Summer Student Research Program
Project Description

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PROJECT TITLE (200 Characters max):
A genome-wide overexpression screen to identify proteins that inhibit the growth of *Trypanosoma brucei*, the causative parasite of human and animal African trypanosomiasis.

HYPOTHESIS:
To understand essential biological processes in *T. brucei* and utilize them to identify potential targets for the development of anti-trypanosomal chemotherapy, we generated a *T. brucei* gene overexpression (OE) library representing 83% of ~7,250 single copy genes. This OE library can complement the existing RNAi library by identifying genes that show phenotypes (*e.g.*, cell lethality, drug resistance, defects in the control of the parasite's immune evasion mechanisms) when they are overexpressed. We screened ~ $4x10^5$ of individually targeted trypanosome clones for growth deficiency and found ~ 200 candidate ORFs. We will validate some of these candidates (34 selected) by generating new OE vectors, targeting them in reporter cell lines, and examining their effects on trypanosome cell growth and immune evasion.

PROJECT DESCRIPTION **(Include design, methodology, data collection, techniques, data analysis to be employed and evaluation and interpretation methodology)**
We will first generate OE validation vectors for candidate ORFs by TA cloning. The backbone of these vectors contains rDNA space sequences for targeting, blasticidin or hygromycin resistance gene (BSD or HYG) for selection, and a cloning region for an ORF, whose expression can be driven by T7 polymerase. To this, we engineered in two AhdI cleavage sites (AhdI digestion will create two ends with a 5′ T overhang) and an epitope tag (N- or C-terminal 3xHA or His6, or no tag) (plasmids, pSUN85-89). PCR-amplified ORFs with 3′A overhangs can be ligated with a pSUN vector digested with AhdI enzyme. ORFs should be in frame with an epitope at the N or C-terminus. We have already generated validation constructs for about 50% of our selected candidates. However, we have found that the efficiency of cloning is low because of partial digestion by AhdI enzyme. To increase the cloning efficiency, we will increase the length between two AhdI sites in these pSUN vectors using a site-directed mutagenesis or Gibson assembly technique. A student will first participate in these cloning experiments. Modified vectors will be confirmed by Sanger-sequencing and restriction enzyme digestion. Using these vectors, a student will generate additional ORF OE validation vectors. If time permits, a student will participate in trypanosome cell transfection experiment. ORF OE vectors will be transfected in our reporter strains to confirm their effects on trypanosome cell growth.

SPONSOR'S MOST RECENT PUBLICATIONS RELEVANT TO THIS RESEARCH:

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Carter M, Gomez S, Gritz S, Larson S, Silva-Herzog E, Kim HS, Schulz D, Hovel-Miner G. A Trypanosoma brucei ORFeome-Based Gain-of-Function Library Identifies Genes That Promote Survival during Melarsoprol Treatment. mSphere. 2020 Oct 7;5(5):e00769-20. doi: 10.1128/mSphere.00769-20. PMID: 33028684; PMCID: PMC7568655.

THIS PROJECT IS: ☐ **Clinical** ■ **Laboratory** ☐ **Behavioral** ☐ **Other**

THIS PROJECT IS CANCER-RELATED ☐ **NO**
Please explain Cancer relevance

THIS PROJECT IS HEART, LUNG & BLOOD- RELATED ☐ **NO**
Please explain Heart, Lung, Blood relevance

THIS PROJECT INVOLVE RADIOISOTOPES? ☐ **NO**

THIS PROJECT INVOLVES THE USE OF ANIMALS ☐ **NO**
PENDING ☐ **APPROVED** ☐ **IACUC PROTOCOL #**

THIS PROJECT INVOLVES THE USE OF HUMAN SUBJECTS? ☐ **BO**
PENDING ☐ **APPROVED** ☐ **IRB PROTOCOL # M**

THIS PROJECT IS SUITABLE FOR:

UNDERGRADUATE STUDENTS	☐	ENTERING FRESHMAN	☐
SOPHMORES	☐	ALL STUDENTS	☐

THIS PROJECT IS WORK-STUDY: **Yes** ☐ **or** **No** ☐

THIS PROJECT WILL BE POSTED DURING ACADEMIC YEAR: May be.
FOR INTERESTED VOLUNTEERS: **Yes** ☐ **or** **No** ☐

WHAT WILL THE STUDENT LEARN FROM THIS EXPERIENCE?

Molecular Cloning is one of basic techniques required for all biology experiments. Through this project, students can learn following techniques: oligo design for PCR amplification, PCR amplification techniques, agarose gel electrophoresis to visualize PCR amplified products, restriction enzyme digestion for confirming of PCR products or plasmids that they generate, preparation of Sanger-sequencing samples. They will also learn how to use DNA star software for oligo design, map drawing, sequence alignment etc. Students may be able to learn how to do transfection of an ORF DNA into trypanosome cells to generate transgenic parasite and determine the effect of the ORF OE in parasite cell growth. Students will participate in lab meeting and journal club meetings and present their work at the end of program. Thus, they will learn how to communicate, how to work as a member of a team, how to present their work effectively.