RNA Splint:

RNA Splint for protein split and assembly - luxI as inspiration and CM^R for proof of concept

Our system is made for the detection of substances in very low concentration. It is based on a signal transduction and amplification between the individual bacterium. The major risk in systems like this one is that a false positive result will activate the whole system, rapidly. In order to solve this problem, we came up with few methods. One of those methods is the split of a key component protein, which activates the system: LuxI. It allows the communication of an activation signal to between all of the cells in the sample. We thought it would be feasible to solve it by ensuring the production of LuxI only when a toxin is in the sample. To avoid the leakiness of the promoters we have decided to split the LuxI gene so each part will be regulated under a different promoter (of the same type). This way the probability for leakiness of the two promoters in the same cell and assembly of the two parts of the protein is very low. That way LuxI will be produced only when the cell detects a toxin.

To screen for the positive colony with the positive RNA splint and the functionality of LuxI, the detector strain assay will be done (the same screening method from gate 1)

LuxI-Inspiration:

- RNA splint: bridges between A protein RNA and B protein RNA. The splint enables the ligation of two RNA molecules. In order for the ligation of the 2 RNA molecules to occur a T4 RNA ligase is needed. T4 RNA Ligase is the enzyme from the phage that makes this ligation between two RNA molecules, it recognizes a specific site (this will be addressed later on). This is the preferable method.
- The design using luxI:

  הגייט שאבי עשה

CM Resistance:

RNA splint is an in-vitro method described for the ligation of 2 RNA molecules. (M.R. Stark, J.A. Pleiss, (2006), RNA)

- For a simple and robust screen CM split is preferred over the split of luxI

החלק השני שאבי עיצב (עם הגייט המלא-הארוך)

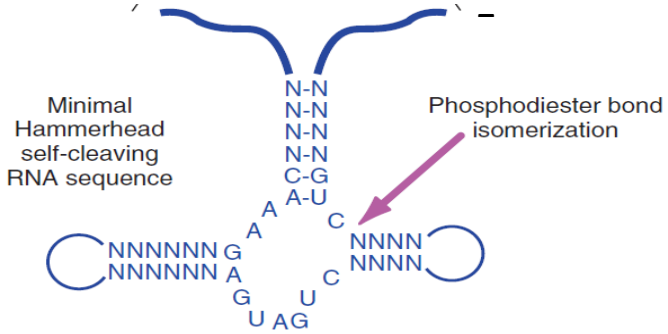
Some background about the components needed for the described system:

- Role of T4 Ligase in nature: to repair tRNA damage during the invasion of the bacteriophage (maybe cause of different anti codon usage in the phage) (“Thus, reprocessing could be yet another T4 device to adapt the translation apparatus to post-infection codon usage”) (**Amitsur et al., 1987**), (**C. Kiong Ho, Li Kai Wang 2004**)
- Problem: Ribosome will get stuck because of the double strand RNA

- Solution: RNA Helicase-exist in *E-coli* K-12 (http://www.ncbi.nlm.nih.gov/gene/948290)

- The 5' of the mRNA is phosphorylated - it is important for the ligation
- The terminator should be removed from the first part of the CM resistance, or else it will be translated in the middle of the gene-will be done by Hammerhead Ribozyme
- Chloramphenicol acetyltransferase is the protein for the resistance - **There is a His residue in the C-terminus that is important for the mechanism of the enzyme** so it is possible to knock the activity out by splitting the sequence
- We used Rnl1 for this ligation (ligates tRNA)(T4 nucleic acid ligases: Bullard & Bowater 2006) http://www.ncbi.nlm.nih.gov/nuccore/29345244?from=136340&to=137464&sat=4&sat_key=40961403&report=fasta
- It's enough to use 7 bases for each side (14 total) we did 20bp totally (E. Paredes et al. / Methods 54 (2011) http://www.sciencedirect.com/science/article/pii/S1046202311000442
- GTN for the Ribozyme cut, N needs to be **purine** (but could be any base except G)
- TTC is the loop of tRNA-Lys (UUC) - Needs TTCN for the T4 Ligase
- The first part ends with TTCGTC
- In luxI there is TTCTC so it is supposed to be suitable for the approach
- This is Type 3 Ribozyme.

(Christian Hammann, Marcos De La Pena, (2012), The ubiquitous hammerhead ribozyme. RNA)



(William G. Scott (RNA Technologies 2010))

(http://www.tulane.edu/~biochem/nolan/lectures/rna/ham.htm)