The proposed translation products of the *ncr*A show a strong homology with *nre*B from pTOM9 of *Alcaligenes xylosoxydans* 31A, there was 77 % amino acid identity.

F307

Analysis of Reverse Transcriptase Gene (pol) of the Korean-type Bovine Leukemia Virus (BLV)

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Bovine leukemia virus (BLV) is an etiological agent of chronic diseases in cows worldwide. The BLV is one of retroviruses that contain a multi-functional enzyme, reverse transcriptase produced from the pol gene in its genome. We have sequenced some regions in the pol gene of 13 BLV proviruses found in the Southern province of Korea from samples that turned out to be BLV positives by a PCR analysis. On the 5' side of the BLV pol gene (polymerase region), it was found that there were four leucines located at every 7 amino acid. They can form a leucine zipper motif that was not same as the pol gene of Japanese BLV isolate. The sequencing result of the proviral pol gene in Korean-type BLV also revealed some mutations leading to amino acid changes such as AAT (Asn)→AAA(Lys), CCT(Pro)→CTC(Leu), and non-sensible variations i.e., TCT(Ser)→TCC(Ser) and ACG (Thr)→ACA(Thr). On the 3' side of the pol gene (integrase region), some nucleotide sequences were mutated and led to amino acid changes. Among them, a mutation, GAA(Glu)→GAC(Asp) occurred in many Korean-type BLV proviruses was very interesting because the amino acid was regarded as one of the most conserved amino acids in the retroviral integrase. It was also notable that the mutation on any leucine residue did not occur, inspite of its frequent appearance. Furthermore, it was found that the mutation rates in the analyzed regions of the Korean-type BLV pol gene were variable from BLV10C (2.01%-2.81%) to BLV6C (3.21%-4.02%) although the values were far less than those of other countries' BLV.

F308

Single Point Mutation at the 26th Amino Acid in Spp2p of *Saccharomyces cerevisiae* is Responsible for the Dominant Negative Phenotype

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The Spp2p of *Saccharomyces cerevisiae* is known to promote the first transesterification reaction of the pre-mRNA splicing and interacts with the Prp2p which is also involed in the pre-mRNA splicing. As an approach to searching for the functional domain of the Spp2p, we have tried to isolate dominant negative mutations of the *SPP2* gene of *S. cerevisiae*. First, we constucted a YEp-GAL1-*SPP2* plasmid in which *SPP2* gene expression is inhibited by glucose but induced by galactose. Then, libraries of mutant *spp2* genes were generated by subculturing the *dnaQ E. coli* strains CGSC 6485 and CGSC 6862 harboring the YEp-GAL1-*SPP2* plasmid for 500 generations at 37° C. Because the *E. coli* strains have no proof-reading activity of the DNA polymerase III at nonpermissive temperatures, mutations would be randomly generated in the *SPP2* DNA during the generations. Ura^- yeast cells were transformed to Ura^+ with mutant library DNAs isolated from the *E. coli* cells. We then screened yeast transformants which cannot grow upon shifting to a galactose-containing

medium. The mutant plasmid DNA designated M3 was retrieved from one of the screened transformants, and its base sequence was analyzed. We found that only one base had been altered at the 76th nucleotide of the *SPP2* reading frame from thymine to cytosine, resulting in a codon change of Ser26Pro. Currently, we are isolating mutant *spp2* genes which have other amino acid codons at the 26th codon.

F309

The Autonomously Replicating Sequence (ARS) of the *Coriolus versicolor*

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Fragments containing ARSes were cloned from the genomic DNA library of the fungus *Coriolus versicolor*. Fifteen recombinant plasmids from the library showed high frequencies of transformation in yeast *Saccharomyces cerevisiae*. They were maintained stably under selective conditions, but were gradually lost from yeast cells at different rates under nonselective conditions, indicating that they contain eukaryotic origins of DNA replication and exist as extrachromosomal plasmids. Base sequences of three ARS DNAs among the 15 cloned fragments revealed that all of them contain the 11 bp [(A/T)TTTA(T/C)(A/G)TTT(A/T)] consensus sequence of the budding yeast ARS. and the other elements. In order to construct a shuttle vector for *E. coli*, *S. cerevisiae*, and *C. versicolor* we generated recombinant plasmids consisting of one of these ARS fragmants, the origin sequence for ColE1 replication, the yeast *URA3* marker, and the phosphinothricin resistance gene. We are currently focusing on the isolation of phosphinothricin resistant transformants of *C. versicolor* by introducing the ARS-containing recombinant plasmids into the protoplasts of *C. versicolor*.

F310

Genomic cloning and characterization of *HpaD* gene encoding 2,3-dioxygenase from *Pseudomonas* sp.DJ-12.

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2,3-dioxygenase (*Hpa*D gene) catalyzes the ring-fission reaction of aromatic substrate. *Hpa*D gene was cloned from the chromosomal DNA of *Pseudomonas* sp. DJ-12 and it's nucleotide sequence analyzed. The 40kb fragment containing *Hpa*D gene was inserted into the BamH1 site of pWE15 cosmid vector and designated as pKC1. The 3.4 kb fragment containing *Hpa*D gene was made from pKC1 with subcloning. The open reading frame (ORF) corresponding to the *Hpa*D gene consists 864 base pair with ATG initiation codon and TGA termination codon. The putative RBS gene fragment is located on the 10bp upstream of the initiation codon. The gene analysis was carried out by comparison of the gene with another strains. Sequence homology was revealed 55.6% with another strain such as *Escherichia, Klebsiella* and *Salmonella*.

F311

Molecular Cloning and Expression of Fe-Containing Superoxide Dismutase from *Neospora caninum*