Improved thermophilic genome editing tools for engineering *Clostridium thermocellum*

U.S. DEPARTMENT OF ENERGY | Office of Science

Background

- Discovery and validation of functional thermophilic genetic tools are needed to accelerate genome editing in the cellulolytic chassis bacterium *Clostridium thermocellum.* Standard tools do not function at thermophilic temperatures.

Approach

- Characterized the native Type I-B CRISPR-Cas system as well as evaluated thermophilic Type II systems to enable CRISPR-based genome editing
- Tested potential thermophilic homologs of Lambda Red and RecE/T recombineering systems to improve homologous recombination efficiencies

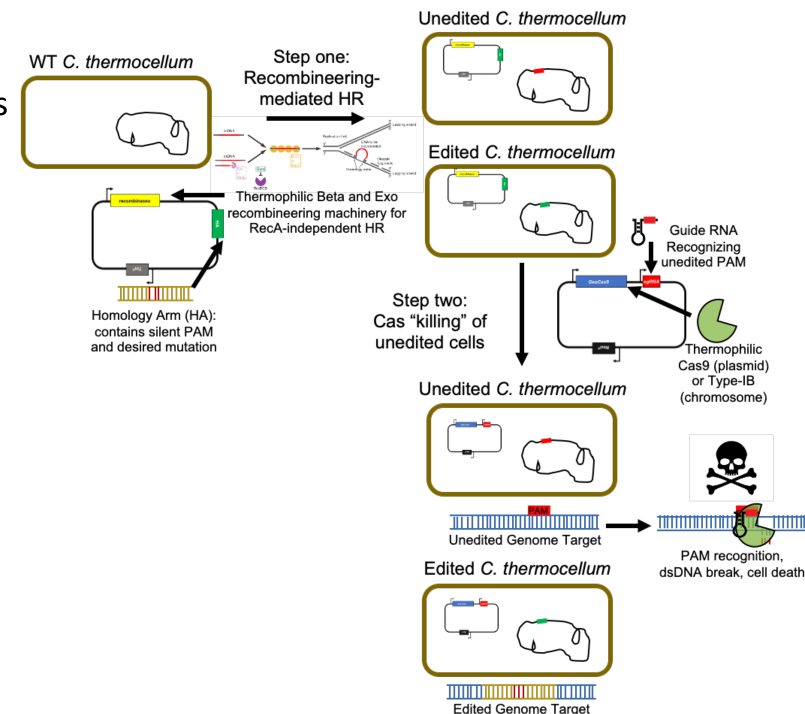
Outcome

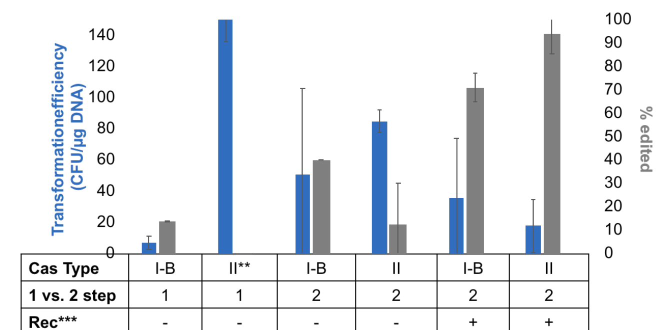
- Identified functional protospacer adjacent motif (PAM) sequences for efficient targeting for cutting by the Type I-B system
- A Type II system previously isolated from *Geobacillus* was found to be highly effective for genome targeting and cutting
- Lambda Red beta/exo homologs from *Acidithiobacillus caldus* improved homologous recombination efficiencies by 35-fold
- Developed a markerless, 2-step system for generating mutants with up to 94% efficiency

Significance

- These tools allow for accelerated specifically targeted mutant generation (knockouts, knockins, SNPs) in *C. thermocellum* and potentially other thermophiles

2-step CRISPR-based Genome Editing





Cas Type	I-B	II**	I-B	II	I-B	II
1 vs. 2 step	1	1	2	2	2	2
Rec***	-	-	-	-	+	+

*Correct transformants = TE * % edited colonies
** Cas9 nickase variant was used
***Rec = *A. caldus* Exo/Beta recombineering machinery

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Walker, J.E., et al., "Development of both type I-B and type II CRISPR/Cas genome editing systems in the cellulolytic bacterium *Clostridium thermocellum*", Metabolic Engineering Communications, 10, 2020. https://doi.org/10.1016/j.mec.2019.e00116

March 2020