Protocol #:

UNIVERSITY OF WISCONSIN LA CROSSE UWL

IBC Biosafety Protocol Application

Section I: Principal Investigator & Project Overview

A. Principal Investigator (PI)

Name[1]:	[redacted]	Department:	[redacted]
Email:	[redacted]	Employee Classification:	Faculty

B. Project Overview

Project Title: Structure and Function of DcrB, an Enterobacterial Copper Resistance Protein

Course Number & Name[2]: [redacted]

Project Type: ☒ Research ☒ Teaching

Application Type: ☒ New ☐ Renewal ☐ Revision[3]

If a renewal or revision:
Protocol number:
Summarize change(s): Click or tap here to enter text.
List revised protocol section(s): Click or tap here to enter text.

Funding:

Is this project associated with external award(s)? ☒ Yes ☐ No If yes, complete information below.

Sponsor	Status	Award # (if current)	Start Date	End Date
[redacted]	☐ Current ☒ Pending	[redacted]	[redacted]	[redacted]
Click or tap here to enter text.	☐ Current ☐ Pending	Click or tap here to enter text.	Click or tap to enter a date.	Click or tap to enter a date.

Location(s):

List all locations where biological materials will be used, stored, or handled. Add lines if needed.

Building	Room Number	Containment and/or Storage Equipment (e.g., biosafety cabinet refrigerator, freezer, dewar)
[redacted]	[redacted]	Biosafety cabinet, refrigerator (short-term storage), -20 °C freezer (long-term storage), and -80 °C freezer (long-term storage). Primary research lab space for using, storing, and handling all biological materials described below.
[redacted]	[redacted]	Shared-use autoclave room containing 3 steam sterilizers for disinfection of biohazardous waste
[redacted]	[redacted]	Only used for handling cultures of non-pathogenic Escherichia coli harboring recombinant DNA

[1] Only a UWL faculty or staff member may be listed as the PI on a Biosafety Protocol Application. All other project personnel, including students, must be listed in Section IV. Personnel.

[2] If lab courses involve recombinant materials, they are subject to NIH Guidelines, and a protocol is required. Lab courses not involving other biological materials but not recombinant materials may submit a protocol but are not required to do so.

[3] For revisions to research elements, biological materials used, and/or locations, complete this form. For personnel or award modifications, submit the IBC Personnel & Award Modification Form.

C. Project Summary

Provide a brief description of the research project(s) in which the materials and/or organisms addressed in Section II will be used.

This project seeks to understand structure-function relationships for a novel copper ion resistance protein, named DcrB, in *Salmonella enterica* serovar Typhimurium, a major bacterial cause of food-borne illness in humans. We have shown that the lipoprotein DcrB confers resistance to high Cu^{2+} in *Salmonella*. We determined the first three-dimensional structure of a DcrB protein, and we identified structural features that are important for the function of DcrB in copper resistance. This project seeks to understand how these features of DcrB are essential for its function. [redacted] We will use site-directed mutagenesis to probe the structural and biochemical properties for each feature of DcrB. We will use X-ray crystallography to determine how changes to these features impact the structure of DcrB; we will use biophysical experiments to investigate the influence of these features on the thermodynamics of folding of DcrB; and we will use genetic experiments in *Salmonella enterica* serovar Typhimurium to determine whether variants of the DcrB protein are functional in copper resistance.

Section II: Biological Materials

A. Recombinant Materials

Complete this section if working with any recombinant or synthetic DNA/RNA materials. Provide the following information, and expand the table if needed:

- Gene name(s) and acronym(s)
- All pertinent biological activities of the encoded protein(s) (e.g., normal biological function, oncogenic potential, toxicity) – If unknown, indicate "unknown" and explain. Address the suspected nature of the gene, if any.
- Biological source/origin (genus and species)
- Risk group (RG) of the source organism(s) – see ABSA Risk Group Database
- Vector(s) (bacterial plasmid, virus, or other vector)
- Host(s) (genius, species, strain, tissue, cell line) that the recombinant material might be inserted into
- Risk group (RG) of the host – see ABSA Risk Group Database

Name of Gene or Gene Fragment	Nature of Gene	Source Organism(s)	RG of Source Organism	Vector(s)	Host Administered to	RG of Host
1. *dcrB (STM3580)*	Encodes inner membrane lipoprotein DcrB, which plays a role in cell envelope biogenesis, maintenance of cell envelope integrity, membrane homeostasis, and copper resistance. UniProt ID: Q7CPJ3	*Salmonella enterica* serovar Typhimurium	2	pBR322	*Salmonella enterica* serovar Typhimurium strain ATCC 14028	2
2. *His6-dcrB-del37*	Encodes folded domain of inner membrane lipoprotein DcrB: Plays a role in cell envelope biogenesis, maintenance of cell envelope integrity, membrane	*Salmonella enterica* serovar Typhimurium	2	pET-22b(+)	• *Escherichia coli* strain BL21(DE3) • *Escherichia coli* NEB® 5-alpha	1

	homeostasis, and copper resistance. UniProt ID: Q7CPJ3					
3. *SePhoN-GSSHis8*	Encodes a non-specific acid phosphatase enzyme. UniProt ID: P26976	*Salmonella enterica* serovar Typhimurium	2	pET-22b(+)	• *Escherichia coli* strain BL21(DE3) • *Escherichia coli* NEB® 5-alpha	1

a. For each material listed in the table above, indicate all categories from the NIH Guidelines, Section III that apply.

1. For pBR322-*dcrB,* Section III-D-1-a (Experiments involving the introduction of recombinant or synthetic nucleic acid molecules into Risk Group 2 agents) applies.
2. pET-22b(+)-*His6-dcrB*-del37, Section III-D-2-a (Experiments in which DNA from Risk Group 2 or Risk Group 3 agents...is transferred into nonpathogenic prokaryotes) applies.
3. pET-22b(+)-*SePhoN-GSSHis8*, Section III-D-2-a (Experiments in which DNA from Risk Group 2 or Risk Group 3 agents...is transferred into nonpathogenic prokaryotes) applies.

b. Attach plasmid maps as part of the protocol in Section VI: Attachments. *Plasmid maps are attached.*

B. Microorganisms, Viruses, & Prions

Complete this section if working with any prokaryotes, fungi, virus, viral vectors, or prions. Provide the following information, and expand the table if needed:

- Organism name(s) (genius, species, strain), name of virus(es), and/or prion name(s) and natural host(s)
- Whether the agent is a human, animal, and/or plant pathogen (and if a plant pathogen, whether it is indigenous to Wisconsin) – if none apply, enter "n/a"
- Risk group (RG) – see ABSA Risk Group Database
- Biosafety level (BSL) – see UWL Biosafety Manual, Summary of Biosafety Levels
- Any rDNA (plasmid, virus, DNA fragment, or other vector)
- Host (genius, species, strain, cell lines) exposed to the microbial agent

Organism, Name of Virus, or Prion Name & Natural Host	**Human, Animal, or Plant Pathogen**	**RG**	**BSL**	**rDNA Added?** *(If yes, indicate identity from II.A.)*	**Administered to host?** *(If yes, reference protocol section)*
1. *Salmonella enterica* serovar Typhimurium strain ATCC 14028	Human and animal pathogen	2	2	Yes (pBR322-*dcrB*)	No
2. *Escherichia coli* BL21(DE3)	No	1	1	Yes (pET-22b(+)-*His6-dcrB-del37* and pET-22b(+)-SePhoN-GSSHis8)	No
3. *Escherichia coli* NEB® 5-alpha	No	1	1	Yes (pBR322-*dcrB*, pET-22b(+)-*His6-dcrB-del37*, and pET-22b(+)-SePhoN-GSSHis8)	No

a. If the identity of your microbial agents, viruses, or prions is unknown, please explain.

Not applicable: I am using well-characterized laboratory strains of each organism.

b. If any of the microbial agents, viruses, or prions are pathogenic, indicate the host(s) organism(s) at risk of infection.

Salmonella enterica serovar Typhimurium can infect humans, other mammals, birds, and reptiles, including domestic livestock, pets, and wild animals. Because animals are not used in this research and are not allowed in lab, only lab personnel are at risk of infection in the lab setting.
c. Will any of the microorganisms be grown in volumes of 10 liters or more? If so, indicate which and the volume.
No
d. In protocol section III.A., address the following information for each microorganism, virus, or prion: Describe the safety procedures personnel will use to protect themselves from exposure and appropriate response if accidental exposure occurs. Address both collection and research if applicable. *See Section III.A. for the requested information.*

C. Human and Non-Human Animal Tissues, Cell Lines, & Blood Products: NONE

Complete this section if working with any human-derived materials *or* non-human animal-derived materials that are infectious, potentially infectious, or recombinant. Provide the following information, and expand the table if needed:

- Type of material used (species, strain, technical name)
- Source
- Risk group (RG) – see ABSA Risk Group Database
- Biosafety level (BSL) – see UWL Biosafety Manual, Summary of Biosafety Levels
- Any vector (bacterial plasmid, virus, or other vector) that will be delivered into the sample – if applicable, indicate identity from other protocol sections
- Host (genius, species, strain) to which the samples will be applied – if applicable, reference protocol section

Type of Material	Source	RG	BSL	Exposed to biological material or rDNA?	Administered to host?
1. not applicable					
2.					

a. Does the material contain a known infectious agent?
☐ Yes ☐ No
b. If administering nucleic acids, toxins, nanoparticles, microbes, viruses, or other biohazardous material to animals, describe the route of delivery.
c. In protocol section III.A., address the following information for each material listed above: Describe the safety procedures personnel will use to protect themselves from exposure and appropriate response if accidental exposure occurs.
d. **All work involving live (non-fixed) human-derived materials (e.g., blood or blood components, tissues, secretions), cell lines, and/or bloodborne pathogens must comply with the OSHA Bloodborne Pathogens Standard** (29 CFR 1910.1030). If this is applicable to your project, contact Environmental Health & Safety to ensure compliance with annual training and other UWL Bloodborne Pathogens Program requirements.

D. Biological Toxins: NONE

Complete this section if working with any toxin(s) of biological origin. Provide the following information, and expand the table if needed:

- Biological toxin name(s) and acronym(s) if appropriate, biological source/origin (genus species)
- Median lethal dose (LD_{50}) as ng/kg
- Risk group (RG) – see ABSA Risk Group Database
- Biosafety level (BSL) – see UWL Biosafety Manual, Summary of Biosafety Levels
- Whether toxin is a CDC Select Agent (see CDC Select Agents and Toxins List)
- If the gene encoding the toxin will be cloned into a vector (bacterial plasmid, virus, or other vector), or host (genius, species, strain) that the toxin or recombinant materials containing the toxin gene might be inserted into
- Maximum amount administered to each type of recipient at one time (e.g., 0.1 ng)

Biological Toxin and Source Organism	LD_{50} (ng/kg)	RG	BSL	Select Agent? (Y/N)	Administered to host? (Y/N; if yes, reference protocol section)	Max Amount Administered (at one time)
1. not applicable						
2.						

a. In protocol section III.A., address the following information for each biological toxin: Describe the safety procedures personnel will use to protect themselves from exposure and appropriate response if accidental exposure occurs.

b. For CDC Select Agent Toxins, address the following information in protocol section III.A. Include information for both collection and research if applicable.

i. Maximum amount of toxin inventory and how you will document inventory (permissible amounts of select toxins are listed on the CDC website)
ii. How the toxin will be stored securely
iii. For toxins that will be reconstituted from a powder, how select toxins will be reconstituted (should be conducted inside containment, e.g., chemical fume hood, biological safety cabinet)
iv. How each toxin will be inactivated (Appendix H of the BMBL describes inactivation procedures)
v. List aerosol generating activities and how an exposure risk will be mitigated
vi. Indicate if sharps will be used in procedures involving toxins.
vii. If administering the toxin to live animals, describe the route of delivery and maximum dose.

E. Vertebrate & Invertebrate Animals: NONE

Complete this section if working with any vertebrate or invertebrate animals administered biological materials. Work with vertebrate animals additionally requires IACUC review and approval before initiating work. Provide the following information, and expand the table if needed:

- Animal common name and genus species
- Risk group (RG) – see ABSA Risk Group Database
- Animal Biosafety Level (ABSL) – see UWL Biosafety Manual; BMBL Section V, and NIH Guidelines, Appendix M
- Whether animal is transgenic
- Biological materials administered to the animals – type of materials, quantity, and method of administration
- Housing – type of housing for animals (e.g., static microisolators, rack system), and building(s) and room number(s) where animals will be housed

Vertebrate or Invertebrate (Common name; Genus species)	**RG**	**ABSL**	**Transgenic?** (Y/N)	**Biological Materials Administered** (Type, Quantity, Method of Administration)	**Housing** (Type, Building & Room Number(s))
1. not applicable					
2.					

a. Indicate routes of shedding for any biological material administered (e.g., feces, urine, saliva, respiratory droplets, bites).

b. List PPE used to reduce exposure risk when personnel handle the animal(s) listed.

c. Indicate the period of infectivity and shedding for any biological materials administered.

d. List aerosol generating activities involving biological materials and how an exposure will be mitigated.

e. Address any additional information that would facilitate a complete biosafety review.

F. Plants & Soils: NONE

Complete the relevant table(s) in this section if working with any of the following:

- Plants that are recombinant (transgenic), exotic, or grown in association with pathogenic or recombinant microbes or pathogenic or recombinant small animals (insects, etc.)
- Foreign soils or domestic soils from counties listed under federal quarantine by the USDA to another US location (see the USDA APHIS federal domestic soil quarantines map)

For Plant Biosafety Levels, see the UWL Biosafety Manual and NIH Guidelines, Appendix L.

Plant (Common name, Genus species)	**Plant Biosafety Level**	**Transgenic?** (Y/N)	**Biological Materials Administered**	**Growth Location** (e.g., greenhouse, growth chamber location)
1. not applicable				

2.				

Soil Source Location	Foreign or Quarantined Domestic?	Destination Location	Estimated Quantity	"Active" or Sterilized Soil?
1. not applicable				
2.				

a. If applicable, describe how pathogenic organisms will be stored.
b. If applicable, describe when and how pathogenic organisms will be disposed of at the termination of the study.

Additional permits and approvals:

- The import of foreign plant material requires a permit from the USDA. If applicable, approved permits must be attached to this protocol. See USDA APHIS plant import information.
- The import of foreign soils into the continental US requires a permit from the USDA. If applicable, approved permits must be attached to this protocol. See USDA APHIS soil import and permit information.
- The movement of domestic quarantined soil requires authorization by the local APHIS office. If applicable, approval must be attached to this protocol. Contact USDA APHIS PPQ for information regarding quarantine status, soil regulations, or movement eligibility.

Section III: Safety Precautions & Waste Disposal

A. Safety Precautions

Address the following information as applicable:

- Describe the methods for handling materials and/or organisms addressed in Section II.
- If you will be employing Biosafety Level 2, 3, or 4 materials, provide additional information about investigator experience, adequacy of facility design and containment equipment, personnel practices, decontamination and disposal, staff training, and chemical hygiene considerations.
- Address any additional required information as directed in relevant subsections from Section II.
- All laboratories using hazardous chemicals must take actions to minimize exposure to hazardous chemicals as defined in the UWL Chemical Hygiene Program (CHP) and Hazard Communication Policy. The Chemistry & Biochemistry and Microbiology Departments have department-specific CHPs. Hazardous chemical means any chemical that is classified as a physical hazard or a health hazard, a simple asphyxiant, combustible dust, pyrophoric gas, or hazard not otherwise classified. Contact Environmental Health and Safety for additional information.

The strain of *Salmonella enterica* serovar Typhimurium used in this lab (ATCC 14028) can cause gastroenteritis and diarrheal illness in healthy adults. According to the CDC BMBL 6th edition information on this strain of *Salmonella,* "Ingestion and parenteral inoculations are the primary laboratory hazards." (Section VIII-A: Bacterial Agents: *Salmonella* serotypes, other than *S. enterica* serotype Typhi (S. Typhi)). The BMBL also states that, when working with *Salmonella, "*It is recommended that special emphasis be placed on personal protective equipment, handwashing, manipulation of faucet handles, and decontamination of work surfaces to decrease the risk of LAI (laboratory-aquired infection)." (Section VIII-A: Bacterial Agents: *Salmonella* serotypes, other than *S. enterica* serotype Typhi (S. Typhi)). The following procedures follow this recommendation.

Research-Specific Methods for Handling Biohazardous Microorganisms

- Streaking of bacteria, including *Salmonella enterica* sv. Typhimurium, non-pathogenic *Escherichia coli* BL21(DE3), and non-pathogenic *Escherichia coli* NEB 5-alpha, on solid media in 10 cm plastic Petri plates will be performed on the open bench next to a Bunsen burner flame using autoclaved wooden toothpicks. Toothpicks will be discarded in biohazard waste containers.
- Plates will be incubated in microbiological incubators present in the lab, typically at 37 °C. To document bacterial growth, plates will be photographed within the laboratory. Plates will then be disposed of as solid biohazardous waste (see Section III.B below)
- *Salmonella enterica* sv. Typhimurium cultured in liquid media will be contained in glass test tubes with plastic caps at no more than 2 mL volume. These cultures will be incubated with orbital shaking. These incubators are located within the laboratory. Upon completion of each experiment, any remaining culture will be disposed of as liquid biohazardous waste. (see Section III.B. below)
- Non-pathogenic *Escherichia coli* BL21(DE3) harboring recombinant DNA will be used for recombinant protein production. These strains will be grown in liquid media in glass test tubes with plastic caps at 5 mL volume or in

glass Fernbach flasks at no more than 1 L volume and incubated with orbital shaking at 37 ºC. These incubators are located within the laboratory. Upon completion of each experiment, any remaining culture will be disposed of as liquid biohazardous waste and flasks will be disinfected.

- Non-pathogenic *Escherichia coli* NEB® 5-alpha will be used for production of recombinant DNA plasmids. These strains will be grown in liquid media in glass test tubes with plastic caps at 5 mL volume. Purification of recombinant plasmid DNA will be done using commercially available kits (such as GeneJET Plasmid Miniprep Kit) using manufacturer's protocol.
- Measurements of cell density will be taken using a Genesys 10S spectrophotometer located in the laboratory. The test tubes used for growth fit the measurement cell of the spectrophotometer. After measurements, bacterial cultures will be disposed of as liquid biohazardous waste.
- Centrifugation of *Salmonella enterica* sv. Typhimurium will be performed in rotors with aerosol-containing biosafety lids. Cultures will be centrifuged in plastic, disposable tubes with aerosol-containing lids. After centrifugation, supernatants will be disposed of as liquid biohazardous waste, and tubes as solid biohazardous waste.
- Electroporation to introduce recombinant DNA will use a plastic cuvette, which will then be disposed of as solid biohazardous waste.
- All work surfaces must be disinfected daily by wiping down with 10% bleach (prepared fresh daily) (see below, "Decontamination and Disposal".
- All procedures involving the manipulation of infectious *Salmonella* cultures that have a high potential of generating aerosols are conducted within a Biosafety Cabinet (BSC). These procedures relevant to this research include pipetting, opening containers of infectious materials, mixing, opening centrifuge rotors containing sealed plastic tubes of infectious materials, vortexing, and electroporation.
- Mouth pipetting is prohibited. All pipetting must be done using mechanical devices.
- All containers of biohazardous organisms, including Petri dishes, test tubes, centrifuge tubes, and flasks, will be labeled with the corresponding strain number to ensure proper identification of biohazards.
- Additional information about investigator experience, adequacy of facility design and containment equipment, personnel practices, decontamination and disposal, staff training, and chemical hygiene considerations is described below.

Investigator Experience with BSL2

The PI [redacted] has 20 years of experience working at Biosafety Level 2 (BSL2) and has 13 years of experience working specifically with *Salmonella enterica* serovar Typhimurium. The PI was trained to work with *Salmonella enterica* sv. Typhimurium during his postdoctoral research with [redacted]

Adequacy of Facility Design and Containment Equipment

The primary research space ([redacted]) has the following design features and containment equipment:

- Access to the laboratory is controlled. Laboratory doors are locked via key card swipe locks, and only authorized and trained personnel will have swipe card access.
- Doors are kept closed when not entering or exiting. Doors automatically lock upon closing.
- Laboratory Emergency Information door card must be posted at the entrance to the laboratory. The card is reviewed and dated annually for accuracy.
- A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory. The sign includes the biohazards in use and the name and phone number of the laboratory supervisor or other responsible personnel. The sign is reviewed and dated annually for accuracy.
- For hazards communication, all persons, including visitors and service personnel, entering the lab must be advised of the potential hazards and meet specific entry/exit requirements, such as donning and doffing of PPE.
- The lab contains two biosafety cabinets that are certified annually.
- Aerosol-tight centrifuge rotors with biocontainment lids are available for centrifugation of biohazardous materials.
- Stocks of biohazardous organisms and recombinant materials are stored long-term in –80 ºC freezers in vials inside a clearly marked sturdy box for long-term storage or in a refrigerator in lab on Petri dishes within a secondary container marked with the universal biohazard symbol. The outside of the freezers and refrigerator is marked with a universal biohazard symbol. The PI maintains an inventory of all strains and recombinant materials.

The supporting spaces ([redacted]) have the following design features. These spaces are used for growth of non-pathogenic *Escherichia coli* harboring recombinant DNA ([redacted]) or steam sterilization of solid biohazardous waste in autoclaves ([redacted]).

- Access to the laboratory is controlled. Laboratory doors are locked via key card swipe locks, and only authorized and trained personnel will have swipe card access.
- Doors are kept closed when not entering or exiting. The door to [redacted] automatically locks upon closing.

- Laboratory Emergency Information door card must be posted at the entrance to the laboratory. This card is reviewed and dated annually for accuracy.
- A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory. The sign includes the biohazards in use and the name and phone number of the laboratory supervisor or other responsible personnel. This sign is reviewed and dated annually for accuracy.
- For hazards communication, all persons, including visitors and service personnel, entering the lab must be advised of the potential hazards and meet specific entry/exit requirements, such as donning and doffing of PPE.

Personnel Practices

Minimal Personal Protective Equipment (PPE) Requirements for Entry into Laboratory

- Closed-toe shoes and clothing that covers the leg down to the shoe must be worn in the laboratory at all times.
- Eye protection (safety glasses or chemical goggles) must always be worn in the laboratory. Contact lenses may be worn with discretion and in combination with eye protection. Depending on the activities, it may be appropriate to use safety glasses with side shields, goggles, and/or a splash shield.

Additional PPE Requirements for Handling Biohazardous Materials

- Laboratory coats must be worn. To avoid bringing hazardous materials out of the laboratory, lab coats must be removed before exiting and remain in the laboratory. Lab coats should be laundered onsite or through a laundry service and never taken home for cleaning.
- Disposable gloves must be worn whenever there is the potential for contact with hazardous materials. They further serve to maintain the integrity of the material being handled. Gloves must be removed in a manner that prevents contamination of hands. Gloves should be removed before exiting the laboratory. Disposable gloves should not be reused.
- Use of the above PPE is particularly important because ingestion of contaminated materials is one of the primary laboratory hazards when working with *Salmonella*.

Lab Practices

- For hazards communication, all persons, including visitors and service personnel, entering the lab must be advised of the potential hazards and meet specific entry/exit requirements, such as donning and doffing of PPE.
- All personnel will don minimal entry PPE as listed above prior to entry.
- No food or drink is allowed in lab. No eating or drinking is allowed. This is particularly important because ingestion of contaminated materials is one of the primary laboratory hazards when working with *Salmonella*. All food or drink containers must be left outside of the lab prior to entry.
- Don extra PPE according to activities being performed.
- Mouth pipetting is prohibited; pipetting devices must be used.
- Procedures for safe handling of non-medical sharps (e.g., pipettes, broken glassware) must be followed. The proposed research does not involve the use of medical sharps (e.g., needles, scalpels), which minimizes the risk of parenteral inoculation, the other primary laboratory hazard when working with *Salmonella*.
- Plastic ware should be substituted for glass wherever possible.
- Potentially infectious materials must be placed in a durable, leak-proof container during collection, handling, processing, storage, or transport within the laboratory.
- Procedures must be performed to minimize the creation of splashes and/or aerosols.
- All procedures involving the manipulation of infectious materials that have a high potential of generating aerosols are conducted within a Biosafety Cabinet (BSC). These procedures relevant to this proposed research include pipetting, opening containers of infectious materials, mixing, opening centrifuge rotors containing sealed plastic tubes of infectious materials, vortexing, and electroporation.
- Centrifugation will be performed using autoclavable, aerosol-tight rotors and safety cups. The PI's lab is equipped with centrifuge rotors that have biocontainment lids.
- Spills involving infectious materials must be contained, decontaminated, and cleaned up by laboratory personnel properly trained and equipped to work with infectious material.
- Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety protocol.
- Decontaminate work surfaces after completion of work and after any spill or splash of potentially hazardous material with an effective disinfection method.
- Persons must wash hands with soap and warm water before leaving the laboratory. Special care must be taken to avoid manipulating faucet handles with gloved hands or with biohazardous materials. Proper hand-washing is particularly important because ingestion of contaminated materials is one of the primary laboratory hazards when working with *Salmonella*.

- Practices to prevent spills and potential exposures during movement of materials in hallways, elevators, or public spaces are in place. These include using a cart to transport materials and transporting materials in a closed primary container and in a secondary container that is durable, leak proof, labeled, and surface disinfected.
- Animals and plants are not permitted in the laboratory. Note that the proposed research does not involve the use of animals or plants.
- Practices to reduce pests, mold, fire hazards are in place (e.g., reduce cardboard, reduce clutter).

Decontamination and Disposal

- Surfaces and equipment will be chemically disinfected with a freshly made solution of household bleach (sodium hypochlorite) at 10% (v/v) final concentration or 70-90% (v/v) ethanol. At least 10 minutes of contact time must be used for disinfection. The 10% bleach solution must be made fresh daily.
- Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
- Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
- Proper decontamination is particularly important because ingestion of contaminated materials is one of the primary laboratory hazards when working with *Salmonella*.
- All cultures, stocks, and other forms of potentially infectious or recombinant materials must be inactivated using an effective method by laboratory personnel before disposal. (see "Decontamination Procedures" above)
- Disposal of biohazardous waste is described below in Section III B

Staff Training

The PI is responsible for ensuring that all personnel working with biohazardous or recombinant materials in their laboratory complete the required biosafety training listed in Section VI.A below. Laboratory specific training for all personnel is also listed below in Section VI.A.

Chemical Hygiene Considerations

As a member of the Department [redacted], the PI follows the safety procedures and practices described in the departmental Chemical Hygiene Plan. Students working in lab also receive specific chemical safety training through a course offered at the beginning of each semester by the Department [redacted].

Describe the safety procedures personnel will use to protect themselves from exposure and appropriate response if accidental exposure occurs.

- The PPE and Lab Practices described above will be used to protect personnel from exposure.
- The response to a spill or accidental exposure is described below in "Spill and Exposure Response."

Spill and Exposure Response

Spill Response Kit

Basic equipment is concentrated disinfectant (chlorine bleach), a package of paper towels, household rubber gloves, an empty spray bottle for preparing fresh solutions of 10% bleach, biohazard bags, and forceps to pick up broken glass. Contents of the kit will be kept in a plastic container in the cabinet under the sink. The outside door of the cabinet will be labeled with the words "Biological Spill Kit".

Biosafety Level 2 (BSL2) Spill Response

1. Avoid inhaling airborne material, while quickly leaving the room. Notify others to leave. Close the door, and post a warning sign outside of the door.
2. Remove contaminated clothing, turn exposed areas inward, and place in a biohazard bag.
3. Wash all exposed skin with soap and disinfectant.
4. Inform the PI.

Clean-up of BSL2 Spill

1. Allow aerosols to disperse for at least 30 minutes before re-entering the laboratory. Assemble clean-up materials (disinfectant, paper towels, biohazard bags, and forceps).
2. Put on protective clothing (lab coat, face protection, utility gloves if necessary).
3. Cover the area with disinfectant-soaked paper towels, and then carefully pour disinfectant around the spill. Avoid enlarging the contaminated area. Use concentrated disinfectant as it is diluted by the spill. Allow at least a 30 minutes contact time.
4. Handle any sharps objects with forceps and discard in a sharps container. Wipe surrounding areas (where the spill may have splashed) with disinfectant.
5. Soak up the disinfectant and spill, and place the materials into a biohazard bag.

6. Spray the area with 10% household bleach solution (make the 10% bleach solution fresh daily as needed). After at least a 30 minute contact time, wipe down with bleach-soaked paper towels. Place all contaminated paper towels and any contaminated protective clothing into a biohazard bag and autoclave.
7. Wash hands and exposed skin areas with soap and water.

Medical Assessment and Exposure Monitoring

Individuals possibly exposed to biohazardous *Salmonella enterica* sv. Typhimurium used in this research must seek immediate medical assessment. The following information about the strain used in this research should be shared with a medical provider:

- According to CDC BMBL 6th edition, disease caused by the non-typhoidal strain of *Salmonella enterica* used in this research "usually presents as acute enterocolitis (fever, severe diarrhea, abdominal cramping), with an incubation period ranging from six to 72 hours, most often lasting four to seven days and patients tend to recover without treatment.
- Antimicrobial therapy is not recommended for uncomplicated *Salmonella*-related gastroenteritis" (Section VIII-A: Bacterial Agents: *Salmonella* serotypes, other than *S. enterica* serotype Typhi (S. Typhi)). Also, according to the *2017 Infectious Diseases Society of America Clinical Practice Guidelines for the Diagnosis and Management of Infectious Diarrhea* (Clinical Infectious Diseases, Volume 65, Issue 12, 15 December 2017, Pages e45–e80, https://doi.org/10.1093/cid/cix669), antimicrobial therapy is not recommended for uncomplicated infections with non-typhoidal *Salmonella enterica.*
- Certain groups are at risk of invasive infection, including "persons >50 years old with suspected atherosclerosis, persons with immunosuppression, cardiac disease (valvular or endovascular), or significant joint disease." For these groups, ciprofloxacin is listed as an effective antimicrobial therapy.
- The wild-type strain used in this research has no engineered antibiotic resistance, and mutant derivatives have genetically engineered resistance to chloramphenicol or kanamycin. *Salmonella* strains harboring recombinant DNA (pBR322-*dcrB*) will have plasmid-encoded resistance to beta-lactam antibiotics, including ampicillin and carbenicillin.

B. Waste Disposal & Terminal Inactivation

In the table below, describe the method of disposal of hazardous substances, animal wastes and carcasses, and residual human substances (e.g., incineration, autoclaving, chemical disinfection). If chemical disinfectant is used, state kind and concentration. Is autoclave monitored with a biological indicator (e.g., spore strips)?

Substance	Disposal method	Description of procedure
Petri dishes containing solid agar-based bacteriological media inoculated with *Salmonella enterica* sv. Typhimurium, including strains harboring recombinant DNA; or with non-pathogenic *Escherichia coli* harboring recombinant DNA from *Salmonella enterica*; plastic pipet tips used for pipetting small volumes (<1000 µL) of cultures of *Salmonella enterica* sv. Typhimurium.	Steam sterilization by autoclaving	Solid biohazardous waste is collected in orange biohazard bags inside of study plastic buckets marked on the outside with the universal biohazard symbol. When no more than ¾ full, the bag is closed, sealed with autoclave indicator tape, and transported in a leak-proof container on a cart to be autoclaved in Rm 4011 Prairie Springs Science Center, which is a shared use autoclave facility. The bag containing the biohazardous waste is sterilized in a Beta Star steam sterilizer (autoclave) for 90 minutes at 121 ºC at 15.7 psi. Detailed procedures for autoclaving, including sterility monitoring with spore strips, autoclave training procedures, and disposal of sterilized waste, are attached with Supporting Materials (Section VI.B.): Autoclave Standard Operating Procedure.

Liquid media containing *Salmonella enterica* sv. Typhimurium including strains harboring recombinant DNA; or with non-pathogenic *Escherichia coli* harboring recombinant DNA from *Salmonella enterica.*	Chemical disinfection followed by disposal in a sanitary drain within the lab	Liquid media is treated with bleach (sodium hypochlorite) at 10% final concentration for at least 30 minutes to disinfect. This procedure exceeds the minimum treatment (0.2% bleach for 10 minutes) that reliably kills *Salmonella* [Rutala WA *et al.* (1998) Stability and bactericidal activity of chlorine solutions. *Infect Control Hosp Epidemiol*. 19: 323–327.] The 10% bleach solution is made fresh daily. After treatment, the disinfected liquid media will be disposed in a sanitary drain within the lab followed by a water rinse of the sink with sufficient volume of water to remove all disinfected media from the sink and the sink trap.

Section IV: Personnel

In the table below, identify all personnel, including students, who will be working on the biological materials described in this protocol. Copies of required CITI training completion certificates for the PI and all individuals listed below must be included as an attachment (see Section VI. Attachments). For training requirements, see the IBC website.

Teaching laboratory courses: Students enrolling in laboratory courses do not need to be listed below and are not required to complete CITI training modules unless directed by the instructor. Instead, summarize the training provided to students who will be involved in the course (e.g., hands-on training, instructor-based training, online learning) and include the summary as an attachment (see Section VI. Attachments).

Name	Personnel Type	Project Role (e.g., PI, co-PI, research assistant)
[redacted]	[redacted]	Senior key personnel, research assistant
	Choose an item.	
	Choose an item.	
	Choose an item.	
	Choose an item.	
	Choose an item.	
	Choose an item.	
	Choose an item.	
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	Choose an item.	
	Choose an item.	
	Choose an item.	

Section V: PI Assurances

I certify that the information contained in this application is accurate and complete. I am familiar with and agree to abide by the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (current edition), CDC Biosafety in Microbiological and Biomedical Laboratories (current edition), and University of Wisconsin-La Crosse (UWL) Biosafety Manual. Also, I agree to abide by the following requirements:

a. I will not initiate any biological research subject to the guidance and guidelines mentioned above until that research has been registered, reviewed, and approved by the UW-La Crosse (UWL) Institutional Biosafety Committee (IBC). The purview of the UWL IBC includes biological research involving recombinant or synthetic nucleic acids; biological agents and pathogens; human cells, tissues, materials and embryonic stem cells; non-human animal-derived cells, tissues, materials, or samples that are infectious, potentially infectious, or recombinant; animals or plants that are recombinant, exotic, and/or grown in association with pathogens, biological toxins, and/or recombinant materials; select agents and toxins; biological toxins; dual use research of concern (DURC) agents and toxins; and the use of any of these in animal or plant research.
b. I will assure that personnel, including animal care staff or other laboratory support staff, have received appropriate information, including signage, about the biological hazards of the research outlined in this application by making available copies of approved protocols, Biosafety Manuals, and Biological Research Registrations that describe the potential biohazards and precautions to be taken to prevent exposures or release to the laboratory or the environment.
c. I will ensure that laboratory personnel understand the procedures for dealing with incidents and spills of biological materials and know the appropriate waste management procedures.
d. I will work with appropriate university personnel to comply with all training and shipping requirements for the transport of hazardous biological materials (e.g., export controls regulations, US Department of Transportation (DOT) 49 CFR 171-178, International Civil Aviation Organization (ICAO), International Air Transport Association (IATA), US Department of Agriculture (USDA) 9 CFR 122).
e. I will comply with the OSHA Bloodborne Pathogen Standard 29 CFR 1910.1030 if my research includes human cells, tissues, materials, or embryonic stem cells.
f. I will ensure that all laboratory personnel working with biological materials are listed on this application.
g. I will assure that I along with all laboratory personnel have completed all required biosafety training and that their training records are up to date.
h. I assure that all laboratory spaces associated with the research and/or instruction described in this application are listed.
i. I am familiar with and understand my responsibilities as a Principal Investigator as outlined in Section IV-B-7 of the NIH Guidelines.
j. I will assure adequate supervision of personnel and will correct work errors and conditions that could result in breaches of the guidelines and regulations pertaining to this research as listed above.

I understand that failure to adhere to all related requirements may result in penalties outlined in federal and state regulations, sponsor guidelines, and institutional policies such as the IBC Noncompliance Policy.

[redacted]	
Principal Investigator Signature	**Date**

Section VI: Attachments

A. Training

Attach copies of the required CITI training completion certificates for the PI and all individuals, including students, listed in Section IV. Personnel. For training requirements, see the IBC website.

Teaching laboratory courses: Students enrolling in laboratory courses are not required to complete CITI training modules unless directed by the instructor. Instead, summarize the training provided to students who will be involved in the course (e.g., hands-on training, instructor-based training, online learning) and include the summary as an attachment or in the space below.

The CITI training certificates for [redacted] are attached.

Students working on this project will be enrolled [redacted] during the academic year. Students enrolled in these courses will receive the following biosafety training:

- Students will be required to complete the following CITI training modules prior to working with biohazardous materials: Biosafety Course Overview (13314), Laboratory-Acquired Infections (13454), Risk Management: Work Practices (13898), Risk Management: Personal Protective Equipment (13458), Risk Management: Emergency and Spill Response (13459), Work Safely with Sharp Instruments (13899), Disinfection and Sterilization (13900), and NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (13493)
- All personnel, including students, handling biohazardous material will receive an electronic copy of this biosafety protocol in a shared lab folder on a cloud platform such as Microsoft OneNote.
- The PI will personally review this protocol with new personnel, including students.
- A paper copy of the protocol will be posted on a bulletin board in lab.
- The PI will provide hands-on safety instruction for biohazardous procedures to all personnel, including students.
- The PI will ensure that laboratory personnel, including students, demonstrate proficiency in standard and special microbiological and laboratory techniques/practices before working with biohazardous materials.

B. Supporting Materials

Attach plasmid maps and/or any other supporting materials as instructed by applicable subsections in Section II.

The following supporting materials are attached:

- plasmid map for pBR322-*dcrB*
- plasmid map for pET-22b(+)-*His6-dcrB-del37*
- plasmid map for pET-22b(+)-*SePhoN-GSSHis8*
- Standard Operating Procedure for autoclave usage