



Loss of susceptibility loci in tobacco for the development of durable resistance to black shank.

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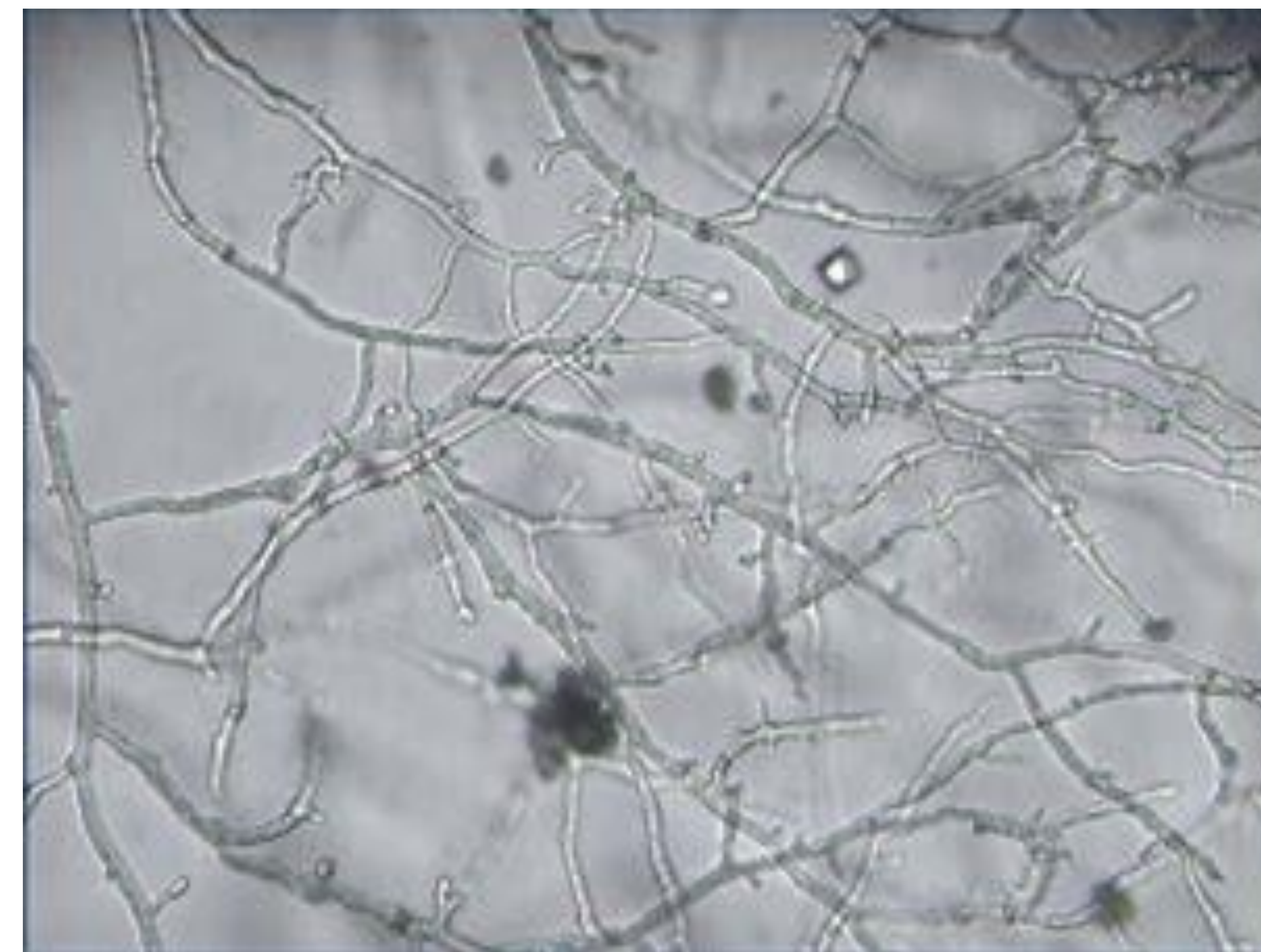
CORESTA CONGRESS

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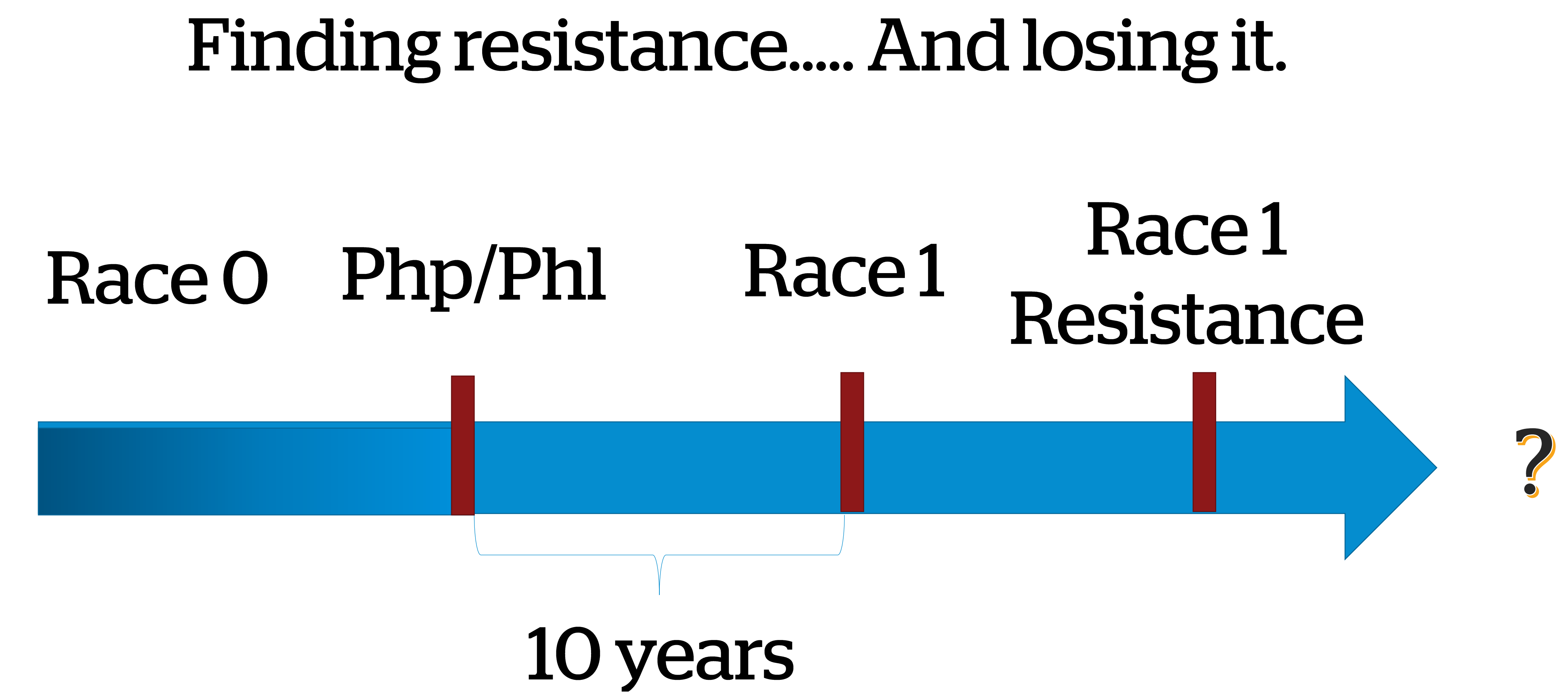
Overview

- Why is durable black shank resistance still important?
- Resistance vs Loss of Susceptibility (LOS)
- Effectoromics methodology
- Identification of possible susceptibility genes
- Conclusions and future directions



Black Shank Disease

- **Black Shank: A devastating disease caused by the oomycete pathogen *Phytophthora nicotianae*.**
- **Resistant lines were developed utilizing the Ph gene leading to a resistance breaking race to emerge.**



Race 0/1 Resistant

Race 0 resistant

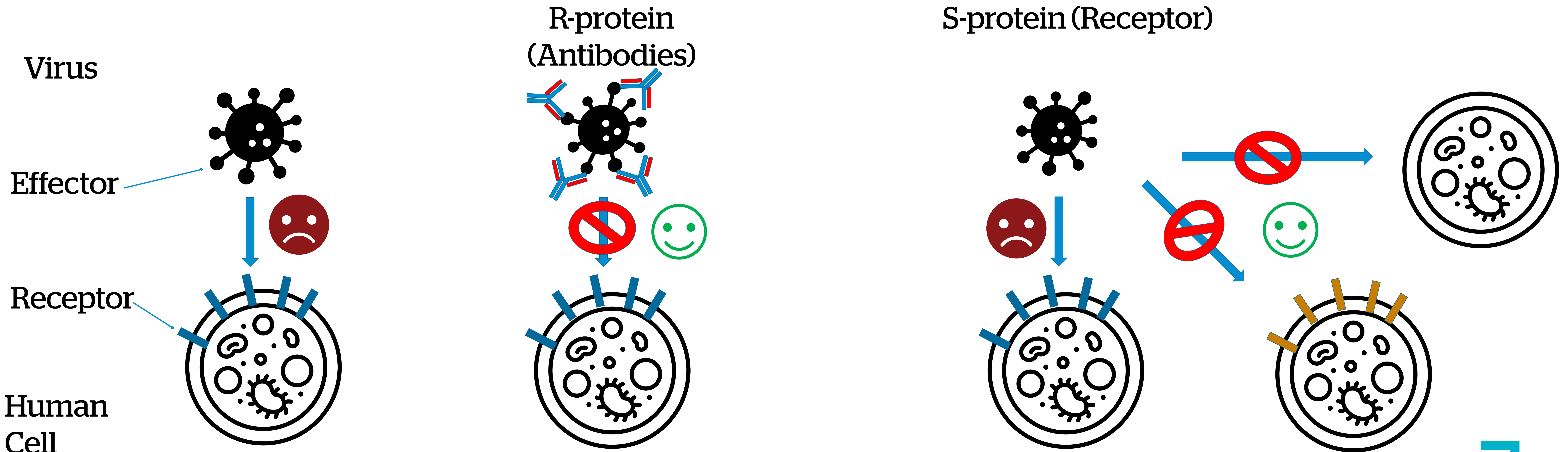
Resistance (R) Genes and Susceptibility (S) Genes

•R Genes

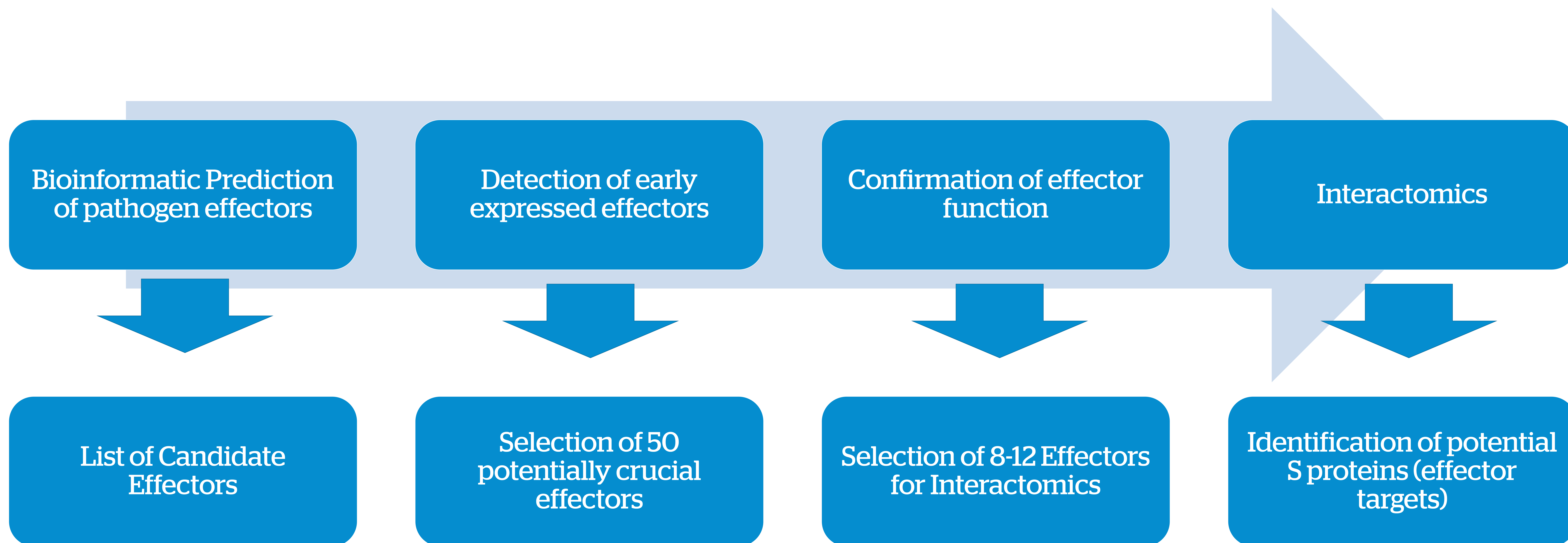
- Protein that conveys resistance by interacting with pathogen virulence factors or the pathogen itself diminishing their efficacy

•S Genes

- Protein that facilitates the infection process or supports compatibility with the pathogen



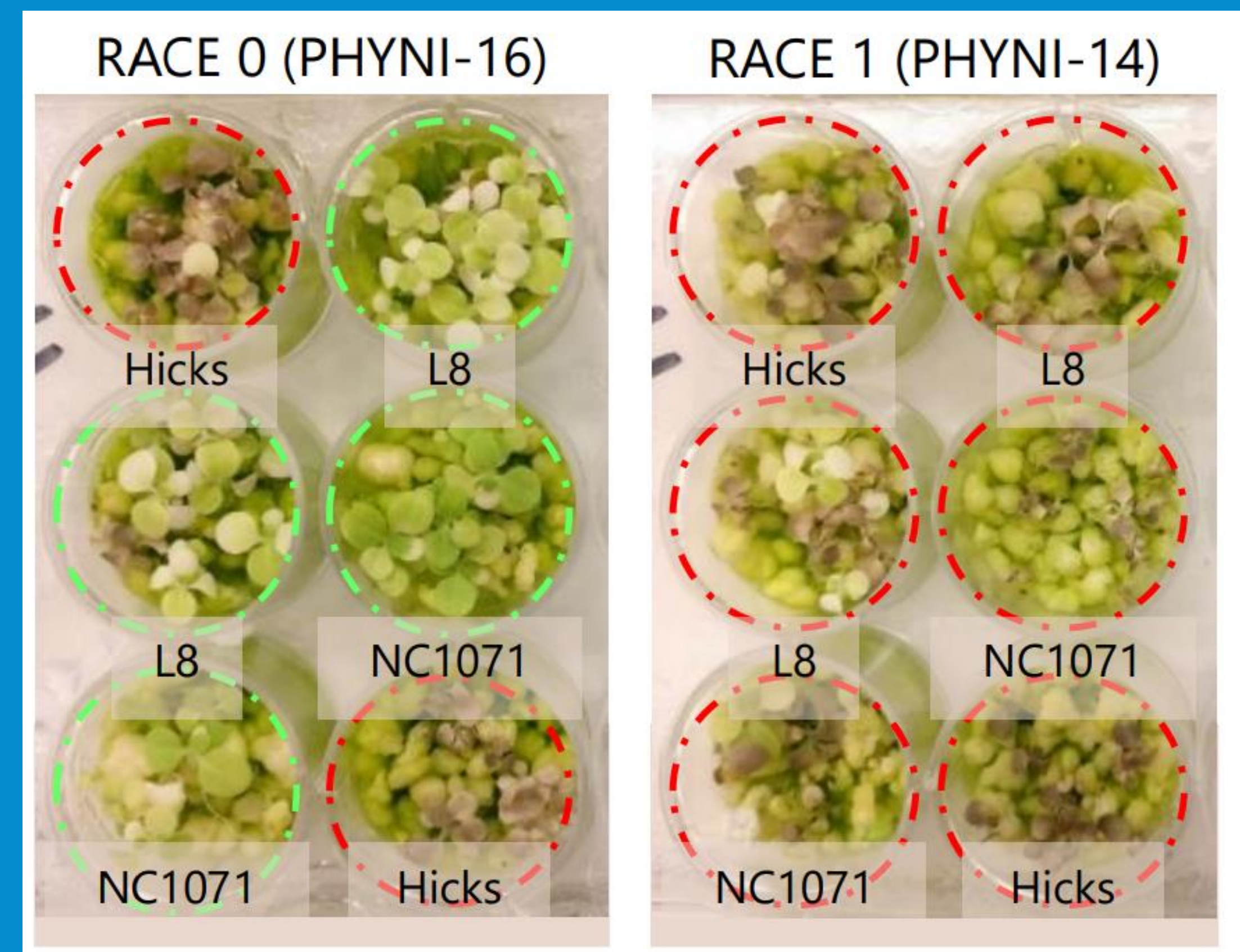
Loss of Susceptibility / Effectoromics Approach



Bioinformatic Prediction of Pathogen Effectors

Strain	Location		Race
PHYNI-02	Indonesia	Deli	non virulent
PHYNI-03	Puerto Rico	-	R0?
PHYNI-04	USA	Nottoway, VA	R0
PHYNI-05	USA	Franklin, VA	R0
PHYNI-06	USA	Cumberland, VA	R0
PHYNI-07	USA	Mecklenburg, VA	R0
PHYNI-08	USA	Dinwiddie, VA	R0
PHYNI-09	USA	Pittsylvania, VA	R1
PHYNI-10	USA	Dinwiddie, VA	R0
PHYNI-11	USA	Mecklenburg, VA	R1
PHYNI-12	USA	Brunswick, VA	R1
PHYNI-14	USA	Yancey Co, NC	R1
PHYNI-15	USA	Buncombe, NC	R0
PHYNI-17	USA	Davie, NC	R0
PHYNI-18	USA	Sampson, N C	?
PHYNI-19	USA	Yancey, NC	R1?
PHYNI-20	USA	Haywood, NC	R1
PHYNI-21	USA	Buncombe, NC	R1
PHYNI-22	USA	Alamance, NC	R1
PHYNI-24	Australia	Mareeba, Queensland	R0
PHYNI-25	Australia	-	?
PHYNI-26	Australia	-	?
PHYNI-27	Australia	Queensland	R0?
PHYNI-28	South Africa	Prinsloo	R0
PHYNI-29	Japan	-	R0
PHYNI-30	Japan	-	?
PHYNI-31	South Africa	N. Transvaal	R0
PHYNI-32	New Zealand	New Zealand	non virulent
PHYNI-33	Indonesia	Java	R0

Race Determination

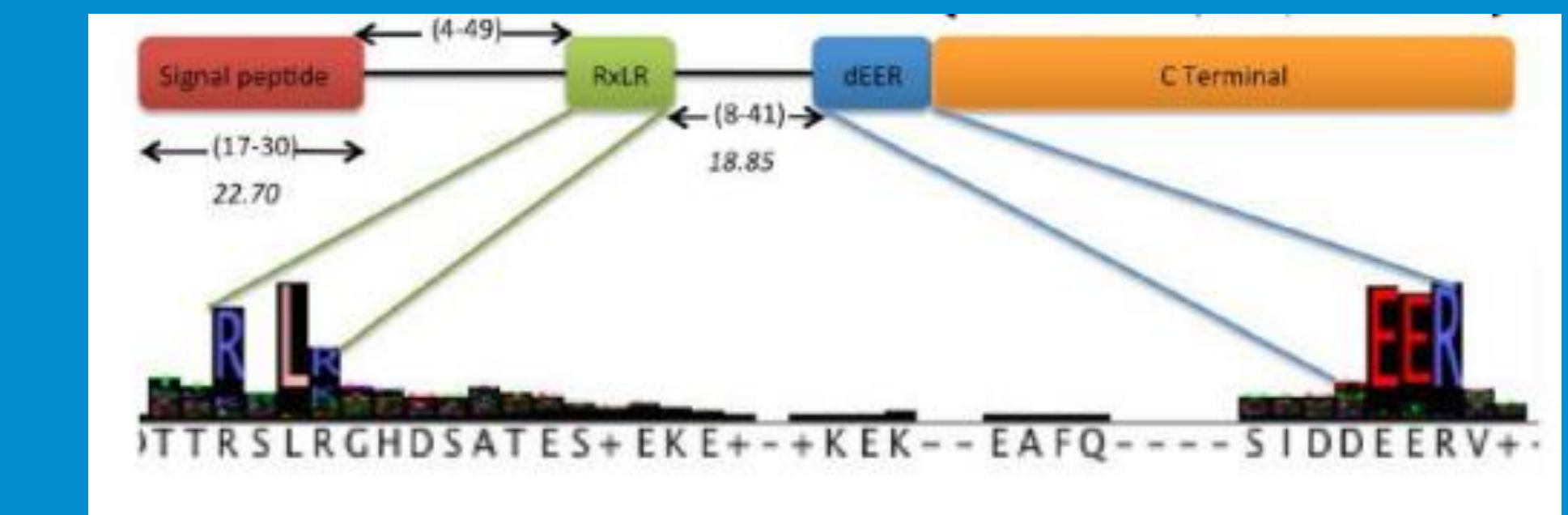


RNA-seq



- 3, 6, 18, 24, 30 hours post infection
- Mapping to both *Phytophthora* and Tobacco genomes

Effector Mining



- Utilized common effector gene structures
- Conservation among strains
- Early gene expression during infection

Genome Sequencing - 29 strains

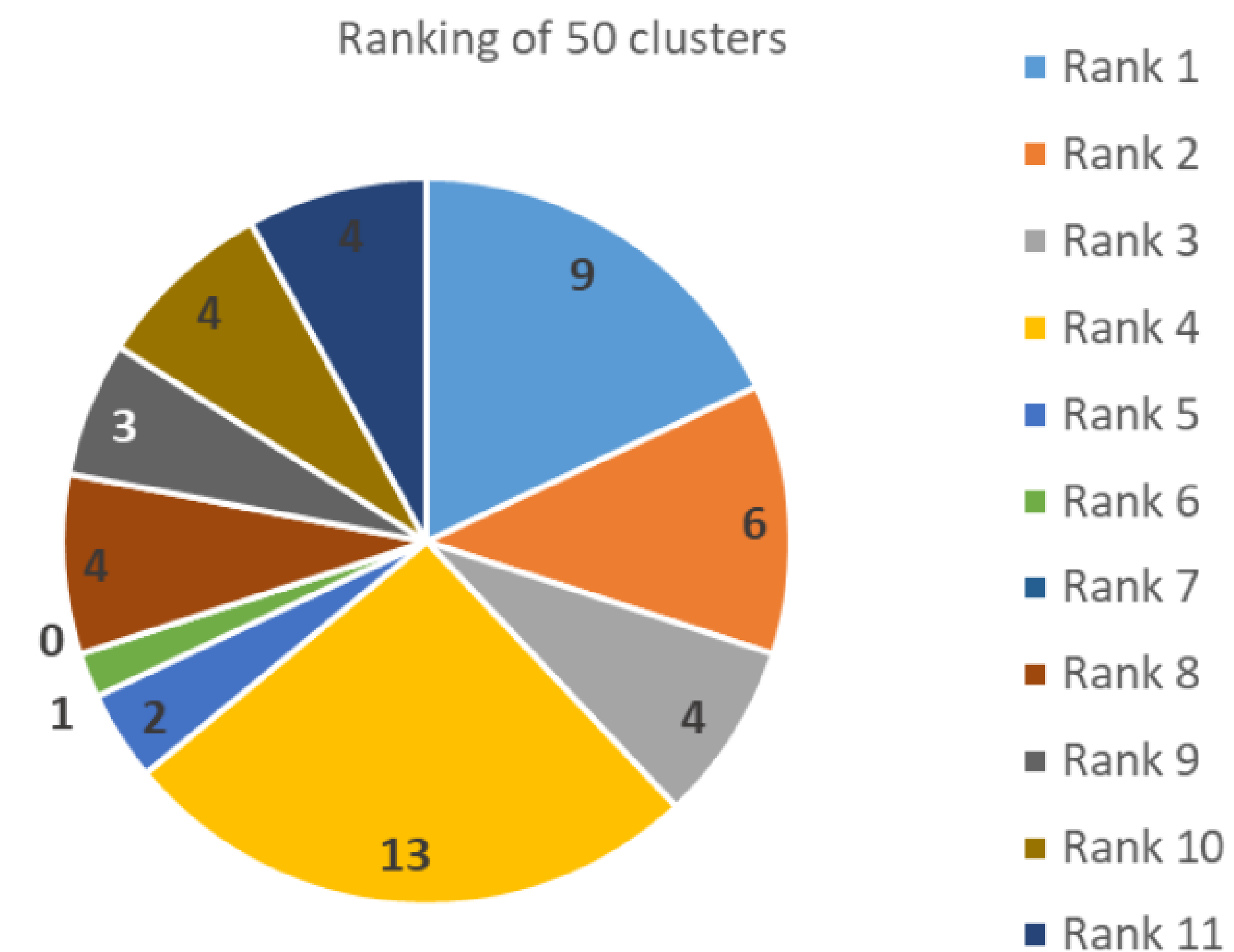
- PacBio Sequencing
- *De novo* assembly
- Annotated
- Reference quality single chromosome obtained
- * PHYNI-32 was determined to be *P.*

Selection of Putative Effectors for Validation

- Average of 417 predicted effectors per strain
- No significant difference in numbers of effector genes between races
- 50 putative effectors were selected based on conservation and gene expression

Effector clusters and rankings

	Rank 1 + 2	Rank 3 + 4	Rank 5 + 6	Rank 7 + 8	Rank 9	Rank 10 + 11
Distinct strains	28	27	28	27	26	26
TP1-TP3 expression	y	y	-	-	y/n	y
Pinf gene included	-	-	-	-	y	-

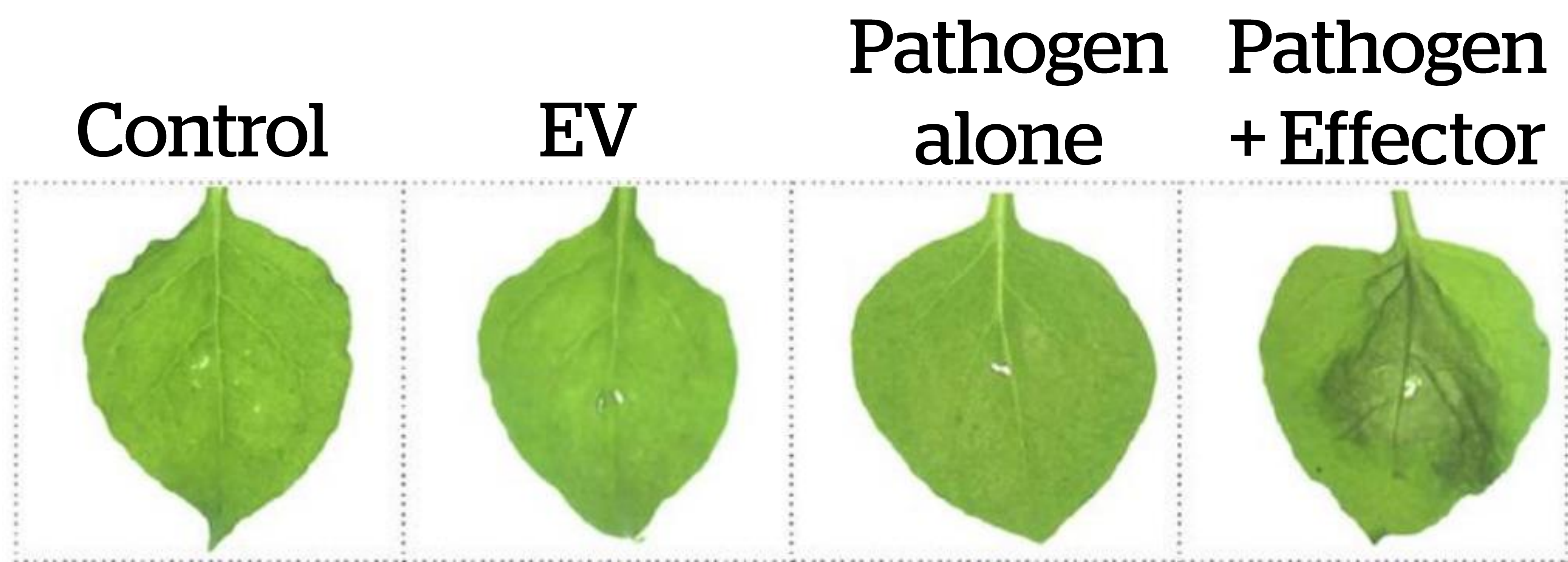


- 87 % of selected effectors are expressed in early infection
- 70 % of selected effectors > 95 % identity among strains

Which of the putative effectors actually function in virulence?

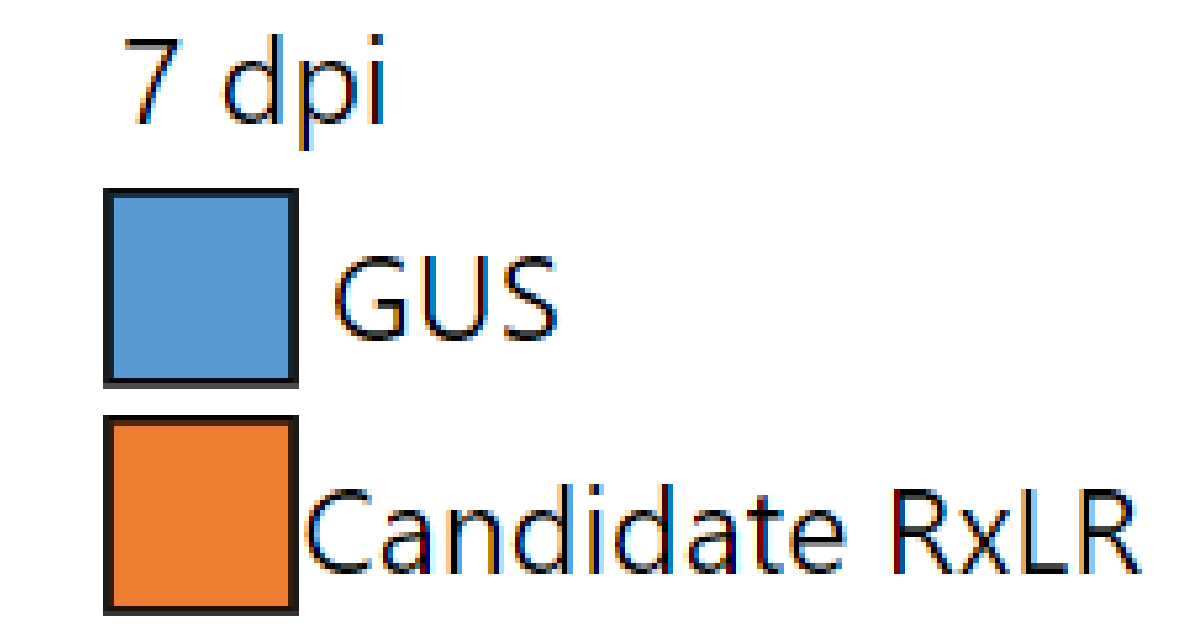
Confirmation of Effector Function

- 50 effector genes
- Cloning into overexpression constructs
- Agroinfiltration
- Inoculation with *P. infestans*
- Score lesion size

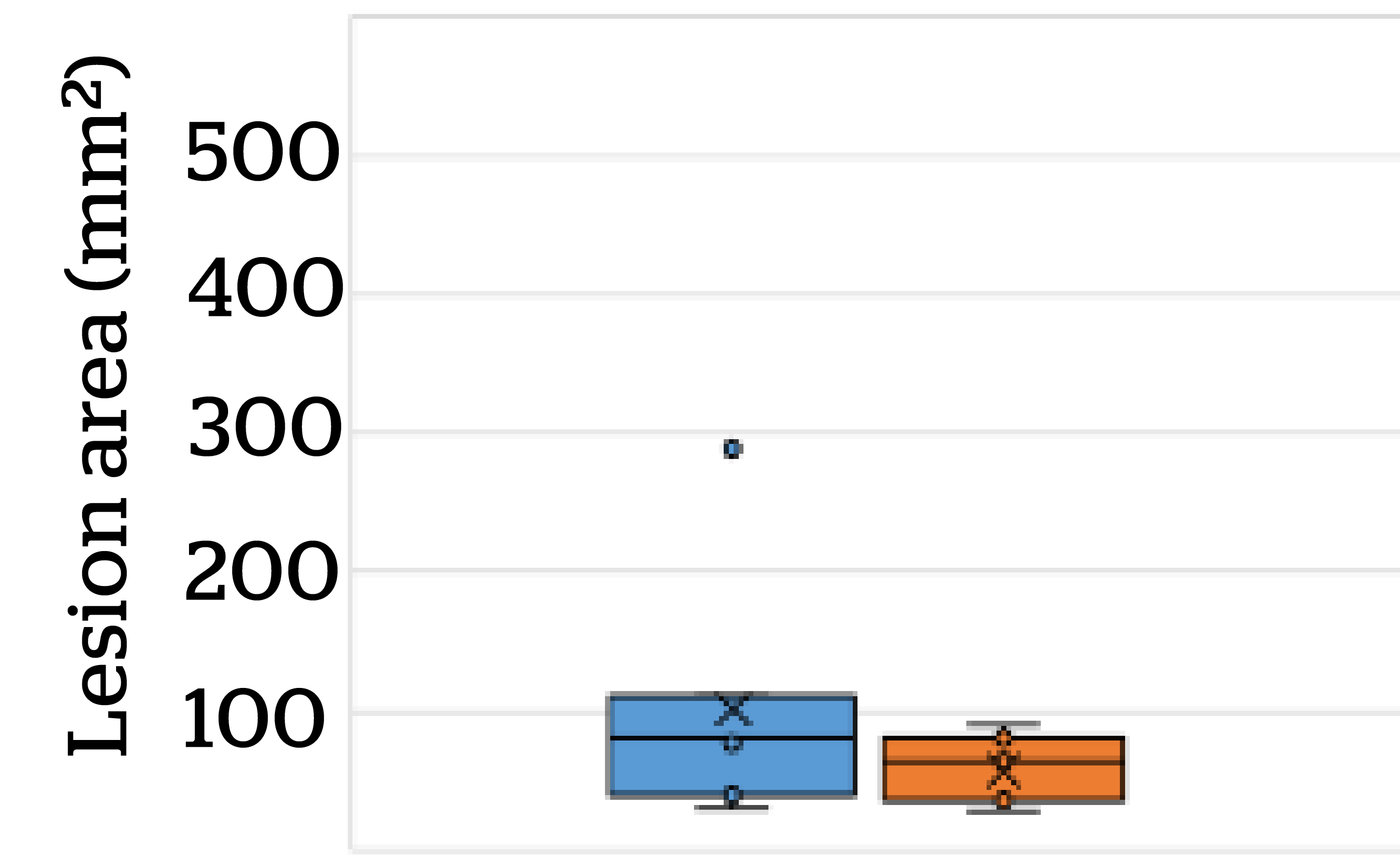
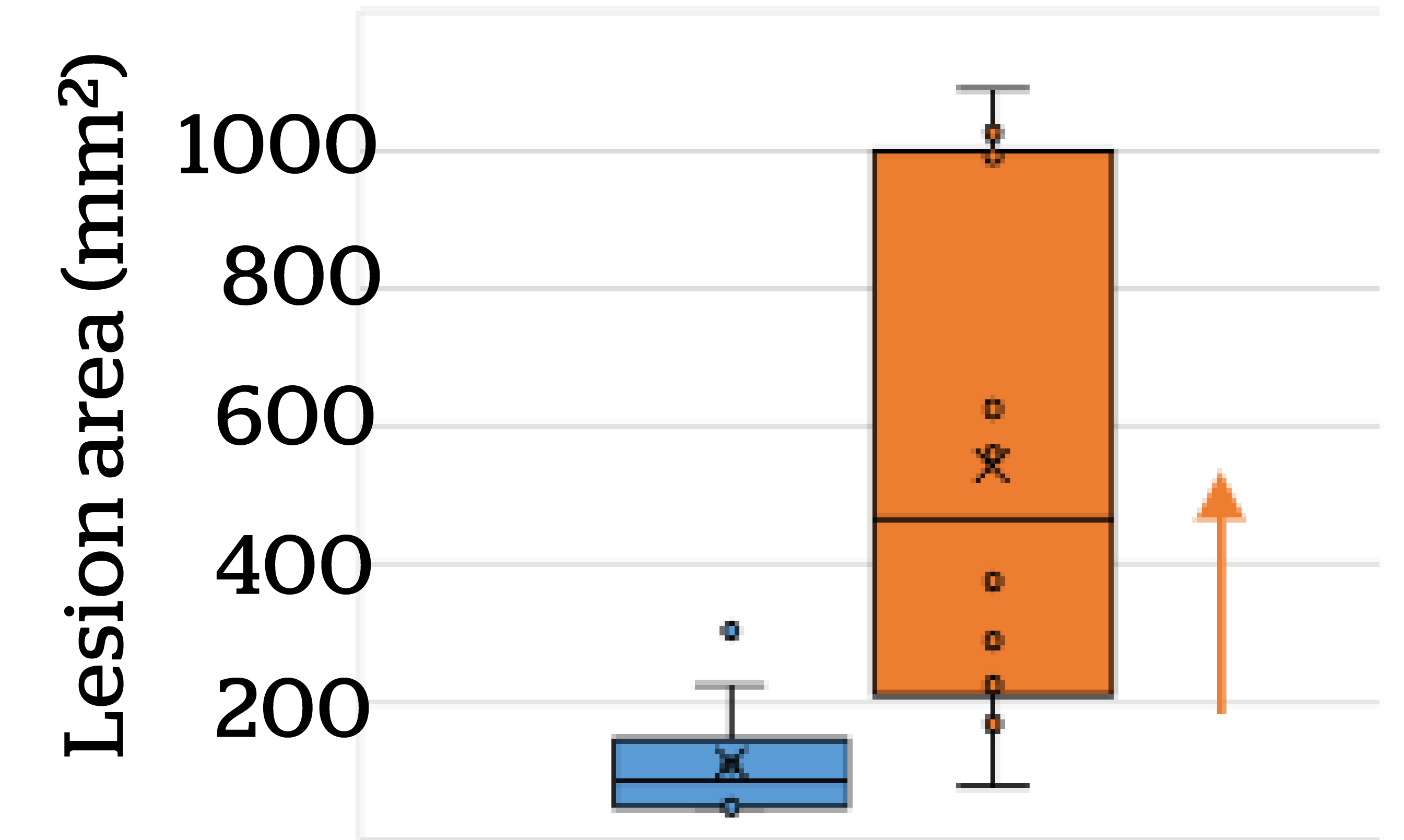


12 effectors selected for Interactomics based on conservation, gene expression, and a strong effect on virulence.

Positive effect



No effect

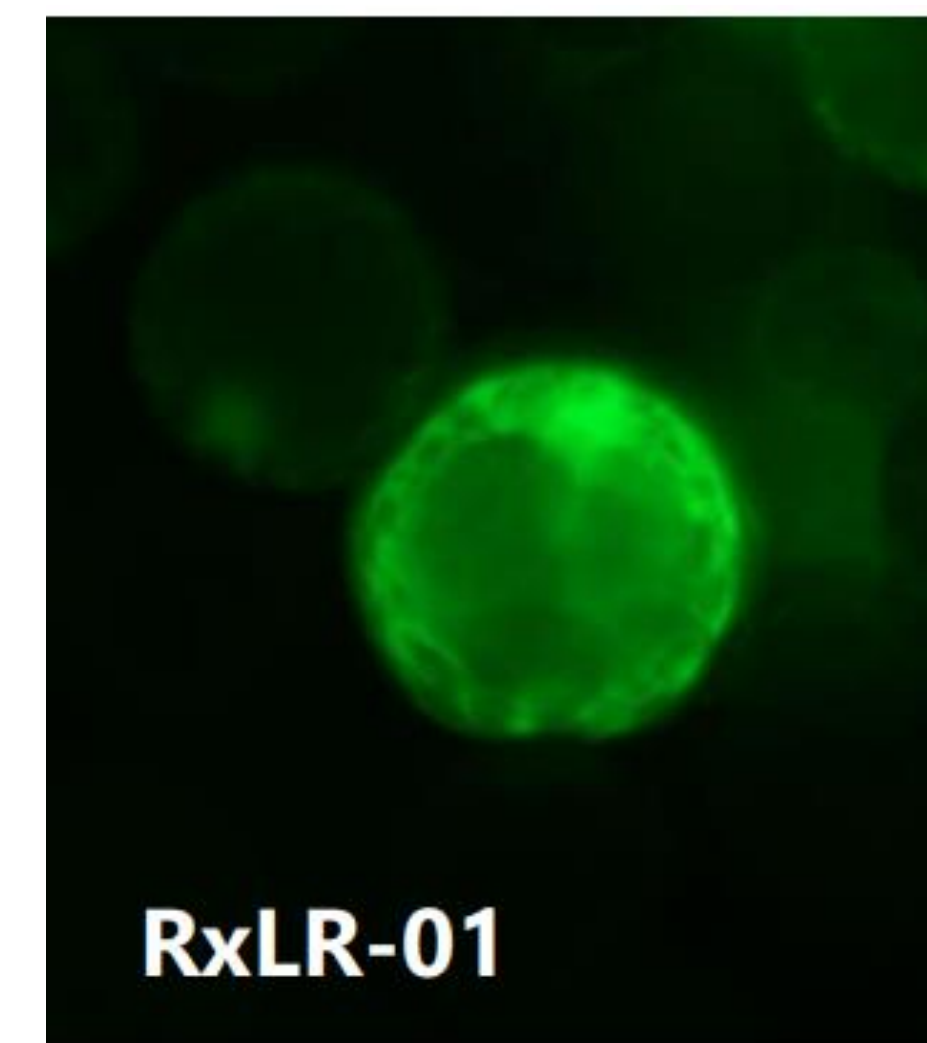


Interactomics - Using Pathogen Proteins to Find Interacting Plant Proteins

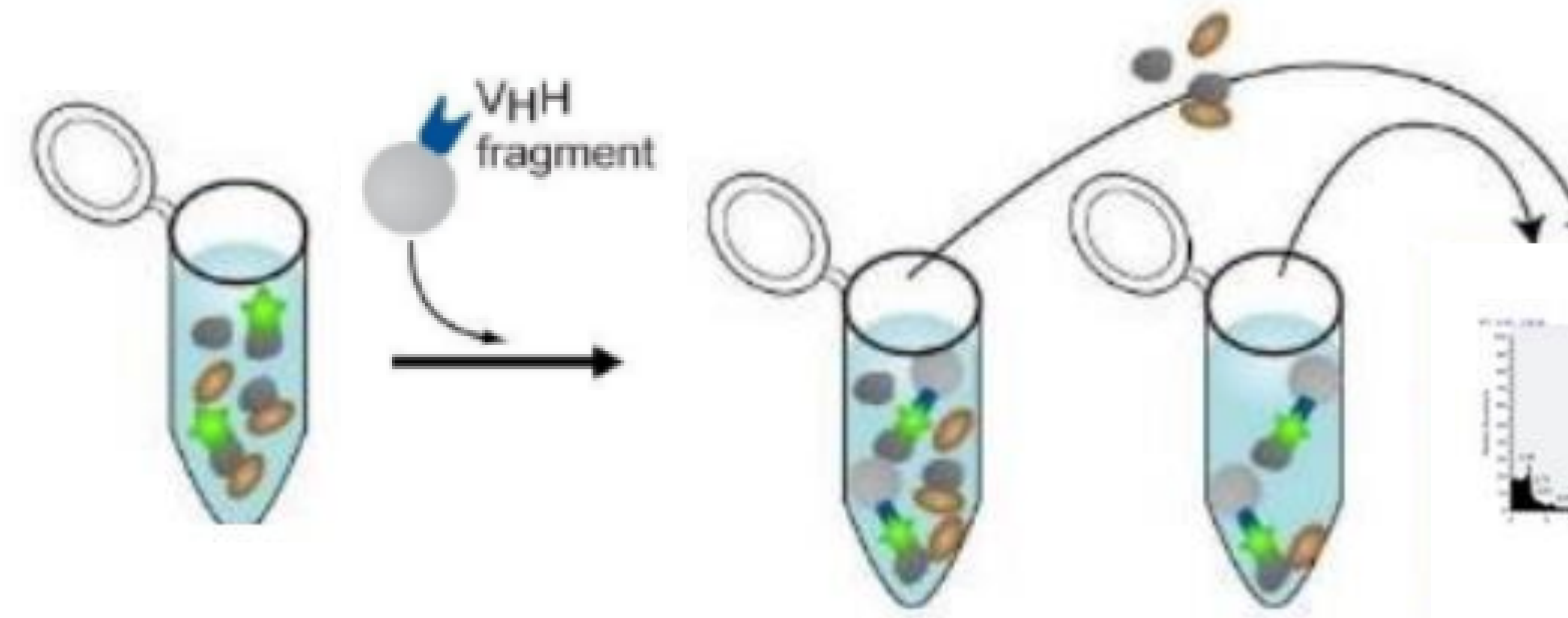
Yeast 2 Hybrid Approach Library construction



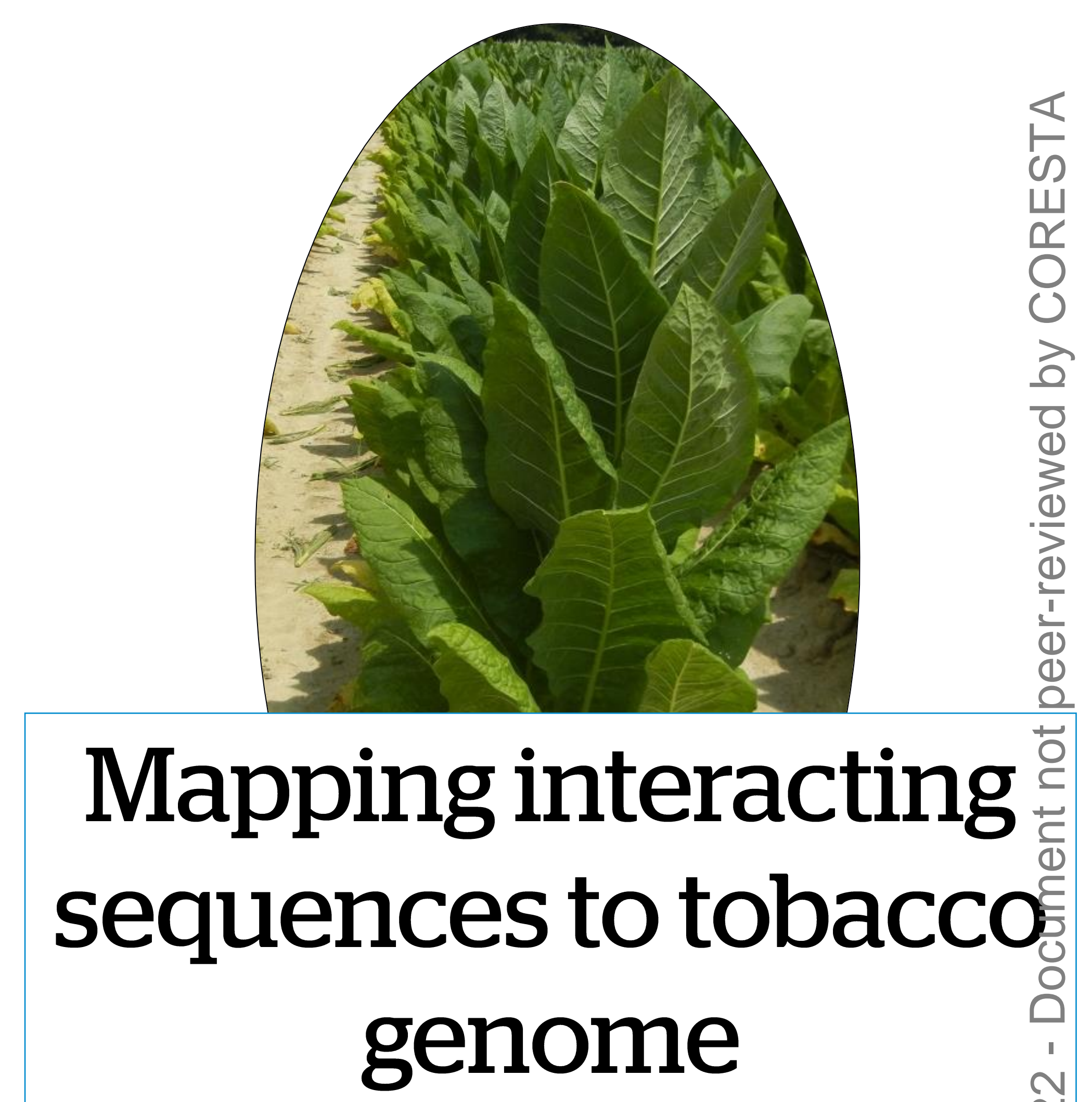
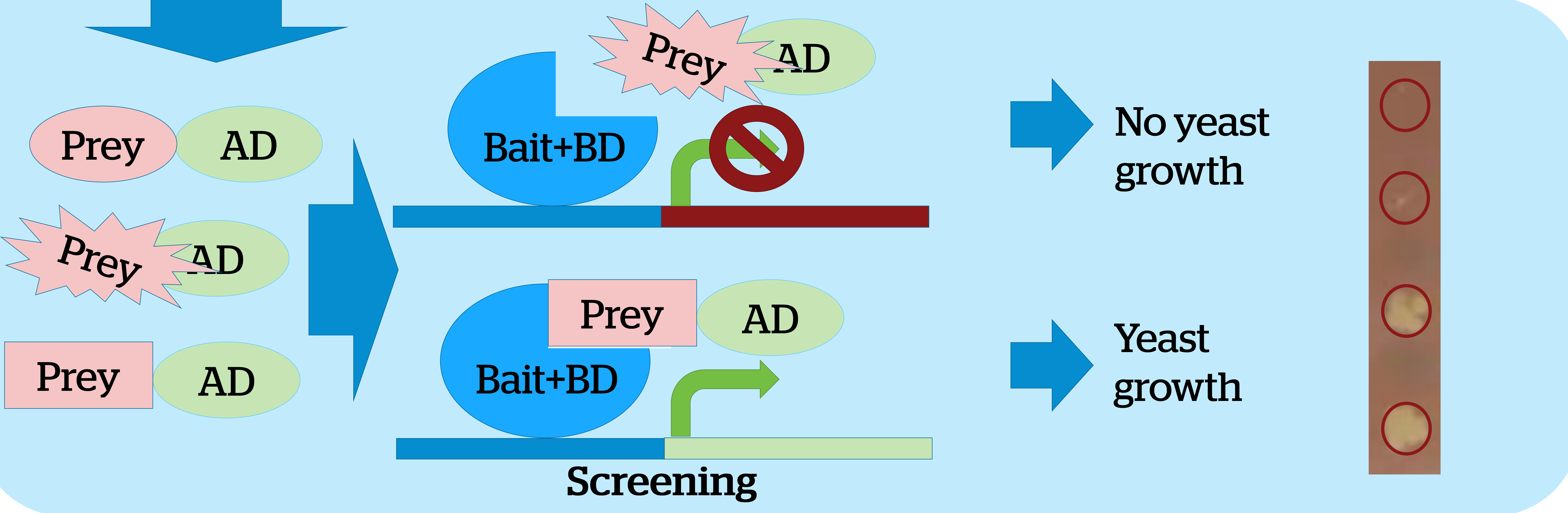
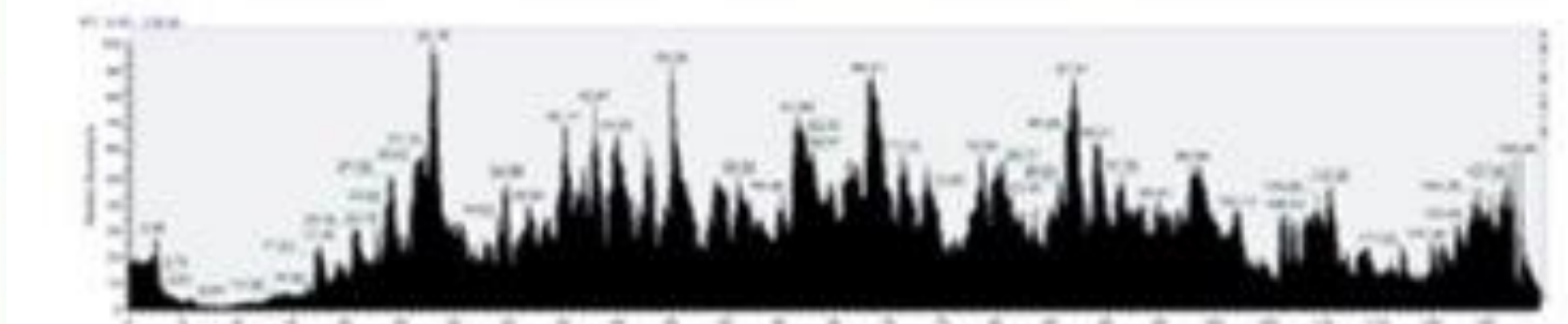
Protoplast Transformation



Pull down approach



Mass Spec

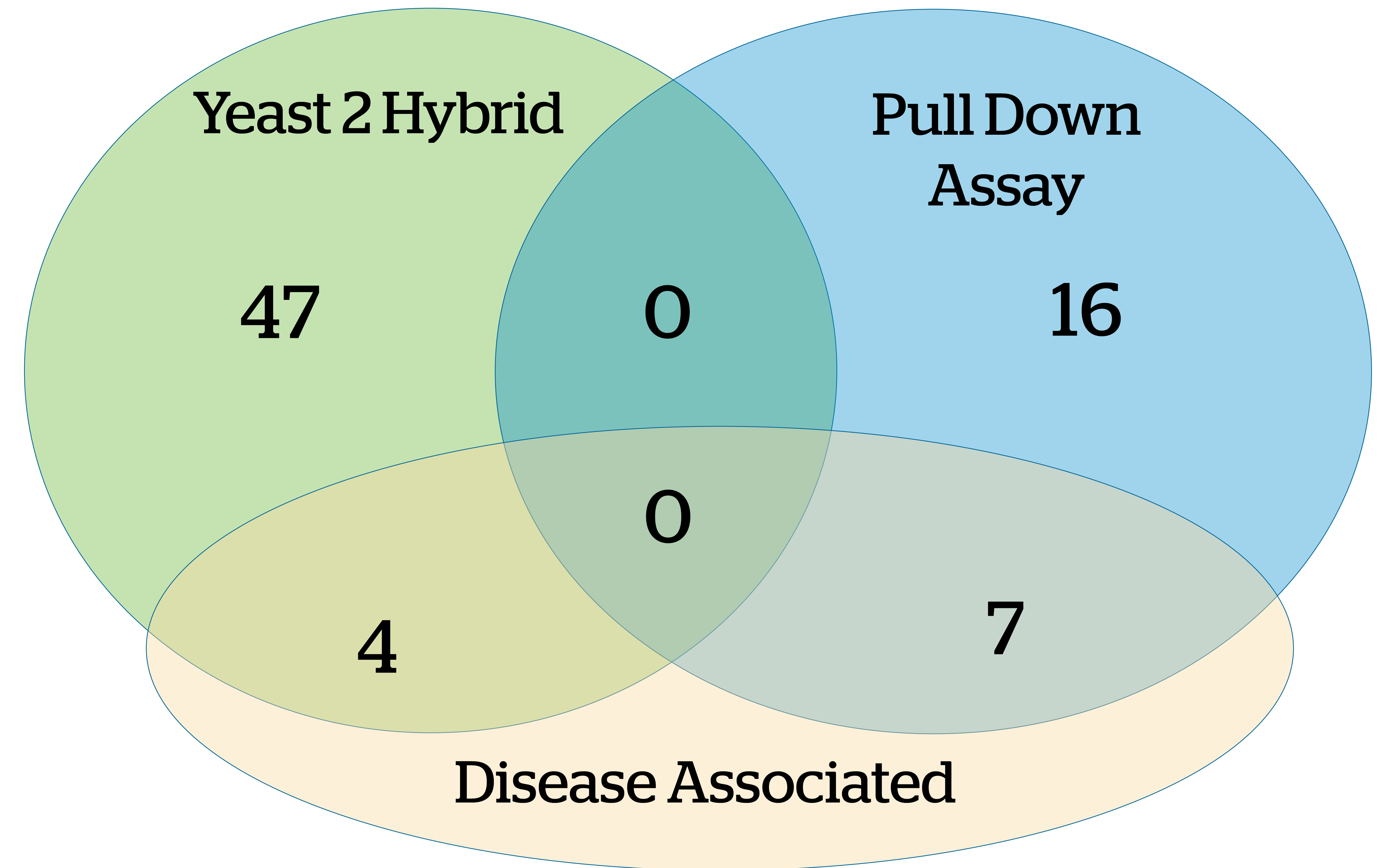
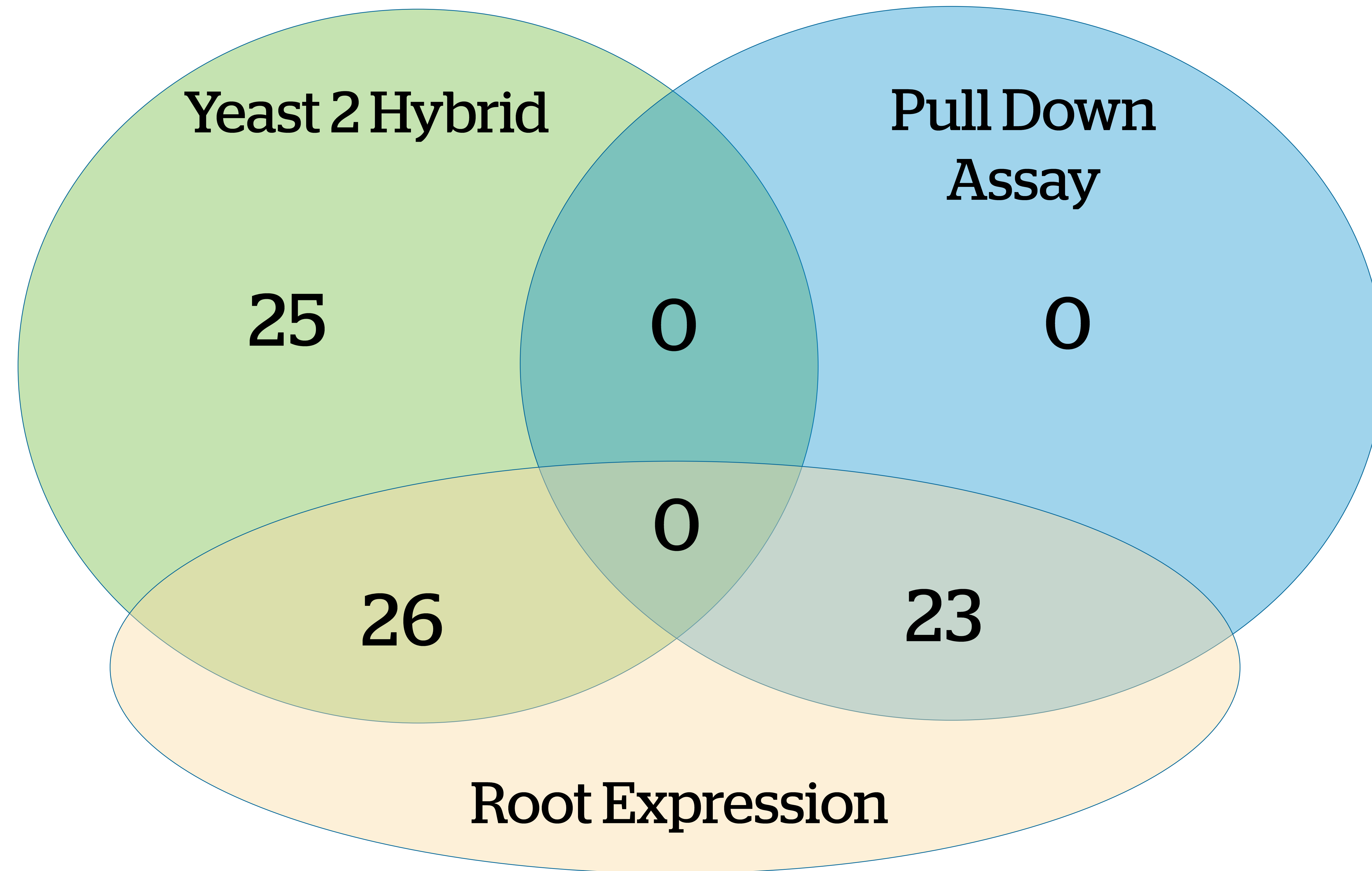


Identification of Putative S-genes

74 potential S-proteins
No common targets identified

Expressed in Roots

Disease Associated



Conclusions and Future Directions

- **Conclusions**

- Generated 29 Strain specific *Phytophthora* genomes
- Identified > 400 possible effectors for each strain
- Enabled further identification of strain specific virulence factors
- Identified 74 Possible S-proteins with 11 predicted to have a role in biotic stress
- Genes are not enriched in any specific genomic loci

- **Future Directions**

- Mutational screening of possible targets would enable elucidation of functional mechanisms
- Further data mining of *P. nicotianae* genomes may determine race specific markers

 Questions?

