Notes for Workshop 3: Restriction Enzyme Digest, Gibson Assembly, & Ligation Reaction Notes

Restriction Enzyme Digest:

- The iGEM backbone includes four specific restriction enzyme cut sites: EcoRI, XbaI, SpeI, and PstI. As all BioBrick parts are required to not include these specific cut sites, they may be used to combine individual BioBricks to generate combination parts.
- XbaI and SpeI are particularly useful as they form a scar. Sticky ends produced by the two enzymes are complementary, but the ligated product cannot be cut by either enzyme.

XbaI

SpeI

Possible Scars:

TCTAGT	ACTAGA
AGATCA	TGATCT

- To insert a BioBrick in front of another part already located within the plasmid backbone, cut the insert with E and S and the backbone with E and X.
- To insert a BioBrick behind another part already located within the plasmid backbone, cut the insert with S and P and the backbone with X and P.

Calf Intestinal Alkaline Phosphatase (CIAP):

- After completing restriction enzyme digestion, it is important to prevent re-circularization and re-ligation of the plasmid. Otherwise, the plasmid may close up on itself, preventing insertion of the desired DNA inset.
- The CIAP enzyme prevents such ligation as it cleaves the phosphate off of the 5’ end of DNA. Thus, the digested DNA cannot ligate back onto itself as the phosphate is required in order to covalently bond.
- Meanwhile, the desired DNA inset has the phosphates on its 5’ ends, so the inset and the plasmid backbone may be ligated, inserting the gene. Additionally, as ligase uses ATP to complete the ligation reaction, it is able to remove an ATP phosphate to fill in the gap as it adds the gene of interest into the plasmid.
- It is extremely important to ligate either the plasmid or the inset, but not both as to do so would prevent ligation.

Ligation Reaction:

- In order to insert the desired DNA inset within the plasmid vector, the digested fragments must be combined through a ligation reaction.
- Naturally, DNA ligase is used to repair single-stranded and sometimes even double-stranded DNA breaks. Synthetic biologists, however, use DNA ligase for ligation reactions. As the complementary base pairs of sticky ends hydrogen bond together, DNA ligase completes the phosphodiester bonds of the backbone to complete the recombination.
- As with all enzymes, it is important to carry out the reaction at the enzyme's optimal temperature.

Gibson Assembly:

- Single reaction DNA fragment assembly method which can combine over 10 DNA fragments together. This is beneficial as by combining the DNA fragments in one step, time is saved and less reagents are used. No restriction digest and ligation reaction are necessary, and no restriction site scars remain.
- DNA fragments must overlap by 20-40 base pairs. When combined with enzymes (5' exonuclease, DNA polymerase, DNA ligase) and incubated at 50 degrees Celsius for an hour, the fragments are combined in order.
    - 5' exonuclease starts at the 5' end and breaks apart the DNA strand. This produces single-stranded DNA which allows the complementary sequences of fragments anneal.
    - DNA polymerase adds nucleotides, eliminating any gaps in the DNA.
    - DNA ligase joins the DNA fragments with overlapping complementary ends.