CALIFORNIA STATE UNIVERSITY
EAST BAY

SUPPLY GRANT APPLICATION
Center for Student Research

INSTRUCTIONS TO ORIGINATOR

Student supply grants from the Center for Student Research are very competitive. Supply grants are capped at $500 and only awarded for allowable proposed costs. Students will be responsible for costs exceeding $500 or the approved amount. In exceptional cases, requests may exceed $500. Please type the information in the form and be as detailed as possible.

Student Name: _____ **Date:** 3/2/2017

Faculty Mentor Name: _____

In the space below, describe in as much detail as possible the supplies you would like to purchase and how they will be used to support your research project. On a separate page, provide an itemized budget of the requested supplies and be sure to include estimates of taxes and shipping as applicable.

I would like to purchase supplies to manipulate my gene of interest in cancer cells and determine resulting differential gene expression for markers of cell death, proliferation, survival and migration. I will knock-down my gene of interest using silencing RNA in order to decrease its expression in cancer cells. This protocol requires that cells be cultured in OptiMEM, a special medium for growing cells for growing cells. I will also knock-out and completely disrupt my gene of interest using CRISPR gene editing. This protocol requires a Site-Directed Mutagenesis Kit to construct the appropriate plasmid vector for targeting my gene of interest in cancer cells. To create this vector, I must use site-directed mutagenesis to insert the necessary guide RNA, that will target my gene of interest, into the CRISPR vector. I will then transfect cancer cells with the CRISPR plasmid DNA so that my gene of interest cannot be expressed.

After performing the know-down and knock-out procedures, I will use quantative PCR to determine any changes in the expression levels of genes that play a role in tumorigenesis. This protocol requires a Reverse Transcription Kit to converted RNA into cDNA. To verifity gene expression levels, I will perform Western Blotting. This allows the detection of specific proteins and a measurement of protein amount. To perform Western Blot, all of the proteins in a cell lysate are blotted onto a membrane. A primary antibodie against the protein encoded by my gene interest will be use to bind this protein. Then a secondary antibody, against the primary antibody, will be used for detection. This secondary antibody is conjugated to the horseradish peroxidase ennzyme (HRP). For visualization via hemiluminescence, this protocol requires the substrate for HRP.

For my research, I am requesting the following:
iScript™ Reverse Transcription Supermix, 25 x 20 µl rxns, 100 µl
Opti-Mem I medium, 100 mL
SuperSignal West Pico PLUS Chemiluminescent Substrate for HRP, 20 mL
Q5® Site-Directed Mutagenesis Kit

By signing below, students and faculty mentors affirm that the requested supplies are necessary and will support the student research project and that the faculty mentor recommends funding of this proposal.

Student Signature **Date** 3/2/2017

Faculty Mentor Signature **Date** 3/2/2017

Tobey Colston					
CSR Research Supplies Itemized Budget					
Winter 2017					
Product	Company	Catalog #	Price	Quantity	Cost
iScript™ Reverse Transcription Supermix, 25 x 20 µl rxns, 100 µl	BioRad	1708890EDU	$ 120.00	1	$ 120.00
4–20% Mini-PROTEAN® TGX™ Precast Protein Gels, 10-well, 30 µl	BioRad	4561093S	$ 22.00	2	$ 44.00
Opti-Mem I medium, 100 mL	ThermoFisher Scientific	31985062	$ 20.54	1	$ 20.54
SuperSignal West Pico PLUS Chemiluminescent Substrate for HRP, 20 mL	ThermoFisher Scientific	34579	$ 42.50	1	$ 42.50
DEPC-Treated Water, 100 mL	ThermoFisher Scientific	AM9915G	$ 32.41	1	$ 32.41
Q5® Site-Directed Mutagenesis Kit	NEB	E0554S	$ 188.00	1	$ 188.00
SUBTOTAL					$ 447.45
SALES TAX (10%)					$ 45.00
SHIPPING					$ 52.50
TOTAL					$ 499.95