Lecture and Dialog on Synthetic Biology

Drew Endy (endy@stanford.edu)

Goals for Today

A. Consider engineering of biology workflow.

B. Review DNA synthesis chemistry, including capping & deprotect.

C. Grok limitations of de novo synthesis (e.g., coupling efficiency).

D. Understand assembly of genes from oligos, including error filtration.

E. Introduce assembly of "long length" DNA from "gene" length DNA.

F. On the importance of decoupling design & construction (e.g., short or long).

G. Introduce Assignment #1

A. Engineering of Biology Workflow

1. The process of engineering a synthetic biological system involves several steps, of which the editing, synthesis or construction of genetic material is only one step. Representative steps might be labeled: "Design," "Construction," "Testing," "Analysis/Debug."

2. Today, typically, many or all of these steps are carried out by one person or team (this varies somewhat as a function of academic or industry research), meaning that one person or team needs to be expert in all these steps. For comparison, buildings are typically designed by an expert designer (e.g., an architect) and constructed by a different, expert builder. As a second comparison, microprocessors are typically designed by one team of design experts and constructed by a second team of fabrication experts.

3. To an engineer, what is the consequence of being able to "decouple" design from construction? To a geneticist? Let's return to these questions at the end of today's class.

B. DNA Synthesis Chemistry

4. Practically, synthesis lets you start with phosphoramidites comprising the four bases of DNA, along with the information specifying a DNA sequence(s) to be constructed and assemble the genetic material from scratch. If you are familiar with the food replicators from the TV show Star Trek, DNA synthesis is sort of the same thing (i.e., "matter compilers" for genetic material, the stuff that contains most of the known information coding for much of the living world!).

5. The process of synthesizing and constructing DNA can be organized as a function of the length of the DNA fragments to be produced. In order of increasing length these

fragments can be called: “oligonucleotides,” “genes,” and “genomes.” More on gene and genome construction below.

6. Oligonucleotides is often abbreviated “oligos.”

C. Grok Limitations of Oligonucleotide Synthesis

7. Oligo synthesis is a four step process, carefully designed to allow for the desired base to be added at each step in elongating the oligonucleotide chain, but one and only one of each base. Newly added bases are “protected” so that only a single base can be added at a time. Oligo chains that don’t receive a new base are “capped” so that deletion mutants are produced.

8. The process of oligo synthesis based on the Caruthers’ et al. chemistry is very impressive, but not perfect. For example, a well run oligo synthesizer might have an overall “coupling efficiency” of ~99%. (Coupling efficiency refers to what fraction of each growing oligonucleotide gets the correct base, following each four step elongation cycle).

9. Thus, you can’t synthesize at reasonable yields oligonucleotides above some length. You can estimate the final yield via a simple probability analysis: yield = (coupling efficiency)^N, where N is the number of bases comprising the oligo. So, w/ a 99% coupling efficiency, you’d get yields of 99% (N=1), 90% (N=10), 77% (N=25), 60% (N=50), 36% (N=100), <1% (N=500).

10. Thus, you need some way to build longer fragments, such as genes, from oligos.

D. Building Genes From Oligos

11. Pim Stemmer figures out one interesting way to assemble longer fragments of DNA from oligonucleotides. His method is called “PCR Assembly.” Via this method, overlapping oligonucleotides are combined in a single multi-stage PCR reaction, allowing for a longer fragment of DNA to be assembled quickly. (Stemmer et al, “Single-step assembly of a gene and entire plasmid from large numbers of oligodeoxyribonucleotides.” Gene. 1995 Oct 16;164(1):49-53.)

12. PCR assembly can work well, but also can run into big problems. For example, “cross talk” in the hybridization of oligos. As a second example, errors introduced by the polymerases used in the PCR process.

13. However, methods are available to detect and filter errors in the assembly of genes from oligos. For example, point mutations (single base pair errors, arising either in the oligo synthesis or gene assembly stages) can be detected and filtered out. The way this works is for single stranded DNA to be hybridized against sequences that should be the same, but that may have errors. These single base mutations can be detected by natural proteins (e.g., MutS) that bind to single base mismatches in double stranded DNA. If the MutS protein is attached to a bead or can be otherwise re-

moved from solution, errors in individual DNA molecules can be removed from a population of DNA molecules.

E. *Building Genomes from Genes (move to start of Thursday's class).*

F. Impact of DNA Construction

G. For Next Time

14. Please complete and turn in Assignment #1 by the beginning of Thursday's class.