j5: DNA assembly design automation for metabolic pathways

Hillson, N.J.
Joint BioEnergy Institute, U.S.A.

Introduction

The production of clean renewable biofuels from cellulosic starting material requires concerted feedstock engineering, deconstruction of plant matter into simple sugars, and microbial fermentation of the sugars into biofuel. These three efforts share significant molecular biological challenges, including the construction of large enzymatic libraries (e.g. vast collections of glycosyl transferases, cellulases, and efflux pumps), the generation of combinatorial libraries (e.g. multi-functional enzyme domain fusions; variations in copy number, promoter and ribosomal binding site strength), and the concurrent assembly of multiple biological parts (e.g. the incorporation of an entire metabolic pathway into a single target vector). The recent emergence of foundational technologies, including repositories of biological parts, biological computer-aided design (BioCAD) tools, and automated DNA-assembly methods, promises to greatly facilitate the execution of these tasks and to increase the scope of what is readily experimentally achievable.

Results and Conclusions

We have developed two on-line software tools, j5 and DeviceEditor, that automate the design of sequence agnostic, scar-less, multi-part assembly methodologies and translates them to robotics-driven protocols. Given a target library to construct, the software provides automated oligo, direct synthesis, and cost-optimal assembly process design, and integrates with liquid-handling robotic platforms to set up the PCR and multi-part assembly reactions. This work reduces the time, effort and cost of large scale cloning and assembly tasks, as well as enables research scales otherwise unfeasible without the assistance of computer-aided design tools and robotics.

This work conducted by the Joint BioEnergy Institute was supported by the Office of Science, Office of Biological and Environmental Research, of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.

Author publications

1. Iniesta, A.A.*, Hillson, N.J.*, and Shapiro, L. (2010) Polar Remodeling and Histidine Kinase Activation, Which Is Essential for *Caulobacter* Cell Cycle Progression, Are Dependent on DNA Replication Initiation. *J. Bacteriol. 192*, 3893-3902.
2. Iniesta, A.A.*, Hillson, N.J.*, and Shapiro, L. (2010) Cell pole-specific activation of a critical bacterial cell cycle kinase. *Proc. Natl. Acad. Sci. U.S.A. 107*, 7012-7.
3. Christen, B.*, Fero, M.J.*, Hillson, N.J., Bowman, G., Hong, S., Shapiro, L., and McAdams, H.H. (2010) High-throughput identification of protein localization dependency networks. *Proc. Natl. Acad. Sci. U.S.A. 107*, 4681-6.