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Increasing the mobility of Cas9 transcripts, delivered by viral vectors

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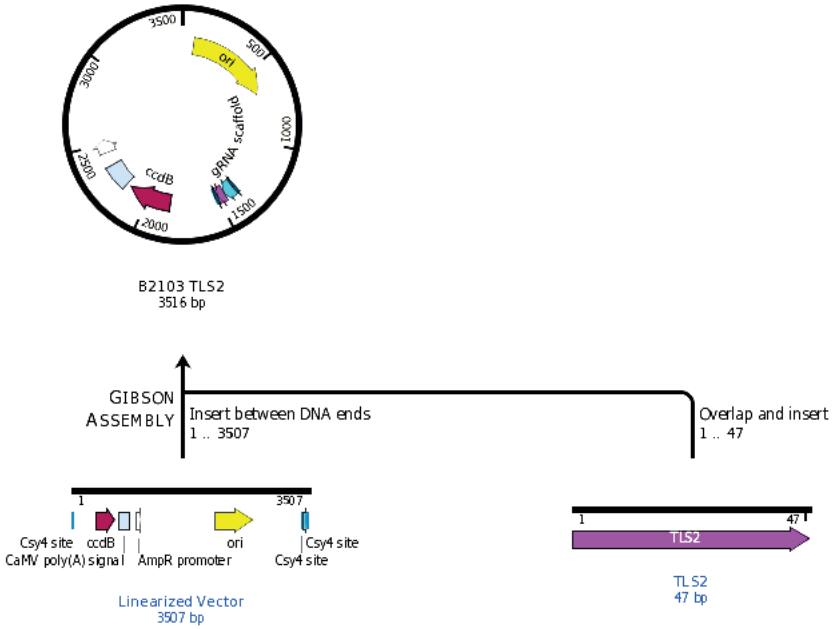
Motivation and aim: Regeneration from explants is one of the biggest technical bottlenecks in genome editing of agricultural crops. Elite lines often resist tissue culture, and it can take years to optimize conditions for successful regeneration *in vitro*. Delivery of CRISPR/Cas components using virus-derived vectors is a new, rapidly developing area of research in the field of genome editing. But plant viruses rarely infect the germline, so they usually aren't passed to the next generation via seeds. Therefore, existing approaches for virus-induced genome editing (VIGE) also require tissue culture step. This problem could be overcome by increasing the mobility of the Cas nuclease via making a fusion protein with mobility-related signal.
Results: A literature study has shown that two types of mobility-related signals are used in plant genome editing: Flowering Locus T (FT) and the tRNA-like structure (TLS). FT is a small protein which travels through the phloem from the leaves to the shoot apical meristem, where it activates flowering genes. TLS motif mimics the shape and features of a tRNA, and is present in many RNAs that move through the phloem. In VIGE they were previously fused only to gRNA scaffolds, and not Cas proteins, probably due to the limited cargo size of the viruses. However, successful grafting of crop shoots onto roots of a model plant expressing mobile Cas and gRNA, resulting in genome-edited seeds, suggests that this approach may be suitable for generating transgene-free genome-edited crops. New vectors with larger cargo capacity have also been proposed recently, including geminiviral and bunyaviral vectors.
Our aim was to fuse either FLT or TLS motifs to both Cas9 and gRNA scaffold in BeYDV vector (Addgene plasmids # 91120, #91013 and #91061) and TSWV vector (Addgene plasmids # 196325 and # 196296). Vectors were linearized by PCR, and the mobility-related sequences were cloned after Cas9 stop codon and after gRNA scaffold using Clon Express MultiS One Step Cloning Kit (Vazyme, China) as shown on Figure.



Cloning of a TLS motif into B2103 vector

The resulting vectors were cloned to *A. tumefaciens* strain EHA105, and then agrofiltrated into tobacco leaves. Our preliminary results show that Cas9 tanscripts can be discovered in other leaves, far from infiltration site. Further experiments will be carried out with gRNA targeting the PDS gene of different crops.
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