Determining the Roles of Candidate Genes in Citrus-HLB Interactions and Creating HLB-Resistant Citrus Cultivars

UF UNIVERSITY of FLORIDA
IFAS
Citrus Research and Education Center

Project Director
Fred G. Gmitter Jr.

USDA-NIFA-SCRI
Citrus Disease Subcommittee Meeting

- Fred Gmitter: UF-Citrus Research and Education Center
- Zhanao Deng: UF-Gulf Coast Research and Education Center
- Yi Li: University of Connecticut
- Richard McAvoy: University of Connecticut

Research Team Leaders

UF UNIVERSITY of FLORIDA IFAS Citrus Research and Education Center

HLB-Resistant Citrus Scions and Rootstocks

THE NEED

UF UNIVERSITY of FLORIDA
IFAS
Citrus Research and Education Center

- Breeding and selection

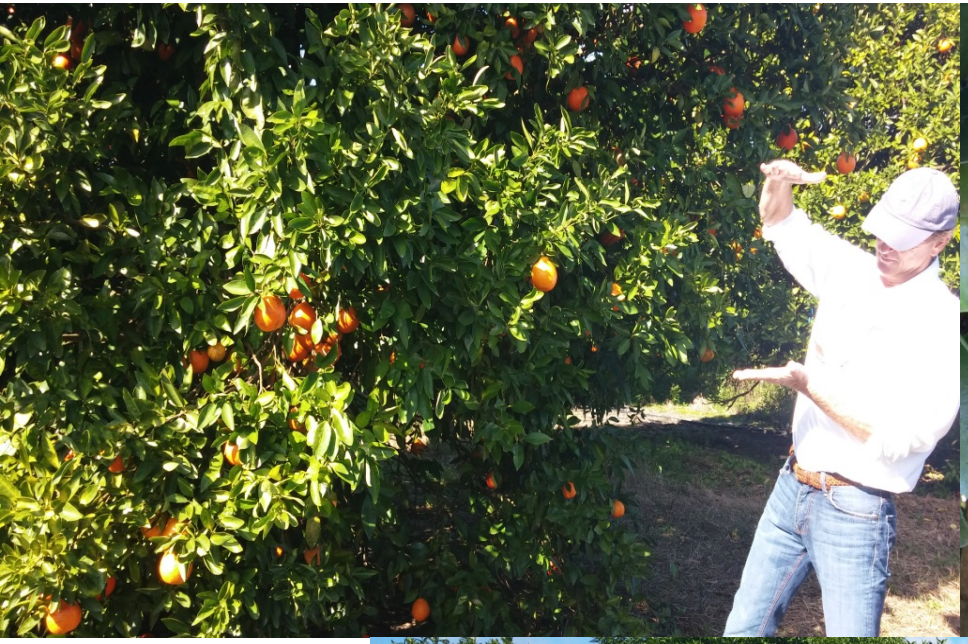
- Genetic transformation to develop plants that produce resistance-inducing molecules

  - Genes from other organisms
  - Artificial gene constructs
  - Citrus-derived gene constructs

- GMO, or NO?!

Options

HLB Tolerance of LB8-9; Sugar Belle®



Pita-2 from Rice

Inoculum budstick

Healthy growth, 2 yr

Transgenic HLB (–) tree expressing the LIMA antibacterial peptide.

Transgenic HLB (–) tree expressing Arabidopsis NPR1

Engineer HLB Resistance/Tolerance with Genes from Citrus or Citrus Relatives

THE GOAL

UF UNIVERSITY of FLORIDA
IFAS
Citrus Research and Education Center

- Validate candidate gene expression
- Identify sequence polymorphisms/dissect gene structure and organization
- Understand the roles of candidate genes
- Develop CRISPR-mediated technologies
- Precision editing to produce HLB-resistant citrus

Research Objectives

UF UNIVERSITY of FLORIDA IFAS Citrus Research and Education Center

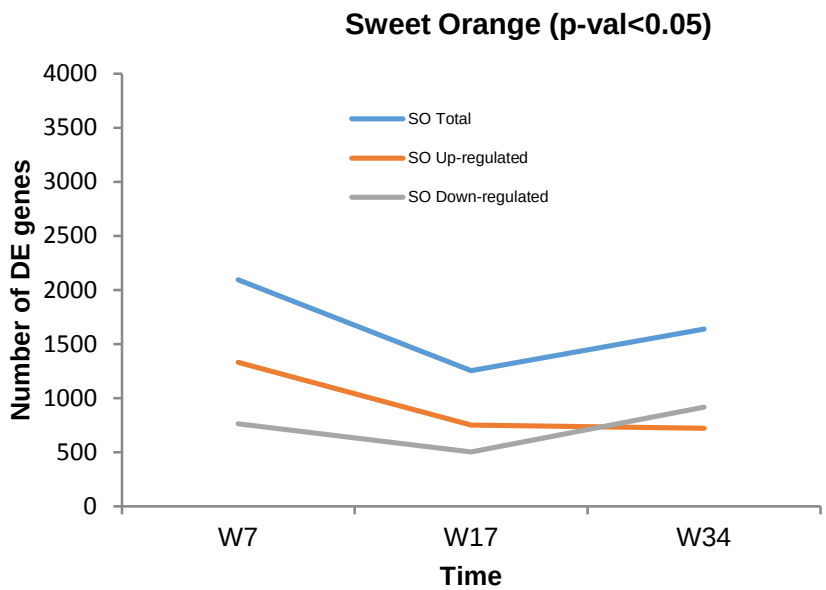
- Genetic linkage studies
  - Segregation of DNA sequence polymorphisms with HLB resistance/tolerance in constructed bi-parental populations

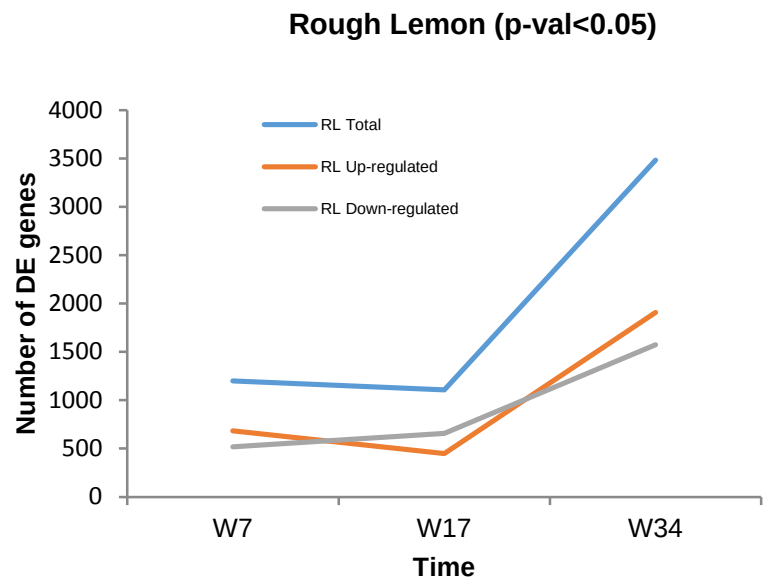
- Genetic association studies
  - Association of DNA sequence polymorphisms with HLB resistance/tolerance among existing varieties & germplasm

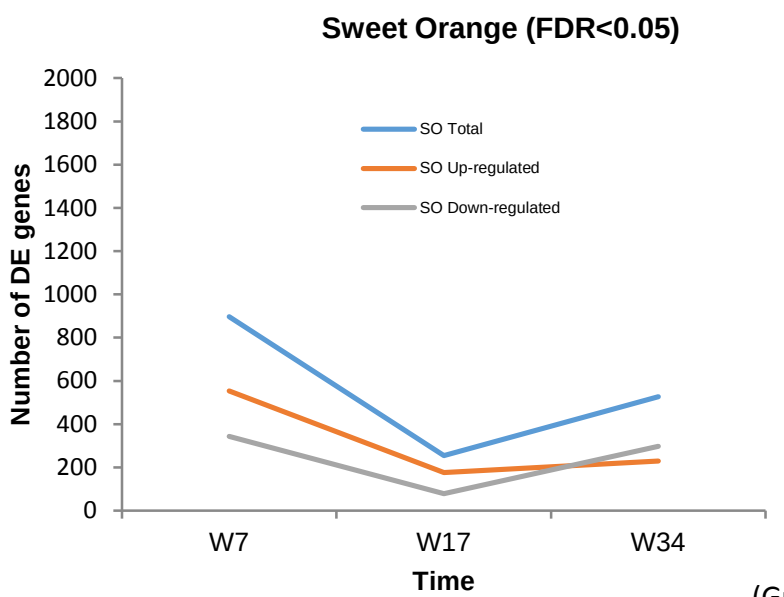
- Gene expression studies
  - Gene expression differences among citrus varieties or species
  - Gene expression differences before & after *C*Las inoculation

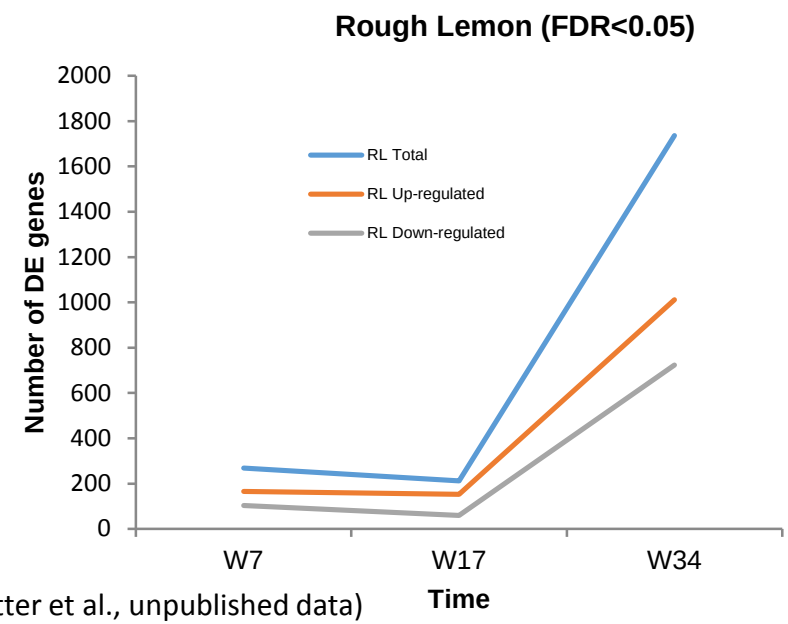
Identify Candidate Genes

Differentially expressed genes in SO and RL

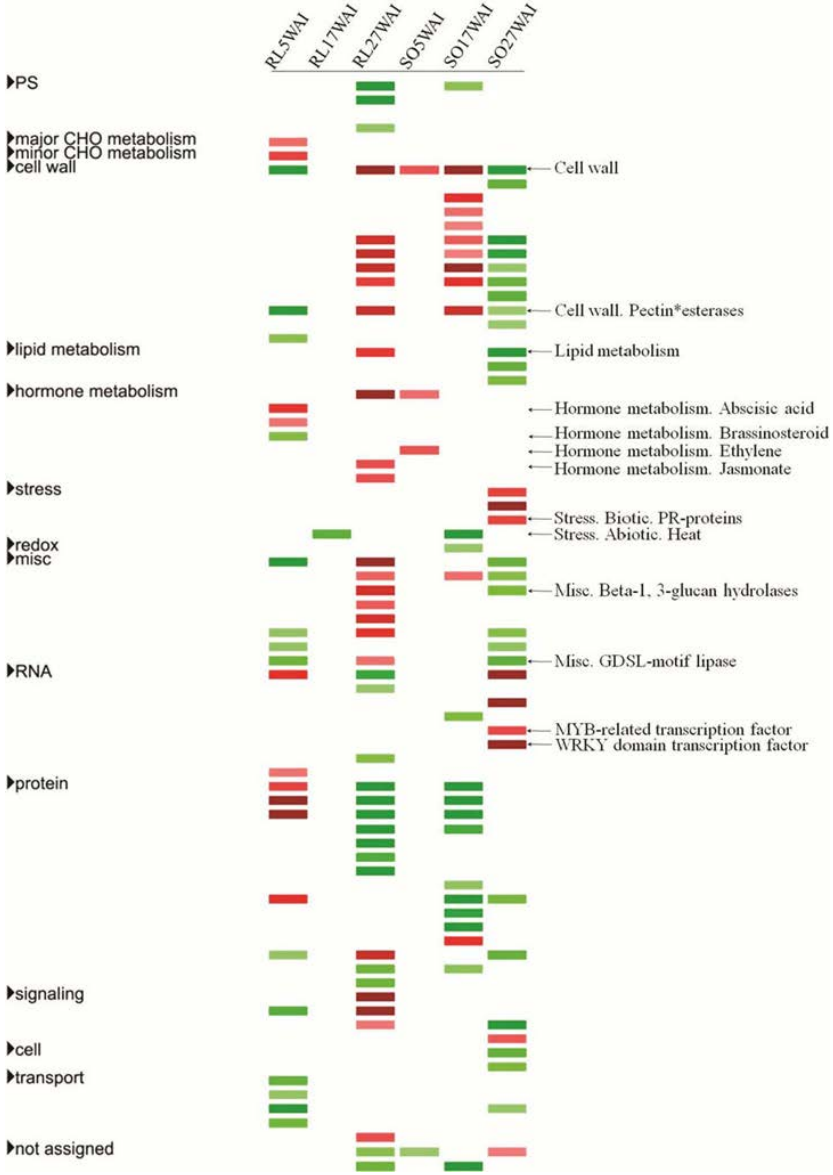








(Gmitter et al., unpublished data)



Gene expression patterns at 5W, 17W, and 27W differ in RL and SO:

- ❑ Cell wall metabolism
- ❑ Lipid metabolism
- ❑ Stress response
- ❑ Protein metabolism
- ❑ Signaling pathways
- ❑ Nutrient transport
- ❑ More …

(Fan et al., 2012)

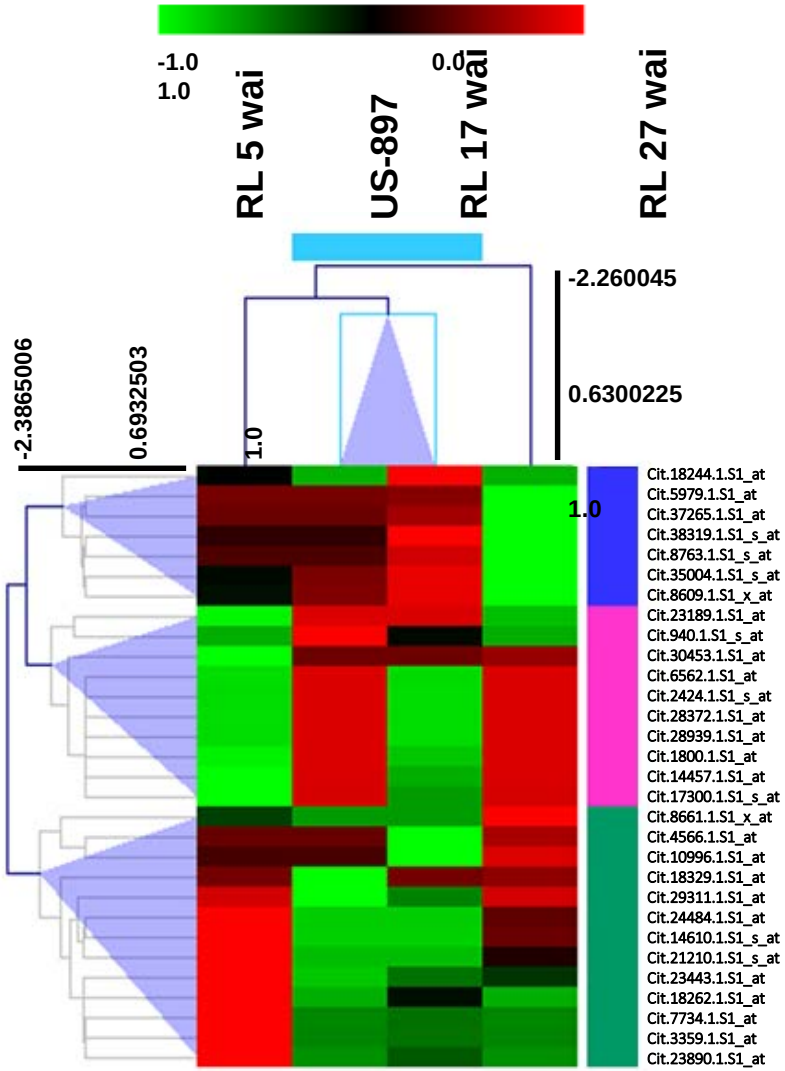
Identify Candidate Genes from Expression Studies (1)

UF UNIVERSITY of FLORIDA IFAS Citrus Research and Education Center

- 22 datasets from all available studies
- 7,412 probe sets differentially expressed
- Two more methods used: LIMMA, RankProd
- 3,122 probe sets identified by both methods
- 318 probe sets differentially expressed in resistant (R) samples
- 288 probe sets differentially expressed in 1 R data set
- 30 probe sets in more than 2 R data sets

Identify Candidate Genes from Expression Studies (2)

UF UNIVERSITY of FLORIDA IFAS Citrus Research and Education Center

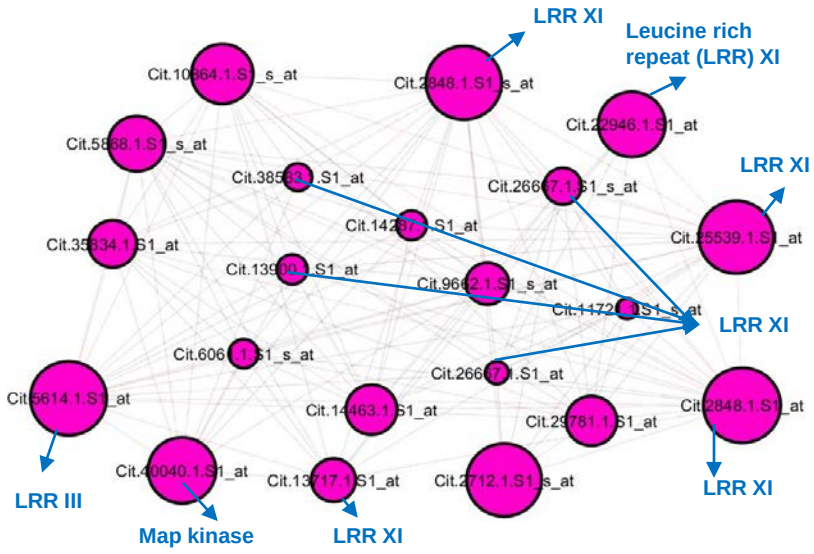


Clustering of 30 R-specific probe sets

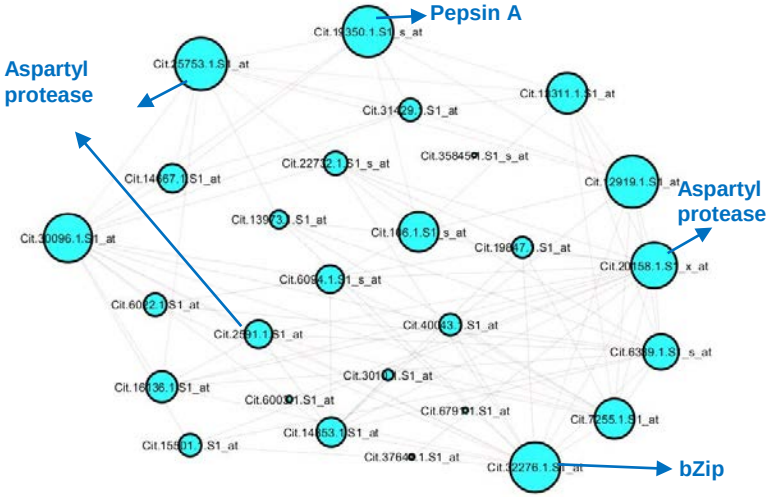
RL = Rough Lemon

US 897 = *Poncirus* hybrid US 897

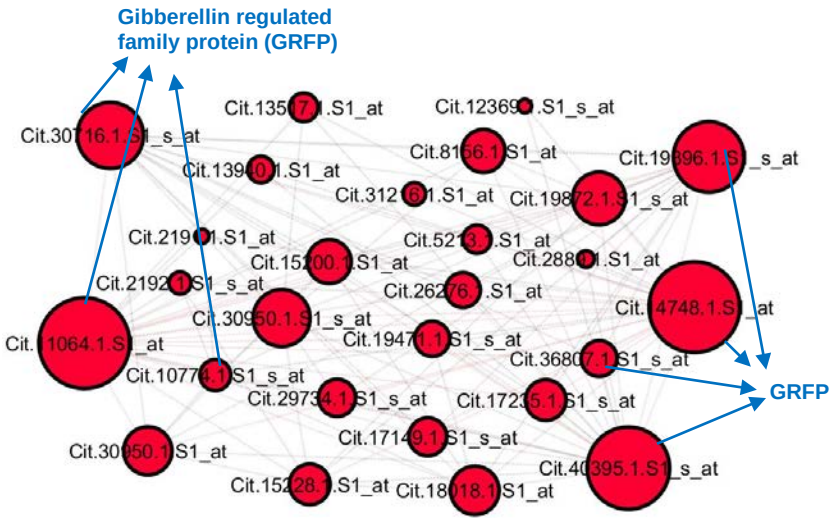
WAI = weeks after inoculation



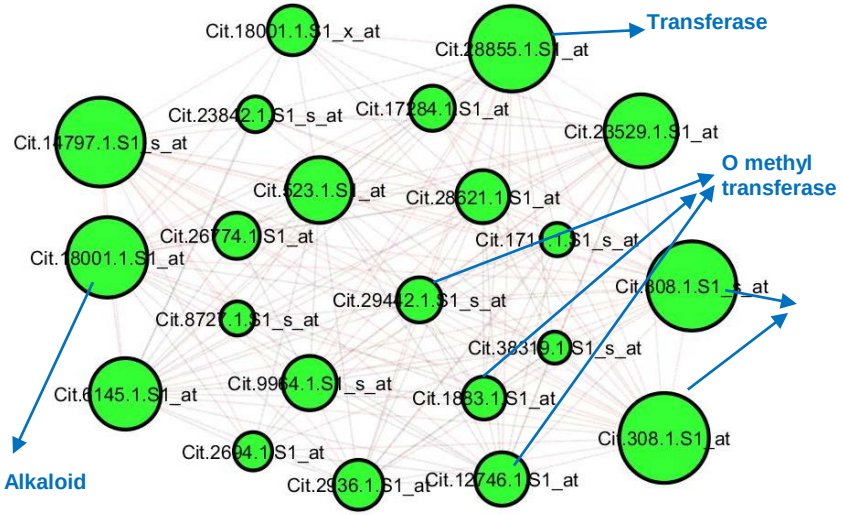
Signaling



RNA and transcription factor



Hormone metabolism



Secondary metabolism

Grafted citrus cultivars, ranging from tolerant to sensitive to HLB, are ready

- Flying Dragon (*Poncirus*)
- Rough lemon
- *Citrus latipes*
- SFB7R2T47 (*Poncirus* hybrid)
- B11R5T68
- 7-6-27
- LB8-9
- US-897
- Valencia

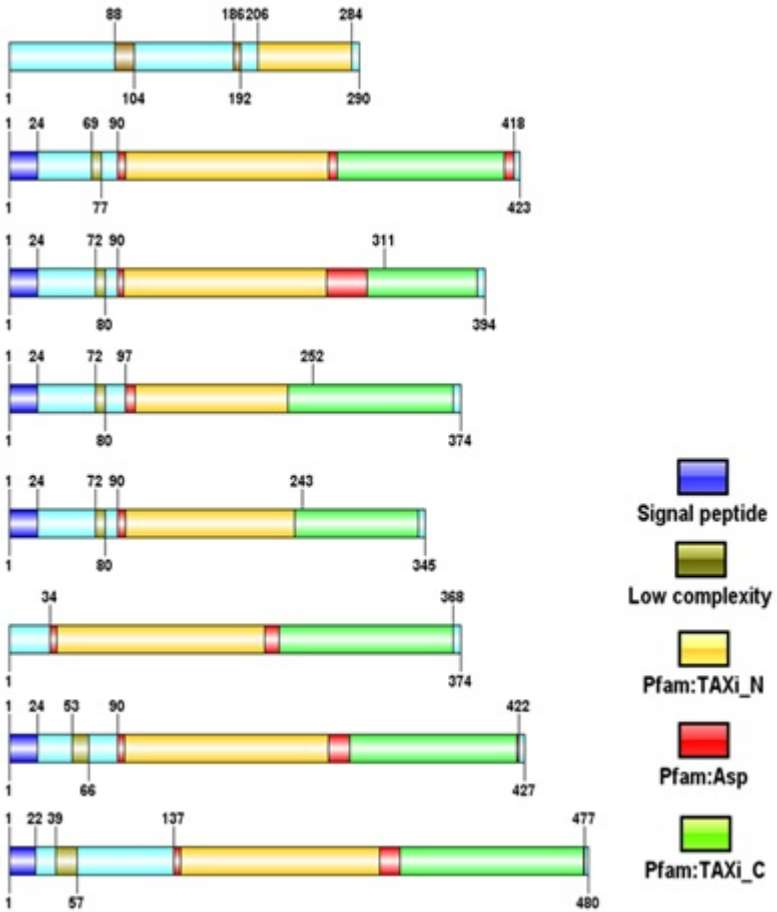
Materials for Gene Validation

UF UNIVERSITY of FLORIDA IFAS Citrus Research and Education Center

- 8 to 9 members in sweet orange, Clementine, & *Poncirus* genomes
- 2 members expressed in *Poncirus* leaves, increased expression after *C*Las inoculation
- Similar genes conferred resistance to bacteria in Arabidopsis & rice
- Secreted to intercellular spaces in those plants
- Suspected to produce a mobile signal
- Induced local & systemic defense responses
- Being cloned for transformation

1st Group of Candidate Genes

Functional Domains in Predicted Proteins



- NB-LRR genes confer resistance to bacteria, fungi, viruses, & nematodes, and also to aphids
- Partial sequencing of the genomes & transcriptomes of *Poncirus* & *Poncirus* hybrid varieties
- ~ 614 copies of NB-LRR genes in *Poncirus*
- 19 NB-LRR genes up-regulated in two *Poncirus* varieties in response to *C*Las inoculation
- Real-time PCR analysis on-going

2nd Group of Candidate Genes

UF UNIVERSITY of FLORIDA IFAS Citrus Research and Education Center

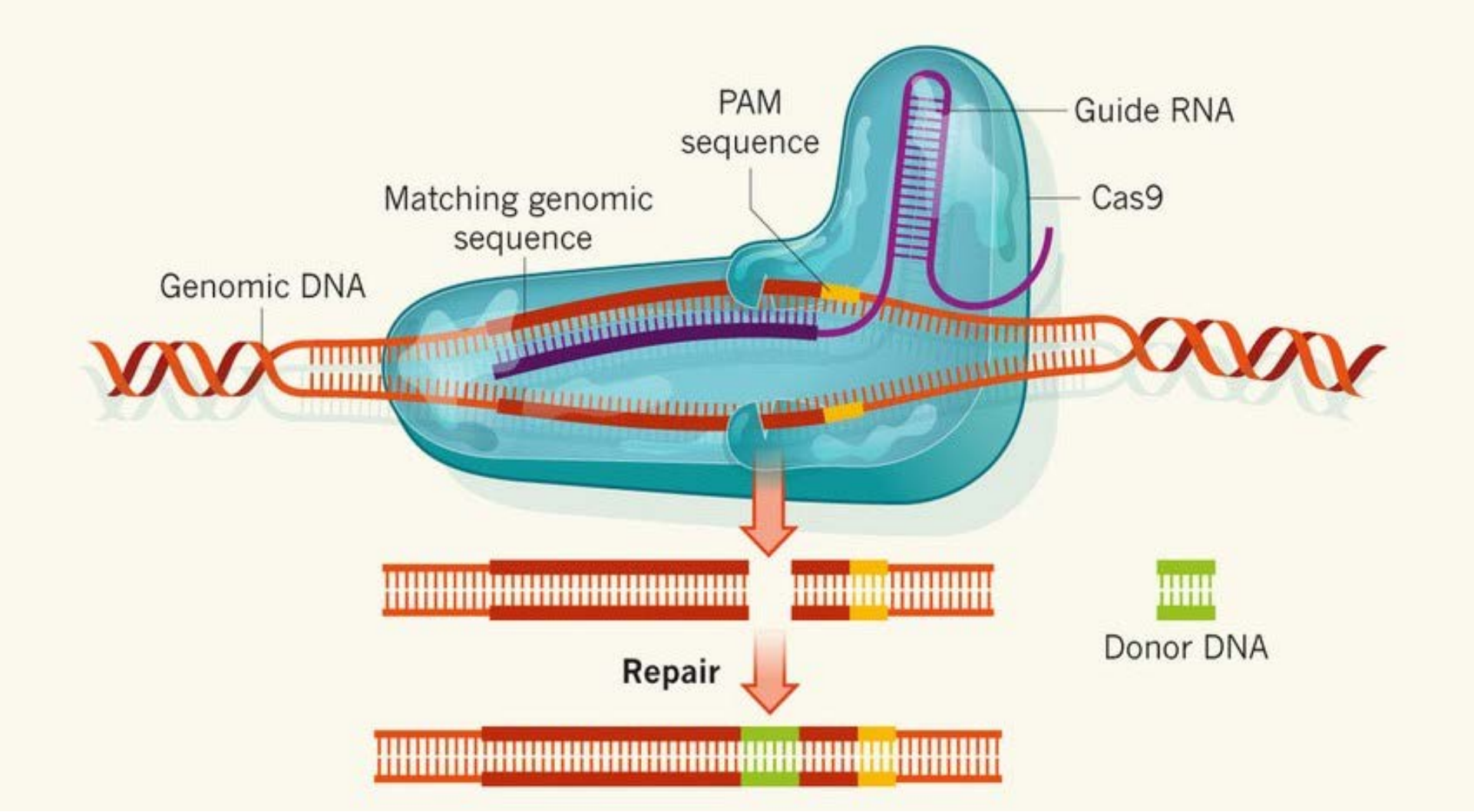
- Highly expressed in Rough Lemon after *C*Las inoculation
- 16-33 genes in sweet orange & Clementine genomes
- 11 commonly induced in *Poncirus*
- 2 genes induced in *Poncirus* varieties after *C*Las inoculation
- Increased expression after aphid infestation
- Reportedly this group of genes has defense functions

3rd Group of Candidate Genes

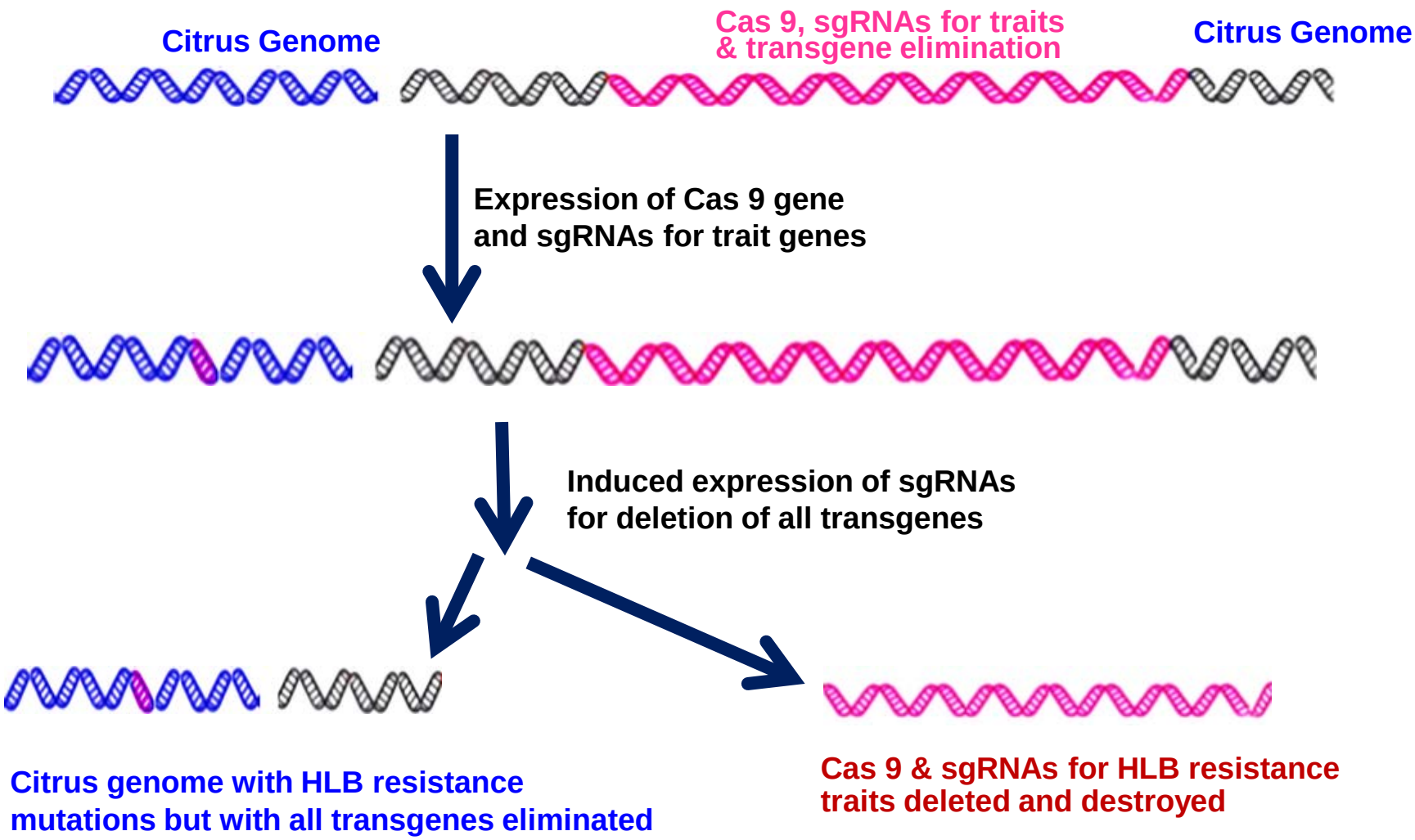
UF UNIVERSITY of FLORIDA
IFAS
Citrus Research and Education Center

Objective 4

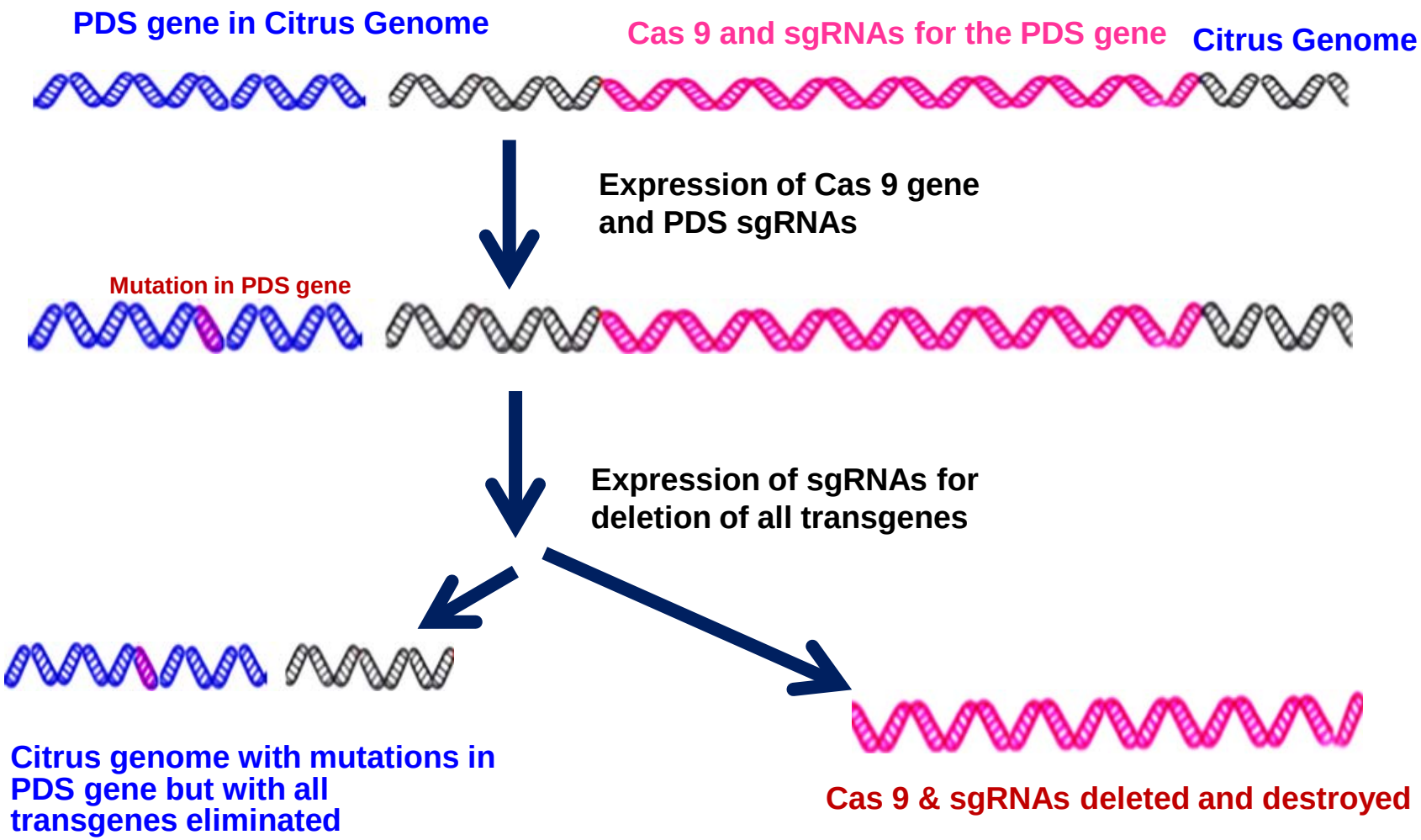
Develop CRISPR-mediated technologies suitable for breeding of non-transgenic HLB-resistant citrus



Our Approach to Create
Non-Transgenic HLB Resistant Citrus Cultivars



A Proof of Concept: **We are using PDS gene as a target gene to create easily detectable albino mutant plants and using these albino mutants to test efficiencies of deleting all transgenes**



A Proof of Concept

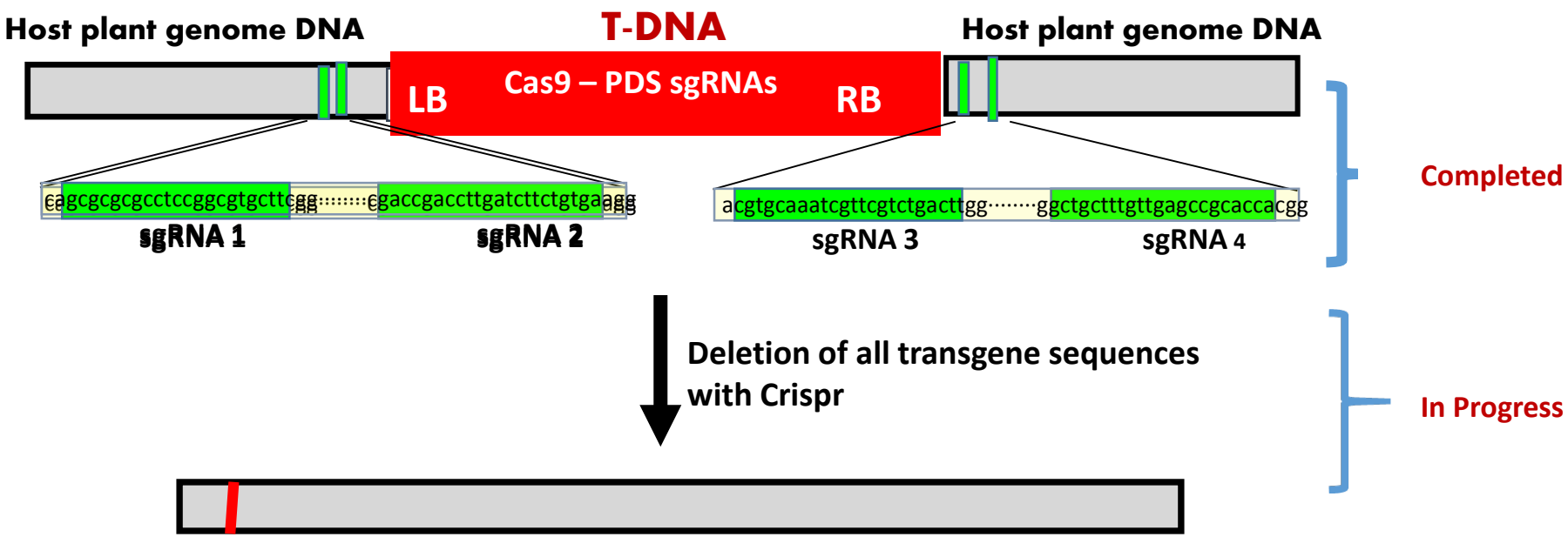
We have produced albino mutants of citrus with CRISPR

We have produced albino tobacco mutants with CRISPR

A Proof of Concept: To delete all transgene sequences from the genome of albino plants, we have cloned and sequenced the border sequences of the T-DNA inserts in albino plants, and designed sgRNAs on each side of the flanking sequences. We are in the process of testing efficiencies of deleting all transgenes from the mutant plants.

Future Experiments: Once we demonstrate that our strategies to eliminate all transgenes from the citrus genome work well, we will create mutations in HLB resistance candidate genes and then eliminate all transgenes from the citrus genome.

Significance: All transgene sequences will be eliminated from the citrus genome once desirable mutations are created with CRISPR.



Host plant genome with a mutation in PDS gene but has no transgenic sequences

USDA-NIFA-SCRI

CRDF

FCPRAC

TEAM MEMBERS

THANK YOU!

UF UNIVERSITY of FLORIDA
IFAS
Citrus Research and Education Center