GENOME EDITING AND SYNTHESIS PLATFORMS WHICH FACILITATE THE CONSTRUCTION OF CELL FACTORIES

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We have developed the platform technologies such as genome editing and a large gene cluster synthesis systems and are going to integrate to set up the automated systems for efficient construction of microbial cell factories.

By tethering the DNA deaminase activity to nuclease-deficient CRISPR/Cas9 system, we have developed a genome editing tool that enables targeted point mutagenesis. An AID orthologue PmCDA1 was attached to nuclease-deficient mutant of Cas9 (D10A and H840A) to perform highly efficient and target-specific nucleotide editing. This hybrid system, termed Target-AID, induced cytosine point mutation in 3-5 bases range at the distal site within target sequence. Use of nickase Cas9 (D10A), which retains single-strand cleaving activity, greatly increase the efficiency, although it also occasionally induces insertion/deletion (indel) in higher eukaryotes. Uracil-DNA glycosylase inhibitor further increase the efficiency and reduced the indel formation. The toxicity associated with Cas9 has been greatly diminished, enabling application of this technique to wider range of organisms including yeast, bacteria, animals and plants. In addition, by tethering Glycosilase activity to nuclease-deficient CRISPR/Cas9 system, we have developed a genome editing tool that enables targeted randam mutagenesis.

We have also developed an efficient DNA assembly method, namely, Ordered Gene Assembly in *B. subtilis* (OGAB) method. OGAB method can assemble more than 50 DNA fragments in one-step using *B. subtilis*. Thanks to this high processability, even in construction of long DNA (~100 kb), material DNA fragments can be kept in chemical DNA synthesis-friendly and sequencing-friendly small size (< 2 kb). Since there is no *in vitro* DNA synthesis step that may cause unexpected mutation(s), long DNA by OGAB method using sequence-confirmed material DNA thus contains essentially no mutation. We are now constructing user friendly DNA synthesis system by integrating new automation system, such like a liquid handling robot that is specifically developed for OGAB method

These technologies might lead to new pipelines through which functional genomes are cleated with much faster speed to construct microbial cell factories to produce variety of biofuels and chemicals.