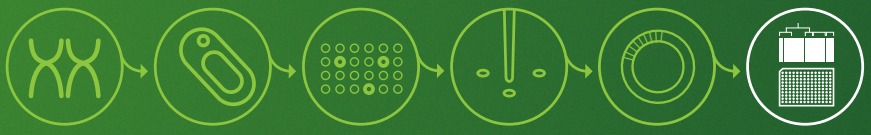
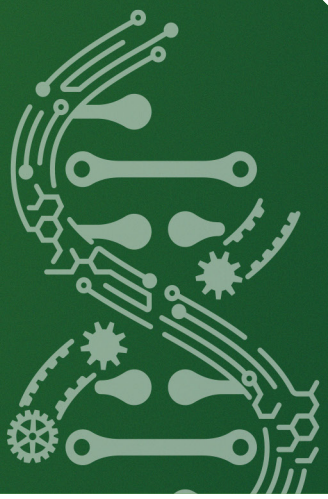
Hudson ROBOTICS

SYNTHETIC BIOLOGY SERIES

Expression Analysis and Screening





The conventional approach to strain improvement involves mutagenesis, followed by expression analysis and screening of mutants to find the most productive clones. Expression analysis and screening for the improvements induced by the mutation leads to either repetition of the mutagenesis process, or termination of the experiment and collection of the clone, or clones, with the desired properties. Regardless, the molecular basis for increased productivity covers many loci and is rarely investigated or discovered.

An alternative is the synthetic approach using recombinant DNA technology. This approach involves cloning the desirable gene or gene cluster, followed by DNA sequencing and bioinformatics analysis of the sequence to identify the structural and regulatory regions. These regions can be modified or replaced with different promoters to increase product yield.

The "Test" Phase of the Process

Synthetic biology is often depicted as a circular process, and expression analysis and screening are more likely a point in the DBTL (design, build, test, learn) cycle, in which results are assessed, and the outcomes are used to inform the details of the next rotation.

In the "test" component of the cycle, sequence-verified constructs are transformed into a cell and assayed for function. Depending on the result, changes to the construct design can be made, and further iterations of the test cycle repeated, until a DNA construct is obtained that produces the desired function. This "DBTL" cycle has become the backbone of synthetic biology and has put a premium on automated processes and methods that can shorten the development cycle and increase throughput.

Synthetic Genomics' Solution

Expression analysis and screening can be handled as an off-line process, as in the case of the "digital-to-biology converter."[1] The researchers who built the digital-to-biology converter used Hudson Robotics instrumentation to automate the front end of the process by creating a system that would construct clones, ligate plasmids, and transform bacteria to produce the desired product. Their synthesis of GFP (green fluorescent protein) was visualized by SDS-PAGE, and they determined the DNA sequence responsible by Sanger sequencing. They used a similar process to verify abatanercept and trastuzumab production and noted that these forms of the molecules were inactive because the expression system they used did not induce defined folding.

Other researchers have integrated the processes of expression analysis and screening into the synthetic biology process to produce a system that is capable of all but the "learning" part of the DBTL cycle, expressed as inputs into an oligonucleotide library design. In addition to handling the laborious, repetitive aspects of laboratory operation, such as pipetting and microplate stacking, robotic automation applies precision and accuracy to the processes and eliminates sources of human error. In addition, the ability to trace a clone from its screening result all the was back through its incubated plating or gridding images and its original position and DNA sequence in the clone library is essential to the learning process in synthetic biology.

Systems Integration at the USDA

An early instance of a total automation solution is the integrated plasmid-based robotic workcell of the USDA (United States Department of Agriculture)[2] designed using Hudson Robotics instrumentation. These researchers used a high-throughput growth assay, a binding assay, or a biochemical assay to identify strains with desired qualities. Due to the large numbers of samples to be tested, the high-throughput processes utilized 96-well or 384-well microplates, with each well containing a different sample to test, and instrumentation that automated the processing of the microplates. However, even concentrating the test format into a microplate footprint required processing of potentially thousands of microplates before successful outcomes were achieved.

Expression analysis and screening is an extension of plasmid-based functional proteomics, which requires rapid plasmid preparation methods to obtain adequate quantities of high-quality plasmid to conduct all required steps in the process, from creation of plasmid libraries to functional testing of expressed proteins. Plasmid libraries are composed of several thousand unique genes, so automation is essential.

The ideal system, said the authors of the paper describing the system, was "an automated integrated programmable platform capable of producing cDNA libraries, colony picking, isolating plasmid DNA, transforming yeast and bacteria, expressing protein and performing appropriate functional assays."

To accomplish the assay with yeast ORFs (open reading frames), plasmids were collected and clonase reactions were performed to move the yeast ORF insert into *in vitro* and *in vivo* expression vectors in yeast and bacteria. Expressed proteins were spotted by the pipette arm of the liquid handler on assay microplates, moved into position on the liquid handler deck and assayed for function. The researchers noted that in addition to the agar plate assay coupled with the imaging system described, the robotic workcell could be programmed to perform enzyme assays in solution and collect the results with a UV/VIS plate reader.

Another example of an integrated operation scripted for the plasmid-based robotic workcell used the functional protein assay in a multifunctional setting for high-throughput screening of mutants of CelF (CUGBP Elav-like family) to identify plasmids containing optimized clones expressing mutants with improved activity at lower pH. The automated track-based operation included picking of colonies into a 96-well plate, inoculation from the 96-well plate into four 24-well deep microplates in quadruplicate, *in vitro* protein production and evaluation of the expressed protein for temperature and pH stability using an azo-CMC plate assay.

For pH testing, the study's authors used azo-CMC (carboxymethyl cellulose dyed with remazolbrilliant blue) microplates at pH 5.8, 5.0, 4.5, and 4.0, loaded into the passive stacker, delidded them with the gripper arm, moved them to the liquid handler, spotted the protein, and moved the microplates into the incubator.

For temperature stability testing, they placed microplates containing protein in an incubator at 50 °C or a thermal cycler. The heated proteins were then spotted and moved to an incubator.

All the microplates were incubated for 10 hours at 37 °C then moved out of the incubator and digitally photographed. The activity of the protein mutants was determined by their reaction with azo-CMC on the microplates. All microplate manipulations were accomplished in a hands-off, automated fashion.

Customer Solutions

Hudson Robotics customers use a number of systems for expression analysis and screening. Among them are microplate handlers, microplate tracks and stacks, pipetting platforms, microplate heaters, shakers, and peelers, incubators, bar code readers, and software that makes all these components work together in a coordinated robotic system.

Our view is that synthetic biology is based on the same principles of engineering that make the development of manufacture of our component systems possible. While there is no one perfect synthetic biology workflow or workstation that will suit all projects, Hudson Robotics can help you design the most efficient and effective system to achieve your goals.

1. Boles, K. S. et al. Digital-to-biological converter for on-demand production of biologics. *Nature Biotechnology* **35**, 672–675 (2017).

2. Hughes, S. R., Butt, T. R., Bartolett, S., Riedmuller, S. B. & Farrelly, P. 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**, 292–307 (2011).

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10 Stern Avenue
Springfield, NJ 07081-2905
1.973.376.7400

info@hudsonrobotics.com
www.hudsonrobotics.com

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