

Science Education Collection

Synthetic Biology

URL: <https://www.jove.com/science-education/5792>

Abstract

This video presents synthetic biology and its role in bioengineering. Synthetic biology refers to the methods used to genetically modify organisms in order to make them capable of producing large quantities of a product. This product could be a protein that the cell already makes, or a new protein that has been encoded in a newly-inserted DNA sequence.

Here, we discuss how an organism's genetic material is modified using transformation or transfection. Then, the process is shown in the laboratory, and the applications of the technique discussed.

Transcript

Synthetic Biology is a field that combines biology and engineering to create or redesign biological entities organisms or pathways, the idea is similar to chemical synthesis in chemistry where well known reactions are used to synthesize new chemical compounds, the end goal can vary, from the creation of new biological drug molecules to the modification of organisms to make them able to break down wastes. This video discusses the basic principles of synthetic biology and some techniques used to construct biological modules. Finally, we present some real world applications of this evolving field.

The primary objective of this emerging field, is to use biology and bio-engineering as a tool to create new molecules and organisms. Much like an electrical engineer, creating a functional circuit from individual electrical components, a key goal of synthetic biology, is to create a programmable microorganism from scratch using individual cell components. However, this is still far from achievable at this point, primarily because biological processes are less understood, this goal is made more achievable by recent advances, such as next generation DNA sequencing, using DNA sequencing researchers can identify the functions in DNA sequence in specific genes in organisms that possess certain desirable traits. Then, the exact DNA sequence can be synthesized in large amounts and then used to genetically modify a cell using transfection. Transfection is the process of inserting genetic material such as DNA or RNA into mammalian cells. When performed on bacterial cells, the technique is called transformation. In this process, DNA is often complexed with positively charged carrier molecules or condensed within a positively charged liposome or polymer particle such as polyethylenimine. The positively charged complex attaches to the negatively charged cell membrane and then enters the cell via endocytosis, which is a process by which molecules enter a cell via a membrane bound vesicle called endosomes. Once inside the cell, the genetic material leaves the endosome and eventually enters the nucleus, where the cell's machinery is able to make mRNA and then protein from it. Now that we have introduced the basics of synthetic biology, let's take a look at some transfection techniques commonly used in the laboratory.

Electroporation is a technique that involves the use of an electrode to create tiny pores in the cell membrane, thereby allowing DNA to pass through. First, the bottom of each well of a 48 well plate is coated with 250 microliters of antibody and buffer with calcium and magnesium. The plates are then incubated at 37 degrees. Next, the RNA is prepared for transfection. One microliter of RNA stock solution is aliquoted into a microcentrifuge tube for each separate transfection. With the tubes remaining on ice. The antibody coated plates are then washed with buffer before the cell media is added. The cells are detached from the bottom of the tissue culture wells, pooled in a centrifuge tube pelleted and resuspended. The cells are counted and their viability assessed. Then a small volume of cells is added to each aliquot of RNA the cells in RNA are then loaded into a pipette electrode tip and electrolytic buffer added to a glass cuvette. The cuvette is placed into the holder, and the pipette electrode placed in the cuvette. The cells are electroporated using a pulsed voltage of about 1500 volts. After the electroporation is complete, the cells are mixed with a cell culture media in a culture plate and either used or stored.

Another technique is the heat shock method, which uses heat to create openings in the cell membrane. First, the appropriate media and agar are prepared and sterilized. Then the cooled agar containing antibiotic is poured into plates and allowed to cool to room temperature. Next a water bath is set to 42 degrees Celsius and the chemically competent cells thawed on ice. One to five microliters of one nanogram per microliter of cold plasmid is added to the thawed cells and mixed gently. Then the cell and plasma mixture is returned to the ice for 30 minutes. After this incubation on ice, the cell mixture is placed in the water bath to heat shock it for 30 seconds. The cell and plasmid mixture is then immediately placed on ice and fresh media added. Then the cell mixture is placed in a shaking incubator at 37 degrees Celsius for one hour, so that the cells can recover. Next the cells are cultured on the agar plates by adding 20 to 200 microliters of the cultured bacteria to the plate and then spreading. The plates are then incubated overnight. The following day, the agar plates should have colony growth indicating that the cells have taken in the plasmid. These colonies can now be used for further experimentation.

Now that we have introduced some common transfection and transformation methods, let's take a look at some applications of this novel field. Genetically engineered bacteria could be used for environmental cleanup like degrading oil residues. Using synthetic biology techniques custom organisms could be engineered to break down specific environmental pollutants. This could incur lower cleanup costs than typical labor intensive cleaning methods. Synthetically constructed molecular scaled biological systems could also be created to diagnose and treat specific diseases like cancer. These organisms could be created to respond to the characteristic signatures or antibodies of cancer cells. Also, they could aid in the treatment of infected cells by programmed targeting.

You've just watched Jove's Introduction to Synthetic Biology. You should now be familiar with the goals of this novel field and some techniques used to enhance and eventually create organisms to combat current world problems. Thanks for watching.