



**CALIFORNIA STATE SCIENCE FAIR
2012 PROJECT SUMMARY**

Name(s) Preethi Padmanaban	Project Number S0520
Project Title GM Plants: Manipulating Extracellular pH to Reduce Antibiotic Transformation Frequency in E. coli	
Abstract Objectives/Goals Genetically modified plants usually contain a gene of interest as well as an antibiotic marker gene, which can be transferred to soil bacteria, causing a myriad of problems. The objective of this project is to determine a soil pH that would reduce genetic transformation while being conducive to plant growth. Methods/Materials E. coli was grown in an LB broth culture, then made competent using solutions of MgCl(2) and CaCl(2). Tris-HCl buffer solutions of pH 5-9 were added to agar plates and LB broth. The plasmid used was p-ARAr, a vector with RFP and ampicillin resistance. The E. coli was transformed and grown for 40 hours in each respective pH. Additional trials with ampicillin, arabinose, and the buffer were plated. Smaller intervals of pH and dilutions were later tested to narrow down results. Results The E. coli grown naturally in each pH showed the most colony-forming units at pH 7. The bacteria transformed and grown at pH 8 had the highest number of transformants per plate. Those at pH 5, 6, and 9 had considerably fewer successful colonies. The bacteria grown at a 1:100 dilution had a higher frequency of transformed bacteria across pH levels, as compared to the undiluted bacteria. Conclusions/Discussion The optimal pH for transformation in E. coli is between pH 8.25 and pH 8.5. This range would thus be the range to avoid in soil. As transformation was observed most frequently at a lower population density, it is likely that soils with a higher concentration of bacteria and microbes would have a lower risk of gene transfer. Disregarding the extreme pH levels of 5 and 9, the targeted soil pH would be pH 6, as this would both reduce transformation frequency and allow GM plants to grow normally.	
Summary Statement E. coli was transformed and grown at a range of pH levels to determine the pH value that is least conducive to genetic transformation, simulating the scenario of GM plants transferring resistance genes to environmental bacteria and plants.	
Help Received Used high school lab equipment under guidance of Ms. Huong Tran; Received reagents, plasmid, and bacteria from Dr. Katy Korsmeyer of SCCBEP.	