Weed Genetics Project Update

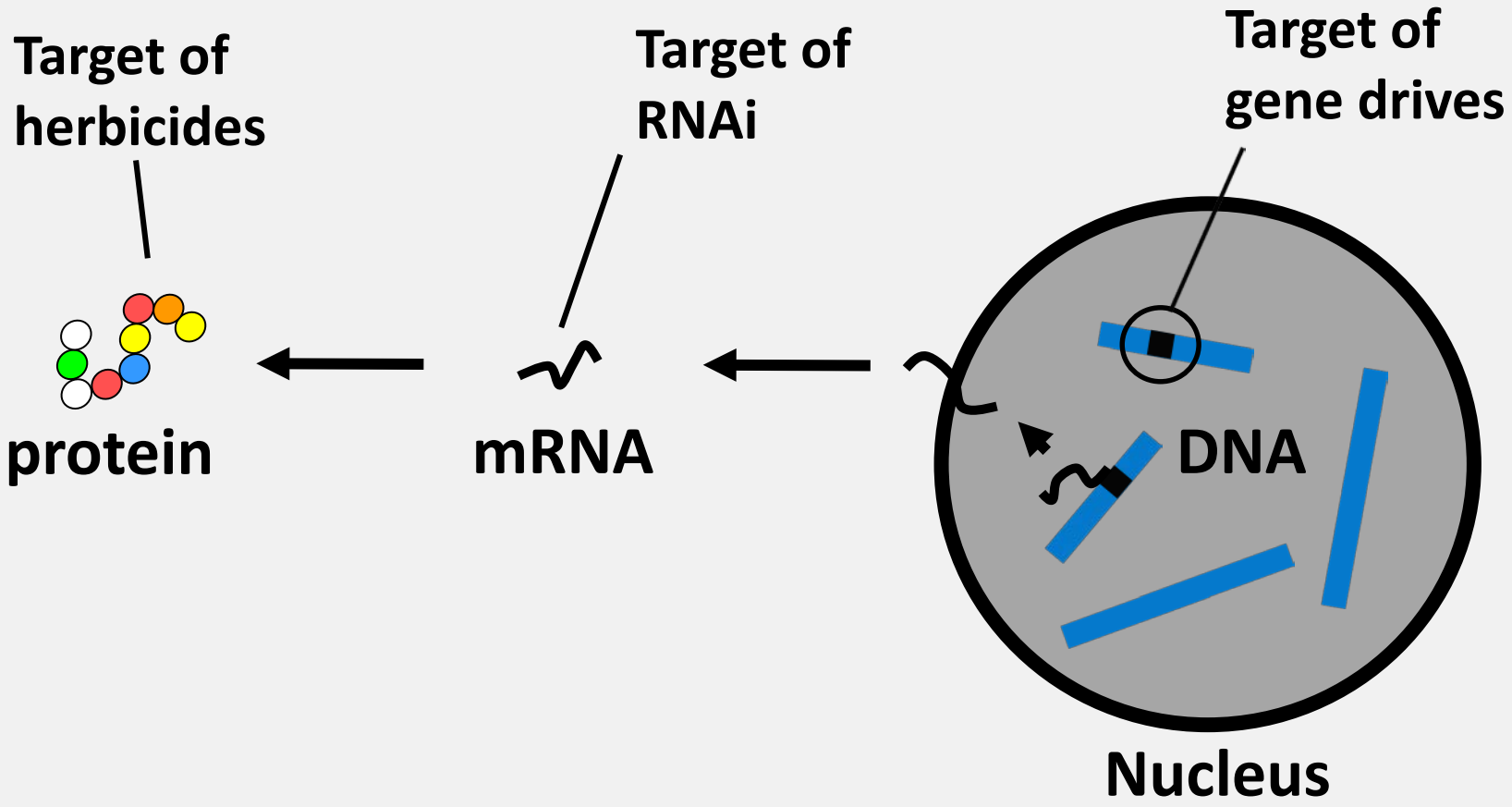
Michael Christoffers, Ph.D.

Department of Plant Sciences

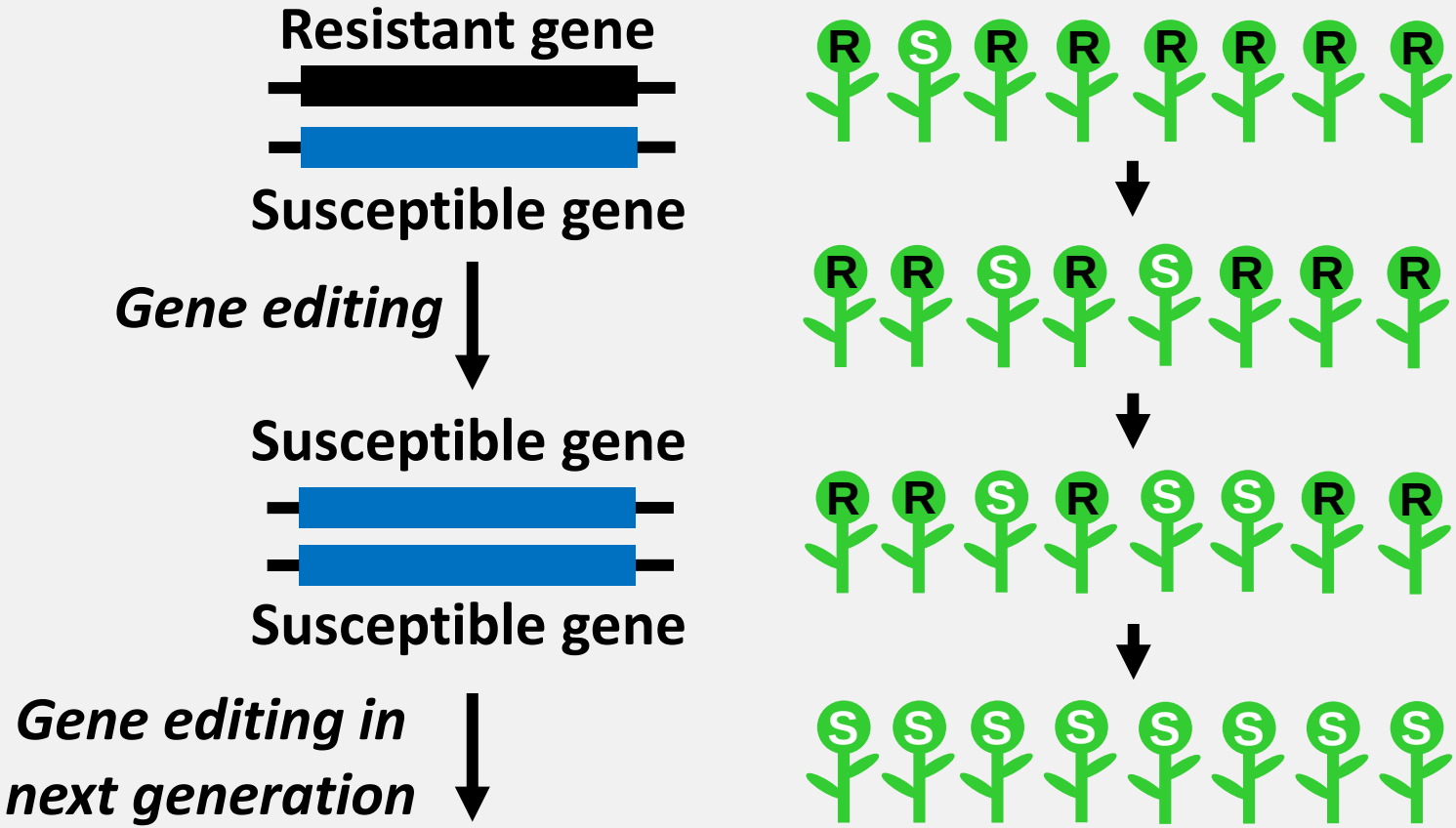
North Dakota State University

NDSU NORTH DAKOTA STATE UNIVERSITY

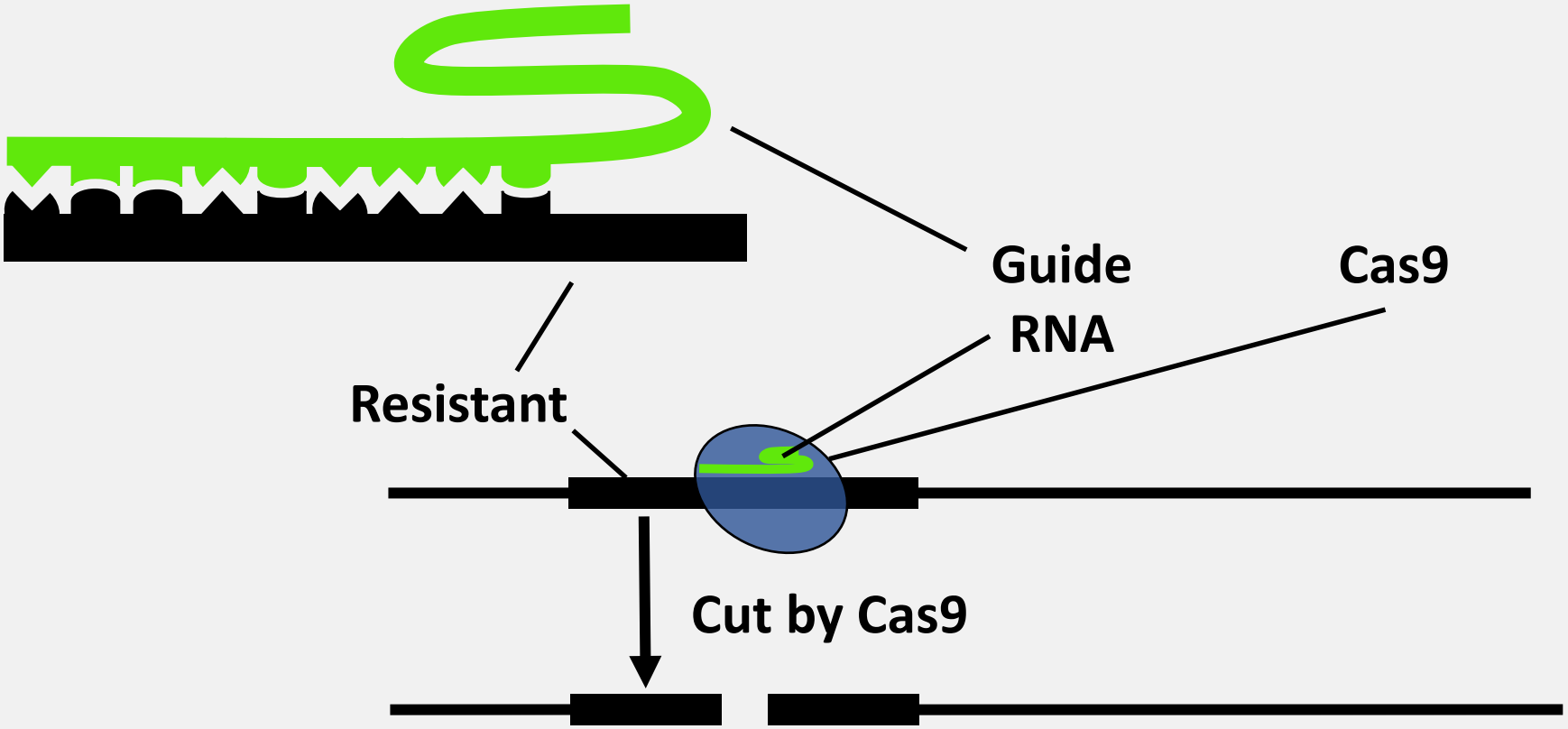
Genetic Biocontrol



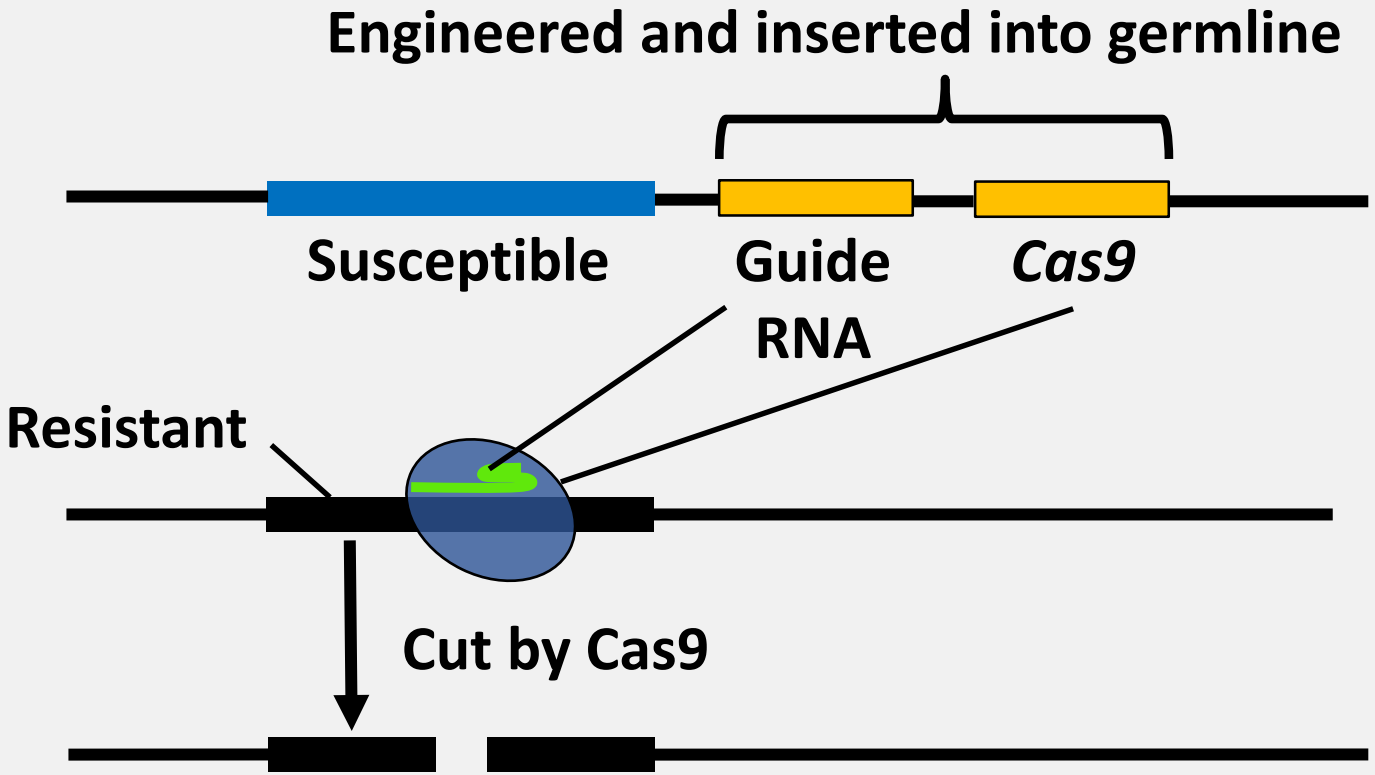
Example: Reversion of Pesticide Resistance



Example: Reversion of Pesticide Resistance



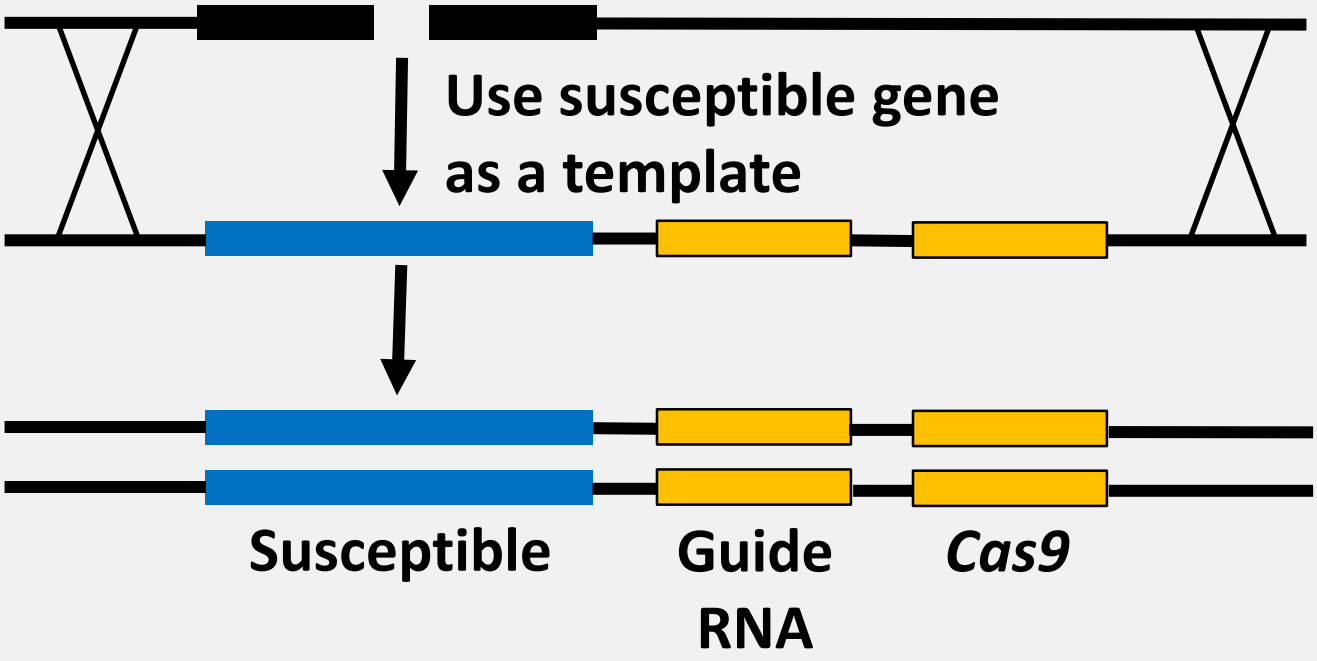
Example: Reversion of Pesticide Resistance



Example: Reversion of Pesticide Resistance

Natural repair of the cut, one of two possibilities:

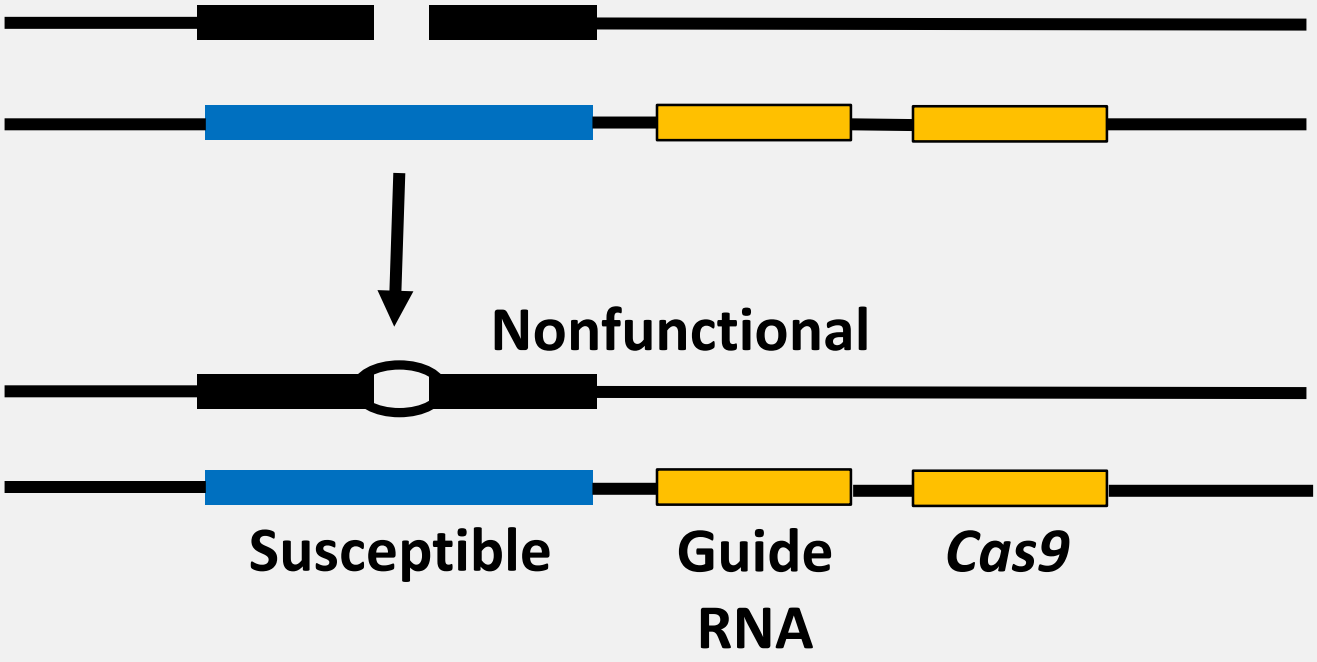
① Use susceptible gene as a template



Example: Reversion of Pesticide Resistance

Natural repair of the cut, one of two possibilities:

② Join the broken ends together (common in plants)



New Models for Plant/Weed Gene Drives

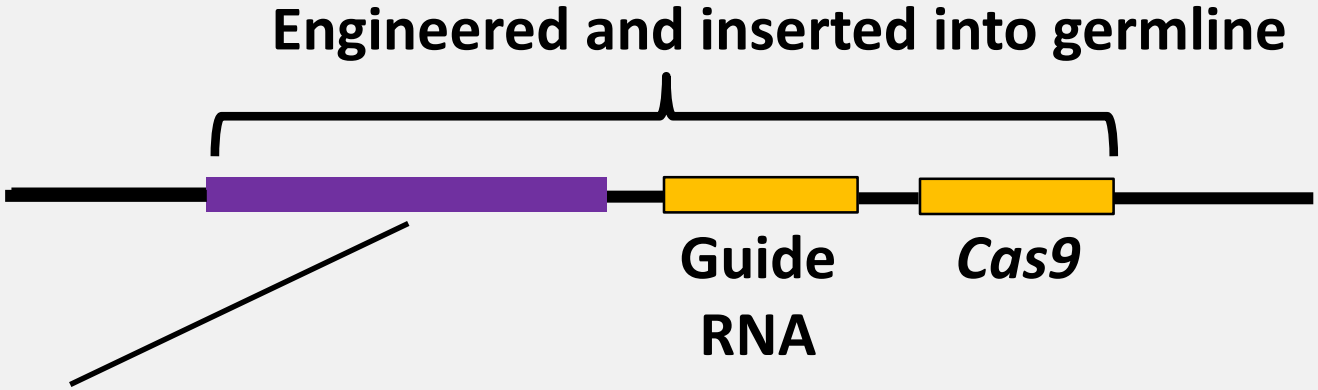
Toxin–Antidote [CRISPR-Assisted Inheritance utilizing *NPG1* (*CAIN*)]

- **Yang Liu et al., Chinese Academy of Sciences and Peking University**
- **Target gene: *NPG1* for pollen development in *Arabidopsis***
- ***Nature Plants* 10:910–922 (2024)**

Cleave and Rescue (*ClvR*)

- **Georg Oberhoffer et al., California Institute of Technology**
- **Target gene: *YKT61* for pollen and ovule development in *Arabidopsis***
- ***Nature Plants* 10:936–953 (2024)**

New Models for Plant/Weed Gene Drives



Functional version of target gene but <u>not recognized by guide RNA</u>

Nonfunctional

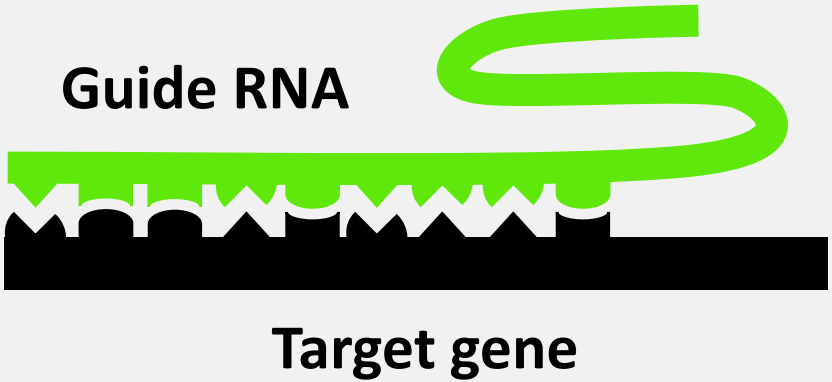
\+

Functional (plant is “rescued”)

Critical Research

Specific and efficient targeting of gene by guide RNA is critical

- **In our research, the target gene is waterhemp acetolactate synthase (*ALS*)**



Editing of the ALS Gene of Yeast

Herbicide susceptible

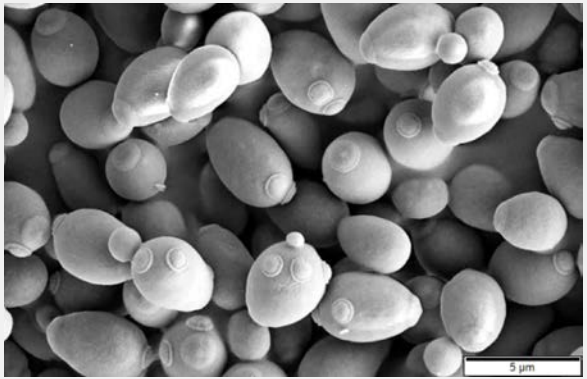
Herbicide resistant

Tryptophan

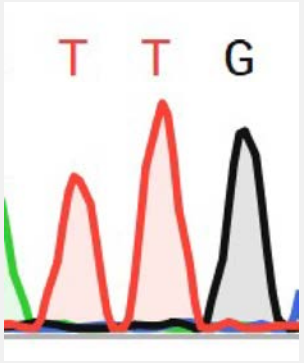
Leucine

T G G

Gene editing

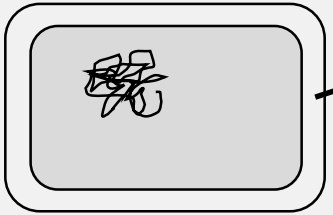


Saccharomyces cerevisiae, SEM image by MD Murtey and P Ramasamy [CC BY 3.0], via Wikimedia Commons

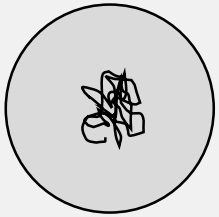


Peter Beerbower, Ph.D. research

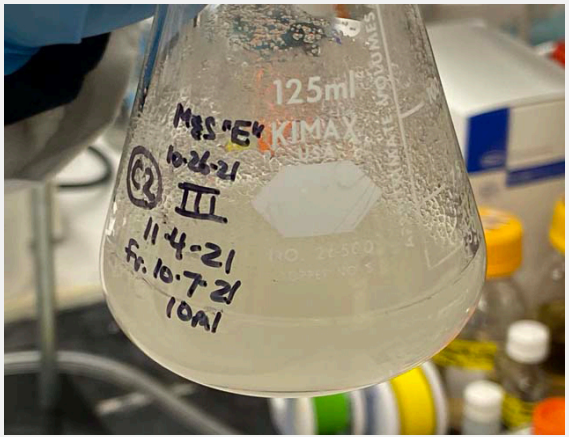
Production of Waterhemp Protoplasts



Cell wall

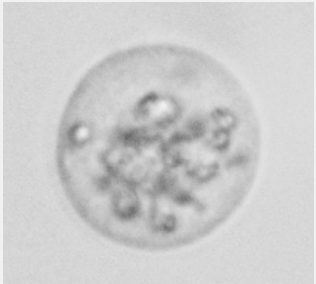


Protoplast



Waterhemp cell culture

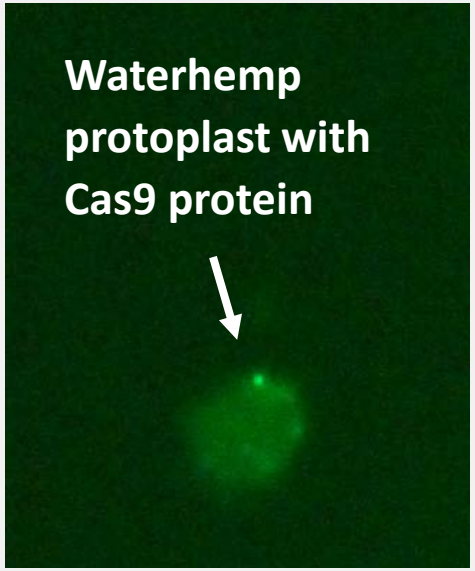
Degrade (remove) cell walls



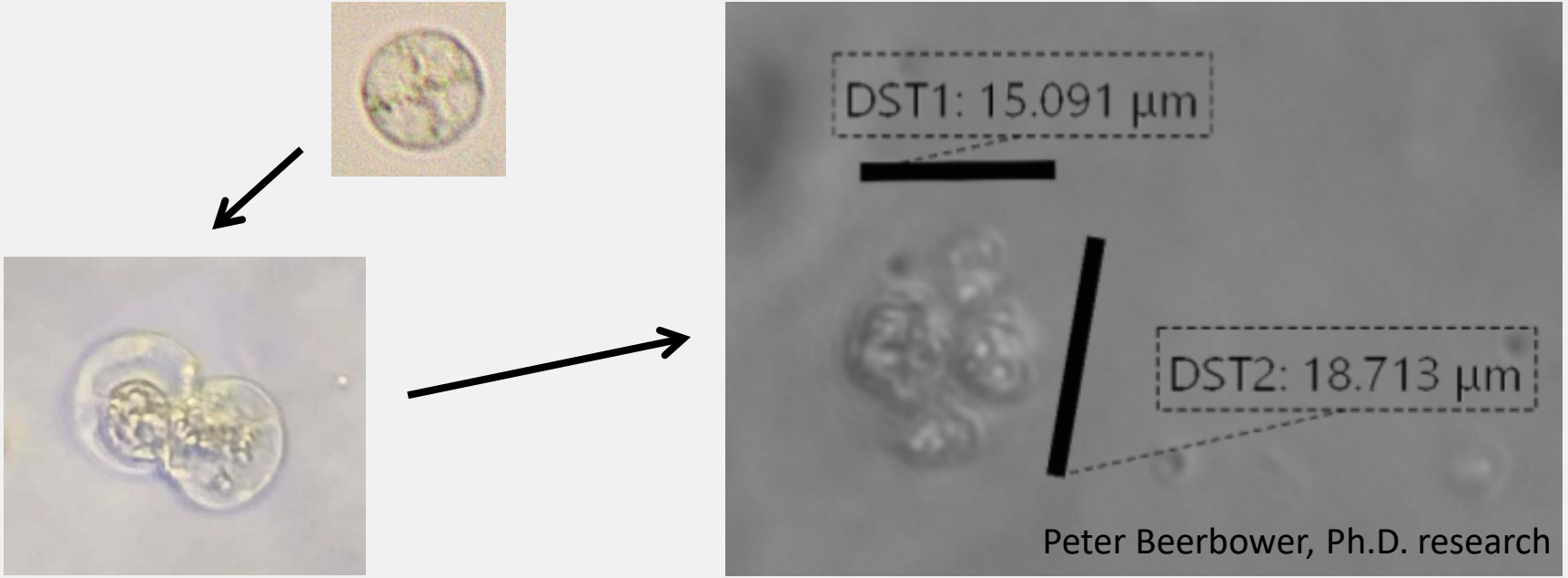
Waterhemp protoplast

Targeting Waterhemp *ALS*

- 1 of 4 tested guide RNAs successfully targeted and cut *ALS* in isolated waterhemp DNA
- We did not achieve successful targeting and repair of *ALS* in waterhemp protoplasts
- Waterhemp protoplasts also would not express a transgene and would not divide, likely because they were under oxidative stress



Breakthrough: Waterhemp Protoplast Cell Division



We have achieved waterhemp protoplast recovery and cell division by treating cells with 2-aminoindane-2-phosphonic acid (AIP)

Next Steps

- **Evaluate transgene expression and targeting of *ALS* in waterhemp protoplasts treated with AIP**
- **Achieve continued cell division beyond just a few divisions**
- **Extend research to Palmer amaranth**

Acknowledgments

- Robert Sabba and Peter Beerbower
- ND Agricultural Experiment Station
- ND Corn Utilization Council
- ND Soybean Council
- ND State Board of Agricultural Research and Extension – Soybean
- NDSU RCA Research Support Grant

- This research was supported, in part, by the intramural research program of the U.S. Department of Agriculture, National Institute of Food and Agriculture, Hatch project accession number, 7006983. The findings and conclusions in this preliminary presentation have not been formally disseminated by the U.S. Department of Agriculture and should not be construed to represent any agency determination or policy.