IGEM EPFL – CheY CheZ & Luciferase complementation assay experiment
Constructs Design

Split Luciferases :

	N Terminus	C Terminus
Renilla Luciferase	1-110	111-311
Firefly Luciferase	1-416	394-550

- The two parts of the split Renilla Luciferase have been amplified from a plasmid containing full Renilla Luciferase (rLuc from Waldor Laboratory [paper]).

- The two parts of the split Firefly Luciferase habe been amplified from Cambridge 2010 team's Bba_K325108 (EPIC Firefly Luciferase).

- E. Coli CheY and CheZ were amplified respectively from UT Dallas 2011 team's BBa_K569017 and SYSU China 2011 team's BBa_K629003 .

- Primers were designed to perform a Gibson assembly. We put the two fusion proteins for each split luciferase in the same vector.

- The N terminus part of each split was fused to the C terminus of CheY, while the C terminus part of each split was fused to the C terminus of CheZ, with a flexible linker between each fused proteins.

- The start codon was removed from the N terminus part of the splits. At the end of the N terminus part was added a stop codon. Between the two fusion proteins was added a second RBS site.

- For the controls, we created plasmids containing the two parts of each split luciferase (Renilla and Firefly) without fusion protein. Stop and start codon were added if not already present at the extremities of both parts of the split.