Promoter Library

We synthesized the theoretical ideal promoter (K1084031) based on plasmid and consensus sequence. After that, designed 4096 different promoters by mutating 6 bases at -35 region. We picked up 10 of them (Fig. 1) and measured their strength. Finally, we selected 5 of them for POK (Fig. 2).

RBS Library

We constructed new RBS library, S02, SD4, SD6, SD8 (Fig. 3) based on Vibomg (2007). We measured our RBSs translation efficiency by β-Galactosidase assay.

Promoter Selector

Promoter Selector can optimize the strength of promoters. It consists of five plasmids. Each plasmid has different promoters with different color for identification (Fig. 5). This kit uses “Golden Gate Assembly” reaction. Digest and ligate your protein coding sequence and all our Promoter Selector together. All the protein coding sequence will be inserted in the plasmid. Transform the ligated DNA to E. coli.

Result of Promoter Selector

As a demonstration of our Promoter Selector, we optimized the expression of Kanamycin resistance protein. If the Kanamycin concentration was high, the colony with strong promoter would survive. On the contrary, in low Kanamycin condition, colony with weaker promoter would survive. Actually around 300 colonies had appeared on LBKC plate (Fig. 9). All colors of expressed by Promoter Selector vector were appeared. From the result and the experimental fact, the existence of Km resistance gene in Promoter Selector’s Bsal cloning section is partially confirmed. Our Promoter Selector was successfully assembled. However it was not all to adopt all, and there were no difference among the numbers of expressed colonies.

Future

You can combine Promoter Selector and RBS library. That will result in 320 patterns (Fig. 12). Therefore, you can get wider range of protein expressions. In the case of producing valuable molecules inside the cell, it is important to select the RBS which has the largest yield. By using our kit, you can optimize various enzymes instead of color protein. Therefore, this kit gives us a possibility to regulate metabolic reaction.

Assembly Method - Golden Gate Assembly with Bsal -

Bsal

In those kits, we use a restriction enzyme, Bsal. The property of Bsal is a key of our two kits. Bsal recognizes GTGTC sequence but Bsal’s recognition site is different from its cutting site. By designing cutting sites, you can create 4096 kinds of overhangs and assemble DNA as desired! You can insert all CDS to our kits without mistaking

GGA

You can digest and ligate at same time in one tube. Overhangs that you designed make it possible. Overhangs are created by Bsal digesting. Different color means different overhang. So, you can assemble DNA as desired.

Result of RBS Selector

64 RBS Selectors! We decided to use color expression for CDS (erfluc, arluc, and amr2AP) as a demonstration of our Golden Gate Assembly using tandem-RBS. If those colors were expressed in one colony, several colored colonies would be observed by the differences of RBS strength. After assembling, such construct like (Fig. 10) will be assembled, and three kinds of protein might be expressed. The RBS exists in the upstream of each CDS will become clear by the electrophoresis of colony PCR.

Conclusion: From the colony PCR result, in blue colony, it is considered that the SD2 RBS is in the upstream of amr2AP. Additionally, SD4 connected with erfluc, SD6 connected with arluc. In Green colony, SD4 exists both upstream of erfluc and arluc, then, SD4 exists upstream of amr2AP. From this results, we considered that our tandem-RBS and CDS are partially assembled in Golden Gate Assembly.

Electrophoresis result of colony PCR. If band exists in a lane, this means the protein exists downstream of the lane’s RBS.

RBS Selectors (RS) consists of “Tandem RBS” (K1084031), which includes 4 kinds of RBS for 3 CDS, and its acceptor vector.

1. Tag Bsal site to CDS(s) of your interest. (Primer Designer will help you!) 2. Mix RS and CDS(s), then digest and ligate them using Golden Gate Assembly! The RBS will be randomized so Transform and spread it on the plate.

3. After a 24h, you will get many colonies. Each of them has 1 of 64 patterns of RBSs! There must be a favorite combination of RBSs on the plate! Please perform some assays and select the best one!

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