SAGE Technology Developed for Engineering Non-model Microbes Licensed by Two Companies

Background

- Developing precise, efficient, and high-throughput genetic engineering tools is critical, especially for non-model microorganisms.
- Serin recombinase, a site-specific recombinase, is a single subunit enzyme that can be used for genetic engineering for most organisms since it does not require host proteins.

Approach

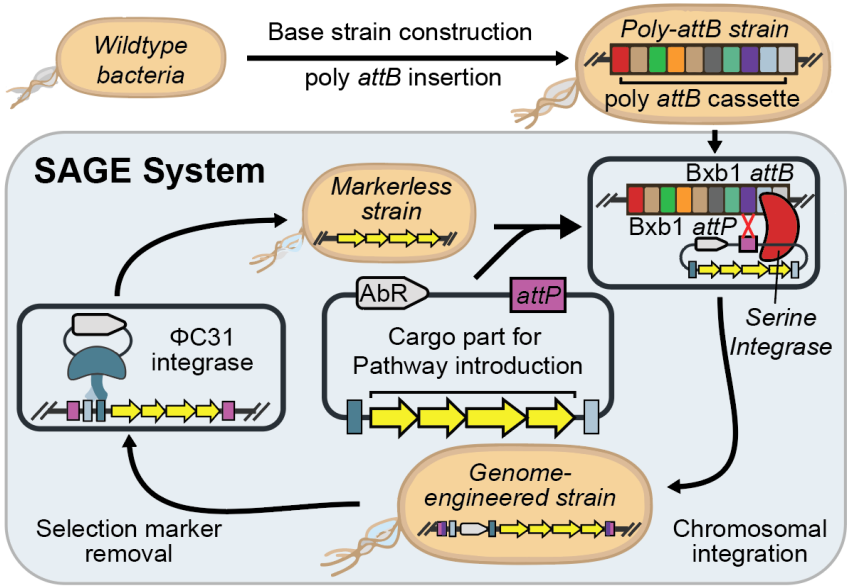
- We developed Serine recombinase-Assisted Genome Engineering (SAGE) toolkit consisting of:
  - "landing pad" containing ten orthogonal recombination sites (unique 10 attB sites) and
  - high-efficiency serine recombinases.
  - These are in nonreplicating and conditionally replicating plasmids with multiple options for selection and counterselection markers.
- First, the "landing pad" is integrated into the chromosome of a microorganism. Second, a plasmid containing the desired genetic "cargo" (ie, promoter libraries) is transformed and transiently expressed a recombinase. This can be done iteratively.

Results

- We demonstrated robust SAGE performance in five bacteria representing multiple taxonomic groups including an undomesticated sorghum rhizosphere isolate.
- We demonstrated that genomic integration of DNA with SAGE generates stable cell lines expressing heterologous genes/libraries.

Significance

- The SAGE system is simple and has broad applicability including non-model organisms; other DOE projects now are using SAGE tools extensively.
- SAGE reliably generates stable cell lines which is critical for industrial application; SAGE tools have been licensed by (1) Kiverdi for use in *Cupriavidus necator* and (2) Cemvita in FY23.



Overall process for SAGE-based strain engineering

Companies and other DOE projects using SAGE

Elmore JR, et al. *Science Advances*, 2023. 9(10): p. eade1285. DOI: 10.1126/sciadv.ade1285