Intro to Synthetic Biology
HSSP Summer 2018

Last week, we learned about using gene circuits to visualize about concrete designs for biological vectors. These concepts can help us design systems that can be created in the lab. Knowing this, how do we actually design these systems?

Most biological pipelines have three main components: design, creation, and insertion. Let's use an example of wanting to inactivate a gene in yeast to show this.

Design consists of isolating proper components to include in your system. In this case, we will use the CRISPR/Cas9 system to engineer this change. This system has a common Cas9 nuclease, but we need to design the gRNA.

- Since your gRNA needs to match the DNA of the organism, it would be helpful to select candidates from existing databases. Here are some examples:
  - Saccharomyces (yeast): https://www.yeastgenome.org/
  - E. coli: https://ecocyc.org/
  - Arabidopsis thaliana (plant): https://www.arabidopsis.org/
- In order to confirm that a selected region is a good candidate, you can use online tools to help confirm your results. Here is an example: https://crispr.dbcls.jp/
  - Insert your region of genome into the search box, select the specific organism, and search. Results will be organized from most to least efficient.
- Once you have created your final gRNA, you can send the design off to a company for them to create. The gRNA that you have designed will henceforth be referred to as an olignucleotide.

Creation consists of synthesizing the actual vector that you will use to create your desired change. Most standard vectors have multiple components:

- Promoter - a promoter is a region of DNA placed before all of these other components that helps to facilitate transcription. Promoters are important because they help make sure everything is expressed in the cell. The exact promoter in use depends on the organism type that the vector will be in.
- Oligonucleotide - this is what was previously designed by you (in the case of the CRISPR/Cas9 system, this would be your gRNA)
- Primers - primers are short DNA sequences that help proper enzymes attach to express the vector. (Note that this is different from the promoter). Specific primer design depends on many factors such as melting point and organism type, and is a little complicated, so we won't cover it in detail.
- Selectable marker - a selectable marker is a region of DNA that allows resistance to a certain type of antibiotic (in our example, let's use kanamycin). Only a cell with a selectable marker will be able to survive the presence of the specific antibiotic (in this case, kanamycin). Selectable markers are important because they help to isolate which of the cells have the vector and which don't.

Although it is possible to individually create each of the above components and put them together, it is rather time consuming and not usually done in practice. Instead, restriction enzymes are used with certain premade vectors that already contain ideal promoters and selectable markers. Restriction enzymes cut at specific sequences and can "open" the vector, allowing you to add your specific oligonucleotide and primers. Once you've added these, you will need to re-ligate the vector closed.

Insertion consists of introducing your fully designed vector into the organism of choice. Depending on the organism, there are multiple ways to do this:

- For simple organisms (such as bacteria and yeast):
    - Electroporation consists of using specific cells (such as DH5 E.coli cells) that, when given an electric charge, temporarily open the cell membrane. Once you add your vector to these cells, you use a specific machine to give an electric pulse so the vector can enter the cell.
    - Heat shock can also be used in some cases. This happens when a cell has been exposed to ice for a very long time and then is suddenly exposed to very high temperatures (or vice versa). This works because the sudden shift in temperature makes the cell membranes weaker, allowing for your vector to easily be transmitted.
    - **Note: once you have inserted the vector into the cell via one of two methods described above, you can use the selective marker to validate. If you plate the bacteria or yeast on media specifically containing that antibiotic, any colonies that grow will have the selectable marker and therefore contain the rest of the vector, which is the desired outcome.
- For more complex organisms (such as plants or animals), other techniques are needed since they are much larger in size.
    - Injection via needle is useful for mice and other animals of that size.
    - A technique for plants specifically is agroinfiltration. A specific type of bacterium, *Agrobacterium tumefaciens*, is introduced with your specific vector (using the techniques described above). Once the vector is in the bacterium, a syringe is used to inject the bacteria within the leaf itself. From there, expression will proliferate.