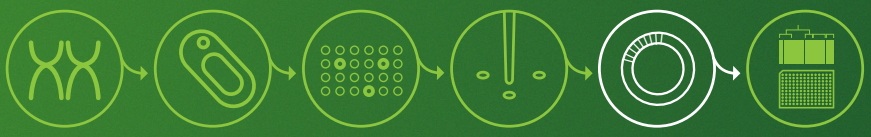
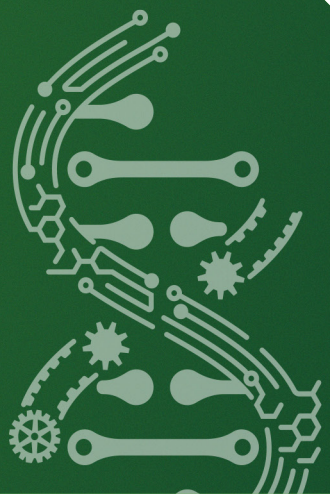
Hudson ROBOTICS

SYNTHETIC BIOLOGY SERIES

Plasmid Preparation





Synthetic biology is an iterative process which borrows the "Design-Build-Test-Learn" (DBTL) paradigm from engineering. The ultimate goal in synthetic biology is to make an organism that is *better* at something specific, whether investigators are starting *de novo*, or starting out with an existing organism and designing improvements that will turn it into something more useful.

Plasmid Prep Requirements

When introduced into a host organism by transformation a plasmid will be replicated, creating numerous copies of the DNA fragment under analysis. Functional evaluation may include hundreds of clones, so the process of plasmid preparation must proceed at rate that suits the investigator's timetable for this stage in the process. The plasmids produced must be of sufficient quality and purity that the downstream functional assays can be performed without compromise. Finally, the process of plasmid preparation must produce the plasmids in sufficient quantities, from low milliliter starting volumes of culture, to allow several parameters to be measured in the functional assay. Especially in early cycles, the back-trace to the best clones with which to start the next iteration may depend on multiple functional parameters considered in union.

USDA's Experience

In 2011, the USDA found with rapid gene assembly and mutagenesis strategies, gene open reading frames could be synthesized, cloned into plasmid vectors, transformed into yeast strains, and screened to identify those clones producing proteins that give increased fuel production and enable use of biomass as a feedstock. They found that a 1.3 mL starting bacterial culture yielded 5.4 µg of plasmid in an automated process using systems from Hudson Robotics.[1]

Their 4x96 well plate process, including storage, pelleting of bacterial cultures, and sterile tip transfer produced plasmids in about six hours. The quality and quantity of plasmids enabled DNA sequencing, *in vitro* transcription and translation, and transformation of bacterial and yeast strains for protein expression. Significantly, in a measure used by many to evaluate the performance of robotic systems, the quality of plasmid preparation was equivalent to manual operation. The investigators at USDA used this robotic system to optimize cellulases, bioinsecticides, and xylose utilization by yeast, and for catalyzing the production of biodiesel from corn oil.

Automation

Plasmid preparation done manually is low-throughput and expensive, with several steps per sample and hundreds of samples. It is therefore, a target process for automation. Automation yields a hands-free workflow with none of the errors associated with manual processing, rapid plasmid preparation, and quality and quantity equivalent to manual preparation. For investigators wishing to automate plasmid preparation, Hudson Robotics offers a Plasmid Prep Workcell which consists of the SOLO automated pipettor, a FilterPress™ automated 96-well filterplate position pressure manifold, and a microplate stacking nest. High-throughput capability can be enhanced by adding a PlateCrane robotic arm and microplate hotels. The Plasmid Prep Workcell can stand alone or be integrated with other components as part of an automated synthetic biology workstation.

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1. Hughes, S. R., Butt, T. R., Bartolett, S., Riedmuller, S. B., & Farrelly, P. (2011). Design and construction of a first-generation high-throughput integrated robotic molecular biology platform for bioenergy applications. *JALA: Journal of the Association for Laboratory Automation, 16*(4), 292–307. https://doi.org/10.1016/j.jala.2011.04.004

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