E. curi: A way of deactivating antibiotic resistance genes in E. coli

By Jonathan Huang, freshman majoring in Health Promotion

A Summer Dedicated to Research

Originally hailing from Fountain Valley, California, Kevin Le is a sophomore at USC double majoring in math and physics. Last summer, Kevin participated in IGEM (Internationally Genetically Engineered Machine), a national team synthetic biology competition, along with several other undergraduate researchers under the instruction of Dr. Sean Curran. What drew Kevin to IGEM was "I felt that its mission was very important and resonated well with what I like to see in science, an innovative fostering of a relationship between the scientific community and society."

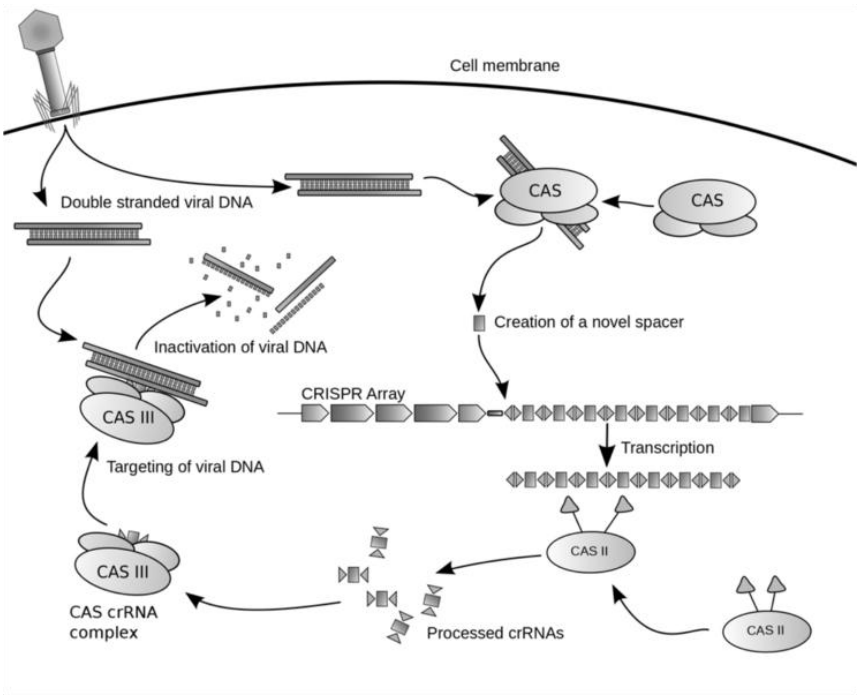
The essential aim of IGEM is to create a database of DNA parts so that people can pick and choose parts to engineer together and create tools for novel technologies. Each team must not only use already existing parts, but also develop new parts to add to the database. During the beginning of the summer, Kevin and his teammates brainstormed nine ideas for a project and then narrowed them down to three to

begin in wet lab. Quickly, however, the team realized that one of those ideas had by far the most potential: thus project *E. curi* was born.

Resistance No More

CRIPR (Clustered Randomly Interspaced Palindromic Repeats) is a system that helps bacteria protect their genome and destroy foreign DNA. It functions as a biological defense mechanism much similar to the human immune system. The aim of the team's research was to exploit the CRISPR system to develop a method for deactivating the antibiotic resistance genes in E. coli. His team isolated the CRISPR system from the bacteria and used restriction enzymes to cut up the CRISPR genome. Then, through horizontal gene transfer, a process in which genetic material is inserted into neighboring bacteria from an external source, they inserted a "spacer", or additional gene sequences, into the genome, re-assembled the CRISPR system and injected the system back into the original bacteria.

This new CRISPR system acted analogously to a Trojan Horse. Although the system was meant to assist in defense, the added gene sequence caused proteins associated with CRISPR to destroy the bacteria's antibiotic resistance genes. Over the summer, Kevin and his team tested different versions of modified CRISPR systems on several antibiotics including ampicillin. Laboratory techniques that Kevin performed regularly included DNA transformation, plasmid mini-prep, horizontal gene transfer and PCR (polymerase chain reaction).



How CRISPR Works (courtesy of USC IGEM wiki page)

The research did not always go smoothly. To periodically check that the modified CRISPR system worked, Kevin and his teammates would perform optical density readings on the cultures to check the decay rate of the bacteria. In one instance, the team saw unpredicted decay for a certain culture of cells. After a long night of pondering potential sources of error, the team came to the conclusion that perhaps there were DNA sequences in the CRISPR genome that recognized sequences in the intrinsic bacterial genome. Then they set to work to locate these specific sequences using the search function on the online genomic record for the particular bacteria. Their hypothesis turned out to be true, and by modifying the CRISPR genome the team was able to avoid the problem of accidentally destroying the bacteria's genome along with the antibiotic resistance genes. Clever problem solving and hard work ethic characterized the nature of the research that Kevin and the rest of his team performed during the summer.

The Ride Never Stops

Even after summer, there was further work for Kevin to do. He prepared for the presentation at the national IGEM conference in Indianapolis, where USC's team had one of the most original ideas. Currently he is making even further progress in determining how to modify the CRISPR system to efficiently deactivate different types of antibiotic resistance genes. Although there is still much to investigate, modification of the CRISPR system seems a promising method for impeding phages from destroying bacteria and combating plasmids that transfer antibiotic-resistant genes to other bacteria, functions that connote powerful implications in medical and environmental fields.

However, Kevin's achievements are not just limited to the research lab. He recently won USC's Science Film Festival and is part of the USC Math Team and SC Outfitters. In addition to his many extracurricular activities, his hobbies include philosophy, international relations, music and basketball. His career goal is either become a physicist or work for the U.S. government to discover new clean energy solutions. There is no doubt that, given the tremendous amount research experience that Kevin gained through IGEM, he is well on his way to achieve any of his scientific career pursuits.