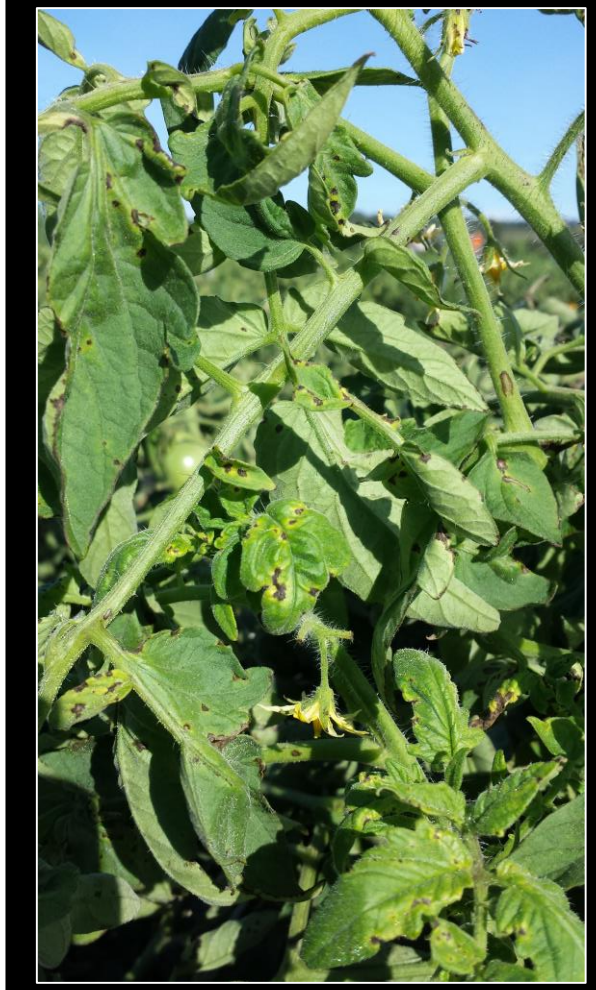
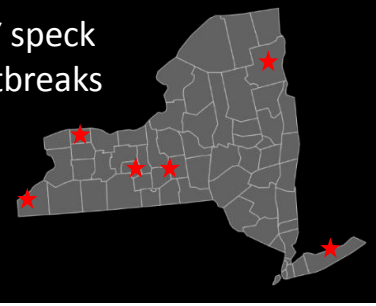
# A Screen of Genetically Diverse Tomato Accessions Reveals a Range of Unusual Responses to *Pseudomonas syringae* pv. *tomato*

**Robyn Roberts,** Samantha Mainiero, Adrian F. Powell, Sarah R. Hind, Susan R. Strickler, and Gregory B. Martin

Boyce Thompson Institute

# Bacterial Speck of Tomato: An Economically Important Disease

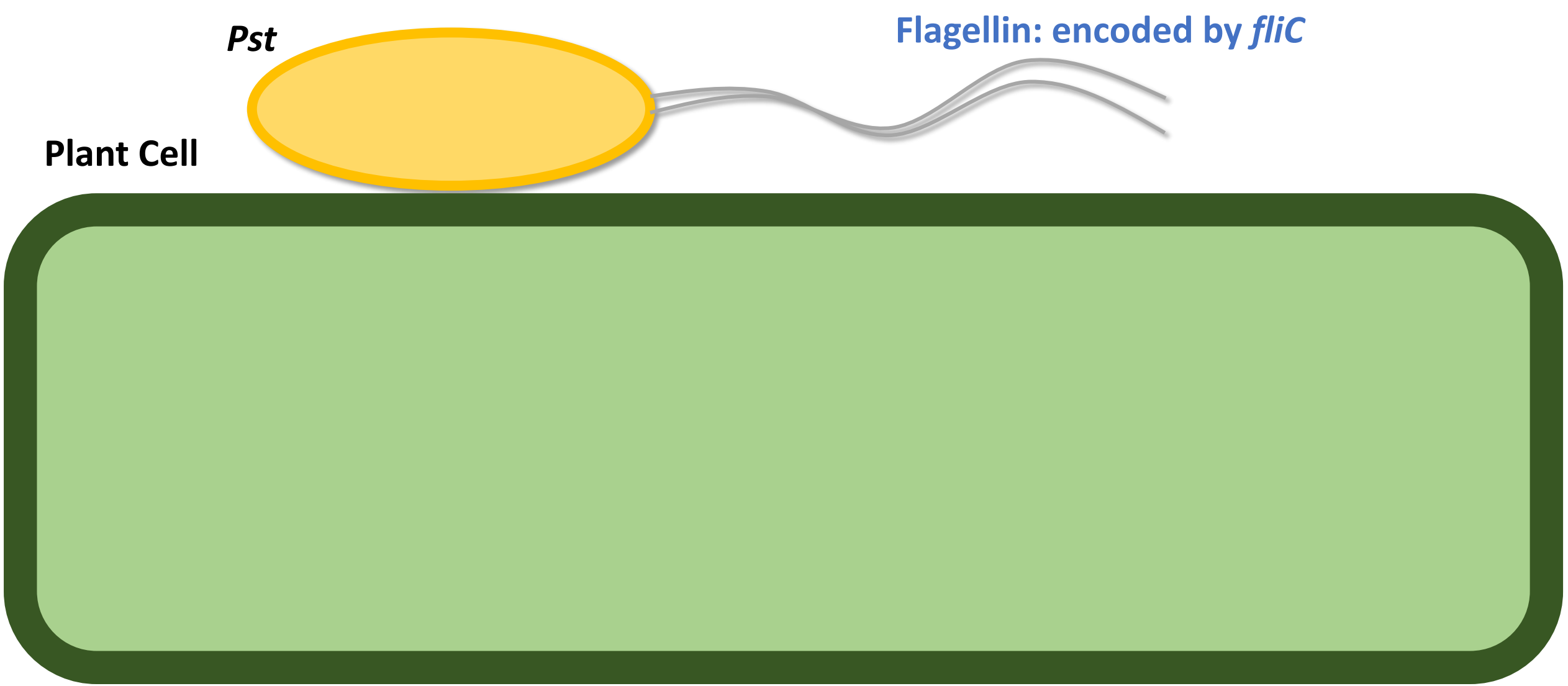




**Bacterial speck disease on tomato in New York,**

# Pattern Triggered Immunity

***Pseudomonas syringae* pv. tomato (*Pst*)**



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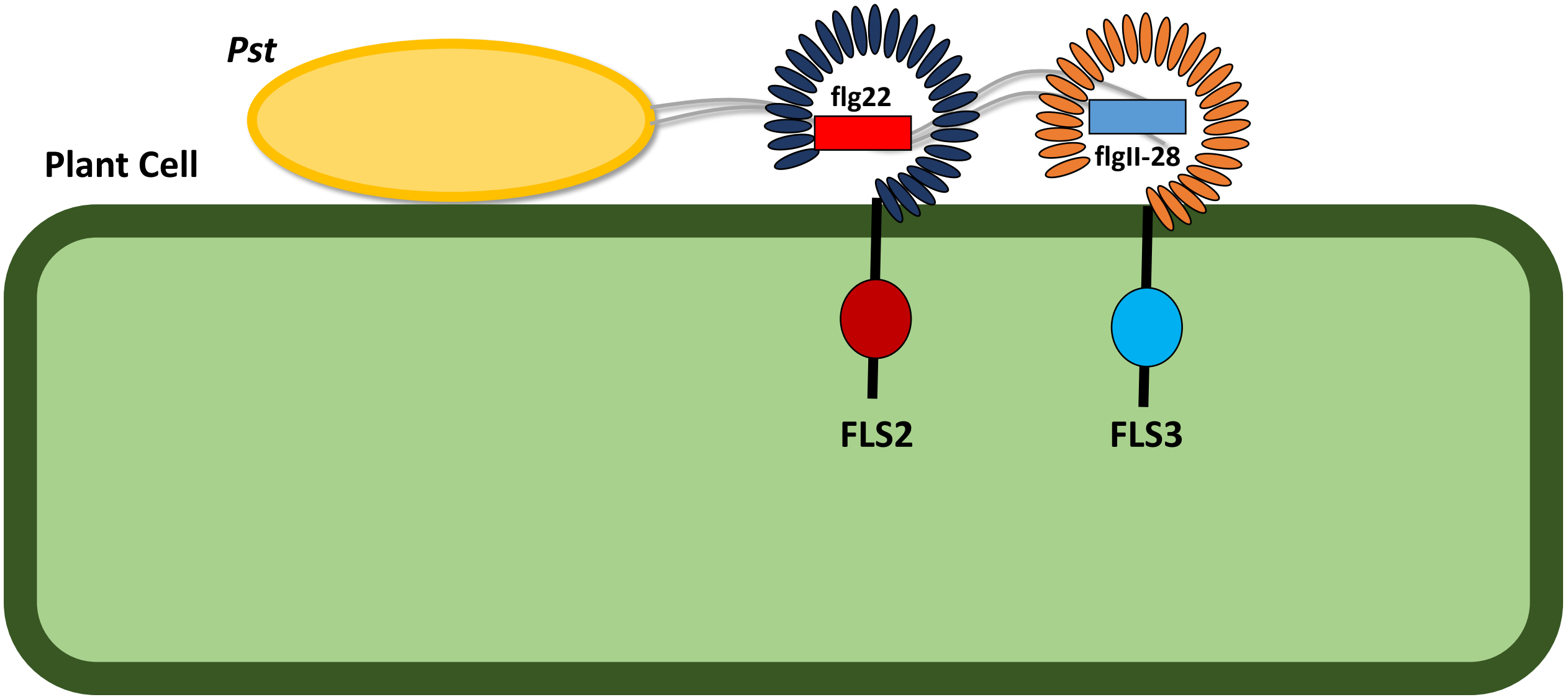

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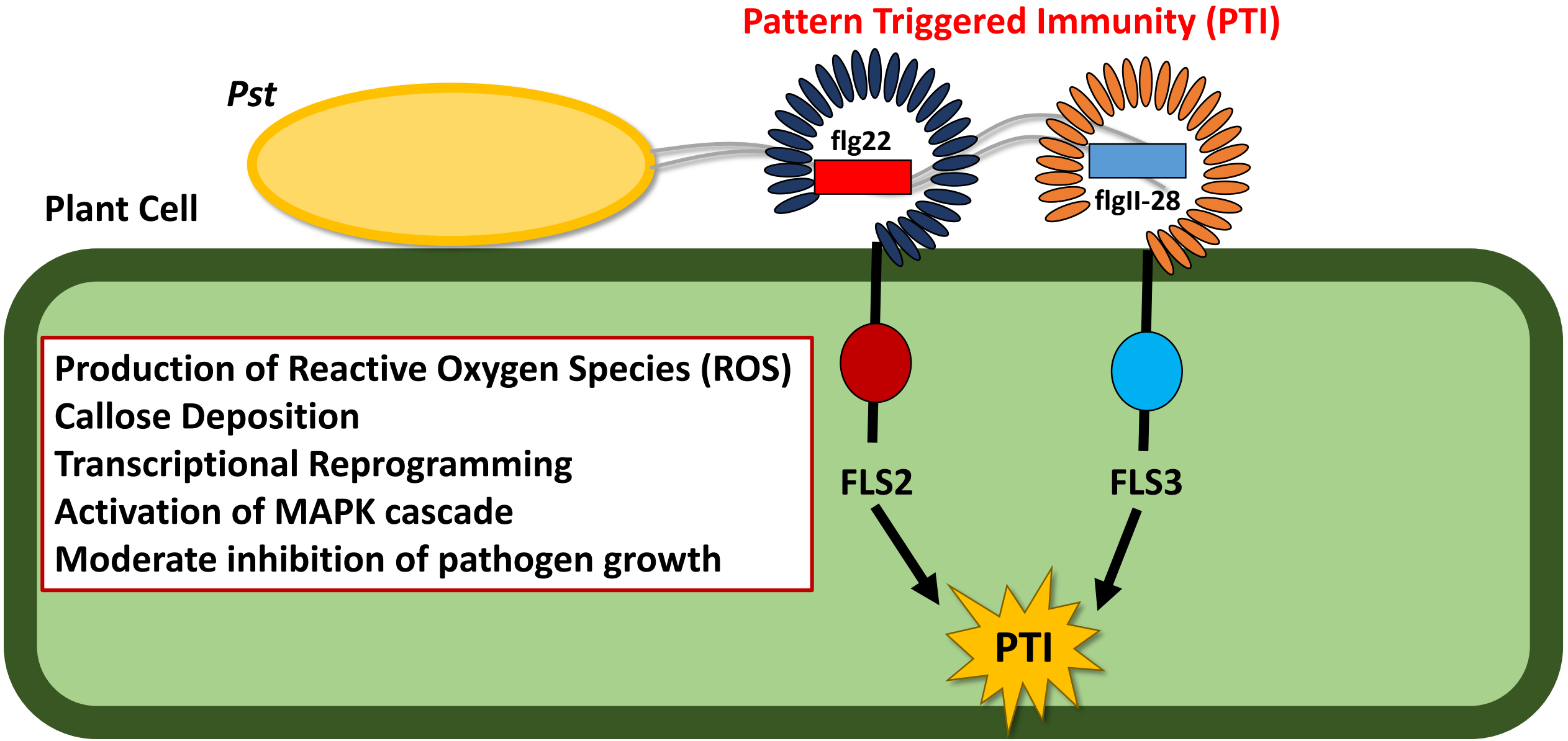
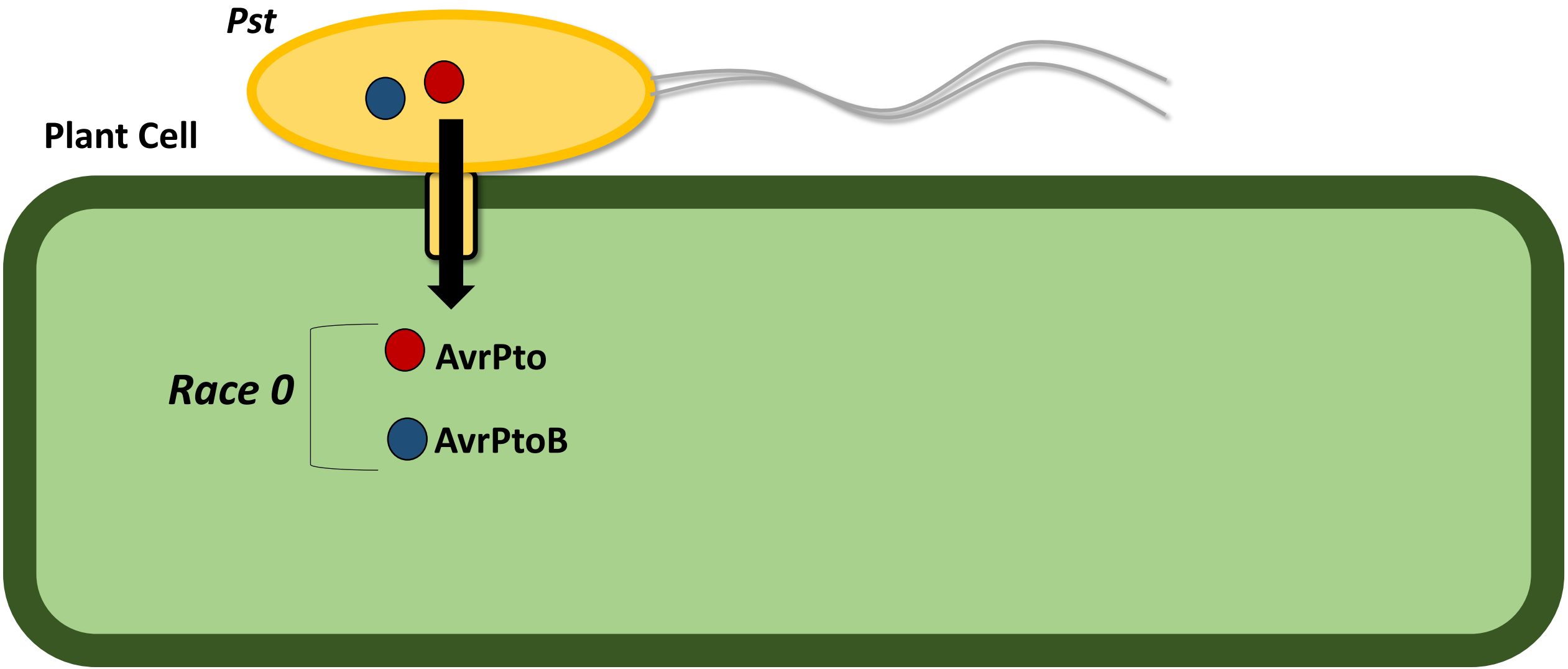


# Effector-Triggered Immunity



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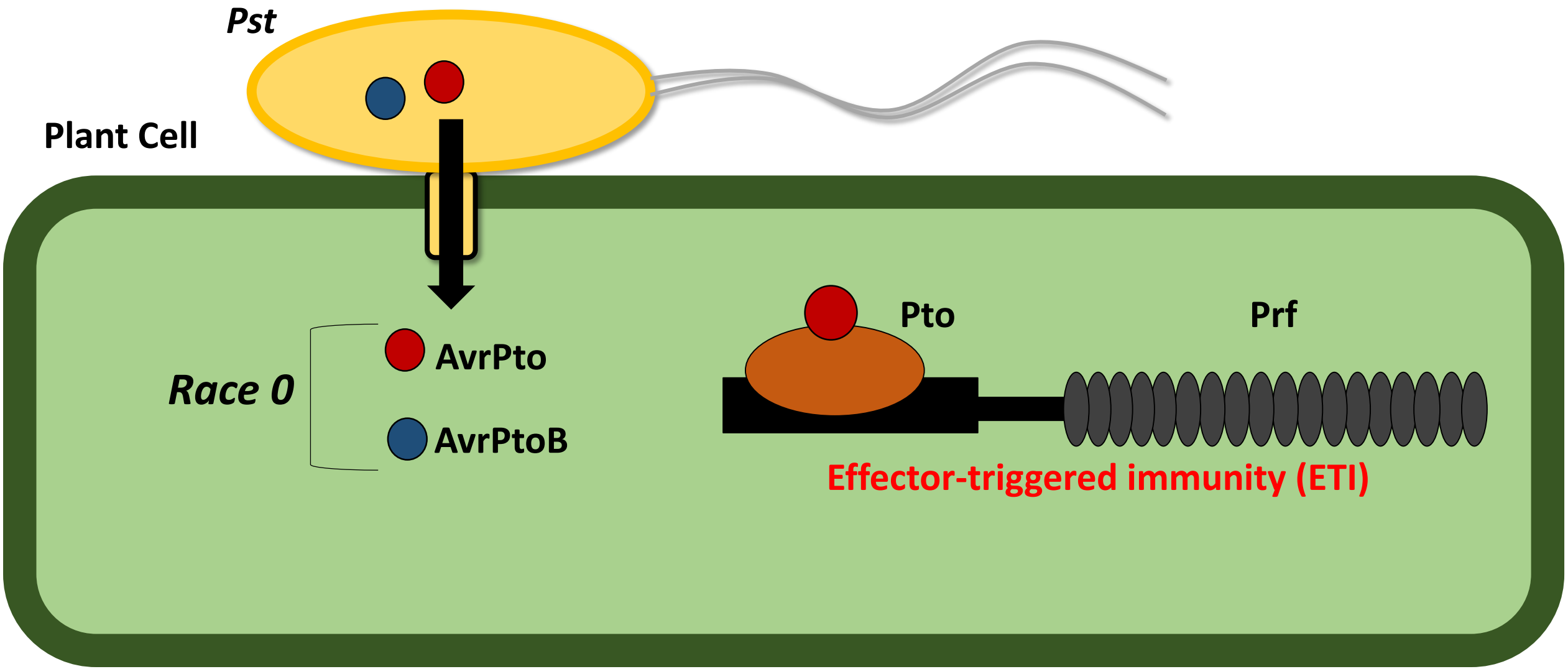

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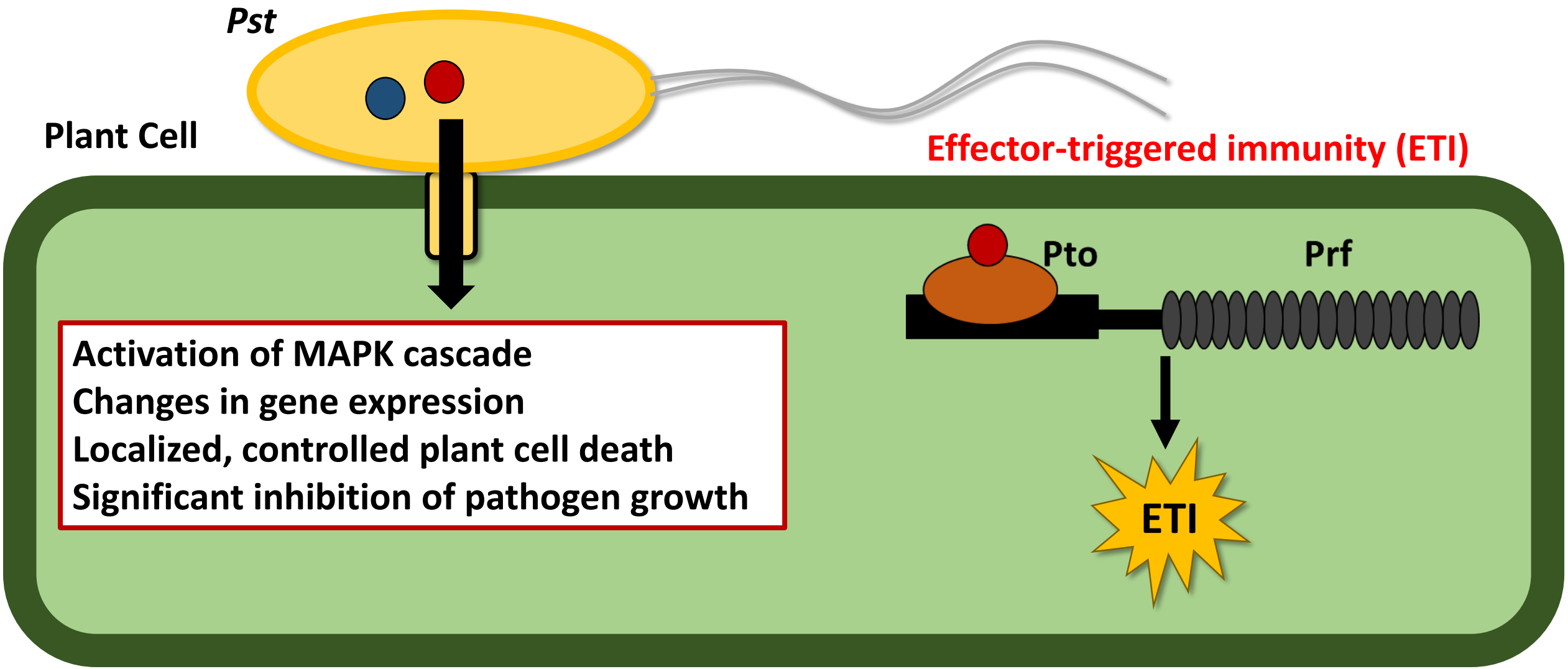

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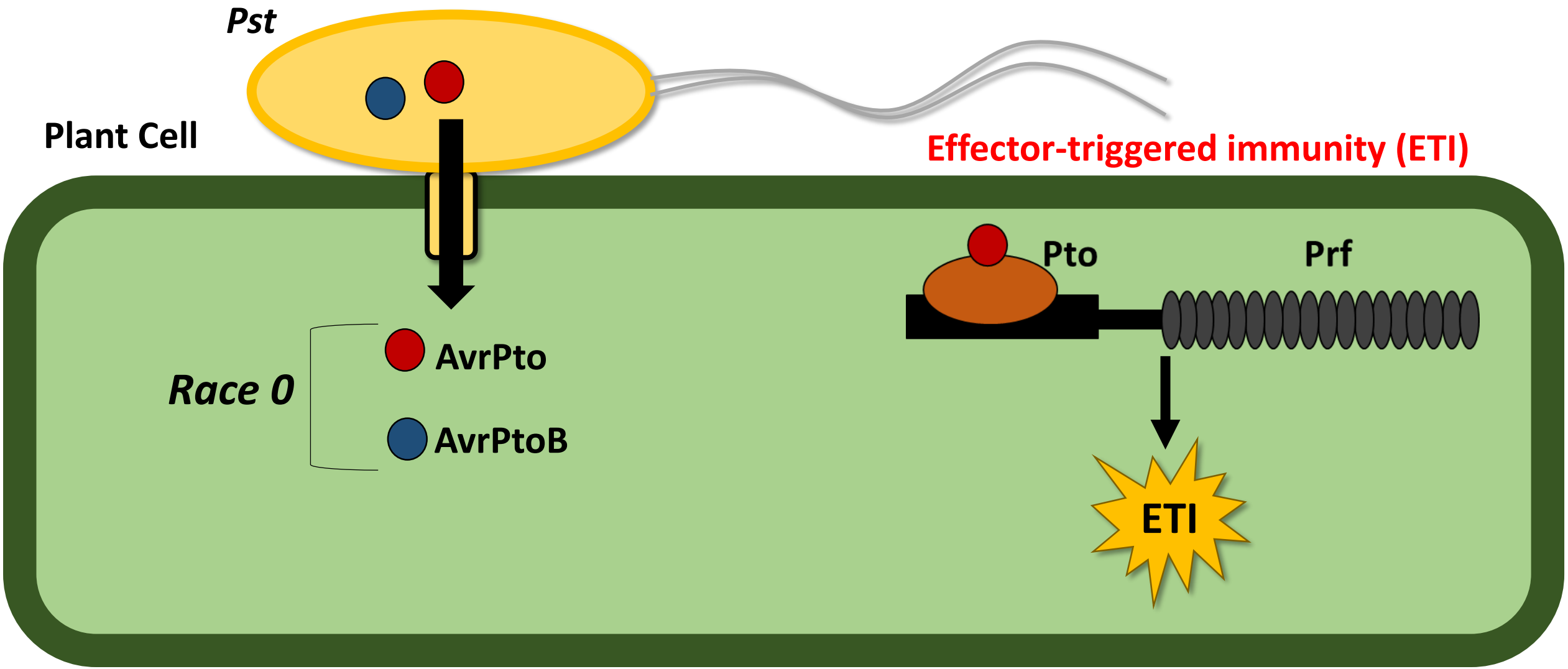

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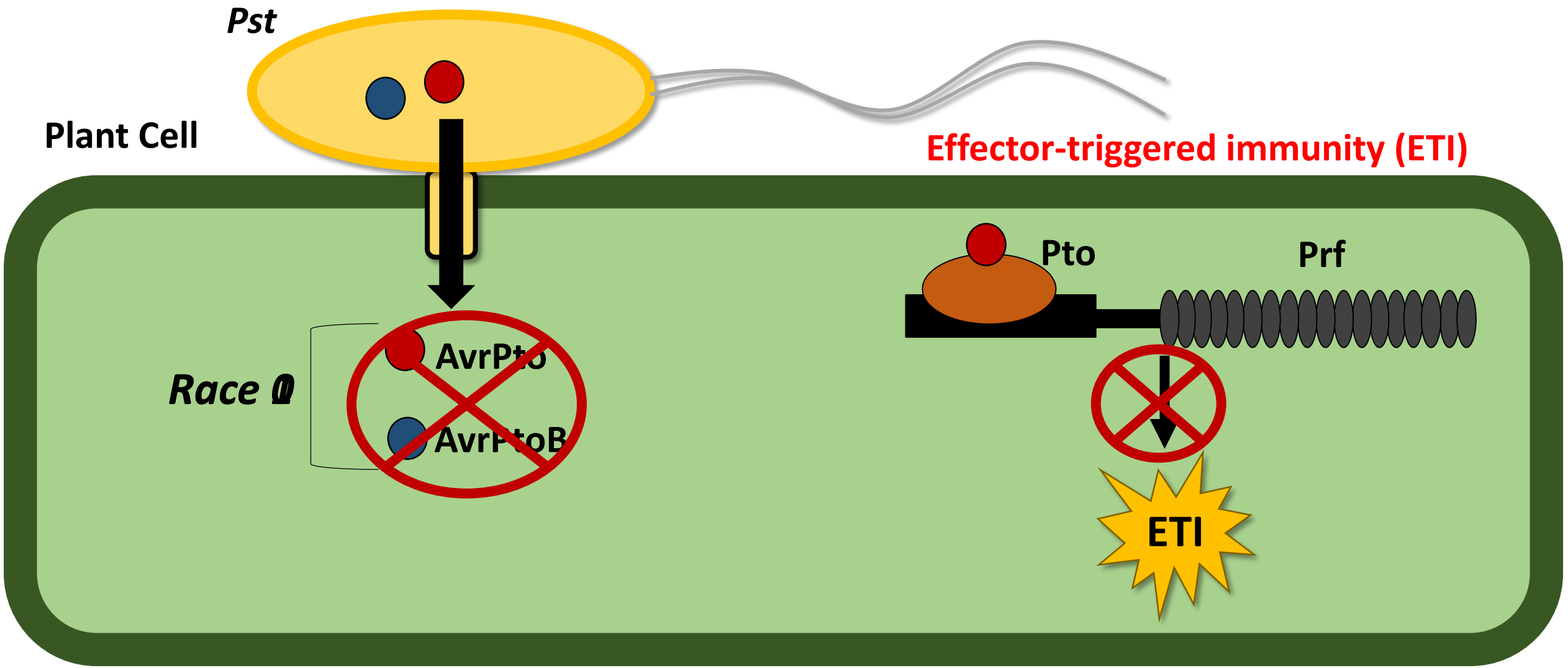

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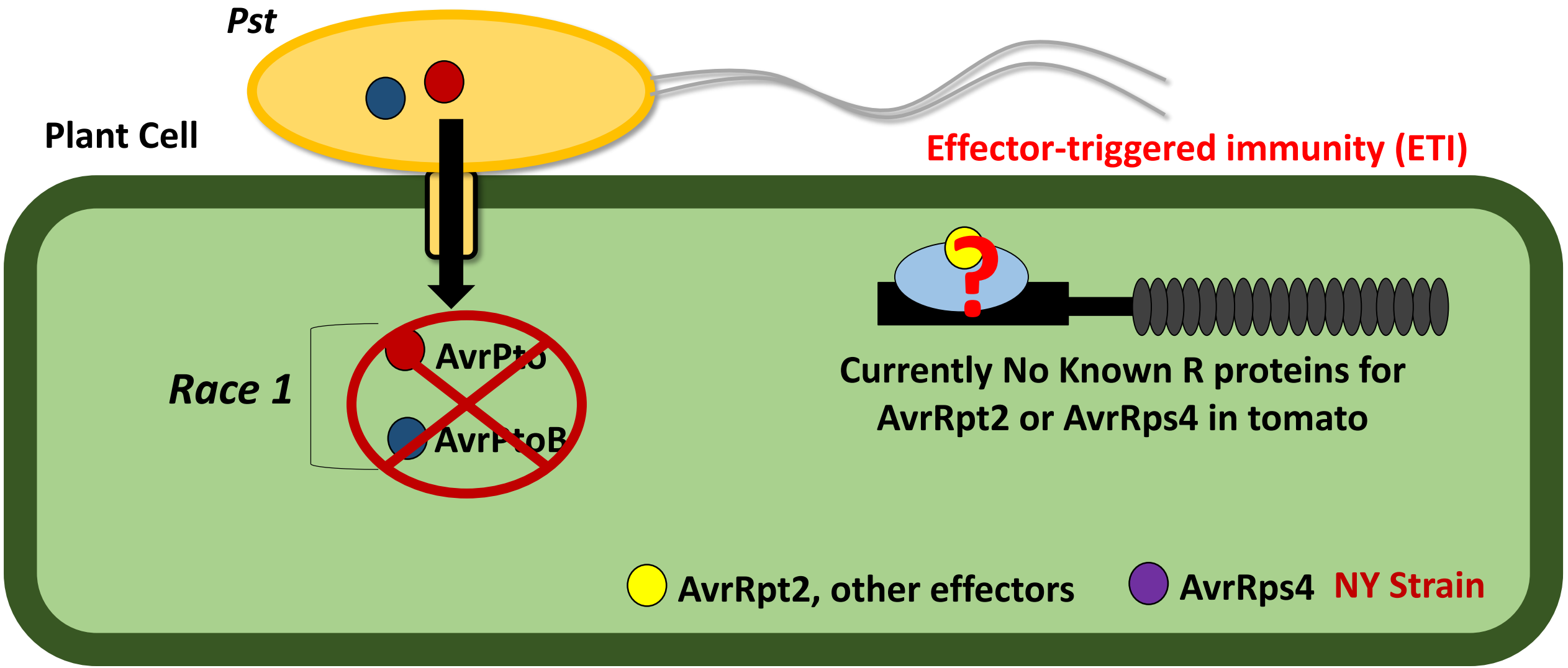


# Project Rationale

**Currently no resistance to *Race 1* strains**

- Identify new sources of resistance
  - PTI- has potential to be effective against other pathogens
    - Use *Pst* as a probe for novel PTI resistance
- Identify (and eventually eliminate) susceptibility genes/factors
- Characterize and understand the molecular mechanisms behind resistance and susceptibility

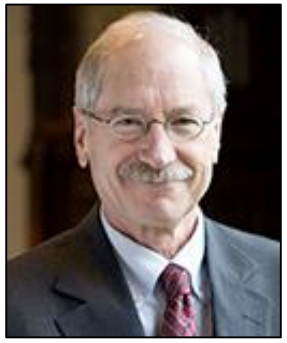
# Project Goal

Conduct a screen of genetically diverse tomato accessions, including heirlooms, breeding lines, and a wild species (*Solanum pimpinellifolium*), to look for new sources of resistance to *Pst*

# Leveraging natural variation in tomato to identify, characterize, and deploy new sources of disease resistance

A collaborative project, funded by the *National Science Foundation*

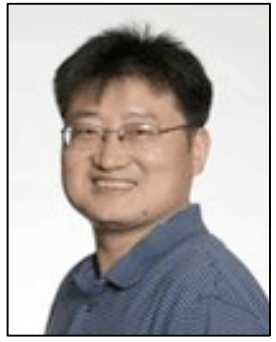
## Plant-Microbe Biology



Alan Collmer
Cornell

Greg Martin
BTI / Cornell

## Bioinformatics / Web Resources



Zhangjun Fei
BTI

Susan Strickler
BTI

Magdalen Lindeberg
Cornell

## Tomato Breeding

Sam Hutton
University of Florida

Dilip Panthee
North Carolina State University

## Collaborators

Chris Smart
Cornell

Dani Zamir
Hebrew University

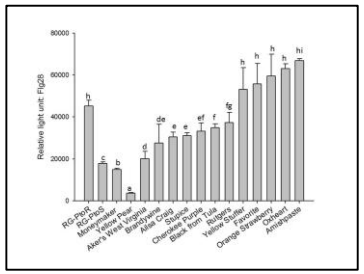
Martha Mutschler
Cornell

Margaret McGrath
Cornell

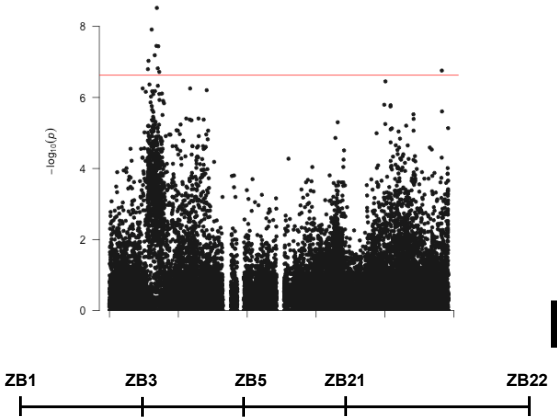
David Francis
Ohio State

# Leveraging natural variation in tomato to identify, characterize, and deploy new sources of disease resistance

Screen ~200 accessions for novel sources of resistance

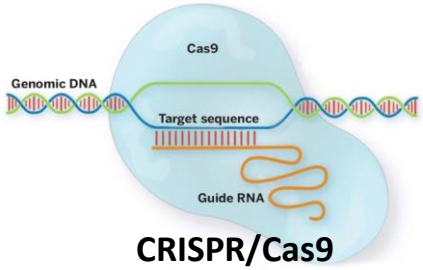


Investigate molecular functions of novel resistance




ID genes and develop markers for breeding programs

Introduce novel resistance into breeding lines



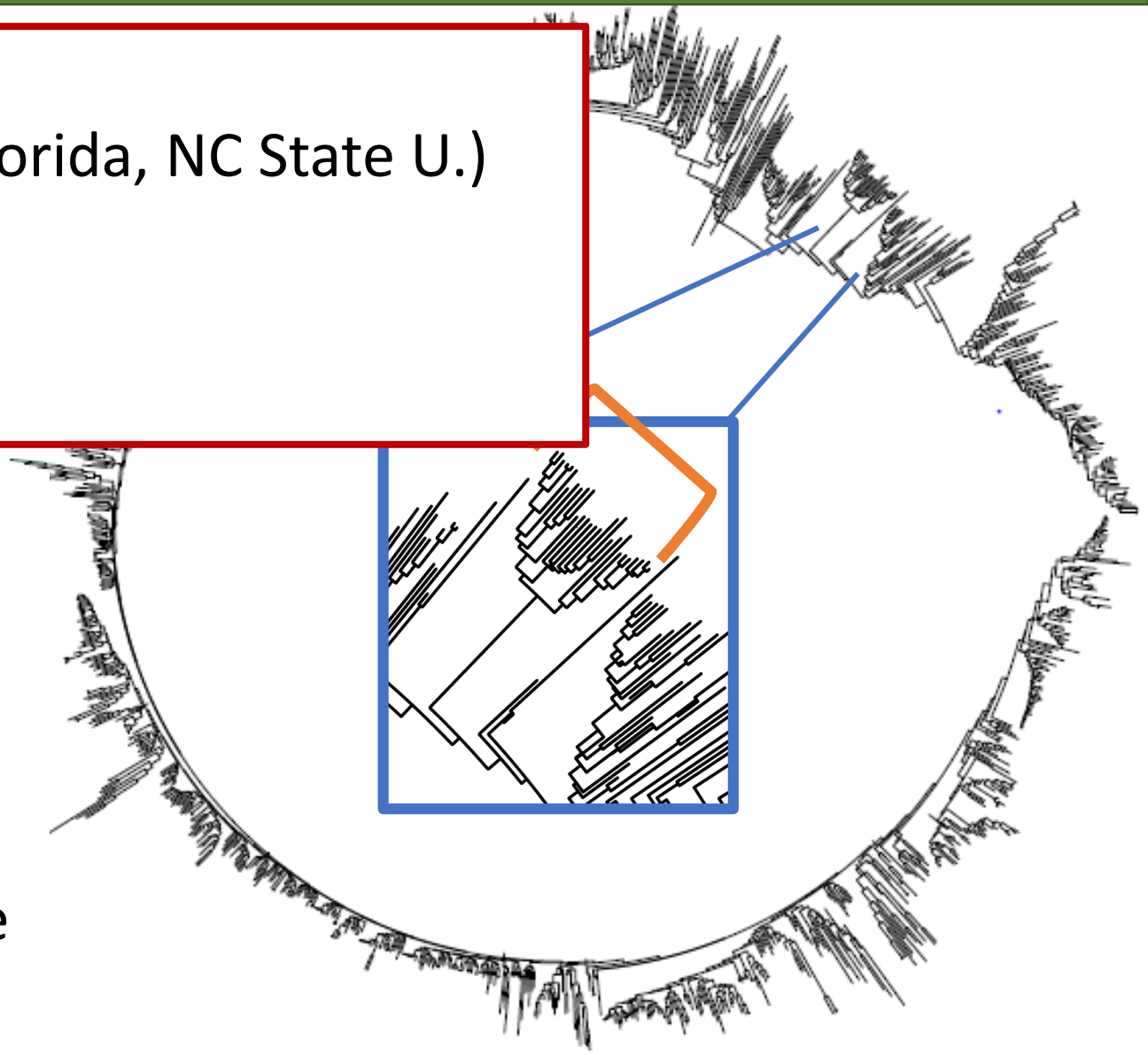

CRISPR/Cas9

Develop ~150 CRISPR lines in immunity associated genes and characterize for immune responses

# Natural Variation in Tomato

Susan Strickler, BTI

Heirlooms/Landraces
Breeding Lines (Cornell, U. Florida, NC State U.)
var. Cerasiforme
Processing and Fresh Market
*S. pimpinellifolium*

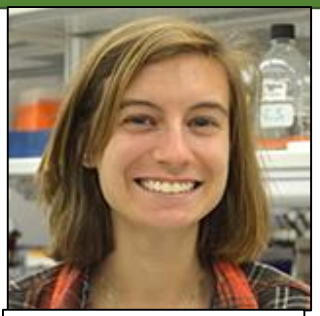


- Genome sequence data for **1,430 tomato accessions** was mined to identify **6,225 SNPs** to use in construction of a neighbor joining tree.

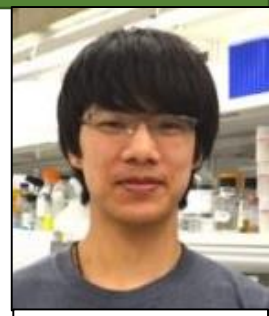
- **216 accessions** representing each clade were chosen for screening.

# Natural variation in the tomato immune response

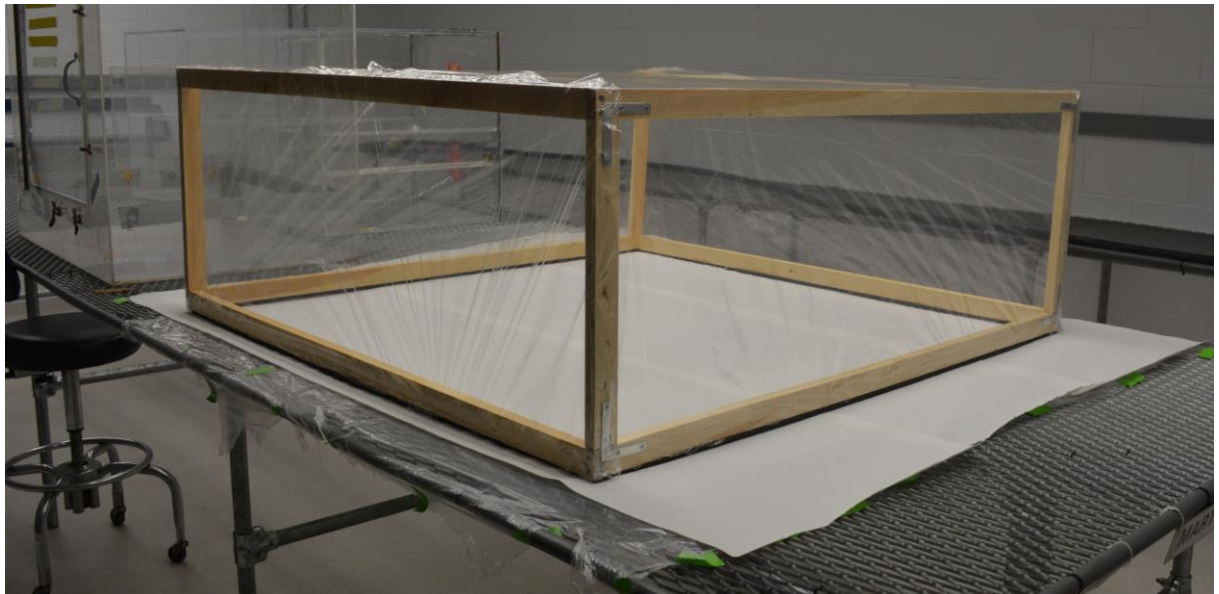
- Spray inoculated 3-week-old plants in 100% humidity using $10^8$ cfu/mL
  - Pst25 (*avrPto*; NY strain) } **ETI**
  - DC3000 *ΔavrPtoΔavrPtoB* } **ETI**
  - DC3000 *ΔavrPtoΔavrPtoBΔfliC* } **PTI** (flagellin)
- Scored plants for responses at 3 and 6 days post inoculation and photographed



Samantha Mainiero, Research Specialist



Alex Liu, Undergrad



# Disease Rating

**1**
**No Symptoms**

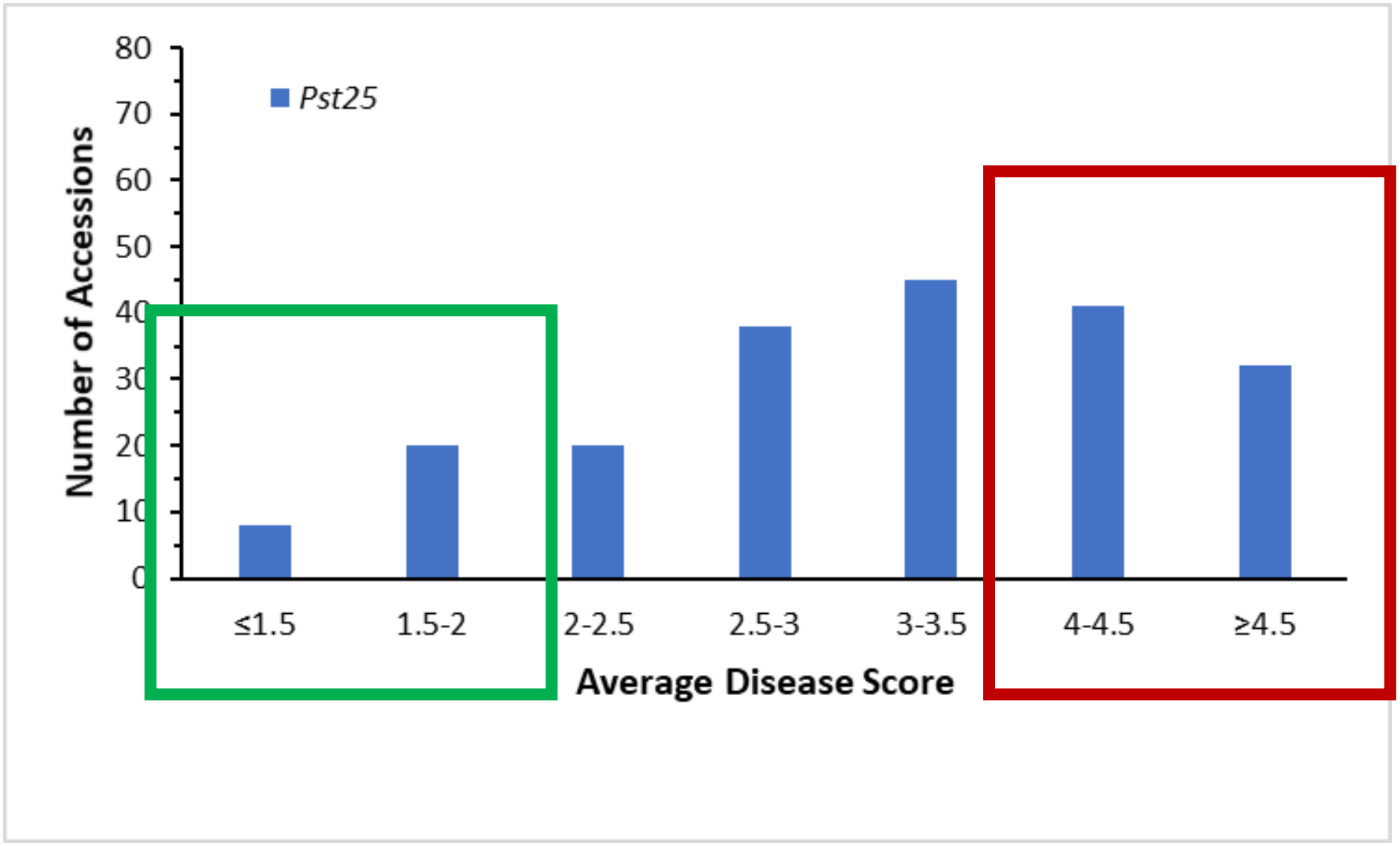
**2**
**Mild Symptoms/ Specks**

**3**
**Moderate Symptoms/ Specks**

**4**
**Severe Specks and/or Leaf Necrosis and Leaf Drop**

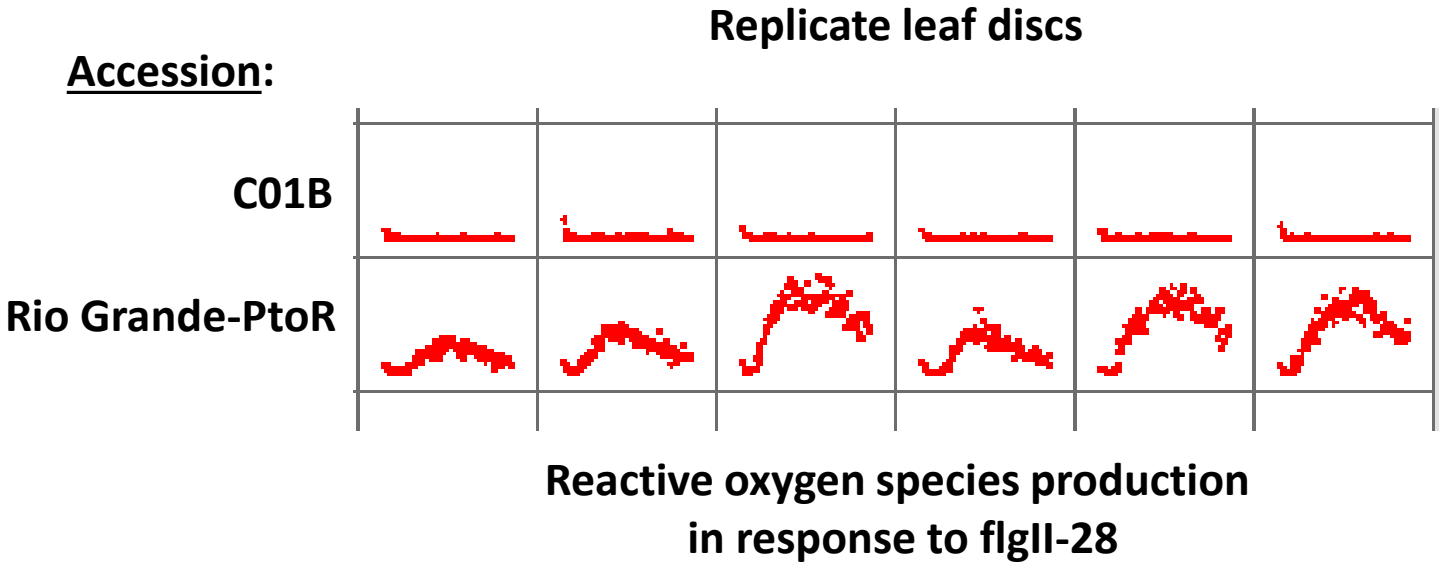
**5**
**Very Severe Necrosis and/or Leaf Drop**

# Natural variation in Responses to *Pst*



# Natural Variation in Pattern-Triggered Immunity

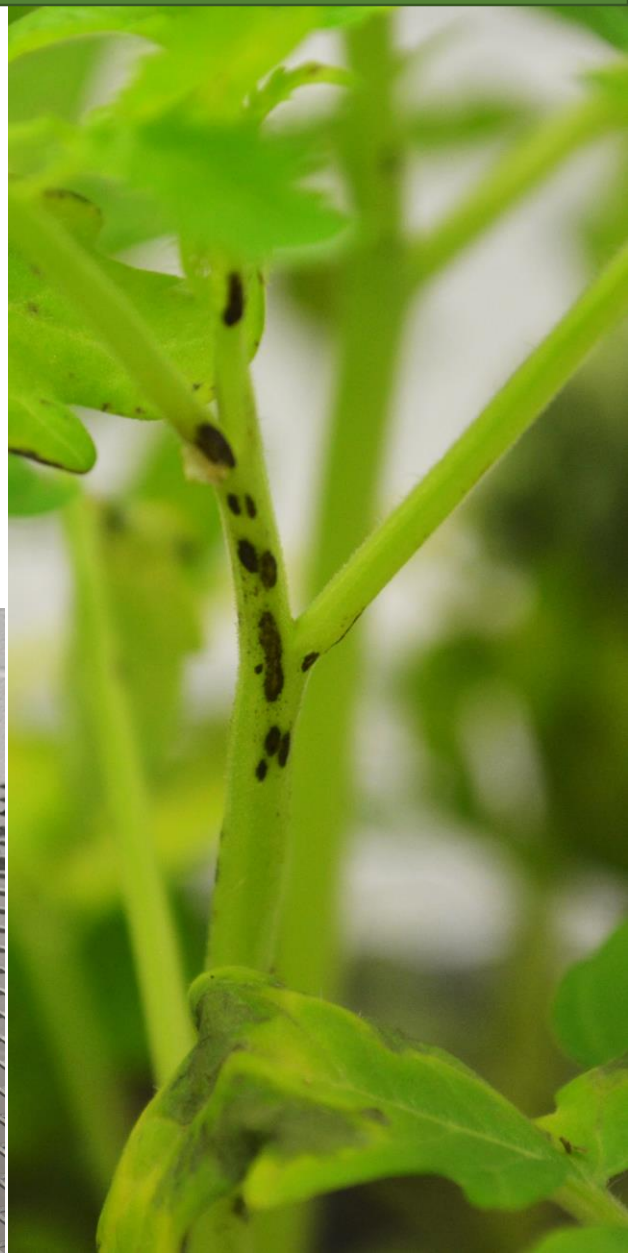
- ~100 accessions that showed increased or reduced responses to flagellin
- Reactive oxygen species assays to see which flagellin peptides might be recognized
  - ~20 accessions have unique responses to flg22 and/or flgII-28



# Natural Variation in Effector-Triggered Immunity

- 28 candidate accessions from initial screen
  - Resistance to Race 1

- Vacuum infiltrated accessions to confirm resistance phenotypes



# Example of an ETI-like Phenotype

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**DC3000 Δ*avrPto/avrPtoB/fliC* (*Race 1 Pst*)**

**C03A**

**Rio Grande-PtoR**

**4 dpi, ~$10^5$ cfu/mL**

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**C03A**

**Rio Grande-PtoR**

**4 dpi, ~$10^5$ cfu/mL**

# Example of an ETI-like Phenotype

**Pst25 Δ*avrPto/avrRpt2***
***(*NY Strain engineered to be *Race 1 Pst)***

**CXT**

**Rio Grande- PtoR**

**4 dpi, ~$10^5$ cfu/mL**

# Unusual Host Responses to *Pseudomonas syringae* Strains

Necrosis

"Other" (Fungal-like?)

Coronatine

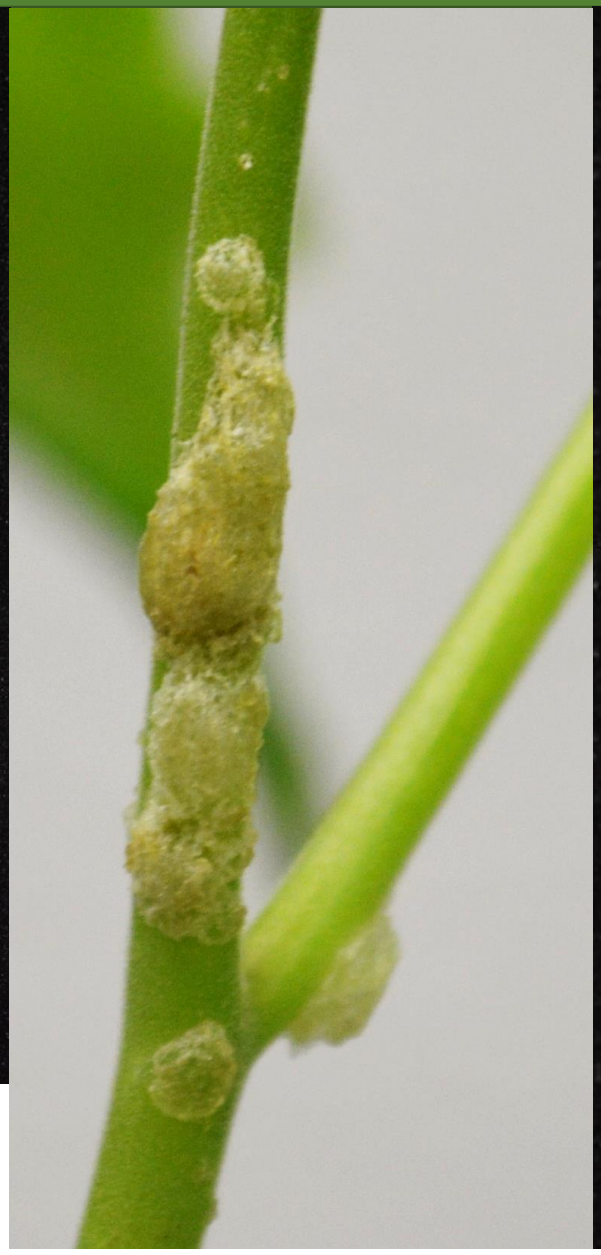
"Galls"

Necrotic spots

Shothole

Water soaking

# Unusual Phenotypes: “Galls”



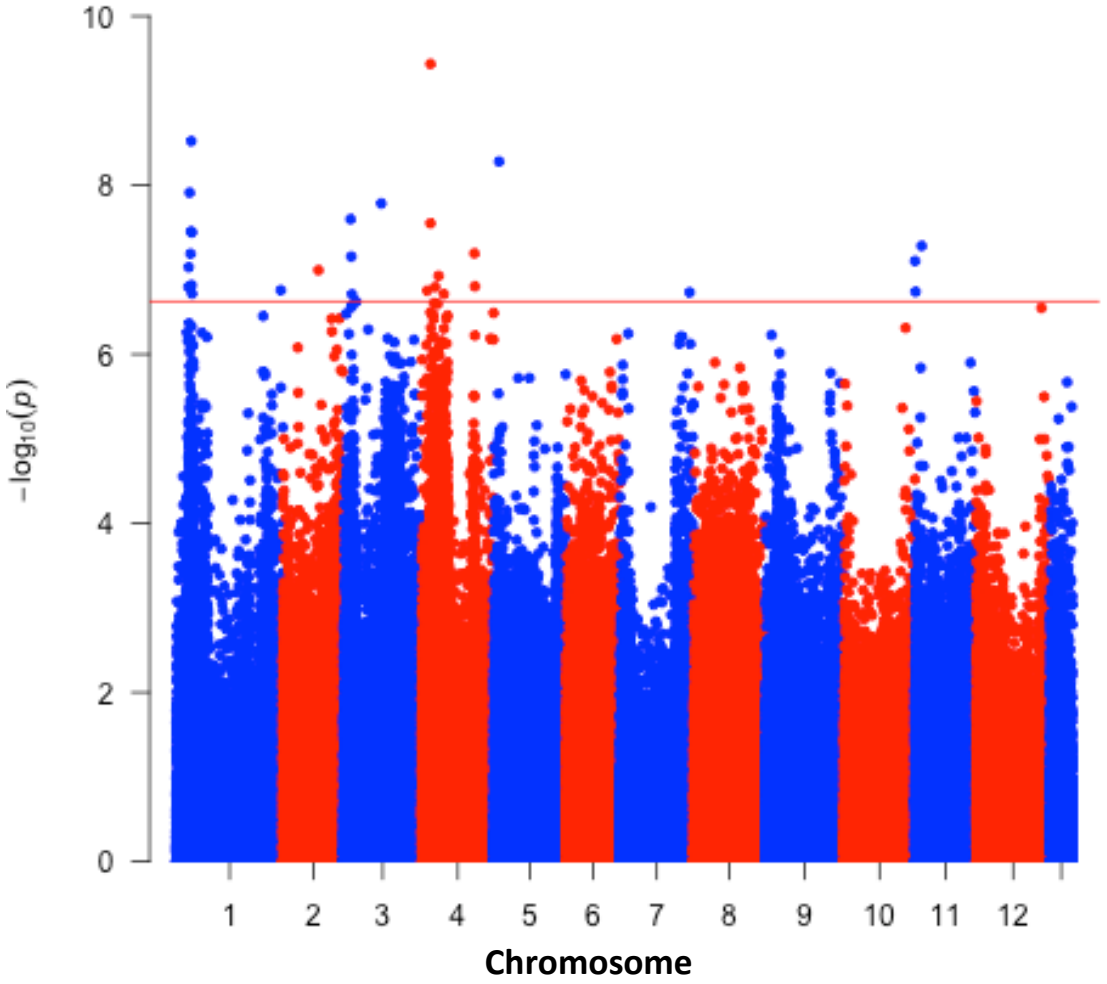
14 +galls, 38 –galls (52 total)

3:1 segregation, recessive

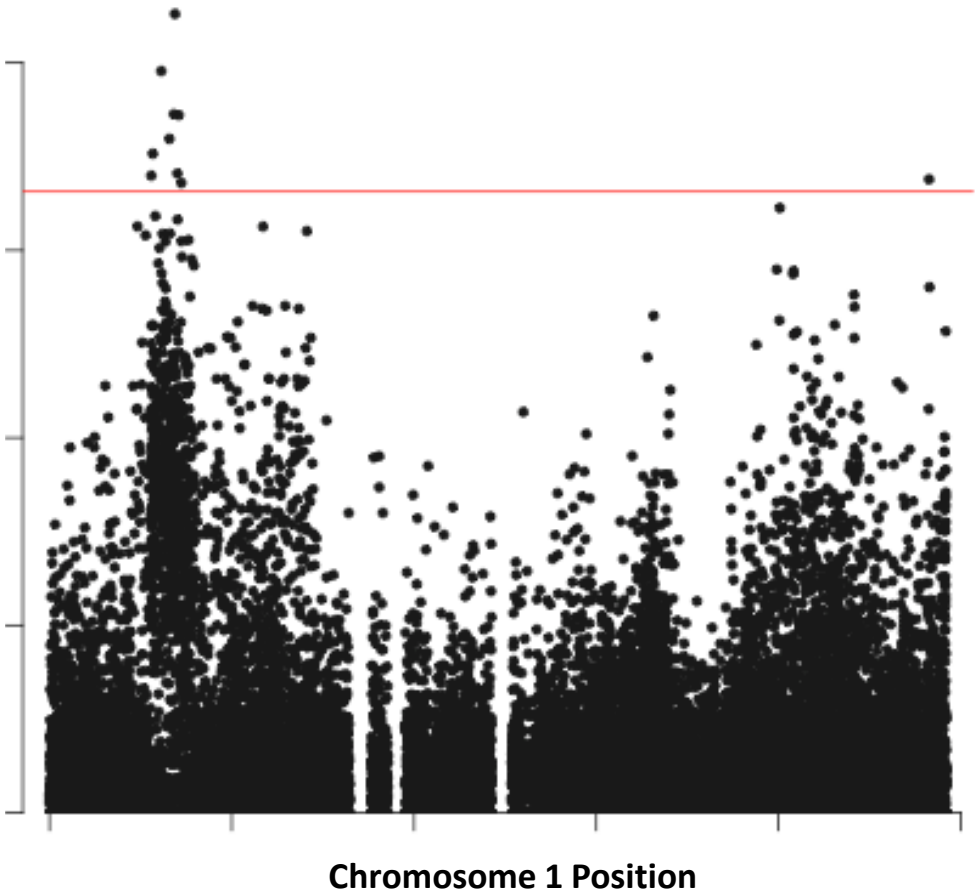
# Gall Phenotype GWAS

Adrian Powell, Postdoctoral Researcher

**Chromosomes 1-12**




**Chromosome 1**



# Next Steps

**Follow-up assays:**

- Bacterial population assays
- Screening F2 populations
- MAPK activation assays
- Reporter genes for ETI and PTI (qRT-PCR)
- Test with other *Pst* strains with diverse effector repertoires
- Develop markers for “gall” phenotype

# Field Experiments 2018

## New York

***Bacterial Speck***

**Greg Martin**
**BTI / Cornell**
**(Freeville, NY)**

***Bacterial Spot***
***Bacterial Canker***

**Chris Smart**
**Cornell**
**(Geneva, NY)**

***Bacterial Speck***

**Margaret McGrath**
**Cornell**
**(Long Island, NY)**

## Ohio

***Bacterial Spot***

**David Francis**
**Ohio State**

## North Carolina

***Bacterial Speck***
***Bacterial Spot***

**Dilip Panthee**
**North Carolina State University**

## Florida

***Bacterial Spot***
***Bacterial Wilt***

**Sam Hutton**
**University of Florida**

## Martin Lab

**Greg Martin**
**Samantha Mainiero**
Fabian Giska
Ning Zhang
Fabio Rinaldi
Carolina Mazo
Holly Roberts
Noam Eckshtain Levi
Kai Shi

***Undergraduates***
**Alex Liu**
**Annie Geiger**
Sarah Lynn
Kellie Damann
Sahara Richardson
Mishi Vachev (2017 REU)
Lydia Zamidar
**Nick Glynos**
**Nicole Avellanet**

***Former Lab Members** (past year)*
**Sarah Hind**
Christine Kraus

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## Collaborators

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Chris Smart- Cornell
Magdalen Lindeberg- Cornell
**Suzy Strickler- BTI**
**Adrian Powell- BTI**
Jim Giovannoni- BTI
Surya Saha-BTI
Martha Mutschler-Cornell
Lukas Mueller- BTI
Boris Vinatzer- Virginia Tech
Zhangjun Fei- BTI
Frank Schroeder- BTI
Guido Sessa-Tel Aviv Univ.
Lirong Zeng-Univ. Arkansas
Dilip Panthee-NC State Univ.
Sam Hutton- Univ. Florida
David Francis- Ohio State Univ.
Dani Zamir- Hebrew Univ.

National Science Foundation

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