Towards Transgene-Free Genome Editing in Onion

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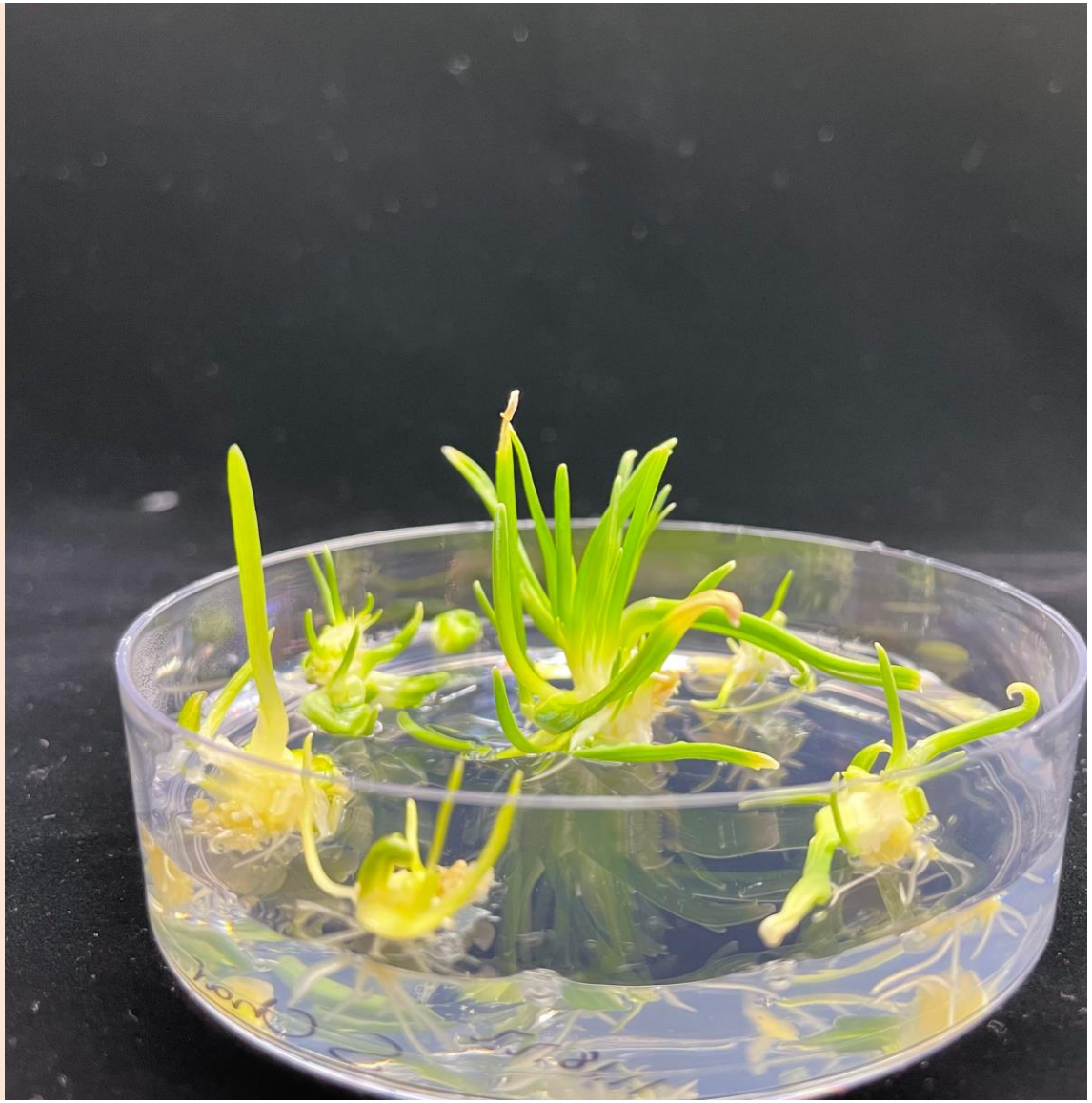
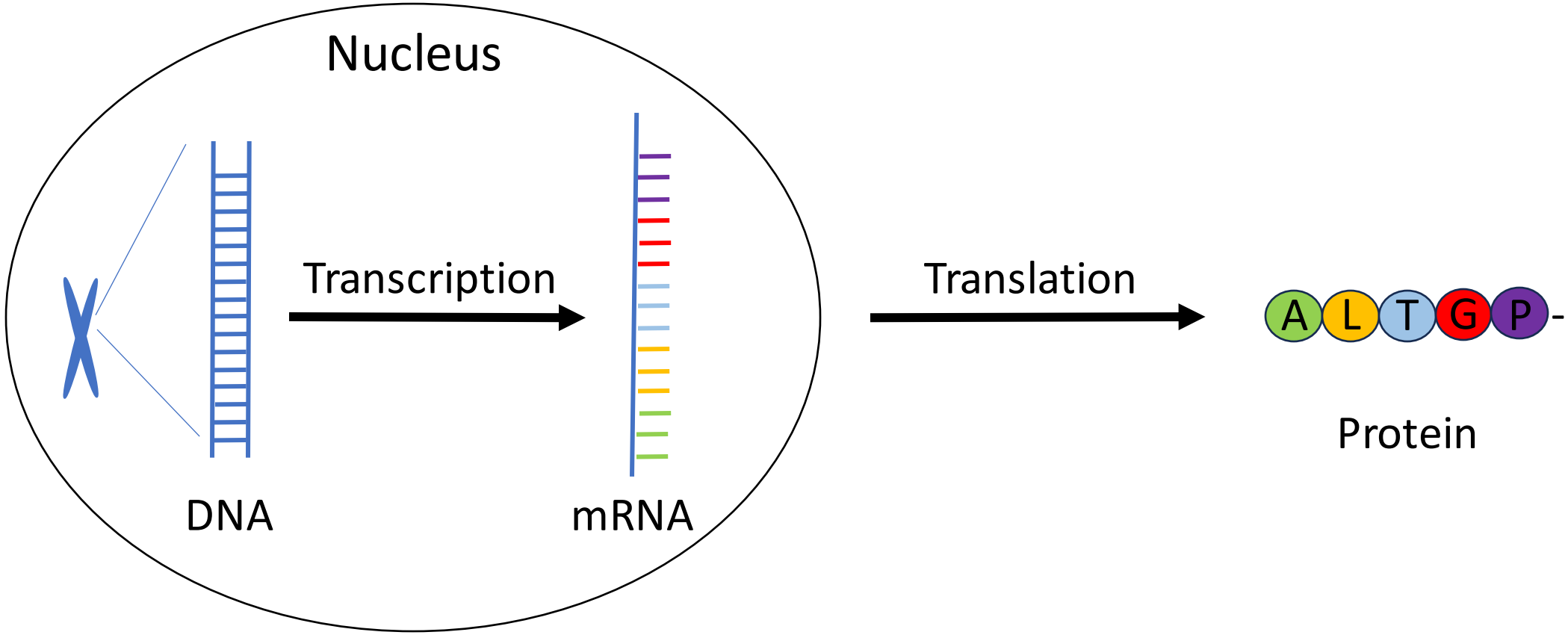


Preview

1. Genetics review
2. Gene editing
3. Results

Part 1: Genetics

Central Dogma



Change DNA → Change protein

Part 2: CRISPR Gene Editing

Gene editing checklist

STEP 1: DEVELOP PLANT TISSUE CULTURE SYSTEM

STEP 2: PERFORM GENE EDITING IN LIVING CELLS

STEP 3: REGENERATE EDITED CELLS INTO PLANTS

Havey, M. J. (2020). Genetic Mapping of Chartreuse Bulb Color in Onion. *Journal of the American Society for Horticultural Science J. Amer. Soc. Hort. Sci.*, *145*(2), 110-119

Chartreuse onion

Chartreuse onions

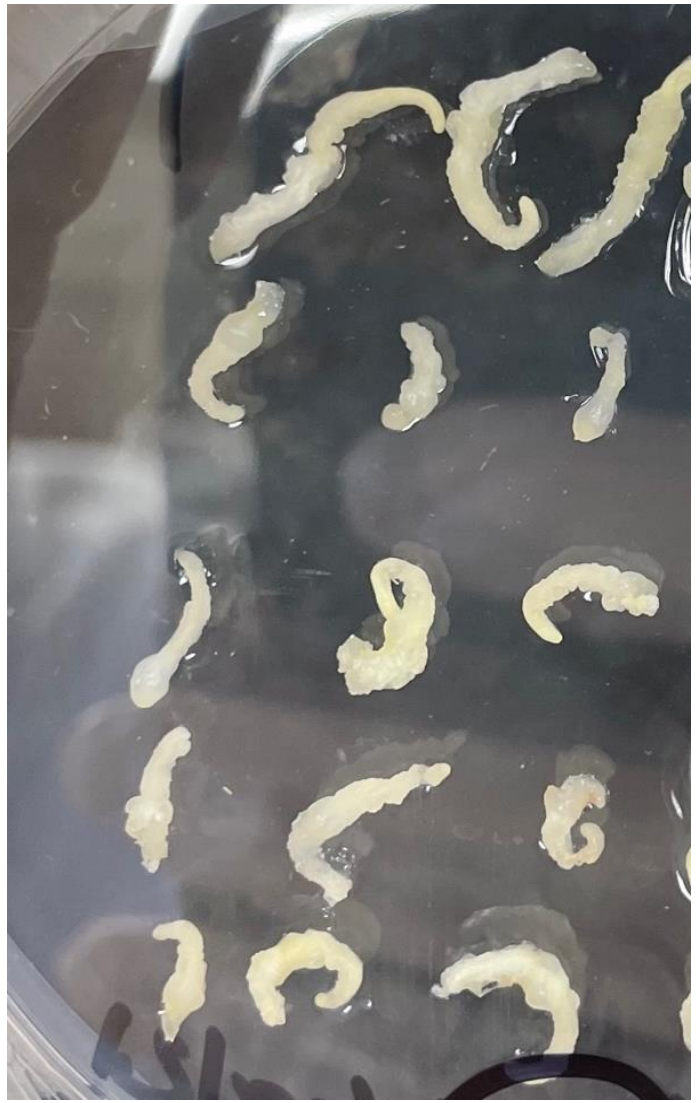
grown in greenhouse

Immature embryos

surgically removed ~28 days after pollination

1 week old embryos

Callus induction

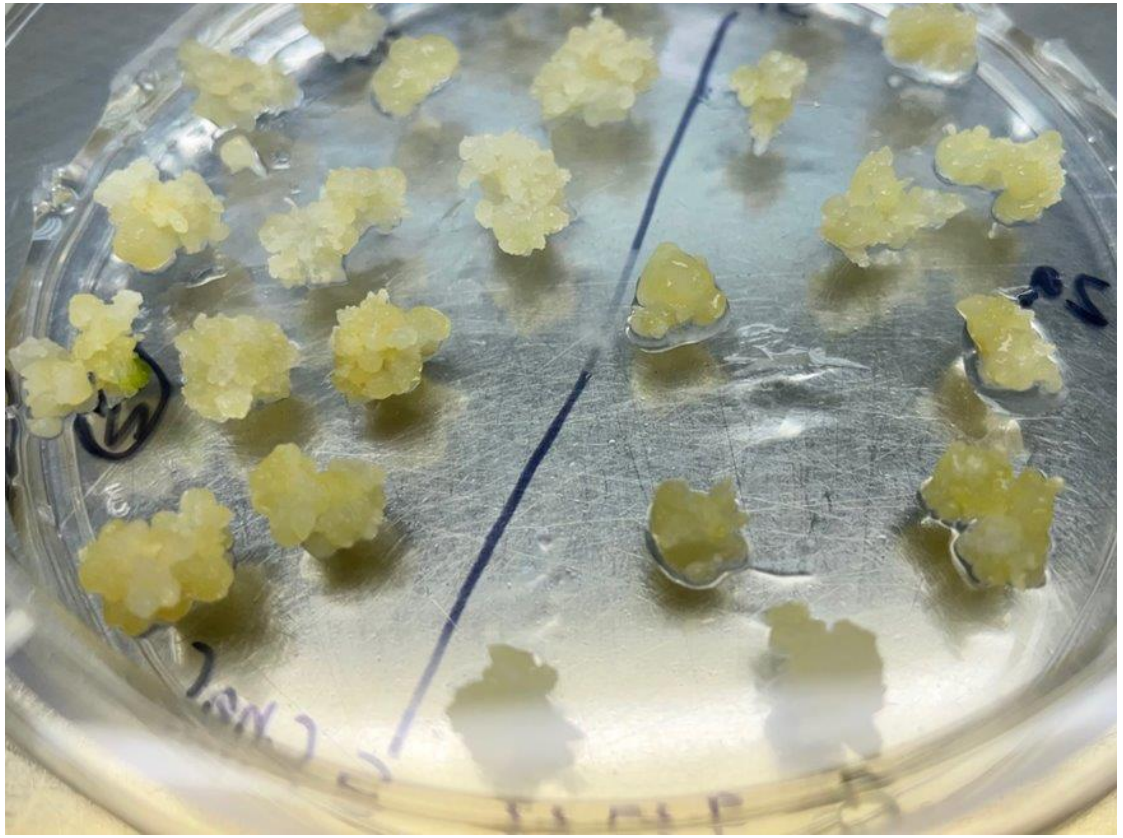


4 weeks old

Friable callus

5-6 weeks old

Shoot induction media

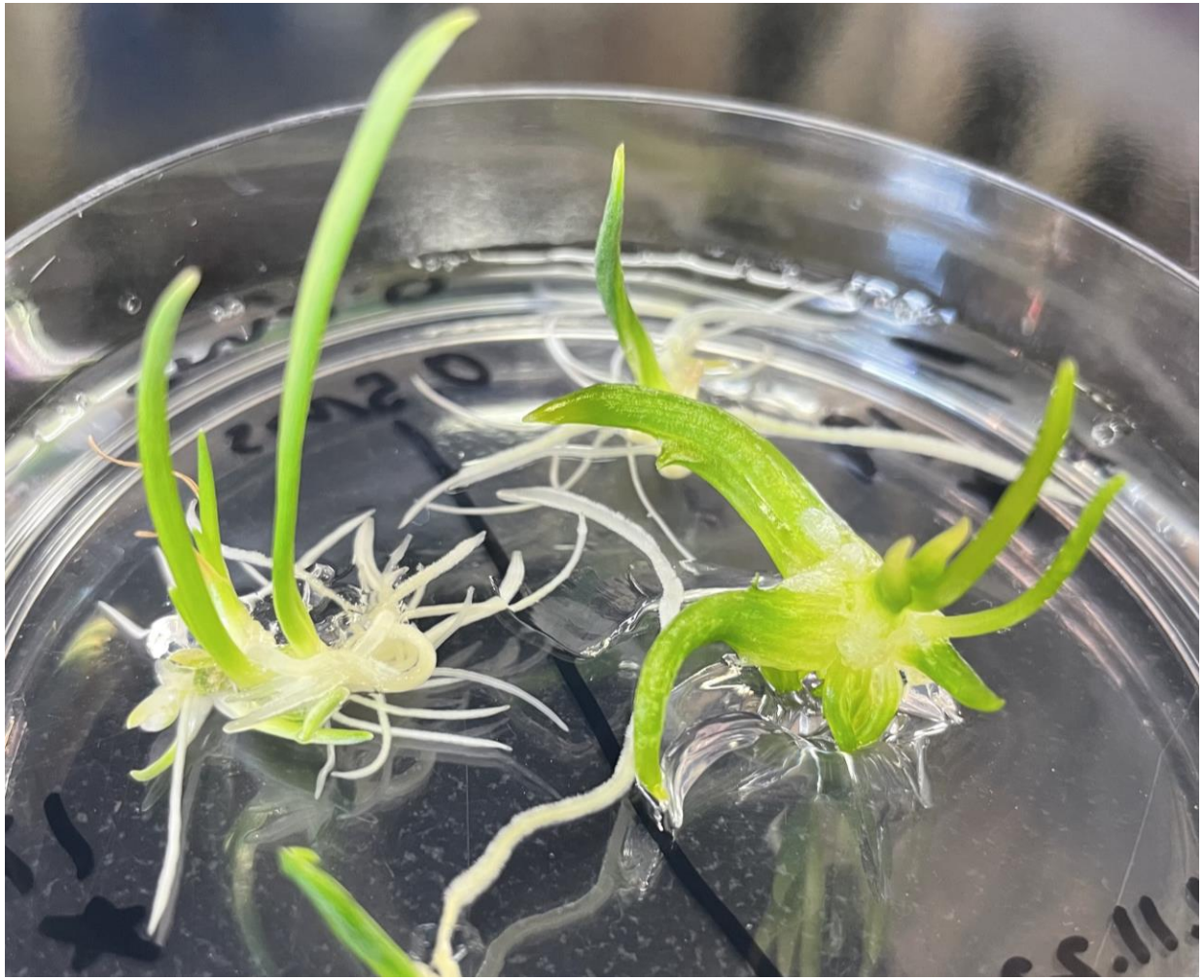


7-8 weeks

Shoot production

Plants
ready for soil!

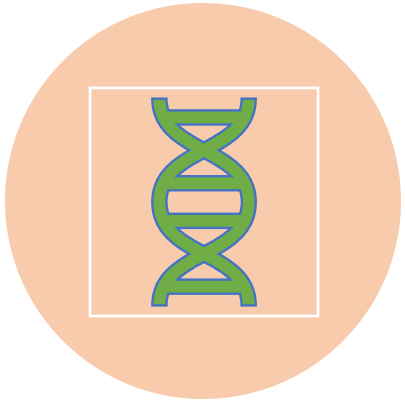
2.5-3 months



Gene editing checklist

STEP 1: DEVELOP PLANT TISSUE CULTURE SYSTEM

✓



STEP 2: PERFORM GENE EDITING IN LIVING CELLS

STEP 3: REGENERATE EDITED CELLS INTO PLANTS

JOURNAL ARTICLE

Insights from the first genome assembly of Onion (*Allium cepa*)

Richard Finkers ✉, Martijn van Kaauwen, Kai Ament, Karin Burger-Meijer, Raymond Egging, Henk Huits, Linda Kodde, Laurens Kroon, Masayoshi Shigyo, Shusei Sato ... Show more

G3 Genes|Genomes|Genetics, Volume 11, Issue 9, September 2021, jkab243,
https://doi.org/10.1093/g3journal/jkab243

Published: 13 July 2021 **Article history** ▾

Gene editing mechanism

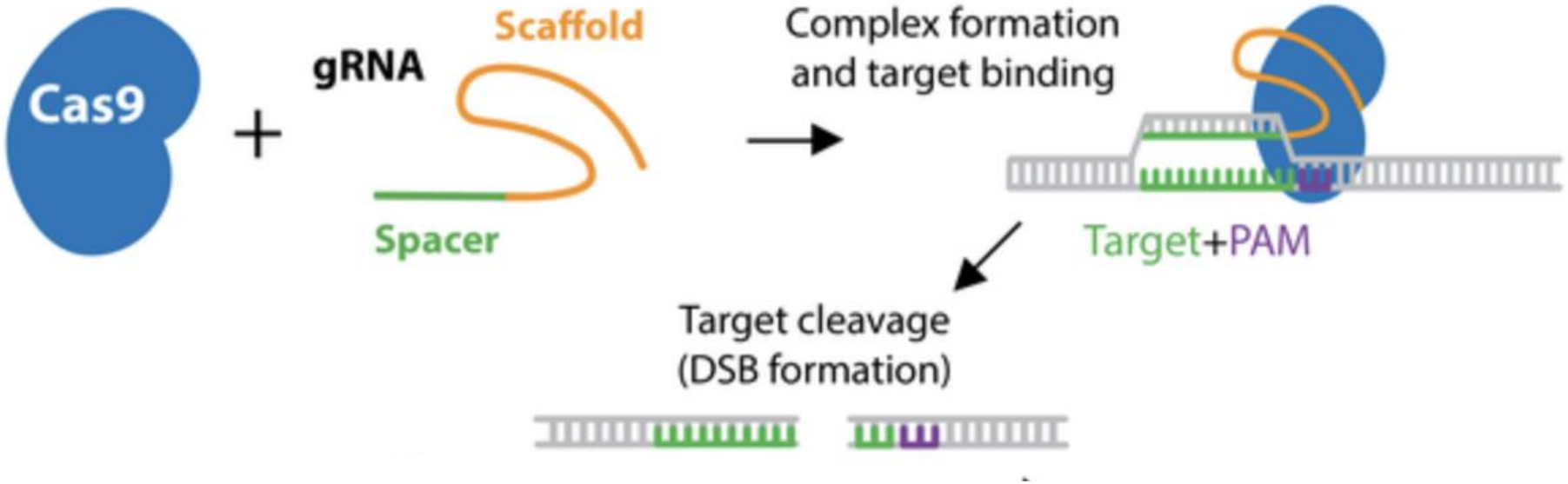


Image from Addgene.com

Gene editing mechanism

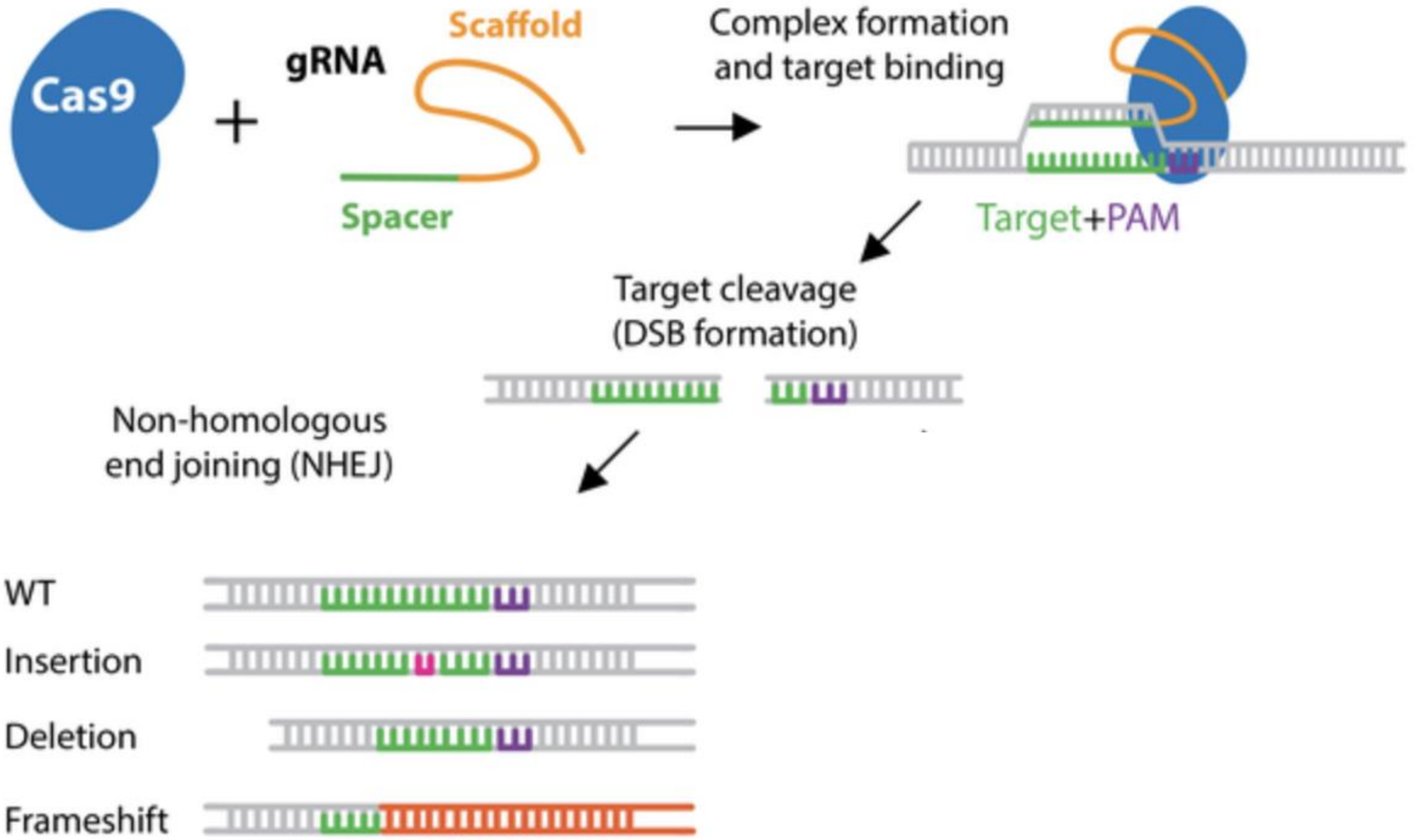


Image from Addgene.com

Gene editing mechanism

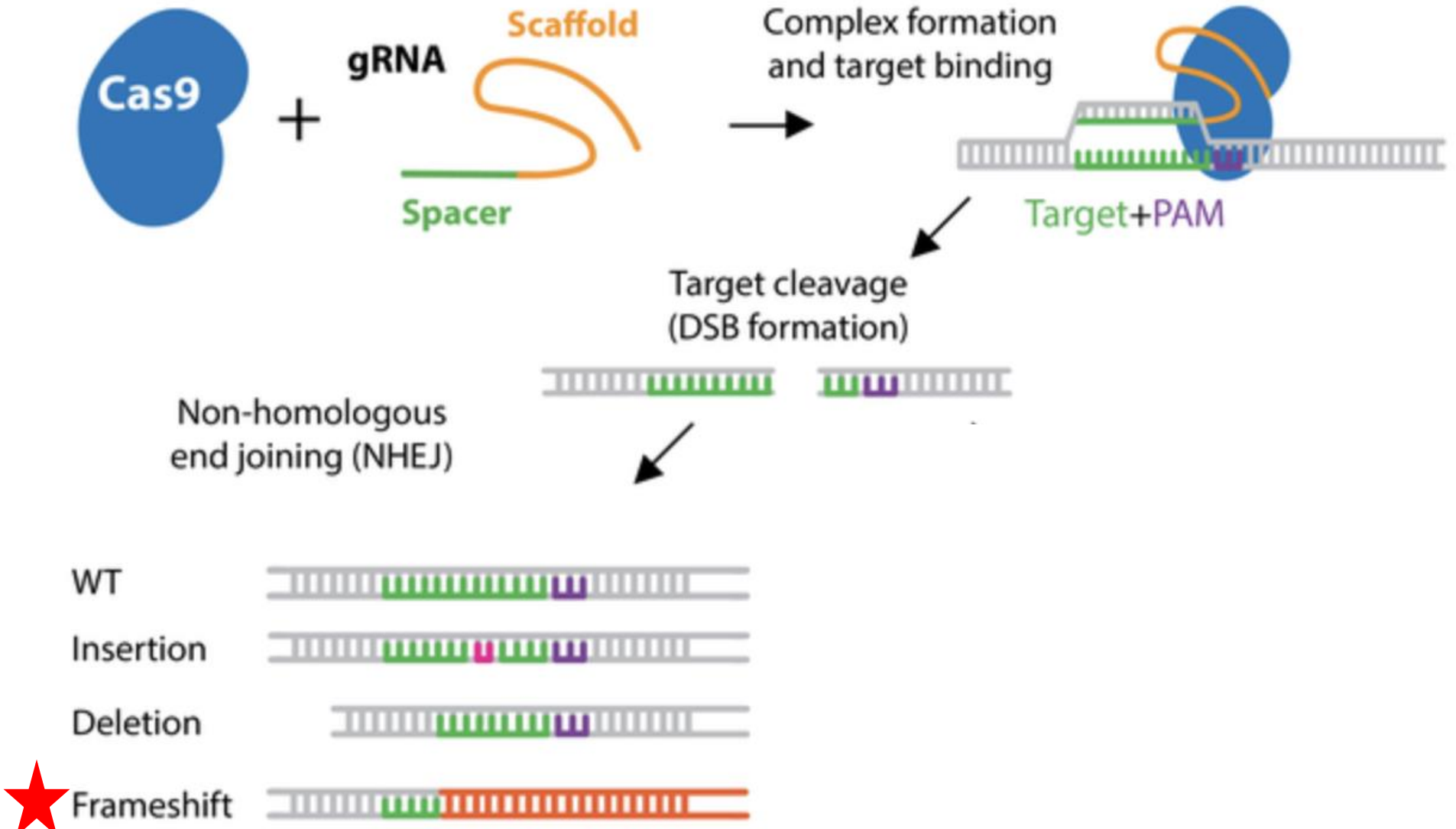


Image from Addgene.com

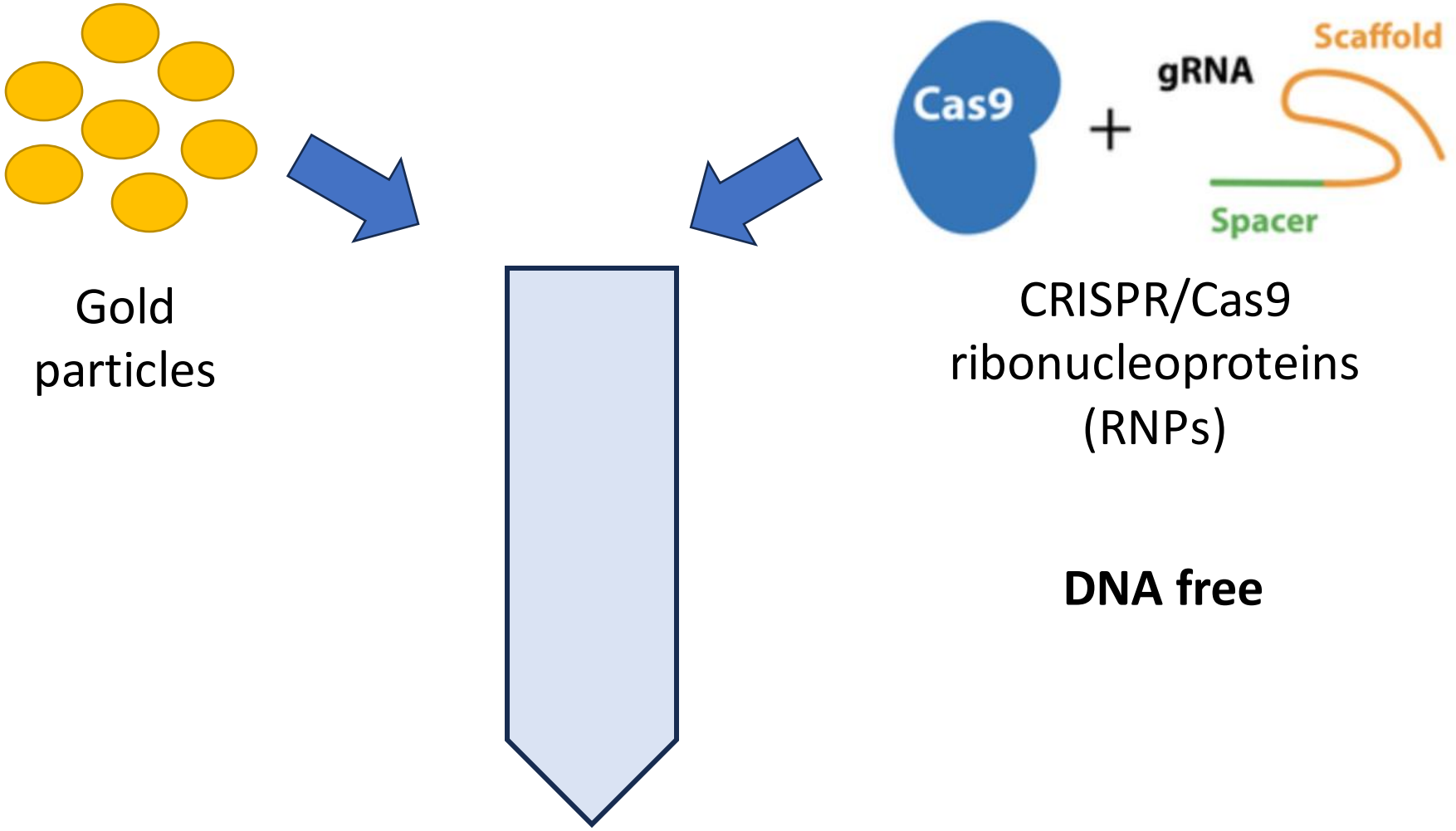
Gene gun – Biolistic Delivery

Sanford, J.C. The development of the biolistic process. *In Vitro Cell.Dev.Biol.-Plant* **36**, 303–308 (2000)

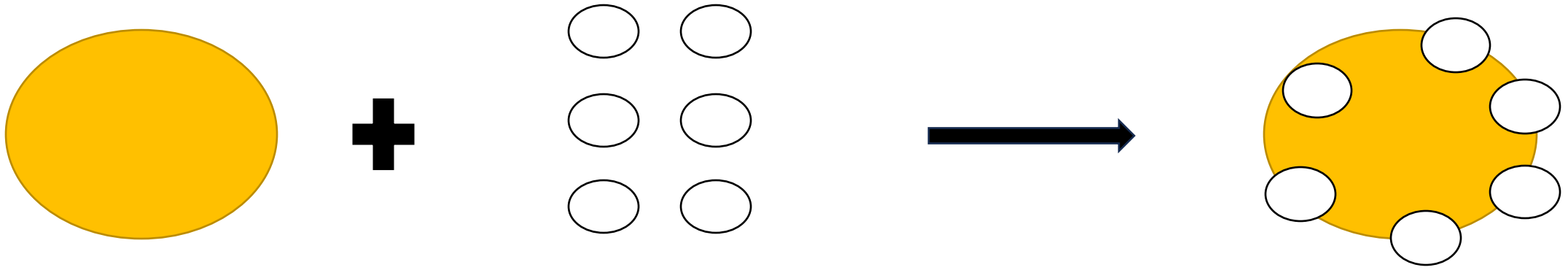
John C. Sanford Describing Early Research

I took it back to my lab, along with the tungsten particles, and began to experiment with onion. Through a series of experiments I was able to show that onion cells could survive particle penetration, and that foreign material, including DNA, could be delivered into such living cells (Sanford et al., 1987).

Prepping components for delivery



RNPs adhere to gold



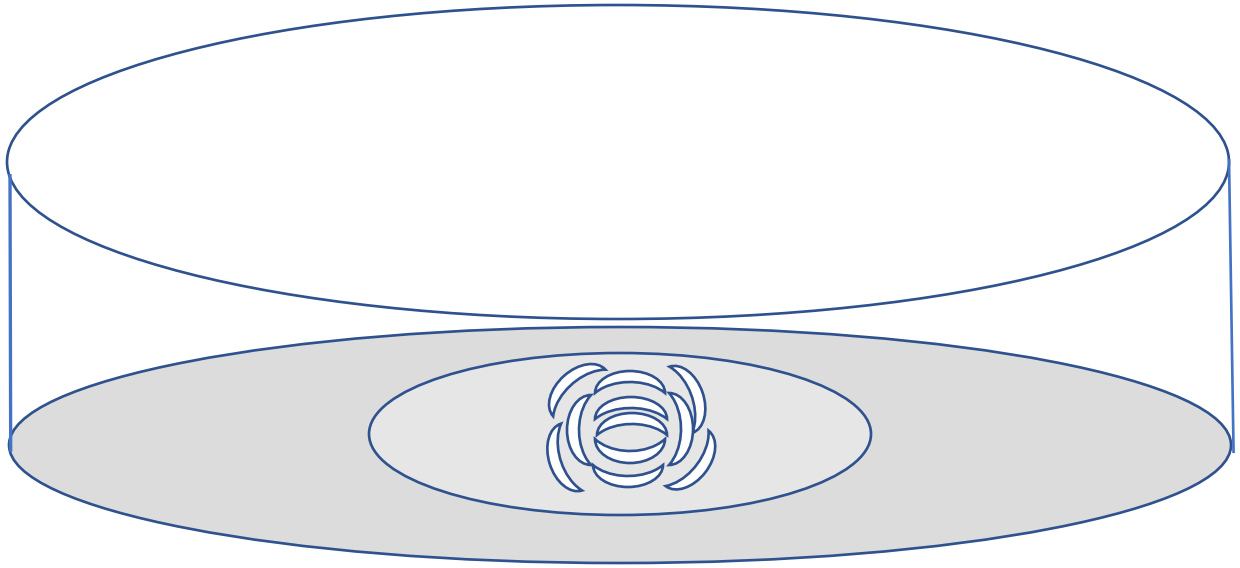
Gold particle

RNPs
(Cas9 + guide complex)

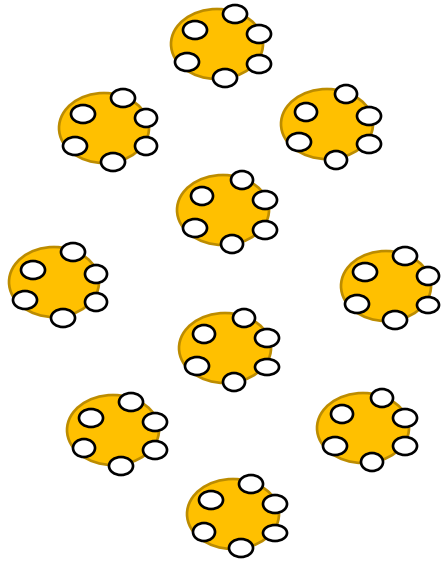
RNP coated particle

Immature embryos

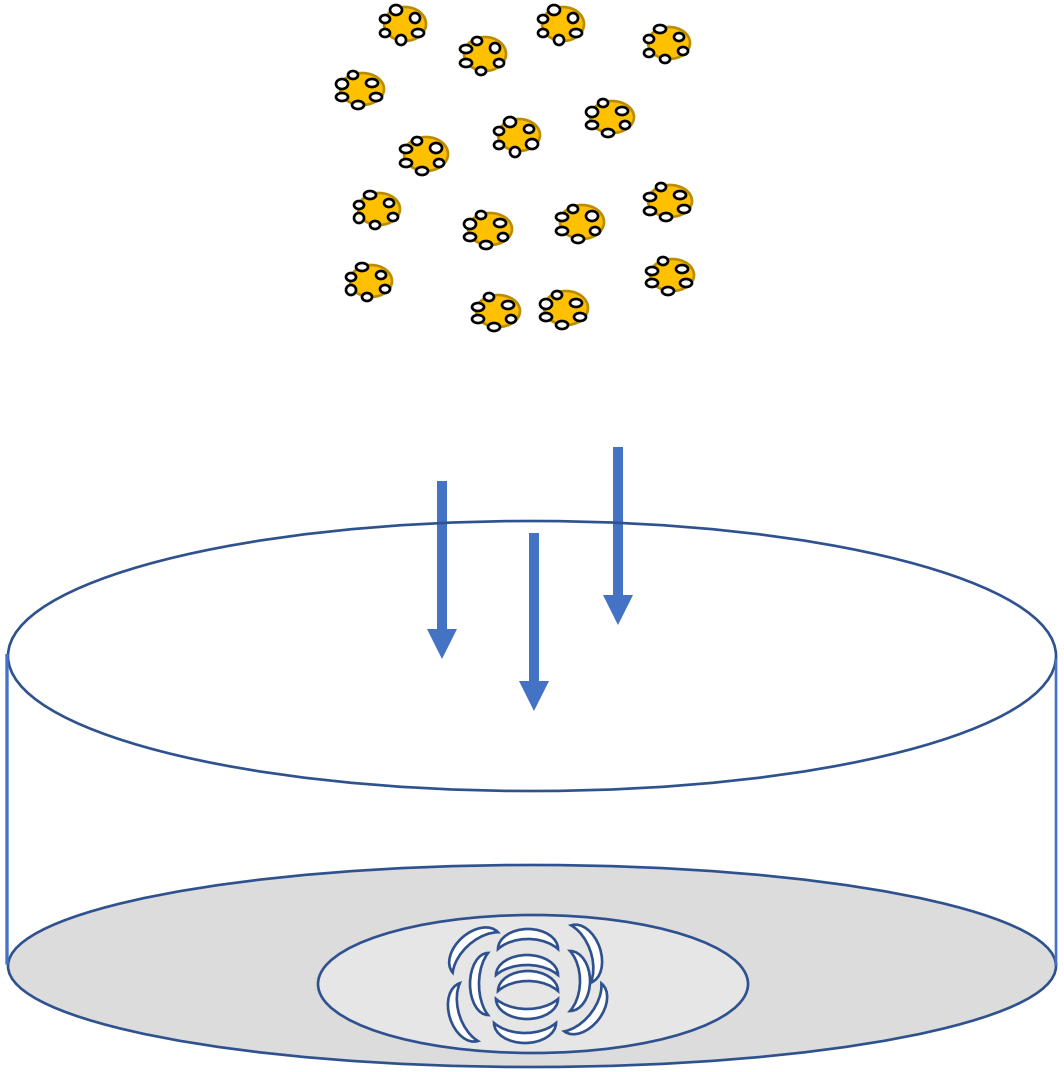
Immature Embryos



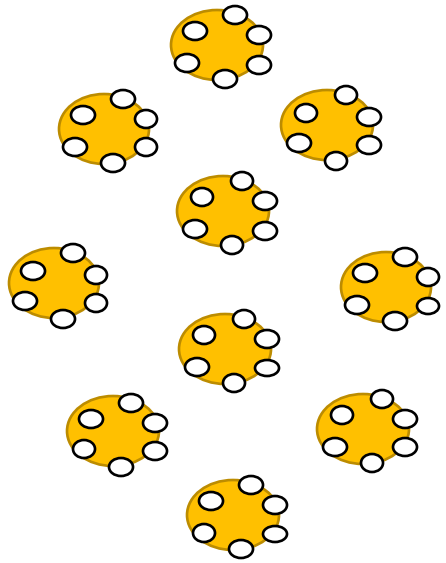
RNP Delivery- Particle bombardment



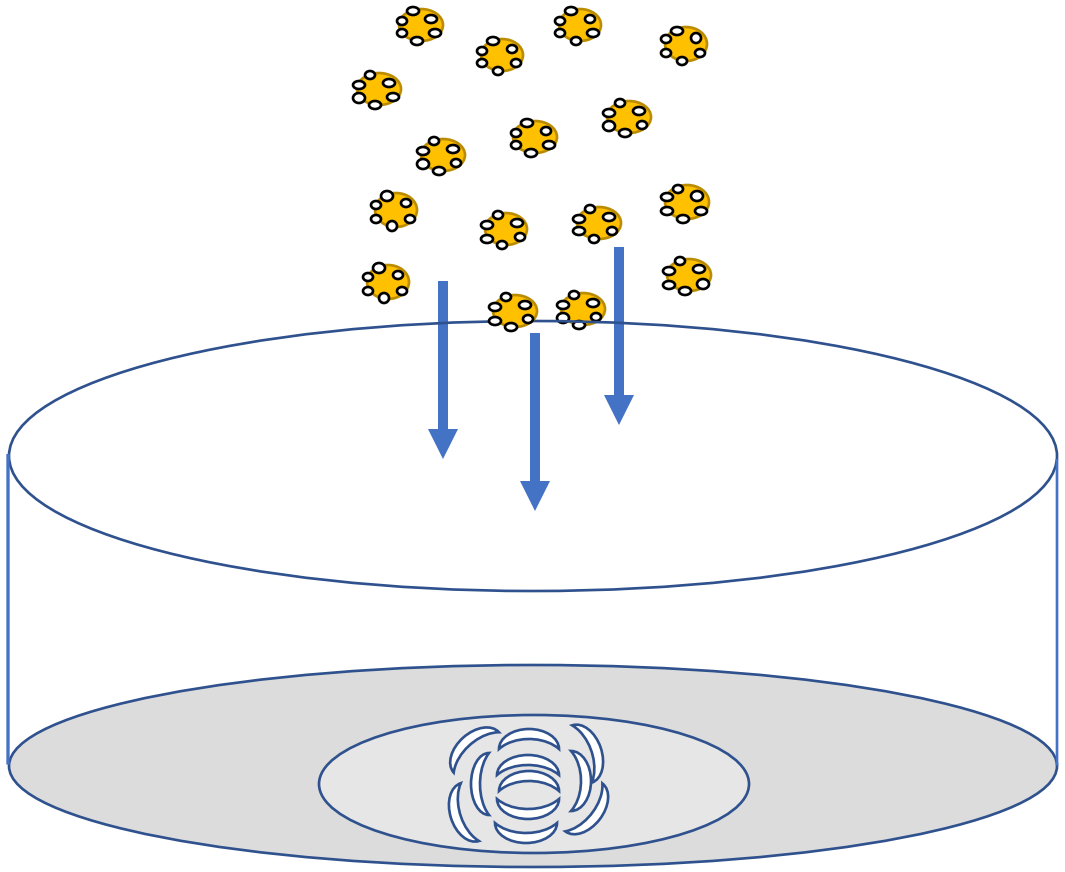
RNP coated gold particles



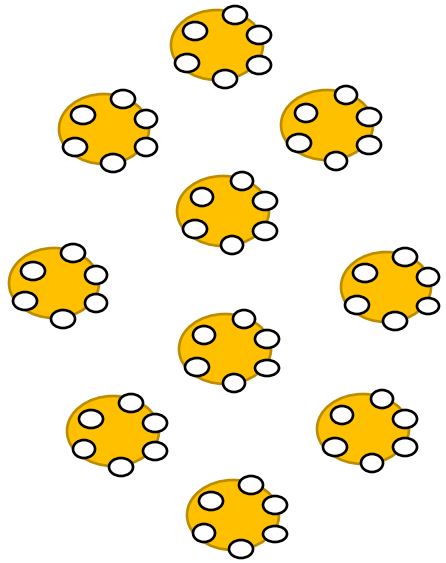
RNP Delivery- Particle bombardment



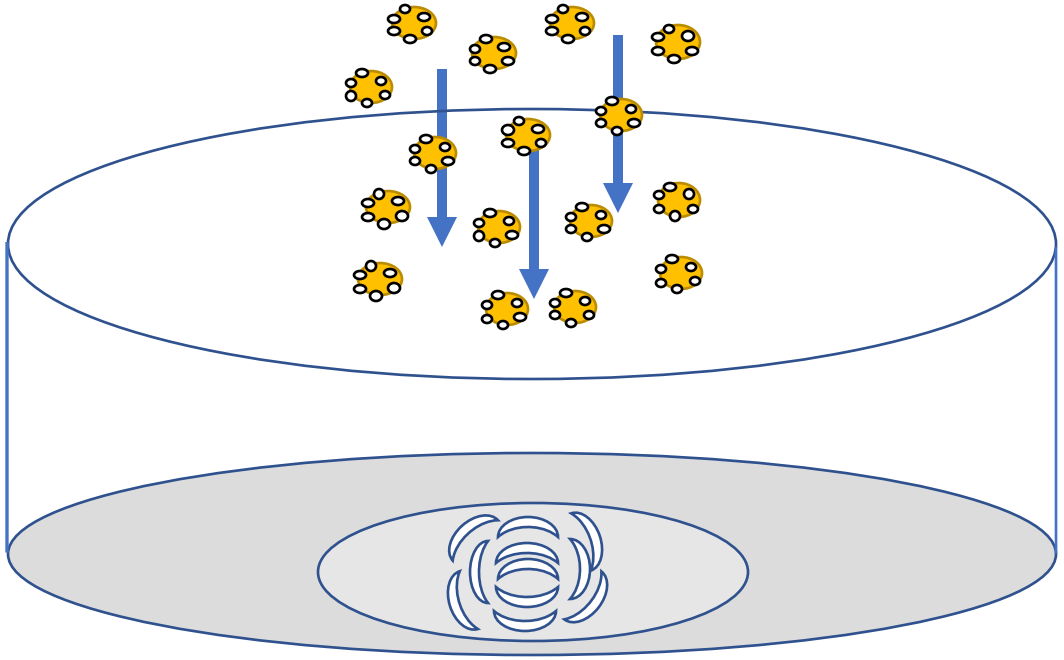
RNP coated gold particles



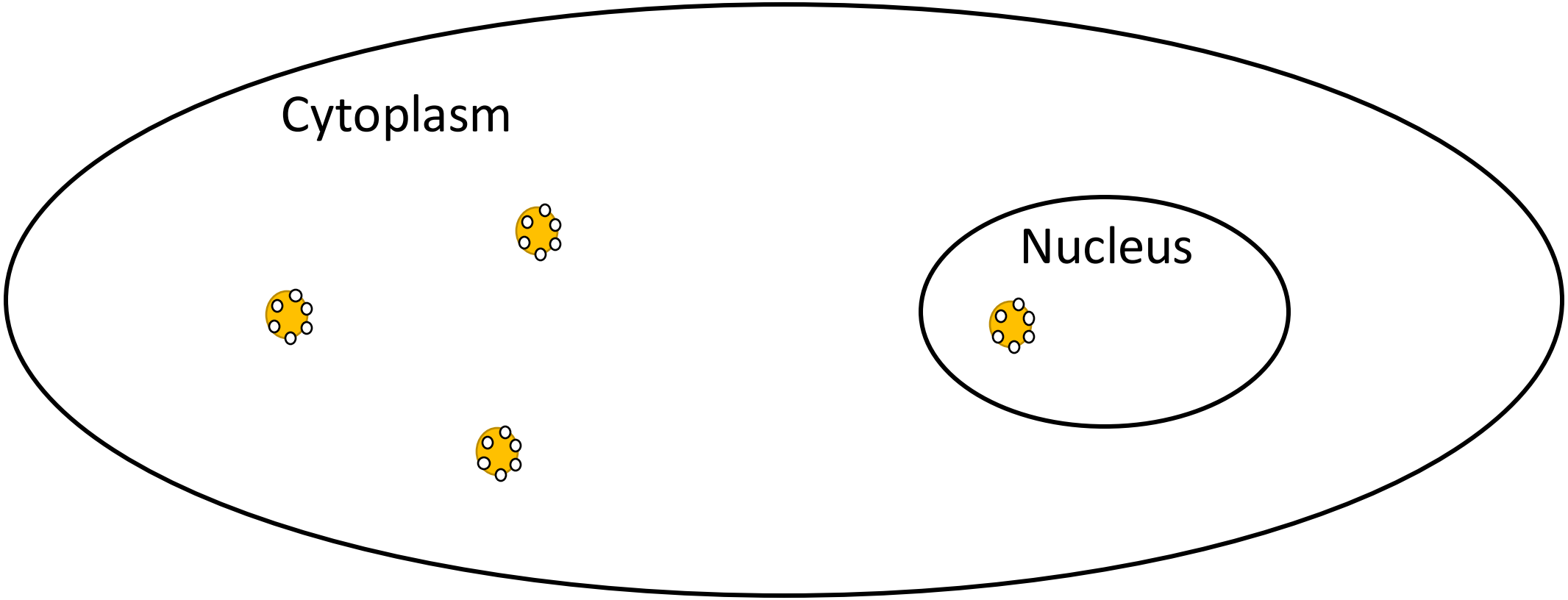
RNP Delivery- Particle bombardment



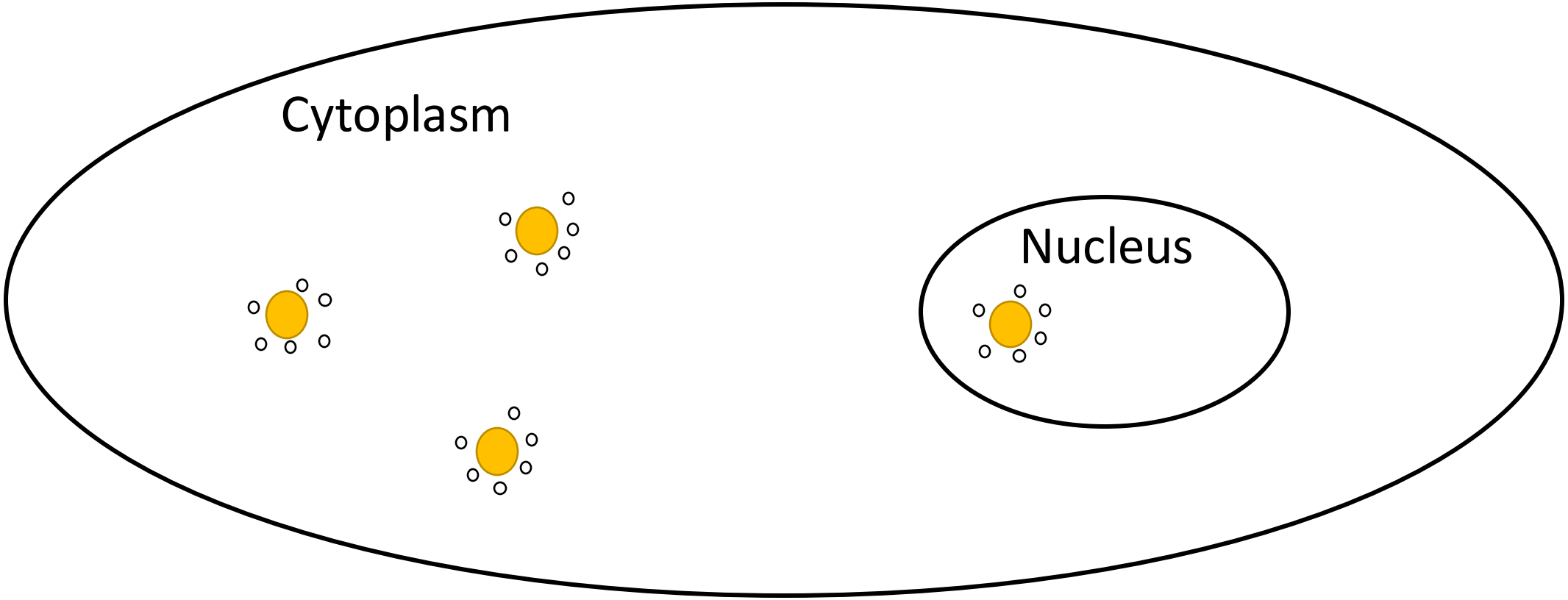
RNP coated gold particles



Bombarded Cell

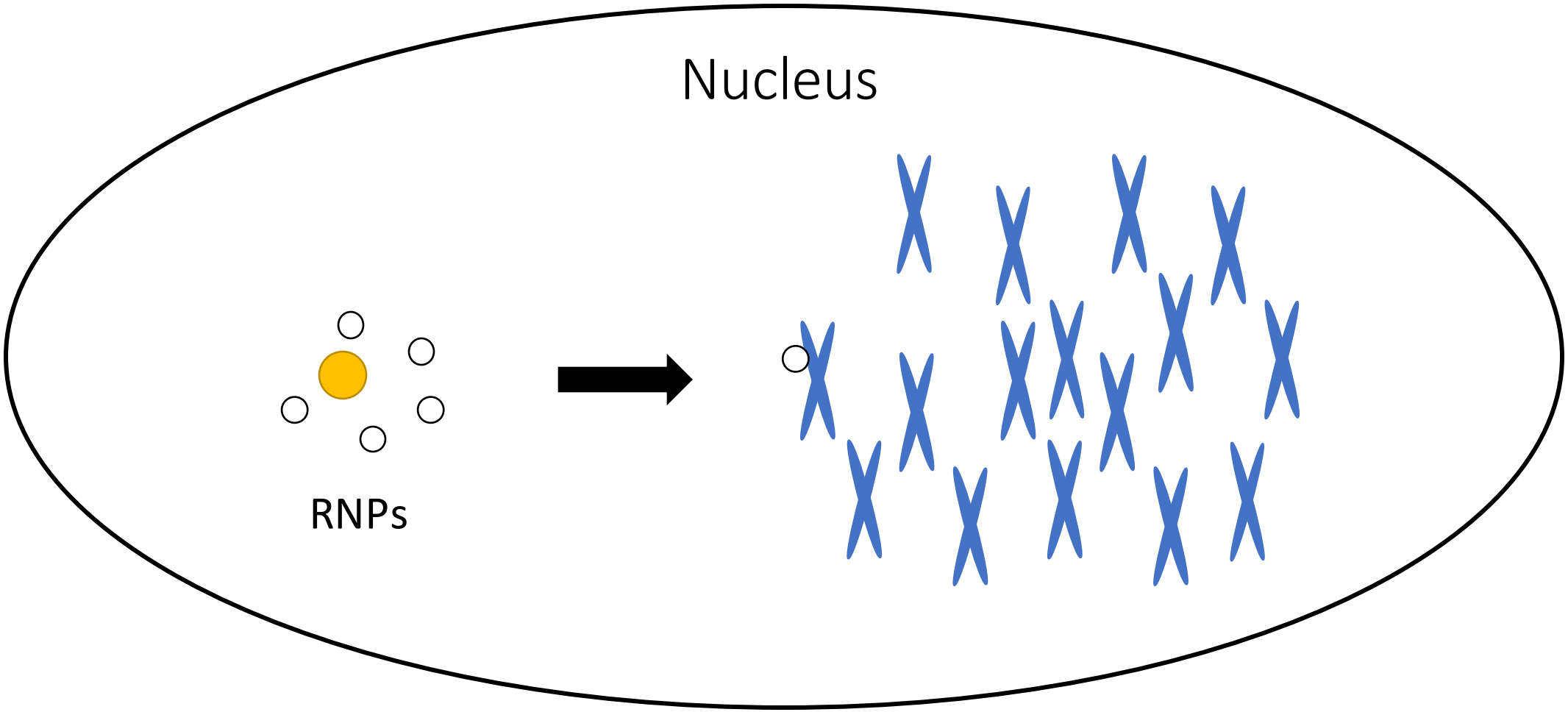


Bombarded Cell



RNPs dissociate

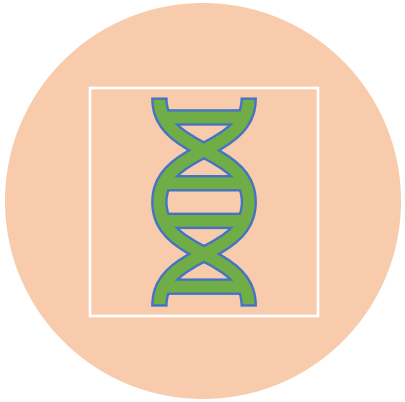
Cytoplasm



Gene editing checklist

STEP 1: DEVELOP PLANT TISSUE CULTURE SYSTEM

✓



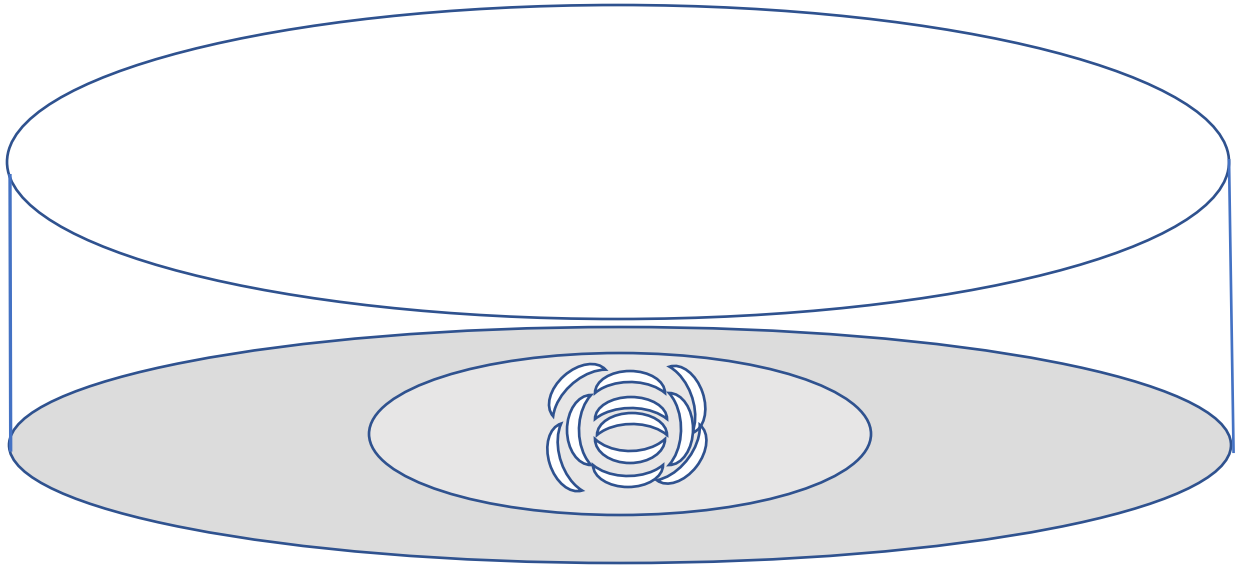
STEP 2: PERFORM GENE EDITING IN LIVING CELLS

✓

STEP 3: REGENERATE EDITED CELLS INTO PLANTS

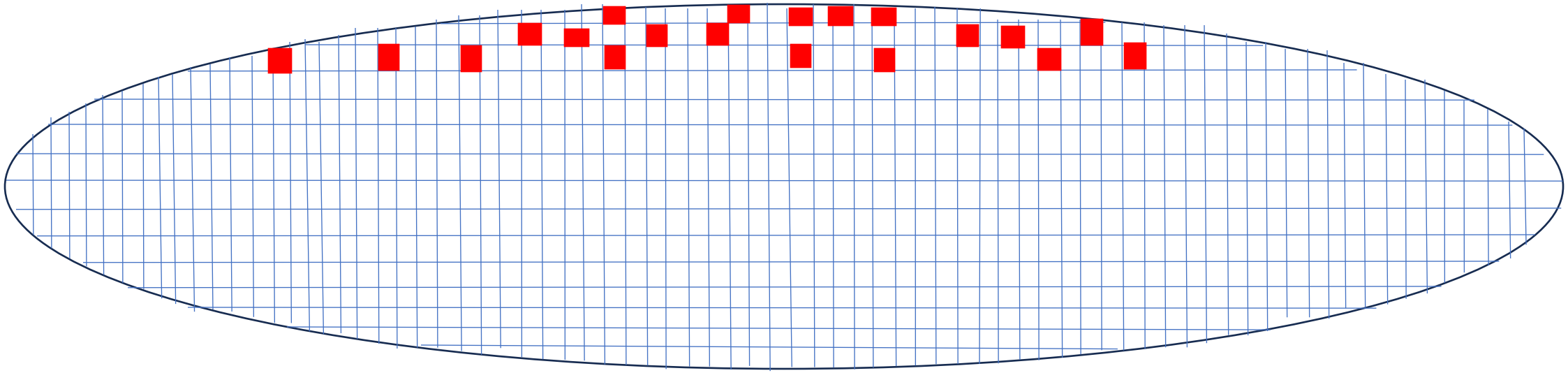
Immature embryos

Immature Embryos



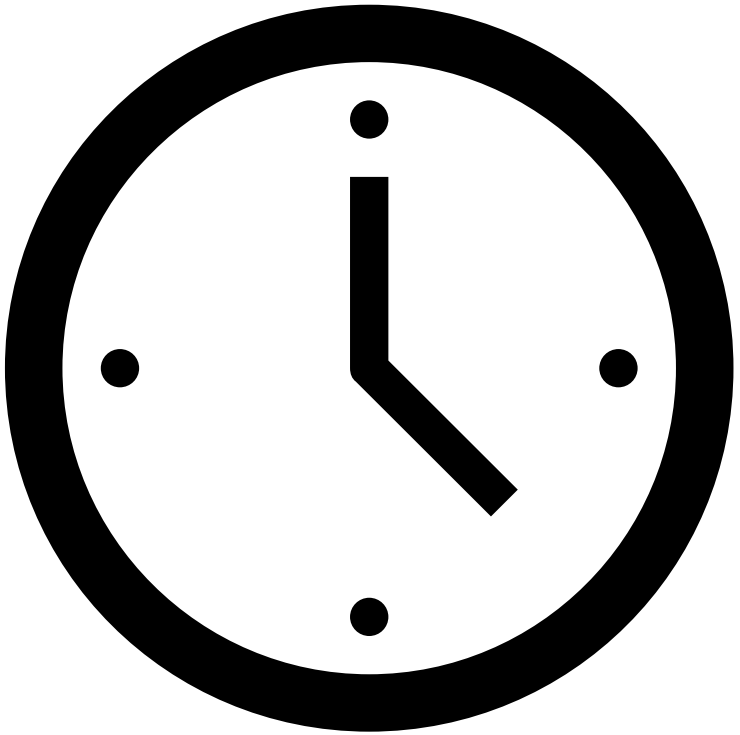
Edited Cells

Unedited Cells



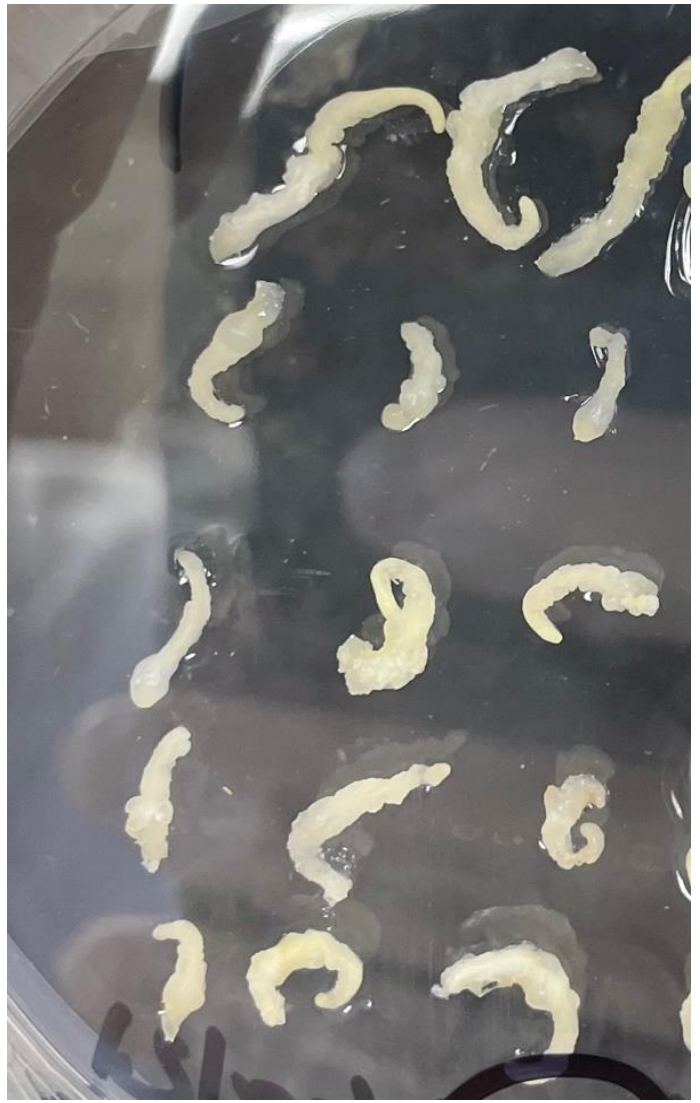
Bombarded Immature Embryo

1-2 weeks later…



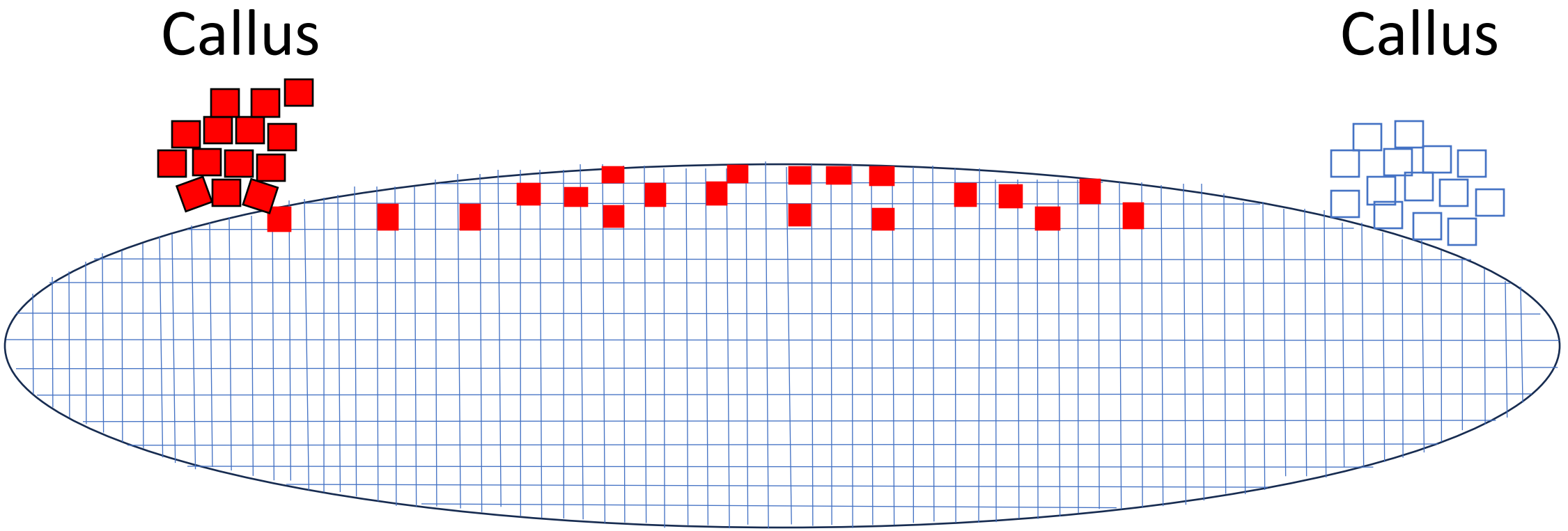
1 week old

Callus induction media



Edited Cells

Unedited Cells



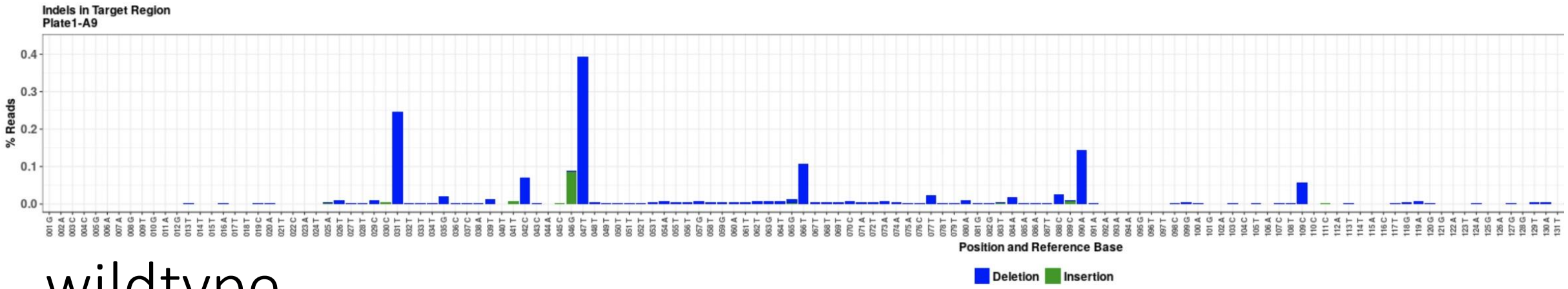
Bombarded Immature Embryo

Part 3: Results

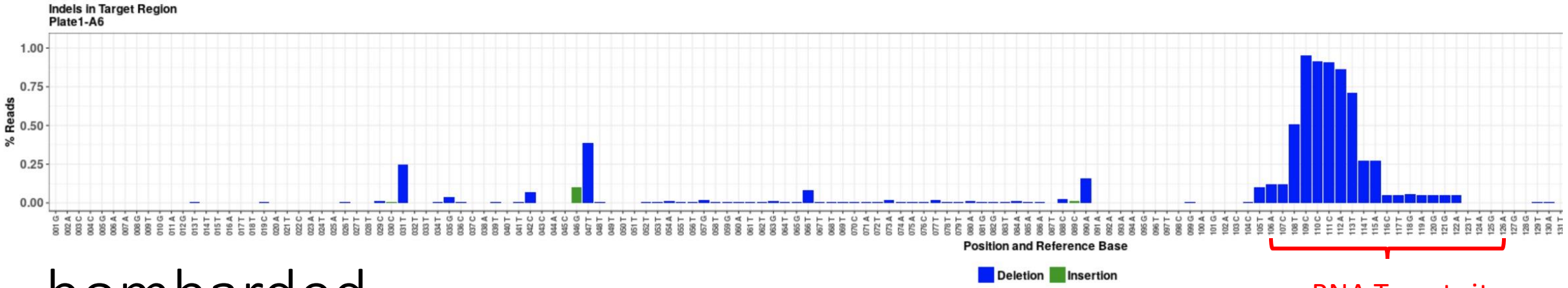
Experimental design

1. Bombard embryos with RNPs targeting onion gene
2. Incubate several weeks
3. Extract DNA from bombarded tissue
4. PCR amplify target gene
5. Perform amplicon sequencing to measure gene editing

CENH3 amplicon results



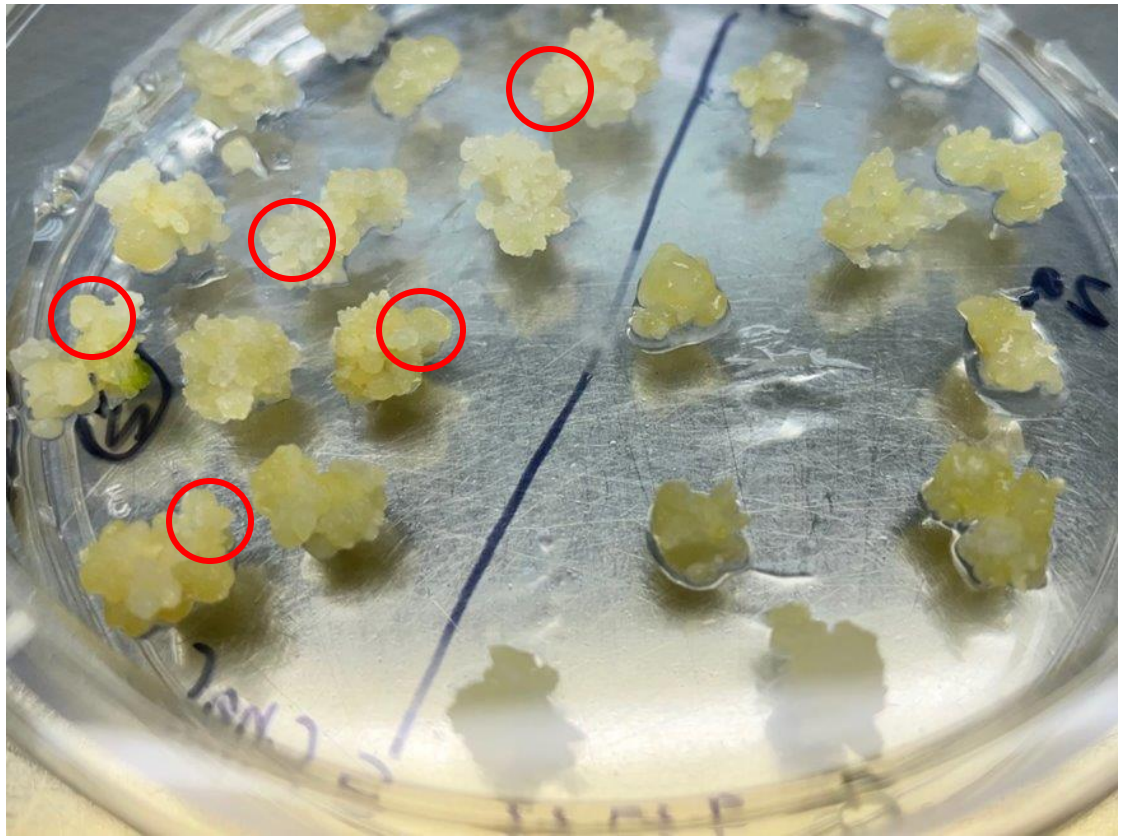
wildtype



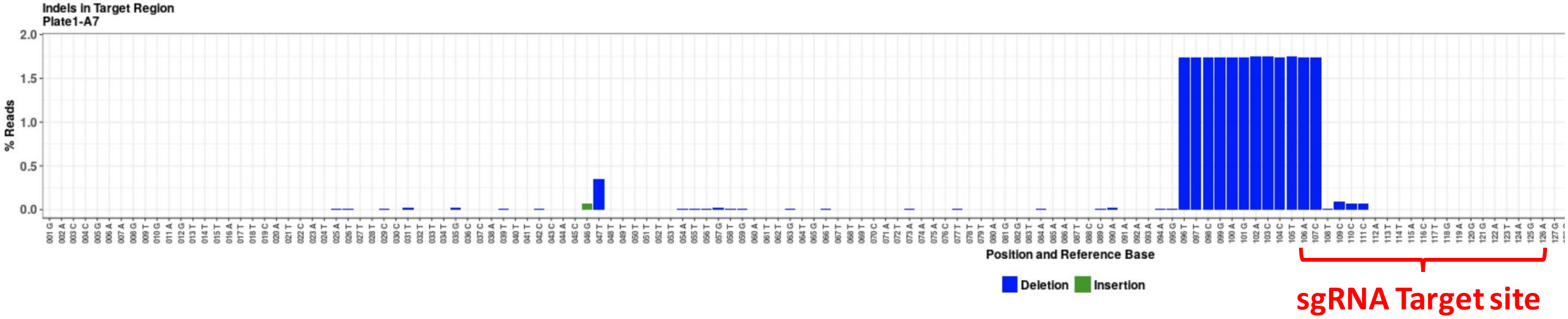
bombarded

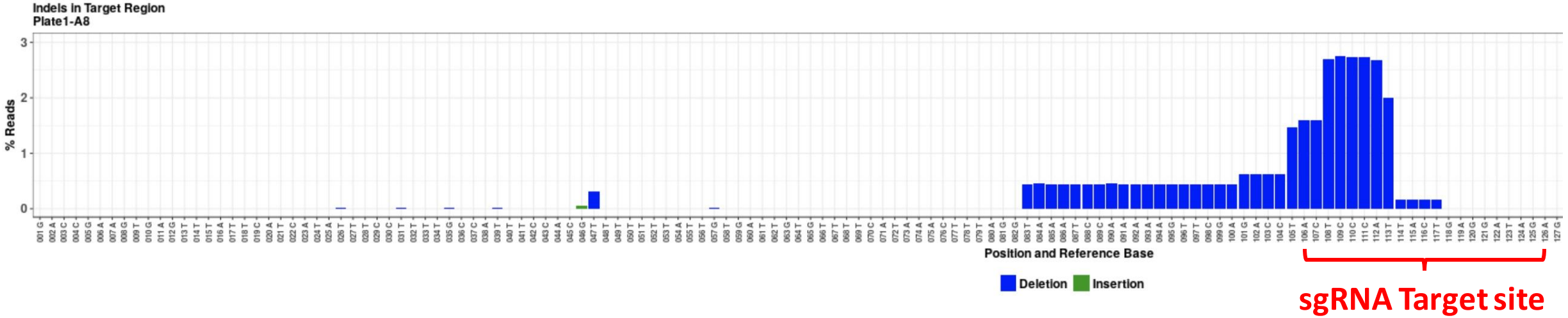
Sequencing target gene from 5 week old tissue

1. Pool tissue from different pieces of callus
2. Extract DNA
3. PCR amplify target gene
4. Perform amplicon sequencing

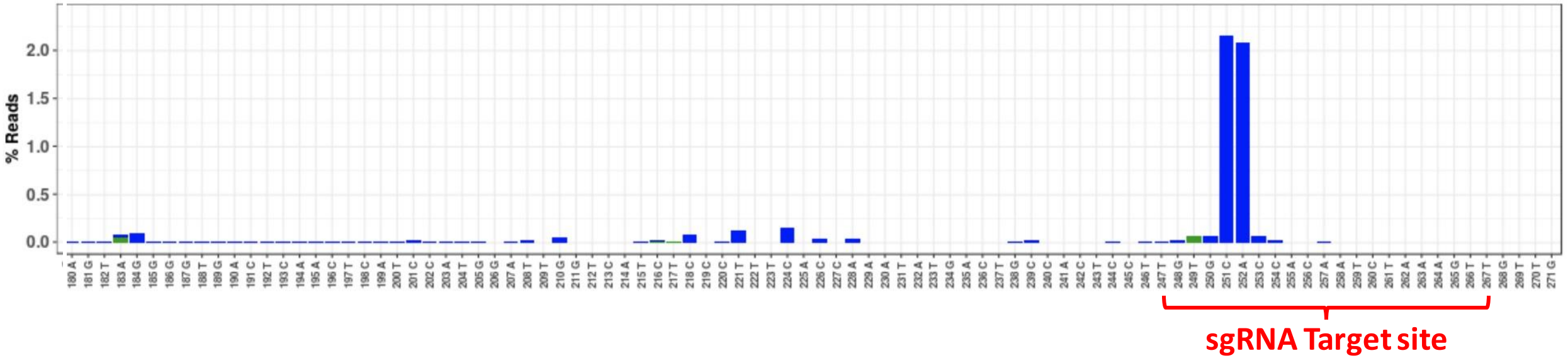


CENH3 editing

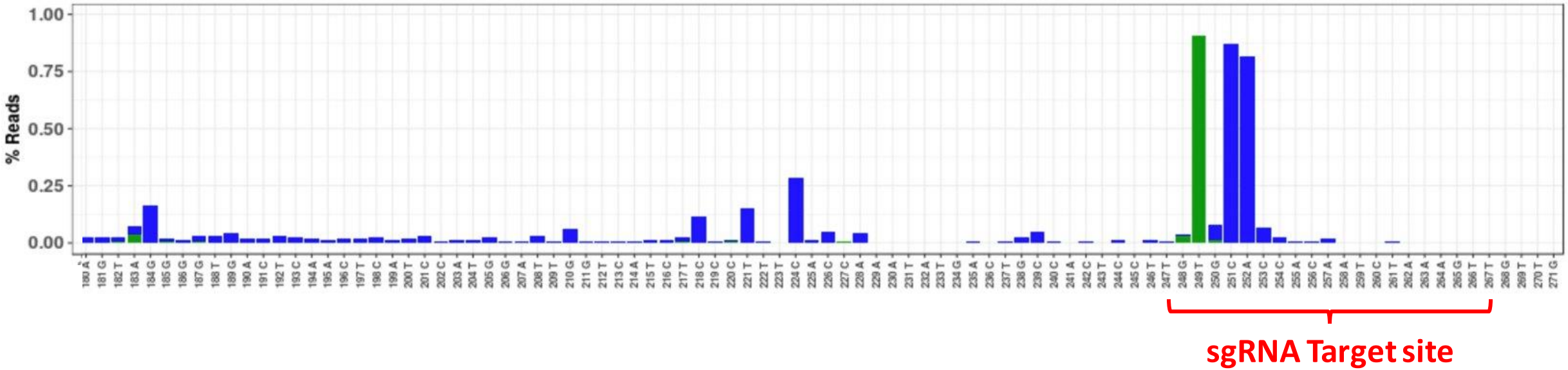




Alliinase editing



Alliinase editing



Gene editing checklist

STEP 1: DEVELOP PLANT TISSUE CULTURE SYSTEM

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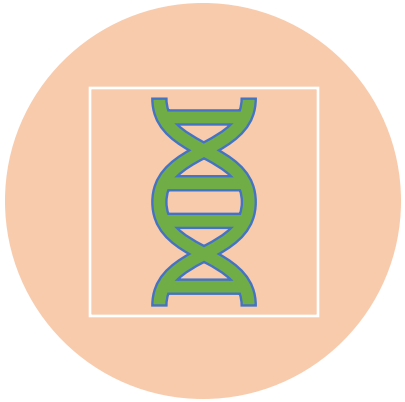
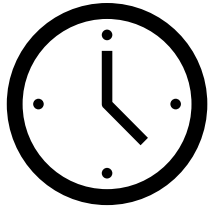


STEP 2: PERFORM GENE EDITING IN LIVING CELLS

✓

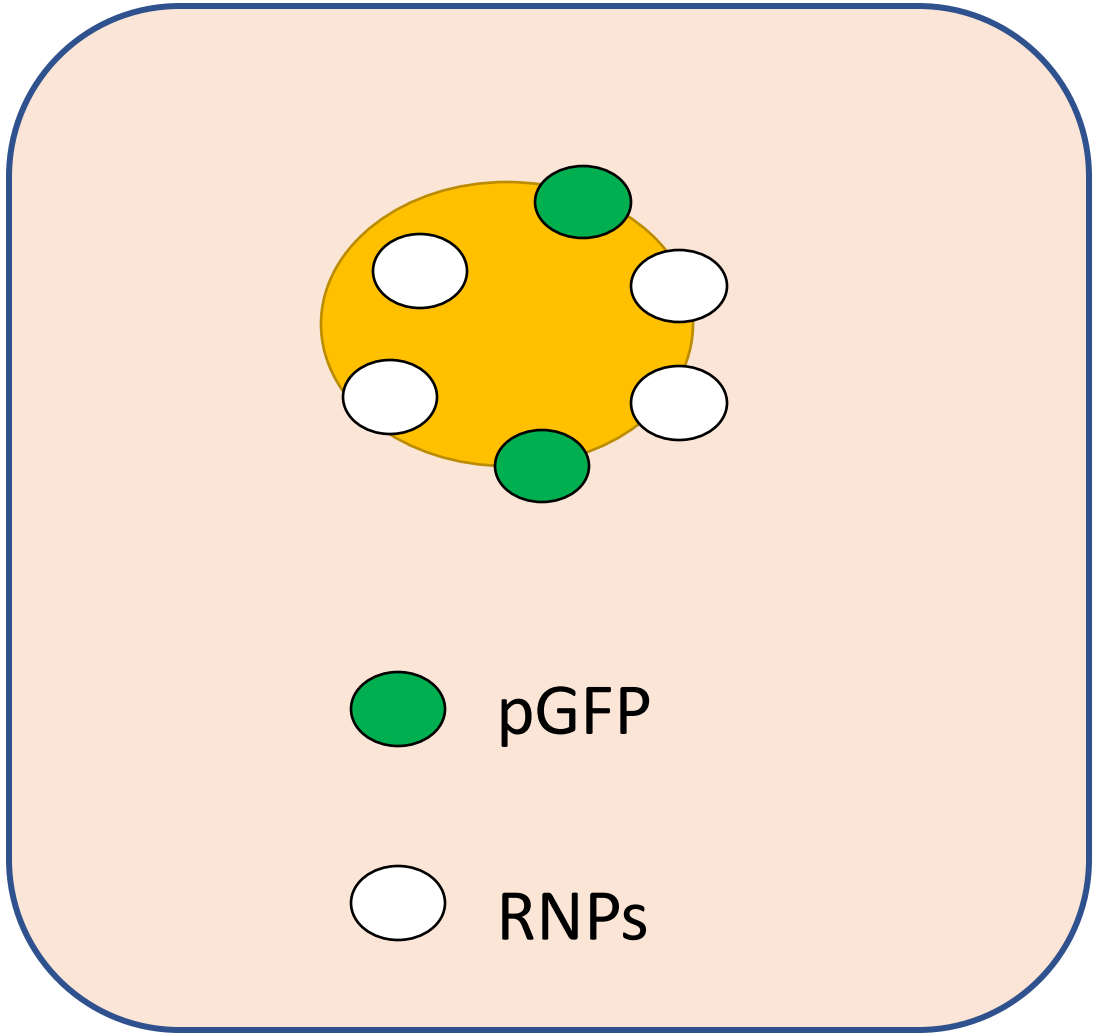
STEP 3: REGENERATE EDITED CELLS INTO PLANTS

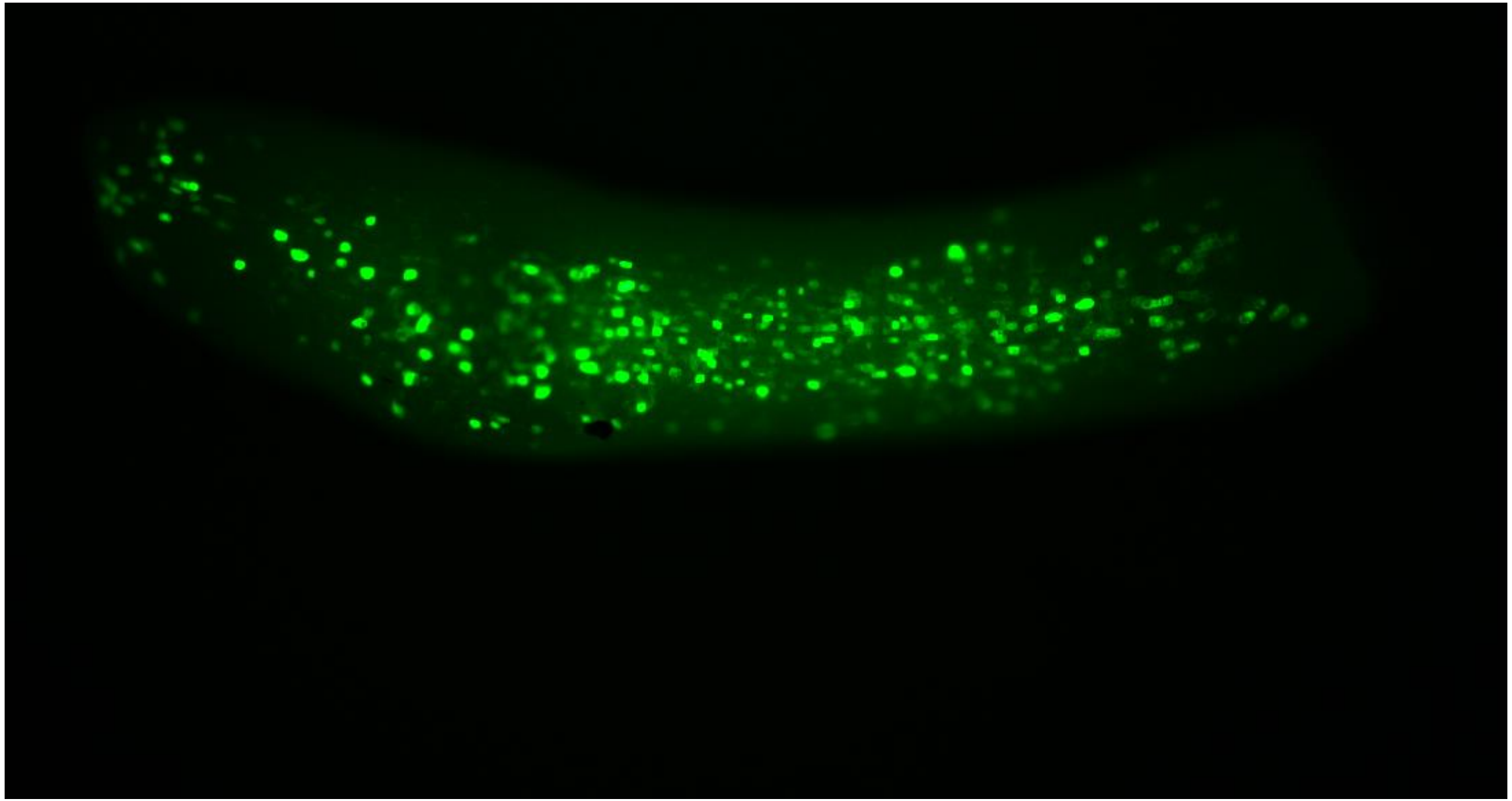


Screening regenerated plants for editing

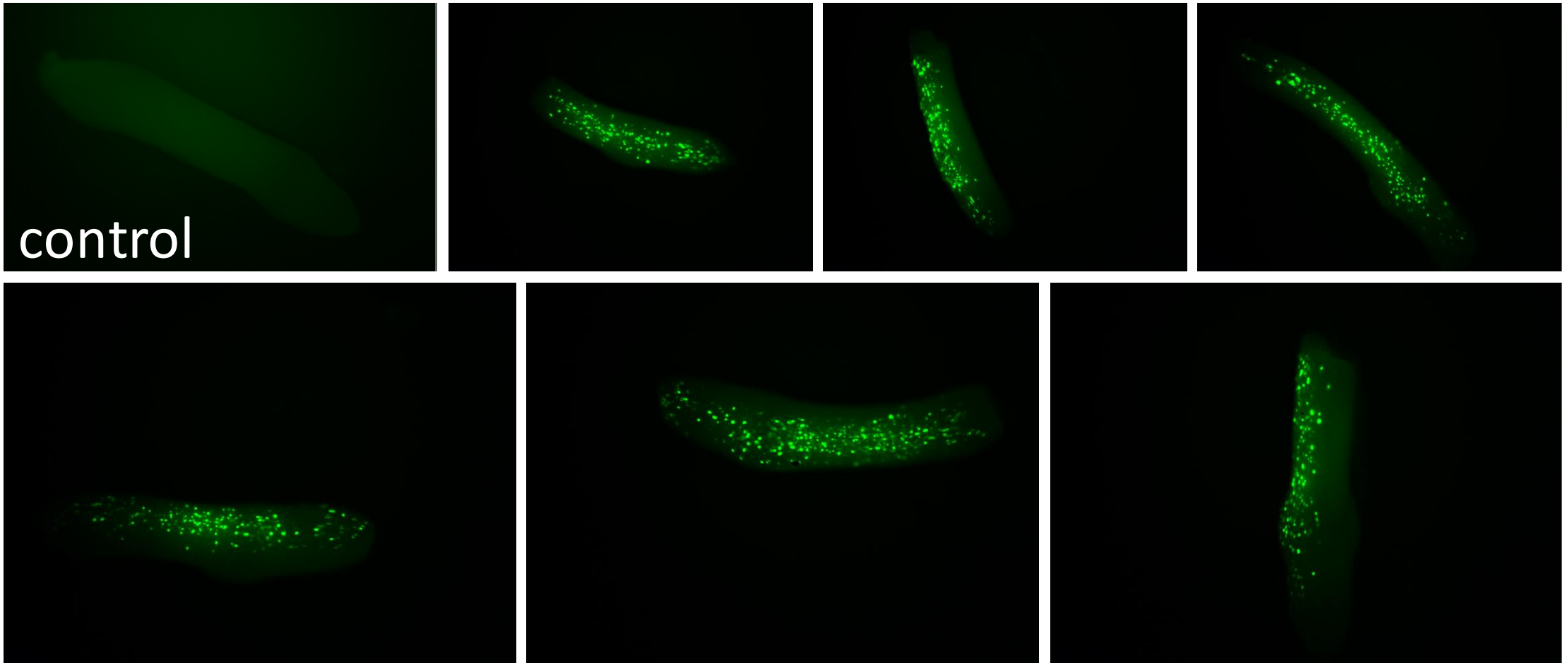
- Currently have callus from 10 bombardment experiments at various stages of regeneration.
- 70 to 100 immature embryos per bombardment
- Screen regenerated plants
- Expect 400-600 plants

Co-bombardment

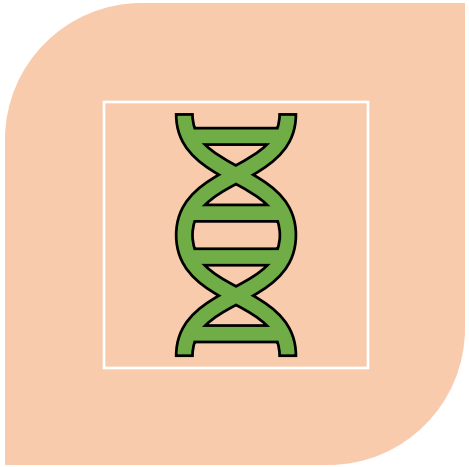




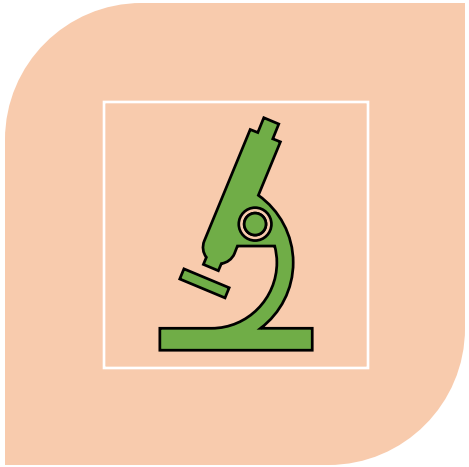
GFP visualizes bombardment



Future directions



INCREASE EFFICIENCY OF GENE EDITING IN OUR SYSTEM



DEVELOP METHODS TO ENRICH FOR EDITED CELLS THAT GO ON TO REGENERATE

Acknowledgements

- Funding provided by National Science Foundation (NSF)
- My advisors Patrick Krysan and Mike Havey
- Christy Stewart, lab technician
- Collaborators at University of Warwick
- Colin Eady's group for tissue culture recipes!

Plant Breeding & Plant Genetics Program
University of Wisconsin – Madison