Quantifying CRISPR/Cas9 as a method for preventing Geminivirus replication

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Purpose

Assess the feasibility of using the CRISPR/Cas9 system to generate geminivirus resistance in plants by targeting a site in the viral genome that is essential for viral rolling circle replication.

Hypothesis

For the purposes of this experiment, the null hypothesis (H0) will be that the CRISPR/Cas9 system will not be able to efficiently attenuate replication of the virus. The research hypothesis will be that if a CRISPR/Cas9 cassette is constructed to express a gRNA that targets the virus replication of origin site, then CRISPR will be able to cleave the site, allowing for NHEJ, and effectively stop viral replication. A significance level of 0.05 will be used for data analysis.

Project Overview

The *Geminiviridae* family is at cause for multiple economic agricultural losses. Geminiviruses all share a single stranded DNA (ssDNA) genome and a common site of replication: TAATATTAC. The CRISPR/Cas9 is a relatively new and quick technique that can be used to target this replication site and cause a double stranded break (DSB) at the target site once the virus is converted to double strand DNA by host DNA polymerase. This break can be repaired by nonhomologous end joining (NHEJ), resulting in novel DNA insertions and deletions. The resulting viral mutation may halt production of the Rep protein, inhibiting viral replication. The efficiency at which replication was inhibited was qualified by quantitative, real-time PCR.

Variables/Research

Controlled Variables	Independent Variable	Dependent Variable
• Time allowed for virions to spread, age and growth factors of *N. benthamiana plants*	• The presence of a gRNA targeting the Ori of the pTRANS201	• The viral titer present in *N. benthamiana* leaves identified by qPCR

Materials

Materials	Quantity
pRGEB31 w/ gRNA	15 µL
pTRANS201	15 µL
N. Benthamiana seeds	165 seeds
A. Tumefaciens (AGL0)	2 mL
E. Coli (OneShot Top10 Cells)	2 mL
Propane –1,2,3-triol	50 mL
Antibiotics: Kanamycin and Rifampin	20 µL each

Procedure

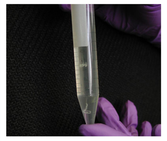
Step 1

Transformation of AGL0 and Top10 cells followed by preparation of glycerol stocks

Step 2

Agroinfiltration of *N. Benthamiana* plants proceeded by a two day waiting period

Step 3



Extract DNA from *N. benthamiana* leaves and create cDNA

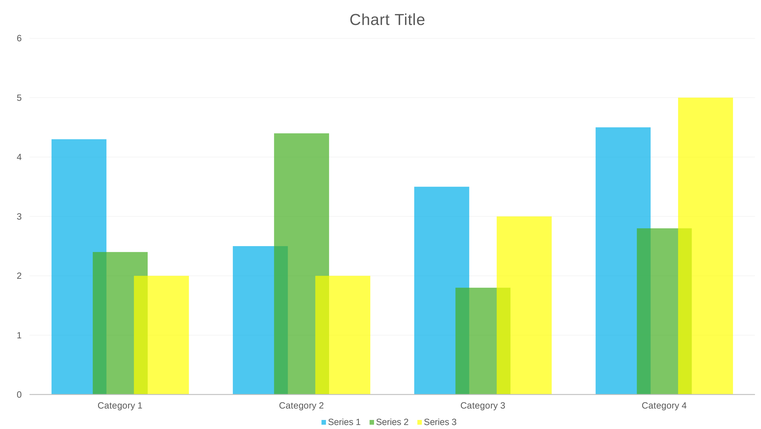
Step 4

Analyze results after following quantitative, real time PCR protocols

Data / Observations

- Observation 1
- Observation 2
- Observation 3

Results



- Include results based on experiments
- Result 2
- Result 3

Conclusion

- Brief summary of what was discovered based on results
- Indicate and explain whether or not the data supports hypothesis

Works Cited

- Include print and electronic sources in alphabetical order