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: *The effects of pH and osmotic stress on Escherichia coli virulence*

Course Number & Name[2]: [redacted]

Project Type: ☒Research ☒Teaching

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

If a renewal or revision:
Protocol number: [redacted]
Summarize change(s): Rooms, plasmids used, and procedures used have been updated
List revised protocol section(s): *In vitro testing of gene regulation*

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
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.
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]	Nanodrop Machine CFX Connect Real-Time Machine Autoclaves Biosafety Cabinet II, freezers (-20 and -80 C), refrigerator Centrifuges Spectrophotometer
[redacted]	[redacted]	
Click or tap here to enter text.	Click or tap here to enter text.	Click or tap here to enter text.

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.

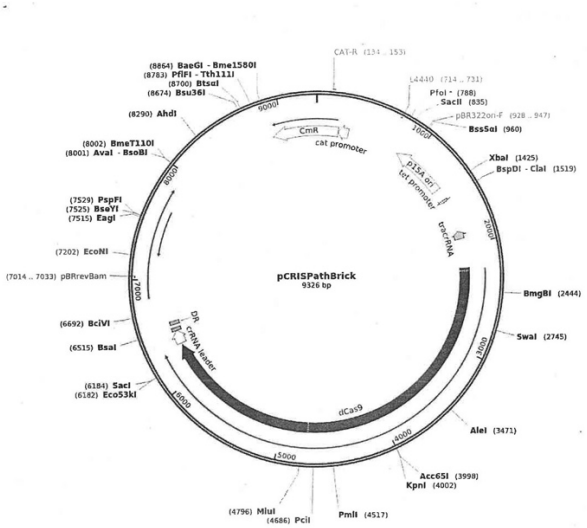
Research Objectives:
We are seeking an amendment to IBC protocols [redacted] that will allow us to use uropathogenic *Escherichia coli* in [redacted] for assays to determine virulence *in vitro*. The amendment will also cover rDNA or RNA usage in [redacted] In this amendment, we wish to work with the uropathogenic *E. coli* strains [redacted].

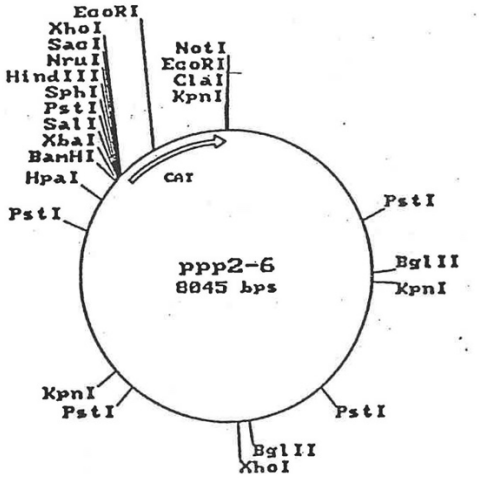
[1] Only a UWL faculty or staff member may be listed as the PI on a Biosafety Protocol Application. All other project personnel, including students, must be listed in Section IV. Personnel.
[2] If lab courses involve recombinant materials, they are subject to NIH Guidelines, and a protocol is required. 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.

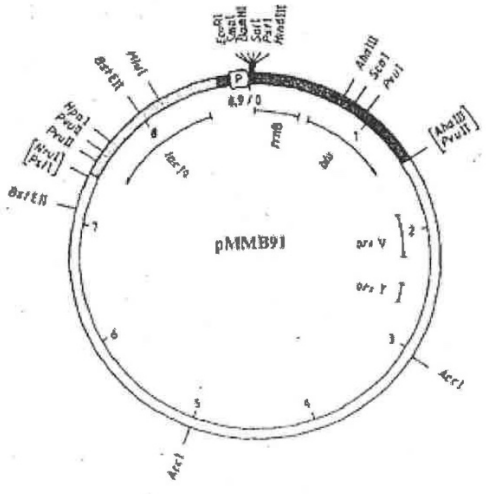
The PI has 38 years of experience working with uropathogenic *E. coli* and other BSL-2 bacterial species. In the past, the PI has followed NIH guidelines regarding safety precautions for the BSL-2 organisms. The PI has had 38 years of experience doing molecular manipulation with BSL-2 bacteria.

Construction of recombinant plasmids

For creation of *E. coli* recombinant plasmids, virulence factor genes will be PCR amplified from *E. coli* strain NU149 or UTI89 DNA in Room 4014 PSSC. Each PCR product will be designed to have restriction endonuclease sites flanking the DNA for ligation into plasmids pPP2-6, pCRISPPathBrick, or pMMB91. The DNA will be cut with the appropriate restriction endonuclease and ligated to plasmid DNA also cut with the appropriate restriction endonucleases. Ligated DNA will be transformed into *E. coli* DH5α cells, selecting for ampicillin resistance for the pMMB91 plasmid or chloramphenicol for the pPP2-6 and pCRISPPathBrick plasmids. Transformants containing the right virulence factor gene will be verified by restriction endonuclease digests. Upon verification, purified plasmid DNA will be electroporated into *E. coli* strains NU149 or UTI89. Electroporation cuvettes are plastic single use and disposed of into an autoclave bag. Colonies will be selected on Luria agar (LA) plates containing chloramphenicol or ampicillin. Plates will be discarded into an autoclave bag for autoclaving after use. Previous studies have shown *E. coli* can develop resistance to both chloramphenicol and ampicillin naturally.







Growth of the bacteria

The wild-type *E. coli* strains will be propagated on LA plates or in Luria–Bertani (LB) without antibiotic. Strains containing recombinant plasmids with the pMMB91 backbone will be grown on LA or in LB with 100 μg/mL of ampicillin (Sigma Aldrich). Strains with the recombinant plasmid pPP2-6 or pCRiSPPathBrick will be grown on LA or in LB with 12.5 μg/mL of chloramphenicol (Sigma Aldrich). Broth cultures will be autoclaved in their glass tubes when done. Agar plates will be discarded into autoclave bags after use to be autoclaved. Both uropathogenic *E. coli* strains pose a minimal aerosol risk.

In vitro testing of gene regulation

To measure differences in transcription for specific genes, total RNA will be extracted from *E. coli* NU149 or UTI89 cells grown with shaking to mid-exponential phase (OD_{600} 0.5–0.8) at 37 ^{0}C in pH 5.5/low osmolality LB medium, pH 5.5/high osmolality (800 mOsm NaCl) LB medium, pH 7.0/low osmolality medium or pH 7.0/high osmolality (800 mOsm NaCl) LB medium in Room 4014 PSSC. The bacteria will be grown in 25 mm glass tubes in a volume of 10 ml. Cell growth will be measured on the spectrophotometer [redacted]. Bacterial samples will be decanted into a beaker containing Quatsyl after measuring the absorbance and let stand in the Quatsyl for 15 min. Bacterial broth cultures will be pelleted by centrifugation in closed bottles or tubes with aerosol-containing lids [redacted]. A commercial RNA extraction kit (Roche) with 50 μl lysozyme (10 mg/ml) added to help break the cell wall in [redacted]. Plasticware used in the procedure will be placed in an autoclave bag. Purity of the RNA preparations will be assessed using the Nanodrop machine [redacted]. Used micropipette tips will be discarded into an autoclave bag for autoclaving. Nanodrop machine will be wiped with 70% ethanol after use. For each RNA sample, 2 μg of RNA will be converted to cDNA using a SuperScript First-Strand Synthesis kit (Invitrogen) following the protocol recommended by the manufacturer [redacted]. With the cDNA preparations, quantitative reverse transcribed-polymerase chain reaction assays will be performed using an iTaq SYBR green Kit (BioRad) [redacted]. These samples will be run on the CFX Connect Real-Time System thermal cycler in [redacted]. Samples will be discarded into an autoclave bag when done. Personnel will wear nitrile gloves during each step.

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. Non-toxin and non-oncogene virulence factor genes (see Appendix A)	Non-toxin (see Appendix A)	*E. coli* NU148 and UTI89	2	pPP2-6, pCRISPPathBrick & pMMB91	*E. coli* DH5α	1
2. Non-toxin and non-oncogene virulence factor genes (see Appendix A)	Non-toxin (see Appendix A)	*E. coli* NU148 and UTI89	2	pPP2-6, pCRISPPathBrick & pMMB91	*E. coli* NU148 and UTI89	2

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

For plasmids with the pPP2-6, pCRISPPathBrick, and pMMB91 backbones, Section III-D-1-a (experiments involving introduction of recombinant or synthetic nucleic acid into Risk Group 2 *E. coli* strains NU149 and UTI89) applies.
For plasmids with the pPP2-6, pCRISPPathBrick, and pMMB91 backbones, Section III-D-2-a (experiments involving introduction of recombinant or synthetic nucleic acid from Risk Group 2 *E. coli* strains NU149 and UTI89 into nonpathogenic prokaryote *E. coli* strain DH5α) applies.
Genes that will be studied are all non-toxin and non-oncogenic (see Appendix A).

b. Attach plasmid maps as part of the protocol in Section VI: Attachments.

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. Uropathogenic *E. coli* strains NU149 and UTI89	Yes	2	2	Yes, non-toxin virulence genes on pPP2-6, PMMB91, or pCRISPPathBrick plasmid	No
2. *E. coli* strain DH5α	No	1	1	Yes, non-toxin virulence genes on pPP2-6, PMMB91, or pCRISPPathBrick plasmid	No

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

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

[redacted]

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 information requested.

C. Human and Non-Human Animal Tissues, Cell Lines, & Blood Products

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. NA					
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

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. NA						
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

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. NA					
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

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. NA				
2.				

Soil Source Location	**Foreign or Quarantined Domestic?**	**Destination Location**	**Estimated Quantity**	**"Active" or Sterilized Soil?**
1.				
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 *E. coli* strains NU149 and UTI89 were isolated from patients with urinary tract infections. Both strains have a low risk of aerosol transmission. Emphasis is placed on personal protection, handwashing, and decontamination of work surfaces to decrease the risk of laboratory-acquired infection.

Investigator BSL-2 Experience

The PI has 38 years of experience working with uropathogenic *E. coli* and other BSL-2 bacterial species. In the past, the PI has followed NIH guidelines regarding safety precautions for the BSL-2 organisms. The PI has had 38 years of experience doing molecular manipulation with BSL-2 bacteria. Physical containment will be for a Biosafety Level 2 organism, following Appendix G-II-B of the NIH Guidelines.

Personnel Safety Practices

1. Laboratory personnel will always wear a laboratory coat, safety glasses, and nitrile gloves.
2. Closed-toe shoes and clothing down to their ankles is also required.

Adequacy of Facility Design and Containment Equipment

1. The PI's laboratory [redacted] has controlled access. Doors are shut and locked allowing only authorized users entry.
2. Emergency laboratory information is posted on the exterior of the laboratory.
3. A biohazard sign is posted on the exterior doors.
4. Non-authorized visitors are appraised of the biosafety hazards.
5. The laboratory houses two Biosafety Cabinets II that are certified annually.
6. Centrifuge rotors have aerosol-tight lids.
7. Stock cultures of the bacteria and recombinant nucleic acids are housed in either the -80 C or -20 C freezer[redacted].
8. Working cultures of bacteria and recombinant nucleic acid are housed in the refrigerator [redacted].
9. A biohazard sign is posted on the outside of each piece of equipment.
10. An inventory is kept of bacterial strains.

Laboratory Safety Practices

1. Laboratory personnel put on laboratory PPE upon entry
2. No eating, drinking, applying makeup, or smoking is allowed in the laboratory.
3. Access to the laboratory is restricted and the laboratory door is kept shut and locked when unoccupied.
4. No mouth pipetting.
5. Disinfection with Quatsyl after use of all bench tops will be done.
6. Procedures are done to minimize aerosol transmission.
7. Procedures that have a high potential of creating an aerosol will be performed in a Biosafety Cabinet II in the laboratory.
8. A spill protocol is in place in the laboratory detailing how to clean up all spills that result (see Appendix B).
9. All material contaminated with whole cells or nucleic acids from the bacteria will be autoclaved. Contaminated disposable materials will be closed before being removed from the laboratory. Decanted supernatants will be chemically disinfected as noted below.

10. Special care is taken to avoid skin contamination with organisms containing recombinant DNA through use of protective gloves.
11. Personnel wash their hands with soap and water before exiting the laboratory.
12. Rodent and insect control plans are in place for the building.
13. A standard operating procedure is posted in the laboratory in case of exposure to needle sticks and cuts.
14. Laboratory dry waste is collected in a large autoclavable bag and sealed shut when it becomes ¾ full. The bag is put into a large plastic tray and transported to the autoclave room using a rolling cart to be autoclaved for 90 min.
15. Spill and injury Incidents are reported to the PI.

Decontamination and Disposal

1. Surfaces and equipment are decontaminated with Quatsyl described in Section III-B.
2. Equipment is decontaminated with Quatsyl before it is repaired or removed from the laboratory.
3. All cultures and recombinant DNA are inactivated by either autoclaving or treatment with Quatsyl as described below.

Staff Training

1. All students in the laboratory have to watch a basic laboratory safety video and score 80% on a safety quiz before working in the laboratory.
2. Students have an official autoclave training check list completed before using the autoclaves.
3. Other laboratory and biological safety procedures will be taught by the PI to ensure that proper safety precautions are followed.

Chemical Hygiene Considerations

The PI follows the departmental Chemical Hygiene Plan. Students are required to pass a safety quiz that in part deals with chemical safety.

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
E. coli liquid and agar cultures as well as contaminated glassware	Autoclaving	Autoclaving of all contaminated material in a Beta Star autoclave, monitoring with autoclave spore strips
E. coli spills or decanted broth cultures	Chemical disinfection	Apply Quatsyl at 2 ml/4 liters concentration for 15 min.

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]	[redacted]
	Choose an item.	
	Choose an item.	
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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.

Protocol #:

[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.

B. Supporting Materials

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

Appendix A

List of non-toxin and non-oncogenes that will be studied for this project:

fimA	Encodes bacterial structural protein FimA
fimB	Encodes bacterial regulatory protein FimB
fimE	Encodes bacterial regulatory protein FimE
fliC	Encodes bacterial structural protein FliC
ompR	Encodes bacterial regulatory protein OmpR
envZ	Encodes bacterial regulatory protein EnvZ
gadA	Encodes bacterial enzyme protein GadA
gadB	Encodes bacterial enzyme protein GadB
gadE	Encodes bacterial regulatory protein GadE
gadX	Encodes bacterial regulatory protein GadX
gadW	Encodes bacterial regulatory protein GadW
cya	Encodes bacterial enzyme protein Cya
crp	Encodes bacterial regulatory protein Crp
rpoS	Encodes bacterial regulatory protein RpoS
adiA	Encodes bacterial regulatory protein AdiA
omrA	Encodes bacterial small regulatory RNA OmrA
omrB	Encodes bacterial small regulatory RNA OmrB
frzR	Encodes bacterial regulatory protein FrzR
yicJ	Encodes bacterial structural protein YicJ
yicI	Encodes bacterial enzyme protein YicI
xylA	Encodes bacterial enzyme protein XylA
xylR	Encodes bacterial regulatory protein XylR
nhaC	Encodes bacterial structural protein NhaC
aspC	Encodes bacterial enzyme protein AspC
putA	Encodes bacterial regulatory protein PutA
putP	Encodes bacterial structural protein PutP
rcsB	Encodes bacterial regulatory protein RcsB
rcsC	Encodes bacterial regulatory protein RcsC
rcsD	Encodes bacterial regulatory protein RcsD

Appendix B

[redacted] Lab Spill Protocol

For spills on inanimate surfaces:

1. Avoid inhaling airborne material as you leave the room, notify others in the laboratory, and close the door to wait for 30 min.
2. Allow aerosols to disperse for at least 30 min before returning to the laboratory
3. Put on personal protective equipment (lab coat, safety glasses, and nitrile gloves) and squirt Quatsyl on affected inanimate surface.
4. Let Quatsyl sit for 15 min on affected surface.
5. Wipe up Quatsyl/spill with paper towel and dispose in infectious waste receptable with autoclave bag.
6. Notify Dr. [redacted]

For spills on skin or laboratory clothing:

1. Avoid inhaling airborne material.
2. Remove contaminated clothing, squirt Quatsyl on the affected clothing, and place in biohazard bag to be autoclaved.
3. Wash hands and affected skin surface with soap and water as well as notify others in the laboratory.
4. Notify Dr. [redacted]
5. File an incident report if there is overt exposure.