Introduction of EvolvR

1. Introduction of EvolvR

The capacity to diversify genetic codes advances our ability to understand and engineer biological systems[1]. Several methods for generating mutant libraries exist, but none provide a means to continuously diversify all nucleotides within a user-defined genomic region. EvolvR, a CRISPR-Cas9 based targeted mutagenesis method developed by the Dueber Lab at Berkeley, provides a new approach for generating novel genetic variants in bacteria.(from https://blog.addgene.org/targeted-mutagenesis-with-evolvr)

By fusing error prone polymerase Poll3M to nCas9, the nick site, introduced by sgRNA targeting and nCas9 nicking, serves as the initiate point for error prone PolI3M function. Due to its modular nature, the Dueber Lab created a few versions of EvolvR. PolI5M, generated by two additional mutations to PolI3M which increased EvolvR's mutagenesis rate to ~10-3 mutations per nucleotide per generation.

Here we use PolI5M and eSpnCas9 to target the music sequence, expecting to generate mutated music sequence and "listen to the mutation".

2. Construction of EvolvR

We use GoldenGate Assembly to construct EvolvR. By synthesizing eSpnCas9-linker-PolI5M 1kb per 1kb, we add GoldenGate tag and linked the parts. After that, the part was linked to standard pSB1C3 vector by T4 DNA ligase mediated ligation.

3. Experimental progress of EvolvR

- Constructing pEvolvR
- Ligating EvolvR
- Cutting pSB1C3 vector
- Linking EvolvR and pSB1C3 vector
- Introduce pEvolvR to bacteria
- Sequencing for the editing outcome

[1] Halperin, S.O., Tou, C.J., Wong, E.B. et al. CRISPR-guided DNA polymerases enable diversification of all nucleotides in a tunable window. Nature 560, 248-252 (2018).