

# 2018 PAID SUMMER PROGRAMS

## INFORMATION MEETING: JANUARY 27, 2018\*

**Analysis of gene expression in sub clones of an osteocytic cell line**  
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**Abstract**  
Osteoclasts, the bone forming cells, and osteoclasts, the bone resorbing cells, are necessary to maintain bone mass in human and mice models. The function of these actions are determined by osteocytes, the most abundant cell type. Over the years the only osteocytic cell line that has been completely characterized is the MLO-Y4. However, these cells do not express sclerostin, the product of the SOST gene and a molecule that is essential for osteocyte function. Normally sclerostin decreases bone formation. If there are lower levels of sclerostin then there is higher bone formation. Previous work of Dr. Plotkin's laboratory created sub-clones of the MLO-Y4 osteocytic cells expressing green fluorescent protein (GFP). The purpose of my project is to determine the level of protein and gene expression and, in particular, any of the sub-clones expresses SOST/sclerostin. Currently we are working on protein gene expression to check the levels of SOST/sclerostin. I have started having different subclones, and have analyzed the cellular morphology and the expression of GFP. I have also isolated protein and RNA from 4 subclones. Once we collect protein and mRNA from at least 3 subclones we will use Western blotting to measure protein expression and the qPCR to measure gene expression.

**Objective**  
The aim is to determine the levels of SOST/ sclerostin in the sub clones after being cultured. This is done by determining gene and protein expression.

**Methods**  
**Establishment of MLO-Y4 cells stable transduced with green fluorescent protein:**  
-The plasmid containing the retroviral GFP construct was transiently transfected into the packaging cell line Phoenix Eco (3) using Superfect (Qiagen, Santa Clarita, CA).  
-Supernatants containing retroviral particles were collected 24-48 h after transfection, and used to infect cell cultures.  
-Subconfluent MLO-Y4 osteocytic cells were exposed to viral supernatants in the presence of 8 µg/ml polybrene for 8 h and then incubated in fresh culture medium for 18 h.  
-Transduced cells were selected by culturing them in the presence of 400 µg/ml of G418 for three weeks.  
**Experimental Procedure:**  
**Cell culture** - MLO-Y4 GFP expressing osteocytic cells were cultured and expanded using 10cm collagen coated plates. Cells from expanded clones were trypsinized and plated in replicates of 6.  
**RNA extraction** - mRNA was isolated and quantified from expanded clones.  
**Quantitative PCR** - After quantifying each sample, mRNA was normalized and cDNA was generated using PCR.  
**Protein Extraction** - Protein lysates were removed from a 10 cm plate by use of lysis buffer. Cells were scraped off and pipetted in the appropriate labeled tubes. Concentrations of each sample of protein were quantified with protein assay.

**Clone Morphology**  
Figure 7: Clone 5  
The cells in clones 5, 6, 10, 11, and 12 were cultured in 96-well plates. The cells were very star-shaped when alive (left) and appeared to have rounded cell bodies when dead (right).  
Figure 8: Clone 13  
The cells in clone 13 were cultured in 96-well plates. The cells were very star-shaped when alive (left) and appeared to have rounded cell bodies when dead (right).

**Results**

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See page 2 for location & parking. Other details at:

[www.IndyProjectSEED.org/application](http://www.IndyProjectSEED.org/application)

*\*This meeting is for students in grades 9-12 from Indianapolis and Marion County Public Schools, interested in exploring a career in science. The meeting is sponsored by the Indianapolis Project SEED, the Future Scientist Program and Project STEM. Other program directors will present also. Seats are limited, Click & Register Today!*

**\*Location and Parking:** The IUPUI Campus Center is located at 420 North University Boulevard, Indianapolis, IN 46202.

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Enter campus on New York Street, turn right onto Patterson, the next street after University. Patterson ends at the Vermont Garage (G) next to the Campus Center (CC)

