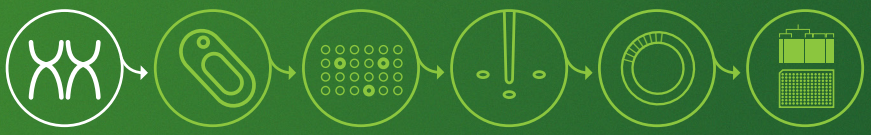
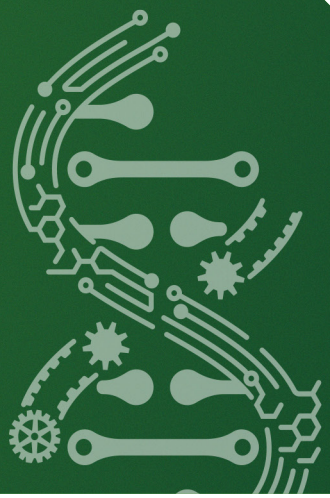
Hudson ROBOTICS

SYNTHETIC BIOLOGY SERIES

Gene Synthesis





The synthetic biology pipeline consists of gene synthesis, transformation, colony gridding, colony picking, plasmid prep, and expression analysis. This is a practical overview of the first step, gene synthesis.

Designing Oligonucleotide Libraries

Traditionally, strain improvement, the end goal of much of synthetic biology, entailed mutagenesis, followed by screening mutants for desired improvements, followed by further mutagenesis. In general, the molecular basis for increased production would cover many loci and would never be uncovered. By using recombinant DNA technology, an alternative method is to clone the desired gene or gene cluster, followed by DNA sequencing and bioinformatics analysis of the sequence to identify structural and regulatory regions. Specifically mutating these regions or replacing them with different promoters could then result in improvements.

It is impossible to predict which codon changes might lead to an improvement in the final functionality of the gene product. Consequently, synthetic biologists build *in silico* oligonucleotide libraries in hope of finding at least one oligonucleotide that will result in the desired change in the candidate organism's genome. This type of library involves designing single-stranded nucleotides that assemble into a sequence that bears a mutation when pooled together as *ex silico* oligonucleotides.

One systematic method of library construction is "amino acid scanning mutagenesis" (AASM). AASM allows the synthesis of all possible mutations across a gene open reading frame (ORF), replacing each amino acid in the encoded polypeptide or protein with an alternate amino acid. In this manner, every codon is considered.[1]

The synthetic biologist wishes to avoid redundancy but ensure the likelihood of introducing a positive mutation. Downstream, every oligonucleotide requires a column and a series of laboratory steps for its production, which is costly and time consuming. Starting with the right oligonucleotide library can shave years of frustration and hundreds of thousands of dollars off the cost of a synthetic biology project.

Homology Path Software

Using the principles of DNA shuffling and dynamic re-use, Homology Path software designs oligonucleotide libraries, designed for each ORF, that minimize the number of homologous regions while preserving the required variable regions. DNA shuffling brings about rapid directed evolution from a relatively small number of variants. The software produces oligonucleotide coverage maps illustrating the continuous oligonucleotide coverage for each ORF and oligonucleotide pooling instructions for the liquid handler. Oligonucleotides can be ordered from a vendor or produced in-house.

Oligonucleotide Production

Synthetic oligonucleotides are usually made by column-based synthesis. By adding trichloroacetic acid, dimethyltryptamine (DMT) - protected nucleoside phosphoramidites on a solid support (synthesis column) are deprotected. Next, a DMT-protected phosphoramidite is added to the previous nucleoside phosphoramidite, which couples to the 5' hydroxyl of the previous base and forms a phosphite triester. The unreacted 5' hydroxyl groups are acylated to render any unextended sequences inert in subsequent rounds of chain elongation, thereby reducing the likelihood of deletion errors in the oligonucleotide sequence. Through oxidation with an iodine solution, the phosphate triester linkage between the monomers is converted into a phosphate linkage, resulting in a cyanoethyl protected phosphate backbone.

The synthesis cycle is repeated for the next base in the sequence by removing the 5' terminal DMT protecting group. The support-attached phosphoramidite is thus activated for the next phosphoramidite monomer to extend the chain. Following the synthesis of the desired sequence from 3' to 5', the oligonucleotide is chemically cleaved from the solid support, and the protecting groups on the bases and backbone are removed.[2]

The yield of full-length oligonucleotides decreases with increasing nucleotide length, with a theoretical limit of 300 nucleotides and a practical limit of 200 nucleotides. Fortunately, this process is highly amenable to automation.[3]

Gene Synthesis and Assembly

Gene synthesis is the production of gene-length pieces of DNA (250 – 2000 bases) directly from single-stranded DNA oligonucleotides made by phosphoramidite chemistry either by an oligonucleotide vendor or in-house. Because every oligonucleotide synthesized is made on an individual synthesis column, the oligonucleotide needed to assemble a gene must be added together into an assembly pool post-synthesis.

To facilitate the assembly of a synthetic double-stranded DNA (synthon) from single-stranded oligonucleotides, adjacent oligonucleotides are designed to contain overlapping sequences and produce synthons from 200 to 2000 base pairs in length.

Synthons can then be assembled together by numerous methods to make larger DNA constructs. The most common method of assembly is polymerase cycle assembly (PCA). The resulting overlapping sections are assembled into larger pieces of DNA, cleaned, and cloned into an expression vector. Cleaning is based on the formation of double-stranded DNA by heating and cooling the mixture and allowing heteroduplex DNA to form. The positions that contain mismatches can be removed by the protein MutS or an endonuclease cocktail.[4]

The steps involved in oligonucleotide production, gene synthesis, and assembly can be done in-house or “farmed out,” in part or in whole, to a speciality vendor. Concerns about data security often enter into this decision. Many synthetic biologists are averse to sending DNA or DNA data for processing in countries lacking in strong intellectual property protections, or anywhere that data might be compromised. For groups with such concerns, concrete legal assurances must be provided or a capable in-house program for synthetic biology must be constructed.

Automation

The laboratory operations of synthetic biology are of moderate complexity but very high in volume. Not only is there a tremendous amount of liquid handling involved, but materials must be moved between workstations. It is not advisable to move materials manually because of contamination risk. The movement of experimental materials by hand may also increase the likelihood of human error from spills and drops, or from simple confusion.[5]

The goal of automation is to execute repetitive tasks in order to maximize the effectiveness of a DNA sequence of a target organism. While gene synthesis is the first step, synthetic biologists must keep the other steps in mind when designing an automated system.

Track-based systems offer fast, linear response, no manual handling of plates, and the capability of doubling up on rate-limiting steps. Robot arms allow integration of any instrument that can be remotely controlled. Robot arms are larger and take up more lab space than a track-based system, but additional arms can be added. A hybrid system consisting of track-based and robot arm systems working together in the same workflow offers the possibility of greater throughput, greater capacity, and space efficiency. In all cases, automated workflows and systems can be designed to meet a requirement that prohibits manual microplate handling.[6]

Hudson Robotics Solutions

Hudson Robotics has automated the gene assembly process. Synthetic biologists may choose to integrate their existing instrumentation into a gene synthesis workflow, or explore Hudson's offerings. We have systems that can be integrated with commercial automated oligonucleotide synthesizers, as well as subsequent deprotection, purification, and normalization. A software plug-in, Oligonucleotide Synthesis, downloads desired oligonucleotide formulations from a local file or the internet, and converts them into instructions for an oligonucleotide synthesizer and operator prompts to ensure that required materials are present.

Hudson Robotics has developed a multi-functional software integration for the LGC/BioAutomation MerMade™ Synthesizer to take advantage of automated oligonucleotide synthesis. The MerMade Oligonucleotide is designed to synthesize DNA in a column format using standard or modified chemistry. Hudson's MerMade plug-in for SoftLinx™ converts downloaded oligonucleotide library files into digital instructions for the MerMade instrument, and also controls deprotection and purification steps.

Deprotection and purification are carried out using the Hudson Robotics SOLO™ instrument, the positive pressure FilterPress™ and Deprotection and Purification software. Synthesized oligonucleotides are eluted from their solid substrate, the SOLO and FilterPress work to deprotect them in a fully automated series of steps. The synthesizer columns are rinsed under positive pressure, deprotection reagents are added, and the collection plate is sealed if necessary. Plates are unsealed after the deprotection reaction is complete. The entire process is hands-free and automated.

In continuation of the automated, hand-free process, SoftLinx software from Hudson Robotics follows the downloaded instructions to direct the automated pipettor to pool the appropriate oligonucleotides to enable the final assembly process. PCR carried out automatically with heating and cooling, and SoftLinx directed pipetting of the appropriate reagents, create the completed gene assembly. SoftLinx also has the ability to adapt its instructions for the SOLO pipettor to the results of an absorbance reader, yielding a normalized concentration of DNA in every plate well.

The final product is ready for transformation.

Endnotes

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6. Hudson Control Group. Systems and methods for automated proteomics research. United States Patent No. US 2007/018456 A1.

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