

## BACKGROUND

*Agrobacterium tumefaciens* is a bacterial plant pathogen that causes crown gall disease and tumors in the tissues of plants. They accomplish this through using two major growth hormones, cytokinin and auxin. This can cause nurseries to lose product and sales and can adversely affect a plants health.

## THE PROJECT

This project aims to study the role that cytokinin and auxin genes have in the virulence of the bacteria. To do this, we created mutant *Agrobacterium* by deleting these genes, adding new ones, or rearranging their order on the bacterial chromosome.



Checking bacterial transformations for fluorescent proteins that indicate first transformation was a success

Lindsay Sandusky Summer 2019 Beginning Researchers Support Program

## GENERATING MUTANTS

- Using the pJQ 200 suicide plasmid, we generate in-frame deletions of *Agrobacterium tumefaciens*. We'll be amplifying and assembling the upstream and downstream fragments that flank the target genes into the plasmid.
- This plasmid is introduced into *Agrobacterium* via electroporation, and the DNA is integrated into the chromosome via a crossing-over event. This is the first recombination event. As the bacterial cells replicate, a small percentage of these cells will undergo a second recombination event that creates mutants.
- We screen for these mutants by growing them on an LB with sucrose plate and confirm their mutation via colony polymerase chain-reactions (PCR).
- With these mutants, we are able to add genes in any order or combination and decipher which are needed for virulence.

## FUTURE RESEARCH

After a few months practice generating bacterial mutants and learning the skills to screen for their successful recombination, I will begin to generate mutants with different combinations and deletions of the three cytokinin and auxin genes. I can then begin to study the questions of how these genes operate by doing complementation assays to assess the virulence of these mutants.

## ACKNOWLEDGEMENTS

I would like to thank the ER Jackman Friends and Alumni Fund for funding this research, Chih-Feng Wu, Jeff Chang and the rest of the Chang lab for their support.

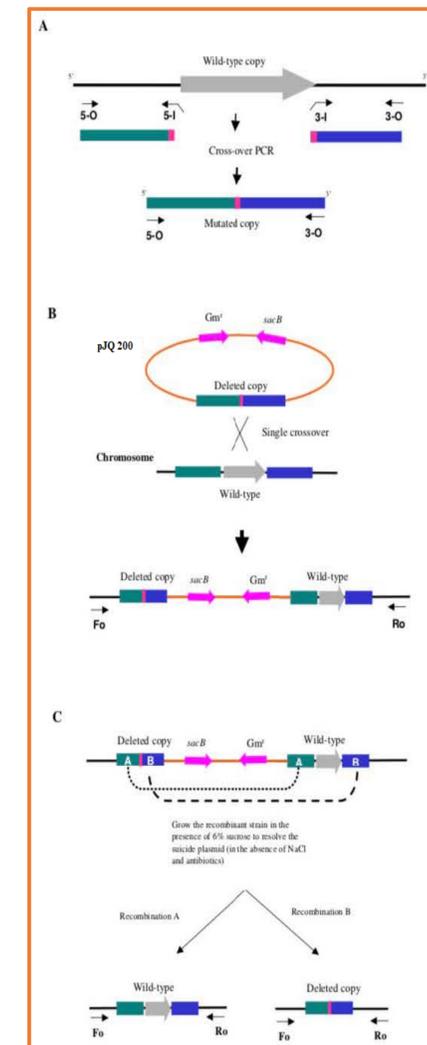


Figure 1. The recombination process.

Adapted from Gao *et al.*, 2006.