



# CALIFORNIA SCIENCE & ENGINEERING FAIR 2018 PROJECT SUMMARY

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<b>Project Title</b> <b>Optimizing Long-term Gene Expression Using Chromatin Insulators in Stably Integrated Multi-gene Constructs</b>	
<b>Objectives/Goals</b> Current gene constructs need an effective mechanism able to maintain expression units in open chromatin configuration, both prohibiting cross talk between nearby promoters in multi-gene constructs and enabling long-term expression. Chromatin insulators have barrier insulation and enhancer-blocking activity that are capable of regulating gene transcription and insulating desired fragments of DNA in gene constructs. This research determined the optimal chromatin insulator for use in the field of synthetic biology. <b>Abstract</b> <b>Methods/Materials</b> Three structurally identical E. coli plasmids containing CAG-GFP sequences were constructed, each utilizing a different type of chromatin insulator: chromatin insulator 2 (ci-2), chicken hypersensitive-site 4 (cHS4), and no chromatin insulator. Each plasmid was amplified, purified, then stably integrated into its own line of HEK293T cells. The cell lines were selected for their respective plasmid using Puromycin and Zeocin to guarantee successful stable integration. Each cell line was sorted to obtain 100% of the GFP-positive cells. The percent of GFP-positive cells was measured each week using fluorescence activated cell sorting to analyze the level, intensity and uniformity of GFP expression over time for a period of 4 weeks (data collection still occurring). <b>Results</b> Cells containing ci-2 maintained bright and constant GFP expression intensity and uniformity in signal strength over the 4-week period. These cells also maintained ideal expression levels, constant at 99.7% cells GFP-positive. Cells with no chromatin insulator showed deviation in GFP signal intensity and dropped in expression level from 100% at week 1 to 92.1% at week 4. Cells containing cHS4 showed poor uniformity in GFP intensity and dropped from 100% GFP expression at week 1 to 80.4% at week 4. <b>Conclusions/Discussion</b> It was determined that chromatin insulator ci-2 is significantly more effective than the widely-used cHS4. These findings have application in synthetic biology as functional and effective chromatin insulators would allow gene therapy and synthetic gene constructs to be more accurate and precise, both in research and clinical settings.	
<b>Summary Statement</b> I found that chromatin insulator 2 is ideal for optimizing long-term gene expression for use in gene therapy and DNA assembly methods as compared to the widely-used cHS4.	
<b>Help Received</b> I conducted my research in the Zhao Lab at University of California, Irvine under the supervision of Dr. Jan Zimak, who trained me to carry out all procedures and use equipment in accordance to university and state safety standards.	