What about CRISPR?

How gene editing could be used to enhance disease resistance in American chestnut

Jared Westbrook
Director of Science
The American Chestnut Foundation
Chestnut Chat
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THE AMERICAN CHESTNUT FOUNDATION®

Motivation

Could we edit the American chestnut genome to have the disease resistance of Chinese chestnut but otherwise retain all of the characteristics of American chestnut?

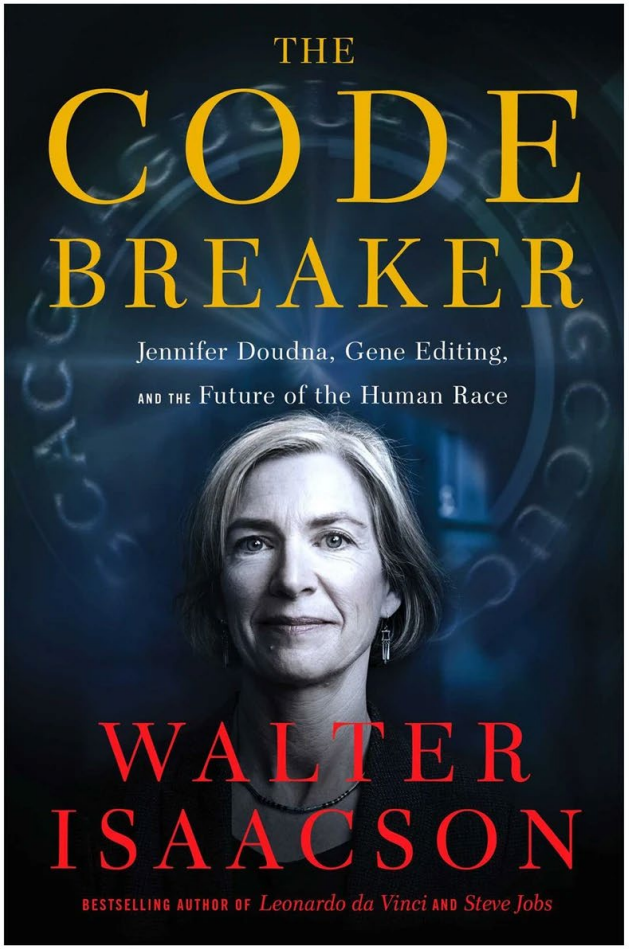
American chestnut

- Not resistant to blight
- **Dominant canopy tree**

Chinese chestnut

- **Resistant to blight**
- **Resistant to phytophthora root rot**
- Orchard tree

Recommended reading



Potential applications of gene editing for American chestnut restoration

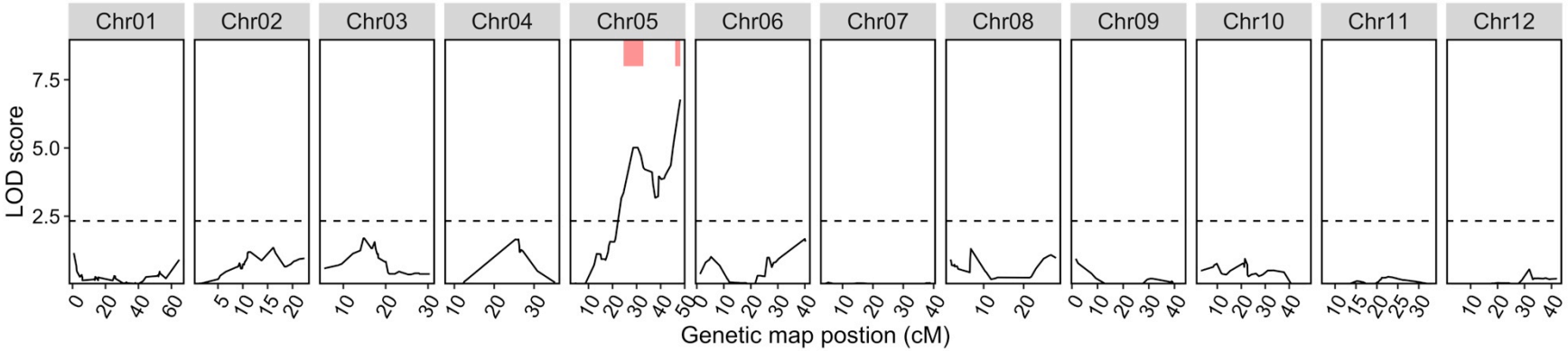
If necessary, blight resistance of OxO lines could be improved by adding/activating resistance genes or knocking out American chestnut susceptibility genes

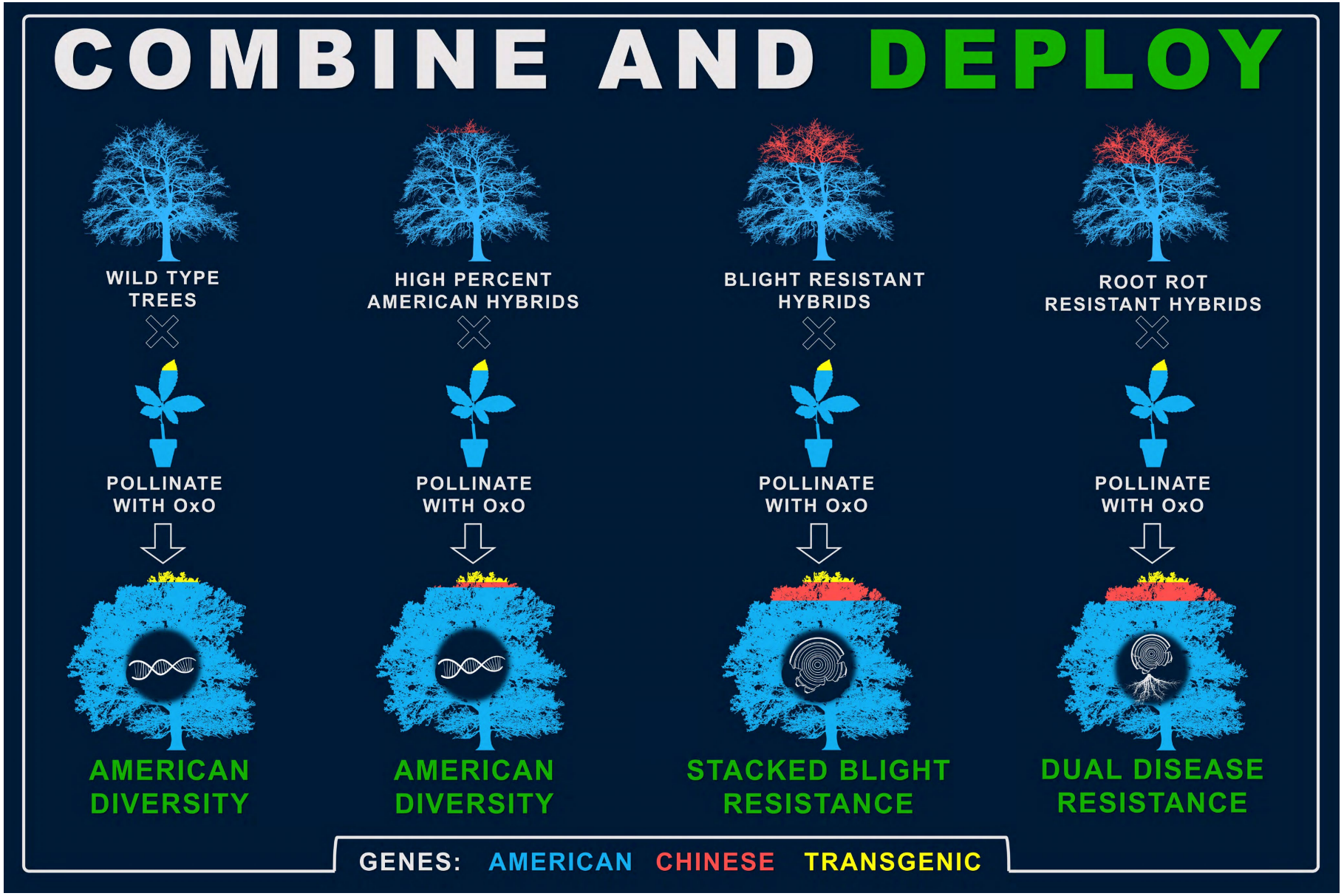
Natural canker on Darling 58 T1
(Photo by Erik Carlson)

Potential applications of gene editing for American chestnut restoration

Increase the Phytophthora root rot resistance of American chestnut

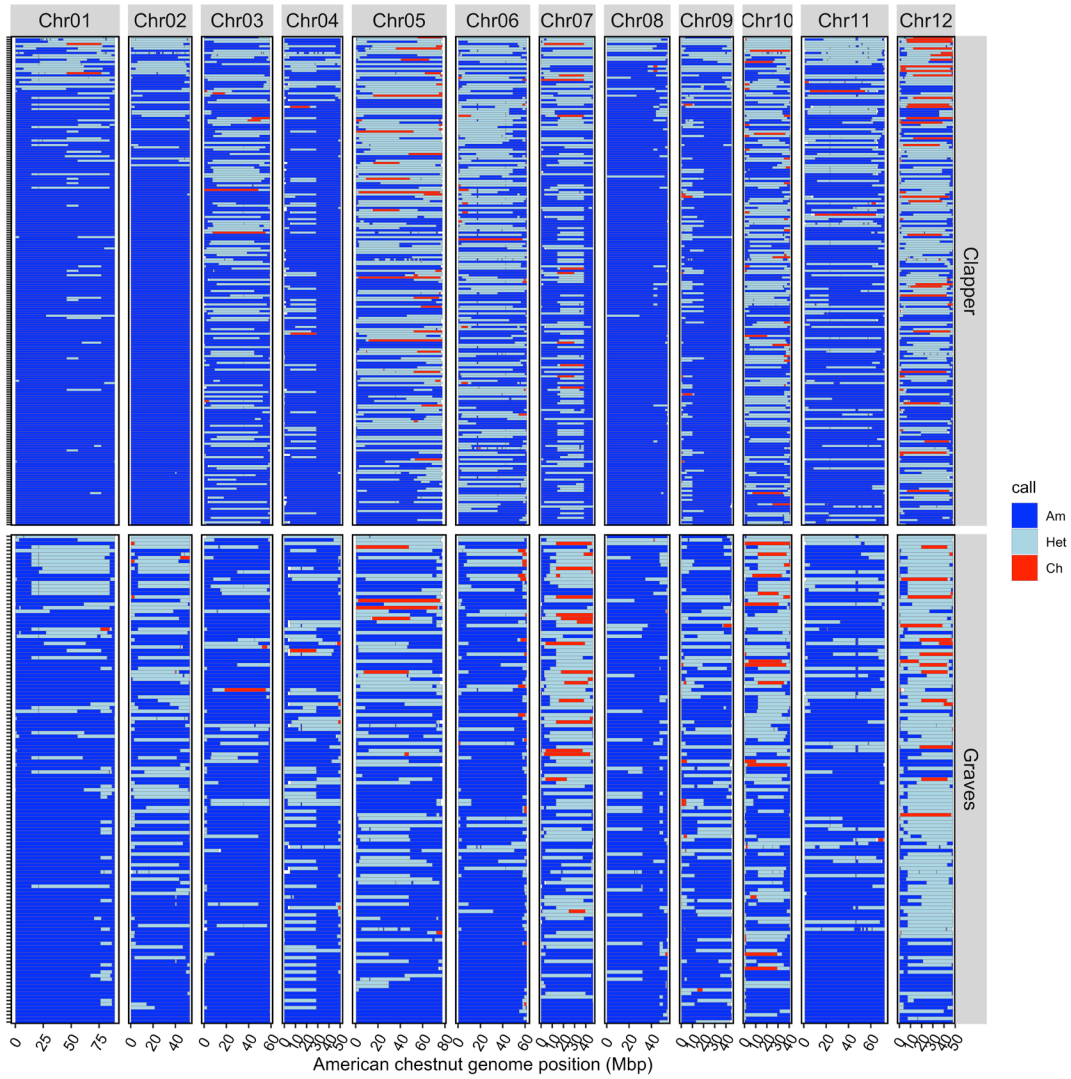
Two regions on Chromosome 5 explain a total of 20% of variation in PRR resistance





Infographic by Vasiliy Lakoba

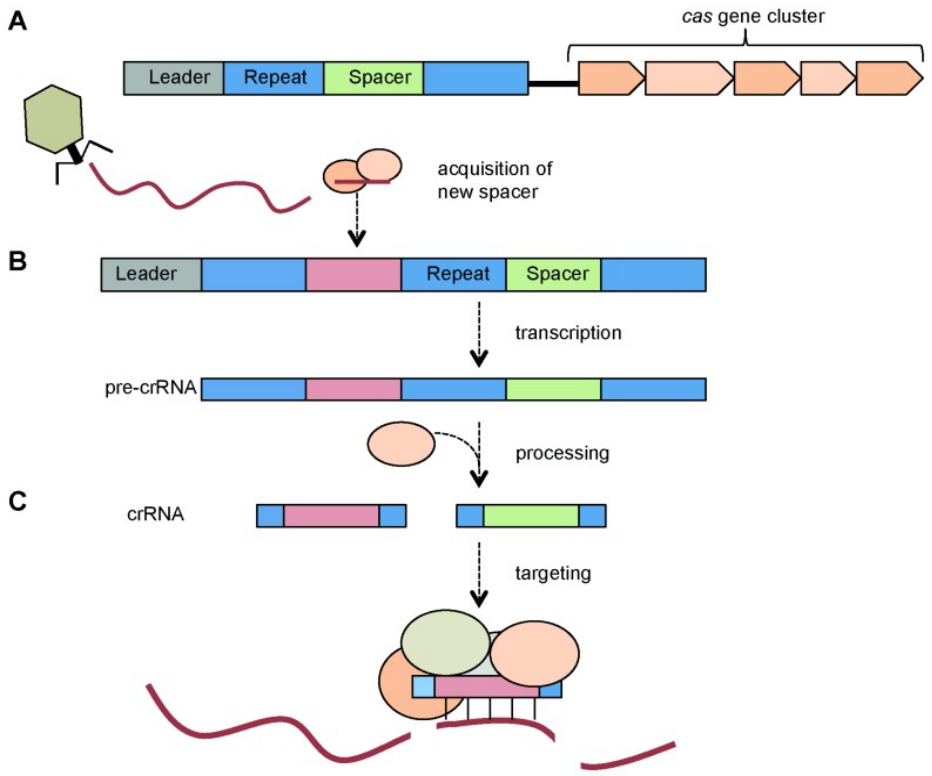
Gene editing (or transgene insertion) enables precise changes to enhance disease resistance without linkage drag of genes unrelated to disease resistance



Breeding enables stacking of multiple resistance genes at one without requirement of knowing the individuals genes involved in resistance

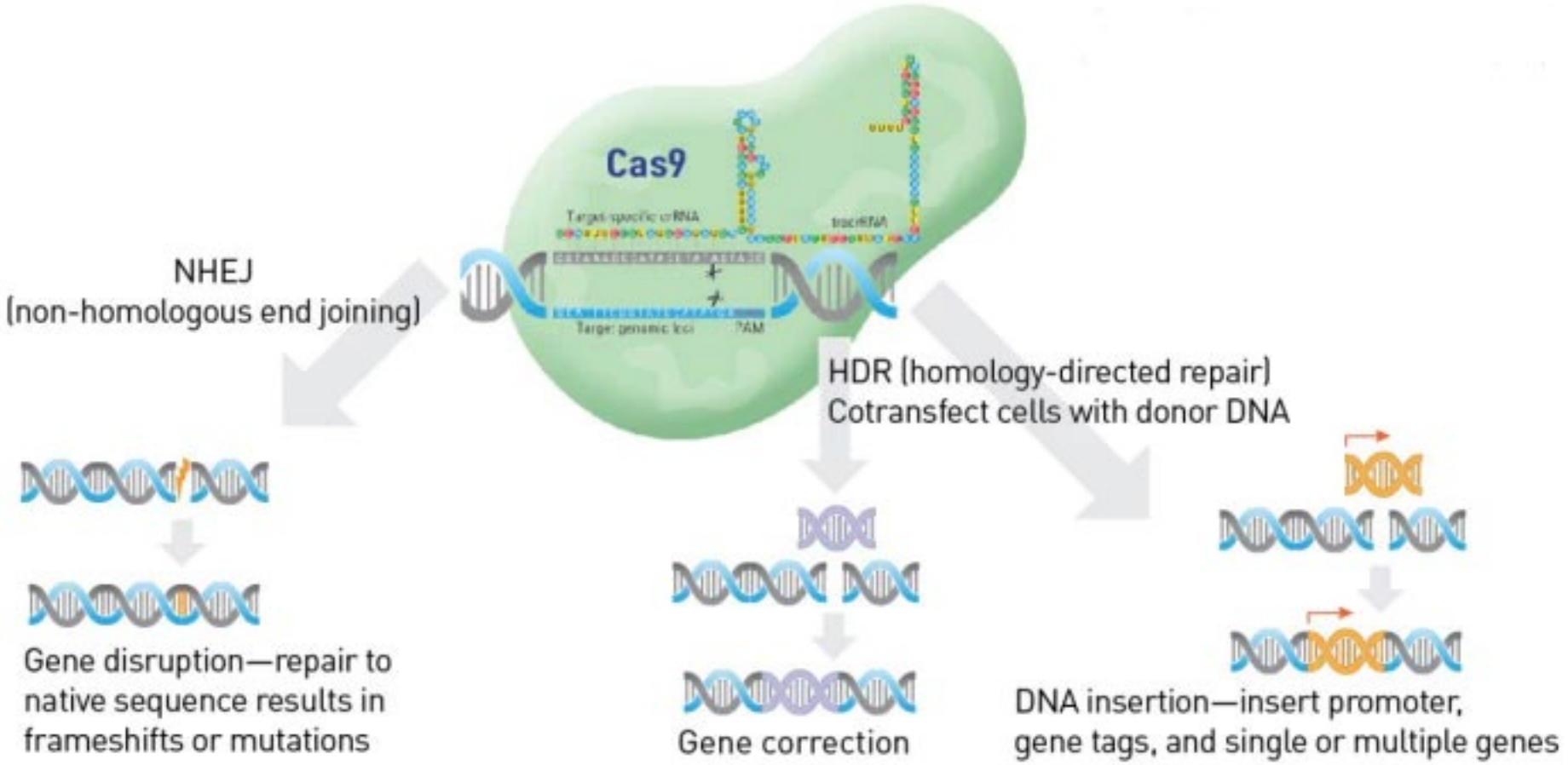
Origin of CRISPR

Bacterial immune system against viruses (phages)



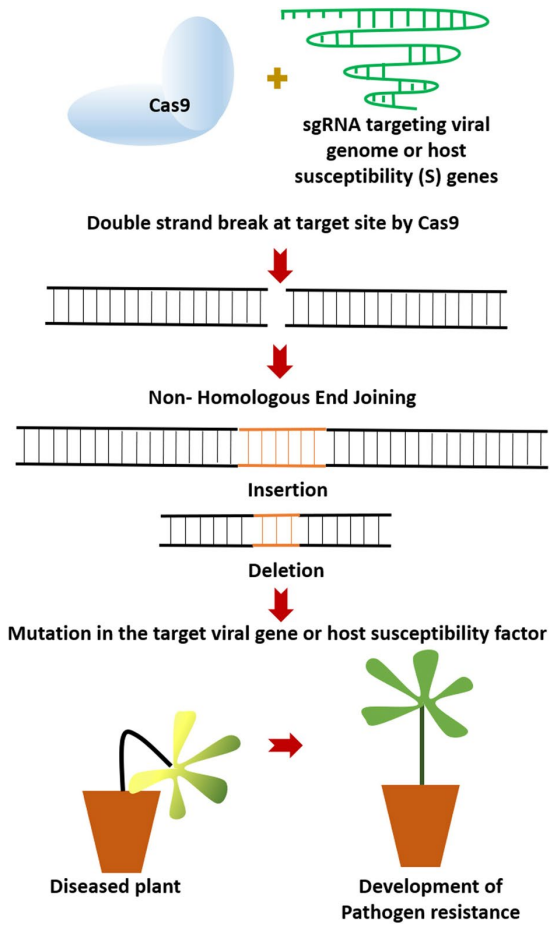
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3497052/#idm140504867563648title

CRISPR for gene editing



Gene editing strategies

Knockout susceptibility genes with CRISPR



Do mutations in susceptibility genes account for ”resistance” in American chestnut?

Pros:

- Targeted gene knockouts technically feasible with CRISPR
- May not be regulated by USDA

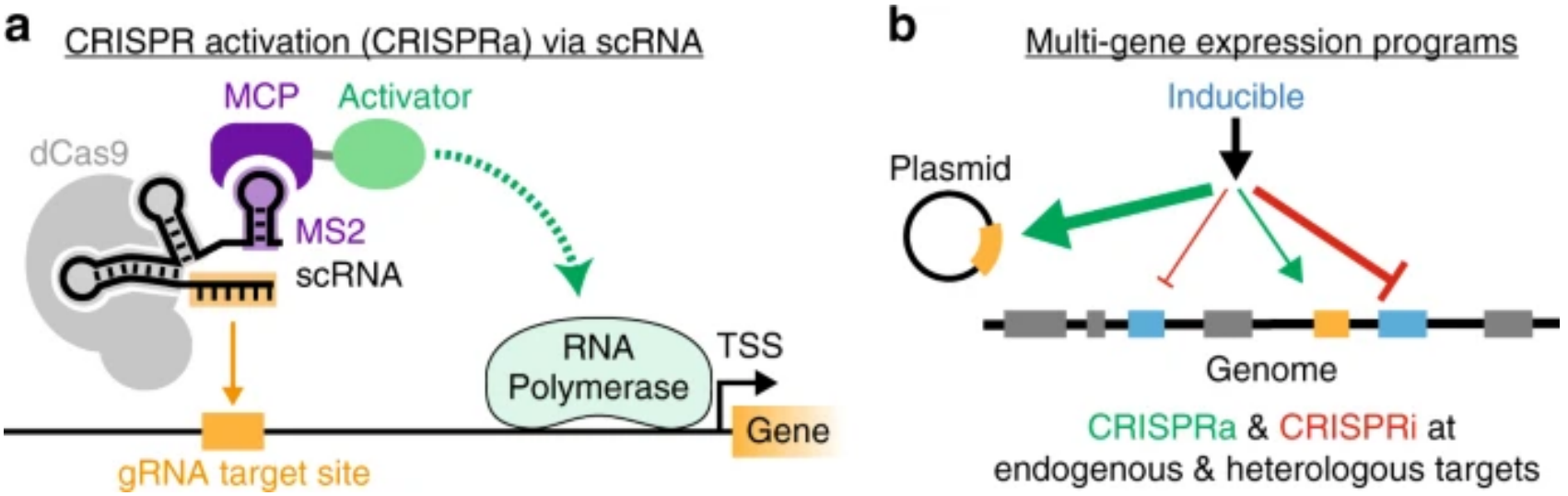
Cons:

- Need to screen large randomly mutagenized populations to identify susceptibility genes (if they exist).
- Knockouts of genes involved in programmed cell death may increase susceptibility to biotrophic fungi

https://www.frontiersin.org/articles/10.3389/fpls.2018.02008/full

Gene editing strategies

- Activation or repression of gene expression
- Facilitated by dead Cas proteins fused to transcriptional activators/repressors



https://www.nature.com/articles/s41467-018-04901-6

Pros:

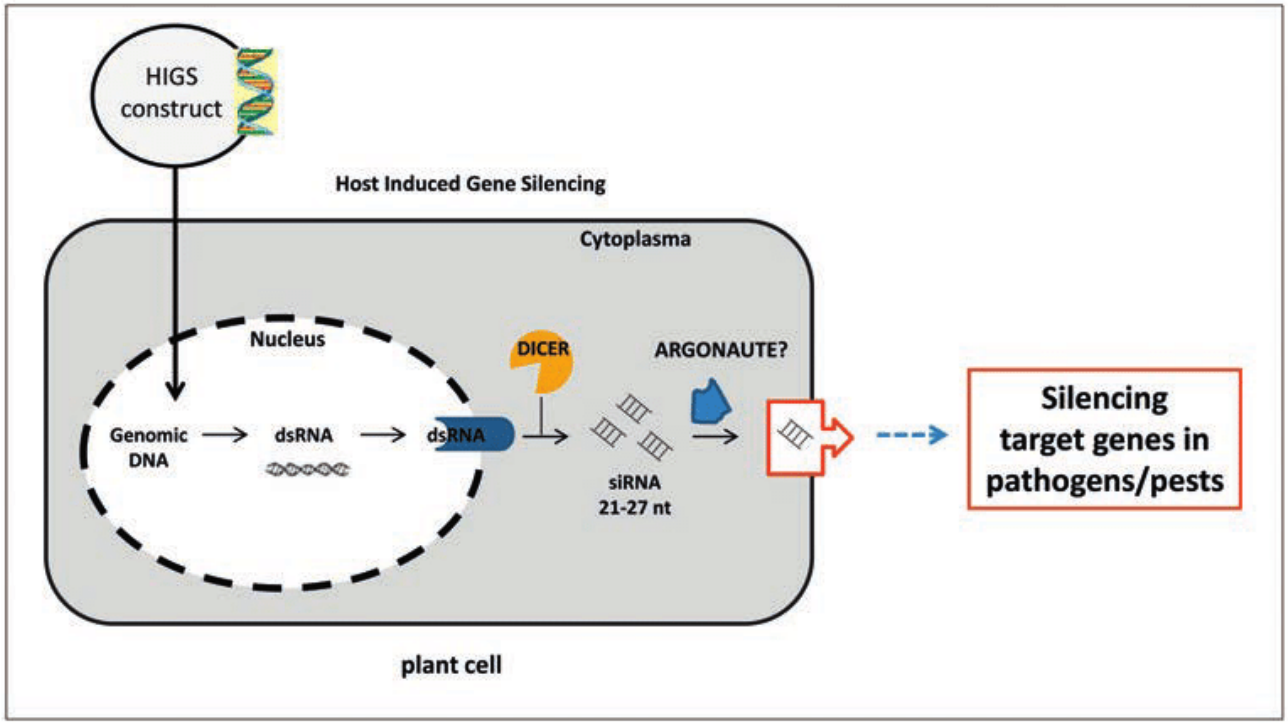
- Possible to change expression of multiple genes at one time

Cons:

- May be regulated as a transgenic plants due to insertion of the CRISPR gene activation construct

Gene editing strategies

Host induced gene silencing



Pros:

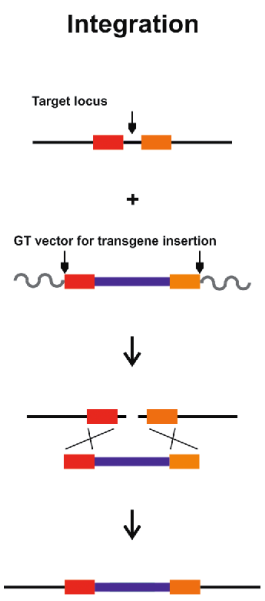
- Target a few key pathogenicity or development genes in host (simplifies breeding)

Cons:

- May not be effective if chestnut blight rapidly kills cells and prevents expression of dsRNA in host
- Regulated as transgenic plant

https://www.researchgate.net/publication/338236242_The_Agronomic_Potential_of_Gene_Silencing_Applications

Gene editing strategies



Tandem insertion of multiple resistance genes

Pros:

- Additive effects on resistance?
- Simplifies breeding with wild trees
- May not be regulated by USDA
- Could be accomplished with Agrobacterium mediate insertion rather than CRISPR

Cons:

- low rate of gene insertions with homology directed repair

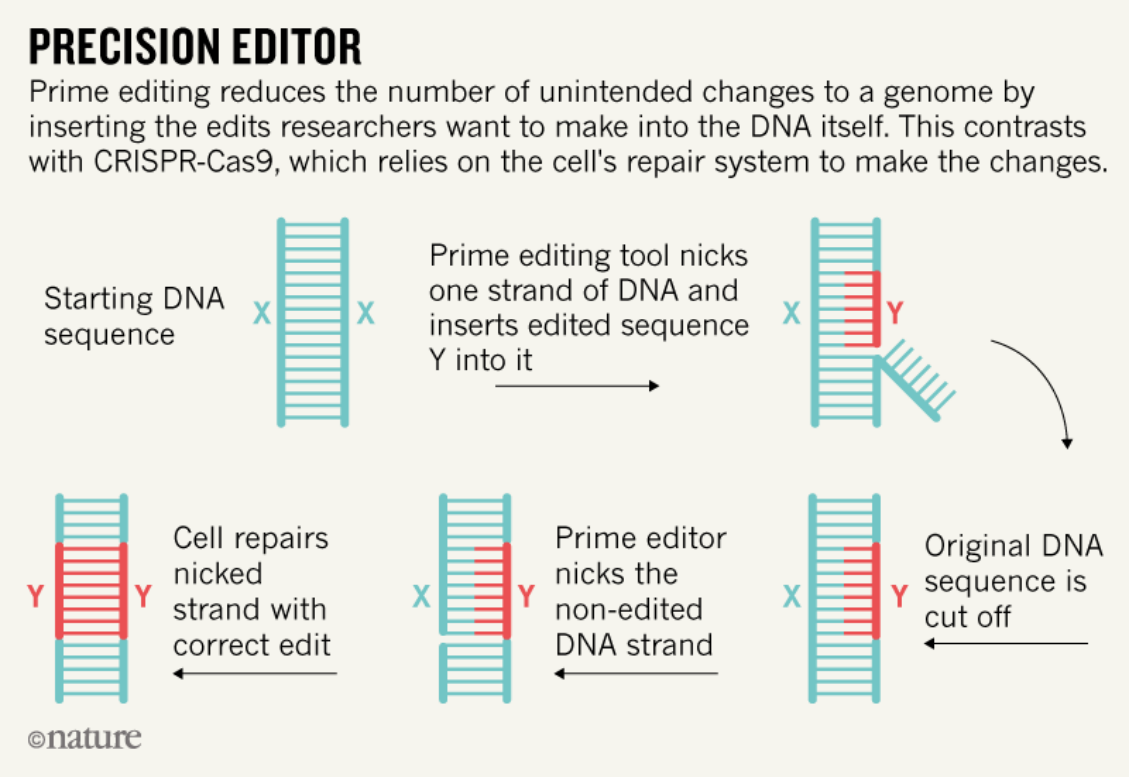
Blight resistance gene A

Blight resistance gene B

Root rot resistance gene

Gene editing strategies

Replace multiple American chestnut alleles with Chinese chestnut alleles with Prime editing



https://www.nature.com/articles/d41586-019-03164-5

Pros:

- Possible to to make multiple types of genetic changes at once (e.g. expression, protein coding sequence)
- May not be regulated
- Make precise edits with no linkage drag

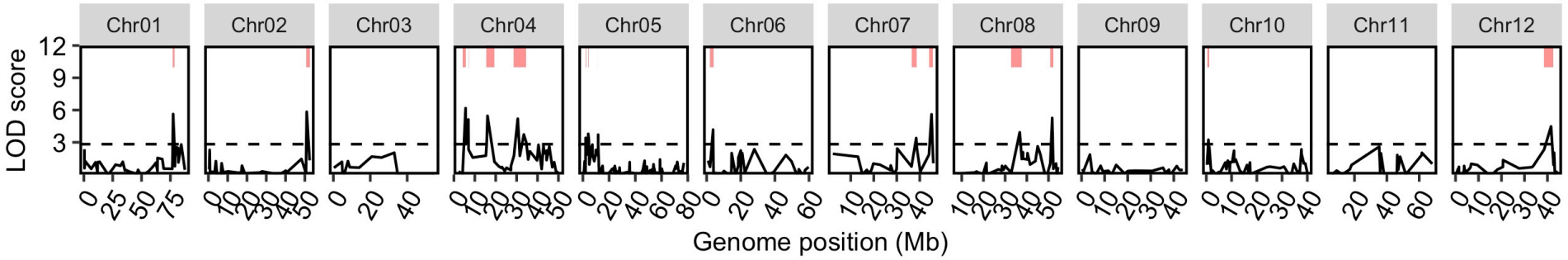
Cons:

- Need to have detailed knowledge of the genes and specific changes to make
- Need to screen large segregating populations for inheritance of edits?

Bottlenecks: Resistance/susceptibility gene discovery

We do not yet know what genes to edit and what specific changes to make

Many QTLs for blight resistance, each with small effect

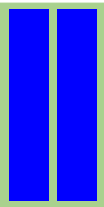


Each QTL may contain 10s to hundreds of genes

Strategy for discovering candidate genes for blight and root rot tolerance

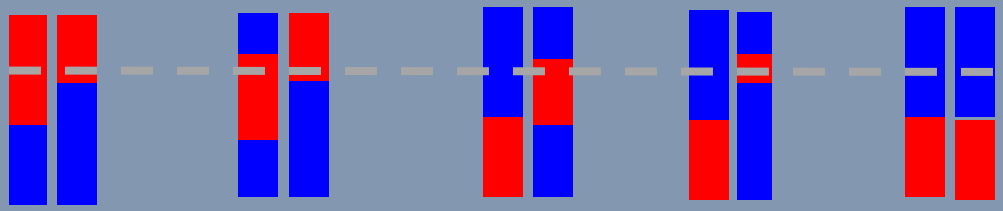
Step 1: Assemble chestnut reference genomes

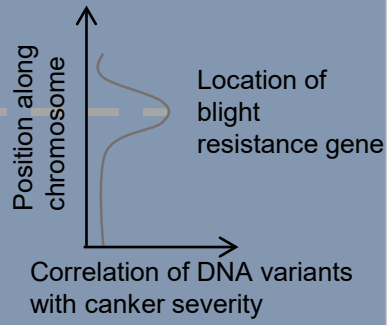
Chinese chestnut source of resistance



American chestnut

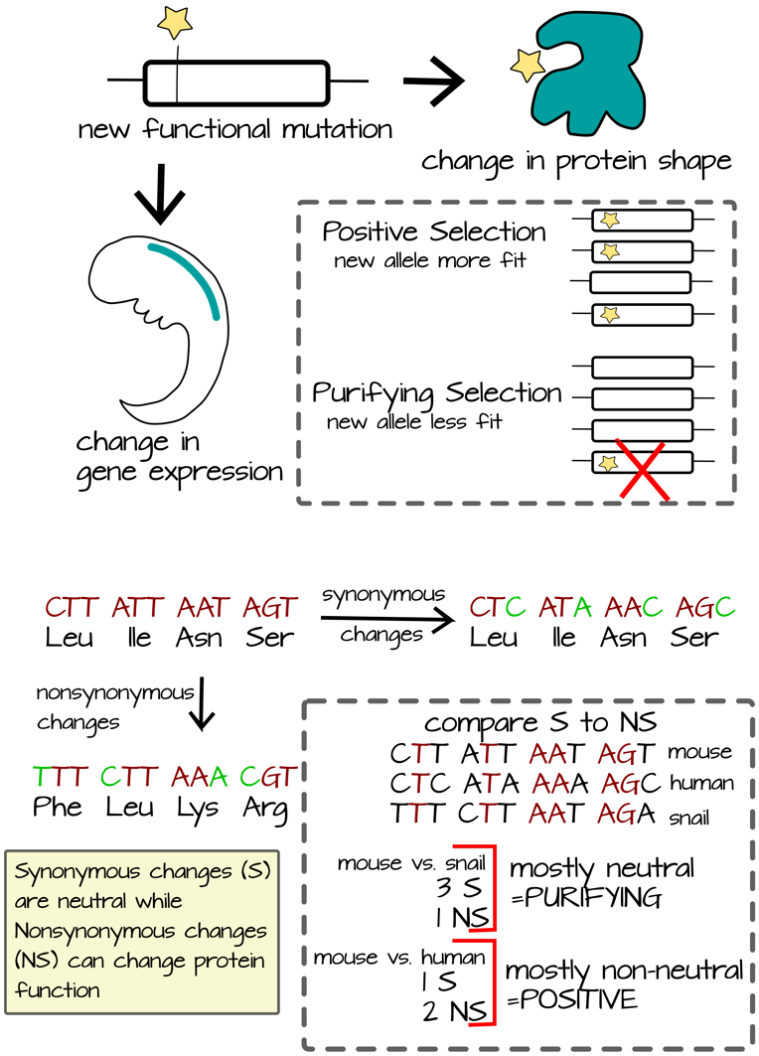
Step 2: Scan hybrid genomes for regions correlated with blight tolerance





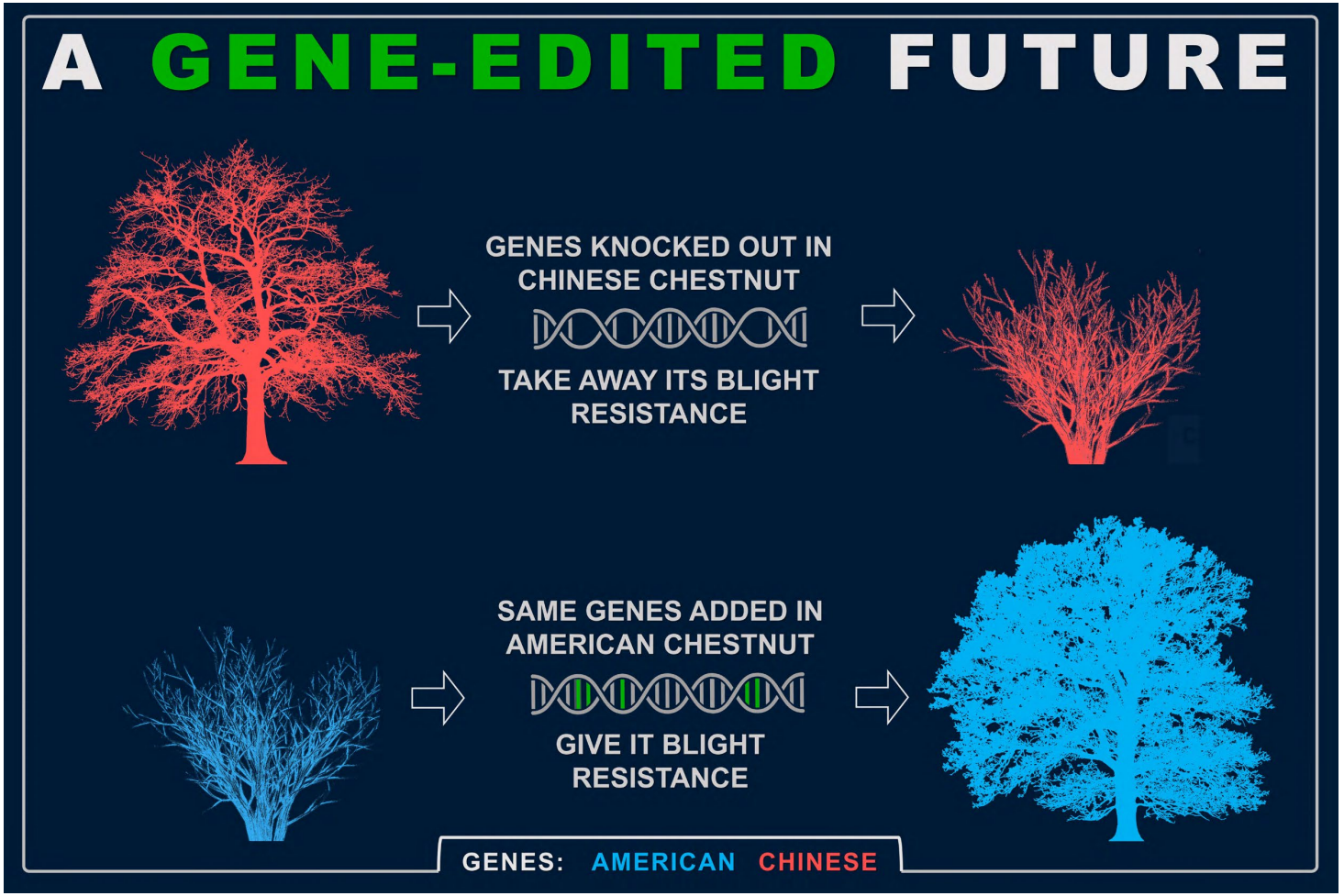
Step 3: Compare gene expression in Chinese chestnut and American chestnut stems after blight infection

Candidate genes: within QTL intervals and demonstrate signatures of selection in Chinese chestnut



https://scholarlycommons.pacific.edu/open-images/29/

Confirm candidate gene function in resistance with knockouts in Chinese chestnut?



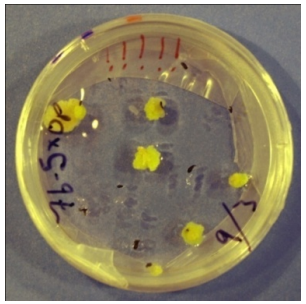
Infographic by Vasiliy Lakoba

Bottlenecks: Tissue culture and transformation

Embryogenesis and genetic transformation is slow, laborious, with differing success among genotypes of American chestnut

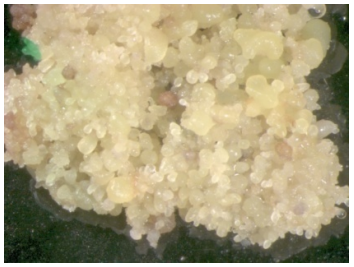
Seeds collected

4 months



Cultures started

4 months



"Captured" SE cultures

3 months

Somatic embryo production

5 months

Somatic seedlings in vitro

2 months

Somatic seedlings hardened-off to greenhouse

7 months

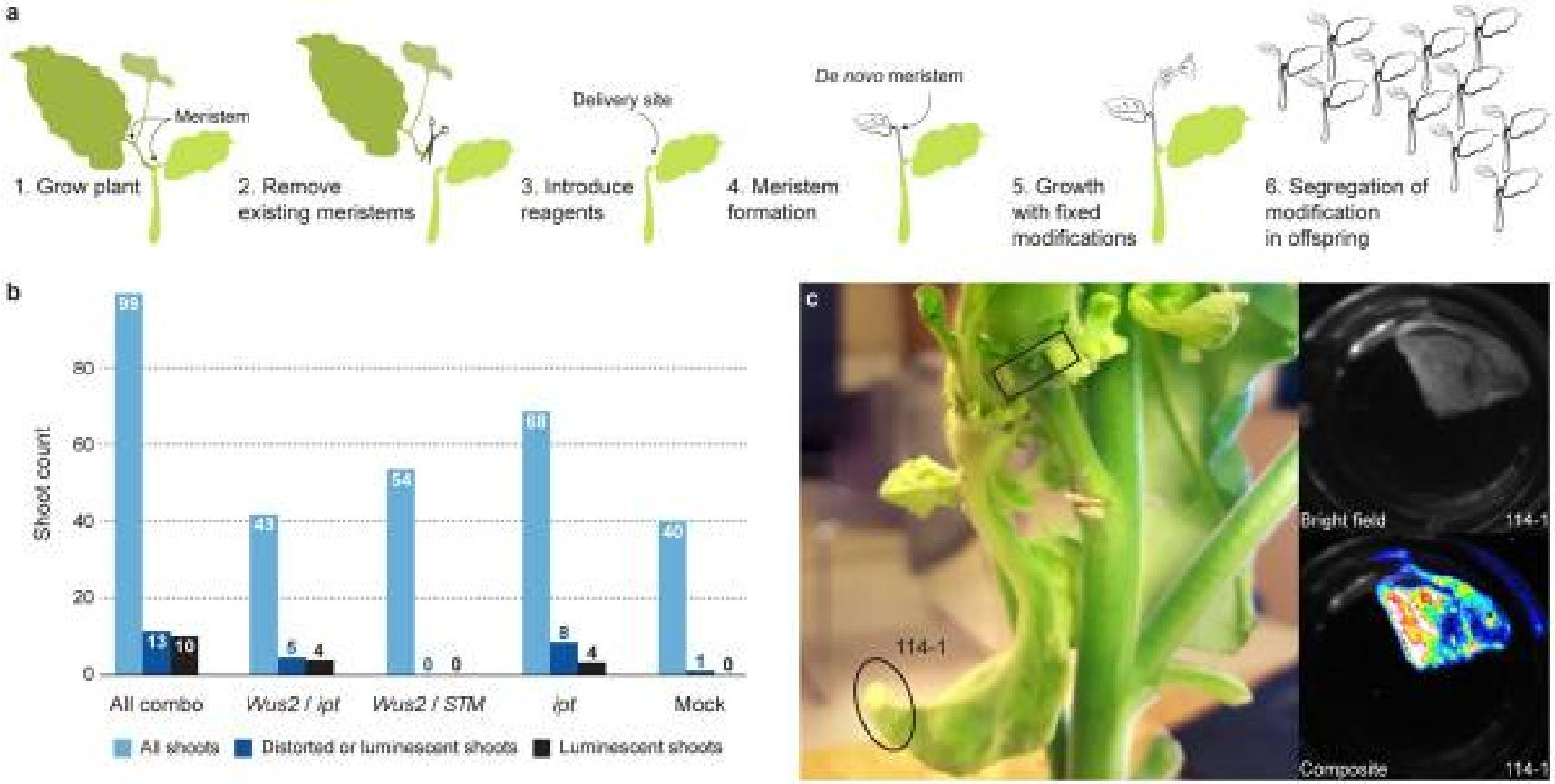
Somatic seedlings moved to shade house

6 months

Somatic seedlings planted

Slide from Scott Merkle

Bypass tissue culture bottleneck by inducing and transforming meristematic tissue?



Mahar et al. 2020. Nature Biotechnology 38(1). 84-89.

Bottlenecks: Measuring blight resistance

Assessing blight resistance in the field is a multiyear process

Main stem alive/dead

Presence/absence
Cankers > 15 long

Presence/absence
Exposed wood

Presence/absence
Sunken cankers

Presence/absence
Sporulation

A non-destructive early screening method for blight resistance?

Which cup to drink from?

Gene editing strategies:

- Knockouts of host susceptibility genes
- Change expression of resistance/susceptibility genes
- Host induced gene silencing
- Tandem insertions of resistance genes
- Base editing to find and replace American chestnut susceptibility with Chinese chestnut resistance

Bottlenecks to overcome:

- Resistance gene discovery/confirmation
- Tissue culture
- Measuring blight resistance
- How will the edited plants be regulated?