

Thermophilic site-specific recombination system for rapid insertion of heterologous DNA into the *Clostridium thermocellum* chromosomes

Background/Objective

- Metabolic engineering of *Clostridium thermocellum* is limited by a lack of efficient genetic tools and slow strain construction methods. Our goal was to simplify and accelerate DNA insertion into the chromosome to enable higher throughput strain construction.

Approach

- We developed a thermostable Serine recombinase Assisted Genome Engineering (tSAGE) approach for gene insertion in *C. thermocellum* using a site-specific recombinase from *Geobacillus* sp. Y412MC61.

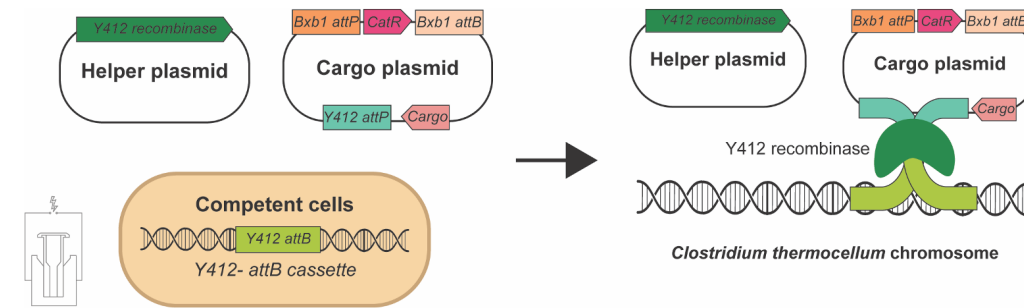
Results

- tSAGE-based chromosomal insertion of plasmid DNA occurred at similar transformation efficiency to a replicating plasmid, with a maximum of 5×10^3 CFU/ μ g.
- We chromosomally integrated and characterized 17 reporter genes, 46 constitutive promoters of varying strengths, 4 inducible promoters, 5 riboswitches, and a ribosome binding site spacer library in *C. thermocellum*.
- Xylose- and 2-aminopurine-inducible gene expression circuits show low background signal and high inducibility.

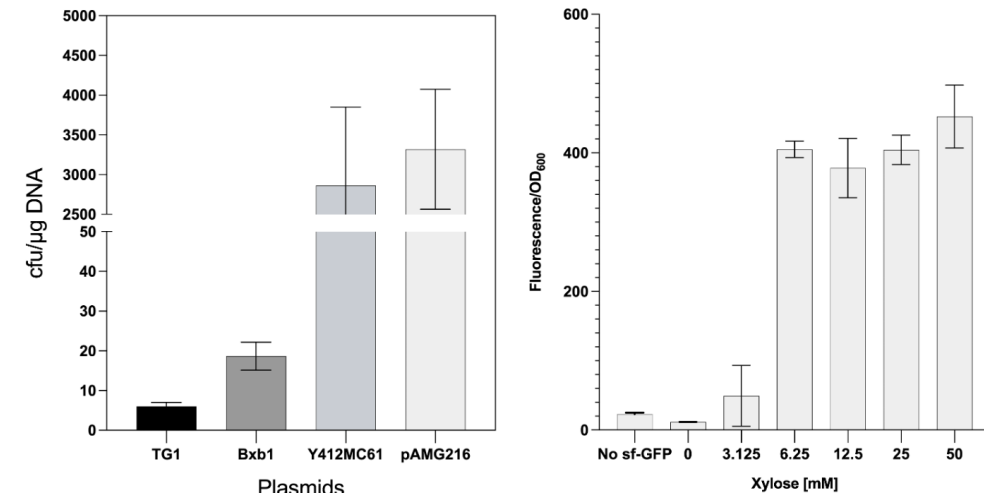
Significance/Impacts

- tSAGE is a fast and efficient single-step method for inserting heterologous DNA into the *C. thermocellum* chromosome, taking only two to three days to complete. tSAGE has thus opened the possibility for high throughput strain construction in *C. thermocellum*.
- The genetic toolbox developed here will aid in accelerating *C. thermocellum* strain engineering for producing sustainable fuels and chemicals from plant biomass.

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Thermostable Serine recombinase Assisted Genome Engineering. After inserting an attB “landing pad” into the *C. thermocellum* chromosome, a recombinase-expressing helper plasmid can insert the cargo plasmid into *C. thermocellum* chromosome.



Left: tSAGE transformation efficiency is similar to using a replicating plasmid. **Right:** tSAGE was used to develop a chromosomally integrated xylose inducible promoter.