IDENTIFICATION AND STUDIES OF DNA INTEGRATION SITES IN TRANSGENIC MDBK SINGLE CELL LINES

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Exogenous DNA can be inserted into genomic DNA by retroviral integration, transposon jumping, as well as gene transfer techniques such as cell transfection assays. The sites of such insertions of exogenous DNA are usually identified by preparing genomic libraries and isolating clones containing both the exogenous DNA and flanking sequences. In this report we obtained several transgenic cell lines from a MDBK (Madin Darby Bovine Kidney) single cell. The established MDBK culture lines were plated at 3 x 10^4 / ml in a 24 well plate and transfected with the plasmid vector pCIneo-?, comprising the *neo* and ?-*gal* genes under control of the cytomegalovirus promoter, utilizing the Lipofectamine™ Reagent (Invitrogen). Twenty days after transfection and selection with geneticin (G418: 400 ?g/ml), single transgenic cells were isolated and expanded to allow isolation 36 ?g of DNA. Genomic DNA from clones cultures were recovered and the flanking sequences were isolated by plasmid rescue in *Escherichia coli*. Since the transgene integration into bovine genome is randomly, the PCR-based method to clone the flanking chromosomal DNA at the site of insertion of foreign DNA is a simple and useful method for this purpose. The extension of this technique by thorough studies might bring new approaches to construction of bovine gene-targeting vectors. Gene targeting is a more powerful method of genetic manipulation and requires essentially the same procedures of transfection and drug selection of cultured cells. The goal to numerous biomedical benefits, for example, ablation of xenoreactive transplantation antigens, inactivation of genes responsible for neuropathogenic disease and precise placement of transgenes designed to produce proteins for human therapy, have incentive commercial and medical sectors to invest in this new technology according to the appropriated integration loci in a more efficient and certain manner to produce the desirable variants.

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