BRC Science Highlight
April 2019

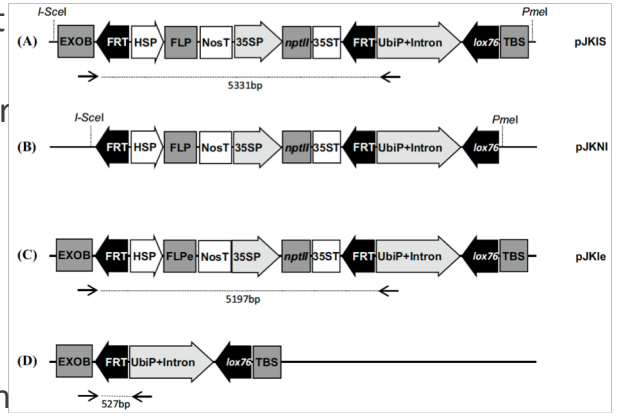
Generation of a Selectable Marker Free, Highly Expressed Single Copy Locus as Landing Pad for Transgene Stacking in Sugarcane

Background/objective

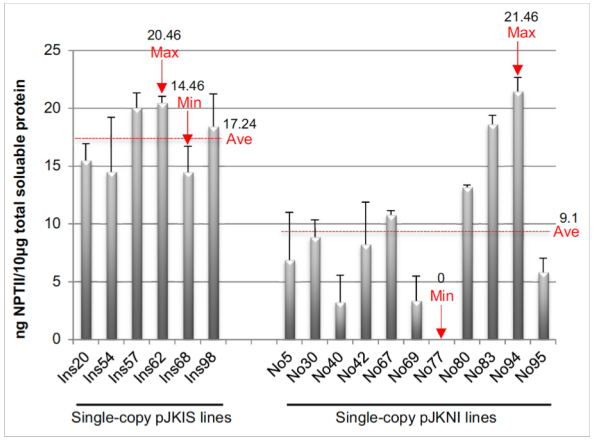
Transgenic sugarcane technology will complement breeding in the development of advanced cultivars and will benefit the global sugar and biofuel industries. In this study, we generated a highly expressed single copy locus with sequences for site specific recombination as a landing pad for transgene stacking.

Approach & Results

- Genetic constructs carrying different site-specific recombination systems and a selectable marker (*npt*II) being flanked by insulators or not, were introduced into sugarcane, followed by selection of single copy events.
- Flanking the transgene (*npt*II) expression cassette with insulators resulted in higher transgene expression, along with reduced line to line variation in single copy events as revealed by NPTII ELISA, Southern blot, and TaqMan® qPCR analysis.
- Heat inducible expression FLP and FLPe recombinases under transcriptional control of the heat shock protein promoter was confirmed by quantitative real-time RT-PCR.
- Excision of the *npt*II selectable marker gene from transgenic sugarcane lines was supported by FLPe/*FRT* site-recombination to create selectable marker free plants and was confirmed by Sanger sequencing of PCR amplicons from single copy events.



Vectors and strategies for improving transgene performance and site specific recombination.



NPTII expression levels in single copy sugarcane lines with (left) or without (right) insulators flanking the transgene cassette.

Significance

- Using insulators in constructs for generating transgenic sugarcane enhances predictable and stable transgene expression, which is critical for genetic improvement of this important feedstock for the emerging bioeconomy.
- The study provides valuable resources for future gene stacking using site-specific recombination or genome editing tools.

*Zhao, Y., et al. 2019. "Generation of a selectable marker free, highly expressed single copy locus as landing pad for transgene stacking in sugarcane." **Plant Mol Biol** . DOI: 10.1007/s11103-019-00856-4*

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