NC STATE

Disrupting Upstream ORFs for Enhanced Protein Production in Plants

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GENETICS & GENOMICS ACADEMY
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Background

- Plants must dynamically regulate gene expression to coordinate growth, development, and stress responses
- Upstream open reading frames (uORFs) are RNA regulatory elements present in ~30-50% of plant transcripts and can influence gene expression
- Despite their widespread prevalence, relatively few uORFs have been experimentally validated
- Disrupting uORFs could advance strategies for enhancing valuable plant traits

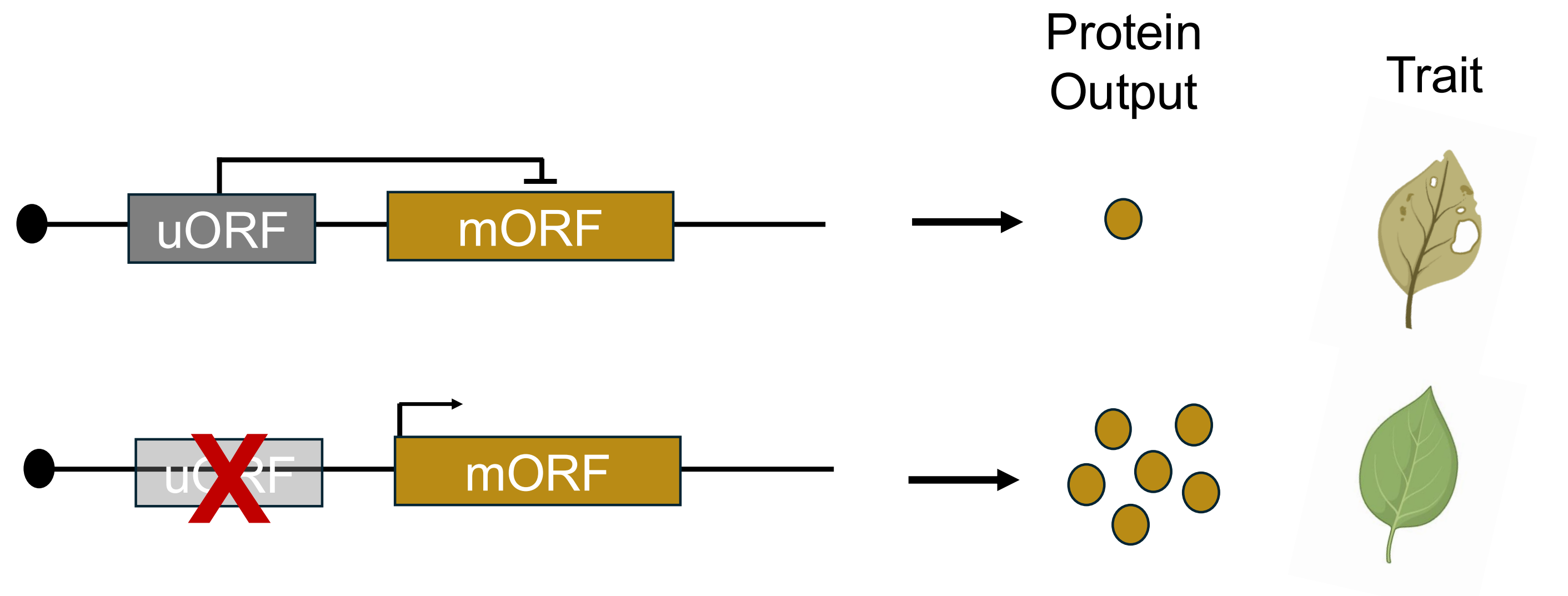


Figure 1. uORF inhibitory removal for increased protein output at the main open reading frame (mORF)

Objective and Hypothesis

- **Objective:** Identify and experimentally validate functional uORFs that regulate gene expression
- **Hypothesis:** Disruption of uORFs will increase protein output at the mORF

EIN2: Central regulator in ethylene signaling

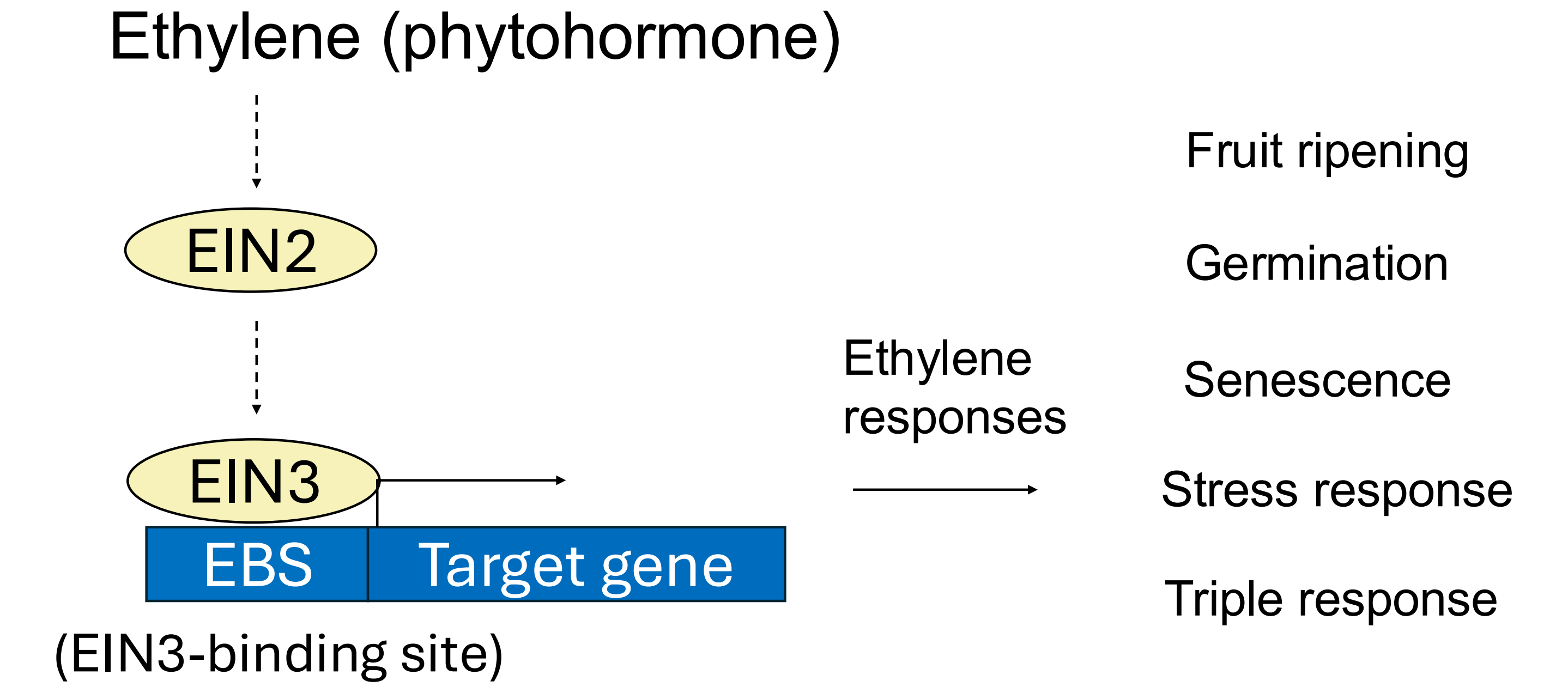


Figure 2. Ethylene signaling triggers numerous plant growth and development responses

Experimental Workflow

Aim 1: Identify candidate regulatory uORFs

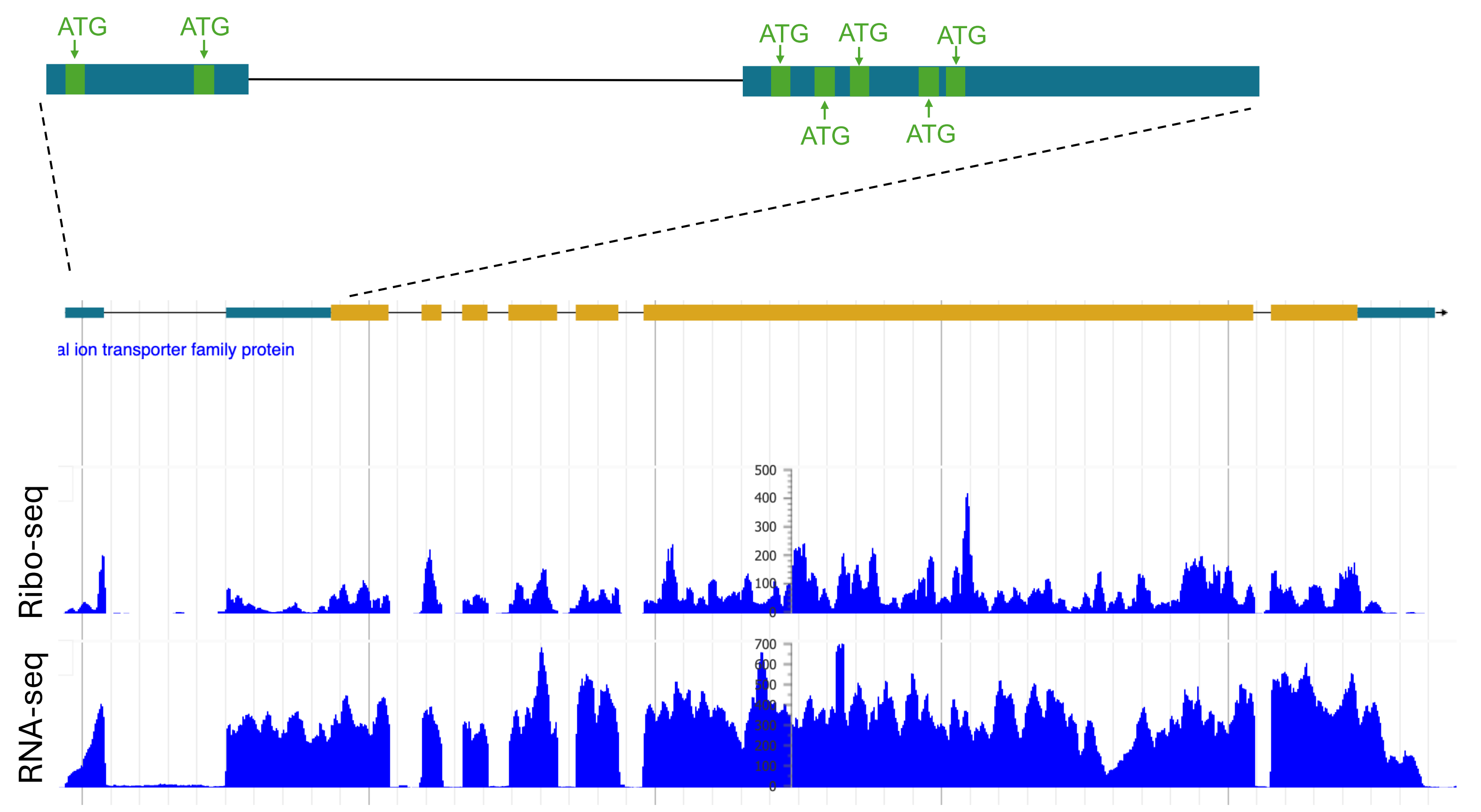


Figure 3. Ribo-seq and RNA-seq data reveal active translation of multiple uORFs of *EIN2* in Arabidopsis

Aim 2: Evaluate function using transient reporter assays

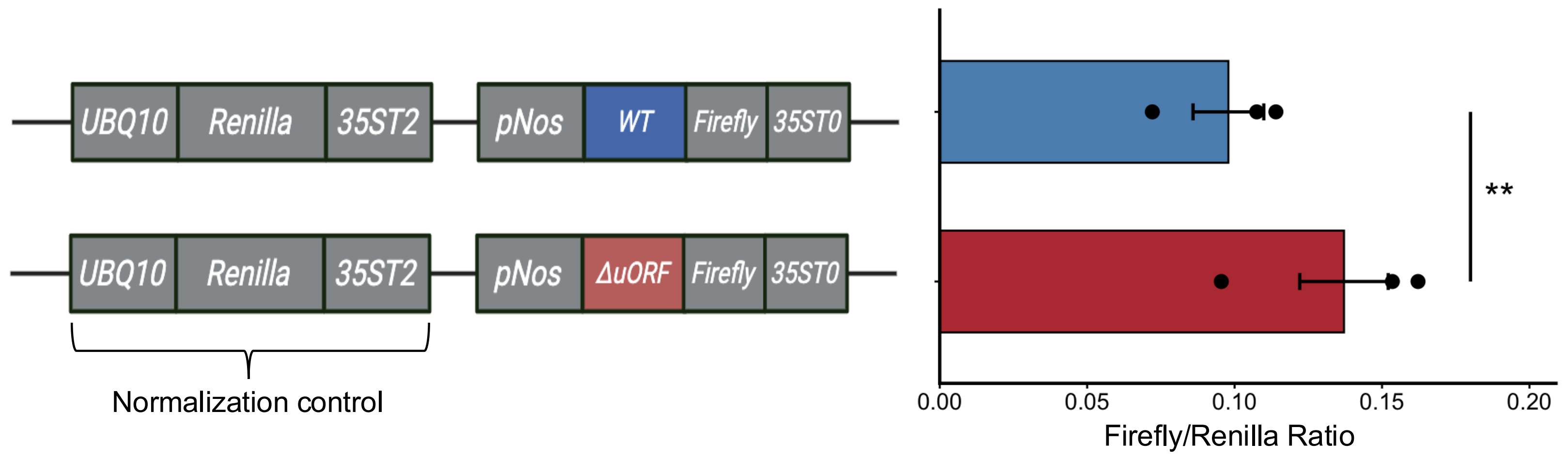


Figure 4. Disruption of *EIN2* uORFs increases normalized *Firefly* reporter gene expression in tobacco transient assays

Aim 3: Validate function in stable, genome-edited plants

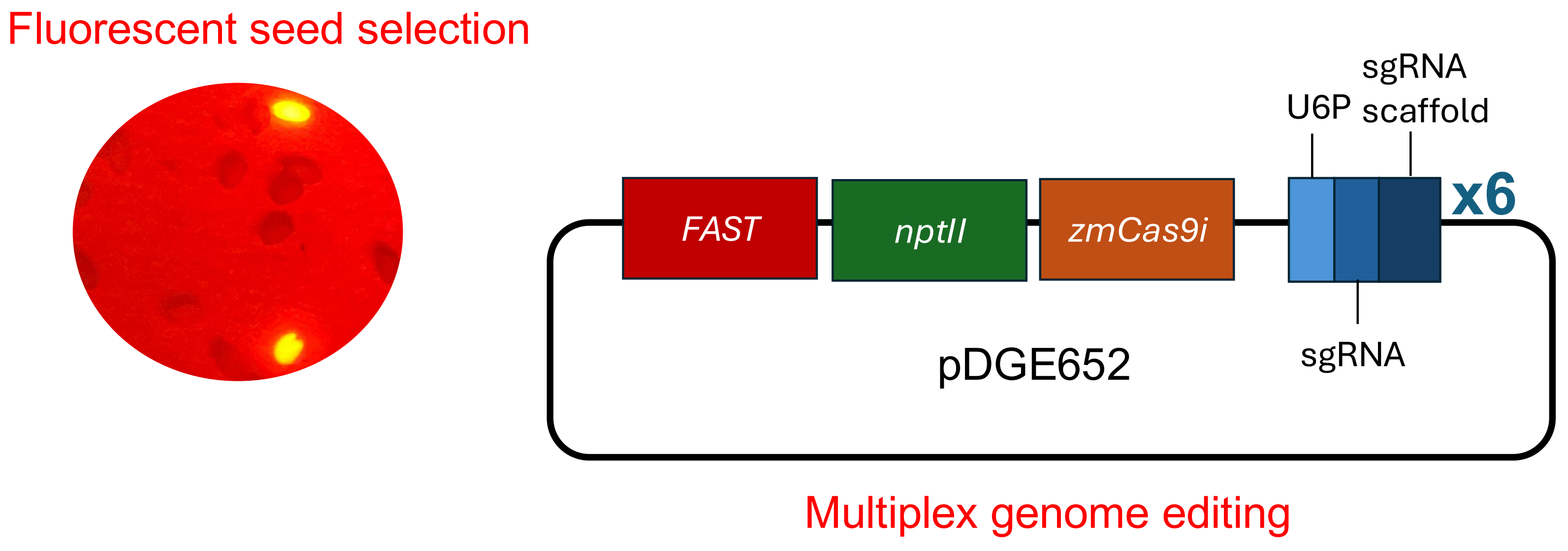


Figure 5. Schematic representation of the CRISPR-Cas9 vector targeting the uORFs of the *EIN2* gene and fluorescent seed selection

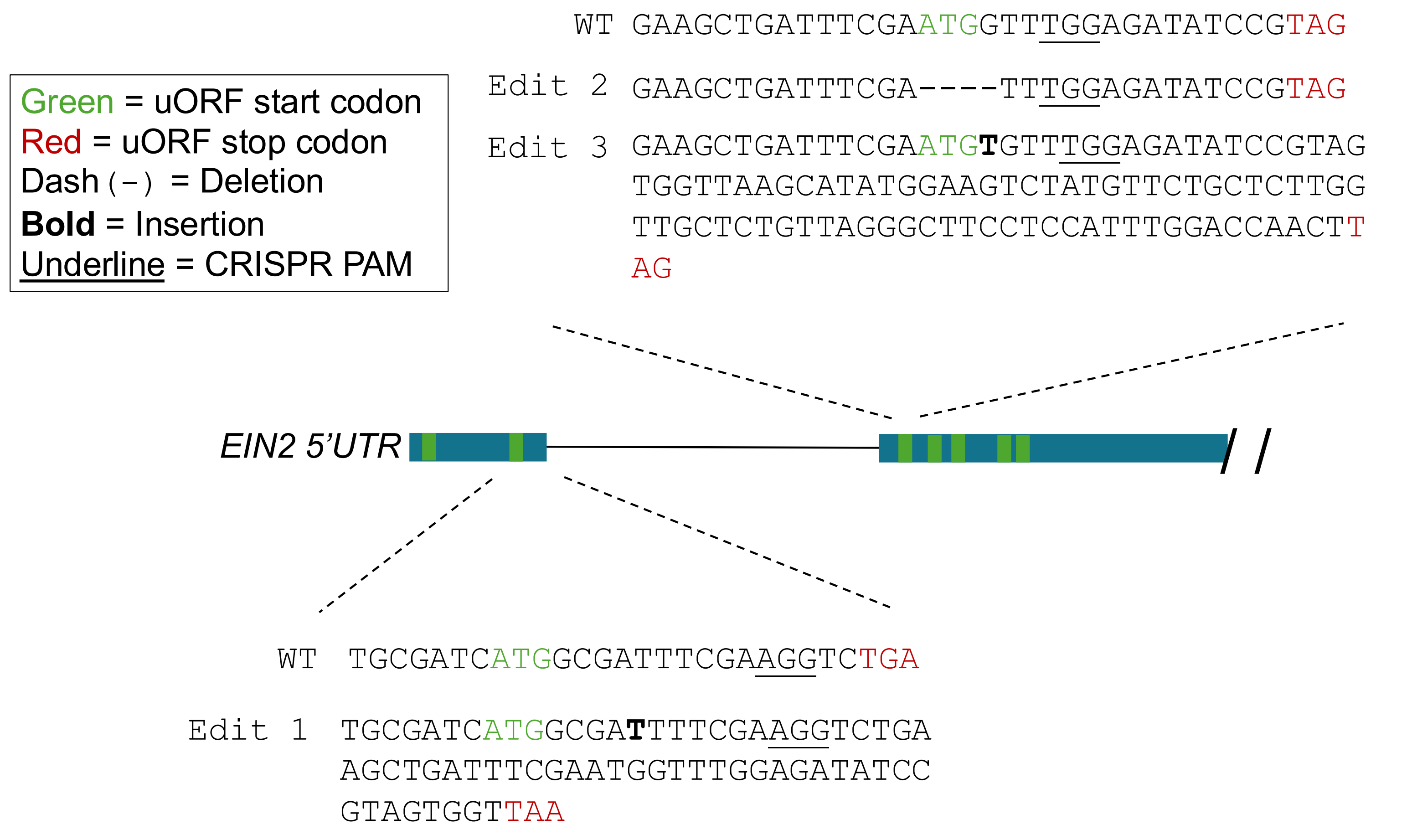


Figure 6. Genome editing generated insertions and deletions that disrupt *EIN2* uORFs

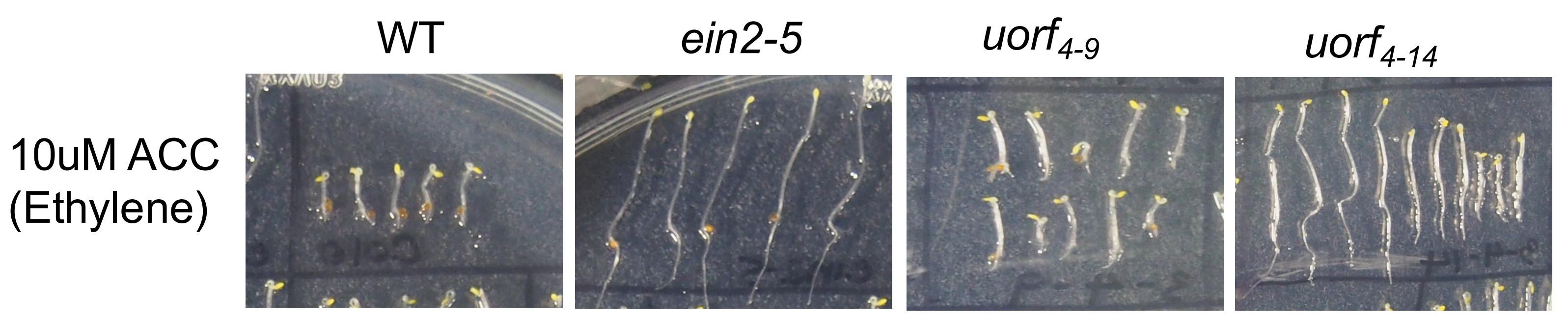
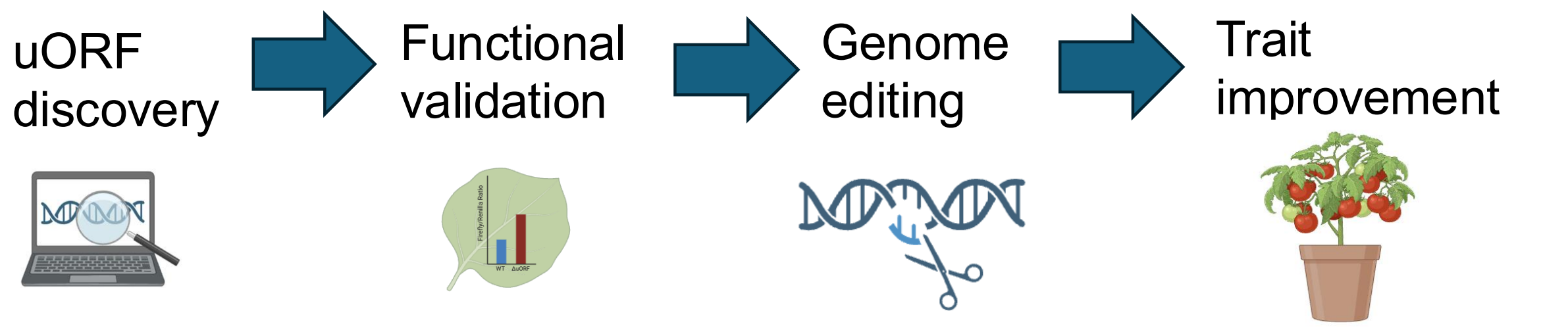


Figure 7. Disruption of *EIN2* uORFs alters ethylene responses

Discussion and Future Work

- Loss of *EIN2* uORFs resulted in increased normalized reporter output in transient assays
- Screening of the CRISPR genome-edited 5’UTR lines uncovered prominent phenotypic responses
- Future work will link specific edits with observed phenotypes, as well as extend the approach to other candidate genes (e.g., *EIN3*)
- This work can inform the development of uORF-editing strategies in plant biotechnology applications



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