Washington State University

Biosafety Approval Form (BAF)

**Section 1a: Basic contact information – BAF Administration**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Application status: | | | | | | | | | | ORSO # (if avail.): | | | XXXX | | |  | | For IBC use only – Do not mark in this area | | | | | | | |
| X | New Submission | | | | | | | |  | |  | | | | | | BAF #: | |  | | | | | |
|  |  | | | | | | | |  | |  | | | | | |  | |  | | | | | |
|  | Renew BAF # | | | |  | | |  | | | | | | | | | Received Date: | | | Version Date: | | | | |
|  |  | | | |  | | | |  | | | | | | | |  | |  | | | | |  |
|  | Amend BAF # | | | |  | | | If checked, highlight the changes. | | | | | | | | | | | | | | | | |
|  | | | |  | | | | | | | | | | | | | | | | | | | | | |
| Project Title: | | | | Dissecting mechanisms of disease during host-pathogen interactions | | | | | | | | | | | | | | | | | | | | | |
| Principal Investigator: | | | | | | | Sal Monella | | | | | | | | Title & WSU ID #: | | | | Assistant Professor, 11111111 | | | | | | | |
| Department: | | | | XXXX | | | | | | | | | | Building: | | | XXXX | | | | | Office# | | XXX-XXXX | | |
| Phone: | | | 5-XXXX | | | | | Fax: | |  | E-mail address: | | | | [XXXX@wsu.edu](mailto:XXXX@wsu.edu) | | | | | | | | | | | |
|  | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Co-Investigator: | | | | |  | | | | | | | | | | Title & WSU ID#: | | | |  | | | | | | | |
| Department: | | | |  | | | | | | | | | | Building: | | |  | | | | | Office# | |  | | |
| Phone: | | |  | | | | | Fax: | |  | E-mail address: | | | |  | | | | | | | | | | | |
|  | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lab contact: | | | | Alexander Yersin | | | | | | | | | | | Title & WSU ID #: | | | | Postdoctoral Fellow, 4444444 | | | | | | | |
| Department: | | | | XXXX | | | | | | | | | | Building: | | | XXXX | | | | | Lab # | XXX-XXXX | | | |
| Phone: | | | 5-XXXX | | | | | Fax: | |  | E-mail address: | | | | [XXXX@wsu.edu](mailto:XXXX@wsu.edu) | | | | | | | | | | | |

|  |
| --- |
| **Principal Investigator’s Certification:**  I certify that I have read the following statements and agree that I and all listed participants will abide by those statements:   1. Ensure that listed personnel have received or will receive appropriate training in safe laboratory practices and procedures for this project *before any work begins* and at least annually thereafter. Also, all listed personnel who have occupational exposure to bloodborne pathogens will be trained annually (EH&S provides this training). 2. Follow health surveillance practices as required in the biosafety laboratory manual and inform those working on the protocol about appropriate emergency assistance information for their location(s). 3. Inform EH&S (335- 3041) and the BSO of any research-related accident or illness as soon as possible after its occurrence. For USDA-ARS employees, contact Occupational Safety and Health Specialist (335-7766). Complete and submit a university incident report form. See [SPPM S25.20](https://policies.wsu.edu/prf/index/manuals/2-00-contents/2-24-reporting-accidental-injuries-work-related-illnesses/) for instructions on filling out this form; 4. Submit in writing a request for approval from the IBC of any significant modifications to the study, facilities, or procedures; and 5. Adhere to all applicable federal, state, local, and WSU regulations, guidelines, or contracts, as IBC approval does not supersede other regulations, guidelines, or contracts. |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Signatures\*: | | | | | | | | | | | | | |
|  | Principal Investigator: |  | | | | | | | | Date: |  | | | |
| \* Signatures are only necessary if not submitted from Principal Investigator’s WSU email | | | | | | | | | | | | | | |
| **For IBC use only:** | | | **IBC-BSL level approval** |  | **BSL-1** | |  | **BSL-1+** |  | **BSL-2** |  | **BSL-2+** |  | **BSL-3** |
| Do not mark in this area | | | **IBC-ABSL approval** |  | **ABSL-1** | |  | **ABSL-1+** |  | **ABSL-2** |  | **ABSL-2+** |  | **ABSL-3** |
|  | | | **IBC BSLP approval** |  | **BSL-1P** | |  | **BSL-1+P** |  | **BSL-2P** | | | | |
|  | | | **IBC Coordinator/BSO Signature:** | | |  | | | | | | | | |

**Section 1b: Basic contact information - Lab Personnel**

List all project personnel (PI, Associates, and technicians who will be involved in conducting the procedures or have access to the biological materials). This information is intended to inform the IBC of the training and background of key personnel. This is an IBC oversight activity required by the NIH Guidelines.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **NAME** | **WSU ID #** | **ROLE ON PROJECT**  **(e.g., PI, Graduate Student, Postdoctoral Scholar)** | **TRAINING & EXPERIENCE RELATED TO PROCEDURES PERFORMED** | | | | | |
| Sal Monella, PhD | 11111111 | **Principal Investigator (PI)** |  | **Beginner** |  | **Intermediate** | X | **Advanced** |
| Cam Pylobacter, BS | 22222222 | Technician |  | **Beginner** | X | **Intermediate** |  | **Advanced** |
| Steph Aureus, BS | 33333333 | Graduate student |  | **Beginner** |  | **Intermediate** | X | **Advanced** |
| Alexander Yersin, PhD | 44444444 | Postdoctoral Fellow |  | **Beginner** |  | **Intermediate** | X | **Advanced** |
|  |  |  |  | **Beginner** |  | **Intermediate** |  | **Advanced** |

Additional rows may be added with the TAB key

**Section 2: Description of research and facilities used (This section must be completed)**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **A.** | **In Lay Language, briefly describe your Goals and Aims of the research project, as if addressed towards a general audience (do not provide a grant proposal). Provide definitions or explanations of technical terms and jargon:**  Our lab focuses on the molecular mechanisms involved in disease associated with enteric pathogens. The goal of our work is to identify bacterial and host factors that contribute disease. To accomplish this goal, we have three aims: 1) Identify effector proteins that are delivered to host cells; 2) Characterize the phenotypes of a defined group of mutants; and 3) Assess the contribution of effector proteins in disease in mice.  The research is primarily focused on *Salmonella enterica* serovar Typhimurium, which is one of the most common causes of food poisoning in the USA. Infection usually occurs by ingestion of infected food or water. Most persons infected with serovar Typhimurium develop diarrhea, fever, and abdominal cramps 12 to 72 hours after infection. The illness usually lasts 4 to 7 days, and most persons recover without treatment. In fact, antibiotic treatment is not recommended for gastroenteritis. Occasionally, the diarrhea may be so severe that the patient needs to be hospitalized. In very rare cases, the Salmonella infection may spread from the intestine to the blood stream, and then to other body sites and can cause death unless the person is treated promptly with antibiotics. The elderly, infants, and those with impaired immune systems are more likely to have a severe systemic infection. | | | | | | |
| **B.** | **Briefly describe your Plan of Work describing all activities in respect to biological agents used to accomplish the goal and aims described above. Include a brief explanation of research methods, microbiological practices, and laboratory procedures in lay language. Provide definitions or explanations of technical terms and jargon:**  We are primarily interested in understanding the pathogenesis of *Salmonella enterica* infection. We study bacterial virulence factors that enable *Salmonella enterica* to penetrate the intestinal mucosa, colonize intracellularly and eventually escape from mammalian cells. We also study how the host can control and eventually eliminate *Salmonella* infections. We primarily infect tissue culture epithelial cells with Salmonella enterica as a model of the intestinal epithelium-bacterial interaction. We also use other enteric bacteria (enteropathogenic *Escherichia coli*, *Yersinia enterocolitica*, *Campylobacter jejuni*) to determine whether *Salmonella* uses unique pathogenic mechanisms, or mechanisms common to many gastrointestinal pathogens. We will employ conventional molecular biology methods to generate gene knockouts and complemented strains for testing both *in vitro* and *in vivo*. Tissue culture cells have been purchased from ATCC. Bacterial strains were originally purchased from the *Salmonella* Genetic Stock Center, ATCC, collaborators, or provided by other researchers at WSU. Bacteria are streaked on LB agar plates from frozen -80°C stocks and inoculated into capped tubes or Erlenmeyer flasks and grown in incubators with bacterial shakers in the lab. Tissue culture cells are grown in CO2 incubators. Prior to infection, cells are transferred to designated CO2 incubators for bacterial infections. Cells are infected with bacteria in the biological safety cabinet in the lab at defined timepoints. Infected cells will be lysed for DNA, RNA, or protein isolation or colony forming unit (CFU) analysis. Alternatively, cells will be fixed in paraformaldehyde on coverslips for microscopic analysis.  For in vivo studies, wild type and KO mice will be inoculated with *Salmonella enterica* and observed for 7 days. Bacterial culture, which will consist of wild-type and various mutant strains, will be prepared and administered in the vivarium. Once challenged the mice will be observed for up to 24 hours, at which time all surviving mice will be euthanized using CO2 asphyxiation. Analysis will include blood collection, bacteriology, and histology. Tissues will also be homogenized in a biosafety cabinet and plated for CFU analysis. | | | | | | |
| **C.** | **Will vertebrate animals be involved?** | **No** |  | **Yes** | X | ASAF Number: | In Progress or XXXX | |
|  | | | | | | | | |
| **D.** | **Will non-vertebrate animals be involved?** | **No** | X | **Yes** |  |  |  | |
|  | | | | | | | | |
| **E.** | **Will human subjects directly or indirectly (samples) be used?** | **No** | X | **Yes** |  | IRB Number: |  | |
|  | | | | | | | | |
| **F.** | **Will plants be used?** | **No** | X | **Yes** |  |  | | |

**G. Appropriate Biosafety Levels**:

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 1. | **Laboratory Containment:** | BSL-1 |  | BSL-2 | | X | BSL-3 |  | other |  |  |
|  |  | If ‘Other,’ explain: | | |  | | | | | | |
|  |  |  | | |  | | | | | | |
| 2. | **Animal Containment:** | ABSL-1 |  | ABSL-2 | | X | ABSL-3 |  | other |  |  |
|  |  | If ‘Other,’ explain: | | |  | | | | | | |
|  |  |  | | |  | | | | | | |
| 3. | **Plant Containment:** | BSL1-P |  | BSL2-P | |  | BSL3-P |  | other |  |  |
|  |  | If ‘Other,’ explain: | | |  | | | | | | |

**H Location of facilities where biological agents are used for this project (See Example in Red):**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Campus** | **Name of Building or Field Plot** | [**Facility ID #**](https://facilities.wsu.edu/facilities-services-administration/space-management/facility-roster/)**:** | **Room #(s)** | **BSL**  **(1-3)** | **ABSL**  **(1-3)** | **BSL-P**  **(1-3)** | **Species of Animals housed in these rooms**  **or location** | **Species of Plants housed in these rooms or location** |
| WSU Pullman | Allen Center | 0822 | 412 | 2 |  |  | No | No |
| WSU Pullman | Bustad | 807 | 516 |  | 2 |  | Yes | No |
|  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |

Additional rows may be added with the TAB key

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| --- | --- | --- | --- | --- | --- | --- |
| **I. Please check Yes or No for each of the following categories that apply (do not leave any blank):** | | | | | | |
| Yes | X |  | No |  |  | **Infectious agents (animal, human, or plant);** If yes, complete **section 3.** |
|  |  |  |  |  |  |  |
| Yes | X |  | No |  |  | **Generating and handling recombinant or synthetic nucleic acids (r/sNA) or using cells, organisms and viruses containing such molecules;** If yes, complete **section 4.** |
|  |  |  |  |  |  |  |
| Yes | X |  | No |  |  | **Human or primate blood, body fluids, cells, cell lines, and tissues;** If yes, complete **section 5**. |
|  |  |  |  |  |  |  |
| Yes |  |  | No | X |  | **Transgenic plants;** if yes, complete **sections 4 & 6.** |
|  |  |  |  |  |  |  |
| Yes | X |  | No |  |  | **Transgenic animals;** If yes, complete **sections 4 & 7.** |
|  |  |  |  |  |  |  |
| Yes |  |  | No | X |  | **Select agents and/or toxins;** If yes, complete **sections 3 & 8.** |

**Section 3: Infectious agents and biological toxins**

**For definition of BSL classifications refer to Biosafety in Microbiological and Biomedical Laboratories (**[**BMBL**](https://www.cdc.gov/labs/BMBL.html)**) or the** [**NIH Guidelines**](https://biosafety.wsu.edu/documents/2023/01/nih-guidelines-04-2019.pdf) **for use of r/sNA.**

1. **List all Agents and toxins (additional rows may be added with the TAB key)**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Infectious Agent** | **Toxin** | Human Hazard\* | | Animal Hazard | | **Plant Hazard** | | **Human Vaccines Available** | | **BSL Level** | | |
| **YES** | **NO** | YES | NO | YES | NO | YES | NO | 1 | 2 | 3 |
| *Salmonella enterica* serovar Typhimurium |  | **X** |  | X |  |  | **X** |  | **X** |  | **X** |  |
| *Salmonella enterica* serovar Typhi |  | **X** |  |  | X |  | **X** | **X** |  |  | **X** |  |
| Enteropathogenic *Escherichia coli* |  | **X** |  | X |  |  | **X** |  | **X** |  | **X** |  |
| *Shigella flexneri* |  | **X** |  | X |  |  | **X** |  | **X** |  | **X** |  |
| *Chromobacterium violaceum* |  | **X** |  | X |  |  | **X** |  | **X** |  | **X** |  |
| *Providencia alcalifaciens* |  | **X** |  | X |  |  | **X** |  |  |  | **X** |  |
| *Yersinia enterocolitica* |  | **X** |  | X |  |  | **X** |  |  |  | **X** |  |
| HeLa cells (HPV genomic fragment) |  | **X** |  |  | X |  | **X** | **X** |  |  | **X** |  |

\*If organism is non-pathogenic to humans check NO.

1. **Briefly answer the following questions in the boxes provided below (if applicable):**

|  |  |
| --- | --- |
| **1.** | **Briefly describe experimental design and goals with the infectious agents and/or toxins:** |
| We plan to infect mammalian cells with the above-mentioned organisms and monitor bacterial and host protein, RNA, DNA levels and cytokine responses, and intracellular trafficking of bacteria. Cells will also be lysed and plated on LB agar to enumerate intracellular bacteria or fixed and processed for transmission electron microscopy or immunofluorescence microscopy. In some instances, we will use small inhibitory RNA to deplete specific mammalian genes and monitor the outcome of bacterial infection. Alternatively, Salmonella gene deletion or gene replacement mutants will be used to infect mammalian cells and eventually mice. The goal of such experiments is to identify bacterial and host factors that contribute to the outcome of infection. |
| **2.** | **What is the source of the agent, toxin, or potentially biohazardous material?** |
| Wild type stocks of *Salmonella enterica* serovar Typhimurium, *S.* Typhi and *C. violaceum* have been purchased from *Salmonella* Genetic Stock Center (SGSC), ATCC and BEI Resources, respectively. Enteropathogenic *E. coli* has been provided by XXX collaborator McGill University in Montreal, Canada. *S. flexneri* has been provided by XXX collaborator at Harvard University. *P. alcalifaciens* has been provided Los Angeles County Department of Public Health. Y. enterocolitica has been provided by XXX collaborators at Paul G. Allen School for Global Health. Gene deletion or gene replacement mutants have been created by myself or collaborators. |
| **3.** | **What is your assessment of the biohazardous potential? Include vaccines that will be required (if applicable).** |
| The bacteria used in our studies can cause disease in humans and animals. In humans, the diseases caused by these organisms are characterized by bloody diarrhea with a fever, nausea, vomiting, dehydration and intestinal cramping (gastroenteritis) or by fever alone (typhoid fever, *S.* Typhi only). Immunocompromised individuals (cancer patients, AIDS patients, etc) can develop severe systemic disease that is life threatening. Infection with *Salmonella* serotypes can be successfully treated with antibiotics (ciprofloxacin or third generation cephalosporins). There is a commercially available vaccine for serovar Typhi, which will be administered to workers if we start working with this bacterium. *Enteropathogenic E. coli, Salmonella enterica serotypes, S. flexneri, C. violaceum, P. alcalifaciens and Y. enterocolitica* are categorized as risk group 2. Accordingly, we will use BSL-2 practices in our laboratory. |
| **4.** | **What containment procedures will be used when transporting agents within and between facilities? Provide an example of the double-containment used when transporting agents outside of the primary laboratory facility.** |
| Within the lab, bacterial cultures and infected cells will be carried by workers wearing lab coats and gloves. If samples containing live bacteria are to be taken out of the lab, transportation will be on foot. Samples will be in a closed, leak-proof, durable, labeled primary container and placed inside a Thermo Scientific Nalgene BioTransport carrier (double containment). A biohazard sticker will clearly be posted on this secondary carrier. |
| **5.** | **What is the method of terminal inactivation of the biological agent (autoclave, chemical inactivation, compost, steam sterilization technology – STI, incineration)?** |
| Autoclaving and for certain items, incineration. |
| **6.** | **What is the disposal method for inoculated animals (including animal bedding and waste) or plants?** |
| All animals will be incinerated. |
| **7.** | **List all materials that require federal permits & include copies of these permits with this application.** |
|  |

**Section 4: Recombinant or Synthetic Nucleic Acid (r/sNA) molecules**

Note: Descriptions in the table below are summarized from the [**NIH Guidelines**](https://biosafety.wsu.edu/documents/2023/01/nih-guidelines-04-2019.pdf), contact the BSO for questions.

1. NIH Review Category & Subcategory: ***Check all the categories, subcategories and information that apply.***

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Category** | | | **Oversight** | **Includes / Subcategories** | | | | | | |
| **III-A** | | | **NIH Director & IBC** | Studies that involve the deliberate transfer of drug resistance to microorganisms (not known to acquire the trait naturally) that can compromise the use of the drug to control the microorganism and its disease in humans, veterinary medicine or agriculture. Contact BSO for special instructions. | | | | | | |
|  |  |  |
|  | | |
| **III-B** | | | **NIH/OSP & IBC** | Studies that involve the deliberate formation of recombinant or synthetic nucleic acid molecules containing genes for the *biosynthesis* of toxin molecules lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight Contact. BSO for special instructions. | | | | | | |
|  |  |  |
|  | | |
| **III-C** | | | **IRB & IBC** | Experiments involving human gene transfer (recombinant or synthetic DNA, or DNA or RNA derived from recombinant or synthetic nucleic acid molecules, into one or more human subjects. Contact BSO for special instructions. | | | | | | |
|  |  |  |
|  | | |
| **III-D** | | | **IBC approval before initiation** |  | | | | | | |
|  | X | **D-1**: Experiments using Risk Group 2 or higher agents as host-vector systems (*e.g.,* lentiviral) | | | | |
|  | |
|  | **X** |  |  | X | **D-2**: Experiments in which DNA from Risk Group 2 or higher agents is cloned into non-pathogenic prokaryotic or lower eukaryotic host-vector systems | | | | |
|  | | |  | |
|  |  |  | | | | |
|  |  | **D-3:** Experimentsin which r/sNA is introduced with infectious or defective viruses in the presence of helper virus in tissue culture systems. | | | | |
|  |  |
|  | |  | Yes |  | No | Experiment is likely to enhance pathogenicity |
|  | | | | | | |
|  | |  | Yes |  | No | Experiment extends the host range |
|  |  |  | | | | |
|  | X | **D-4:** Experiments involving *whole* *animals* in which the animal's genome has been altered by stable introduction of recombinant or synthetic nucleic acid molecules, or nucleic acids derived therefrom, into the germ-line and experiments involving viable recombinant *or* synthetic nucleic acid molecule-modified *microorganisms tested on whole animals*. (This section does not include generation or breeding transgenic rodents, see Section III-E). For more information, see [Animal experiments covered under the NIH guidelines](https://biosafety.wsu.edu/documents/2023/01/nih-guidelines-animal-activities-table.pdf). | | | | |
|  | |
|  | |  | Yes | X | No | Fraction of viral genome utilized may lead to productive infection. |
|  | | | | | |
|  | |  | Yes | X | No | r/sNA source is greater than 2/3 eukaryotic viral genome. |
|  | | | | | |
|  |  | **D-5:** Experiments to genetically engineer plants by recombinant or synthetic nucleic acid molecule methods, to use such plants for other experimental purposes (*e.g.,* response to stress), to propagate such plants, or to use plants together with microorganisms or insects containing recombinant or synthetic nucleic acid molecules (typically risk group 2 or higher: BSL2 or BSL2-P) | | | | |
|  |  |
|  |  | **D-6:** Experiments involving more than 10L of culture of organisms containing r/sNA molecules.  **D-7:** Experiments involving influenza viruses generated by recombinant or synthetic methods. | | | | |
|  |  |
|  |  |
|  |  |
| **III-E** | | | **IBC approval simultaneous with initiation** |  | | | | | | |
|  | x | **E-1:** Experiments involving the formation of r/sNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (cells must lack helper virus for the families of defective viruses used). This category is used for all routine r/sNA cloning or gene expression with low risk agents (*e.g.,* *E. coli* cloning strains). | | | | |
|  | **x** |  |  |  |
|  | | |
|  | | |  | |  |  |  |  |  |
|  | x | **E-2:** Experiments involving nucleic acid molecule-modified whole plants, and/or experiments involving recombinant or synthetic nucleic acid molecule-modified organisms associated with whole plants (typically group 1: BSL1 or BSL1-P). | | | | |
|  |  |
|  |  | **E-3:** Experiments involving the generation of risk group 1 (*e.g., ABSL-1*) rodents in which the animal's genome has been altered by stable introduction of recombinant or synthetic nucleic acid molecules, or nucleic acids derived therefrom, into the germ-line (transgenic rodents). See III-D4 for experiments requiring BSL-2 or higher containment and practices. | | | | |
|  |  |
| **III-F** | | | **WSU policy requires BAF submittal for review and IBC approval simultaneous with initiation** |  |  | Exempt by NIH Guidelines, including synthetic nucleic acids that: (1) can neither replicate nor generate nucleic acids in any living cell (e.g., oligonucleotides or other synthetic nucleic acids that do not contain an origin of replication), and (2) are not designed to integrate into DNA, and (3) do not produce a toxin that is lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight. (Provide descriptions of experiments that use r/sNA molecules and information from the NIH Guidelines that verifies exemption in the narrative of section 2). | | | | |
|  |  |
|  | **x** |  |  |  |
|  | | |

**Recombinant r/sNA information below to be filled out for Category III-A through III-F work.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| 1. **Please check Yes or No for each of the following statements:** | | | | | | |
| **1.** | Yes | X |  | No |  | I am inserting or using foreign DNA or RNA into a vector, organism, or cell/cell line to clone or express it. |
| **The DNA or RNA to be cloned or expressed:** | | | | | | |
| **2.** | Yes |  |  | No | X | Is from a Risk Group 3 agent. |
|  |  |  |  |  |  |  |
| **3.** | Yes |  |  | No | X | Represents more than two-thirds of the genome of a Risk Group 1 or 2 organisms that have not been determined to be exempt by NIH Guidelines. |
|  |  |  |  |  |  |  |
| **4.** | Yes |  |  | No | X | Encodes a known oncogene. |
|  |  |  |  |  |  |  |
| **5.** | Yes |  |  | No | X | Encodes a control element that may extend the host range. |
|  |  |  |  |  |  |  |
| **6.** | Yes |  |  | No | X | Encodes molecules or genes for the biosynthesis of such molecules known to be toxic to vertebrates at  LD50 of less than 100 ng/kg body weight. |
| **The vector I am using for introducing a foreign DNA or RNA into the host:** | | | | | | |
| **7.** | Yes |  |  | No | X | Is from a Risk Group 3 agent. |
|  |  |  |  |  |  |  |
| **8.** | Yes | X |  | No |  | Is a Risk Group 1 or 2 virus that infects eukaryotic cells and contains more than two-thirds of the viral genome. |

**If you checked Yes for any of the above statements, please complete the following r/sNA information.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | | | **Biosafety Level** | | |
| 1. **Host, vector, and gene biosafety information (as applicable):** | | | **1** | **2** | **3** |
| 1. Host(s): | *Salmonella enterica, Shigella flexneri* and non-pathogenic *Escherichia coli* (Top10), mice (C57Bl/6) | | X | X |  |
| If applicable, include plants/animals to be transformed | |  |
| 2. Vector(s)\*: | Plasmids; pACYC177, pACYC184, pBAD18, pBAD30, | | X |  |  |
|  |  | |
| 3. Gene(s), DNA, or RNA involved (e.g., cloned or expressed)#: | Genes associated with virulence (e.g., invA, iroB, spiC, pipD, int1); KO murine model (TLR4-/-) | | X |  |  |
|  |  | |
| 4. DNA/RNA source(s): | PCR products from *Salmonella* and *Shigella* | | X |  |  |
|  |  | |

\* List the type of vector systems used (e.g., plasmid, lentiviral, adenoviral, CRISPR/Cas9 vectors, etc.) and provide vector names or descriptions (e.g., backbone, resistance markers, etc.). If multiple vectors are used, provide a representative example of vector names for each type. # Provide names of genes or families with relevant examples. **Section 5: Human or Primate Blood, body fluids, cells, cell-lines, and tissues**

A. Human blood, body fluids, cells, and tissues must be treated as though containing infectious agents.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| 1. |  | **Blood** |  |  | **Vendor/Collaborator:** |  |
|  |  |  |  |  |  |  |
| 2. |  | **Body fluids** | **List type:** |  | **Vendor/Collaborator:** |  |
|  |  |  |  |  |  |  |
| 3. |  | **Cells/tissues** | **List type:** |  | **Vendor/Collaborator:** |  |
|  |  |  |  |  |  |  |
| 4. |  | **Primary Cell Lines** | **List type:** |  | **Vendor/Collaborator:** |  |
|  |  |  |  |  |  |  |
| 5. | x | **Established Cell lines** | **List type:** | HEK  HELA | **Vendor/Collaborator:** | ATCC |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| 6. | Do any of the cells, tissues, or cell lines have characterized agents? | x | Yes |  | No | **If Yes, Fill out Section 3** |

7. Use of human or primate blood, body fluids, cells, and tissues may require a Bloodborne (BBP) Pathogen Exposure Control Plan (ECP), bloodborne pathogen training, and offer to provide personnel vaccinations (contact EH&S at 335-3041).

|  |  |  |  |
| --- | --- | --- | --- |
| a. | Has a BBP ECP been completed? | x | Yes |
|  |  |  |  |
| b. | Has your BBP ECP been reviewed and approved by the BSO? | x | Yes |
|  |  |
| c. | Are systems in place to ensure lab personnel BBP training? | x | Yes |
|  |  |
| d. | Are systems in place to ensure lab personnel are offered required Hepatitis-B Virus (HBV) vaccinations? (Not applicable for established cell lines except those of liver origin) |  | Yes | x | N/A |
|  |  |  |  |

Please note: Use and/or collection of human blood, body fluids, cells, or tissues may require human subjects’ approval from the WSU [IRB](http://www.irb.wsu.edu/).

**Section 6: Transgenic plants**

The [NIH Guidelines](https://biosafety.wsu.edu/documents/2023/01/nih-guidelines-04-2019.pdf) require that transgenic plant activities be reported to the IBC. Transgenic plant projects requiring only BSL-1 containment may be initiated simultaneously with submission of this form to the IBC. All other transgenic plant research requires IBC review and approval prior to initiation of the research. Please refer to the NIH Guidelines or contact the BSO for additional information. Note: The Agricultural Research Center (ARC) has requested to be kept informed of all transgenic plant research outside the laboratory at WSU. The ORA will forward a copy of any BAF that includes research with transgenic plants to be introduced into the field to the ARC for their records. This will not affect review and approval by the IBC.

**A. *Please check Yes or No for each of the following categories and fill in appropriate boxes***

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Yes |  | No | 1. | Transgenic whole plants will be maintained in the laboratory, greenhouse, or growth chamber. | | | | | | | | | | | | | | | | |
|  |  |  |  |  |  | | | | | | | | | | | | | | | | |
|  | Yes |  | No | 2. | Transgenic whole plants will be introduced into the field. | | | | | | | | | | | | | | | | |
|  | | | |  | |  | | | | | | | | | | | | | | | |
| a. | | If a pharmaceutical or bioactive industrial compound will be synthesized in a food or feed crop, state | | | | | | | | | | | | | | | |
| the reason for choosing that crop: | | | |  | | | | | | | | | | | |
|  | | | | | | | | | | | | | | | |
| b. | | Describe information gained from lab and growth chamber or greenhouse experiments that would be | | | | | | | | | | | | | | | |
| relevant to assessing potential risks from field tests: | | | | | | |  | | | | | | | | |
|  | | | | | | | | | | | | | | | | | |
| c. | | Describe procedures to monitor for and eliminate any volunteer plants: | | | | | | | | |  | | | | | | |
|  | | | | | | | | | | | | | | | | | |
| d. | | How close is the field planting to other plants where gene flow or cross pollination could occur? | | | | | | | | | | | | | | | |
|  | | | | | | | | | | | | | | | |
|  | Less than 100 feet |  | Less than one mile | | | |  |  | | | | | | | |
|  | |  | |  |  | | | |  |  | | | | | | | |
|  | |  | Less than 100 yards |  | Greater than one mile | | | |  |  | | | | | | | |
|  | |  | |  |  | |  | | | | | | | | | | |
|  | |  | Not applicable |  | Explain: | |  | | | | | | | | | |
|  | |  | |  |  | |  | | | | | | | | | | |
| e. | | Have bordering farms been made aware of the transgenic field release? | | | | | | | | | | Yes |  | No |  |  | |
|  | |  | | | | | | | | | |  |  |  |  |  | |
| f. | | I agree to adhere to all federal guidelines as outlined in the attached copy of the APHIS approval/permit | | | | | | | | | | Yes |  | No |  |  | |
|  |  |  |  |  | |
|  | |  | | | | | | | | | |  |  |  |  |  | |
| g. | | A planting map with GPS coordinates and bordering field planting information has been attached. | | | | | | | | | | Yes |  | No |  |  | |
|  |  |  |  |  | |
|  | |  | | | | | | | | | |  |  |  |  |  | |
|  | Yes |  | No | 3. | Is the recombinant plant a noxious weed? | | | | | | | | | | | | | | | | |
|  |  |  |  |  |  | | | | | | | | | | | | | | | | |
|  | Yes |  | No | 4. | Can the recombinant plant interbreed with weeds in the area? | | | | | | | | | | | | | | | | |
|  |  |  |  |  |  | | | | | | | | | | | | | | | | |
|  | Yes |  | No | 5. | Does the recombinant plant have recognized potential for detrimental environmental impact on managed or natural ecosystems? | | | | | | | | | | | | | | | | |
|  |  |  |  |
|  |  |  |  |  |  | | | | | | | | | | | | | | | | |
|  | Yes |  | No | 6. | Does the recombinant DNA work contain a complete genome of a non-exotic infectious agent? | | | | | | | | | | | | | | | | |
|  |  |  |  |  |  | | | | | | | | | | | | | | | | |
|  | Yes |  | No | 7. | Does the recombinant DNA work contain the genome of an exotic infectious agent? | | | | | | | | | | | | | | | | |
|  |  |  |  |  |  | | | | | | | | | | | | | | | | |
|  | Yes |  | No | 8. | Could this work reconstitute the genome of an infectious agent in a plant? | | | | | | | | | | | | | | | | |
|  |  |  |  |  |  | | | | | | | | | | | | | | | | |
|  | Yes |  | No | 9. | Does this work involve exotic infectious agents with potentially detrimental environmental impact? | | | | | | | | | | | | | | | | |
|  |  |  |  |  |  | | | | | | | | | | | | | | | | |
|  | Yes |  | No | 10 | Contains an exogenous toxin? If yes, please describe: | | | | | | |  | | | | | | | | | |

**B. Please complete the questions below:**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| 1. | What physical and or biological containment conditions will be implemented? | | |  | |
|  |  | | | |  |
| 2. | How will biological material be decontaminated or inactivated? | | | |  |
|  | | | | | |
|  | Autoclave | | | |
|  | | | | |
|  | Chemical | | | |
|  | | | | |
|  | Composting | | | |
|  | | | | |
|  | Desiccation | | | |
|  | | | | |
|  | Chopping / Mincing | | | |
|  | | | | |
|  | Steam Sterilization Technology - STI | | | |
|  | | | | |
|  | | | | |
|  | Incineration | | | |
|  | | | | |
|  | Other – Please Explain |  | | |

**Section 7: Transgenic animals**

**A. This section is to be completed for any transgenic vertebrate or non-vertebrate animal.**

**Exception:** Projects involving the purchase, transfer and use of transgenic rodents in BSL-1 experiments are not required to fill out this form. Breeding of BSL-1 rodent colonies within the same genetic lineage is also exempt if:

* Both parental rodents can be housed under BSL-1 containment; **and** neither parental transgenic rodent contains either of the following genetic modifications:
  + Incorporation of more than one-half of the genome of a eukaryotic virus from a single family of viruses; **or**
  + Incorporation of a transgene that is under the control of a gammaretroviral terminal repeat (LTR); **and**
* The transgenic rodent that results from this breeding is not expected to contain more than one-half of an exogenous viral genome from a single family of viruses.

|  |
| --- |
| x |

**This research utilizes only rodents that meet this exception.**

|  |
| --- |
|  |

**This exception does not apply to the animals involved in this research.**

**B. For transgenic animal research (not excepted as described above) complete the following section:**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 1. | Source of animal: | | | | Jackson Labs | | | |
|  |  | | | | | | |  |
| 2. | Animal decontamination upon termination of experiment:   |  |  |  | | --- | --- | --- | |  | | | |  | | |  | Composting | |  | | | x | Steam Sterilization Technology - STI | |  | | |  | Incineration | |  | | |  | | | | | | | | |  |
|  | | |  |  | | | | |
|  | Other- Please specify: | |  | | |
|  | |  | | | |  | | |
|  |  | | | | | |  | |
| 3. | If breeding BSL-1 rodent colonies with other genetic lines describe the genetic make-up of both lineages that will be used for breeding: | | | | | | | |
| N/A | | | | | |

|  |  |
| --- | --- |
| **C.** | **Briefly list all transgenic animals utilized in this research:**  TLR4 KO C57/BL/6 mice |

**Section 8: Select agents and toxins**

Select agents require registration with the Federal Select Agent Program (FSAP) administered by WSU ORA. Both the CDC and USDA APHIS oversee the program and approve research utilizing these agents. Additional information on Select Agents and Toxins can be found on the [Biosafety Website](https://biosafety.wsu.edu/potentially-biohazardous-materials/) or the [FSAP](https://www.selectagents.gov/index.htm) (A list of excluded agents and toxins are found [here](https://www.selectagents.gov/sat/exclusions/index.htm)). Contact the BSO prior to submitting a BAF.

**A. PLEASE CHECK BOXES BELOW INDICATING SELECT AGENTS & TOXINS USED IN YOUR LABORATORY.**

**HHS Select Agents and Toxins**

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | | |
|  | Abrin (over 1000mg) | | |
|  | *Bacillus cereus* Biovar *anthracis* | | |
|  | Botulinum neurotoxins (over 1mg)  Botulinum neurotoxin producing species of *Clostridium* | | |
|  |
|  | Short, paralytic alpha conotoxins containing the following amino acid sequence X1CCX2PACGX3X4X5X6CX7 (over 100 mg)  *Coxiella burnetii*  Crimean-Congo haemorrhagic fever virus | | |
|  |
|  |
|  | Diacetoxyscirpenol (over 10,000mg)  Eastern Equine encephalitis virus | | |
|  |
|  | Ebola viruses  *Francisella tularensis* | | |
|  |
|  | Lassa fever virus  Lujo virus | | |
|  |
|  | Marburg virus | | |
|  | Mpox virus | | |
|  | Reconstructed 1918 influenza virus | | |
|  | Ricin (over 1000mg) | | |
|  | *Rickettsia prowazekii* | | |
|  | SARS-associated coronavirus (SARS-CoV) | | |
|  | SARS-CoV/SARS-CoV-2 chimeric viruses resulting from any deliberate manipulation of SARS-CoV-2 to incorporate nucleic acids coding for SARS-CoV virulence factors | | |
|  | Saxitoxin (over 500mg) | | |
|  | South American haemorrhagic fever viruses. | | |
|  |  |  |  |
|  |  |  | Chapare |
|  |  |  | Guanarito |
|  |  |  | Junin |
|  |  |  | Machupo |
|  |  |  | Sabia |
|  | Staphylococcal enterotoxins A,B,C,D,E subtypes (over 100mg)  T-2 toxin (over 10,000mg)  Tetrodotoxin (over 500mg) | | |
|  |
|  |
|  | Tick-borne encephalitis complex (flavi) viruses | | |
|  |  |  |  |
|  |  |  | Far Eastern subtype |
|  |  |  | Siberian subtype |
|  | Kyasanur Forest disease virus  Omsk hemorrhagic fever virus  Variola major virus (Smallpox virus) | | |
|  |
|  |
|  | Variola minor virus (Alastrim) | | |
|  | *Yersinia pestis* | | |

**Overlap Select Agents and Toxins**

|  |  |
| --- | --- |
|  |  |
|  | *Bacillus anthracis*  *Bacillus anthracis* Pasteur strain |
|  |
|  | *Brucella abortus* |
|  | *Brucella melitensis* |
|  | *Brucella suis* |
|  | *Burkholderia mallei* |
|  | *Burkholderia pseudomallei* |
|  | Hendra virus |
|  | Nipah Virus |
|  | Rift Valley fever virus |
|  | Venezuelan equine encephalitis virus |

**USDA Select Agents and Toxins)**

|  |  |
| --- | --- |
|  |  |
|  | African horse sickness virus |
|  | African swine fever virus |
|  | Avian influenza virus |
|  | Classical swine fever virus |
|  | Foot and mouth disease virus |
|  | Goat pox virus |
|  | Lumpy skin disease virus |
|  | *Mycoplasma capricolum* |
|  | *Mycoplasma mycoides* |
|  | Newcastle disease virus |
|  | Peste Des Petits Ruminants virus |
|  | Rinderpest virus |
|  | Sheep pox virus |
|  | Swine vesicular disease virus |

**USDA Plant Protection and Quarantine (PPQ) Select Agents and Toxins**

|  |  |
| --- | --- |
|  |  |
|  | *Coniothyrium glycines (formerly Phoma glycinicola and Pyrenochaeta glycines)*  *Peronosclerospora philippinensis (Peronosclerospora sacchari)* |
|  |
|  | *Ralstonia solanacearum*  *Rathayibacter toxicus* |
|  |
|  | *Schlerophthora rayssiae* |
|  | *Synchytrium endobioticum* |
|  | *Xanthomonas oryzae* |