

**RECOMBINANT DNA APPLICATION
TO THE CSULB INSTITUTIONAL BIOSAFETY
COMMITTEE**

PLEASE DO THE FOLLOWING:

- 1. FILL OUT THE ELECTRONIC APPLICATION.**
- 2. SEND VIA EMAIL TO JOHN DE LA CUESTA,
JDLC@CSULB.EDU. AFTER REVIEW, HE WILL
PRINT IT OFF & GET SIGNATURES.**

**THE INSTITUTIONAL BIOSAFETY COMMITTEE (IBC)
WILL DISCUSS YOUR APPLICATION AS SOON AS
POSSIBLE.**

PLEASE CALL X55623 IF YOU HAVE QUESTIONS

THANK YOU.

APPLICATION TO USE RECOMBINANT DNA (rDNA)

California State University, Long Beach
Institutional Biosafety Committee

Name:

Dept:

Date:

This research is subject to annual review and renewal. This form must be typed.

SECTION 1: RESEARCH DESCRIPTION

Describe in two or three paragraphs the work to be conducted in your laboratory directly related to rDNA (include general experimental procedures and any microbial, parasitic etc. agents involved):

SECTION 2: CHECK AND IDENTIFY AS APPLICABLE TO THE RESEARCH DESCRIBED ABOVE

Use of Recombinant DNA

Confined to microbial environment, in laboratory

Intentional release to environment

Transgenic Animals, identify

Transgenic Plants, identify

Other; specify

Microbial/Infectious Agents

Viruses, identify:

Bacteria, identify:

Fungi, identify:

Prions, identify:

Parasitic Agents, identify:

Pre-exposure immunization required:

Use of Animals

Invertebrates, identify:

Vertebrates, identify: AWB Protocol #:

Use of infectious agents in animals

Use of animals that are potential reservoirs of zoonotic diseases

SECTION 3: USE OF RECOMBINANT DNA (rDNA)

Provide the following information for the use of rDNA:

- a) Nature of inserted DNA sequence, including the species of origin, gene product and function (if known):
- b) Host(s) and vector(s) to be used:
- c) Will an attempt be made to express the foreign gene? Yes No

SECTION 4: RISK ASSESSMENT AND RISK GROUP (RG)

Check the Risk Group (RG) of the agent (bacteria, virus etc.) being used. **For Risk Group classification go to page 7 of this document or: <http://www.absa.org/riskgroups/index.html>**

RG1	RG2	RG3	RG4	No bacterial, viral, parasitic etc. agent
will be used				

SECTION 5: PHYSICAL CONTAINMENT AND BIOLOGICAL SAFETY LEVEL (BSL)

Check the BSL of the agent (bacteria, virus etc.) being used. **(For Biosafety Level classification go to: <http://www.cdc.gov/biosafety/publications/bmb15/BMBL5 sect IV.pdf>)**

Exempt	BSL-1	BSL-2	BSL-3 Practices/BSL-2 Facility
BSL-3			

If you created a transgenic animal or plant, what special measures will you take to ensure its containment?

SECTION 6: LOCATIONS FOR USE AND STORAGE OF INFECTIOUS AGENTS AND rDNA

List the location(s) where work will be conducted and where materials will be stored.

Building	Room Number	

Does this project involve CSULB-managed funds for the use of rDNA at a site other than CSULB?

Yes No

If yes, please list the site(s) here:

SECTION 7: DECONTAMINATION PROCEDURES

Read the standard practices and physical containment procedures as defined for your BSL level in [appendix G](#) of the [NIH Guidelines](#). Do you agree to abide by these guidelines with respect to standard procedures and physical containment?

Yes No

SECTION 8: DISPOSAL OF CONTAMINATED MATERIALS

Do you agree to dispose of all contaminated materials according to the [CNSM Biohazard Control Program](#) (decon first via autoclave, chlorox etc. as appropriate; sharps into sharps containers; BSL 2 waste into designated red biohazardous waste containers etc.)?

Yes No

SECTION 9: TRAINING OF PERSONNEL

Describe how personnel have been trained in the handling of agents to be used (e.g. hands on training in the lab, lab meeting etc.).

SECTION 10: ACCIDENTAL EXPOSURE

Do you agree to **contact CNSM Safety/ Campus Environmental Health and Safety immediately** in the event an employee, student, or coworker becomes ill and/or exhibits symptoms and signs consistent with an infection by an organism used in this research?

Yes No

Annual Renewals: Have there been any adverse events related to work with this organism over the past year?
Yes No

If yes, please describe:

DEPARTMENTAL APPROVAL

Note to applicant: The CNSM Safety Office will obtain this signature for you if you wish.

The Department Chair/Director must read the protocol and sign below indicating department approval before IBC review may occur. Note: If the Department Chair/Director is an investigator on this application, this approval must be obtained from the next highest level of administrative authority.

I hereby confirm that I have read the Protocol Narrative and can certify that: 1) the research is appropriate in design; 2) the investigator (or faculty sponsor) is competent to perform (or supervise) the study; and 3) there are sufficient funds available to support performance of this research. My signature below denotes Departmental Approval of this study as submitted.

Name

Department Chair/Director

Signature

Department Chair/Director

Date signed

INVESTIGATOR'S ASSURANCE

California State University, Long Beach
Institutional Biosafety Committee

Project Title: ____

1. All persons conducting this work (including my collaborators) have completed the CNSM Safety Program Training and have received instruction on the specific hazards associated with the work and the specific safety equipment, practices, and behaviors required during the course of the work and use of these facilities.
2. Any spill of biohazardous material, any equipment or facility failure (e.g., ventilation failure), and/or any breakdown in procedure that could result in potential exposure of laboratory personnel and/or the public to biohazardous material will be reported to CNSM Safety and Campus Safety & Risk Management immediately.
3. Any proposed changes in my work that would result in an increased level of biohazard will be reported to the IBC **before the change is implemented.**
4. No work that requires IBC approval prior to initiation will be initiated or modified until approval is received from the IBC.
5. If this project involves rDNA molecules, I have read and understand my responsibilities as Principal Investigator outlined in Section IV-B-4 of the *NIH Guidelines*, and will comply with these responsibilities.
6. I certify that the information provided within this application is accurate to the best of my knowledge. I also understand that, should I use the project described in this application as a basis for a funding proposal (either intramural or extramural), it is my responsibility to ensure that the description of the work in the funding proposal is identical in principle to that contained in this application.

7. I certify that all work on this project will be performed in accordance with all policies and procedures of the CNSM Safety Program and in accordance with NIH guidelines as appropriate for the risk group and BSL of the agents involved.

Name of Lead Researcher

Signature of Lead Researcher

APPENDIX B. CLASSIFICATION OF HUMAN [INFECTIOUS] AGENTS ON THE BASIS OF HAZARD

This appendix includes those biological agents known to infect humans as well as selected animal agents that may pose theoretical risks if inoculated into humans. Included are lists of representative genera and species known to be pathogenic; mutated, recombined, and non-pathogenic species and strains are not considered. Non-infectious life cycle stages of parasites are excluded.

This appendix reflects the current state of knowledge and should be considered a resource document. Included are the more commonly encountered agents and is not meant to be all inclusive. Information on agent risk assessment may be found in the *Agent Summary Statements* of the CDC/NIH publication, *Biosafety in Microbiological and Biomedical Laboratories* (see Sections V-C, V-D, V-E, and V-F, *Footnotes and References of Sections I through IV*). Further guidance on agents not listed in Appendix B may be obtained through: Centers for Disease Control and Prevention, Biosafety Branch, Atlanta, Georgia 30333, Phone: (404) 639-3883, Fax: (404) 639-2294; National Institutes of Health, Division of Safety, Bethesda, Maryland 20892, Phone: (301) 496-1357; National Animal Disease Center, U.S. Department of Agriculture, Ames, Iowa 50010, Phone: (515) 862-8258.

A special committee of the American Society for Microbiology will conduct an annual review of this appendix and its recommendation for changes will be presented to the Recombinant DNA Advisory Committee as proposed amendments to the *NIH Guidelines*.

Appendix B - Table 1. Basis for the Classification of Biohazardous Agents by Risk Group (RG)

Risk Group 1 (RG1)	Agents that are not associated with disease in healthy adult humans
Risk Group 2 (RG2)	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are <i>often</i> available
Risk Group 3 (RG3)	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions <i>may be</i> available (high individual risk but low community risk)
Risk Group 4 (RG4)	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are <i>not usually</i> available (high individual risk and high community risk)

Appendix B-I. Risk Group 1 (RG1) Agents

RG1 agents are not associated with disease in healthy adult humans. Examples of RG1 agents include asporogenic *Bacillus subtilis* or *Bacillus licheniformis* (see Appendix C-IV-A, *Bacillus subtilis* or *Bacillus licheniformis* Host-Vector Systems, Exceptions), *Escherichia coli* K-12 (see Appendix C-II-A, *Escherichia coli* K-12 Host Vector Systems, Exceptions), adeno-associated virus (AAV) types 1 through 4, and recombinant AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and are produced in the absence of a helper virus.

Those agents not listed in Risk Groups (RGs) 2, 3 and 4 are not automatically or implicitly classified in RG1; a risk assessment must be conducted based on the known and potential properties of the agents and their relationship to agents that are listed.

Appendix B-II. Risk Group 2 (RG2) Agents

RG2 agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available.

Appendix B-II-A. Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia

- Acinetobacter baumannii* (formerly *Acinetobacter calcoaceticus*)
- Actinobacillus*
- Actinomyces pyogenes* (formerly *Corynebacterium pyogenes*)
- Aeromonas hydrophila*
- Amycolata autotrophica*
- Archaeobacterium haemolyticum* (formerly *Corynebacterium haemolyticum*)
- Arizona hinshawii* - all serotypes
- Bacillus anthracis*
- Bartonella henselae*, *B. quintana*, *B. vinsonii*
- Bordetella* including *B. pertussis*
- Borrelia recurrentis*, *B. burgdorferi*
- Burkholderia* (formerly *Pseudomonas* species) except those listed in Appendix B-III-A (RG3))
- Campylobacter coli*, *C. fetus*, *C. jejuni*
- Chlamydia psittaci*, *C. trachomatis*, *C. pneumoniae*
- Clostridium botulinum*, *Cl. chauvoei*, *Cl. haemolyticum*, *Cl. histolyticum*, *Cl. novyi*, *Cl. septicum*, *Cl. tetani*
- Corynebacterium diphtheriae*, *C. pseudotuberculosis*, *C. renale*
- Dermatophilus congolensis*
- Edwardsiella tarda*
- Erysipelothrix rhusiopathiae*
- Escherichia coli* - all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen, including *E. coli* O157:H7
- Haemophilus ducreyi*, *H. influenzae*
- Helicobacter pylori*
- Klebsiella* - all species except *K. oxytoca* (RG1)
- Legionella* including *L. pneumophila*
- Leptospira interrogans* - all serotypes
- Listeria*
- Moraxella*

- Mycobacterium* (except those listed in Appendix B-III-A (RG3)) including *M. avium* complex, *M. asiaticum*, *M. bovis* BCG vaccine strain, *M. chelonae*, *M. fortuitum*, *M. kansasii*, *M. leprae*, *M. malmoense*, *M. marinum*, *M. paratuberculosis*, *M. scrofulaceum*, *M. simiae*, *M. szulgai*, *M. ulcerans*, *M. xenopi*
- Mycoplasma*, except *M. mycoides* and *M. agalactiae* which are restricted animal pathogens
- Neisseria gonorrhoeae*, *N. meningitidis*
- Nocardia asteroides*, *N. brasiliensis*, *N. otitidiscaviarum*, *N. transvalensis*
- Rhodococcus equi*
- Salmonella* including *S. arizonae*, *S. choleraesuis*, *S. enteritidis*, *S. gallinarum-pullorum*, *S. meleagridis*, *S. paratyphi*, A, B, C, *S. typhi*, *S. typhimurium*
- Shigella* including *S. boydii*, *S. dysenteriae*, type 1, *S. flexneri*, *S. sonnei*
- Sphaerophorus necrophorus*
- Staphylococcus aureus*
- Streptobacillus moniliformis*
- Streptococcus* including *S. pneumoniae*, *S. pyogenes*
- Treponema pallidum*, *T. carateum*
- Vibrio cholerae*, *V. parahemolyticus*, *V. vulnificus*
- Yersinia enterocolitica*

Appendix B-II-B. Risk Group 2 (RG2) - Fungal Agents

- Blastomyces dermatitidis*
- Cladosporium bantianum*, *C. (Xylohypha) trichoides*
- Cryptococcus neoformans*
- Dactylaria galopava (Ochroconis gallopavum)*
- Epidermophyton*
- Exophiala (Wangiella) dermatitidis*
- Fonsecaea pedrosoi*
- Microsporium*
- Paracoccidioides braziliensis*
- Penicillium marneffeii*
- Sporothrix schenckii*
- Trichophyton*

Appendix B-II-C. Risk Group 2 (RG2) - Parasitic Agents

--*Ancylostoma* human hookworms including *A. duodenale*, *A. ceylanicum*

--*Ascaris* including *Ascaris lumbricoides suum*

--*Babesia* including *B. divergens*, *B. microti*

--*Brugia* filaria worms including *B. malayi*, *B. timori*

--*Coccidia*

--*Cryptosporidium* including *C. parvum*

--*Cysticercus cellulosae* (hydatid cyst, larva of *T. solium*)

--*Echinococcus* including *E. granulosus*, *E. multilocularis*, *E. vogeli*

--*Entamoeba histolytica*

--*Enterobius*

--*Fasciola* including *F. gigantica*, *F. hepatica*

--*Giardia* including *G. lamblia*

--*Heterophyes*

--*Hymenolepis* including *H. diminuta*, *H. nana*

--*Isospora*

--*Leishmania* including *L. braziliensis*, *L. donovani*, *L. ethiopia*, *L. major*, *L. mexicana*, *L. peruvania*, *L. tropica*

--*Loa loa* filaria worms

--*Microsporidium*

--*Naegleria fowleri*

--*Necator* human hookworms including *N. americanus*

--*Onchocerca* filaria worms including, *O. volvulus*

--*Plasmodium* including simian species, *P. cynomologi*, *P. falciparum*, *P. malariae*, *P. ovale*, *P. vivax*

--*Sarcocystis* including *S. sui hominis*

--*Schistosoma* including *S. haematobium*, *S. intercalatum*, *S. japonicum*, *S. mansoni*, *S. mekongi*

--*Strongyloides* including *S. stercoralis*

--*Taenia solium*

--*Toxocara* including *T. canis*

--*Toxoplasma* including *T. gondii*

--*Trichinella spiralis*

--*Trypanosoma* including *T. brucei brucei*, *T. brucei gambiense*, *T. brucei rhodesiense*, *T. cruzi*

--*Wuchereria bancrofti* filaria worms

Appendix B-II-D. Risk Group 2 (RG2) - Viruses

Adenoviruses, human - all types

Alphaviruses (Togaviruses) - Group A Arboviruses

--Eastern equine encephalomyelitis virus

--Venezuelan equine encephalomyelitis vaccine strain TC-83

--Western equine encephalomyelitis virus

Arenaviruses

--Lymphocytic choriomeningitis virus (non-neurotropic strains)

--Tacaribe virus complex

--Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)

Bunyaviruses

--Bunyamwera virus

--Rift Valley fever virus vaccine strain MP-12

--Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)

Calciviruses

Coronaviruses

Flaviviruses (Togaviruses) - Group B Arboviruses

--Dengue virus serotypes 1, 2, 3, and 4

--Yellow fever virus vaccine strain 17D

--Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)

Hepatitis A, B, C, D, and E viruses

Herpesviruses - except Herpesvirus simiae (Monkey B virus) (see Appendix B-IV-D, *Risk Group 4 (RG4) - Viral Agents*)

--Cytomegalovirus

--Epstein Barr virus

--*Herpes simplex* types 1 and 2

--*Herpes zoster*

--Human herpesvirus types 6 and 7

Orthomyxoviruses

--Influenza viruses types A, B, and C

--Other tick-borne orthomyxoviruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)

Papovaviruses

--All human papilloma viruses

Paramyxoviruses

--Newcastle disease virus

--Measles virus

--Mumps virus

--Parainfluenza viruses types 1, 2, 3, and 4

--Respiratory syncytial virus

Parvoviruses

--Human parvovirus (B19)

Picornaviruses

--Coxsackie viruses types A and B

--Echoviruses - all types

--Polioviruses - all types, wild and attenuated

--Rhinoviruses - all types

Poxviruses - all types except Monkeypox virus (see Appendix B-III-D, *Risk Group 3 (RG3) - Viruses and Prions*) and restricted poxviruses including Alastrim, Smallpox, and Whitepox (see Section V-L, *Footnotes and References of Sections I through IV*)

Reoviruses - all types including Coltivirus, human Rotavirus, and Orbivirus (Colorado tick fever virus)

Rhabdoviruses

--Rabies virus - all strains

--Vesicular stomatitis virus - laboratory adapted strains including VSV-Indiana, San Juan, and Glasgow

Togaviruses (see Alphaviruses and Flaviviruses)

--Rubivirus (rubella)

Appendix B-III. Risk Group 3 (RG3) Agents

RG3 agents are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available.

Appendix B-III-A. Risk Group 3 (RG3) - Bacterial Agents Including Rickettsia

--*Bartonella*

--*Brucella* including *B. abortus*, *B. canis*, *B. suis*

--*Burkholderia (Pseudomonas) mallei*, *B. pseudomallei*

--*Coxiella burnetii*

--*Francisella tularensis*

--*Mycobacterium bovis* (except BCG strain, see Appendix B-II-A, *Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia*), *M. tuberculosis*

--*Pasteurella multocida* type B -"buffalo" and other virulent strains

--*Rickettsia akari*, *R. australis*, *R. canada*, *R. conorii*, *R. prowazekii*, *R. rickettsii*, *R. siberica*, *R. tsutsugamushi*, *R. typhi* (*R. mooseri*)

--*Yersinia pestis*

Appendix B-III-B. Risk Group 3 (RG3) - Fungal Agents

--*Coccidioides immitis* (sporulating cultures; contaminated soil)

--*Histoplasma capsulatum*, *H. capsulatum* var.. *duboisii*

Appendix B-III-C. Risk Group 3 (RG3) - Parasitic Agents

None

Appendix B-III-D. Risk Group 3 (RG3) - Viruses and Prions

Alphaviruses (Togaviruses) - Group A Arboviruses

--Semliki Forest virus

--St. Louis encephalitis virus

--Venezuelan equine encephalomyelitis virus (except the vaccine strain TC-83, see Appendix B-II-D (RG2))

--Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)

Arenaviruses

--Flexal

--Lymphocytic choriomeningitis virus (LCM) (neurotropic strains)

Bunyaviruses

--Hantaviruses including Hantaan virus

--Rift Valley fever virus

Flaviviruses (Togaviruses) - Group B Arboviruses

--Japanese encephalitis virus

--Yellow fever virus

--Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)

Poxviruses

--Monkeypox virus

Prions

--Transmissible spongiform encephalopathies (TME) agents (Creutzfeldt-Jacob disease and kuru agents)(see Section V-C, *Footnotes and References of Sections I through IV*, for containment instruction)

Retroviruses

--Human immunodeficiency virus (HIV) types 1 and 2

--Human T cell lymphotropic virus (HTLV) types 1 and 2

--Simian immunodeficiency virus (SIV)

Rhabdoviruses

--Vesicular stomatitis virus

APPENDIX G. PHYSICAL CONTAINMENT

Appendix G specifies physical containment for standard laboratory experiments and defines Biosafety Level 1 through Biosafety Level 4. For large scale (over 10 liters) research or production, Appendix K (*Physical Containment for Large Scale Uses of Organisms Containing Recombinant DNA Molecules*) supersedes Appendix G. Appendix K defines Good Large Scale Practice through Biosafety Level 3 - Large Scale. For certain work with plants, Appendix P (*Physical and Biological Containment for Recombinant DNA Research Involving Plants*) supersedes Appendix G. Appendix P defines Biosafety Levels 1 through 4 - Plants. For certain work with animals, Appendix Q (*Physical and Biological Containment for Recombinant DNA Research Involving Animals*) supersedes Appendix G. Appendix Q defines Biosafety Levels 1 through 4 - Animals.

Appendix G-I. Standard Practices and Training

The first principle of containment is strict adherence to good microbiological practices (see Appendices G-III-A through G-III-J, *Footnotes and References of Appendix G*). Consequently, all personnel directly or indirectly involved in experiments using recombinant DNA shall receive adequate instruction (see Sections IV-B-1-e, *Responsibilities of the Institution--General Information*, and IV-B-4-d, *Principal Investigator*). At a minimum, these instructions include training in aseptic techniques and in the biology of the organisms used in the experiments so that the potential biohazards can be understood and appreciated.

Any research group working with agents that are known or potential biohazards shall have an emergency plan that describes the procedures to be followed if an accident contaminates personnel or the environment. The Principal Investigator shall ensure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan (see Sections IV-B-4-d and IV-B-4-e, *Principal Investigator*). If a research group is working with a known pathogen for which there is an effective vaccine, the vaccine should be made

available to all workers. Serological monitoring, when clearly appropriate, will be provided (see Section IV-B-1-f, *Responsibilities of the Institution--General Information*).

The *Laboratory Safety Monograph* (see Appendix G-III-O, *Footnotes and References of Appendix G*) and *Biosafety in Microbiological and Biomedical Laboratories* (see Appendix G-III-B, *Footnotes and References of Appendix G*) describe practices, equipment, and facilities in detail.

Appendix G-II. Physical Containment Levels

The objective of physical containment is to confine organisms containing recombinant DNA molecules and to reduce the potential for exposure of the laboratory worker, persons outside of the laboratory, and the environment to organisms containing recombinant DNA molecules. Physical containment is achieved through the use of laboratory practices, containment equipment, and special laboratory design. Emphasis is placed on primary means of physical containment which are provided by laboratory practices and containment equipment. Special laboratory design provides a secondary means of protection against the accidental release of organisms outside the laboratory or to the environment. Special laboratory design is used primarily in facilities in which experiments of moderate to high potential hazard are performed.

Combinations of laboratory practices, containment equipment, and special laboratory design can be made to achieve different levels of physical containment. Four levels of physical containment, which are designated as BL1, BL2, BL3, and BL4 are described. It should be emphasized that the descriptions and assignments of physical containment detailed below are based on existing approaches to containment of pathogenic organisms (see Appendix G-III-B, *Footnotes and References of Appendix G*). The National Cancer Institute describes three levels for research on oncogenic viruses which roughly correspond to our BL2, BL3, and BL4 levels (see Appendix G-III-C, *Footnotes and References of Appendix G*).

It is recognized that several different combinations of laboratory practices, containment equipment, and special laboratory design may be appropriate for containment of specific research activities. The *NIH Guidelines*, therefore, allow alternative selections of primary containment equipment within facilities that have been designed to provide BL3 and BL4 levels of physical containment. The selection of alternative methods of primary containment is dependent, however, on the level of biological containment provided by the host-vector system used in the experiment. Consideration will be given by the NIH Director, with the advice of the RAC, to other combinations which achieve an equivalent level of containment (see Section IV-C-1-b-(2)-(a), *Major Action*).

Appendix G-II-A. Biosafety Level 1 (BL1) (See Appendix G-III-M, *Footnotes and References of Appendix G*)

Appendix G-II-A-1. Standard Microbiological Practices (BL1)

Appendix G-II-A-1-a. Access to the laboratory is limited or restricted at the discretion of the Principal Investigator when experiments are in progress.

Appendix G-II-A-1-b. Work surfaces are decontaminated once a day and after any spill of viable material.

Appendix G-II-A-1-c. All contaminated liquid or solid wastes are decontaminated before disposal.

Appendix G-II-A-1-d. Mechanical pipetting devices are used; mouth pipetting is prohibited.

Appendix G-II-A-1-e. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only.

Appendix G-II-A-1-f. Persons wash their hands: (i) after they handle materials involving organisms containing recombinant DNA molecules and animals, and (ii) before exiting the laboratory.

Appendix G-II-A-1-g. All procedures are performed carefully to minimize the creation of aerosols.

Appendix G-II-A-1-h. In the interest of good personal hygiene, facilities (e.g., hand washing sink, shower, changing room) and protective clothing (e.g., uniforms, laboratory coats) shall be provided that are appropriate for the risk of exposure to viable organisms containing recombinant DNA molecules.

Appendix G-II-A-2. Special Practices (BL1)

Appendix G-II-A-2-a. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.

Appendix G-II-A-2-b. An insect and rodent control program is in effect.

Appendix G-II-A-3. Containment Equipment (BL1)

Appendix G-II-A-3-a. Special containment equipment is generally not required for manipulations of agents assigned to BL1.

Appendix G-II-A-4. Laboratory Facilities (BL1)

Appendix G-II-A-4-a. The laboratory is designed so that it can be easily cleaned.

Appendix G-II-A-4-b. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix G-II-A-4-c. Laboratory furniture is sturdy. Spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix G-II-A-4-d. Each laboratory contains a sink for hand washing.

Appendix G-II-A-4-e. If the laboratory has windows that open, they are fitted with fly screens.

Appendix G-II-B. Biosafety Level 2 (BL2) (See Appendix G-III-N, *Footnotes and References of Appendix G*)

Appendix G-II-B-1. Standard Microbiological Practices (BL2)

Appendix G-II-B-1-a. Access to the laboratory is limited or restricted by the Principal Investigator when work with organisms containing recombinant DNA molecules is in progress.

Appendix G-II-B-1-b. Work surfaces are decontaminated at least once a day and after any spill of viable material.

Appendix G-II-B-1-c. All contaminated liquid or solid wastes are decontaminated before disposal.

Appendix G-II-B-1-d. Mechanical pipetting devices are used; mouth pipetting is prohibited.

Appendix G-II-B-1-e. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only.

Appendix G-II-B-1-f. Persons wash their hands: (i) after handling materials involving organisms containing recombinant DNA molecules and animals, and (ii) when exiting the laboratory.

Appendix G-II-B-1-g. All procedures are performed carefully to minimize the creation of aerosols.

Appendix G-II-B-1-h. Experiments of lesser biohazard potential can be conducted concurrently in carefully demarcated areas of the same laboratory.

Appendix G-II-B-2. Special Practices (BL2)

Appendix G-II-B-2-a. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.

Appendix G-II-B-2-b. The Principal Investigator limits access to the laboratory. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

Appendix G-II-B-2-c. The Principal Investigator establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific entry requirements (e.g., immunization) may enter the laboratory or animal rooms.

Appendix G-II-B-2-d. When the organisms containing recombinant DNA molecules in use in the laboratory require special provisions for entry (e.g., vaccination), a hazard warning sign incorporating the universal biosafety symbol is posted on the access door to the laboratory work area. The hazard warning sign identifies the agent, lists the name and telephone number of the Principal Investigator or other responsible person(s), and indicates the special requirement(s) for entering the laboratory.

Appendix G-II-B-2-e. An insect and rodent control program is in effect.

Appendix G-II-B-2-f. Laboratory coats, gowns, smocks, or uniforms are worn while in the laboratory. Before exiting the laboratory for non-laboratory areas (e.g., cafeteria, library, administrative offices), this protective clothing is removed and left in the laboratory or covered with a clean coat not used in the laboratory.

Appendix G-II-B-2-g. Animals not involved in the work being performed are not permitted in the laboratory.

Appendix G-II-B-2-h. Special care is taken to avoid skin contamination with organisms containing recombinant DNA molecules; gloves should be worn when handling experimental animals and when skin contact with the agent is unavoidable.

Appendix G-II-B-2-i. All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

Appendix G-II-B-2-j. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably autoclaved, before discard or reuse.

Appendix G-II-B-2-k. Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Institutional Biosafety Committee and NIH/OBA. Reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

Appendix G-II-B-2-l. When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically depending on the agents handled or the function of the facility.

Appendix G-II-B-2-m. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

Appendix G-II-B-3. Containment Equipment (BL2)

Appendix G-II-B-3-a. Biological safety cabinets (Class I or II) (see Appendix G-III-L, *Footnotes and References of Appendix G*) or other appropriate personal protective or physical containment devices are used whenever:

Appendix G-II-B-3-a-(1). Procedures with a high potential for creating aerosols are conducted (see Appendix G-III-O, *Footnotes and References of Appendix G*). These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of materials whose internal pressures may be different from ambient pressures, intranasal inoculation of animals, and harvesting infected tissues from animals or eggs.

Appendix G-II-B-3-a-(2). High concentrations or large volumes of organisms containing recombinant DNA molecules are used. Such materials may be centrifuged in the open laboratory if sealed beads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

Appendix G-II-B-4. Laboratory Facilities (BL2)

Appendix G-II-B-4-a. The laboratory is designed so that it can be easily cleaned.

Appendix G-II-B-4-b. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix G-II-B-4-c. Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix G-II-B-4-d. Each laboratory contains a sink for hand washing.

Appendix G-II-B-4-e. If the laboratory has windows that open, they are fitted with fly screens.

Appendix G-II-B-4-f. An autoclave for decontaminating laboratory wastes is available.

Appendix G-II-C. Biosafety Level 3 (BL3) (See Appendix G-III-P, *Footnotes and References of Appendix G*)

Appendix G-II-C-1. Standard Microbiological Practices (BL3)

Appendix G-II-C-1-a. Work surfaces are decontaminated at least once a day and after any spill of viable material.

Appendix G-II-C-1-b. All contaminated liquid or solid wastes are decontaminated before disposal.

Appendix G-II-C-1-c. Mechanical pipetting devices are used; mouth pipetting is prohibited.

Appendix G-II-C-1-d. Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the work area.

Appendix G-II-C-1-e. Persons wash their hands: (i) after handling materials involving organisms containing recombinant DNA molecules, and handling animals, and (ii) when exiting the laboratory.

Appendix G-II-C-1-f. All procedures are performed carefully to minimize the creation of aerosols.

Appendix G-II-C-1-g. Persons under 16 years of age shall not enter the laboratory.

Appendix G-II-C-1-h. If experiments involving other organisms which require lower levels of containment are to be conducted in the same laboratory concurrently with experiments requiring BL3 level physical containment, they shall be conducted in accordance with all BL3 level laboratory practices.

Appendix G-II-C-2. Special Practices (BL3)

Appendix G-II-C-2-a. Laboratory doors are kept closed when experiments are in progress.

Appendix G-II-C-2-b. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.

Appendix G-II-C-2-c. The Principal Investigator controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

Appendix G-II-C-2-d. The Principal Investigator establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures entering the laboratory or animal rooms.

Appendix G-II-C-2-e. When organisms containing recombinant DNA molecules or experimental animals are present in the laboratory or containment module, a hazard warning sign incorporating the universal biosafety symbol is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the Principal Investigator or other responsible person(s), and indicates any special requirements for entering the laboratory such as the need for immunizations, respirators, or other personal protective measures.

Appendix G-II-C-2-f. All activities involving organisms containing recombinant DNA molecules are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench.

Appendix G-II-C-2-g. The work surfaces of biological safety cabinets and other containment equipment are decontaminated when work with organisms containing recombinant DNA molecules is finished. Plastic-backed paper toweling used on non-perforated work surfaces within biological safety cabinets facilitates clean-up.

Appendix G-II-C-2-h. An insect and rodent program is in effect.

Appendix G-II-C-2-i. Laboratory clothing that protects street clothing (e.g., solid front or wrap-around gowns, scrub suits, coveralls) is worn in the laboratory. Laboratory clothing is not worn outside the laboratory, and it is decontaminated prior to laundering or disposal.

Appendix G-II-C-2-j. Special care is taken to avoid skin contamination with contaminated materials; gloves should be worn when handling infected animals and when skin contact with infectious materials is unavoidable.

Appendix G-II-C-2-k. Molded surgical masks or respirators are worn in rooms containing experimental animals.

Appendix G-II-C-2-l. Animals and plants not related to the work being conducted are not permitted in the laboratory.

Appendix G-II-C-2-m. Laboratory animals held in a BL3 area shall be housed in partial-containment caging systems, such as Horsfall units (see Appendix G-III-K, *Footnotes and References of Appendix G*), open cages placed in ventilated enclosures, solid-wall and -bottom cages covered by filter bonnets or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet in radiation lamps and reflectors.

Note: Conventional caging systems may be used provided that all personnel wear appropriate personal protective devices. These protective devices shall include at a minimum wrap-around gowns, head covers, gloves, shoe covers, and respirators. All personnel shall shower on exit from areas where these devices are required.

Appendix G-II-C-2-n. All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

Appendix G-II-C-2-o. Vacuum lines are protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.

Appendix G-II-C-2-p. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix G-II-C-2-q. Spills and accidents which result in overt or potential exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, and NIH/OBA. Reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.

Appendix G-II-C-2-r. Baseline serum samples for all laboratory and other at-risk personnel should be collected and stored. Additional serum specimens may be collected periodically depending on the agents handled or the function of the laboratory.

Appendix G-II-C-2-s. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow the instructions on practices and procedures.

Appendix G-II-C-2-t. Alternative Selection of Containment Equipment (BL3)

Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified may be conducted in the BL3 laboratory using containment equipment specified for the BL2 level of physical containment. Experimental procedures involving a host-vector system that provides a one-step lower level of biological containment than that specified may be conducted in the BL3 laboratory using containment equipment specified for the BL4 level of physical containment. Alternative combination of containment safeguards are shown in Appendix G-Table 1, *Possible Alternate Combinations of Physical and Biological Containment Safeguards*.

Appendix G-II-C-3. Containment Equipment (BL3)

Appendix G-II-C-3-a. Biological safety cabinets (Class I, II, or III) (see Appendix G-III-L, *Footnotes and References of Appendix G*) or other appropriate combinations of personal protective or physical containment devices (e.g., special protective clothing, masks, gloves, respirators, centrifuge safety cups, sealed centrifuge

rotors, and containment caging for animals) are used for all activities with organisms containing recombinant DNA molecules which pose a threat of aerosol exposure. These include: manipulation of cultures and of those clinical or environmental materials which may be a source of aerosols; the aerosol challenge of experimental animals; the harvesting of infected tissues or fluids from experimental animals and embryonate eggs; and the necropsy of experimental animals.

Appendix G-II-C-4. Laboratory Facilities (BL3)

Appendix G-II-C-4-a. The laboratory is separated from areas which are open to unrestricted traffic flow within the building. Passage through two sets of doors is the basic requirement for entry into the laboratory from access corridors or other contiguous areas. Physical separation of the high containment laboratory from access corridors or other laboratories or activities may be provided by a double-doored clothes change room (showers may be included), airlock, or other access facility which requires passage through two sets of doors before entering the laboratory.

Appendix G-II-C-4-b. The interior surfaces of walls, floors, and ceilings are water resistant so that they can be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate decontaminating the area.

Appendix G-II-C-4-c. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix G-II-C-4-d. Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix G-II-C-4-e. Each laboratory contains a sink for hand washing. The sink is foot, elbow, or automatically operated and is located near the laboratory exit door.

Appendix G-II-C-4-f. Windows in the laboratory are closed and sealed.

Appendix G-II-C-4-g. Access doors to the laboratory or containment module are self-closing.

Appendix G-II-C-4-h. An autoclave for decontaminating laboratory wastes is available preferably within the laboratory.

Appendix G-II-C-4-i. A ducted exhaust air ventilation system is provided. This system creates directional airflow that draws air into the laboratory through the entry area. The exhaust air is not recirculated to any other area of the building, is discharged to the outside, and is dispersed away from the occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the laboratory) is proper. The exhaust air from the laboratory room may be discharged to the outside without being filtered or otherwise treated.

Appendix G-II-C-4-j. The high efficiency particulate air/HEPA filtered exhaust air from Class I or Class II biological safety cabinets is discharged directly to the outside or through the building exhaust system. Exhaust air from Class I or II biological safety cabinets may be recirculated within the laboratory if the cabinet is tested and certified at least every twelve months. If the HEPA-filtered exhaust air from Class I or II biological safety cabinets is to be discharged to the outside through the building exhaust air system, it is connected to this system in a manner (e.g., thimble unit connection (see Appendix G-III-L, *Footnotes and References of Appendix G*)) that avoids any interference with the air balance of the cabinets or building exhaust system.

CNSM BIOHAZARD CONTROL PROGRAM INCLUDING BIOHAZARDOUS WASTE

CNSM BIOHAZARD CONTROL PROGRAM: The CNSM Biohazard Control Program is based on the California Health and Safety Code Sections 117600-118360 and the CDC/NIH Guidelines for Biosafety in

Microbiological and Biomedical Laboratories 5th edition (2007). The guidelines indicate the Biosafety Level (BSL) for each microbial agent; the levels range from 1-4. The Biosafety Level dictates the method of disposal, use of certain lab practices, techniques, safety equipment and facilities. BSL-1 organisms normally do not cause disease in healthy humans (e.g. *Penicillium*); BSL-2 agents are associated with human disease (e.g. *Cryptococcus neoformans*, *Shigella*, any human body fluid, etc.); BSL-3 agents may cause serious or possibly lethal disease, with a potential for aerosol transmission (e.g. HIV, Yellow fever virus, etc.); BSL-4 agents pose a high risk of aerosol transmitted laboratory infections and life-threatening disease (e.g. Ebola virus). Please note that Level 4 organisms are not permitted in the College and Level 3 organisms may not be brought onto College property without prior written permission from the Dean and CNSM Safety Office personnel. The CDC/NIH Guidelines are available for review in the CNSM Safety Office (Micro 001) and are available on-line (http://www.cdc.gov/OD/OHS/biosfty/bmb15/BMBL_5th_Edition.pdf).

The purpose of the CDC/NIH Guidelines is to protect students, employees and the general public from exposure to biohazardous materials. If you work with biohazardous materials, you should ensure that everyone in your program is trained and that the CDC/NIH Guidelines will be observed as appropriate. For example, the **SAFE WORK PRACTICES** listed below must be consistently followed to reduce the likelihood of exposure when using biohazardous agents:

- Avoid hand to face contact, and don't use sharp items (needles, razor blades etc.) unless you must.
- Handle needles & sharps (pasteur pipets, slides, capillary tubes, broken glass, etc.) carefully.
- Use engineered sharps protection (needle w/protective device attached) when drawing human blood.
- Dispose of sharp items in red ("Medical waste") needle boxes if the sharps are biohazardous, contaminated with human blood or blood products, or were used in research involving the treatment or immunization of human beings or animals. Use beige needle boxes for all other sharps. Contact the **CNSM Safety Office (x55623)** to obtain free sharps containers or to arrange for container disposal.
- Use rigid plastic disposal containers for sharps; never use bags.
- Never bend or break needles.
- Never recap needles if at all possible; store syringe needle side down in test tube instead. If you must recap the needle, place cap in a container (ex. Styrofoam), open end up, then with ONE HAND place the needle into the cap. NEVER use two hands to recap, you might stick yourself!
- Wash hands after handling biohazardous materials, even when gloves were worn
- Develop and use a method of decontamination based on surfaces and type of contamination e.g. wipe benches down before and after use with a 5% bleach solution.
- Employ **Universal Precautions**: treat all human body fluids as infectious for HIV (see "Special Biohazards" on next page for more information).

ENGINEERING CONTROLS must be used whenever appropriate; examples include biological cabinets, mechanical barriers, needle boxes, engineered sharps protection on needles etc. If a biological cabinet is required per the CDC/NIH guidelines, it must be certified according to OSHA's Title 8, CCR 5154.1(a).

Ensure that everyone concerned uses **PERSONAL PROTECTIVE EQUIPMENT (PPE)** when needed to shield skin, clothing and mucous membranes from contact with infectious materials. The PPE must be appropriate and fit properly; consider:

- types of fluid or tissue involved
- potential exposure volume
- probable route of exposure e.g. eyes via splash; if the potential for a splash to the eye exists, properly fitting and fully enclosed, indirect vented chemical splash goggles must be worn
- working conditions e.g. aerosol production might require biological cabinet use

Biohazardous waste produced in a teaching or research lab cannot legally be treated and disposed of as regular trash on the premises. The waste shall be placed in a leak-proof container that is double-lined with

red biohazard bags. CNSM safety will provide the container and bags. Call the CNSM Safety Office for the appropriate container.

Biohazardous waste—As defined in the California Health and Safety Code section 117635 is:

Laboratory waste, including, but not limited to, the following:

Cultures and stocks of *infectious agents* from research and industrial laboratories. Wastes from the production of bacteria, viruses, spores... and [contaminated] culture dishes and devices used to transfer, inoculate, and mix cultures.

These regulations define “*Infectious agents*” to include any microorganism, bacteria, mold, parasite, or virus, including, but not limited to, organisms managed as **Biosafety Level 2 (BSL2)**, 3 or 4. The Chief of the Medical Waste Management Program at the California Department of Public Health has concurred with this definition.

Some of the cultures we work with in microbiology, mycology, molecular biology, biochemistry and research labs are at BSL 2 level.

Remember, NEVER put sharps in trash bags of any kind; always use rigid containers such as cardboard containers or the **free** sharps containers provided by the CNSM Safety Office.

HOUSEKEEPING is another important issue for biohazard areas - keep your area clean. OSHA's general sanitation laws in Title 8, section 3362, state that the workplace must be clean and sanitary, and be in a condition not liable to give rise to harmful exposure. Make sure corridors and eyewash/shower units are not blocked.

SPECIAL BIOHAZARDS:

MEDICAL WASTE: If you or those you supervise **immunize animals or work with human tissues or human blood-derived products, you produce medical waste.** If you perform research pertaining to the diagnosis, treatment or immunization of humans or animals, you are probably producing Medical Waste. Medical waste may NOT be autoclaved and/or disposed of on campus property. The regulations for the collection and disposal of medical waste are quite stringent; improper handling could result in serious fines from the City of Long Beach. **Please call the safety office immediately (x55623) if you think you might have medical waste.** We will set up your program for you, and supply you with all the necessary information and free medical waste bags, collection containers, etc. We will also coordinate the waste pick-up and disposal for you.

BLOODBORNE PATHOGENS: If you or those you supervise (including students) **work with any human tissue or fluid - except urine, saliva or cheek cells - your work is regulated by the Cal/OSHA bloodborne pathogen standard.** Improper handling could result in serious fines from the city of Long Beach. Please call the safety office immediately (x55623) if you think your work might fall under the bloodborne pathogen standard.