Sheets/Heikal Group Laboratory Safety Plan (LSP) Toolkit

04012024

The Laboratory Safety Plan (LSP) is a supplement to the UMN Chemical Hygiene Plan. It is a template for research laboratories at UMN to state their lab-specific safety policies, procedures, and documentation. This web toolkit will give you specific guidance as to the content required in an LSP and will give you resources to help you complete those sections.

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Administrative

Lab Safety Contacts

Laboratory safety contacts should be identified and their contact information (i.e. name, phone number, e-mail address, etc.) should be kept on file in your LSP.

PI	Name: Erin Sheets, Ahmed Heikal Phone Number: 218-343-0405 (EDS), 218-343-0624 (AAH) E-mail: edsheets@d.umn.edu, aaheikal@d.umn.edu
Lab Safety Officer (LSO)	Name: Erin Sheets (see above) Phone Number: E-mail:
Department Safety Officer (DSO) **[Link to DSO Master List]**	Name: Greg Mielke Phone Number: 218-726-8308 E-mail: gmielke@d.umn.edu
UHS Research Safety Partner **[Link to RSP Contact page]**	Name: Rachael Perriello, Andrew Kimball Phone Number: 218-726-7273 (RP), (218) 726-6764, (763) 226-7011 (AK) E-mail: rachaelp@d.umn.edu, akimball@d.umn.edu

Resources:

UHS Lab and Research Safety page

Role-Based Safety Guidance (Roles and Responsibilities)

Department Safety Officer (DSO) Master List

Laboratory Working Hours and Working Alone Policy

The UMN Chemical Hygiene Plan states that researchers should limit work after hours (i.e. nights and weekends) to non-hazardous activities such as data analysis and report writing. If hazardous materials or equipment must be used during non-working hours or when the user is alone, training must be provided and documented by their PI as part of their lab-specific training. Any work alone or after-hours requires the PI's approval. Persons under 18 years of age are not allowed to work alone at any time.

Graduate students have 24/7 access to the lab. If doing experiments, a partner is required to be present in the lab. All laser-based experiments require a partner in the lab. Data analysis or inoculating a bacterial culture can be done alone. Undergraduate researchers cannot work alone in the lab. They are allowed to work in the computer lab for data analysis alone.

Resource: Working Alone Fact Sheet

Personal Protective Equipment (PPE) Requirements

All UMN workers are required to wear long pants/skirts and closed toe/heel shoes while in an area where hazardous materials are stored and used. Some laboratories require additional PPE depending on the hazards and the work conducted there.

Safety glasses and gloves are required whenever working in the main laboratory or when sitting at desks and others are working. When working with rDNA, E. coli or mammalian cells, laboratory coats are also required. Laser work requires the appropriate laser safety glasses.

Resource: PPE Selection Guide

Door Signage

The required UMN comprehensive lab sign provides emergency responders, Facilities Management staff, and visitors with information regarding potential laboratory hazards, required precautions for entry, and contact information. It fulfills several hazard posting requirements and makes hazard communication at the University of Minnesota more standardized and recognizable.

Signs are posted by the main lab and the laser labs.

Resource: Signage Requirements – UMN CHP

Hazard Communication and Labeling

All chemicals in the laboratory are required to have a label that indicates chemical contents and hazard warnings. Labs are responsible for labeling chemicals that are transferred from manufacturer containers into a secondary container or chemicals that are synthesized in the lab.

Exemptions: Chemicals that will be used within one work shift and will not be unattended during the work period of their intended use.

Bottles are label with initials, date, and contents. These are stored with secondary containment.

Resources:

Non-Manufacturer Container Labeling Fact Sheet

Sample Chemical Abbreviation Key

Hazard Class Labels

Unattended Operations

Chemical Inventory

All laboratories at UMN are required to keep an inventory of their hazardous chemicals and reconcile it annually.

UHS in the process of implementing the chemical inventory module in Chematix, our safety management software. Labs will be contacted by their Research Safety Partner and Department Safety Officer when it is their turn to transfer their chemical inventory into Chematix. If you have questions about this process, contact your Research Safety Partner directly.

The chemical inventory is in Quartzy.

Hazard Assessments

Hazard assessments are important and must be performed on procedures that are new to the laboratory or have not been studied previously. The level of formality and what you need to document will depend on the process being examined.

Use the following resources to perform hazard assessments for your laboratory procedures. Your Research Safety Partner can help you with this process. All hazard assessment forms should be documented here in your LSP.

Resources:

ACS "Identifying and Evaluating Hazards in Research Laboratories" Document

Hazard Assessment in Research Laboratories

Prudent Practices in the Laboratory, Chapter 4 Evaluating Hazards and Assessing Risks in the Laboratory

Laboratory Safety for Chemistry Students by Hill Finster, Wiley 2010

UMN Chemical Hygiene Plan – Experiment Planning and SOPs

Standard Operating Procedures (SOPs)

An SOP is a documented set of instructions, used to standardize a method and communicate hazards for a specific procedure, process, chemical class, chemical or piece of equipment. Below are specific examples of where SOPs may be required. Lab-specific SOP is attached at the end of this document.

Chemical Classes

Commonly used chemical hazard classes are often treated in a similar manner, and a Hazard Class SOP is sufficient to document the safe use and handling of the entire class. University Health and Safety have created hazard class SOP templates with general recommendations of safe use and storage of several hazard classes of chemicals. As always, customize the SOP to fit your own lab-specific limitations.

Examples: flammables, oxidizers, reactives, corrosives, compressed gases, toxics

Individual Chemicals

Certain high-hazard chemicals may require their own SOP, especially if special working procedures are required or if the hazard posed by the chemical requires special emergency treatment upon exposure.

Examples: hydrofluoric acid, osmium tetroxide

Equipment

Some laboratory equipment may require the use of an SOP for safe and proper use. Consider SOPs for equipment that involve high hazard operations (i.e. high/low temperature, high/low pressure, etc.)

Examples: UV lights, rotary evaporators, glove boxes, anaerobic chambers, lasers

Chemical Process or Procedures

Common lab chemical processes or procedures may require an SOP depending on the hazard level and the desire for reproducibility. For hazardous lab processes, an SOP should document the finding of a hazard assessment of the process.

Examples: acid digestion, acid or base cleaning baths, hydrogenation reactions

Resources:

Hazard Class SOP Templates

Animal Work (IACUC) Resource Folder

Emergency Procedures

Lab Emergency Preparedness Plan

The lab Emergency Preparedness Plan (EPP) provides lab occupants with room-specific instructions on what to do in case of emergency. An EPP is required to be customized based on your lab location and policies and posted near lab exits.

Lab Emergencies and Chemical Spills

Review UHS guidance regarding Emergency Procedures and Chemical Spills for examples of where a researcher must call 911 or where a researcher can call the non-emergency number for assistance. For non-emergencies, your lab must decide what you are capable of handling. A good example would be a small spill in the lab where the chemical is not highly toxic or reactive and the spill is easily contained.

Small spills with low toxicity may be handled by researchers in the group. If the researcher feels unsafe in anyway, they know to call EHS, 911 and evacuated the building, depending on the severity.

Incident/Near Miss Reporting Expectations

Please review the UMN Injury or Illness reporting requirements on the UHS Occupational Health – Injury or Illness webpage. An investigation of an incident or near miss should take place as soon after the incident or near miss is recognized. Inclusion of the Department Safety Officer and your Research Safety Partner is recommended.

This is documented in the lab as well as in the lab-specific SOP that everyone is trained on.

Resource: UMN Chemical Hygiene Plan – Emergency Procedures

Training

UHS Required Training

University Health and Safety required training is dependent on the hazards that you are working with. The UHS Training Locator and your UHS Research Safety Partner can help you determine these requirements.

Our training is primarily one-time with the exception of annual blood-borne pathogen training (when we begin working with mammalian cells). Graduate students are the only ones working with the lasers and are required to be trained with UHS303.

Bloodborne Pathogens: Introduction (UHS100)
Bloodborne Pathogens: Advanced (UHS101)
Bloodborne Pathogens Annual OSHA Requirement (UHS110) (Only if working with mammalian cells)
General Laser Safety (UHS303) (Grad students are the only people working directly with lasers)
Hazard Communication (UHS470)
Implementation of NIH Recombinant and Synthetic Nucleic Acids Guidelines (UHS124)

1. DEHS Introduction to Research Safety
2. DEHS Chemical Safety
3. DHES Fire Extinguishers
4. Hazard Communication
5. DEHS Chemical Waste Management
6. Emergency Preparedness

Resources:

UHS Training Locator

Training Requirements Fact Sheet

Lab-Specific Training

It is the responsibility of each PI/Lab Manager to:

- Identify workplace hazards (chemical, physical, and biological)
- Identify affected employees
- Provide employee access to appropriate hazard information (i.e., Safety Data Sheets (SDSs), Standard Operating Procedures (SOPs), etc.)
- Provide training regarding the specific hazards present in an employee's laboratory work area, including methods to control such hazards
- Keep training records for five years (see Documentation)

Training must include required procedures and personal protective equipment to reduce the risk of exposure. Training must be provided at the time of an employee's initial work assignment, prior to assignments involving new potential exposure situations and **annually** thereafter. In the case that only one employee is working in the lab, that employee must review lab-specific training material annually and document that it has been done.

Resources:

Lab-Specific Training Fact Sheet

Lab-Specific Training Document

Other Documentation

Below is a description of other records that you may be required to keep on file, including the duration of time they need to be kept (if applicable). You are not required to keep the documentation in your LSP, but the records must be readily accessible by all lab staff.

Eyewash Fact Sheet (records must be kept for one year)

Autoclave Testing Log (records must be displayed in the autoclave room or available upon request during inspection)

Lab-Specific Training (documentation must be kept for 5 years)

Lab Self Inspection Form (documentation is optional)

Lab Inspection Records – Found in the Chematix System (documentation is optional)

References and Resources

UMN Chemical Hygiene Plan

UMN Chemical Hygiene Plan References

UHS Document Library

Prudent Practices

Waste Disposal Procedures

Research-Specific Standard Operating Procedures (SOP) For Work with_Preparation of plasmids and transfection into mammalian cells

PIs must have written Standard Operating Procedures (SOPs) for research protocols conducted in their laboratories involving the use of hazardous materials and procedures. SOPs must be accessible to all lab staff as an appendix in your Laboratory Safety Plan (LSP) and should be used as a training tool. SOPs developed through DEHS will be posted periodically in Appendix H of the (LSP)

Please fill out this form and place in your Laboratory Safety Plan. For assistance call 726-7273

Investigator's Name: Sheets, Erin_____	**Emergency #: 726-7273, 911**
Investigator's Phone #: __6046_____	**Waste Disposal #: 726-6764 or 7273**
Room & Building #: _ SSB 260, 259, 257, 158, 25_____	**IACUC Protocol #:__NA_____**
SOP Approval Date: _____	**IBC Protocol #: __Pending_____**

1. Describe experiment/Research protocol, including work with animals

Please refer to the attached document, Biosafety Practices for the Transformation of exempt *E. coli* K-12 strains and/or Use of Recombinant *E. coli* K-12 strains, which describes the specific protocols for E. coli transformation, Mini-prep purifications of plasmid DNA, and E. coli expression and purification of fluorescent proteins.

Bacterial stock containing the plasmid of interest is streaked onto a kanamycin or ampicillin containing LB agar plate (50ug/mL). The colonies are grown overnight. The next afternoon, one colony is chosen and 2mL of LB broth is inoculated. Bacteria are allowed to grow, shaking at 37°C overnight. The next morning, the bacteria are centrifuged, the pellet lysed and DNA purified from the bacterial cell lysate. The purity of the DNA is analyzed on the nanodrop spectrophotometer which uses 2 uL of solution (see miniprep protocol details). Lipofectamine, DNA and cell culture medium are combined for the transfection of mammalian cells, inside the BSL2 biological safety cabinet (in SSB 259), which is allowed to proceed for 4-6 hours (see transfection protocol details). The medium is removed and the cells are used fixed and analyzed by fluorescent microscopy. Please note that only imaging experiments (no cell biology, biochemistry or molecular biology) are carried out in SSB 257, 158, and 25. These three rooms are microscopy ONLY rooms. Cells in 35 mm dishes are washed in buffer or media prior to imaging experiments, and then the dishes are placed in a covered tray for transport to the imaging room (SSB 257, 158, or 25.

Alternatively, bacterial expression vectors (pRSET or pBAD) (Invitrogen) are used to express the protein from the plasmid. The soluble protein is collected using affinity chromatography. The soluble protein is studied in solution.

2. List substances to be used, including hazard class (infectious, radioactive, toxic, oxidizer, flammable, Shock Sensitive. etc).

BSL1 bacteria (*E. coli* DH5α, Top10, BL21 Star (DE3), BL21(DE)pLysS)
BSL1 mammalian cells
BSL2 human cells (J.CaM1.6, J.gamma.1, Jurkat clone E6-1, P116 (all derived from Jurkat cells, human acute T cell leukemia cells), MCF7 (human breast adenocarcinoma from pleural effusion cells), and MCF10A (human epithelial cell line from fibrocystic disease cells)
Sodium hydroxide
Glacial acetic acid
Phenol/chloroform (the use of this is optional and not necessary on a regular basis)
70% ethanol for use in washing and drying DNA pellets
10% bleach for clean up of spills and work space
Solucide for clean up of spills and work space (A broad-spectrum disinfectant that is highly effective against a variety of pathogenic microorganisms. Use as a one-step germicidal cleaner and deodorant designed for general cleaning and disinfecting of non-porous inanimate surfaces.)

3. Describe potential hazards for each step, including physical and health hazards

BSL1 bacteria (*E. coli* DH5α, Top10, BL21 Star (DE3), BL21(DE)pLysS), BSL1 mammalian cells and BSL2 human cell lines (J.CaM1.6, J.gamma.1, Jurkat clone E6-1, P116; MCF7; MCF10A): potential spill and aerosol hazard

Sodium hydroxide and glacial acetic acid: chemical burns, do not breath vapors

Phenol/chloroform: flammable, avoid breathing vapors, use adequate ventilation

70% ethanol: do not inhale, skin irritant

10% bleach: do not inhale, destructive to mucus membranes, skin burns

Sharps or broken glass or hard plastic pipets may become a sharps hazard. Red hard-sided biohazard sharps containers are available at each work station. A broken glass box for non-contaminated broken glass is located in the main lab.

4. Identify and list personal protective equipment required and hygiene practices needed to prevent exposure and to conduct the experiment safely

Always wear protective goggles in the laboratory.
Always wear gloves when working in the laboratory.
Wear a lab coat if there is risk of splashes, spills or chemical burns.
Always wear a lab coat and gloves that lab coats will be worn at all times when working with biological materials requiring BSL1 and BSL2 containment.
Always wear closed toed shoes in the laboratory.
Always wear pants or long skirts in the laboratory.
Know the location of the eye wash and the shower

5. Describe engineering controls that will be used to prevent or reduce employee

exposure to hazardous chemicals. This includes ventilation devices such as fume hoods.

Fume hoods are available for use in SSB 260 when volatile chemicals are used or when pipetting acids or bases. The sash will be maintained at the proper height indicated on the cabinet.
All live mammalian and human cell line cultures will be used with biosafety cabinet (SSB 259). The sash will be maintained at the proper height indicated on the cabinet.

6. **Describe any special handling and storage requirements for all hazardous substances involved in the experiment, including restricted areas, access requirements as well as any special procedures such as labeling the fume hood or biosafety cabinet, dating the chemical container.**

Any containers that will be used for hazardous waste collection must be labeled with a yellow hazardous waste sticker. The name of the person who begins collection, the date, the chemical (in plain terms such as “sodium hydroxide” not NaOH) and the % present in the waste container must be on the label. Secondary containment must always be used.

Phenol/chloroform waste should not be allowed to evaporate in the hood. Collect waste in a small bottle and have it picked up on a regular basis.

Live mammalian and human cultures will be used within the BSL2 biological safety cabinet (SSB 259). The cabinet will be cleaned with 70% ethanol before and after use.

Bench tops will be cleaned with 10% bleach before and after use of bacterial cultures.

Any pipets, flasks or other plastic ware in contact with bacterial or mammalian cell cultures must be destroyed by autoclaving at the end of the day. (121°C for at least 60 minutes).

7. Describe how spills or accidental releases will be handled and by whom. List the location of appropriate emergency equipment (spill kit, eye washes, showers, and fire equipment). Any special requirements for personnel exposure should be identified here such as respiratory protection

Small chemical spills should be cleaned up immediately by the person using the chemical. Gloves, goggles and lab coat should be worn. Spill kits are available in the SSB 260 laboratory.

Larger chemical spills or spills involving chloroform or other volatile chemicals, require that the lab be evacuated and the EHSO called for clean up.

Eye wash, safety shower and a fire extinguisher are available near the door in SSB 260. Fire extinguishers are available near SSB 259, 257, 158 and 25, outside the doors in the hall at regular intervals lengthwise along the space.

If a spill occurs with mammalian cell culture or bacterial cultures, the area should be cleared of personnel for 30 minutes. Then the spill should be treated with final concentration of 10% bleach for a minimum of 30 minutes. Clean up requires the use of gloves, lab coat, goggles.

8. Specify decontamination procedures to be used for equipment, glassware, including equipment such as glove boxes, hoods, lab benches, and controlled areas within the lab.

Any glassware or plasticware that is used for rDNA work will be decontaminated by treating with 10% bleach for 30 minutes or autoclaving for 1 hour at 121°C.
Bench tops will be cleaned with 10% bleach before and after rDNA work.
Biosafety cabinets will be cleaned with 70% ethanol before and after use.
Any contaminated equipment should be cleaned with 10% bleach, if possible. Check manufacturer's instructions.

9. Waste Disposal Procedures. Explain how waste will be disposed and type of waste including contaminated lab-ware

All materials used during experiments that involve rDNA or mammalian cell cultures will be autoclaved after use following the University waste disposal guidelines of 121°C for 60 minutes. This includes all plasticware whether disposable or reusable. Also, any glassware used will be decontaminted with 10% bleach, washed well and autoclaved.

All liquid waste must be autoclaved or treated with 10% bleach for 30 minutes before discarding in the sink.

Chemically resistant secondary containment trays are available throughout the lab for storing bottles of chemical waste. Waste should be segregated, bottles must be of an appropriate material for the waste to be collected, the bottle must be capped and waste should be picked up in a timely manner.

Contaminated sharps are to go in hard side red biohazard sharps container for pick up by EHSO. Non-contaminated broken glass should go into the broken glass box.

10. Additional Information

Accident Response Procedures:

If Incident Results in a Hazard Exposure (i.e. face or eye splash, cut or puncture with sharps, contact with non-intact skin):

- Encourage needle sticks and cuts to bleed, gently wash with soap and water for 5 minutes; flush splashes to the nose, mouth, or skin with water; and flush eyes at the nearest eyewash station with clean water for 15 minutes.
- Call 911 or seek medical attention.
  - For urgent care employees may go to St Mary's- Duluth Clinic (SMDC) Occupational Medicine or St Luke's Occupational Health. You may seek medical attention at the closest available medical facility or your own healthcare provider.
- Report the incident to your supervisor as soon as possible, fill out the appropriate documentation.
  - Supervisors must complete and submit the following reports:
    - First Report of Injury
    - Supervisor Incident Investigation Report
  - Employees must complete and submit Employee Incident Report
- Send Incident Report Form to the IBC if exposure has occurred during work on an IBC protocol.
- Report all biohazard exposures to the Office of Occupational Health and Safety (612-626-5008) or uohs@umn.edu.

Note: It is important to fill out all of the appropriate documents to be eligible to collect workers compensation should any complications from the hazardous exposure arise in the future.

11. Employee Signature

This to certify the employee named below have been trained on this SOP, have read and know the location of the department Laboratory Safety Plan and this SOP and understands the hazards and safe work practices as detailed in this SOP.

Name	Employee Id #	Initials	Date

Supervisor Signature & Date:

_____

Biosafety Practices for the Transformation of exempt *E. coli* K-12 strains and/or Use of Recombinant *E. coli* K-12 strains

Procedural Materials and Methods:

Follow lab specific *E. coli* transformation and/or culturing procedures, included here. **Protocols**: E. coli transformation; Mini-prep for plasmid isolation; Fluorescent protein expression and purification

Transforming with OneShot chemically competent bacteria

following Invitrogen One Shot® MAX Efficiency® DH5α-T1^R Competent Cells Catalog No. 12297-016
Version C 6 April 2004 25-0401
ED Sheets 12 April 2010; Revised 7/14/2011 J Stevens
Revised 6 June 2018 ED Sheets

Before you begin

Ensure that you have the appropriate LB agar plates with the correct antibiotic (use within two or so weeks of preparation. These are sealed tightly and stored at 4°C until use.

Only prepare the number of plates you need! For 60 mm diameter Petri dishes, use ~7 mL LB agar per dish; for 100 mm diameter Petri dishes, use ~15 mL LB agar per dish. Autoclave. Add antibiotic when the temperature of the agar is ~50°C and swirl to mix. Pour aseptically, avoiding bubbles. Allow to cool at RT with lid ajar. When cool, store at 4°C in a tightly sealed bag. Use within 2 weeks of preparation. For typical antibiotic concentrations, see below. NOTE that your antibiotic concentration may differ from these.

NOTE: Set the dry block incubator to 42°C for the heat shock step; use 2 mL blocks. Turn on the shaker incubator in the inner sanctum of SSB 244 (the biochem teaching lab). Turn on the incubator in SSB 260 for plate incubations.

1. Warm LB agar plates upside down in 37°C, a minimum of 1 h ahead of time.

2. Thaw tube of 50 µL One Shot DH5a or other appropriate *E. coli* strain of cells on ice.

3. Add 1–5 µL DNA solution to cells. Mix by *tapping gently*—DO NOT PIPET! Cells are fragile. NOTE: For low yield bacteria, use a larger amount of DNA.

4. Incubate on ice, 30 min. **NOTE:** this is a fine time to prepare the hockey sticks from 9 inch glass Pasteur pipets; that is, glass pipets bent at a 90° angle using a flame.

5. Heat shock by incubating at 42°C, 30 s, in the dry block incubator. Do NOT mix or shake. Place on ice.

6. Add 250 µL SOC (or LB) media.

7. Shake on side, 1 h, 225 rpm, 37°C, taped on side in the shaker.

8. Spread 100 µL and 200 µL (for 100 mm dishes) or 20 µL and 100 µL (for 60 mm dishes) of transformed bacteria onto LB agar plates that contain the appropriate antibiotic. **NOTE:** To spread, use the glass hockey sticks that have been sterilized immediately before spreading by using ethanol and flaming. Cool the hockey stick by touch the agar AWAY from the bacteria droplet.

Incubate 37°C, O/N. Store excess transformed bacteria at 4°C until assured that bacteria grew on plates. **NOTE:** The two volumes take into account transformation efficiency and cell density. The goal is to achieve individual colonies and to have a likelihood of getting colonies.

9. Store plates at 4°C, upside down and wrapped with parafilm in the bug box.

10. Verify transformants (at least 4 colonies) using minipreps and analytical restriction digests. Do NOT forget to flash freeze transformed cell stock in liquid nitrogen and store at –80°C. Refer to appropriate protocols.

Antibiotic Concentrations

ommonly Used Antibioticecommended Concentratio

Ampicillin	100 µg/mL
Bleocin	5 µg/mL
Carbenicillin	100 µg/mL
Chloramphenicol	25 µg/mL
Coumermycin	25 µg/mL
Gentamycin	10 µg/mL
Kanamycin	50 µg/mL
Spectinomycin	50 µg/mL
Tetracycline	10 µg/mL

QiaPrep 2.0 Spin mini-prep for plasmid DNA isolation

following QiaPrep Spin protocol (Qiagen 27104)
Revised: ED Sheets 16 July 2017

Read Prior to Experiment (that is, Step #2): *all spins in table-top microcentrifuge; get centrifuge to temperature ahead of time by pushing the snowflake button and setting the desired temperature. This will take approximately 15 minutes to come to temperature. All spins are at 13,000 rpm, room temperature (RT).*

NOTE: P1 is stored at 4°C. If P2 and N3, or any other buffers, have precipitated salt, warm briefly at 37°C **BEFORE** beginning the prep. **NOTE:** All buffers will likely be stored at 4°C for longer storage lifetime; the spin columns will be at RT.

Protocol:
1. Inoculate 1–5 mL LB + antibiotic with single colony or scrape of frozen transformed stock. Grow 12–16 h, shaking O/N, 37°C.

2. Transfer 1.5 mL culture to sterile 2 mL epi-tube (will need additional 2 mL epi-tubes for larger cultures). Spin 13,000 rpm, RT, 3 min. **NOTE:** keep remaining culture as backup (4° C).

3. Resuspend bacteria in 250 µL P1 by vortexing or by pipet, and transfer to sterile 1.7 mL epi-tube. Use only a total of 250 µL P1 if recombining several tubes.

4. Add 250 µL P2, mix by inverting 4–6 times. Do NOT allow the lysis reaction to proceed for more than 5 min! Do NOT vortex, which will shear genomic DNA.

5. Add 350 µL N3, mix **IMMEDIATELY and THOROUGHLY** by inverting 4–6 times. Centrifuge at 13,000 rpm, 10 min, RT.

6. Carefully transfer 800 µL of supernatant to the QiaPrep Spin column. Avoid white precipitated gunk on your tip.

7. Centrifuge at 13,000 rpm, 1 min, RT. Discard flow through.

8. Wash column with 500 µL PB and centrifuge at 13,000 rpm, 1 min, RT. Discard flow through.

9. Wash column with 750 µL PE and centrifuge at 13,000 rpm, 1 min, RT. Discard flow through.

10. Centrifuge column at 13,000 rpm, 1 min, RT to remove residual wash buffer. Discard flow through.

11. Elute DNA. Place column in a clean, sterile 1.7 mL epi-tube. Add 50 µL EB (10 mM Tris Cl, pH 8.5) to center of column. Allow to stand for 1 min. Centrifuge at 13,000 rpm, 1 min, RT.

NOTE: Turn off the table-top microfuge!!!!

*The nanodrop spectrophotometer (238 SSB) can be used to estimate the concentration of DNA in the sample after the experiment is completed (prior to storage steps). Decontaminate with 70% ethanol after use. Use an aliquot of EB buffer that was used to elute the DNA as the blank for the nanodrop spectrophotometer.

FRET sensor purification (bacterial expression)

ED Sheets 27 September 2015
modified 13 August 2017 H Leopold; 30 July 2019 CP Aplin

The following describes the protocol for the purification of the His-tagged Boersma FRET crowding sensor GE. The *E. coli* strain BL21(DE)pLysS has already been transformed with the pRSET vector that contains the gene for GE. The plasmid carries ampicillin-resistance. **NOTE: all of the FRET sensors (macromolecular crowding and ionic strength) as well as the fluorescent protein controls for bacterial expression and solution studies are in pRSET vectors that have been transformed into the *E. coli* strain BL21(DE)pLysS. SnapGene sequences for these have been uploaded in Dropbox.**

Following a modified procedure outlined in Boersma et al. (Nature Methods [(2015] 12:227) and the Life Technologies ProBond Purification System manual. The ProBond resin is a Ni^{2+}-based affinity resin; please refer to the ProBond material on Moodle for additional specifications.

NOTE: *TB* = terrific broth ***supplemented*** with (0.4% v/v) glycerol as described in Boersma et al. (2015) and on the terrific broth bottle.

Culturing bacteria
1. Grow up a starter culture overnight, 37°C, TB + 1 mg/mL Amp from the frozen glycerol stock. A scrape of the frozen glycerol stock into 2 mL culture medium.

KEY POINT: Reserve the shaking incubator ahead of time for the lower temperature growth conditions for the expression. (If the biochem lab shaking incubator is being heavily used, you may be able to reserve one of the shaking incubators in 250 Life Sci.)

2. For a 50 mL culture, inoculate 1 mL into 50 mL [TB + 1 mg/mL Amp]) into a 250 mL baffled culture flasks. The incubator is at 30°C. Read A600 to track cell growth using the NanoDrop spectrophotometer. Read every hour, and then every 30 min as cells enter log-phase. When A600 = 0.060–0.080, induce with 0.1 mM IPTG and shake overnight at 25°C. (It will take ~5 h to reach the appropriate optical density.) Use an aliquot of TB as your blank.

KEY POINT: You will need to set the shaking incubator to 30°C and allow it to come to temperature, which will take ~30 min or so.

KEY POINT: The NanoDrop does not automatically convert the pathlength of your absorbance. Therefore, the computer will read 0.06 when you are at the appropriate optical density.

KEY POINT: You need to wipe the NanoDrop with 10% clorox after A600 (or after each reading if someone else is using) per the NanoDrop instructions.

KEY POINT: DO NOT FORGET TO RETURN THE SHAKING INCUBATOR TEMPERATURE TO 37°C WHEN DONE!!!!

3. **Precool JA25.50 rotor just before you grab the bug flasks!** Proceed to the protein purification.

KEY POINT: DO NOT FORGET TO RETURN THE SHAKING INCUBATOR TEMPERATURE TO 37°C WHEN DONE!!!!

Purification of FRET sensor (Native Protein)

*****KEY POINT: Precool JA25.50 rotor! Binding buffer, wash buffer and elution buffers bring to RT before purification step. Re-equilibrate the column with 3x binding buffer during steps 2 onward (see step 0). DO NOT MIX RESINS for different proteins!**

NOTE: For the SDS-PAGE aliquots, use screw top tubes to boil samples!!! Snap cap epi-tubes tend to pop open during boiling. Use a **black Sharpie** to label the cap and twice on tube (other colors of Sharpies come off during boiling).

NOTE: This protocol is for column volume of 2 mL. All columns **must** be labeled with what is being purified, the date it was poured, and how many times it has been used & regenerated.

NOTE: Mark a dot on the column to mark settled height to track column volume.

0. The 2 mL column must be re-equilibrated with binding buffer (can do during steps 2 onward). 3x 8 mL binding buffer. Invert to resuspend and settle.

1. Resuspend bugs and transfer to Oak Ridge centrifuge tubes and cap (Thermo Scientific 3119-0050). 1 baffled flask/1 centrifuge tube. Balance tubes. Spin 5000 rpm (3000 x g), 5 min, 4°C. **NOTE: Oak Ridge centrifuge tubes are NOT disposable. Decontaminate for 30 min with 10% (v/v) clorox and wash! NOTE: To balance tubes, actually weigh them (include the caps too!) and balance them WITHIN 10 mg. DO NOT use the ancient, rusty balance in the centrifuge room!**

KEY POINT: You ***MUST*** be trained in the use of the centrifuge. See Sheets for training.

2. Resuspend pellets in 8 mL lysis buffer (total), which is prepared immediately before use (prepare 10 mL, add PMSF [or AEBSF] and lyozyme to lysis buffer) and transfer to a 50 mL conical centrifuge tube. Incubate on ice, 30 min.

3. To lyse, sonicate on ice with the probe sonicator, 6 x 10 sec @ intensity level 7, 50% duty cycle, with 10 sec cooling on ice between bursts. Do NOT sonicate in the Oak Ridge centrifuge tube! (Will be very foamy at this point.)

4. Using a 5 mL syringe and 18.5 gauge needle, draw lysate 4-5 times (1 time = 1 up + 1 down) to shear DNA and other debris. NEVER recap the syringe, it should go straight into the biohazard bin as is. Sit on ice for 45–60 min for foam to dissipate (longer is better). Transfer to a new Oak Ridge centrifuge tube. (If running SDS-PAGE on same day, pour lower gel here.)

5. Clarify lysate (Oakridge centrifuge tube), 5000 rpm (3000 x g), 15 min, 4°C. Transfer supernatant to new 15 mL centrifuge tube and **note the volume**. Remove a 10 µL aliquot ("L" lysate) for SDS-PAGE. Lysate on ice or can freeze at –20°C at this point. **NOTE**: that if you do freeze the lysate, you will have a lower yield of protein, as

compared with continuing with the purification on the same day. **NOTE: Oak Ridge centrifuge tubes are NOT disposable. Decontaminate for 30 min with 10% (v/v) clorox and wash! NOTE: To balance tubes, actually weigh them and balance them WITHIN 10 mg. DO NOT use the ancient, rusty balance in the centrifuge room!**

6. Add imidazole to the lysate at a final concentration of 10 mM.

7. Add the lysate to the re-equilibrated (with binding buffer) column. Rock gently at RT, 60 min; keep checking and rotating to keep resin resuspended! Settle the resin. Remove a 10 µL aliquot ("PL" post-lysate) for SDS-PAGE. Remove the supernatant. **NOTE**: this is a good time to do dishes!

8. Wash 4x (total) 6 mL with wash buffer. Invert to resuspend and settle. Remove a 10 µL aliquot ("W1,2,3,4" washes 1–4) for SDS-PAGE. Remove the supernatant. (If running SDS-PAGE on same day, pour upper gel here.)

9. Clamp column in vertical position. Elute with 8 mL elution buffer and collect 1 mL fractions. **NOTE:** pipet the first milliliter with baby pipet to not disturb the column bed and then add gently(!) the remainder elution buffer.

From each fraction (after the purification), remove a 10 µL aliquot ("1,2,...8" fractions 1–8) for SDS-PAGE. The protein likely eluted in fractions 1–2 and you will be able to see the fluorescence (hopefully). Store the protein fractions at 4°C; you will dialyze the fluorescent protein fraction against the appropriate buffer (see step 10). Boil and spin SDS-PAGE aliquots during the re-equilibration or regeneration steps below (step 11); store the SDS-PAGE aliquots at –20°C; 12% separating gel, 5% stacking gel.

10. Dialyze the fraction with the protein against PBS(pH 7.4) (macromolecular crowding probes) OR 10 mM sodium phosphate, pH 7.4, (ionic strength probes) to remove imidazole. To dialyze using the Slide-a-lyzer mini dialysis device (3.5K MWCO, ThermoFisher 88403), dialyze against the appropriate buffer, for 2 h, 4°C, 230 rpm using the Sheets/Heikal orbital shaker. Change the buffer and dialyze O/N, 4°C, 230 rpm using the Sheets/Heikal orbital shaker. (See the product literature for more information [on google drive].) **NOTE:** be sure to not dry out the dialysis membrane or place it on any surface. Transfer the protein to a new epitube and measure the absorbance (see below). Use an aliquot of the original buffer as the spectrophotometric blank.

11. Wash the column with 8 mL 0.5 M NaOH, 30 min, RT, rocking. Invert to resuspend and settle. (Can reuse a clean 15 mL centrifuge tube to prepare this solution.) **NOTE: keep track of number of uses and number of regeneration on the column label!**

> **KEY POINT:** If the resin has been used 3 times, ***regenerate*** per the ProBond manual. If the resin has been regenerated 3x, then use new resin (2 mL), washing with nanowater (2x 8 mL) and binding buffer (3x 8 mL), per the ProBond manual. This will lead to a column bed of ~2 mL.
>
> ***How to regenerate the resin:***

a. 1x 8 mL 0.5 M NaOH; rock 30 min RT. (This is step 10 above)
b. 2x 8 mL H_2O. Invert to resuspend and settle.
c. 2x 8 mL 50 mM EDTA. Invert to resuspend and settle. (Can reuse a clean 15 mL centrifuge tube to prepare this solution.)
d. 2x 8 mL 0.5 M NaOH. Invert to resuspend and settle.
e. 2x 8 mL H_2O. Invert to resuspend and settle.
f. 2x 8 mL 5 mg/mL $NiCl_2 \cdot 6H_2O$. Invert to resuspend and settle. (Can reuse a clean 15 mL centrifuge tube to prepare this solution.)
g. 2x 8 mL H_2O. Invert to resuspend and settle. (This is step 11 below).

12. Wash 2x (total) 8 mL H_2O. Invert to resuspend and settle.

13. Re-equilibrate the column 3x (total) 8 mL in (binding buffer + 0.2% sodium azide (***careful!!***)). Invert to resuspend and settle. Store upright at RT, wrap cap in parafilm. Note the number of times used.

14. Carry out SDS-PAGE analysis of fractions following the SDS-PAGE protocol with Coomassie staining to assess purification.

15. Read A280 and take spectra from 200–600 nm (against elution buffer blank) to get a ballpark estimate of

concentration. **NOTE:** The sequences for the various probes differ slightly, so their molar absorptivities will vary and **are listed in the table below** (following SnapGene). These values do NOT take into account the chromophores, it will be okay to use this for RELATIVE, not absolute, concentrations. **NOTE**: the value for mEGFP, mCerulean, mCitrine, mTurquoise2 will be substantially different; these values may be in the literature somewhere.

16. Flash-freeze 100 µL aliquots of the protein (using liquid nitrogen) and store these well-labeled tubes in the –80°C freezer. MINIMIZE the freeze/thaw cycles for a given tube.

NOTE: the binding, wash and elution buffers were from Boersma et al. (Nature Methods [(2015] 12:227). These solutions are stored at 4°C, unless otherwise noted.

Lysis buffer (0.2 µm filtered):
10 mM sodium phosphate, pH 7.4
100 mM sodium chloride
0.1 mM PMSF or AEBSF (***careful!!***) (10 µL for 10 mL; in 4°C)
1 mg/mL lysozyme (EDS –20°C nondessicator #1) (10 mg for 10 mL)

Binding buffer (0.2 µm filtered):
10 mM sodium phosphate, pH 7.4
100 mM sodium chloride
10 mM imidazole

Wash buffer (0.2 µm filtered):
50 mM sodium phosphate, pH 8.0
300 mM sodium chloride
20 mM imidazole

Elution buffer (0.2 µm filtered):
50 mM sodium phosphate, pH 8.0
300 mM sodium chloride
250 mM imidazole

Imidazole stock is 1 M (0.2 µm filtered)

IPTG stock is 1 M (stored at –20°C, orange "antibiotic" box)

Prepare **500 mM sodium phosphate** (no pH adjustment) and **4 M sodium chloride** stock solutions to facilitate solution preparation. These both should be 0.2 µm filtered.

Hazard Identification and Risk of Exposure to the Hazards:

The potential release of rDNA materials to the environment is the primary hazard.

Exposure Controls Specific to Above Risk of Exposure:

Refer to the attached Biological Decontamination & Spill Cleanup Plan in eProtocol.
Laboratory coats, gloves, and safety glasses are required at all times. Wear safety glasses when splashes and sprays of microorganisms or other hazardous materials are anticipated. Lab coats are worn to prevent contamination of personal clothing.

Decontaminate work surfaces and equipment with appropriate disinfectant after completion of work and after any spill or splash of rDNA materials. Wash hands after experiments and before leaving the lab. Waste containing rDNA materials is disposed of as biohazardous waste.

Biological Waste Disposal Methods:
Follow the attached lab specific Biological Waste Disposal Plan in eProtocol.

Spill Response Procedures:
Follow the attached lab specific Biological Decontamination & Spill Cleanup Plan in eProtocol.

Accident Response Procedures:

If Incident Results in a Hazard Exposure (i.e. face or eye splash, cut or puncture with sharps, contact with non-intact skin):

- Encourage needle sticks and cuts to bleed, gently wash with soap and water for 15 minutes; flush splashes to the nose, mouth, or skin with water; and flush eyes at the nearest eyewash station with clean water for 15 minutes.
- Call 911 or seek immediate medical attention if overtly exposed to recombinant or synthetic nucleic acid molecules or Risk Group (RG) 2 infectious agent(s).
  - **Twin Cities campus:** For urgent care employees may go to HealthPartners Occupational and Environmental Medicine (M/F day time or Urgent Care after hours), or M Health Fairview - UMMC East Bank or West Bank Hospital (24 hrs.). You may seek medical attention at the closest available medical facility or your own healthcare provider. Follow-up must be done by HealthPartners Occupational and Environmental Medicine.
  - **Crookston campus:** For urgent care employees may go to RiverView Health (218-281-9200). You may seek medical attention at the closest available medical facility or your own healthcare provider.
  - **Duluth campus:** For urgent care employees may go to St Mary's- Duluth Clinic (SMDC) Occupational Medicine or St Luke's Occupational Health. You may seek medical attention at the closest available medical facility or your own healthcare provider.
  - **Hormel Institute:** For urgent care employees may go to Mayo Clinic Health System in Austin (507-433-8758). You may seek medical attention at the closest available medical facility or your own healthcare provider.
  - **Morris campus:** For urgent care employees may go to Stevens Community Medical Center (320-589-1313). You may seek medical attention at the closest available medical facility or your own healthcare provider.
  - **Rochester campus:** For urgent care, but non-life-threatening, illnesses/injuries employees should go to the nearest medical clinic (e.g. Methodist Hospital). If off-hours medical attention is required, the employee should be taken to the emergency room at St. Mary's Hospital or Olmsted Medical Center.
- Report the incident to your supervisor as soon as possible. Fill out the appropriate documentation.
  - Employee First Report of Injury
- Report incident to the IBC if exposure has occurred during work on an IBC protocol. Instructions about incident reporting are available on the IBC web site.
- Report all biohazard exposures to the Biosafety & Occupational Health Department (612-626-5008) or uohs@umn.edu.

Note: It is important to fill out all of the appropriate documents to be eligible to collect workers compensation should any complications from the hazardous exposure arise in the future.

References: *(Research papers, UHS Bio Basics Fact Sheets, CDC/NIH BMBL, Canada Pathogen Safety Data Sheets, UHS training requirements, NIH rDNA Guidelines, etc.)*

Biosafety training requirements:

- Implementation of NIH Guidelines (required once for research involving recombinant or synthetic nucleic acid molecules)
- Biological Safety in the Laboratory (required once for research requiring IBC review)
- Lab specific safety training (required annually for all personnel working in the lab)

Revision Date: 1/3/2019

Biological Waste Disposal Template

P.I./Lab Supervisor: Erin D. Sheets

Lab Location: SSB 260, 259, 257, 158, 25

Types of Biological Waste Generated:

- ☒ Liquid Biological Waste (including plant/animal agents)
- ☒ Solid Biological Waste (including plant/animal agents)
- ☐ Animal Waste (e.g. tissue, carcasses, bedding, rDNA-containing animal waste, transgenic animal, etc.)
- ☐ Toxins
- ☒ rDNA (e.g. solid or liquid rDNA waste, transgenic plants & soil, rDNA-containing plant waste, etc.)
- ☒ Sharps
- ☐ Prions
- ☒ Cell Culture / Blood / Body Fluids (e.g. human / NHP / animal cells, blood, serum, body fluids, etc.)
- ☐ Human Organs, Tissue, Body Parts (Pathological Waste)

Please indicate any other types of biological, or mixed biological with other hazardous wastes that will be generated.

NOTE: For each type of waste checked or listed above, indicate the disposal method below.

Liquid Biological Waste Disposal (e.g. rDNA, cell culture / blood / body fluids, any biological material including Risk Group 1-3, plant & animal agents, etc.):

- ☒ Autoclave for 30 minutes at 121ºC on liquid cycle. Test autoclave monthly with integrator per *Autoclaving Biological Waste Fact Sheet* below.
- ☒ Disinfect with 10% (1:9 v/v) bleach for at least 30 minutes.

OR

- ☐ List other proven effective disinfectant /concentration underneath: (minimum 30 minutes contact time). **Note:** some disinfectants are incompatible with bleach therefore should not be mixed.

Solid Biological Waste Disposal (e.g. proteinaceous toxin, rDNA, cell culture / blood / body fluids, any biological material including Risk Group 1-3, plant & animal agents, transgenic plant & soil, gloves, etc.):

- ☒ Autoclave for 60 minutes at 121ºC or 125 ºC. Test autoclave monthly with integrator per *Autoclaving Biological Waste Fact Sheet* below.
- ☐ Place biological waste in the red biohazard bag, fill to no more than ¾ full, seal and place in the designated waste area in the lab.

Animal Waste:

- ☐ Place animal tissue and carcasses in cooler designated by RAR.
- ☐ Handle animal cages, bottles, and bedding per RAR instructions.
- ☐ Describe other animal waste disposal method.

Toxins:

- ☐ Treat with 10% (1:9 v/v) bleach for at least 30 minutes.
- ☐ Place solid waste containing low molecular weight toxin in a yellow waste bag for incineration.
- ☐ Describe other proven effective inactivating agent.

Human Organs, Tissue, Body Parts (Pathological Waste):

- ☐ Call Bequest Program 612-625-1111

Sharps (contaminated with infectious material, rDNA, or biotoxins):

- ☒ Place sharps in sharps container, fill to no more than ¾ full, seal and place in the designated waste area in the lab.

Prions (call Waste Recovery Services at 5-6481 for yellow bag & yellow barrel delivery & pick-up):

- ☐ Place non-tissue low level solid waste (including animal bedding) in yellow waste bag in yellow barrel for incineration.
- ☐ Autoclave liquid waste at 134°C for 1 hour.
- ☐ Wipe instrument for re-use thoroughly clean, immerse in 2N NaOH for 1 hour, rinse with water, autoclave at 134-138°C for 18 minutes.
- ☐ Wipe instrument for disposal clean, soak in 2N NaOH for 1 hour at 20°C, then disposal.
- ☐ Place sharps in sharps container, fill to no more than ¾ full, seal and place in yellow waste bag incineration.
- ☐ Dispose animal tissue & carcasses in animal digester.
- ☐ Describe other proven effective disinfectant.

Other Waste Disposal:

Waste Disposal Reference:

- Autoclaving Biological Waste Fact Sheet, https://bohd.umn.edu/sites/bohd.umn.edu/files/autoclavebiowaste_1.pdf
- Chemotherapy Drug Disposal Fact Sheet, https://bohd.umn.edu/sites/bohd.umn.edu/files/chemo_waste_disposal_fact_sheet.pdf
- Biological Waste Disposal Table, https://bohd.umn.edu/sites/bohd.umn.edu/files/biological_waste_disposal_table_2018_0.pdf
- Inactivation of Toxins, https://bohd.umn.edu/biologically-derived-toxins
- Sharps Disposal, https://bohd.umn.edu/sharps-non-sharps-and-glass-waste-disposal
- Prion Waste Disposal, https://bohd.umn.edu/prion-waste-handling
- Hazardous Chemical Waste Management Guidebook, https://dehs.umn.edu/sites/dehs.umn.edu/files/cwg2017.pdf

Biological Decontamination & Spill Clean-up Plan Template

This template can be used in writing lab specific SOPs and posted in the lab for reference and annual review. This customized template is a required attachment when IBC forms are submitted. **The top section and any Lab Specific Requirements must be filled in. Note: all r/s NA containing waste must be treated as biohazardous waste.**

P.I./Lab Supervisor: Erin D. Sheets **Lab Location: SSB 260, 259, 257, 158, 25**	**Emergency Contact Info: 218-726-6046 (office); 218-343-0405 (cell)** **(report all spills to P.I. or Lab Supervisor and Biosafety Officer)**	
Biological Agent (s) / r/s NA / Biological toxins	**Disinfectant / Concentration / Contact time**	**Routine Decontamination Procedures (BSC surface, bench top, equipment, etc. Not for spill clean-up.)**
Please see the table appended to the end of this document for rDNA plasmids and E. coli strains and mammalian cell lines	☒ **Bleach / 10% / 30 minutes, OR** ☒ **Other proven effective disinfectant:** **Solucide (A broad-spectrum disinfectant that is highly effective against a variety of pathogenic microorganisms. Use as a one-step germicidal cleaner and deodorant designed for general cleaning and disinfecting of non-porous inanimate surfaces.)** **Note:** some disinfectants are incompatible with bleach therefore should not be mixed.	**Benchtop: Use 10% bleach to wipe down benches and work areas both before and after working with cultures.** **Centrifuge and Incubator shaker: Wipe down surfaces before and after working with cultures using Solucide, a broad-spectrum disinfectant that is highly effective against a variety of pathogenic microorganisms. It can be used as a one-step germicidal cleaner and deodorant designed for general cleaning and disinfecting of non-porous inanimate surfaces.**

Spill Response Equipment:

- Written spill procedure including emergency phone numbers
- Disinfectant suitable for biological materials being used
- Paper towels, gloves, shoe covers, safety goggles
- Forceps to pick up sharps, including broken glass
- Sharps container for broken glass, etc.
- Squeegee & dustpan that can be decontaminated
- Biohazard bags (red bags or clear autoclave bags)

Lab Specific Requirements (please describe below):

We do not have red bags on the Duluth campus. We will use clear bags and autoclave the material. Dust pan available in the lab (SSB 260). Spill kit available in the lab (SSB 260).

Small and moderate spills outside the biosafety cabinet:

- Remove any contaminated clothing and put in autoclavable bag. Be aware that autoclaving may damage fabric.
- Notify other workers in the area of the spill and control traffic through area.
- Wear shoe covers and safety goggles if spill is on floor; there may be splashes beyond immediate area of spill.
- Put on gloves then cover spill area with paper towels.
- Pour disinfectant over towels from edges of spill to center, be careful not to splatter.
- Decontaminate all objects in spill area.
- Allow 30 minutes of contact time.
- Pick up any sharps, including broken glass, with forceps and place in sharps container.
- Use squeegee and dustpan to recover any shards of broken glass in contaminated liquid.
- Wipe area with disinfectant and clean paper towels, mop if spill on floor.
- Remove gloves and foot covers before leaving area of the spill, put in biohazard bag, and wash hands.

Lab Specific Requirements (please describe below):

Large spills (>100ml) in or outside of the biosafety cabinet:

- Evacuate room, close doors, prevent others from entering, and wait 30 minutes for aerosols to settle.
- Follow procedures for small and moderate spills.

Lab Specific Requirements (please describe below):

Notify all lab personnel of the spill.

For small spills in a biosafety cabinet:

- Leave the cabinet running.
- Wipe down all supplies and equipment in the cabinet then move them out of the cabinet.
- Cover spill with paper towels, then pour disinfectant over paper towels allowing 30 minutes contact time, then dispose of paper towels in a biohazard bag.
- Wipe down all interior cabinet surfaces with appropriate disinfectant.

Lab Specific Requirements (please describe below):

For moderate spills in a biosafety cabinet, follow general spill procedures plus:

- Leave the cabinet running.
- Wipe down all supplies and equipment in the cabinet then move them out of the cabinet.
- Cover spill with paper towels, then pour disinfectant over paper towels allowing 30 minutes contact time, then dispose of paper towels in a biohazard bag.
- Wipe down all interior surfaces with disinfectant.
- Determine if spill has gone beyond the work surface such as in the grilles or side seams. Disassemble and decontaminate if necessary.
- If the cabinet has a catch basin below the work surface that may be involved in the spill, flood the basin with disinfectant. Do not use alcohol as a large quantity of alcohol presents a flammable hazard. Wipe out basin after 30 minutes.
- Let cabinet run for at least 10 minutes after cleanup.

Lab Specific Requirements (please describe below):

For major spills in a biological safety cabinet:

- Contact the Biosafety Officer (BSO) (612-626-6002) to determine if professional decontamination is indicated.

For any spills of agents that are transmitted by inhalation, evacuate the lab immediately, close the door, restrict access, remove any contaminated clothing, wash exposed skin with soap and water, call the BSO for assistance at 612-626-6002.

If Incident Results in a Hazard Exposure (i.e., face or eye splash, cut or puncture with sharps, contact with non-intact skin, animal bites or scratches):

- Encourage needle sticks and cuts to bleed, gently wash with soap and water for 15 minutes; flush splashes to the nose, mouth, or skin with water; and flush eyes at the nearest eyewash station with clean water for 15 minutes.
- Call 911 or seek **immediate** medical attention if overtly exposed to recombinant or synthetic nucleic acid molecules or RG2 infectious agent(s) in a BSL2 lab.
  - For urgent care employees may go to St Mary's- Duluth Clinic (SMDC) Occupational Medicine or St Luke's Occupational Health. You may seek medical attention at the closest available medical facility or your own healthcare provider.
- Report the incident to your supervisor as soon as possible, fill out the appropriate documentation.
  - Employee First Report of Injury
- If an incident has occurred during work on a protocol approved by the IBC, report the incident to the IBC using eProtocol as soon as possible after accident response procedures have been followed.
- Report all biohazard exposures to the Department of Biosafety and Occupational Health (612-626-5008) or uohs@umn.edu.

Note: It is important to fill out all the appropriate documents to be eligible to collect workers compensation should any complications from the hazardous exposure arise in the future.