

# Nonredundant Minimal Polymerase III Promoters for CRISPR Applications

## Background/Objective

- Current toolkits for multiplex gene editing lack diversity, affecting their overall utility.
- Repetitive use of the same Polymerase III (Pol III) promoter or excessively long promoters in CRISPR constructs can cause silencing, increased genetic load, and reduced editing efficiency.
- Our goal was to develop a diverse set of short, nonredundant Pol III promoters for efficient and stable multiplex CRISPR applications in dicot plants.

## Approach

- Test Pol III promoter deletion series and diverse short promoters for their editing efficiency in *Nicotiana benthamiana* and *Populus tremula* × *alba* reporter lines.
- Assess the impact of mutations in conserved promoter elements on functionality.
- Evaluate activity of synthetic Pol III variants with mutations outside conserved regions.

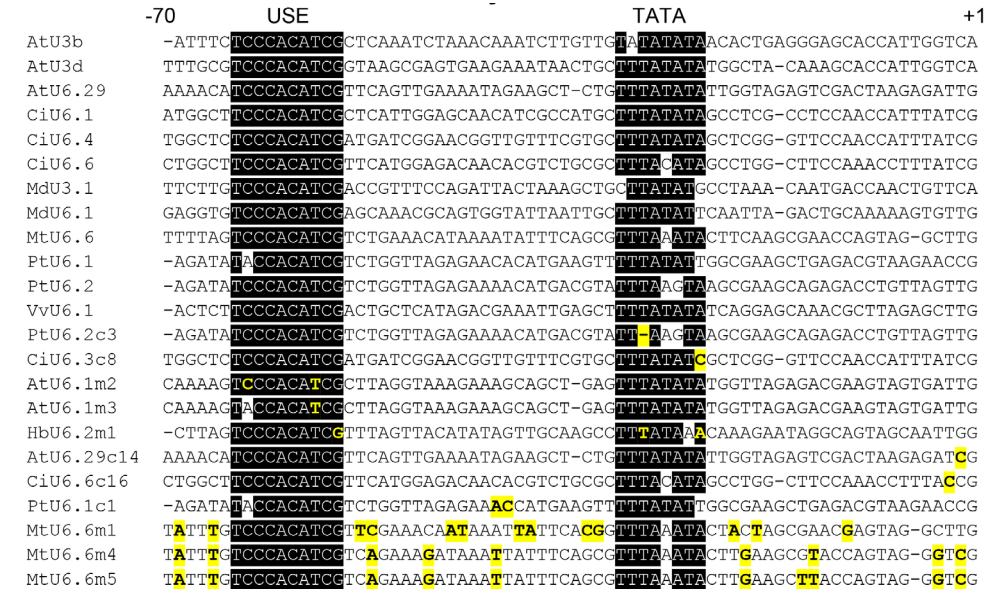
## Results

- Pol III promoters as short as 70 bp, derived from diverse taxa, were functional in both species.
- Mutagenesis confirmed that specific nucleotide changes in the USE (upstream sequence element) or TATA box could abolish or restore promoter activity.
- Synthetic variants with mutations outside conserved regions retained functionality.
- A 4-plex construct successfully edited all target *MYB* alleles in poplar, demonstrating similar editing efficiency among Pol III promoters of varying lengths.

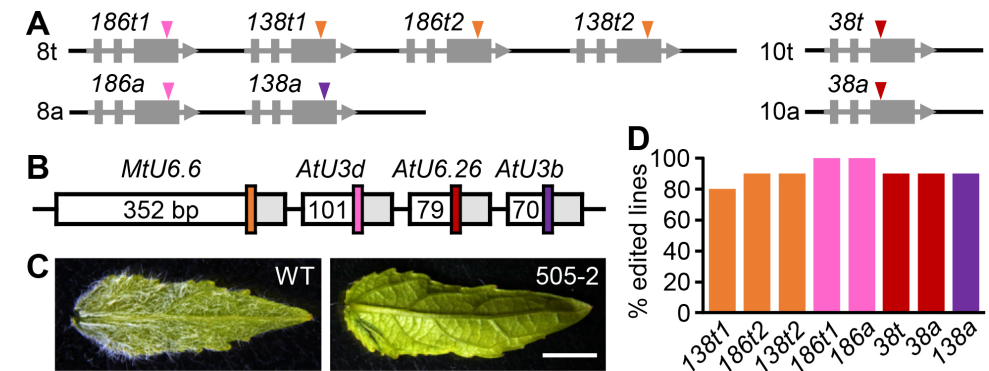
## Significance/Impacts

- Minimal Pol III promoters are sufficient for effective gRNA expression in dicots.
- Refined USE consensus is essential for promoter functionality.
- Conceptual and experimental demonstration of synthetic Pol III promoters.
- Cost-effective and scalable for construct assembly, applicable to monocots with adaptations.
- >20 experimentally Pol III promoters validated to enhance CRISPR multiplexing capabilities.

Deguchi et al. (2025) Plant Physiology 198: kiaf294.



A collection of short Pol III promoters. Conserved elements are shaded in black, mutations in yellow and 3 synthetic variants at the bottom.



A 4-plex construct edited 8 *MYB* alleles (A-B) in poplar, yielding trichomeless leaves (C) and similar efficiency among promoters (D).