

Simultaneous knockdown of six non-family genes using a single synthetic RNAi fragment in *Arabidopsis thaliana*

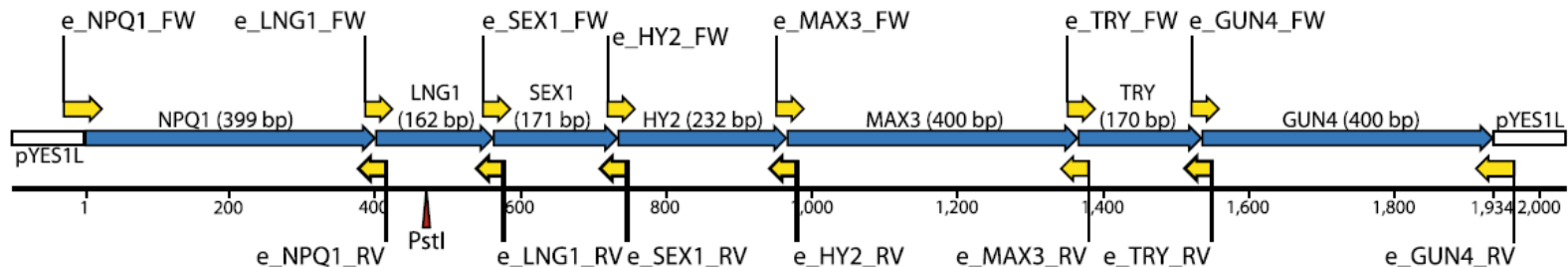
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Background: Genetic engineering resulting in successful establishment of new biochemical or regulatory pathways requires stable introduction or knockdown of one or more genes into the genome. An established way to knockdown gene expression in plants is expressing a hairpin-RNAi construct. Knockdown of multiple genes that do not share homologous sequences is still challenging and involves either sophisticated cloning strategies to create vectors with different serial expression constructs or multiple transformation events that are often restricted by a lack of available transformation markers.

Approach: Synthetic RNAi fragments targeting seven non-family genes were assembled in a single vector using a yeast assembly system.

Outcomes: Transformation of *Arabidopsis* and subsequent expression analysis of targeted genes demonstrated efficient knockdown of all target genes.

Significance: We established a simple and cost-effective method to create constructs to simultaneously knockdown multiple non-family genes. This method can be applied in plant synthetic biology as well as traditional plant and animal genetic engineering.



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