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A CRISPR toolkit for soybean

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CRISPR-mediated genome editing is the newest and cheapest way to create targeted gene knockouts or introductions valuable to soybean breeding and gene discovery research. The technique utilizes endonuclease proteins like Cas9, guided by hair-pinned RNA sequences designed to target specific genomic regions. However, each Cas9 ortholog only cuts targets near a particular protospacer adjacent motif, meaning CRISPR lacks the target flexibility of the older TALEN technology. To overcome this limitation, four different endonuclease proteins were adapted for use in soybean. These are the Cas9 genes from *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Streptococcus thermophilus*, and the Cpf1 gene from *Acidaminococcus spp.* Each of these endonucleases recognizes different protospacer adjacent motifs, so having an assortment of endonucleases provides flexibility when choosing targets for genome editing. The *S. pyogenes* Cas9 has been the most widely used, but the others have their own distinguishing advantages. *S. aureus* Cas9 is the shortest Cas9 gene identified, and hence, is easier to use. Cpf1 from *Acidaminococcus* uses a significantly shorter RNA sequence that is particularly amenable to multiplexing, and *S. thermophilus* is a probiotic bacterium, which requires less federal oversight. Accordingly, the endonucleases were cloned into an expression cassette made of native legume sequences, which includes a nuclear localization signal from the *Glycine max* E1 flowering gene. CRISPR in the legume-sequence expression cassette was shown to be effective at editing DNA in soybean somatic embryos. The efficiency of four Cas9 orthologs were demonstrated by measuring their ability to inactivate a green fluorescent protein in hairy root cultures, relative to both previously validated and inactive controls.