[SPI-1]

Engineering the RNA Genome of Japanese Encephalitis Flavivirus

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Major advances in positive-sense RNA virus research have been facilitated by the development of reverse genetics systems. These systems consist of an infectious cDNA clone that encompasses the genome of the virus in question. This clone is then used as a template for the subsequent synthesis of infectious RNA for the generation of synthetic viruses. However, the construction of infectious cDNA for the Japanese encephalitis virus (JEV) has been repeatedly thwarted by the instability of its cDNA. As JEV is an important human pathogen that causes permanent neuropsychiatric sequelae and even fatal disease, a reliable reverse genetics system for this virus is highly desirable. The availability of this tool would greatly aid the development of effective vaccines as well as facilitate studies into the basic biology of the virus, including the molecular mechanisms of viral replication, neurovirulence, and pathogenesis. We have successfully constructed a genetically stable infectious JEV cDNA containing full-length viral RNA genome. Synthetic RNA transcripts generated *in vitro* from the cDNA were highly infectious upon transfection into susceptible cells, and the cDNA remained stable after it had been propagated in *E. coli* for 180 generations. In summary, we have developed a reverse genetics system for JEV that will greatly facilitate the research on this virus in a variety of different fields.

[SPI-2]

Seguence Analysis of Acidic Laccase in *C. congregatus* and GFP Expression with Acidic Laccase Promoter

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C. congregatus produces several laccase isozymes during its development. A laccase isozyme (acidic laccase) was massively secreted into culture supernatant when *C. congregatus* was tranferred to an acidic liquid medium (pH4.2). The acidic laccase gene is regulated at the transcriptional level and was thought to play a role in protecting this fungus under acidic environment, possibly by neutralizing the acidic condition. In order to understand molecular biological character of acidic laccase, we have analyzed the acidic laccase genomic DNA sequence and have tried to express the laccase promoter-recombinated GFP under acidic conditions. The acidic laccase gene, *lac2* of *C. congregatus* was identified in genomic libraries by using DIG labeled probe which was amplified between copper-binding region I and II in acidic laccase cDNA, *clac2* by PCR technique. Direct comparison with *clac2* showed that *lac2* was interrupted by 8 small introns. Putative eukaryotic regulatory seguences, "CAAT" and "TATA", were observed in the 5'-flanking region of genomic DNA.