UNS-Guided Gibson Assembly

All of our constructs were assembled with the Gibson Assembly method. Many of our primers were designed with the help of NEB's Gibson Primer builder, NEBuilder.NEB.com.

All PCRs to perform Gibson assemblies were done with NEB's Q5 HotStart 2x Master Mix.

We often used the following protocol for our PCRs:
- 1.25 µl Primer 1
- 1.25 µl Primer 2
- 1 µl of 1:10 dilution of miniprep template DNA
- 9 µl Nuclease Free Water (NFW)
- 12.5 µl Q5 HotStart Master Mix

We would often use the UNS primers, specifically P008, P009, P013, and P019, to allow us to do backbone swaps.

We would then run the PCR products on a gel to confirm that they were the correct size, and if so, would perform a DpnI digestion to remove residual template DNA:
- 24 µl PCR product (we used 1 µl to run on the gel)
- 2.7 µl CutSmart Buffer
- 0.5 µl DpnI

After the DpnI reaction, we would either PCR Purify or perform a Gel Extraction, depending on if there were extra multiple bands on the gel that we wanted to remove. We followed these protocols as described by NEB, with the one modification of heating Buffer EB to 50°C before eluting to increase yields.

From here, we proceeded to the Gibson reaction. We used NEB's Hifi DNAAssembly Master Mix for our assemblies. We reduced the reaction volume by half, in order to conserve Master Mix:
- 5 uL 2x Hifi DNAAssembly Master Mix
- 5 uL mix of insert DNA, backbone DNA, and NFW

The exact ratios of DNA and NFW in this mix was calculated based on the length (weight) of the DNA and their concentrations as found with the NanoDrop One following PCR purification. This calculation was based off of NEB's Gibson guidelines.

Finally, we performed a chemical transformation with NEB's 5-alpha, 10-beta, or BL21 Competent E. coli with 2 µl Gibson product.