RNA in Love

Abstract
Anti-sense RNA (asRNA) does not encode any protein, and plays a role of reducing translational efficiency of target mRNA by binding to the target. However, any consistent system to make a stable and highly-efficient asRNA has never been reported yet. This year, we propose a reliable asRNA system based on a clear design concept. To utilize this system, we constructed a BioBrick named “H-stem vector”. By employing this system, we made a BioBrick part that expresses asRNA targeting B0034, the most common RBS part used among iGEMers. We believe that this BioBrick will make a translational suppression easier for all iGEMers and all molecular biologists. Furthermore, we analyzed requirement of asRNA length for efficient translational suppression by testing several species of asRNAs with distinct length. We found that efficiency of the suppression is dependent on an asRNA sequence. The aspect asRNA binds to mRNA specifically seems that a girl falls in love with a handsome boy. So, we named our project “RNA in love”. HokkaidoU Japan is a Cupid to help love of asRNA for mRNA. Don’t leave your eyes from their pure love!

H-stem vector
Since a single-stranded asRNA is fragile to degradation by ribonuclease, production of a great amount of asRNA is required to suppress target mRNA. Recently, Nakashima et al. found that asRNA flanked by paired termini, stem-loop structure, assists stable suppression of expression of target gene[1]. Based on this theory, we designed an original BioBrick, “H-stem vector”, which enables you to utilize such a powerful asRNA. Inserting a certain part of target gene into this vector upside down by NdeI and XhoI, you can express asRNA with paired termini.

Anti-sense B0034
We made a common asRNA construct that can suppress most of target genes generated by iGEMers. The target region of this asRNA is RBS. More than 2/3 of BioBricks use BBa_B0034 as RBS. Thus, we constructed anti-sense RNA targeting B0034.

Anti-sense B0034 (asB0034) fragments were synthesized by annealing oligonucleotides. Unfortunately, the experiments with H-stem vector were unsuccessful. So we showed the results of preliminary experiments using pHN1257 below. First, we evaluated whether asB0034 could repress GFP or mRFP expression (Fig. 5). We found asB0034 works to different kind of CDS. Furthermore, to investigate asB0034 specificity, we used B0034 and B0032 as RBS controlled targets (Fig. 6). We found that the repression level of B0034 was stronger than that of B0032. According to these results, we suggest that we can suppress more than two constructs all at once when we use asB0034 construct.

Length variation
We used B0034-mRFP as the target gene construct and found the most powerful anti-sense.

Each fragment has the sequence from just upstream of RBS to a CDS with distinct position. And then, we ligated DNA fragments with H-stem vector in reverse direction to make asRNA constructs.

We found that 90 nt asRNA showed the most significant suppression among four asRNA constructs. This result indicates that there is an optimal length for asRNA. Although optimal length might be distinct depending on a kind of target gene, you may find the best one by following our method.

Development
We developed a method to make all constructs used above experiment at once. When we amplified DNA fragments corresponding to regions for asRNA, we used a common forward primer and randomized reverse primer with Neol restriction site and 6 arbitrary bases, which binds to any region of target with equal probability. Since Tm of randomized primer is rather low, we used a single-stranded template and DNA polymerase which works at 37°C at initial step of DNA synthesis followed by PCR amplification of DNA fragments containing target sequence. We inserted these DNA fragments into H-stem vector in opposite direction.

Reference