Team Leader (Last name, first name):	LaRock, Christopher
Title of Project:	Safe, sustainable reductions of biohazard and chemical waste during BSL2 pathogen research
Amount:	$2265.75

Brief description of project for the OSI website and communications

This project focused on how we could reduce our biohazardous waste and chemical waste streams. A source of our plastic waste is single-use microtiter pipette tips needed for the accurate measurement of micro-liter volumes. They must be treated as a biohazard, so it has additional energy costs from autoclaving/incineration and transportation. We adapted our experiments to replace a portion of this waste with a sterilizable, stainless steel pin replicator that can be reused indefinitely. Second, a survey of our hazardous chemical waste requiring EHSO disposal reveals that most of it is from isolation of nucleic acids and proteins, and contains heavy metals, guanidinium thiocyanate, or organochlorides. We adapted a magnet-based system for the routine separation of biomolecules to eliminate this waste.

Project objective

For this year, we focused on how we could reduce our biohazardous waste and chemical waste streams. Weekly, we generate several kg of single-use plastic waste that must be treated as a biohazard, so it has additional energy costs from autoclave/incineration and transportation. A large component of this is microtiter pipette tips needed for the accurate measurement of micro-liter volumes. However, roughly half of these tips could be eliminated by developing protocols to instead use a pin replicator that delivers a standardized 3 micro-liter volume but can be reused indefinitely. This project will attempt to adapt our existing standard assays to use this device to reduce waste, while remaining mindful of necessary biosafety precautions. If successful, it will faithfully replicate our current methods for quantifying pathogenic bacteria, while reducing the plastic tip waste needed for this protocol by half. Second, a survey of our hazardous chemical waste requiring EHSO disposal reveals that most of it is from isolation of nucleic acids and proteins, and contains heavy metals, guanidinium thiocyanate, or organochlorides. Adapting to a magnet-based system for the routine separation of biomolecules has the potential to eliminate this waste. If successful, this system will isolate biomolecules with similar purity to our existing methods, with less, and less toxic, waste.

Results and Lessons Learned

Experiment 1: MRSA (Staphylococcus aureus) was cultured in quadruplicate in a 96-well format. Using a multichannel pipettor, 10-fold dilutions will be made in PBS. These dilutions were transferred to agar plates using single use plastic tips (yellow box), or using the stainless steel pin replicator, sterilized with ethanol and flame between samples. The plate will be incubated inverted at 37C and 5% CO2 overnight, colony-forming units enumerated. Technical variation between the quadruplicates for each method was found to be ~5%, and unlikely to vary experimental interpretation. The removal of ethanol by flame was identified as a possible hazard. Sterilization was done in low volume to avoid this. Experimental setup indicated in Figure 1.

Experiment 2: A frozen cell pellet of E. coli expressing recombinant protein will be homogenized, cell debris removed by centrifugation, and the filtered lysate separated for purification by Magnetic Dynabead purification (Figure 2) At the conclusion of each method, samples will be separated by SDS-PAGE electrophoresis, protein content stained with AquaStain (a non-toxic replacement we have already adopted in place of toxic conventional stains), and visualized. Successful performance is indicated by a band of the same molecular weight as the desired protein, with no others in the sample (Figure 3). This was seen (lane indicated M FT), indicating this method is appropriate for the purification of our protein of interest.

Attach materials produced

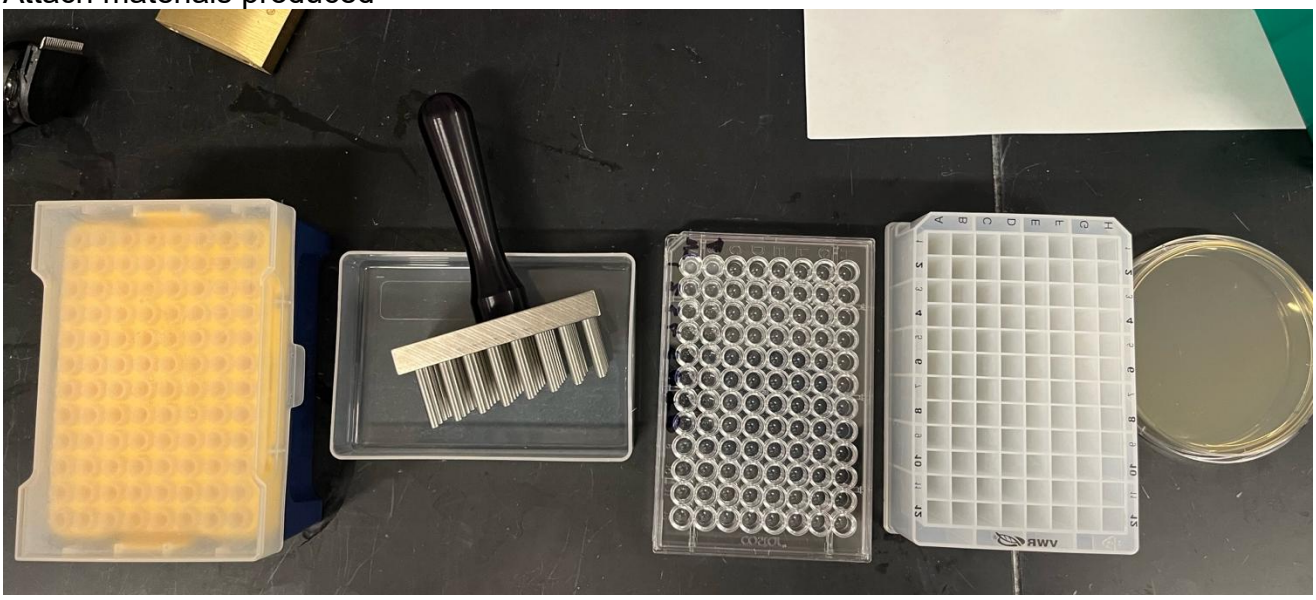

Figure 1. Replica plater setup

Figure 2. Magnetic molecular separator

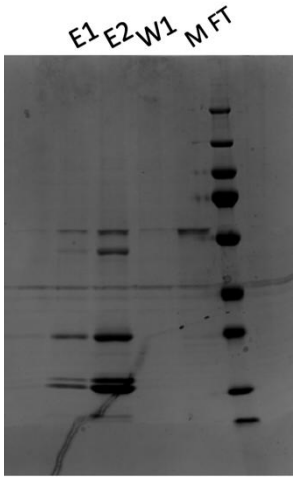

Figure 3. Protein purification results from Aim 2