CENTER of EXCELLENCE for ENGINEERING BIOLOGY

GP-write

Pilot Project Proposal

(Not to exceed two pages)

Name of Project:

Synthetic Screening for Essential Introns and Retroelements in human cell and animals

Proposer and Contact Information:

Yasunori Aizawa (yaizawa@bio.titech.ac.jp)
Tokyo Institute of Technology, Japan

Background & Summary:

In the pre-genome-synthesis era, due to their largeness and repetitiveness, physiological significances of introns and retroelements could not have been systematically evaluated in the context of chromosome within living systems, respectively. We will use the power of the synthetic genome approach to pursue this question in three different systems of model animals (human, mouse & fly) in tight collaboration with experts in mouse and fly phenotype evaluation.

For each animal system, we first focus on one historically well-studied gene capable of being tested by our in-house phenotypic evaluation systems and replace one allele of the target gene including its up- and down-stream intergenic regions with the synthetic fragments that are lack of all introns common to all the splice isoforms and all retroelements non-overlapping to exons. With marking the synthetic allele, we compare transcriptional and epigenetic status between the native and synthetic alleles of the target gene under different circumstances. Moreover, after replacing both alleles into synthetic ones, we perform functional analyses of the gene and pathways that the genes are involved in. If any changes are observed in expressional and functional responses, we systematically put back the introns and/or retroelements to identify which introns and retroelements are significant.

Technical Idea:

The first genes to be explored will be chosen, depending on (1) wealth of our knowledge on its expression and function, (2) our in-house experimental systems for phenotype evaluation, (3) if heterogeneous deletion is known to be non-lethal and to show functional abnormalities (for mouse and fly), (4) gene and intron length, (5) scientific and/or industrial impacts. Our current candidates are myc and p53 for human, dopa decarboxylase (*Ddc*) and tyrosine hydroxylase (TH) for mouse, and hedgehog and Wingless for Drosophila.

Impact of intron and/or retroelement removal will be investigated on cancer-related cell biology by using a duploid human cell line HCT116 (Aizawa) and on neural function, behavior and development by using mice (Keizo Takao, University of Toyama) and flies (Takashi Suzuki, Tokyo Tech). Dr. Takao has a complete battery of ~30 mouse behavior test at the Animal Research Facility that he directs and has used it for >100 lineages of different genetically modified mice so far. Dr. Suzuki and I have long experience and full access to equipment to detect and evaluate expressional and functional changes in fly development and human cell biology, respectively. We will have high chances to detect even slight abnormalities in expressional and functional outcomes from synthetic allele(s) of the genes of our interest.

Our first priority for technology development is to make a "scar-free" marker, which should be the foundation in the GP-Write community. For this, we use Excision only PiggyBac Transposase (SBI, LLC), which can remove 10-100 kbp fragments flanked with PiggyBac Inverted Terminal Repeats (ITRs) without any nucleotide trace on chromosomes, unlike loxP and FRT. In our first design, both mini-HPRT1 (negative selection marker) and ExTrap inducable by the most recently developed Tet-regulated promoter (Tet-ON 3G) are flanked by ITRs. If this succeeds, we wouldn't have to be nervous about where to locate the marker on synthetic fragments, which would make our strategy for genome synthesis much simpler.

Also, we will make a protocol for CRISPR/Cas9 usage for promoting the native and synthetic swapping. For our intron removal, the first and last introns can be the ideal spots for CRISPR cleavage, as they are not present on targeting fragments. But the cleavage sites should not be far from the ends of the targeting fragments. These parameters will be optimized.

We first focus on construction and optimization of the "scar-free" marker and CRISPR/Cas9 system while our attempts to make one synthetic allele of myc and p53 genes in HCT116 cells for the 1st year. After these technical tools are mature, they will be applied to the mouse and fly system. The technical development for fly and mouse containing synthetic genomes will be the basis for the GP-Writers who especially deal with multicellular organisms.

Utility:

The "scar-free" marker and CRISPR protocol will be the common assets for GP-Write community. Also, patterns of dispensable introns and retroelements that we will observe must sophisticate our future approaches for genome synthesis. Fewer retroelements will reduce the risk of amplicon mis-assembly. And lesser introns will make gene structures compact, which simplifies gene shuffling and ORF swapping in our future synthetic genomes. If most of introns and retroelement are found to be dispensable, drastic downsizing of animal genome synthesis will become realistic. Our future genome synthesis would surely be cost-effective and labor-saving.

"Fit" For GP-write:

Our challenge "removal of large regions (introns) and repetitive spots (retroelements, especially young ones) from animal genomes" is practically impossible only by the editing approaches and become possible and feasible only together with "Writing".