experiment classification	Donor nucleic acid (type of nucleic acid)	Identification	Note
(Target gene) Chicken (*Gallus gallus*)	Class 1	Lysozyme C (cDNA)	Completed/Not completed Completed	

(Expression regulatory gene) Baculovirus (host)	Class 1	IE1 promotor (genomic DNA) Polyhedrin promotor (genomic DNA)	Completed	pM15,pM23
(Selectable marker gene) E. coli of the family Enterobacteriaceae	Class 1	Ampicillin-resistant gene (genome DNA)	Completed	Subcloning using *E. Coli*

When changing experimental materials, underline the materials to be added.

Host/vector[13)]

Host	Experiment classification	Vector	Type	Note
Baculovirus Autographa californica nuclear polyhedrosis virus (AcNPV)	Class 1	pVL1392, pVL1393, pORB, pAcSec1, pAcIRES, pIEx/Bac-3 (baculovirus transfer vector; any vector clones the target gene downstream of a polyhedrin promotor. Baculovirus is Amp resistant because of subcloning using *E. coli*.)	Microorganism/Animal/Plant Microorganism	Inactivation of wild-type baculovirus is difficult because of the existence of the polyhedrin gene; however, a recombinant is easily inactivated because polyhedrin gene has been removed.

Characteristics of animals, plants, or cells that possess genetically modified organisms.[14)]	Insect Sf9 cells and HighFive cells will be used in the generation and expression of modified baculovirus, respectively. These cells will be disrupted and will no longer exist after the purification of the target protein.
Table of genetically modified organisms and containment measures.[15)]	See attached
Method of inactivation of genetically modified organisms.[16)]	Genetically modified organisms will be inactivated by autoclaving (121 ºC for 20 min), treatment with sodium hypochlorite (0.1% for 30 min) or 705 ethanol, or UV irradiation.
Note[17)]	This research proposal is designated as a P1 experiment in accordance with the position paper of the Ministry of Education, Culture, Sports, Science and Technology "Containment measures in the use of genetically modified organisms employing nucleic acid extracted from the environment as the donor nucleic acid using certified host-vector system (16 December 2004)".

※Notes

1) For “Project Leader,” the information shall be given on a person who is directly manage a genetic recombination experiment at SPring-8 and have experience for one year or more. However, a student should not be.
2) For “Person in charge of managing experiments,” the information shall be given on a person who is in charge of administration of this application.
   JASRI, RIKEN, JAERI Staff >> Director
   User >> Director of Users Office （A blank is sufficient in case it submits.）
3) Since "Proposal number" is informed when Safety Office receives this application, leave a column blank. Proposal number is required for all the documents for which you will apply in the future.
4) For “Type of Application,” select any items under which your application falls. In case Continuation or Changes, give the previous proposal number.
5) For “Title,” mention a name that expresses the objective and an outline of a genetic recombinant experiment briefly.
6) For “Type,” select all items under which a genetic recombinant experiment falls.
7) For “Outline,” all living modified organisms involved in a genetic recombinant experiment and the categories of containment measures to be taken during a genetic recombinant experiment shall be mentioned so as to show their processes.
8) The experiment is valid for a maximum of three years from approved day.
9) For “Other contact,” if there is any other contact for administrative matters than Project Leader or Deputy Project Leader, give the information on the contact.
10) For “Laboratory ,Experiment area ,Experiment section (include the area of the breeding animals or culture of plants) ,” select all area under which a genetic recombinant experiment falls. If there is no appropriate column, indicate name of facility and room on proper column and attach the information those mentioned in below.
    ①Names and positions of major facilities, equipment and apparatus;
    ②In case an animal or a plant that has no relation with a genetic recombination experiment 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;
11） For “Facility of Storage,” select all area that storage living modified organisms in the process of a genetic recombination experiment. If there is no appropriate column, indicate name of facility and room on proper column and attach the information about them.
12) For “Donor organism/Donor nucleic acid,” the following shall be mentioned about the donor organism and donor nucleic acid of the living modified organism for Genetic recombination experiment every component elements (target genes, expression regulatory genes, drug-resistant genes and marker genes).
    a. General name and taxonomical position (familia, genus, species, strain) of donor organism
    b. General name and type (such as genomic nucleic acid, complementary deoxyribonucleic acid or synthesized nucleic acid) of donor nucleic acid.
    c. Attach copy of 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).
13) For “Recipient organism/Vector,” the following shall be mentioned about the recipient organism and vector of the living modified organism for Genetic recombination experiment.
    a. General name and taxonomical position (familia, genus, species, strain) of recipient organism
    b. General name, code and short explanation about (ex. pUC119 cloning vector for *E.coli*)
    c For “Type,” select Microorganisms, Animals or Plants.
14) For “Characteristics of animal, plant or cell which retains living modified organisms,” in addition to items those mentioned in below, characters expected to be newly given or already given to an animal, a plant or a cell which retains the living modified organism for Genetic recombination experiment in comparison with animals, plants or cells which do not retain the living modified organisms Genetic recombination experiment shall be mentioned.
    a. Taxonomical position and experiment classification of animal, plant or cell which retains living modified organism;
    b. State of distribution in natural environment and environment in which living or growth is possible;
    c. Pathogenicity, production of harmful substances and other properties;
15) For “Combination Living Modified Organism and its Category of Containment Measures,” all donor organisms, donor nucleic acids, vectors, recipient organisms and animals, plants or cells which retains living modified organism involved in a genetic recombination experiment and the categories of containment measures to be taken during the experiment shall be mentioned so as to show processes of the experiment.
16) For “Measure for inactivating living modified organism,” about the containment measures to be taken during Genetic recombinant experiment, mention a measure for inactivating waste products containing the living modified organism and apparatus and appliances to which the living modified organism sticks, and the effectiveness of the measure.
17) When you have received grant of public costs from JASRI, give a note.

Write the combination of nucleic acid donor, donor nucleic acid, vector, host, and possessing organisms along with the containment measures taken in the relevant step of the experiment so that the flow of the experiment is clear. When changing experimental materials, underline the materials to be added.

Table of genetically modified organisms and containment measures

Nucleic acid donor	Donor nucleic acid	Vector	Host etc.	Possessing organisms	Containment Measure Classification	Note
Chicken Baculovirus Baculovirus E. coli of the family Enterobacteriaceae	Lysozyme C (cDNA) IE1 promotor (genomic DNA) Polyhedrin promotor (genomic DNA) Ampicillin-resistant gene (genome DNA)	pVL series (Commercial item: Allele Biotechnology) pORB (Commercial item: Allele Biotechnology) pAcSec1 (Commercial item: Allele Biotechnology) pAcIRES (Commercial item: Allele Biotechnology) pIEx/Bac-3 (Commercial item: Novagen)	Baculovirus (Commercial item: Allele Biotechnology, Oxford Expression Technologies, BD, Sigma, Novagen)	None High Five cell at the time of protein expression	P1	The protein expressed from recombinant baculovirus and High Five cells is purified and crystallized to obtain samples for x-ray diffraction.