Siemens Competition
Math : Science : Technology
National Finalist

Names: Evelyn McChesney & Madeline McCue
High School: Breck School
Mentor: Dr. Yiannis Kaznessis, University of Minnesota
Project Title: *Engineering a Broad-Spectrum Antibacterial Probiotic Via Inclusion of Antimicrobial Peptide-Encoding DNA, Year Two*

This is the second year of a two-year study to engineer probiotics to deliver antimicrobial peptides (AMPs) that show promise as an alternative to antibiotics. The work this year involved designing a digital "blueprint" in SerialCloner for a broad-spectrum *Escherichia coli* Nissle 1917 AMP delivery system designed to secrete two AMPs to target enteropathogenic bacterial infections. The AMPs microcin L and enterocin A were initially chosen because they show antimicrobial activity against gram-negative and gram-positive enteropathogens, respectively.

The first step included designing a plasmid (pMK-P+) with a pMK-RQ-Bb backbone and the strong promoter proTeOn+. Next, because the microcin L secretion machinery was costly, the highly homologous microcin V operon was isolated from an available plasmid (pHK22) and then mutated so it would not the produce microcin V. The microcin V operon was digitally inserted into pMK-P+ to create the pMK-P+-V plasmid. Finally, gene blocks that encode for production of the AMPs microcin L and enterocin A were designed and digitally inserted into the pMK-P+-V plasmid.

Laboratory work using digestion, ligation, and PCR was successful in engineered the pMK-P+-V plasmid. Furthermore; a commercial pHK22 plasmid was successfully mutated to produce the microcin V operon (pHK22Δ).