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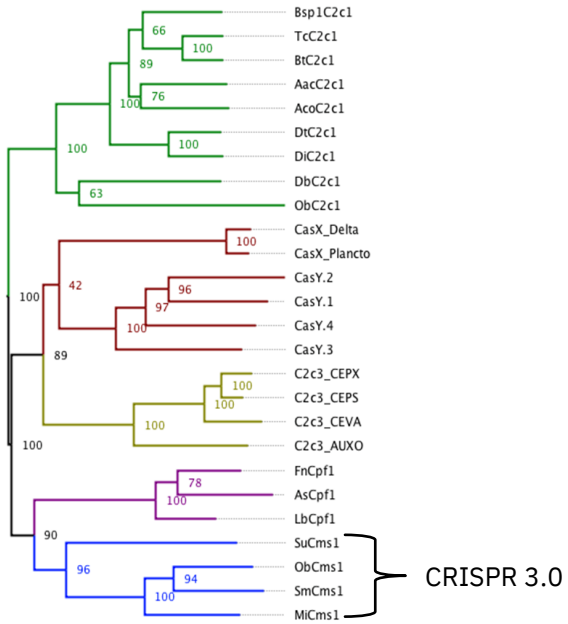
Unlocking Nature's Potential.

Advances in genome editing technology have the potential to dramatically change the landscape of agriculture, industrial biotechnology, and human health. The discovery of bacterial CRISPR nucleases dramatically decreased the technical and cost barriers associated with genome editing relative to the first generation of genome editing tools (TALENs, Meganucleases, and Zinc Finger Nucleases). The simplicity and high efficiency of CRISPR systems have led to rapid progress in the use of these new applications of genome editing across basic and applied sciences. However, the patent rights to the early CRISPR technologies have been highly contested, in many cases stifling innovation and broad adoption. In addition, while several CRISPR systems have been identified, only a limited number of nucleases within each system are actually capable of efficient editing across target organisms. While many CRISPR/Cas9 and Cpf1 (CRISPR 2.0) nucleases are known, only a limited number have been shown to function at a high efficiency and each nuclease has technical and host-specific limitations. There is an urgent need for more options in the CRISPR genome editing space to further drive innovation and greater improvement in the sustainability and resiliency of our food and agricultural system.

The scientific team at Benson Hill Biosystems (BHB) is a leader in the discovery of novel CRISPR nucleases and the application of genome editing in plants. BHB scientists have previously uncovered new CRISPR 2.0 nucleases with unique properties and applications. **Recently, the BHB scientific team, using its advanced *in vivo* nuclease screening process, has discovered a new group of CRISPR nucleases that are functionally active *in vivo* and capable of generating genome edited plants.** This new CRISPR system is named Cms1 (CRISPR from *Microgenomates* and *Smithella*) after the bacterial phyla and genera these nucleases were originally isolated from. BHB is calling this nuclease system CRISPR 3.0 as it is a major advancement in genome editing and provides clear advantages over existing technologies.

Figure 1: Phylogenetic Tree of Type V CRISPR Nucleases



These Cms1 proteins are Class 2 Type V CRISPR nucleases and are evolutionarily distinct from other known nuclease systems (see Figure 1 for a phylogenetic tree). Similar to other Type V CRISPR nucleases, the Cms1 proteins have three RuvC domains, but their organization within the protein and amino acid sequence motifs are clearly differentiated from the other nuclease systems. The RuvC domains contain the active sites involved in the enzymatic cutting of the target DNA and are a useful signature for identifying and classifying these CRISPR nucleases. In addition, these nuclease proteins are significantly smaller than most CRISPR/Cas9 and CRISPR 2.0 proteins, and the lack of a requirement for tracrRNA results in an extremely compact system for delivery to cells for genome editing.

Figure 2: Examples of Targeted Deletions using CRISPR 3.0 Nucleases

Nuclease	PAM	Target Sequence	Indel Size
WT		ACAAGAAGAACTCACCTTTCTGGAGCAACACCTGAAGGAAGGCTTGATGAGCAAGTGCGGCAGCAAAG	
Sm		ACAAGAAGAACTCACCTTTCTGGAGCAACACCTGAAGGA--------TGAGCAAGTGCGGCAGCAAAG	-8
Su		ACAAGAAGAACTCA----------GCGGCAGCAAAG	-42
Mi		ACAAGAAGAACTCACCTTTCTGGAGCAACACCTGAAGGA--------TGAGCAAGTGCGGCAGCAAAG	-8
Ob		AC----------AAG	-65

The Cms1 nuclease from *Smithella* sp. SCADC (SmCms1) and its respective crRNA guide was initially screened for genome editing in rice using biolistic delivery. This nuclease was shown to generate deletion mutations at multiple sites within the rice genome with several AT rich PAM sites. This was the first demonstration of genome editing from a Cms1 nuclease in any system. The effectiveness of this nuclease led the BHB team to discover additional Cms1 nucleases (see Figure 2 for examples of *in planta* edits). **A total of five Cms1 nucleases were tested for the ability to generate targeted mutations in the rice genome. All the tested nucleases were capable of editing at an effective frequency.** This rate of nuclease success in an *in vivo* system is unprecedented in CRISPR nuclease discovery research, and additional work with these nucleases can be expected to further improve editing efficiencies. These nucleases are currently being applied across multiple crop plant species in BHB's and partners' product development pipeline.

CRISPR 3.0 nucleases are a clear improvement over existing technology and increase the options available to researchers across industries. These nucleases are smaller than most CRISPR/Cas9 and 2.0 nucleases and have a simple RNA structure, dramatically simplifying delivery of the core genome editing reagents. In addition, the efficacy of this nuclease family in an *in vivo* system is substantial and suggests translatability across target systems.

BHB received an issued patent from the US Patent and Trademark Office on CRISPR 3.0 on February 20th, 2018. BHB is currently licensing CRISPR 3.0 to commercial and academic partners around the world to leverage the natural genetic diversity of plants and to develop crops and traits that benefit both farmers and consumers. In addition to providing a technology license, BHB is actively involved in helping partners to achieve their product development goals through knowledge transfer and technical support. *For more information regarding the CRISPR 3.0 technology or licensing options, please contact:* genomeediting@bensonhillbio.com.

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