



Signal Integration: Applications of RNA Riboregulator Capabilities

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Introduction

Self-complementary messenger RNA has a high potential for tunable repression of translation. A self-complementary hairpin which includes the Shine-Dalgarno site in the stem will greatly reduce protein expression until the necessary conditions are met for the hairpin to completely unfold.

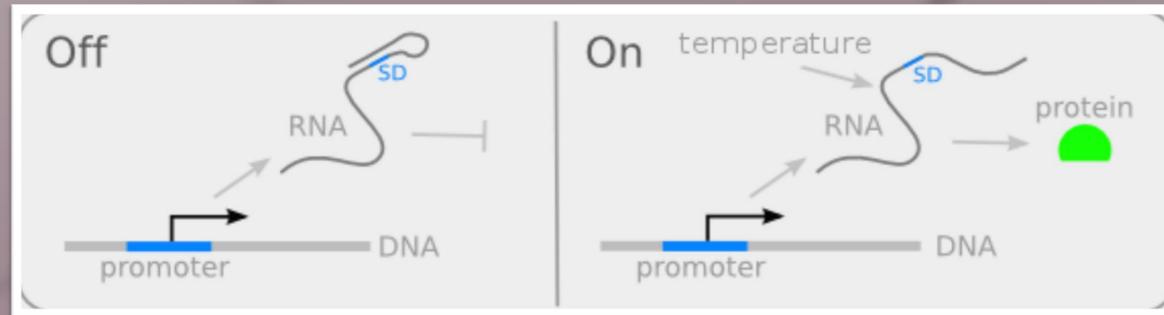


Figure 1. Temperature sensitive hairpin loop

Background – Biothermometer

An RNA hairpin will unfold when exposed to temperatures past the melting point for the sequence. This permits the temperature-sensitive expression of the downstream gene. The TUDelft 2008 iGEM team retrieved natural RNA thermometers from three species, then sequenced and redesigned them to test for a modified temperature range. We worked with their 32° C thermometer as it was found to be their most effective.

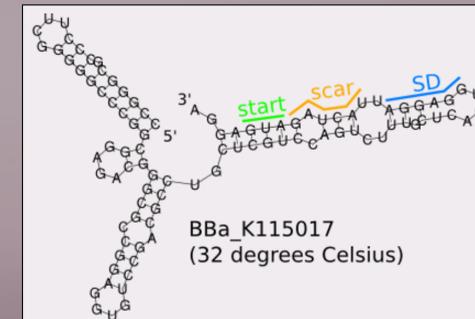


Figure 2. The 32° C temperature sensitive hairpin part of TUDelft 2008.

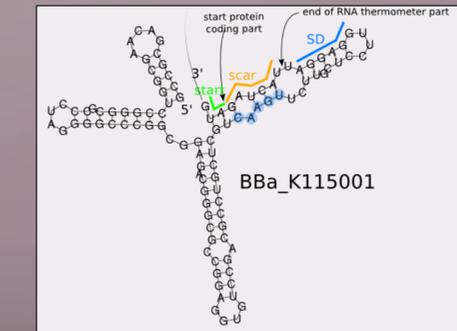


Figure 3. The 37° C temperature sensitive hairpin part of TUDelft 2008.

Background - Ribolock and Ribokey

An RNA hairpin will also unfold when exposed to a sequence of higher specificity. When the expression of such a complementary sequence is controlled by a separate promoter, this permits a condition-sensitive expression of the downstream gene. The condition may be the presence or absence of a metabolite, of an antibiotic or toxin, or of various wavelengths of light. The Berkeley 2006 iGEM team began with the ribosome binding site hairpin (the "ribolock") and the highly specific complementary sequence (the "ribokey") produced by Collins *et al.*, then redesigned the lock and key sequences to reduce background transcription and increase activated transcription.

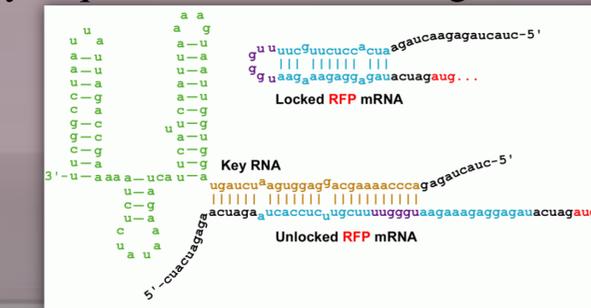


Figure 3. The ribolock and ribokey mechanism of Berkeley 2006.

Methods

Our project has gone through different stages as we found out what actually worked and what didn't. We started with our NAND gate project but were stalled when we failed to get a lambda cI promoter working. We then focused on a limited version of the bio-thermometer project. Unfortunately this ran out of time before it could be completed.

The final project consisted of a simplified biothermometer design. Using the 32° C ribothermometer linked to either RFP or GFP would produce a system with a visual response to differences in temperature. There were two levels for this: K235034 and K235035 used a lactose promoter and repressor to regulate RFP and GFP respectively; and K235036 and K235037 were directly coupled to RFP or GFP respectively.

Three Antibiotic Assembly was used to ligate our parts together.

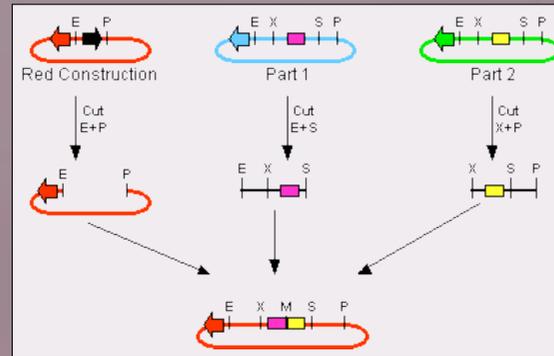


Figure 4. The 3A Assembly Method.

Goal - Ribothermometer Test

We intended to test the 32° C ribothermometer produced by the TUDelft team with fluorescent proteins instead of luciferase. We designed two types of systems. The first placed the fluorescent protein directly under the control of the ribothermometer, and should not fluoresce until the temperature is beyond 32° C. The second placed the fluorescent protein under the control of the lactose promoter, and put the LacI protein under the control of the ribothermometer; this system should only fluoresce at temperatures below 32° C, or when IPTG is present.

K235036 - This should be controlled by the 32° C thermometer and red should be repressed under 32° C.



Figure 5. K235036 composite parts

K235037 - This should be controlled by the 32° C thermometer and green should be repressed under 32° C.



Figure 6. K235037 composite parts

K235032 - This should be inducible by IPTG and produce green.

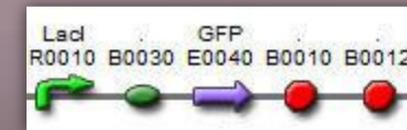


Figure 7. K235032 composite parts

Goal – NAND Gate Test

The logical function "not and" (NAND) is a function that takes two inputs, which may be either 0/false or 1/true, and gives output according to

Input 1	Input 2	NAND Output
0	0	1
0	1	1
1	0	1
1	1	0

Table 1. NAND Gate function

Using BioBrick parts, it should be possible to make very simple NAND gates out of *E. coli* cells. The configuration we intended was RFP under the control of the λ cI promoter, the λ cI repressor under the control of the ribolock and the arabinose promoter, and the ribokey under the control of the lactose promoter. The absence or presence of arabinose and IPTG would be the 0 or 1 inputs, and the absence or presence of red fluorescence would be the 0 or 1 outputs. Replacement of the arabinose or lactose promoters would provide control tests to compare against.

Goal - Ribolock Heat Test

In theory, there is nothing preventing a ribolock from acting like a ribothermometer, as both are hairpin structures. Our intent was to test the repression capability of a ribolock by culturing *E. coli* encoding ribolock-controlled GFP at room temperature, 30° C, 37° C, and 45° C, and measuring the resultant fluorescence.

Results

Early tests had shown that we had functional fluorescence producing BioBricks. We cultured *E. coli* producing ribothermometer-controlled GFP and mCherry overnight at 30° C, 34° C, 37° C, and 42° C, as well as constitutive fluorescence producers, constitutive non-producers, and a lactose-controlled GFP producer both with and without IPTG. The next day fluorescence microscopy was used to test for fluorescence levels. However, only the lactose-controlled GFP producer fluoresced, both with and without IPTG. Neither ribothermometer-controlled fluorescence producer functioned as expected.

A host of problems haunted our work with the ribolock and ribokey. We were able to assemble neither the final NAND gate nor a ribolocked fluorescence producer. Our final product was a functional GFP under the control of a lactose promoter.

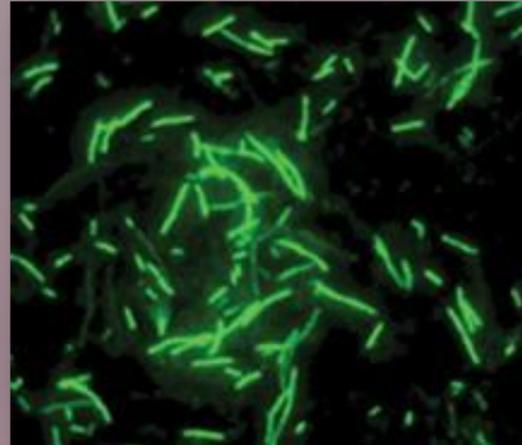


Figure 8. Temperature sensitive hairpin loop

Conclusions

We designed three systems utilizing the regulatory capabilities of mRNA hairpin loops. We were able to produce a working GFP under the control of a lactose promoter, but we were unable to assemble two of our three systems; the one we did assemble failed without enough time remaining in the project to determine what went wrong.

Our suggestions for future iGEM teams include the completion of the NAND gate, and further attempts to digest and ligate component parts for the two types of ribothermometer tests. While we had developed standard RBS controls for both types, we did not have time to assemble them, and they would be necessary for testing once the test parts were properly assembled.

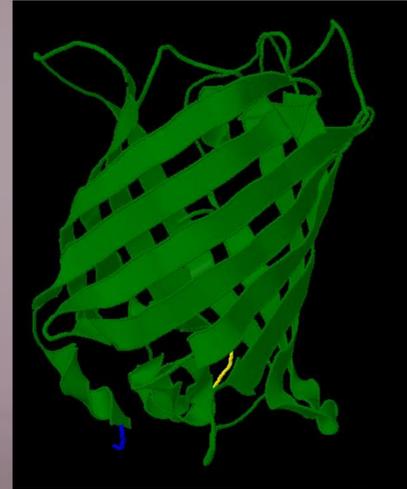


Figure 9. GFP protein structure.

References

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