|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| https://brandp.ecu.edu/wp-content/pv-uploads/sites/67/2017/09/ECU_lockup_stacked_alt_Purple-1.jpg | | Office of Prospective Health/Biological Safety REGISTRATION FOR THE USE OF BIOHAZARDOUS AGENT  **\*\*\*Version 1/25/2023\*\*\*** NOTE: This registration is project/biohazardous agent-specific. Use of a new/different agent, manipulation, or research protocol will require submission of a new registration. | | | | | | | | | |
| Biological Safety Registration #:       (assigned by Biological Safety) Date approved: | | | | | | | | | | | |
|  | | | | | |  | | | | | |
| **Principal Investigator:** | | | |  | | | |  | Date: |  | |
|  | | | |  | | | | | | | |
| **Department:** |  | | | | | | |  | Phone: |  | |
|  | | | |  | | | | | | | |
| **Laboratory Location(s):** | | | | |  | | |  | Building: | |  |
|  | | | | |  | | |  |  | |  |
| Renewal: | | | Yes  No | | | | If yes, approval dates: Are there any changes?  Yes  No | | | | |
|  | | |  | | | |  | | | | |
| AUP #: | | | IRB #: | | | |  | | | | |
| \*\*\*\*Please attach a summary of changes from previous registration and highlight the changes in this current registration.\*\*\*\*\* | | | | | | | | | | | |

**I. PROJECT TITLE / AGENTS** (Principal Investigator, please complete pages 1-8)

Project Title:

1. What materials or agents will be used? (Check all that apply)?:

|  |
| --- |
|  |
| Recombinant DNA or RNA use, genetic recombinant techniques, complete molecules, or organisms (If checked complete Appendix A) | |  | Infectious Agents; bacteria, viruses, fungal, prions, etc. |
| Transgenic organisms, plants, animals?  (Complete Appendix A) | |  | Biotoxins, allergens, or other Biohazardous  Material (Complete Appendix E, if applicable) |
| Viral or host systems Vectors (specify **)**  Complete Appendix A | |  | Human/Non-Human Primate blood, serum, fluid |
|  | |  | Human/Non-Human Primate tissue or cell lines |
|  | |  | NOTE: All human blood/tissue, or cell cultures are to be potentially infectious for bloodborne pathogens regardless of ATCC designation |

B. Is this agent/vector/material potentially infectious or hazardous to humans?  Yes  No

If yes, by what exposure routes?  Injection  Ingestion  Contact  Inhalation

C. Is this agent/vector/material a potential hazard to the environment, agriculture, wildlife, or other plants or animals?

Yes  No

D. Will this work use a Select Agent as defined in Biosafety Manual or CDC web site (most up-to-date)?

Yes  No

E. Status of Project:  Contingent on funding, notification date  Contingent on other (specify)

Will begin, date        Ongoing  Materials or agents ordered  Yes  No

**II. PROTOCOL**

A. Provide a brief overview or “lay abstract” of the proposed research containing sufficient information to ensure adequate review of the protocol by the East Carolina University Biological Safety Program, and determination of compliance with State, and Federal Regulations. Please include the goals of the research and the procedures and materials used to achieve these goals. This form may be reviewed by people outside of your areas of research so please use language that is easy to understand.

**Insert Abstract:**

* 1. What techniques, manipulations or handling of infectious agents, or human tissue, or recombinant genetic materials will be performed? The specific of the procedures should be described here or can be attached in a separate SOP. Please be sure to included safety precautions (for example, all cell-culture will be done in a biosafety cabinet).
  2. What is the source of the biological agent, select agent, or recombinant genetic material, organisms or molecules? How will this material be obtained by your lab?
  3. Where will work be conducted? If more than one room or lab is used, what material is used at each room/lab? (Specify transfer methods and containment if biohazardous material is moved between rooms)
  4. How will the infectious or biohazardous material and waste be treated or inactivated before disposal? This should include disinfectant use and contact time. If waste is autoclaved, please include time and temp.

(Located on page 6 see guidelines in Laboratory Safety Plan template or Biosafety manual <http://www.ecu.edu/cs-dhs/prospectivehealth/Biological-Safety-Office-of-Prospective-Health.cfm>)

* 1. Will experimental animals be exposed?  Yes  No If yes, what species?

By what means? e.g.?  Injection  Ingestion  Topical Application  Inhalation

Physical containment level requested based on *Biosafety in Microbiological and Biomedical Laboratories 6th Edition 2020* and/or the *NIH 2019 Guidelines for Research Involving Recombinant DNA Molecules:*. Consult Biological Safety manual or web site for more information (<http://www.ecu.edu/cs-dhs/prospectivehealth/Biological-Safety-Office-of-Prospective-Health.cfm>). Appendix B summarizes the 2020 BMBL basics.

**Biosafety Level (1-4):       or Animal Biosafety Level (1-4):**

1. Will the material used be subject to any of the following aerosol generating procedures?

Flow Cytometry/ Sonicated  Yes  No

Cell sorter  Yes  No Aerosolized  Yes  No

Centrifuged  Yes  No Aspirated  Yes  No

**(All of these activities may create aerosols)**

If “Yes”, containment procedures must be used to limit aerosol spread:

Specify all containment precautions planned.

Biological Safety Cabinet

Sealed rotor or safety centrifuge? (Give details**)**

Microcentrifuge used in Biological Safety Cabinet

Other: (**)**

If Flow Cytometry/Cell sorter being used, are cells fixed  Yes  No

**If Flow Cytometry/Cell sorter being used, complete Appendix C on pages 21-22.**

C. If a Biological Safety Cabinet will be used, will it be used:

1. Only for the specific aerosol-producing procedures indicated in “B” above?  Yes  No

Specify:

OR

2. a. For **all** manipulations of agents or materials?

(Opening of culture plates may create aerosol)  Yes  No

b. For specific agent manipulation only (e.g., weighing or mixing of lyophilized toxin).

Yes  No

Specify:

3. For animal use:

a. For **all** manipulations of infectious, biohazard-exposed animals?  Yes  No

b. For a specific animal procedure?  Yes  No Specify procedure:

**D.**

1. What personal protective equipment will be used during your work?

Gloves  Aprons

Gowns  Shoe Covers

Lab Coats\*  N-95 Particle Respirator (annual fit test required)

Surgical Face Mask or Face Shield, Eye Protection  Other Respirator:

Ear Protection

Scrub suits  Other:

**\*Lab Coats will not be worn outside of the lab if used as protective apparel; laundering will be provided**

2. Will sharp needles, scalpels, or other sharp instruments be used in the work?  Yes  No

If yes, use safety sharps available in medical storeroom.

Or if safety sharps cannot be used for this work, justify why they cannot be used:

3. a. Will human blood, serum, body fluids or unfixed tissue be used in the work? (This is considered human tissue and triggers BL-2 precautions)  Yes  No

b. Will human cell lines be used in the work? (This is considered human tissue and triggers BL-2 precautions)

Yes  No

c. Will HIV be cultured or propagated in the work?  Yes  No

If yes, are eye wash stations available?  Yes  No

**IF YES TO ANY OF THE ABOVE IN 3**

Have all workers and students received the OSHA required Blood Borne Pathogens Training and/or annual refresher training in the past 12 months?

Yes  No

Are all workers and students immune to Hepatitis B or signed an OSHA-declination form?

Yes  No

The NIH Guidelines specify practices for constructing and handling recombinant DNA or RNA molecules, organisms or viruses containing recombinant molecules, including transgenic animal and knock-out/-in animals. NIH defines *Recombinant DNA molecules* BROADLY to include molecules constructed outside living cells creation of natural or synthetic DNA segments or molecules, or use of host-vector systems, synthetic genomics or other genetic techniques. The NIH Guidelines apply to all research conducted at ECU regardless of funding source. See full NIH Guidelines on our web site and applicable categories in appendix to this form.

**E.** Will this work involve recombinant DNA or recombinant genetic techniques as broadly defined by NIH?  Yes  No

**If Yes: Complete the questions below AND Appendix A**

1. Does this project involve the deliberate transfer of a **drug resistance** trait to microorganisms that are not known to acquire the trait naturally? (NIH Section III-A)  Yes  No
2. Does this project involve the deliberate formation of recombinant DNA containing genes for the biosynthesis of **toxin molecules** lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight kilogram body weight? (NIH Section III-B)  Yes  No
3. Does this project involve experiments using Risk Group 2, Risk Group 3, or Risk Group 4 agents (bacteria, virus, or lower eukaryote)? (NIH Section III-D)  Yes  No
4. Does this project involve the formation of Recombinant DNA molecules containing **no** **more than two-thirds of the** **Genome** of any Eukaryotic Virus? (NIH Section III-E)  Yes  No
5. Does this project involve experiments using whole animals in which the animal's genome has been altered by stable introduction of recombinant DNA into the germ-line (**transgenic animals**)?  Yes  No

6. Does this project involve experiments involving viable recombinant DNA-modified microorganisms tested on whole animals at BSL-2 or higher? (NIH Section III-D)  Yes  No

7. Does this project involve experiments to **genetically engineer plants** by recombinant DNA methods, or to use plants together with microorganisms or insects containing recombinant DNA?

Yes  No

8. Does this project involve experiments involving **more than 10 liters of culture**? (NIH Section III-D)

Yes  No

9. “**Experiments of Concern**” "Recent concerns about bioterrorism and potential dual use technologies have prompted the federal government to identify seven classes of experiments with potential for misuse. Does any of your research fall under one of the following categories?" Would the recombinant DNA work:

a. Demonstrate how to render a vaccine ineffective?  Yes  No

b. Confer resistance to therapeutically useful antibiotic or antiviral agent?  Yes  No

c. Enhance the virulence of a pathogen or render a non-pathogen virulent?  Yes  No

d. Increase transmissibility of a pathogen?  Yes  No

e. Alter (to expand) the host range of a pathogen?  Yes  No

f. Enable the evasion of diagnostic/detection modalities?  Yes  No

g. Enable the weaponization of a biological agent or toxin?  Yes  No

**III. TRAINING DOCUMENTATION**

A. Principal Investigator Training and Experience: Please describe your past training and experience in performing the research procedure described in your proposal:

List below all individuals who will be exposed to or handle biohazardous agents in your work:

Have the individuals listed been trained and demonstrated proficiency on use of the agents and procedure-specific\*\* laboratory safety practices and containment procedures and precautions for the Biosafety level requested? Agent and Procedure Specific Training is to be provided by the Principal Investigator, and should be documented in laboratory records, **Appendix D completed** and summarized below.

Short term students and professional visitors to the laboratory should not be exposed to biohazardous agents until they are trained in the laboratory procedures and familiarized with the safety plan of the laboratory. Non-essential visitors and minor children should not be allowed access to a laboratory or perform procedures which may expose them to infectious, or biohazardous agents.

Blood Borne Pathogens Training is required for all personnel handling human blood, body fluids, unfixed tissues or human cell lines (including short-term students and visitors). Immunization against Hepatitis B must be offered. (Bloodborne Pathogen Training provides a general overview of BL-2 precautions and is highly recommended for all work at BSL-2 or higher but must be supplemented with laboratory specific training.)

Immunization against other agents used may be offered if effective vaccine is available.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Name |  | Position | Agent and Procedure Specific Training completed and documented by PI to date | | BBP Training in past 12 months if “Yes” to D-3 | Faculty | | Staff | | Grad | | Under-grad | |
| **1.** |  |  |  |  | Yes **Date**       No | Yes  No  Unknown |  |  | |  | |  | |
| **2.** |  |  |  |  | Yes **Date**       No | Yes  No  Unknown |  |  | |  | |  | |
| **3.** |  |  |  |  | Yes **Date**       No | Yes  No  Unknown |  |  | |  | |  | |
| **4.** |  |  |  |  | Yes **Date**       No | Yes  No  Unknown |  |  | |  | |  | |
| **5.** |  |  |  |  | Yes **Date**       No | Yes  No  Unknown |  |  | |  | |  | |
| **6.** |  |  |  |  | Yes **Date**       No | Yes  No  Unknown |  |  | |  | |  | |
| **7.** |  |  |  |  | Yes **Date**       No | Yes  No  Unknown |  |  | |  | |  | |
| **8.** |  |  |  |  | Yes **Date**       No | Yes  No  Unknown |  |  | |  | |  | |
| **9.** |  |  |  |  | Yes **Date**       No | Yes  No  Unknown |  |  | |  | |  | |
| **10.** |  |  |  |  | Yes **Date**       No | Yes  No  Unknown |  |  | |  | |  | |

B. Will Comparative Medicine personnel be exposed to Biohazardous agents directly or indirectly?  Yes  No

If yes, specify

C. Serologic surveillance:

*Note*: Baseline sera will be collected and frozen for research at Biosafety Level 4; optional at BL-3, based on Risk Assessment.

**IV. Laboratory Safety Plan**

Sections A and B together constitute your laboratory Biosafety Manual. This manual should be maintained and accessible in your laboratory. It will be reviewed during Biological Safety inspections.

Section A. General guidelines (Check all that apply to customize for your project)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| 1. | | The entry door to the lab will be posted with the Biohazard Symbol, Biosafety Level, Agent, and Contact Information. BL-1 and above. | | | |
|  | |  | | | |
| 2. | | Specimens will be placed in biohazard labeled, well-constructed containers during, handling, processing, and storage. Sturdy leak proof closable secondary containers are used for transport or shipping to prevent leakage or spillage. Appropriate precautions will be used for transfers within or between buildings, using common hallways. | | | |
|  | |  | | | |
| 3. | | Water-resistant lab coats will be used if splashes of infectious fluids are anticipated. Masks and protective eyewear will be worn if mucous-membrane contact with blood or potentially infectious/biohazardous materials is anticipated. | | | |
|  | |  | | | |
| 4. | | Protective clothing will be removed before leaving the laboratory and stored in an area where it will not be a source of contamination for other people or their belongings. Recommended at BL-1/Required at BL-2. | | | |
|  | |  | | | |
| 5. | | Single-use, disposable gloves will be worn at all times when handling specimen or agent. Hands will be washed with a disinfectant soap after removing gloves or immediately if hand is contaminated. Gloves will be removed when exiting the lab and not worn in common areas outside the laboratory. Recommended at BL-1/Required at BL-2. | | | |
|  | |  | | | |
| 6. | | Pipetting or suctioning by mouth is forbidden. Pipetting performed to minimize aerosol creation. BL-1 and above. | | | |
|  | |  | | | |
| 7. | | Eating, drinking, smoking, applying cosmetics or lip balm, or handling contact lenses is prohibited in the work area. No food or drinks will be stored in areas where potentially infected materials are present. BL-1 and above. | | | |
|  | |  | | | |
| 8. | | All workers and students with contact with human blood, tissue in cell culture, or HIV will be instructed with regard to OSHA Blood Borne Pathogens and Standard Precautions prior to work, and at least annually thereafter. This training will be provided or documented by the Office of Prospective Health. | | | |
|  | |  | | | |
| 9. | | All human blood and body fluids or unfixed tissue or cell cultures will be considered to be potentially infectious. | | | |
|  | |  | | | |
| 10. | | The Principal Investigator will establish policies and procedures so that persons will be fully trained about the potential hazards and meet all specific entry requirements (training vaccination, etc.) before entering the lab or beginning work. BL-2 and above. | | | |
|  | |  | | | |
| 11. | | All workers and students will be instructed by the PI or designee regarding the Biosafety procedures required for the agent used and manipulation performed, e.g. BSL2 and BSL3 Training will be provided by PI prior to work and proficiency documented. Biosafety Training slides are available on the Prospective Health web site for the use of the Principal Investigator. BL-2 and above. | | | |
|  | |  | | | |
| 12. | | Class II (or higher) biological safety cabinets will be used whenever potential for aerosol generation exists. BL-2 and above. | | | |
|  | |  | | | |
| 13. | | Work surfaces will be decontaminated with an EPA approved disinfectant after completing work or at least daily, or after a splash or spill event. Large spills should be cleaned up before applying disinfectant to a surface. Appropriate personal protective equipment will be used during cleanup (BL-1 and above). **Please note: When bleach and water are mixed together to create a cleaning or disinfecting solution, the solution is only good for 24 hours.  After the 24 hours, solution begins to lose needed disinfecting properties. Therefore, we recommend the solution be made fresh daily.**  **SPECIFY** disinfectant(s) to be used:  1.10 Bleach Solution  Clidox  70% Ethanol  Other  **(If your disinfectant is not listed above, please attach information about its active ingredients and manufacturer’s recommended contact time here for committee review.)** | | | |
|  | |  | | | |
| 14. | | Use of needles is limited to situations where there is no alternative available. Safety needles or sharps will be used for all work unless incompatible with the procedure to be performed. Needles will not be bent, sheared, recapped or removed from the syringe following use intact needle and syringe will be disposed of into sharps container after use; other used sharps and broken glassware will be placed into approved sharps containers for disposal. BL-2 and above. | | | |
|  | |  | | | |
| 15. | | All concentrated cultures or stocks of biohazardous material will be decontaminated with an EPA approved disinfectant, (BL-1) or will be autoclaved prior to disposal in biohazardous waste containers. Contaminated re-usable equipment or heavily contaminated waste will be decontaminated or autoclaved before disposal. (BL-2 and above). | | | |
|  | |  | | | |
| 16. | | Procedure for Biological Spill: (Mark one) | | | |
|  | |  | | | |
| ***Spill involving a microorganism or material requiring BSL 1 containment:***   * Wear disposable gloves. * Use paper towels with or without detergent or soap solution to wipe up the visible spill * Clean spill area with fresh towels soaked in disinfectant allowing contact time per manufacturer’s instructions and then collect. * Place towels in biohazard bag for disposal. | | | |
|  | |  |  | | |
| ***Spill involving a microorganism or material requiring BSL 2 containment:***   * Alert people in immediate area of spill. * Put on additional protective equipment - *if needed to protect shoes, face eyes, etc.* * Cover spill with paper towels or other absorbent materials to stem its flow. * Avoid splashing. * Use paper towels with or without detergent or soap solution to wipe up the visible spill. * Allow a 20-min contact period if using prepared 1 in 10 dilution of household bleach or follow manufacturer’s directions for contact time if a commercial product is used. * Clean spill area with fresh towels soaked in disinfectant. * Place towels in a red biohazard bag. Decontaminate waste in an autoclave if heavily contaminated. | | | |
|  | |  |  | | |
| ***Spill involving a microorganism or material requiring BSL 3 containment:***   * Alert people in the laboratory of spill to evacuate immediately; unless spill is contained within Biological Safety Cabinet. Attend to any injured or contaminated persons and remove them from exposure. * Shut off any ongoing source of contamination spill or splash. * Close all doors to affected area and post a sign on the entry door “Spill, Do Not Enter”. * The laboratory **should not** be reentered to decontaminate and clean up the spill for at least 30 minutes appropriate respiratory protection is worn during cleanup. The aerosol will be removed from the laboratory air by the exhaust air ventilation system over a period of 1-6 hours depending upon rate of air change if there is not a continuing source of vapor generation. * Call Biological safety at 744-2070 (main), 744-3437 or 744-2237 (M-F, 8-5). Contact ECU Police 744-2247 to call Biological Safety after hours. | | | |
| Section B. Lab-Specific/Procedures. (Please attach any lab specific SOP’s here or copies of AUP procedure which may help us understand your work.)  1.  I have attached an additional separate sheet(s) with my procedure SOP(s).  2. Centrifuge procedure from page 46-47 and 123 – 126 of the Biosafety Manual will be copied and used,  <http://www.ecu.edu/cs-dhs/prospectivehealth/Biological-Safety-Office-of-Prospective-Health.cfm>  Without modification  With the following modifications:  Lab-specific centrifuge SOP attached.  3. Biological Safety Cabinet SOP from Biological Safety manual pages 41 – 45 and 96 – 104 will be copied and used,  <http://www.ecu.edu/cs-dhs/prospectivehealth/Biological-Safety-Office-of-Prospective-Health.cfm>  Without modification  With the following modifications:  Lab-specific Biological Safety Cabinet SOP attached.  4. Autoclave SOP from Biological Safety manual page 50 – 55 will be copied and used,  <http://www.ecu.edu/cs-dhs/prospectivehealth/Biological-Safety-Office-of-Prospective-Health.cfm>  Without modification  With the following modifications:  Lab-specific autoclave SOP attached. | | | |

|  |  |  |  |
| --- | --- | --- | --- |
| **As the Principal Investigator, I shall abide by the ECU Biological Safety Policy and this Laboratory Safety Plan. I will communicate this information to and enforce these practices with all workers and students under my direction and ensure that all personnel under my direction will participate in the Occupational Health Program as indicated.** **I agree to be responsible for the safe conduct of all personnel under my direct supervision.** | | | |
|  | | | |
| I understand that the East Carolina University Biological Safety Committee will review this registration form, and will approve or assign the Biological Safety Level to this project. I will notify the Office of Prospective Health/Biological Safety in writing of any changes in the information contained on this registration form; e.g. personnel changes, lab changes, new procedures. Addition of a new organism or biohazard may require submission of a new registration. | | | |
| I understand and will abide by the NIH guidelines, when recombinant DNA research (as broadly defined in Appendix A) is conducted, I also understand that any significant problems, violations of the NIH Guidelines or any significant research-related accidents or illnesses must be reported to NIH-OBA within 30 days. Accidental spills, loss of containment or personnel contamination incidents are conditions which could trigger reporting. I will report such incidents to Biological Safety as soon as recognized; Biological Safety will notify NIH.  Spills or accidents in laboratories operating at BSL-2 or higher which result in an overt exposure to any laboratory personnel must be reported immediately to NIH-OBA; I will report such incidents to Biological Safety immediately; Biological Safety will contact NIH. | | | |
|  | | | |
| Signed: |  | Date: |  | |
|  | Principal Investigator |  |
| **Please email a copy of this word document to**  [**Yvonne Taylor**](mailto:TAYLORY@ecu.edu) **(taylory@ecu.edu), Office of Prospective Health. Type in your name on the signature line; signature will be obtained after approval is complete.** | | | |

**To be completed by the Office of Prospective Health / Biological Safety**

Biological Safety Level for this Project

BSL 1  ABSL 1

BSL 2  ABSL 2

BSL 3  ABSL 3

BSL 4  ABSL 4

Select Agent?  Yes  No (Appendix D)

Serum Banking?  Yes  No

Serologic Surveillance?  Yes  No

Specific Immunizations?  Yes  No

If yes, specify\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Biosafety Hazard:

Infectious Agent  Human Blood, Tissue, Cells

Biotoxin  Transformed Cells

Allergen  Tumor Cells

Other

And/or NIH Use of Recombinant DNA (or RNA)

NIH Classification(s):

Drug resistance transfer or other III-A

Cloning toxin molecules, LD 50<100µg/kg; DNA transfer “restricted” or other III B

Human gene transfer or other, III-C

Any BL-2 work or other, III-D  Work <BL-2 or other, III - E

Purchase or transfer transgenic rodents for BL-1 work or other III-F  Create transgenic animals or test modified organisms on whole animals for BL-1 work III-E

Other NIH Exempt III-F

Other

(See Appendix for more information on NIH categories)

Laboratory Inspected?  Yes  No

If yes, date inspected

Satisfactory ****Unsatisfactory

**Biosafety Committee Chair Approval: Signed: Date:**

Biosafety Committee Chair

**Biosafety Registration #: Signed: Date:**

Biosafety Officer

**Appendix A**

**rDNA Registration Form**

**See page 21 for details of NIH Classification system**

|  |  |  |  |
| --- | --- | --- | --- |
| 1. | The proposed experiments with recombinant DNA molecules are (check one): | | |
| Exempt under the NIH Guidelines  (NIH Section III-F) | | Non-Exempt according to the NIH Guideline  (See attached Appendix NIH categories III-A, III-B, III-C, III-D, III-E descriptions) |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| 2. | If you checked “Exempt” in question 1, indicate under which criteria the experiments are exempt by marking the unshaded boxes. | | | |
|  | a. | | The experiments involve rDNA molecules that are not in organisms or viruses (Section III-F-1 of the NIH Guidelines). | |
|  | b. | | The experiments involve rDNA molecules that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent (Section III-F-2 of the NIH Guidelines). | |
|  | c. | | The experiments involve rDNA molecules that consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well established physiological means (Section II-F-3 of the NIH Guidelines). | |
|  | d. | | The experiments involve rDNA molecules that consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species) (Section III-F-4 of the NIH Guidelines). | |
|  | e. | | The experiments involve rDNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent (Section III-F-5 of the NIH Guidelines). | |
|  | f. | | The experiments involve rDNA molecules that do not present a significant risk to health or the environment as determined by the NIH Director (Section III-F-6 of the NIH Guidelines). | |
|  |  | f-1 | | Recombinant DNA in Tissue Culture. Experiments involve rDNA molecules containing less than one-half of any eukaryotic viral genome (all viruses from a single family being considered identical), that are propagated and maintained in cells in tissue culture (Appendix C-I-A of the NIH Guidelines). | |
|  |  | f-2 | | *Escherichia coli K-12* Host-Vector Systems. Experiments which use *E. coli* K-12 host-vector systems, with the exception of those experiments listed in Appendix C-II-A of the NIH Guidelines, are exempt provided that: (i) the *E. coli* host does not contain conjugation proficient plasmids or generalized transducing phages; or (ii) lambda or lambdoid or Ff bacteriophages or non-conjugative plasmids shall be used as vectors. Experiments involving the insertion into *E. coli* K-12 of DNA from prokaryotes that exchange genetic information with *E. coli* may be performed with any *E. coli* K-12 vector (e.g., conjugative plasmid). | |
|  |  | f-3 | | *Saccharomyces* Host-Vector Systems. Experiments involving *Saccharomyces cerevisiae* and *Saccharomyces uvarum* host-vector systems are exempt from the *NIH Guidelines*, with the exception of experiments listed in Appendix C-III-A, | |
|  |  | f-4 | | *Bacillus subtilis* or *Bacillus licheniformis* Host-Vector Systems. Any asporogenic *Bacillus subtilis* or asporogenic *Bacillus licheniformis* strain which does not revert to a sporeformer with a frequency greater than 10-7 may be used for cloning DNA with the exception of those experiments listed in Appendix C-IV-A of the NIH Guidelines. | |
|  |  | f-5 | | Extrachromosomal Elements of Gram Positive Organisms Recombinant DNA molecules derived entirely from extrachromosomal elements of the organisms listed in Appendix C-V of the NIH Guidelines (including shuttle vectors constructed from vectors described in Appendix C), propagated and maintained in organisms listed in those Guidelines are exempt. | |
|  |  | f-6 | | Purchase or Transfer of Transgenic Rodents. The purchase or transfer of transgenic rodents for experiments requiring Biosafety Level 1 (BSL1) containment are exempt. (Appendix GIII- M of the NIH Guidelines). | |

**SECTION A**

Biosafety Information

|  |  |  |
| --- | --- | --- |
| 3. | Indicate the risk groups (or class) of all material(s) used in the recombinant DNA experiments in this project by marking the un-shaded box. | |
|  | Risk Group 1 | Agents are *Not* associated with disease in healthy adult humans. |
|  | Risk Group 2 | **Agents are associated with human disease that is rarely serious.**  There are often preventive or therapeutic interventions available. |
|  | Risk Group 3 | Agents are associated with serious or lethal human disease for which preventive or therapeutic interventions *MAY* be available. |
|  | Risk Group 4 | **Agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *NOT* *USUALLY* available.** |

|  |  |  |
| --- | --- | --- |
| 4. | Indicate the biosafety level(s) at which the recombinant DNA work will be performed for this project. | |
|  | BSL-1 | Low risk agents (generally risk group 1), special containment equipment not required   * Work is done on open bench tops. * Biohazard signs must be posted. * Standard microbiological practices are observed Biohazard signs should be posted. |
|  | BSL-2 | Moderate risk agents (generally risk group 2), biosafety cabinets, restrictions to research areas. All BSL-1 containment and practices plus the following:   * Laboratory access is restricted when experimental work is in progress. * Personnel have specific training in handling of agents.   Biological safety cabinets (BSC) or other physical containment devices are used for potential aerosol generation procedures.   * Biohazard signs must be posted. * Specific PPE (personnel protective equipment) and entrance requirements. |
|  | BSL-3 | High risk agents (generally risk group 3), BSL-3 containment facilities, and practices. All BSL-2 containment and practices plus the following:   * Laboratory access is restricted. * Personnel have specific training in handling of agents. * All procedures are performed in biological safety cabinets (BSC). * Biohazard signs must be posted. * Written safety policies provided by the investigator defining laboratory procedures, waste disposal, disinfection and medical surveillance. * Centrifuge safety cups must be used. * Specific facility design parameters must be followed, including requirements for location, ventilation, room integrity and security. |

|  |  |
| --- | --- |
| 5. | Is the highest proposed Biosafety Level lower than the risk group classification for any of the described agents? (e.g. Use of a single gene from a risk group 3 organism may qualify for work at BL-1 or BL-2). Answer Yes or No in the box below. If “Yes, explain the rationale for the use of the lower Biosafety Level. |
|  | |

|  |  |
| --- | --- |
| 6. | Describe the potential biosafety risks of this research proposal. Address the following:  Whether agent(s) may be infectious to humans.  The risks of accidental exposure to personnel.  Whether there is potential for airborne transmission of agent(s).  Precautions to be taken by personnel including any personal protective equipment and/or routine monitoring.  Disposal of agent(s). Describe the specific methods of disposal or inactivation of the agent(s) or contaminated/infectious material(s). |
|  | |

|  |  |
| --- | --- |
| 7. | Describe the Principal Investigator’s experience with all vectors, viruses, organisms, or recombinant DNA materials described in this application |
|  | |

**SECTION B**

Vectors, Hosts, and rDNA Agents: (Maybe NIH Section III-A, III-C, III-D or III-E)

|  |  |
| --- | --- |
| 8. | List all Plasmid and Phage Vectors Used: |
|  | |

|  |  |
| --- | --- |
| 9. | List inserted DNA used. Include the species from which the insert is derived and what gene product is expressed. If no inserts are used, state “None.” |
|  | |

|  |  |
| --- | --- |
| 10. | List known oncogenes, or inserts from above that have oncogenic properties. If none are used, state “None.” |
|  | |

|  |  |
| --- | --- |
| 11. | List host organisms for foreign DNA sequences (e.g. E. coli, S. cerevisiae, fungi, mammalian cells or cell lines). Give any pertinent details. |
|  | |

|  |  |
| --- | --- |
| 12. | List any oligonucleotides used to manipulate gene function (e.g. siRNA) or as adjuvants (e.g. CpG-containing DNA) either in cell culture or in vivo. If none are used, state “None.” |
|  | |

|  |  |
| --- | --- |
| 13. | Are toxins to be expressed and released as part of this research? Answer Yes or No in the box below. If “Yes,” describe the toxic product(s) (including the LD50) that could be produced or released and the containment precautions to be used. |
|  | |

|  |  |
| --- | --- |
| 14. | Is there any potential for increased virulence with insertion of DNA into the vector or organism? Answer Yes or No in the box below. If “Yes,” explain in detail. |
|  | |

**SECTION C**

Viruses and virus Vectors: Maybe NIH Section III-C, III-D, III-E

|  |  |  |
| --- | --- | --- |
| 15. | Does this project involve the use of viruses or viral vectors?   * If “No,” skip questions 16-26, and go directly to Section D (question 27). * If the Virus or viral vector is a lentivirus (e.g. FIV, HIV, SIV, etc) complete questions 16 and 21-26. * For all other Viruses or viral vectors, complete questions 16-20. | |
|  | Yes |
|  | No |

|  |  |
| --- | --- |
| 16. | List viruses and/or viral vectors used:  Specify the virus family and/or subfamily (e.g. herpesvirus, oncogenic retrovirus, adenovirus, adeno-associated virus, etc).  State the species of origin for each virus or vector used. |
|  | |

|  |  |  |
| --- | --- | --- |
| 17. | Is the virus/viral vector able to enter or infect human cells? | |
|  | Yes |
|  | No |
|  | If “Yes”, indicate whether it is a productive or limited infection, and state whether infection can cause disease. | |
|  | | |

|  |  |  |
| --- | --- | --- |
| 18. | Is a helper virus used in this project? | |
|  | Yes |
|  | No |
|  | If “Yes”, describe the helper virus used. | |
|  | | |

|  |  |  |
| --- | --- | --- |
| 19. | Is the virus/viral vector replication-defective? | |
|  | Yes |
|  | No |
|  | If “No”, skip questions 20-26, and go directly to Section D; question 27 (unless also using Lentivirus/Lentiviral Vectors).  If “Yes”, describe the deletions rendering it defective and complete question 20. | |
|  | | |

|  |  |  |
| --- | --- | --- |
| 20. | Has the preparation of replication-defective vectors been tested for the presence of replication competent virus? | |
|  | Yes |
|  | No |
|  | If “Yes”, provide details of the assay used.  If “No”, what is the likelihood of conversion to replication-competent virus? | |
|  | | |

**Lentiviruses and Lentiviral Vectors (III-C or III-D)**

|  |  |
| --- | --- |
| 21. | List the specific virus or strain and species of origin (e.g. HIV, human; FIV, feline). |
|  | |

|  |  |  |
| --- | --- | --- |
| 22. | Is the lentivirus/lentiviral vector obtained from a commercial source? | |
|  | Yes |
|  | No |
|  | If “Yes”, provide the name of the commercial source.  If “No”, provide the source of the lentivirus/lentiviral vector (e.g. the name of the institution or individual supplying the material). | |
|  | | |

|  |  |  |
| --- | --- | --- |
| 23. | Is the lentivirus/lentiviral vector generated from a multi-component system? (e.g. separate plasmids for packaging, envelope and gene transfer) | |
|  | Yes |
|  | No |
|  | If “Yes”, describe the system used. | |
|  | | |

|  |  |  |
| --- | --- | --- |
| 24. | Is the lentivirus/lentiviral vector pseudotyped (e.g. expressing a different envelope gene)? | |
|  | Yes |
|  | No |
|  | If “Yes”, provide whether the pseudotyping alters the host and cell tropism. | |
|  | | |

|  |  |  |
| --- | --- | --- |
| 25. | Is the lentivirus/lentiviral vector replication-defective? Answer Yes or No in the box below. | |
|  | Yes |
|  | No |
|  | If “Yes”, describe the deletions rendering it defective and complete question 26.  If “No”, skip to Section D (Question 27). | |
|  | | |

|  |  |  |
| --- | --- | --- |
| 26. | Has the preparation of replication-defective vector been tested for the presence of replication-competent virus? | |
|  | Yes |
|  | No |
|  | If “Yes”, provide details of the assay used.  If “No”, what is the likelihood of conversion to replication-competent virus? | |
|  | | |

**SECTION D**

Animal Use Information: Maybe NIH Section III-C, III-D, III-E or III-F

|  |  |  |
| --- | --- | --- |
| 27. | Does the work involve animal use? | |
|  | Yes |
|  | No |
|  | If “No”, skip to Section E | |

|  |  |  |
| --- | --- | --- |
| 28. | Has an Institutional Animal Care and Use Committee (IACUC) application been submitted? | |
|  | Yes |
|  | No |
|  | If “Yes”, provide the IACUC protocol number to be linked to this rDNA project.  NOTE: REGISTRATION OF THE PROTOCOL WITH AND APPROVAL BY THE IACUC IS REQUIRED BEFORE THE RESEARCH CAN BE INITIATED. | |
|  | | |

|  |  |  |
| --- | --- | --- |
| 29. | Will animal tissues or cells be used in vitro? | |
|  | Yes |
|  | No |
|  | If “Yes”, explain | |
|  | | |

|  |  |  |
| --- | --- | --- |
| 30. | Will transgenic or gene targeted animals be used? | |
|  | Yes |
|  | No |
|  | If “Yes”, explain | |
|  | | |

|  |  |  |
| --- | --- | --- |
| 31. | Will recombinant agents be administered to live or intact animals? (viral vectors, transfected cells, plasmids)? Answer Yes or No in the box below. | |
|  | Yes |
|  | No |
|  | | |

|  |  |  |
| --- | --- | --- |
| 32. | Do you anticipate that work with animal subjects will be conducted at a different BSL than the *in vitro* portions of the study? | |
|  | Yes |
|  | No |
|  | If “Yes”, provide the BSL for work with or housing of animal subjects, and explain the rationale or justification for the proposed BSL. | |
|  | | |

|  |  |
| --- | --- |
| 33. | Provide the animal species (and strain if applicable) receiving the recombinant DNA material; list each species to be used. |
|  | |

|  |  |
| --- | --- |
| 34. | Describe the route of administration for each recombinant agent used. |
|  | |

|  |  |
| --- | --- |
| 35. | Provide the concentration and volume for each recombinant agent to be administered |
|  | |
| 36. | Will transgenic or knock-in/knock-out colonies be bred or maintained at ECU? |
|  | Yes |
|  | No |

|  |  |
| --- | --- |
| 37. | Will outcrosses of homo-or heterozygote animals be performed? Will transgenic animals be crossed with wild type animals? |
|  | Yes |
|  | No |

For rodents only:

|  |  |
| --- | --- |
| 38. | A. Will either parent contain more than one-half of the genome of an exogenous eukaryotic virus from a single family of viruses or a transgene that is under the control of a gammaretroviral long terminal repeat (LTR)? |
|  | Yes |
|  | No |

|  |  |
| --- | --- |
|  | B. Is the progeny expected to contain more than one-half of an exogenous viral genome from a single family of viruses? |
|  | Yes |
|  | No |

**SECTION E**

Plants

|  |  |
| --- | --- |
| 39. | Will transgenic plants be used or created? |
|  | Yes |
|  | No |

**SECTION F**

Human Use Information: (May be NIH Category III-A, III-B, III-C or III-D)

|  |  |  |
| --- | --- | --- |
| 40. | Does work involve human cell lines (including cell lines such as 293T, HeLa)? | |
|  | Yes |
|  | No |
|  | If “Yes”, list below | |
|  | | |

|  |  |  |
| --- | --- | --- |
| 41. | Will human tissues or primary cells be used *in vitro*? | |
|  | Yes |
|  | No |
|  | If “Yes”, describe the use of the tissues or cells | |
|  | | |

|  |  |  |
| --- | --- | --- |
| 42. | Is this a gene transfer proposal (recombinant materials administered to human subjects)? | |
|  | Yes |
|  | No |
|  | If “Yes”, you must submit a detailed addendum in which each topic of Appendix M in the NIH Guidelines for Research involving Recombinant DNA Molecules is addressed. PATIENT CONSENT FORMS AND PROOF OF SUBMISSION OF PROPOSAL TO NIH MUST ALSO BE SUBMITTED. | |
|  | | |

|  |  |  |
| --- | --- | --- |
| 43. | Does this project involve the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into Human research participants (**Human Gene Transfer or Gene Therapy**)? | |
|  | Yes |
|  | No |
|  |  | |
|  | | |

|  |  |
| --- | --- |
| 44. | Has this study been IRB approved? |
|  | Yes Enter HSRRB Number: |
|  | No |

|  |  |  |
| --- | --- | --- |
| 45. | In what clinical facilities will treatment occur? Specify  Has the head nurse(s) on this unit been informed? | |
|  | Yes |
|  | No |
|  |  | |
|  | | |

|  |  |  |
| --- | --- | --- |
| 46. | Has a procedure or guidelines for handling the agent and any contaminated materials in this unit been developed? | |
|  | Yes Please attach. |
|  | No |
|  |  | |
|  | | |

|  |  |  |
| --- | --- | --- |
| 47. | Have the healthcare personnel involved been educated about the project, the agent and its handling? | |
|  | Yes |
|  | No |
|  | Attach outline of training content. Attach attendance sheet if/when education is delivered. | |
|  | | |

|  |  |  |
| --- | --- | --- |
| 48. | Will use or handling of the agent or care of patients occur at ECU Health? | |
|  | Yes |
|  | No |
|  | If yes, has it been reviewed by Vidant Health Safety and Infection Control? | |
|  | Yes | |
|  | No | |

Appendix B

**SUMMARY OF RECOMMENDED BIOSAFETY LEVELS FOR INFECTIOUS AGENTS**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Biosafety **Level** | **Agents** | **Practices** | **Safety Equipment**  **(Primary Barriers)** | **Facilities**  **(Secondary Barriers)** |
| 1 | Not known to  consistently cause  disease in healthy adults | Standard Microbiological Practices | None required | Open bench top sink required |
| 2 | Associated with human  disease, hazard =  percutaneous injury,  ingestion, mucous  membrane exposure | BSL-1 practice plus:  • Limited access  • Biohazard warning signs  • “Sharps” precautions  • Biosafety manual defining any  needed waste  decontamination or medical  surveillance policies | Primary barriers = Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; PPEs\*: laboratory coats; gloves; face protection as needed | BSL-1 plus:  Autoclave available |
| 3 | Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences | BSL-2 practice plus:  • Controlled access  • Decontamination of all waste  • Decontamination of lab clothing  before laundering  • Baseline serum | Primary barriers = Class I or II BCSs or other physical containment devices used for all open manipulations of agents;  PPEs\*: protective lab clothing; gloves; respiratory protection as needed | BSL-2 plus:  • Physical separation from  access corridors  • Self-closing, double-door  access  • Exhausted air not  recirculated  • Negative airflow into  laboratory |
| 4 | Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission | BSL-3 practices plus:  • Clothing change before  entering  • Shower on exit  • All material decontaminated on  exit from facility | Primary barriers = All procedures conducted in Class III BSCs or  Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit | BSL-3 plus:  • Separate building or  isolated zone  • Dedicated supply and  exhaust, vacuum, and  decon systems  • Other requirements  outlined in the text |

\*PPE-Personal Protective Equipment

**SUMMARY OF RECOMMENDED BIOSFAETY LEVELS FOR ACTIVITIES IN WHICH EXPERIMENTALLY OR NATURALLY INFECTED VERTBRATE ANIMALS ARE USED**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **ABSL** | **Agents** | **Practices** | **Primary Barriers and Safety Equipment** | **Facilities**  **(Secondary Barriers)** |
| 1 | Not known to  consistently cause  disease in healthy adults | Standard animal care and management practices, including appropriate medical surveillance programs | As required for normal care of each species | Standard animal facility   * No recirculation of exhaust air * Directional air flow recommended * Hand washing sink is available |
| 2 | * Associated with human disease * Hazard percutaneous exposure, ingestion, mucous membrane exposure | ABSL-1 practice plus:   * Limited access * Biohazard warning signs * “Sharps” precautions * Biosafety manual * Decontamination of all infectious wastes and of animal cages prior to washing | ABSL-1 equipment plus primary barriers:   * Containment equipment appropriate for animal species * PPEs\*: laboratory coats; gloves; face protection as needed | ABSL-1 plus:   * Autoclave available * Handwashing sink available * Mechanical cage washer recommended |
| 3 | * Indigenous or exotic agents with potential for aerosol transmission; * Disease may have serious health effects | ABSL-2 practice plus:   * Controlled access * Decontamination of clothing before laundering * Cages decontaminated before bedding removed * Disinfectant foot bath as needed | ABSL-2 equipment plus:   * Containment equipment for housing animals and cage dumping activities * Class I, II or III BSC’s available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols   PPEs\*:   * Appropriate respiratory protection | ABSL-2 facility plus:   * Physical separation from * access corridors * Self-closing, double-door * access * Sealed penetrations * Sealed windows * Autoclave available in facility |
| 4 | * Dangerous/exotic agents that pose high risk of life-threatening disease * Aerosol transmission, or related agents with unknown risk of transmission | ABSL-3 practices plus:   * Entrance through change room where personal clothing is removed and laboratory clothing is put on; shower on exiting. * All wastes are decontaminated before removal from the facility | ABSL-3 equipment plus:   * Maximum containment equipment (i.e., Class III BSC or partial containment equipment in combination with full body, air-supplied positive-pressure personnel suit) used for all procedures and activities | ABSL-3 plus:   * Separate building or isolated zone * Dedicated supply and exhaust, vacuum, and decontamination systems * Other requirements outlined in the text |

\*PPE-Personal Protective Equipment

**Classification Summary Page for *NIH Guidelines***

**Section III-A: Experiments that require Institutional Biosafety Committee (IBC) approval, Recombinant DNA Advisory**

**Committee (RAC) review, and NIH Director approval before initiation of experiments.**

Deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally, if such

acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine or agriculture.

**Section III-B: Experiments that require NIH/OBA and IBC approval before initiation.**

Deliberate formation of rDNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD50 of

less than 100 nanograms per kg body weight (e.g., microbial toxins such as tetanus toxin).

**Section III-C: Experiments that require IBC and Institutional Review Board (IRB) approvals, and NIH/OBA registration before**

**initiation.**

Experiments involving the deliberate transfer of (1) recombinant DNA or (2) DNA or RNA derived from recombinant DNA

into one or more human subjects.

**Section III-D: Experiments that require IBC approval before initiation of experiments.**

Experiments involving the introduction of recombinant DNA into Risk Group (RG) 2 or RG3 agents for use in animal

experiments or for modifying cells for use in animal experiments. Examples include gene transfer experiments using viral

vectors including adenoviral vectors, murine retrovirus vectors, or lentiviral vectors. Depending upon the details,

experiments with such agents may be conducted at BL1, BL2, or BL3. Experiments in which DNA from RG-4 agents is

transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment after

demonstration that only a totally and irreversibly defective fraction of the agent’s genome is present in a given recombinant.

**Section III-E: Experiments that require IBC notice simultaneously with initiation.**

Experiments involving the formation of rDNA molecules containing no more than 2/3 of the genome of any eukaryotic virus

(All viruses from a single Family being considered identical.)may be propagated and maintained in cells in tissue culture

using BL1 containment. Human cells used as host cells or used for production of viral vectors require BL2 containment. It

must be shown that the cells lack helper virus for the specific Families of defective viruses used. The DNA may contain

fragments of the genome of viruses from more than one Family but each fragment shall be less than two-thirds of a

genome.

**Section III-F: Experiments that are exempt from *NIH Guidelines.* However, registration with the IBC is required or status verified by Biological Safety from Animal use Protocol.**

III-D-4-c-1 Experiments involving the generation of transgenic rodents that will require BL1 for subsequent research use or status verified by Biological Safety from Animal Use Protocol.

III-D-4-c-2 The purchase or transfer of transgenic rodents. It is not required to register transgenic animals modified only

by gene knock-outs or status verified by Biological Safety from Animal Use Protocol.

III-F-1 Recombinant DNA molecules that are not in organisms or viruses.

III-F-2 Recombinant DNA molecules that consist entirely of DNA segments from a single nonchromosomal or viral

DNA source, though one or more of the segments may be a synthetic equivalent.

III-F-3 Recombinant DNA molecules that consist entirely of DNA from a prokaryotic host including its indigenous

plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or

when transferred to another host by well-established physiological means.

III-F-4 Recombinant DNA molecules that consist entirely of DNA from a eukaryotic host including its chloroplasts,

mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or closely related strain

of the same species).

III-F-5 Recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA

by known physiological processes, though one or more of the segments may be a synthetic equivalent. See

Appendix A-I through A-V of the “NIH Guidelines”.

III-F-6 Recombinant DNA experiments that do not present a significant risk to health or the environment as

determined by the NIH Director, RAC and following appropriate notice and opportunity for public comment.

See Appendix C of the NIH Guidelines.

**Appendix C**

**ECU Flow Cytometry Cell Sorting Questionnaire**

Laboratory Director (Principal Investigator)

Phone Number

E-mail

Investigator (Experimenter)

Phone Number

E-mail

Project Title

Biosafety Authorization Number

Project start date/end date

1. Summary of project: (Provide details of cells to be sorted)
2. Will the sample be fixed before submitting to the laboratory?

**** Yes **** No

**If yes,** describe the fixation protocol, fixative and concentration, and exposure time.

1. List type of sample and source.
   1. Circle or highlight one of the following as applicable:

Clinical sample (human) Cultured cells cell lines or other (ATCC # if applicable)

If it is a clinical sample, has it been screened for bloodborne pathogens?

**** Yes **** No

If yes, for which pathogens?

Could the sample contain other known human pathogens?

****Yes **** No

* 1. For cultured eukaryotic cell specimens, identify source (circle one)

Human Non-human primate Rodent Other:      (specify)

Have the cells been tested for mycoplasma and/or viral infection?

****Yes **** No

* 1. Were the cells transformed using any virus such as SV-40, EBV, CMV, HTLV-1, or *Herpes saimiri*? ****Yes **** No

**If yes**, describe method for determining that no live virus remains in the culture.

For prokaryote specimens, identify source (check one)

****Bacteria ****Fungi ****Parasite

Description (strain, species, subtype, etc): (with ATCC # if applicable)

1. Does the sample contain any other known human or animal infectious agent(s), viral vectors or recombinant DNA materials?

**** Yes **** No **If yes**, list agents or materials.

Has the infectious agent been inactivated or rendered non-infectious?

**** Yes **** No

**If yes**, describe method of inactivation.

1. Does the material express any known oncogenes?

**** Yes **** No

**If yes**, provide details

1. Were the cells otherwise genetically engineered or altered?

**** Yes **** No

**If yes**, describe how they were otherwise genetically engineered or altered.

1. Was a viral vector (adenovirus, retrovirus, lentivirus, herpes virus, etc.) used to transfer genetic information to the cells?

**** Yes **** No

**If yes**, describe method for determining that no live virus remains in the culture.

**If yes**, describe method in detail, attach vector map and show packaging cell line.

I have read and answered the above questions carefully and certify the information provided to be correct.

Signature (Principal Investigator) Date

*Biological Safety Officer Approval*

Signature (Biological Safety Officer) Date

**Appendix D**

**Lab Training Acknowledgement**

By signing below, I acknowledge that I have read the registration and understand the work being outlined, the proper biosafety procedures needed to complete the work, and have had training on relevant biosafety practices and procedures used in my lab work. I also understand that I can ask my supervisor for additional training at any point if I feel it is warranted.

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| --- | --- | --- |
| **Print** | **Sign** | **Date** |
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**Appendix E**

Biotoxin Use SOP

|  |  |  |  |
| --- | --- | --- | --- |
| **Principal Investigator:** |  | **Contact Info:** | Phone number, and email |

|  |  |  |
| --- | --- | --- |
| **Toxin name/class:** |  | |
|  |  | |
| **Total Quantity in the Lab:** | |  | **LD50:** |  |

*Work with biotoxins including rDNA WORK THAT ENCODES FOR TOXINS MUST BE APPROVED BY THE* [*IBC*](http://www.safety.duke.edu/BioSafety/ibc.htm)*.*

Any amount in any attenuation likely remains subject to U.S. export control and/or import compliance regulations.  Notify [ECUExportControls@ecu.edu](mailto:ECUExportControls@ecu.edu) if you intend to ship internationally.

1. **Procedure Description:**

|  |
| --- |
| Describe what the use is for including all *in vitro* and *in vivo* procedures, where they are being done, containment (a biosafety cabinet and/or fume hood is required), and work practice controls. Please provide references for procedures when a biotoxin is used. If applicable a “Warning Use of Toxins Do Not Enter” sign should be put on the door during use. Biosafety will also determine through a risk assessment if a buddy system needs to be in place for certain procedures. Additional information on toxin use, including use in animals, can be found in ECU’s Biosafety Manual located on the Office of Prospective Health’s website and the BMBL. |

1. **Potential Hazards:**

|  |
| --- |
| Describe the potential hazards for working with the biotoxin (e.g., routes of exposure) or attach Safety Data Sheet, and say “See attached SDS.” |

1. **Medical Considerations:**

|  |
| --- |
| Are there any pre-emptive or post-exposure treatments or vaccinations? If so, contact Prospective Health. In the event of an exposure what first-aid measures are taken? |

1. **Personal Protective Equipment (PPE):**

|  |
| --- |
| Describe PPE worn when handling the biotoxin:  At a minimum, lab personnel should wear safety glasses, suitable laboratory PPE to protect hands and arms (such as lab coats, smocks, or coveralls), and disposable nitrile gloves. |

1. **Transportation and Storage:**

|  |
| --- |
| Describe how it is transported and stored.   * Toxin containers must be leakproof and labeled with toxin name and hazard warnings at a minimum. * When toxins are stored in the lab, containers should be sealed, legibly labeled and secured to ensure restricted access. * Refrigerators and other storage containers containing biological toxins should be labeled with contact information for trained, responsible laboratory staff and locked for restricted access. * During transport toxins must be sealed in a leakproof biohazard labelled secondary container. * Consider using low-traffic halls for transport. |

1. **Biotoxin Inactivation and Waste Disposal:**

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| Describe how the biotoxin is inactivated.  Following ECU biosafety and EH&S guidelines please describe how both solid and liquid material and sharps that are potentially exposed to the toxin will be decontaminated and disposed. |

1. **Exposures/Unintended Contact:**

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| * Remove contaminated clothing and/or PPE and flush mucous membranes (eyes, nose, mouth) with water from the nearest eyewash or drench hose. Intact or non-intact skin exposures should be washed immediately with soap and water. * Refer to SDS for any specific procedures.   + Report any exposure immediately by calling the Office of Prospective Health 744-2070.   + Leave the area (for inhalation hazards).   + Change gloves (if gloves become contaminated). |

1. **Decontamination, Spills, and Emergency Procedures:**

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| *Decontamination* – the following information MUST be included (at least one method must be listed):   * Name and concentration of decontamination solution: * Time for effective decontamination: * Physical inactivation (temperature, time):   *Spill Procedures*   * For small spills of dilute solution (inside or outside BSC):   At minimum, safety glasses, lab coat, smock, or coveralls should be worn, along with appropriate gloves to clean up a spill. If splashing may occur, safety goggles and a face shield must be worn in place of safety glasses. Cover spill with paper towel or other disposable, absorbent material. Apply **(list decontamination solution and concentration here)** to the spill, beginning at the perimeter and working towards the center, and allow sufficient time **(enter time in minutes here)** to completely inactivate the toxin.   * For any spill of toxin powder or stock solutions:   Contact the Office of Prospective Health 744-2070 or EH&S at 328-6166.  *BSC/Fume Hood Failure*   * Close or cover all toxin containers. Shut down operations, close hood sash, and evacuate room. Contact the Office of Prospective Health 744-2070. |

**By signing below I certify that myself and my lab staff have read this appendix and been trained on the use of biotoxins in my lab.**

Principal Investigator Date

Biosafety Officer Date

**PermissIble Toxin Amounts** (any amount exceeding what is listed below becomes regulated by the CDC Division of Select Agents and Toxins).

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| **HHS Toxins [§73.3(d)(7)]** | **Amount** |
| Abrin | 1000 mg |
| Botulinum neurotoxins | 1 mg |
| Short, paralytic alpha conotoxins | 100 mg |
| Diacetoxyscirpenol (DAS) | 10,000 mg |
| Ricin | 1000 mg |
| Saxitoxin | 500 mg |
| Staphylcoccal Enterotoxins (Subtypes A, B, C, D, and E) | 100 mg |
| T-2 toxin | 10,000 mg |
| Tetrodotoxin | 500 mg |