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## "Expression and Purification of Proteins Involved in Transfer of Conjugative Plasmids in Escherichia coli"

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## **RCEU21-BYS-TS-01**

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**Project Title** - Expression and purification of proteins involved in transfer of conjugative plasmids in *Escherichia coli*

**Faculty** - Tatyana (Tanya) Sysoeva, Assistant Professor, Department of Biological Sciences, Shelby Center room 369M, University of Alabama in Huntsville, 256-824-6371, [tatyana.sysoeva@uah.edu](mailto:tatyana.sysoeva@uah.edu)

**Proposal Identifier** - RCEU21-BYS-TS-01

**Project Description** - Spread of antibiotic resistance genes amongst bacteria is a huge medical problem of our time that is largely attributed to horizontal gene transfer from one species to another through conjugative transfer of resistance plasmids. Conjugative plasmids carry a large number of transfer genes that are required for successful conjugation: pilus production, DNA processing, replication, transfer itself. In addition, there are several genes that code for proteins essential for establishing the initial contact between the two conjugating bacterial cells. In this project we will focus on expressing and purifying these proteins from model conjugative plasmids (such as F plasmid). For that we will design constructs for expression, test the obtained constructs for induction, and work out the protocols for purification these target proteins. As a result, this project will produce necessary materials for further functional analyses of these essential conjugative protein in vitro using biochemical and structural assays.

**Student Duties, Tangible Contributions, and Specific Outcomes** - This project will require full time presence in the lab for 32-40 hours per week, for 10-12 weeks. During this time the student will learn basic microbiological procedures and several functional assays. In particular, this project will entail preparing solutions and plates; working with uropathogenic *Escherichia coli*; grow biofilms; conduct conjugation assays; and image bacteria using confocal microscope. In addition to practical work, the researcher will read the peer-reviewed scientific articles that is required for understanding and developing of the project at hand. The student will also learn how to document the experimental work and keep laboratory notebook with the detailed description of the performed experiments. The student will be encouraged to prepare a poster for consecutive

presentation at a scientific conference. At the end, the student researcher will be required to write a report with suggestions for future development of the project and analyses of the obtained results that can be used in a future publication. This report will be presented at a lab meeting to share the results and experiences with the whole group.

**Student Selection Criteria** - The student (from freshman to senior) should have completed some basic biology courses at UAH or as AP courses. While a good academic standing is not a requirement, the applicant has to have a sincere interest in the topic of microbiology and willingness to learn molecular techniques.

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**Project Mentorship** - Dr. Sysoeva will provide one-on-one meetings to introduce to the project, supervise literature review, discussions and analyses of the experimental results. In addition, weekly group meetings will be held to share the results and solve any ongoing issues. She will ensure close daily supervision and training in the laboratory setting for the hands-on work at the bench, including safety training and microbiological work. Dr. Sysoeva will guide the student researcher in writing up the report of the obtained results and preparing for the final presentation. In addition, four MS students and an experienced undergraduate researcher working on related projects will be conducting their research in the laboratory and assist with microbiological work, such as media preparation, autoclaving, inoculations. Two of the MS students have experience teaching microbiology. It is particularly important that one of the MS students has recently completed the summer RCEU project and thus is familiar with the program goals and requirements. In addition, one of the advanced MS students has the hands-on experience in inducing and purifying recombinant proteins from *E. coli*.

**Safety and Contingency Plan** - Student awarded will complete required general lab safety and biosafety trainings at the UAH OEHS website. This would include any COVID-19 related training as well. As part of our research group the RCEU student will follow the developed reduced density standard procedures that were approved by the COS earlier in 2020 to allow the re-start of the wet lab work in the summer of 2020.

If due to public health concerns the experimental wet lab work on the UAH campus would not be

allowed, the next RCEU project will take place. We will proceed with bioinformatic analysis of the related protein homologs from diverse *E. coli* plasmids, sequences of which are available in the lab and in the online databases. We will construct multiple sequence alignment (MSA) of the proteins and identify proposed functional and structural residues, conserved and hypervariable regions. Using the established MSAs, we will test for correlations of the conserved residues to predict interacting proteins and their domains *in silico*. The outcome of this bioinformatic project will guide later experimental inquiries and result in teaching the RCEU student basics of protein sequence work, conservation and structure analyses.