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Exploring the Potentials of CRISPR-cas9 Genome Editing for Improved Resistance to Cassava Bacterial Blight (CBB)

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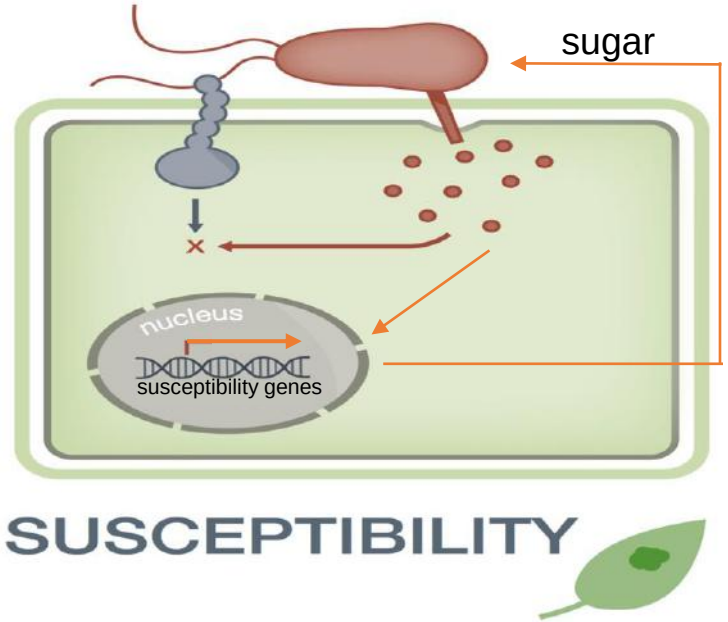
SUNDAY 5TH – THURSDAY 9TH AUGUST 2018

Introduction

Healthy cassava field

After CBB infection



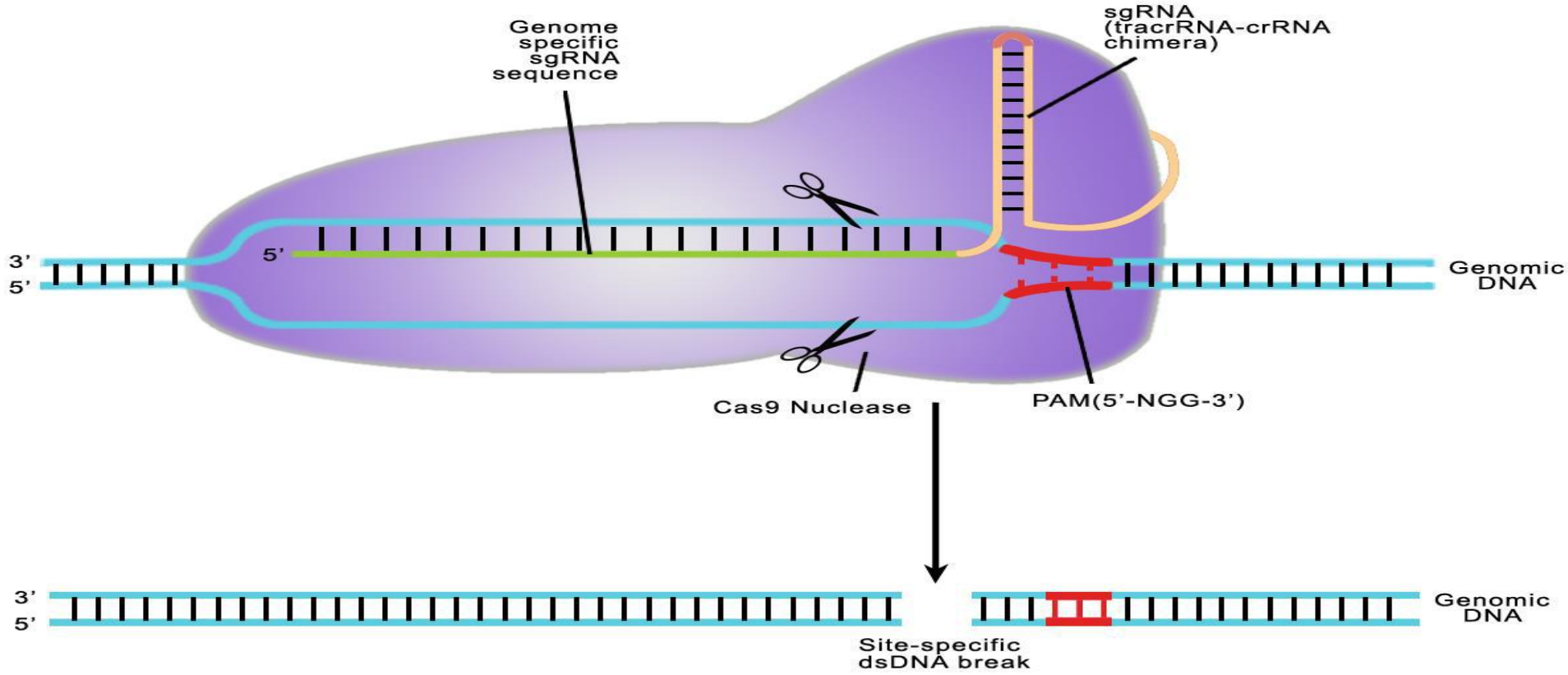
- CBB is caused by *Xanthomonas axonopodis* pv. *Manihotis* (*Xam*)
- Xam delivers a type III effector proteins into the host cell to suppresses or modulates host innate immunity and promote pathogenesis
- One target: modulate expression of susceptibility genes in plant
- *MeSWEET10a* is induced by transcription activator-like (TAL) effectors from *Xam*
- that binds to the Effector Binding Element (EBE) in the promoter

Objectives

1. Validate *MeSWEET10a* gene involvement in African pathosystem
2. Knockout *MeSWEET10a* TAL binding site as a resistance strategy
3. Tag the *MeSWEET10a* gene with a GFP reporter marker as a tool for studying disease progression

Methodology

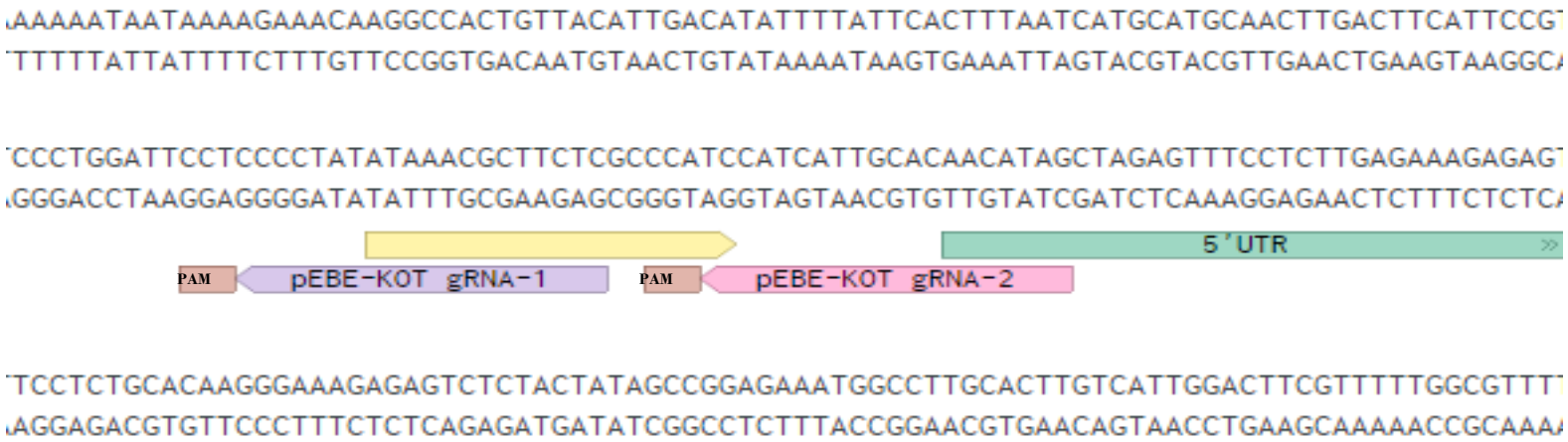
CRISPR genome editing tool



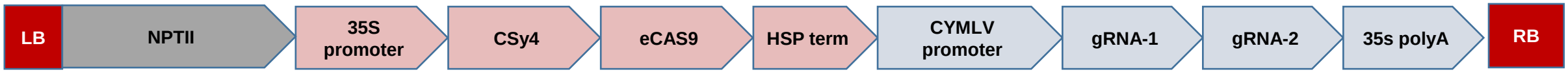
induces a double stranded break that could be repaired leading to insertions or deletions of nucleotide sequence

Methodology

Disruption of EBE site in *MeSWEET10a* gene



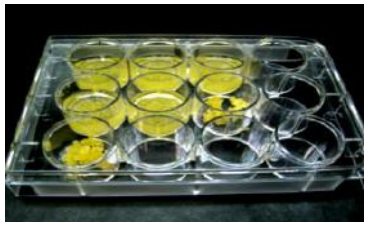
gRNA design for targeting MeSWEET10 EBE binding site



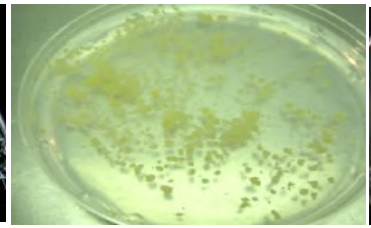
Clone (p8466)

Transformation of cassava FEC with p8466

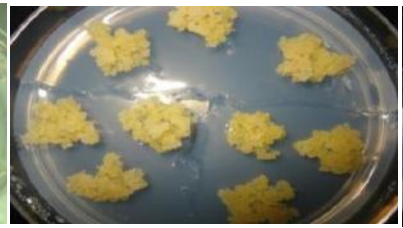
Cassava FEC



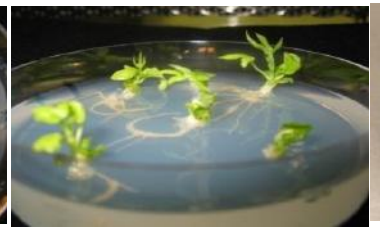
FEC /Agrobacterium inoculation



Selection 27.5 µM paramomycin



Regeneration on MS2 5 µM NAA medium



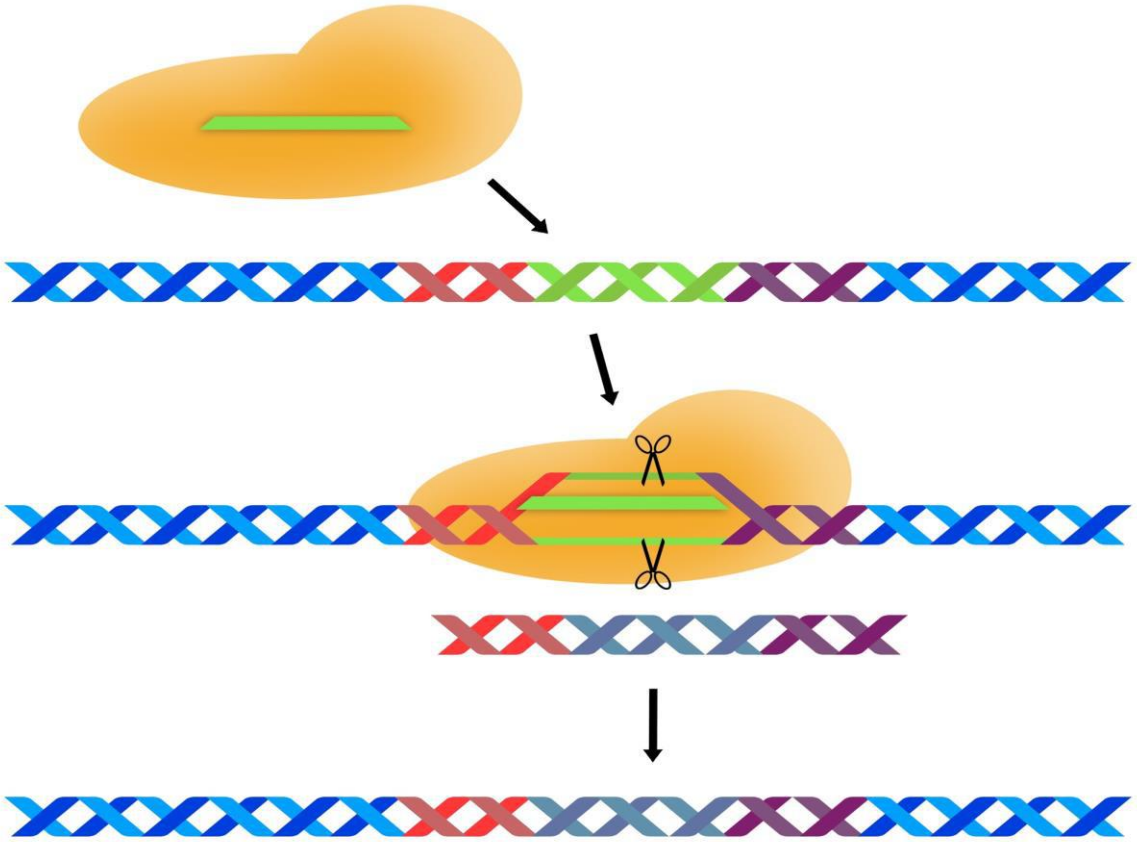
germination on MS2 2 µM BAP

Rooting in MS2 agar

Cassava transformation pipeline

Methodology

Homologous recombination



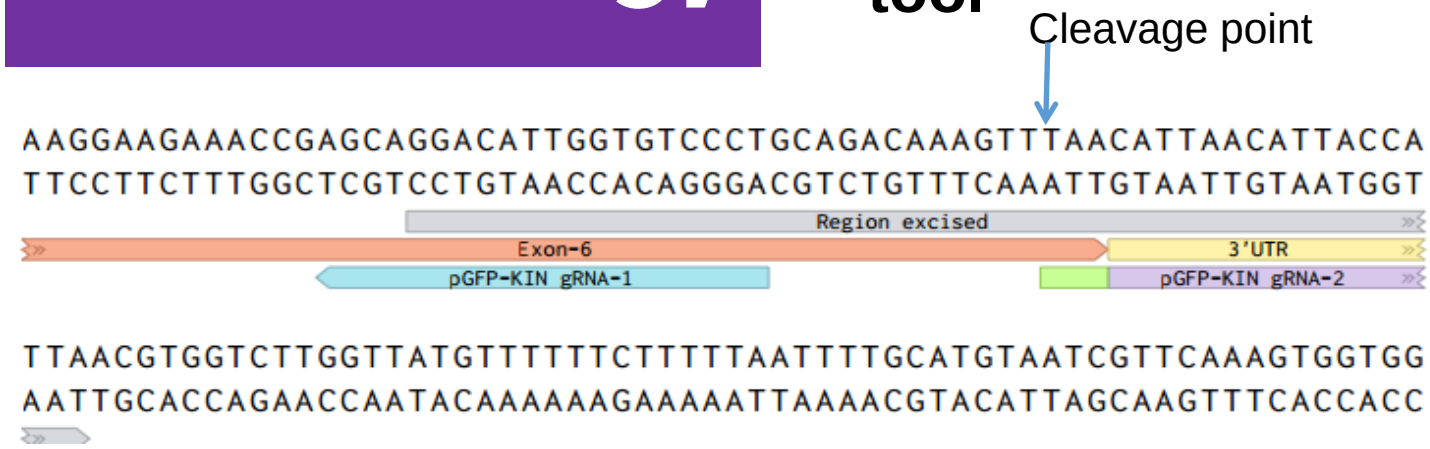
Using *MeSWEET10a* as a readout for infection:

Charpentier & Doudna (2013) doi:10.1038/495050a

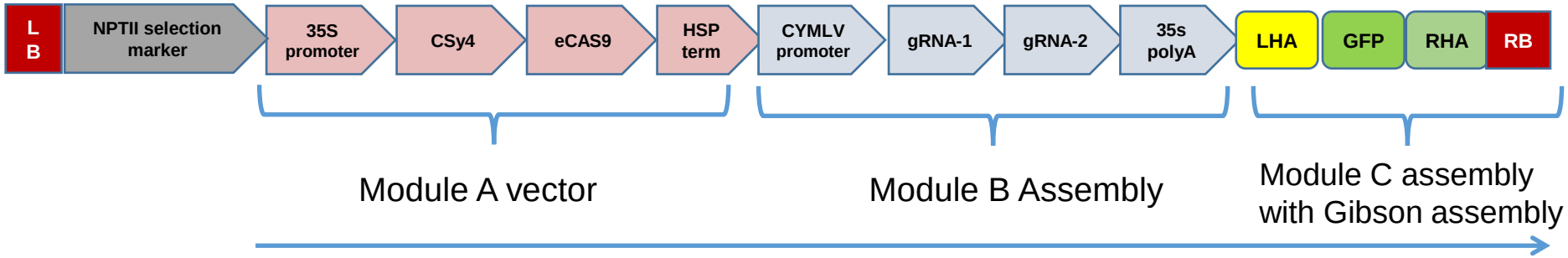
http://www.transomic.com/Products/CRISPR-Genome-Editing.aspx

Methodology

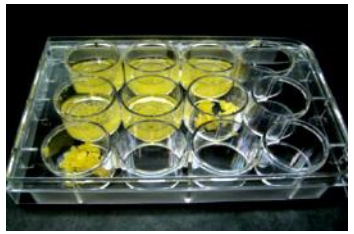
Developing fluorescence based disease model tool



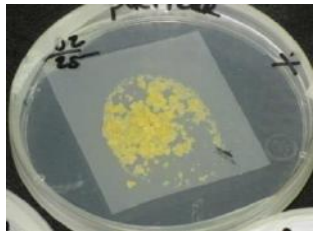
- Fuse GFP at the c-terminal of the MeSWEET10a gene
- Form a heterologous protein
- Induce MeSWEET10a/GFP expression with Xam



Regenerated callus lines recovered from p8469 transformation



Transgenic 8469 callus lines co-cultured with Tal20 in Agrobacterium LBA 4404

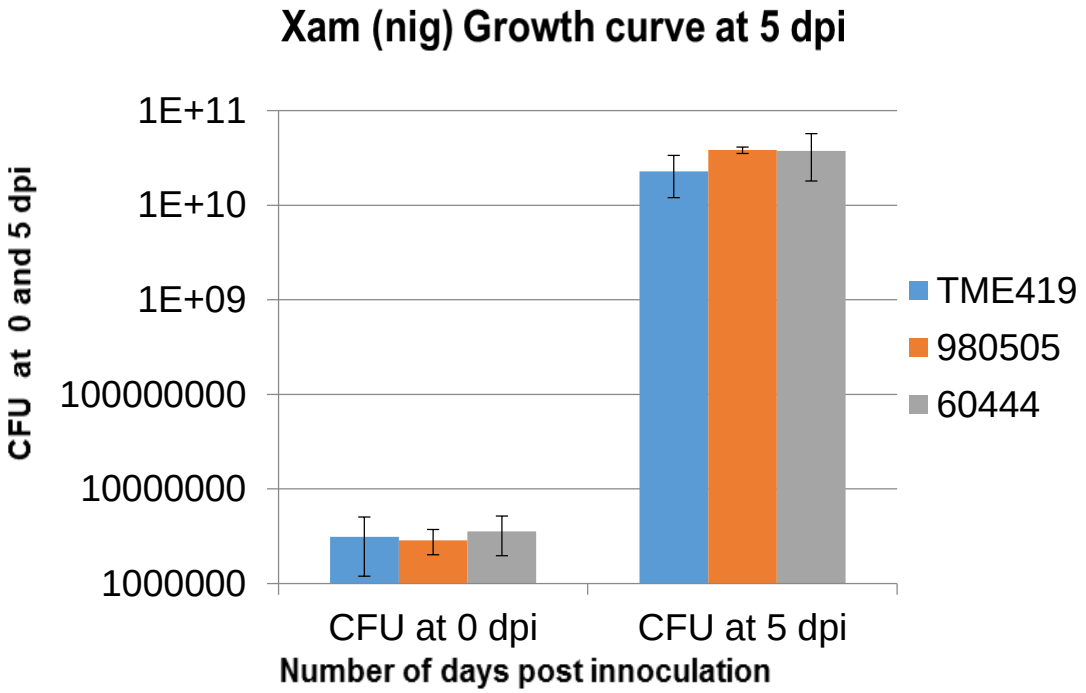


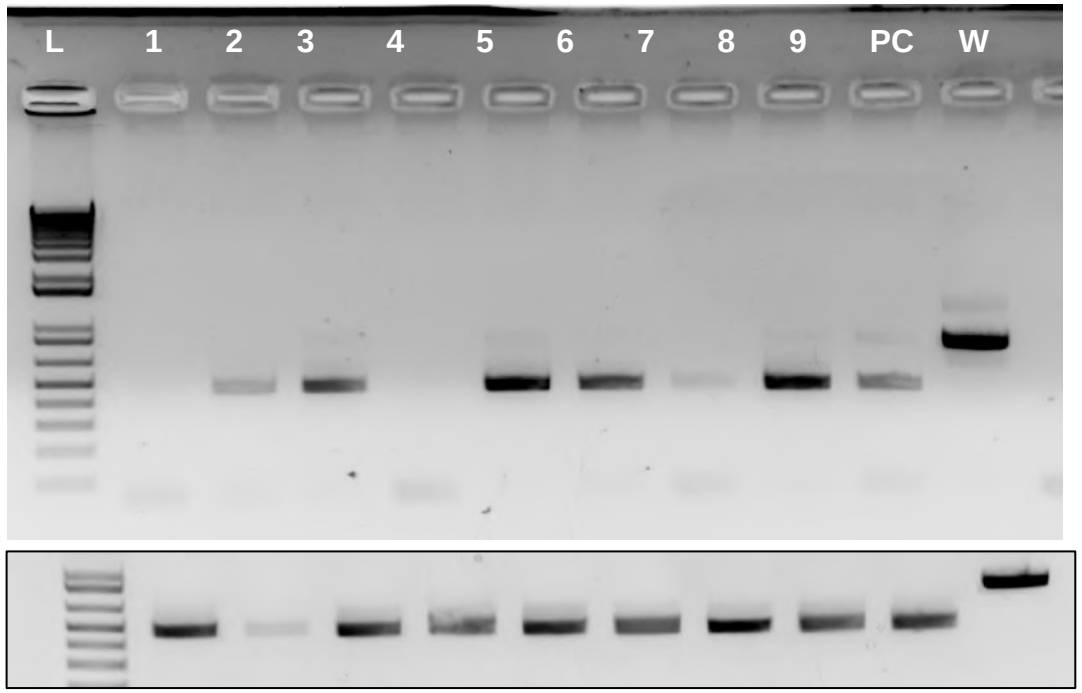
Tal2o inoculated callus lines incubated at 22°C

Excitation at GFP wavelength

Results and Discussion

Goal 1: Validating *SWEET* genes associated virulence in cultivars and strain of interest



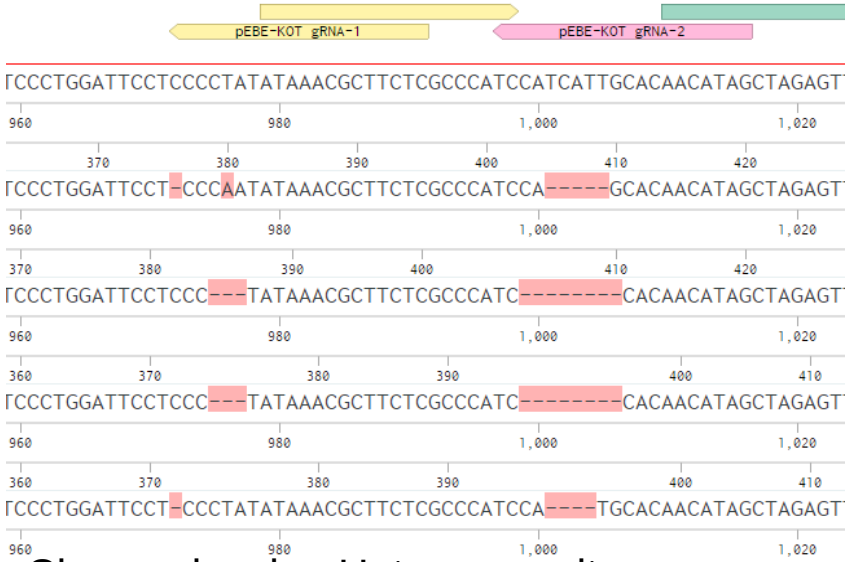


Blue = mock
Red = infected

Results and Discussion

Goal 2: *MeSWEET 10a* gene knockout

Clones showing Homozygosity



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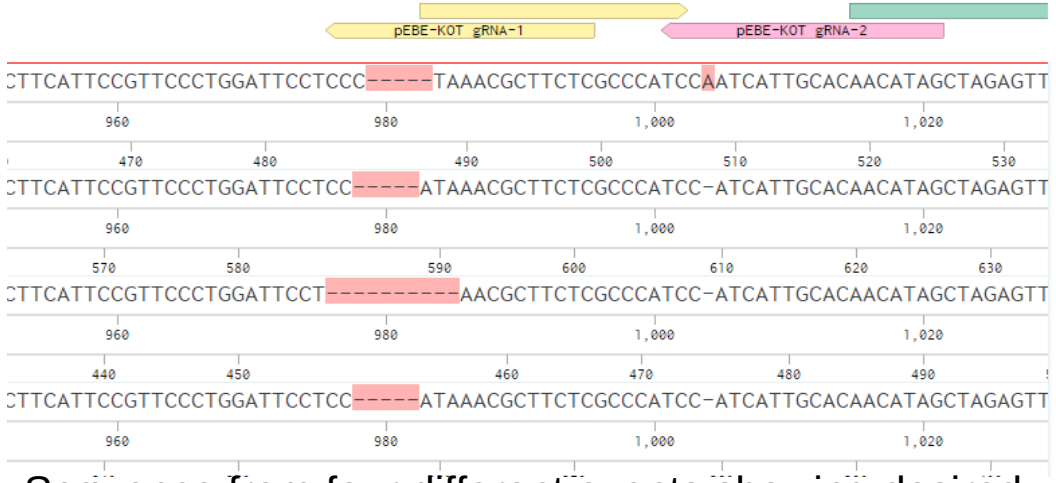
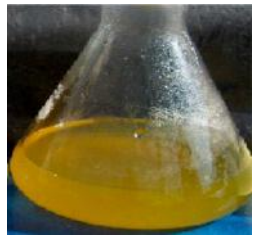


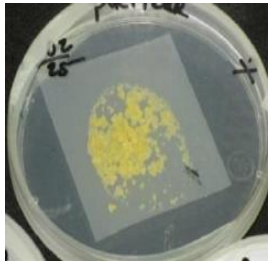
Sequence from four different events showing desired mutation at the EBE

Results and Discussion

Tal20 from Xam & cloned into a binary vector



LBA 4404 harboring Tal 20 clone in binary vector



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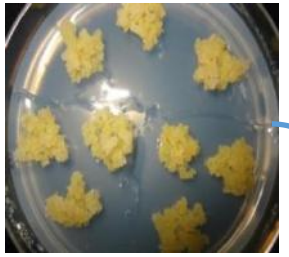
Excitation at GFP wavelength



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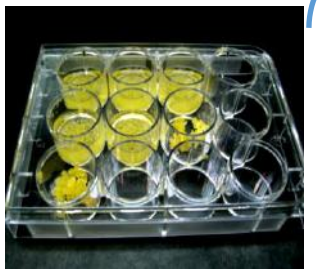

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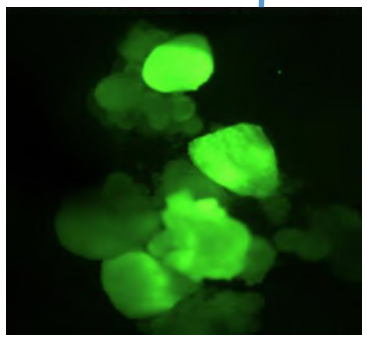
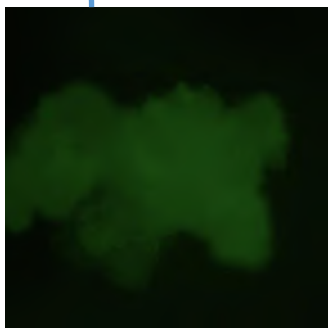
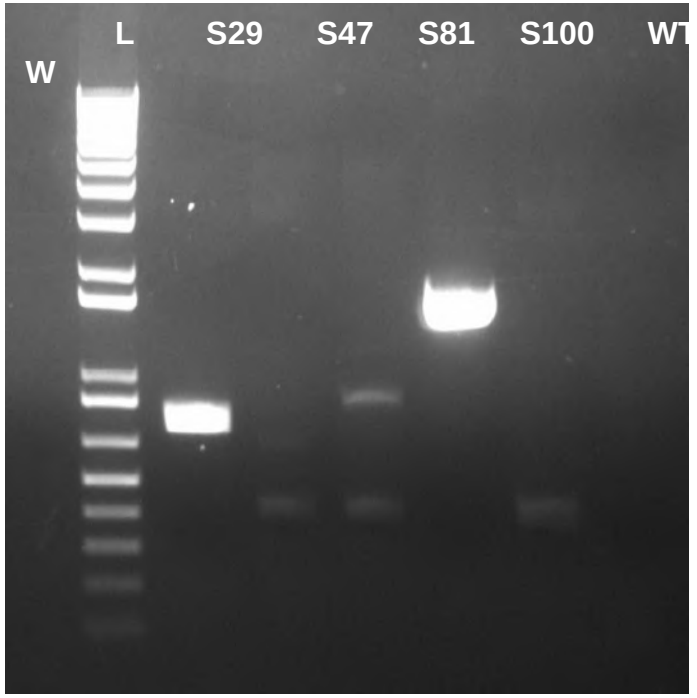


Tal20 treated callus line expressing GFP viewed with fluorescence dissecting microscope



Untreated S-100 event viewed with fluorescence dissecting microscope



Molecular confirmation of targeted GFP integration

selection of products of HR-mediated repair (SureFire)

Conclusions & Recommendations

MeSWEET10 Knock-out

- CRISPR-cas9 tools were effective in producing the desired edits for the EBE knock-out
- 2. Events with homozygous mutations in the EBE are difficult to recover

MeSWEET10/GFP -tag

- 1. 18% (419) and 30% (60444) of GFP knock-in events screened showed GFP expression when induced with Tal20
- 2. 29% of GFP expressing lines has the expected 1.6 kb fragment

Acknowledgements

DONALD DANFORTH PLANT SCIENCE CENTER
DISCOVERY | COMMUNITY | IMPACT

AWARD
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Supervisor

Rebecca Bart
Supervisor

Members of IICI trait improvement lab

Dan Lin
Carrington's Lab

Members of Bart lab