# CALIFORNIA STATE SCIENCE FAIR
# 2015 PROJECT SUMMARY

**Name(s)**
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**Project Number**
35579

**Project Title**
## Optimizing Viral Vectors for Site-Specific Gene Therapy in Hematopoietic Stem Cells

**Abstract**

**Objectives/Goals**
Gene therapy in hematopoietic stem cells (HSC) is a powerful potential treatment for hematologic diseases such as sickle-cell anemia. Changes are retained throughout differentiation. Previous treatments in HSC added genes randomly throughout the genome, possibly disrupting/activating oncogenes. This could be ameliorated through using a site-specific nuclease (i.e. zinc finger nuclease, or ZFN) to cause a break at a predetermined site. Genomic repair mechanisms can be hijacked by adding a repair template to correct/replace a gene. Adeno-associated viral vectors (AAV) are an efficient way to provide the repair template, capable of inserting/repairing genes at frequencies over 20%. Though AAV-ZFN treatments are efficient for site-specific gene therapy, there is some toxicity associated with this, which I aimed to reduce through improving AAV efficiency.

**Methods/Materials**
I took two approaches to modifying AAV: 1) improving the suitability of the template by converting the normally single-stranded DNA genome (ssAAV) to a compact, self-compliment, double-stranded (scAAV) form; and 2) modifying the viral capsid to avoid cellular detection and disposal. For both methods, HSC were treated in vitro with various forms of AAV, then electroporated with ZFN RNA, and allowed to incubate for 96 hours. To compare the efficiency of ssAAV and scAAV, DNA was extracted, and gene addition efficiency was measured through RFLP assays. To test the capsid mutants, GFP expression in HSC was measured by flow cytometry and compared to the efficiency of wild-type AAV capsid.

**Results**
Results show that scAAV is actually less efficient than ssAAV, meaning that changing the genome did not improve efficiency. The capsid mutant experiments are currently underway.

**Conclusions/Discussion**
There are two main possibilities as to why ssAAV is more efficient than scAAV in gene addition: 1) the ssAAV genome contatemerizes greatly during vector genome replication; or 2) the ss configuration of template DNA is more conducive to cellular DNA repair mechanisms.

**Summary Statement**
A single stranded configuration of AAV is more efficient than a self-compliment configuration for gene addition in HSC.

**Help Received**
I used lab equipment at the Keck School of Medicine at USC under the supervision of Dr. Paula Cannon and Dr. Colin Exline.