E303 Molecular Cloning and Sequencing of the Double-Stranded RNA
Genome of Ustilago maydis Virus SH14

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The double stranded(ds) RNA genome of *Ustilago maydis* virus(UmV) strain SH14, containing 10 segments genomic dsRNA, was partially cloned and sequenced. Genome dsRNA were extracted from purified virus particle and H3 segment was purified by agarose gel elution. cDNA was synthesized by reverse transcription followed by PCR amplification using oligonucleotide primers. These amplified cDNA fragments were cloned at Sma I site in pBluescript SK(+). The cDNA clones of UmV H3 genome were about 460, 590 and 700 bp long. The 590 and 700 bp cDNA fragments showed a high degree of amino acid sequence similarity to *Saccharomyces cerevisiae* virus L-A pol and capsid protein region, respectively, upon BLAST searches of the GenBank database. The results of northern hybridization confirmed that all three cDNA clones originated from H3 segment of UmV SH14. The results of sequencing data from other cDNA clones were also discussed.

E304 Expression and purification of immunologically active porcine recombinant $TGF-\beta 1$ and GFP fusion protein from insect cells

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We have generated recombinant baculovirus for the expression of recombinant porcine $TGF-\beta 1$ fusion protein. The recombinant baculovirus was produced by $Bac-To-Bac^{TM}$ baculovirus expression system. In this system the expressed recombinant protein has $6 \times His$ at its amino terminus. The 6xHis has strong affinity for Ni-NTA resin allowing the desired protein to be purified easily. We expressed the recombinant $TGF-\beta 1$ fusion protein which contained green fluorescent protein (GFP) at N terminus of $TGF-\beta 1$ monomer. The fusion protein was detected at maximum level at 72 hours of post-infection at moi of 10 and it was purified using Ni-NTA column. The expressed fusion protein and purified fusion protein has the same molecular weight of 45 kDa on SDS-PAGE. These fusion protein has strong reactivity with a polyclonal antibody against human $TGF-\beta 1$ and GFP polyclonal antibody.