

FLP to the Future: Methylation-Insensitive Gene Excision System for Clean Gene Editing

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Typical Gene Editing Workflow for Row Crops

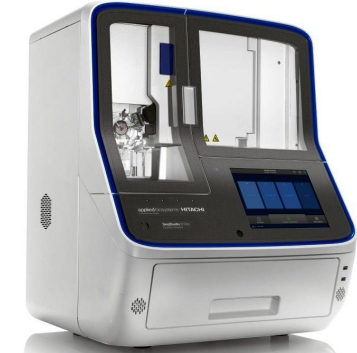
Agrobacterium tumefaciens/ rhizogenes



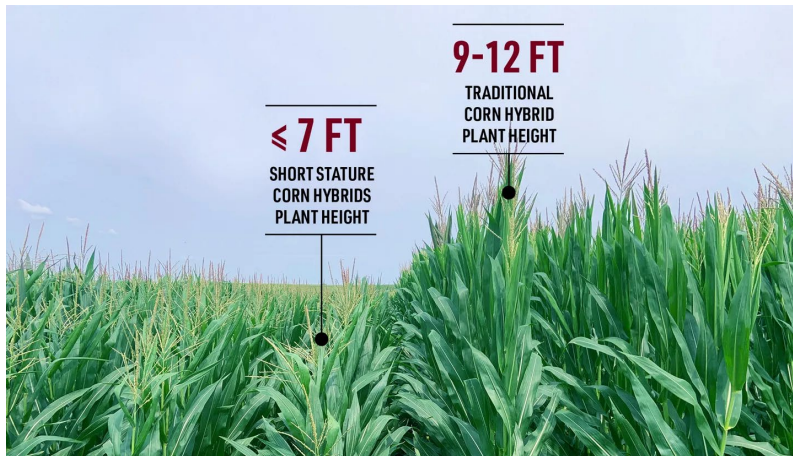
DNA delivery
Cas9/sgRNA transgene



Transformation & Regeneration



Genotyping



Edited Plants
Transgene free

Self-pollination
Segregate out transgenes



Problems in Clonally Propagated Tree Systems

- Time constraint to floral onset (years)
- Loss of phenotype characteristics through self-pollination (hybrid trees)



Stanton et al. 2010 *Populus* Breeding: From the Classical to the Genomic Approach

Excising Transgenes with Site-Specific Recombinases

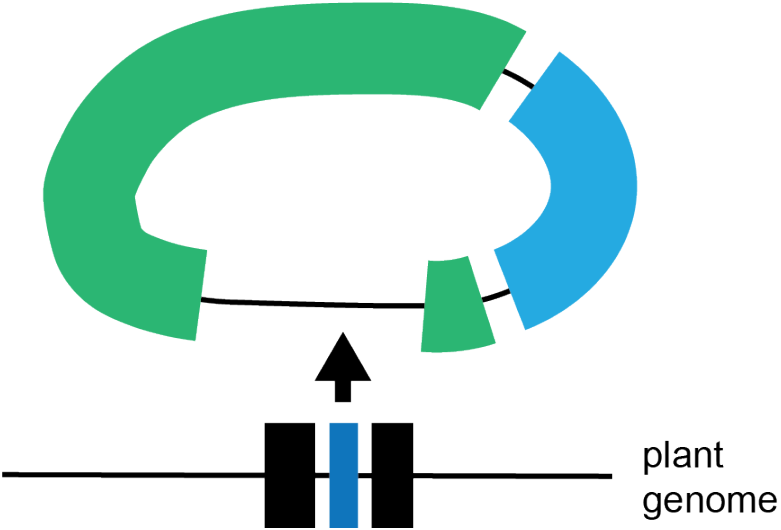
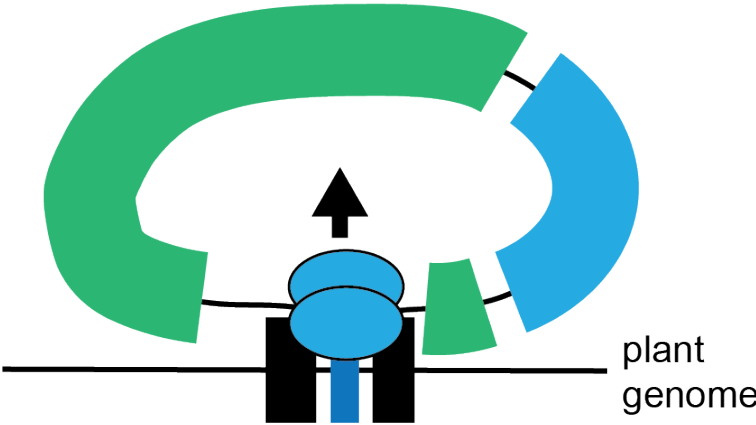
- Reduces off-target gene edits
- Deregulation? (RSR-trait-MOA regulation may allow for footprint approval – potential USDA-SECURE simple edit exceptions)

1 Genomic integration

2 Recombinase processing

3 Excised transgene

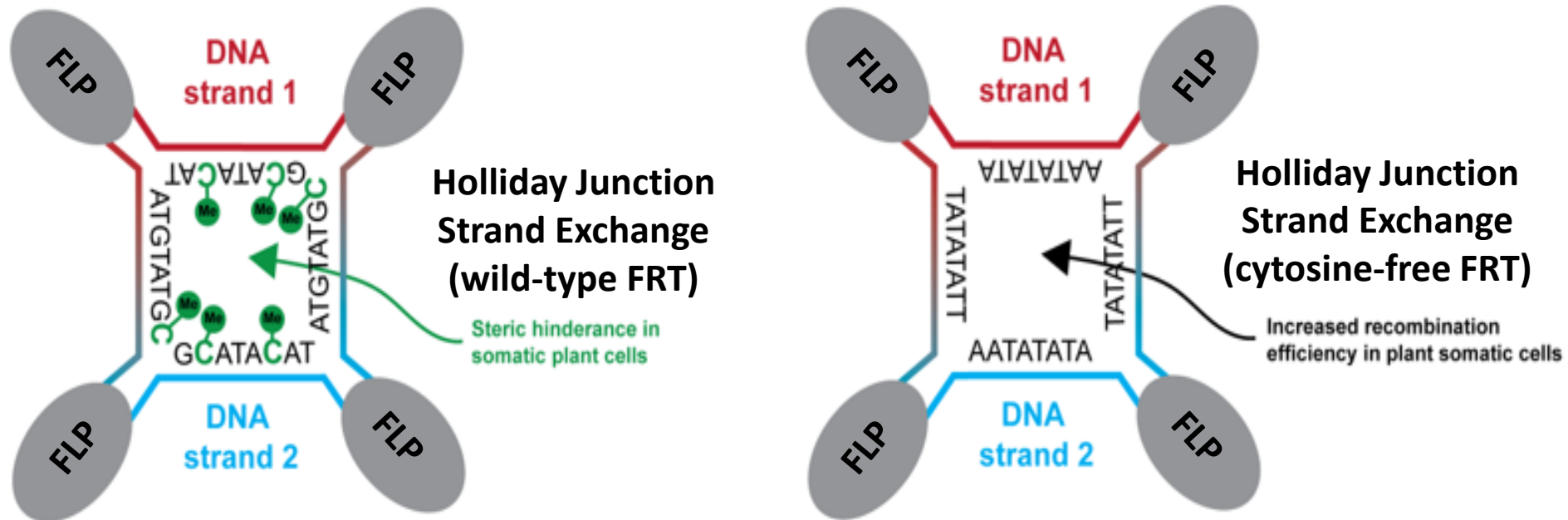
- Cas9/ sgRNAs/ Plant Selectable Marker
- Recombinase
- Recombinase Recognition Sequence
- T-DNA borders



Recombinase Challenges in Plant Systems

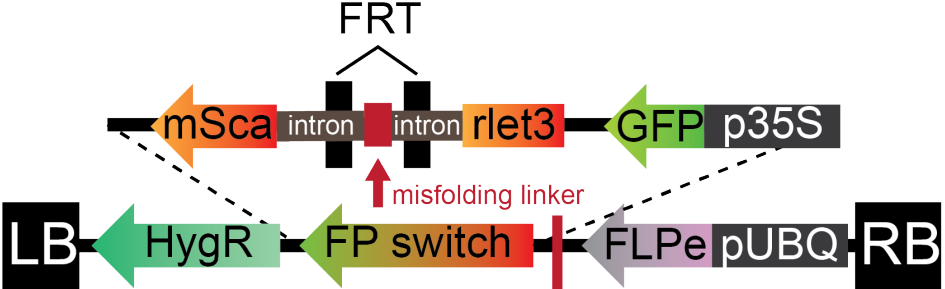
DNA methylation occurring in Cre-expressing cells inhibits loxP recombination and silences loxP-sandwiched genes

Ruochen Liu, Qin Long, Xiuping Zou, You Wang, Yan Pei ✉

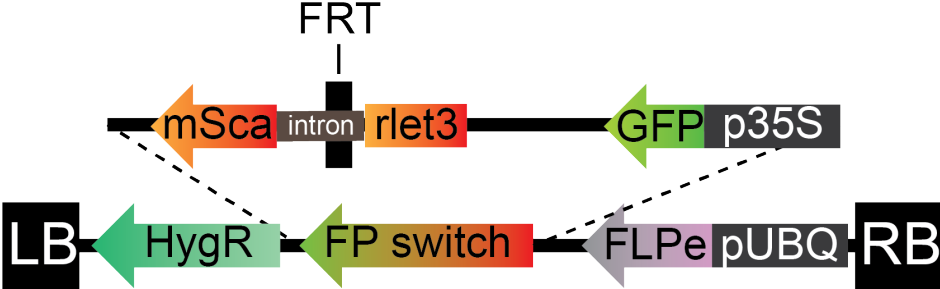


Methylation at recognition site spacer sequence causes steric hindrance at Holliday junction during recombination

Quantify Excision Efficiency – Active Excision Reporter



Excision

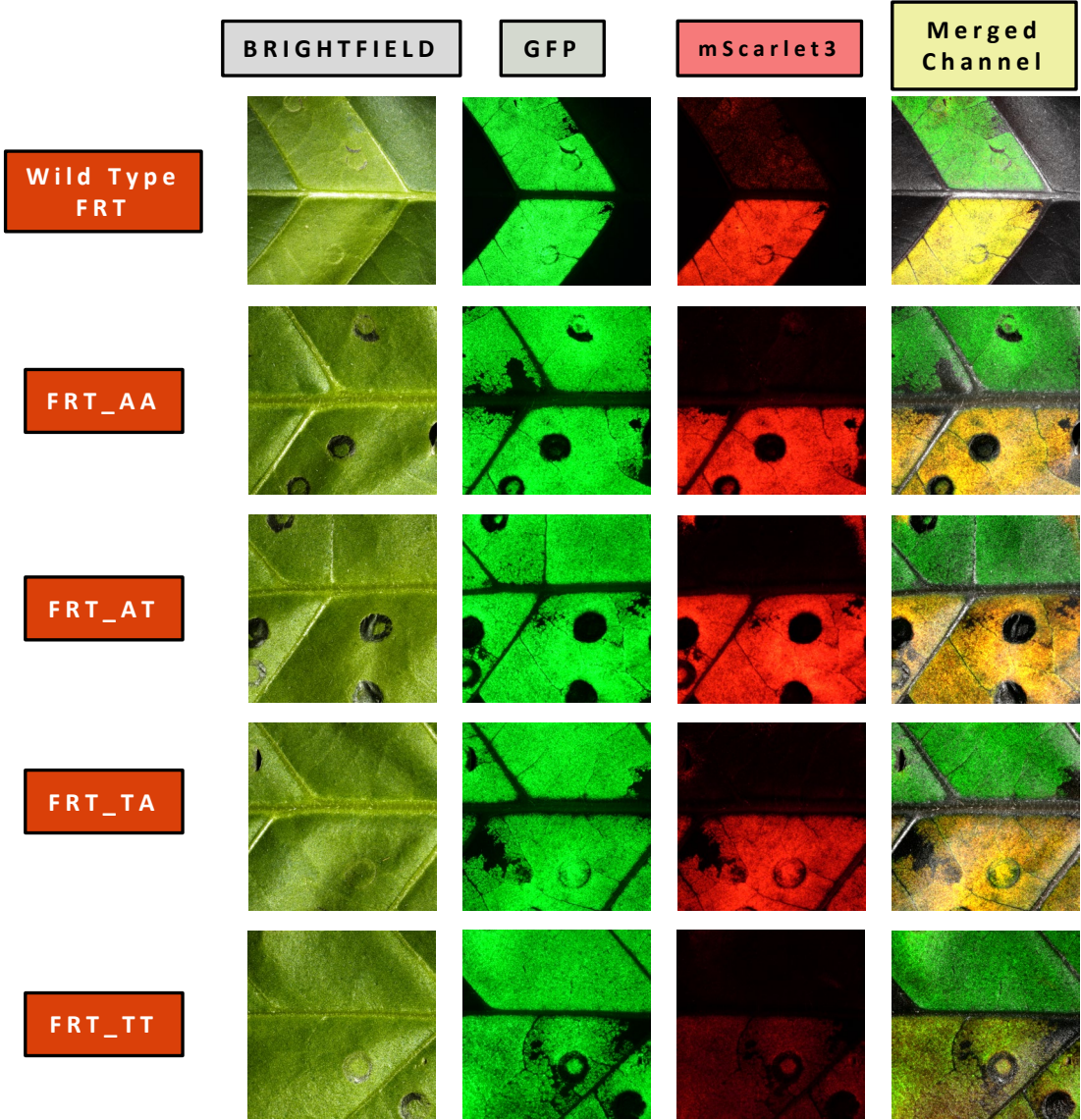


NO RED FLUORESCENCE



RED FLUORESCENCE

Site Efficiency Screen - Agroinfiltration



Top
no FLP recombinase

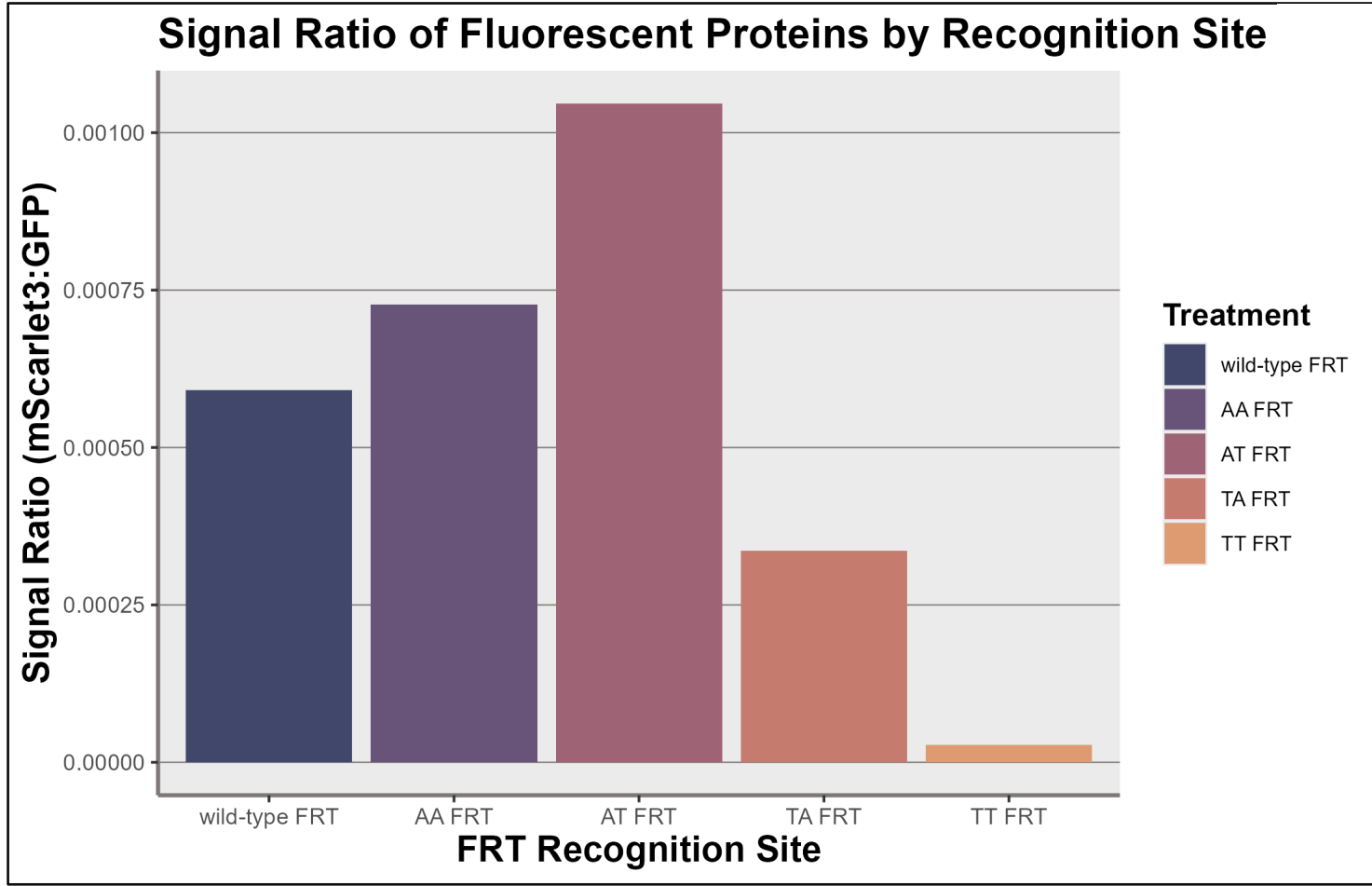
Bottom
with FLP recombinase

Analysis Using GMODetector Phenomics Pipeline

Leaf discs from Agrobacterium infiltration imaged using a fluorescent hyperspectral camera

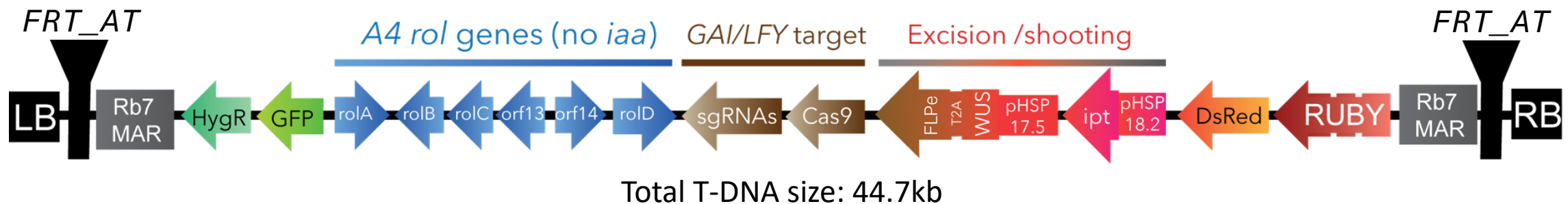


GMODetector
Quantification of fluorescent signal

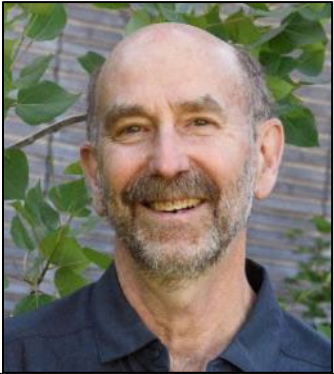


Summary & Future

- “AA” and “AT” FRT sites showed promise in this construct with transient expression
 - Other sites lower-performing, still saw some excision
- Future Goals:
 - Test this construct in stable transformation for hybrid poplar
 - Head-to-head of top-performing cytosine-free FRT site against wild-type FRT site in root-to-shoot construct (below)



Acknowledgements



Steve Strauss
Professor FES



Cathleen Ma
Transformation &
Greenhouse
Experiments



Kate Peremyslova
Transformation
Experiments



Greg Goralogia
Postdoc



Anthony Marroquin
Greenhouse Manager



Chris Willig
Postdoc

Scientific assistance

Robert Alba (Lab Manager)

Colette Richter (FRA)

Victoria Conrad (Undergraduate)

Abigail Lawrence (Undergraduate)

Daniel Casey-Hain (Undergraduate)

Aiden Seidel (Undergraduate)

Michael Nagle (LIBD)

Bill Gordon-Kamm (Corteva)

Todd Jones (Corteva)

Jim Thomson (ARS Albany)

Institutional assistance

Melora Park (FES)

Arthur Myers (COF)

This work is supported by the USDA NIFA – Biotechnology Risk assessment Grant (grant no. 2023-33522-40414) and the Genetic Research on Engineering and Advanced Transformation of Trees (GREAT Trees) cooperative



National Institute of
Food and Agriculture



Oregon State
University