Methods for Metabolic Engineering of Thermoanaerobacterium saccharolyticum

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Background

 Thermoanaerobacterium saccharolyticum is an anaerobic thermophilic bacterium that can ferment C6 sugars, C5 sugars, and C5 sugar polymers. Techniques for genetic modification of this organism have been developed over the last decade but were published across many different periodicals.

Approach

- Best practices were summarized in this chapter for several important techniques, including:
 - 16S analysis
 - Positive and negative selection systems
 - Natural competence transformation protocol
 - Chromosomal modification by homologous recombination
 - Phenotype analysis by fermentation and enzyme assays
 - Heterologous protein expression

Significance

• A single resource for techniques for metabolic engineering of *T.* saccharolyticum will make it easier for researchers unfamiliar with this organism to start using it.

Single-construct Gene insertion Gene deletion Homologous recombination target Description of key steps for chromosomal Internal region of gene target (Int) modification by either gene insertion or deletion in T. saccharolyticum ---- Homologous recombination event Primer

Hon, Shuen, Liang Tian, Tianyong Zheng, Jingxuan Cui, Lee R. Lynd, and Daniel G. Olson. 2020. "Methods for Metabolic Engineering of *Thermoanaerobacterium saccharolyticum*." In Synthetic and Systems Biotechnology, 3:21–43. doi:10.1007/978-1-0716-0195-2 3.

