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CRISPR/Cas9-mediated gene targeting of a cytochrome P450 gene in soybean hairy roots

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Targeted mutagenesis with the CRISPR/Cas9 system has recently become a routine technique. CRISPR/Cas9-mediated mutagenesis has also been achieved in both soybean hairy roots and transgenic plants. Typically, double-strand breaks are generated by the Cas9 nuclease/guide RNA complex at specific sites, and the most common mutations are small indels from error-prone non-homologous end joining pathway. In addition, double-strand breaks could also be repaired by homology-directed recombination, albeit at a much lower frequency, especially in somatic cells. By cloning CRISPR targets in combination with donor DNA/repair templates in a Gateway T-DNA vector, we detected homology-directed recombination events in a cytochrome P450 gene in hairy roots. Toward improving gene-targeting efficiency, we cloned the identical CRISPR targets and donor DNA/templates in a germini viral vector for hairy root transformation. The recombinant germini viral vector functions as an independent replicon upon cleavage off from the T-DNA region, which may lead to higher copy number of donor DNA/template per cell and subsequently higher homology-directed recombination events.