Cas9-mediated genome editing in bacteria using an endogenous CRISPR system

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RNA-guided immunity against invasive genetic elements is encoded as clustered regularly interspersed short palindromic repeats (CRISPR) and their CRISPR-associated sequences (cas genes), which constitute the prokaryotic adaptive immune system. Characterization of CRISPR-cas systems has yielded applications in typing and strain detection, engineered interference against mobile genetic elements, and programmable genome editing tools in a variety of organisms. *Streptococcus thermophilus* is an industrially prolific dairy starter culture, which harbors two Type IIA CRISPR-cas systems active in acquisition of new bacteriophage memory sequences and interference against viruses upon subsequent encounters, likely due to the severe and continuous exposure to phage infections in this environment. Interference mediated by Type IIA systems results in double-stranded DNA cleavage mediated by Cas9, the signature endonuclease gene of Type IIA systems. Furthermore, CRISPR-cas can be programmed for genome editing through specific and directed self-targeting of chromosomal loci. Here we show that the CRISPR1 and CRISPR3 systems in *S. thermophilus* LMD-9 can be repurposed for site-directed genome editing. Guidance of Cas9 to target chromosomal *lacZ* is accomplished through design and transformation of a vector containing a repeat-spacer-repeat array corresponding to the N-terminal sequence of *lacZ* with a protospacer adjacent motif downstream of the target sequence, which is necessary for target recognition and cleavage. To further engineer a specific genome edit, a homologous template excluding the target sequence in *lacZ* was co-transformed with the targeting plasmid, resulting in a 65-bp, out of frame deletion in double crossover recombinant clones which employed the template to circumvent self-targeting.