Notes for Workshop 2: BioBrick and Plasmid Backbone Structure

Genes:

- DNA contains both coding and noncoding regions. While the function of the noncoding regions is still unknown, scientists have dividing coding regions into functional units known as genes. Each gene encodes for the production of a specific protein.
- The term “gene” is often misused. For example, many people refer to a “gene” for blue eyes. However, they are actually referring to an “allele,” or a variant of a gene. In this case, the “gene” would control eye color, and possible “alleles” would be blue, green, hazel, and grown.
- In reality, however, it is not so simple. Most traits, including eye color, are polygenic, meaning they are controlled by multiple genes. Many genes have been identified and mapped to specific chromosomes already, but there is still significant progress to be made before the approximately 25,000 genes within the human genome are fully understood.

Promoters & Repressors:

- Promoters are located upstream of the associated gene, initiating gene transcription.
- Repressors bind to an operator to inhibit gene expression.
- Using promoters and repressors, genes can be organized into various circuits. Two common circuits are the genetic toggle switch and the repressilator, as discussed by Ahmad Khalil and James Collins in “Synthetic biology: Applications come of age.”
- The genetic toggle switch alternates between expression of two genes, A and B. For example, say expression of gene A inhibits expression of gene B, and expression of gene B inhibits expression of gene A. This repression of B by A is inhibited by heat, and the repression of A by B is inhibited by IPTG. Thus, by introducing heat, the switch may be flipped from expressing gene A to expressing gene B, and by introducing IPTG, the switch may be flipped from expressing gene B to expressing gene A.
- The repressilator includes three genes, A, B, and C. As A represses B, B represses C, and C represses A. Thus, if A is being expressed, B is repressed. Because B is repressed, B is not repressing C, so C starts to be expressed. As C is further expressed, it represses A. The cycle then repeats with C being expressed, B beginning to be expressed as A is repressed, and B then repressing C so that B is fully expressed. Thus, an oscillation pattern is observed between expression of the three genes.

Gene Construct Structure:

- Gene constructs include multiple subcomponents in addition to the gene itself. A promoter is located upstream of the gene in order to initiate gene transcription. This is followed by the ribosome binding site (RBS), a short sequence on mRNA upstream of the start codon to which the ribosome binds to initiate protein translation. The gene follows, and the construct ends with a terminator.

- Many constructs, however, also include a reporter gene. This gene remains under the same promoter as the gene of interest so that both are expressed at the same time. In contrast, the genes are placed under separate RBS's so that they are translated individually.
- Reporter Gene: Reporter genes indicate whether a particular gene of interest has been taken up or is being expressed by a cell. They are attached to the regulatory sequence of this particular gene so that they are expressed along with the particular gene of interest. Common reporter genes are GFP and mCherry, which fluoresce green and red, respectively.

Plasmids:

- To insert desired gene constructs into bacterial cells, plasmids are utilized as a vector, a carrier molecules used to transport DNA into cells. Viral vectors are also used to insert exogenous DNA into a cell; DNA from a virus, however, is inserted into the host cell DNA, while plasmid DNA remains separate from the host genome.
- Plasmids are small circular strands of DNA from a bacteria. Naturally-occurring plasmids often code for antibiotic resistance, but biologists have synthesized plasmids for genetic engineering as their small nature makes them easy to manipulate and transfect. DNA fragments from one organism can be cut, spliced into plasmids, and inserted into a different organism. These plasmids are known as recombinant DNA as they include DNA from multiple sources, creating novel DNA sequences otherwise not present in biological organisms.

Plasmid Backbone:

- Within the iGEM competition, DNA sequences with characterized structure and function are known as BioBrick standard biological parts. BioBrick constructs may contain a single element (individual promoter, RBS, gene, or terminator sequence) or else an entire construct (promoter, RBS, gene, and terminator sequences all combined). These BioBricks are stored within the online Parts Registry and are available for all scientists worldwide, providing a common interface for synthetic biology.
- BioBricks are placed on the standard iGEM operation plasmid including the following:
    - Replication Origin - where replication begins on the plasmid. Different replication origins can be used to control how many plasmid copies remain in the cell, how the number of plasmid copies varies among cells, how this plasmid copy number is controlled, and how the plasmid DNA coils.
    - Antibiotic Resistance Marker - a selection marker, allowing you to recognize cells that have taken up the plasmid. Grow all of the bacteria on antibiotic media and the colonies that survive have been transformed. Backbones often confer resistance to ampicillin, kanamycin, chloramphenicol, or tetracycline.
    - Multiple cloning sites [see restriction enzyme notes below]- several restriction enzyme sites in a row on the plasmid backbone provide leeway in where the DNA fragment may be inserted as well as in which enzymes may be used. The BioBrick cloning site includes a BioBrick prefix with EcoRI and XbaI recognition sites (before the BioBrick part) and a BioBrick suffix with SpeI and PstI recognition sites (after the BioBrick part).

Restriction Enzymes:

- Isolated from bacteria and Archaea. Used by these organisms for defense against invading bacteriophages as the enzymes selectively cut up foreign DNA. Meanwhile, methylase enzymes modify the host DNA so that it is not damaged by enzyme cleavage.
- Cut DNA at or near specific recognition nucleotide sequences known as restriction sites. These recognition sequences are palindromes.
- The enzymes make two cuts- one through each side of the sugar phosphate background. These cuts can be aligned to make a blunt cut straight through the DNA. The cuts may also occur several bases away from each other, producing what are known as sticky ends. These sticky ends can recombine with each other through hydrogen bonding, or they may join with recombinant DNA cut by the same restriction enzymes (and thus with the same complementary sequences).
- There are three types of restriction enzymes. These are categorized by their structure and whether the recognition site and cleavage site are the same or different.
- The iGEM plasmid construct contains four main restriction enzymes, including EcoRI, XbaI, SpeI, and PstI.

EcoRI

XbaI

SpeI

PstI