Pilot Project Proposal (Not to exceed two pages) （案）

Name of Project: Long and precise genomic DNA construction using *Bacillus subtilis* （案）

Proposer and Contact Information: Kenji Tsuge (Kobe University), Mitsuhiro Itaya (Keio University) and Akihiko Kondo (Kobe University)
Contact: Kenji Tsuge (ktsuge@port.kobe-u.ac.jp)

Background: In 2006, we performed first whole genome (3.5 Mbp) cloning of bacterium by *Bacillus subtilis* (1). Since then, several instances for bacterial genome-sized long DNA (re)construction appeared, almost all of those used yeast *Saccharomyces cerevisiae*, as an assembly host, due to easy for assembly of long DNA. Yeast assembly system is popular nowadays, but several problems, such like related to GC-content and/or replication origin, unexpected mutation associated with direct use of PCR-amplified DNA as a material, etc, are emerging. We think that *B. subtilis* can hamper these problems and here we propose long and precise genomic DNA construction using *B. subtilis.*

Technical Idea: Ordered Gene Assembly in *B. subtilis* (OGAB) method is an efficient DNA assembly method can assemble more than 50 DNA fragments in one-step using plasmid transformation system of *B. subtilis* (2). 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. Moreover, this system is using bacterium as a host, assembly step and subsequent confirmation step finish within short period (2-3 weeks), comparison with yeast system. However, OGAB method is less easy-to-do compared to other conventional gene assembly method, since this method requires high skill in precise control of molar concentration of material DNAs. In this pilot project, to overcome this situation we are going to develop more user friendly DNA system by integrating new automation system, such like a liquid handling robot that is specifically developed for OGAB method.

Long DNAs thus obtained can be assembled into longer DNA in *B. subtilis* genome by using DOMINO method (3). In this method, a series of DNA fragments, named DOMINOs, which have homologous DNA segments at both ends for recombination between neighbor fragments, are serially assembled in *B. subtilis* genome. Since both OGAB and DOMINO

are completely different assembly manner from yeast system, this combination system may contribute to serve alternative option for conventional long DNA construction method.

As an instance for long DNA construction, we are planning to create new HAC (human artificial chromosome) vector-platform in *B. subtilis* genome, which will make it feasible for construct and transplant long DNA into mammalian cells.

Utility: Construction of long DNA with highest preciseness.

"Fit" For GP-write: System developed in this pilot project may bring opportunities to construct long (~100 kb) and precise genomic DNA within short period, such like 2-3 weeks.

Reference

(1) Itaya, M., Tsuge, K., Koizumi, M., and Fujita, K. Combining two genomes in one Cell: Stable cloning of the cyanobacterium PCC6803 genome in the *Bacillus subtilis* 168 genome. *Proc. Natl. Acad. Sci., USA*, 102, 15971-15976 (2005)

(2) Tsuge, K., Sato, Y., Kobayashi, Y., Gondo, M., Hasebe, M., Togashi, T., Tomita, M., and Itaya, M. Method of preparing an equimolar DNA mixture for one-step DNA assembly of over 50 fragments. *Sci. Rep*. 5, 10655 (2015)

(3) Itaya, M., Fujita, K., Kuroki, A., and Tsuge, K. Bottom-up genome assembly using the *Bacillus subtilis* genome vector. *Nature Methods*, 5, 41-43 (2008)

Submit to: info@engineeringbiologycenter.org