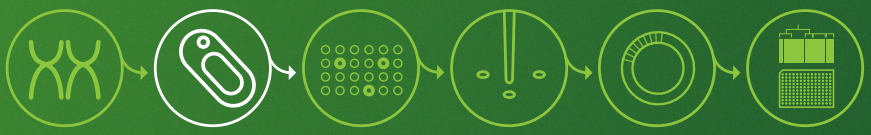
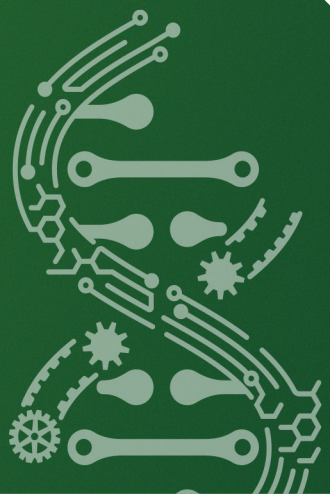
Hudson ROBOTICS

SYNTHETIC BIOLOGY SERIES

Transformation





Advances in the tools of liquid handling and other forms of automation are driving greater utilization of synthetic biology. Most people have heard by now of synthetic hamburger available in supermarkets, but synthetic biology is now also used to construct hormone biosensors[1], clinically important antibiotics[2], medicines, cosmetics and even jet fuel[3]. Automation has led the way and affects all phases of the synthetic biology process. Part of a series on synthetic biology, this article from Hudson Robotics is about the second stage of the process, transformation.

Transformation for Synthetic Biology

Synthetic biology depends on transformation, the introduction of synthetic genes into the genome of the host organism. While transformations, such as those involved in horizontal gene transfer, occur in nature, synthetic biologists must get cells to participate in the transformations they want to have happen. This involves producing competent cells, that is, cells that will take up the synthesized DNA, express it, and replicate it as part of their own genomes.

Advances in Transformation

Competent cells must be manufactured whether the intended host organism is prokaryotic or eukaryotic. Transformation protocols exist for bacteria, yeast, plants, and fungi. Transformation of animal cells, properly called “transfection,” is often transient and is used for gene expression studies. A stable transfection of animal cells is much more difficult to achieve.

Entire organisms have yet to be created from scratch, although entire genomes, such as hepatitis C[4], poliovirus[5], and the bacteriophage φX174[6] have been transformed into competent cells.

Mechanisms of Transfection

Competent bacterial cells are made for chemical transformation by incubating them in a solution containing divalet cations, usually in the form of $CaCl_2$, under cold conditions before exposing the cells to a heat pulse. This weakens the cell surface making possible to DNA to enter the cell. The heat pulse creates a thermal gradient across the cell membrane which forces DNA into cells. For electroporation-based transformation of bacterial cells, the cells are briefly shocked, creating holes in the membrane through which DNA can enter.

In yeast, competent cells are made by enzymatic digestion (50, Wikipedia), or agitation of the cells with glass beads (52). Yeast cells also respond to electroporation, which allows DNA or RNA to enter through holes in the cell membrane.(50)

Among the methods for plant transformation is transfection, wherein a virus that affects the plant is engineered to infect the plant cells with the desired sequence. Plant embryos are the target of gene guns, which shoot tungsten or gold particles coated with the transforming genetic material. Initially, gene guns used .22 caliber cartridges borrowed from nail guns, but modern gene guns use compressed helium as a propellant. More conventionally, electroporation can be used to create competent plant cells.

The transformation mechanisms for fungi are similar to those of plants. However, some fungi are dikaryotes, so the percentage of transformed nuclei decreases with each sporulation.

Hudson Robotics Automated Transformation

Automation increases throughput and reduces errors. Hudson's synthetic biology workstation automates the entire pipeline, from gene assembly through plasmid prep. The instruments composing the workstation can be used by themselves, but for maximum time savings and throughput, researchers combine them into a larger synthetic biology workflow.

Hudson has already developed a number of automated systems that transform bacterial cells using a heat-shock based protocol. These systems can be expanded to include other bacterial transformation protocols, and related automation protocols for yeast, plants, and fungi.

With Hudson Robotics automation, large numbers of organisms can be initiated into the transformation protocol. This is important, because transformation is a matter of percentages. Not all organisms are transformed by transformation protocols, and not all transformed organisms carry the desired clone. Clones carrying markers indicating successful transformation are selected by gridding and picking, which are the next steps in Hudson Robotics' workflow for synthetic biology.

Citations

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