Improve AmilCP, Weekly Notes

The original native AmilCP sequence, was codon optimized with both a codon optimization tool provided by Integrated DNA Technologies (IDT) and another tool called COOL. The three Biobrick parts was ordered as gBlocks from IDT inserted in pUCIDT backbone with Ampicillin resistance.

Week 1 (3/9-9/9)

Each Biobrick part was transformed into commercially competent E.coli DH5-alpha cells from NEB. Transformation did not give satisfactory results for unknown reason.

The three Biobrick parts containing variants of AmilCP were digested and ligated into a pSB1C3 plasmid backbone through 2A assembly.
Size of digested parts were controlled by gel electrophoresis.
After ligation, DNA concentration of the three ligated plasmids was measured with the Qbit.

Plasmid/Biobrick containing codon optimized sequence (IDT): 0,150 ng/uL
Plasmid/Biobrick containing codon optimized sequence (COOL): 0,822 ng/uL
Plasmid/Biobrick containing original native sequence: 0,428 ng/uL

Biobrick parts in psb1c3 backbone were transformed into commercially competent E.coli DH5-alpha cells.

Week 2 (10/9-16/9)

A colony from each transformation plate were inoculated in LB+Amp medium overnight.(3 separate O.N cultures).

Stability assay was started the following day with O.N. cultures. 999 uL of LB with chloramphenicol (25 ug/ml) was inoculated with 1uL culture in eppendorf tubes in 10 replicates for each part. Tubes were incubated in 24 hours to allow 10 generations of growth.

Following day, cultures were spinned down and color comparison of the culture pellets was done through visualization. Culture pellets with strongest color was resuspended and plated on LB Agar+cam plates.
Each culture was diluted 1:1000 with LB+Cam liquid medium in new eppendorf tubes and put on incubation for 24 hours.

Procedure was repeated until cultures had grown for 40 generations. Pictures of culture pellet was taken for 10, 20, 30 and 40 generations in order to analyse the color intensity and deduce the stability of the chromoprotein-encoding plasmid

Week 3 (17/9-23/9)

When consulting Anthony Forster about our results from the stability assay we unfortunately

discovered that we had made a mistake when preparing the first cultures for the stability assay. For all three strains containing one variant of the chromoprotein-encoding plasmid, we started the first 10 cultures for the stability assay from the same O.N culture.
Instead we should have inoculated one single colony directly in the eppendorf tubes (10 replicates for each part) in order to avoid mutations that may affect the result.

Each Biobrick part inserted in pUCIDT backbone with Ampicillin resistance
was once again transformed into commercially competent E.coli DH5-alpha cells.

1 mL of LB with ampicillin (25 ug/ml) was inoculated with one single colony from transformation plates in eppendorf tubes in 10 replicates for each part. In order to allow 10 generations of growth, a 1:1000 dilution was made of the overnight culture and incubated for 24 h.

The following day, comparison of color intensity was done through visualization of centrifuged cultures and the dilution procedure was repeated.

Week 4 (24/9-30/9)

Second round of stability assay was finished after cultures had grown for 40 generations. Already after 10 generations of growth it was clearly visible that the color intensity was best distributed in the cells containing the plasmid with the IDT-optimized AmilCP sequence. The results confirm that the plasmid containing codon optimized part generates better maintenance of protein expression than the plasmid containing the original native part.

Plasmid containing the Biobrick with IDT optimized AmilCP was extracted from O.N culture with GeneJET plasmid miniprep kit from Thermo Fischer Scientific.
The extracted plasmid was then digested and ligated into pSB1C3 backbone through 2A Assembly (according to iGEM protocol of 3A assembly).

The DNA concentration was then measured with Qbit. The ligated pSB1C3 plasmid was transformed into commercially competent DH5-alpha E.coli cells.

Week 5 (1/10-7/10)

O.N culture was made from transformation plates.
Plasmid containing the Biobrick with IDT optimized AmilCP was extracted from O.N culture with GeneJET plasmid miniprep kit from Thermo Fischer Scientific. Two plasmid extractions were made from the same culture. The DNA concentration was then measured with Qbit

Yield:
Extracted plasmid 1: 20,8 ng/uL
Extracted plasmid 2: 21,4 ng/uL

250 ng (12uL) of the extracted plasmid 2 was put in the igem submission kit plate in wells 1A and 1B.