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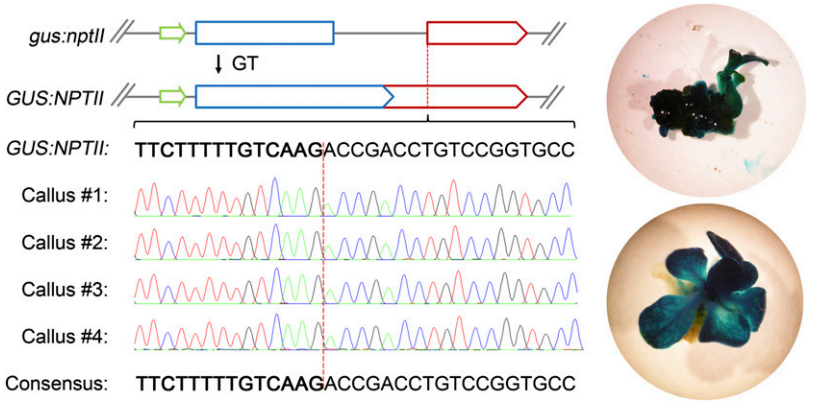
IN BRIEF

Geminivirus Vectors Deliver Reagents for Plant Genome Engineering

It never fails—you want your best scissors, your roll of duct tape, and your finest pen, but they're all in different rooms. The best tools do you no good at all if they're not together, whether it's in your house or in a plant cell. Recent work has generated many novel tools for genome engineering (reviewed in Belhaj et al., 2013). For example, engineered nucleases can generate site-specific double-stranded DNA breaks, which can be repaired by nonhomologous end joining to create large or small deletions or by homology-dependent repair using a template you provide to create specific sequence changes. These methods require delivery of multiple factors to the target cell, for example, the site-specific nuclease and the template for repair. The specificity of zinc-finger nucleases and transcription activator–like effector nucleases (TALENs) depends on the protein sequence, but specificity of the CRISPR (clustered regularly interspaced short palindromic repeats)–associated (Cas) nucleases requires a noncoding guide RNA in addition to the nuclease and the repair template. That's even harder than assembling your scissors, tape, and pen—it's a lot to deliver into a plant cell.

Current delivery systems generally use protoplasts or *Agrobacterium tumefaciens*–mediated transformation, but these methods limit downstream applications, such as plant regeneration. Here, **Baltes et al. (pages 151–163)** develop a system using geminiviruses to deliver nucleases and repair templates. Geminiviruses have small (~3 kb), circular, single-stranded DNA genomes and can move through plasmodesmata, but viruses with larger genomes may move less efficiently, or not at all. First, the authors used *Arabidopsis thaliana* to establish that a geminivirus sequence can serve as a template for homologous repair of a double-stranded break. Next, the authors removed the coat protein and movement protein genes from bean yellow dwarf virus, leaving only the sequences required for replication. They then used *Agrobacterium* to transfer the vector into tobacco (*Nicotiana tabacum*) cells and deliver various nucleases (including zinc-finger nucleases, TALENs, and the CRISPR/Cas system) and showed that these generate double-stranded DNA breaks that the cell fixes by nonhomologous end joining. Finally, they delivered a site-specific nuclease and a template to generate double-stranded breaks that the cell fixes by homology-dependent repair (see figure). By quantifying events, the authors find that virus-based delivery of template and nuclease produced higher repair frequencies than T-DNA–based delivery of the same components. Also, viruses that replicate the template sequence produced higher repair frequencies than non-replicating viruses. In addition to increasing template concentration, pleiotropic effects of the viral replication protein on the plant cell cycle may increase the repair efficiency.



Repair of a transgene and regeneration of callus and plantlets. Left, top: Original transgene containing an operon with a deletion (gray line) in *GUS* (β-glucuronidase) and *NPTII* (neomycin phosphotransferase), with *GUS* in blue and *NPTII* in red. Left, bottom: Repaired transgene with complete *GUS:NPTII* sequences and sequence traces from PCR products from regenerated calli. Right: Callus and plantlet stained for GUS activity, in blue. (*Reprinted from Baltes et al. [2014], Figure 5.*)

Geminiviruses infect monocots and dicots, making them potentially suitable for engineering many crops. Moreover, the authors regenerated modified cells into callus and plantlets (see figure), indicating that this method may enable rapid generation of plants with specific genomic modifications. Therefore, although questions about the sequence capacity of geminivirus vectors remain to be explored, this study in tobacco provides a promising first step toward modifying other crop plants. Now, about those scissors…

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