H302 Isolation of Full Size Human Telomerase Reverse Transcriptase(hTERT) Gene by TAR cloning

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The enzyme telomerase plays a crucial role in cellular proliferation and tumorigenesis. Telomerase represents a ribonucleoprotein complex whose core is composed of a catalytic subunit (hTERT) and a RNA component providing the template for the synthesis of telomeric DNA. Previous studies reported isolation of genomic fragments from hTERT. However, the sequence of intron 6 was not completed in that study, and therefore the sequence of hTERT was deposited in GenBank as two fragments separated by a gap of unknown size. Using the recently developed TAR cloning technique in *S.cerevisiae*, which allows entire genes and large chromosomal region to be specifically and accurately isolated from total genomic DNA. We isolated 1 genomic clones containing the entire hTERT gene by TAR cloning.

H303 Study of Tandem Repeat Polymorphisms in Intronic Regions of the Human Telomerase Reverse Transcriptase Gene

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In this work, the full-length hTERT gene was isolated and the sequence of the previously unknown region in intron 6. We have shown that intron 6 includes a variable number of tandem repeats of a 38 bp sequence(hTERT-VNTR 6-1). Eight alleles of hTERT-VNTR 6-1 were identified among 103 unrelated individuals, ranging from 27 to 47 repeats. hTERT-VNTR 2-2 is another new 61 bp minisatellite repeat found in intron 2 of hTERT. At least four alleles of hTERT-VNTR 2-2 can be distinguished. Previous studies have described polymorphisms for minisatellites hTERT-VNTR 2-1 and hTERT-VNTR 6-2. These, together with another minisatellite found in intron 12, add up to 5 such structures within the hTERT gene. The segregation of hTERT minisatellites was analyzed in families, revealing that the VNTRs are transmitted through meiosis following a Mendelian inheritance. Minisatellites in hTERT were also analyzed in matching normal and cancer tissues from patients with tumors; in one patient with a kidney tumor, the 2 VNTRs in intron 6 had undergone concomitant rearrangements. This observation suggests that chromosomal rearrangements implicating these VNTRs may be associated with the activation of telomerase expression in cancer cells.

H304 Analysis of useful TARget Hooks for Optimization of TAR Cloning

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Transformation-Associated Recombination (TAR) cloning can be possible to directly isolate specific chromosomal region and gene from complex genomes as large circular YAC/BACs. In this work, we studied utility of several TARget hooks(one unique, radial and two unique hooks) and their minimal size. At first, we constructed two TAR cloning vector that contain 381 bp and 63 bp of 3′ HPRT sequence at one end and Alu repeat sequence at the other end. As a result, the frequencies of positive clones among total transformants were almost same as 0.43% and 0.45%, respectively. Thus, it is demonstrated that the minimal size of unique sequence

required for single copy gene isolation by TAR cloning is enough approximately 60 bp. And then we compare the utility of several TARget hooks, using same gene. We found the 10-times high transformation frequency using radial hooks, but there are 4 chimeric DNAs among the 9 positive clones. But when we used two unique hooks, there are no chimeric DNA in 12 positive clones. Therefore, we can used two unique hook for accurate TAR cloning.

H305 Study of Using Aqueous Genomic DNA for Optimization of TAR Cloning

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The TAR cloning method is based on co-penetration into yeast spheroplasts of target DNA along with vector(s) that contain a sequence common to human DNA, followed by homologous recombination between the vector(s) and the human DNA to establish a YAC. The speed and efficiency of TAR cloning, as compared to the more traditional methods of gene isolation, provides a powerful tool for the analysis of gene structure and function. To further improve the TAR cloning technology, we determined the optimal size of genomic DNA for easy penetration into spheroplast. We used the aqueous genomic DNA instead of agarose plug for DNA shearing. The frequency of transformation with sheared human DNA is increased at least 10 times. And then, we checked the inserted DNA size in YACs using randomly picked 30 colonies. Interestingly, 4 groups (0, 9, 18 times pipetted DNA samples and commercial DNA) showed the almost same inserted DNA sizes of ~100 kb by CHEF gel. Moreover, we examined the usefulness of aqueous DNA for the isolation of human single gene.

H401 Isolation and Characterization of the Bacteria Having Algicidal Activity Against Toxic Microcystis sp.

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In order to develop ecotechnology controlling cyanobacterial blooms in fresh waters, we isolated the cyanobacteria-lysing (killing) bacteria from the sediment of lake Seokchon and Pal'tang River-Reservoir. A soft agar-overlay technique was used to isolate the algicidal bacteria. Two strains, designated strain SB01 and SB02, showed an algicidal activity against cyanobacterium *Microcystis aeruginosa* (KCTC-AG 10073). The strains were Gram (+) rod and able to produce a brown pigment (fluorescent) when grown on the BG-11 agar medium supplemented with 0.05% yeast extract. Sequence analysis of the bacterial 16S rDNA compared with sequences in the GenBank nucleotide database. An initial test on the cyanobacteria-lysing activity of the bacteria showed that SB02 have the higher activity than the SB01. This results strongly suggest that the indigenous strains may have a potential in development of ecotechnology controlling harmful cyanobacterial blooms in fresh waters.

H801 Effect of UV-irradiation on Expression of Green Fluorescence Protein in yeast Schizosaccaromyces pombe

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yEGFP is a mutant of green fluorescent protein (GFP) that is optimized for increased fluorescence in yeast. In the