

strategy is that the level of expression of target gene is not sufficient to kill specific cancer cells and regulatory elements expressing the target gene only in cancer cells are hard to obtain. As a means to obviate this critical limitation, we demonstrated the isolation of synthetic regulatory elements showing cancer-cell specific expression from the shuffles of various combinations of the regulatory elements in several well-known cancer cell-specific genes. Out of 7 selected regulatory elements in T cell factor 1 α (TCF1 α) gene enhancer, c-Ha-ras gene promoter, transcription factor E2F binding site in cyclin E and cdc25 phosphatase genes, hexokinase gene promoter, and telomerase catalytic subunit (hTERT) gene promoter, more than 200 combinatory regulatory elements were constructed by random ligation or random PCR. Cancer specific high expressivities of these synthetic regulatory elements were monitored in the transiently transfected cancer cell lines (HeLa, CV1, and HepG2) or normal fibroblasts after subcloning these elements individually into pGL3-Luc vector along with a control pRL-TK vector. Several clones showed promising luciferase activities in HeLa cell line and two of them also showed high luciferase activities in HepG2 cell line.

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Expression of Poly (ADP-ribose) Polymerase During Apoptosis Induced by Ultraviolet Radiation in HeLa S3 Cells

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The present investigation has been performed to elucidate the expression of poly (ADP-ribose) polymerase (PARP) during apoptosis induced by ultraviolet radiation

(UV) in HeLa S₃ cells. Four assays were employed in this investigation; Gel electrophoresis of isolated DNA, quantitative assay of fragmented DNA, morphological assay of apoptotic cells and western blot analysis. Alteration of DNA level on apoptosis was determined by DNA ladder pattern. DNA ladder pattern in HeLa S₃ cells irradiated with UV was observed from 6 hrs to 18 hrs after incubation. The cleavage of PARP and DNA fragmentation during apoptosis was shown from 6 hrs after incubation. And the cleavage of PARP pretreated with 2 J/m² UV and subsequently treated with 50 J/m² UV was lower than treated with 50 J/m² UV alone. And the cell viability pretreated with 2 J/m² following treated with 50 J/m² is higher than treated with 50 J/m² alone.

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Construction and Characterization of Garlic Bacterial Artificial Chromosome Library.

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We had constructed two BAC libraries of Danyang garlic cultivar. First one has been constructed using the pIndigoBAC536 vector. HMW garlic DNA was extracted from leaf protoplasts. Total 129 clones were obtained from transformation of 1/50 fraction of the ligation mixture. Among 129 clones, 47 clones contained relatively large inserts and average size was about 97 kb. Seventeen BAC clones out of 47 showed strong positive hybridization signals on Southern blot analysis using garlic genomic DNA as a probe. Second BAC library was constructed using the pBAC1SACB1 vector, which is designed to give zero background. HMW

garlic DNA was extracted from leaf nuclei. A total of about 10,000 clones were obtained. This BAC library has more than 92% recombinants and 50-150 kb of genomic DNA inserts. Using several candidates from first BAC library as a probe, PFGE Southern blot analysis and FISH analysis were performed to assign the locations of each DNA inserts on Not I -restricted chromosomal DNA and on the chromosomes, respectively. Its experimental result will be discussed.

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Isolation and Characterization of Annexin cDNAs in *Capsicum annuum* L.

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Annexins are a family of structurally related proteins that exhibit Ca²⁺-dependent phospholipid-binding. Also they are involved in variety of plant cellular processes. Most annexins are abundant intracellular proteins, composing often more than 2% of total cellular proteins. They have been implicated in multiple aspects of cell biology including regulation of membrane trafficking, trans-membrane channel activity, inhibition of phospholipase A2, inhibition of coagulation, transduction of mitogenic signal, and settlement of cell-matrix interaction. But the exact biological functions of the annexins are not known yet. By polymerase chain reaction (PCR) using degenerated primers, we isolated partial cDNA encoding hot pepper (*Capsicum annuum* L.) annexin P38. And by cDNA library screening, we isolated two full-length cDNAs related to annexin and annexin P38 of bell pepper. As the results of sequencing analyses, annexin is about 1.2 kb nucleotide encoding 317 amino acid-long peptide that

shows 98% sequence identity and annexin P38 is about 97% sequence identity with bell pepper. Using Southern blot analyses, these cDNA clones were represented small copy number, and in northern and western blot analyses, the potential roles of these cDNA clones in variety of stresses and hormone-induced cellular functions are discussed.

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Organization and Regulation of the *arg* Operons in *Corynebacterium glutamicum*: ArgR Acts as the Arginine Repressor Protein

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We have studied the organization and regulation of the arginine biosynthetic genes in *Corynebacterium glutamicum*. A genomic library for *Corynebacterium glutamicum* was screened for clones carrying arginine biosynthesis genes by complementation of *Escherichia coli* mutants. As based on the complementing and sequencing analysis of the plasmids that carries a cluster of *argCJBDFGH*. It was found that *C. glutamicum* has an arginine repressor ArgR, located in the upstream region of *argG*, homologous to the other bacteria. The gel filtration indicated that molecular mass of the native protein is 110-kDa meaning that ArgR is a hexamer of equal subunits. Gel mobility shift assays revealed that in the presence of arginine, ArgR binds to a site upstream from the *arg* promoters.

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Expression Vectors Containing *ars* (aryl sulfatase) Promoter of *Neurospora crassa*