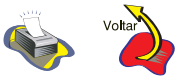

HIGH-FREQUENCY TRANSFECTION OF BOVINE AND OVINE FIBROBLASTS.

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Recombinant proteins are an important staple in the modern pharmacopoeia. The emerging production system of choice for recombinant proteins is the expression in the milk of transgenic dairy animals. The recent achievement of cloning animals utilizing the nuclear transfer, a process which involves the transference of a donor cells nucleus into an enucleated oocyte, has open novel possibilities to achieve transgenic animals at an improved-frequency and reduced costs. The development of an efficient fibroblast transfection system is one of the key steps to generate transgenic embryos. In this study we have evaluated the conditions to develop an efficient bovine and ovine fibroblast transfection system utilizing lipossomes and biolistic. Fibroblasts were isolated from disaggregation of small (2-3 mm) skin tissue fragments from donor animals and cultured in Dulbecco´s modified Eagles Medium (D-MEM), supplemented with 10% fetal serum, 2 mM L-glutamine, 100 µg penicillin/ml and 100 µg streptomycin/ml. The cultures were maintained at 38.5^0C, in atmosphere of 5% CO_2 in air. Before each transference, sub-confluent (70%) cultures were disaggregated by incubation in 0.5% of trypsin and EDTA 0.2% for 5 minutes at 37.5^0C, the total cells were splitted and ressuspended in flasks containning culture medium. After the second transference D-MEM, DMSO and fetal serum at 10% were added to the culture medium. The established fibroblast culture lines were plated at 2 $x10^5$/ml in a culture dish and transfected with the plasmid vector pGAL, comprising the β-galactosidase gene under control of the cytomegalovirus promoter, utilizing different lipossomes (lipofectamine, lipofectin, cellfectin and DMRIE-C, Gibco BRL) or particle bombarded with a high-pressure helium device. Twenty four hours after transfection, the cells were collected, fixed and the expression of the β-galactosidase protein evaluated by histochemical assay. The results have showed a high-frequency expression of the exogenous gene utilizing lipossomes. Under the conditions evaluated, the bombarded cells have showed a low frequency gene expression. The best parameters were utilized to generate stable fibroblasts transfected lines. Fibroblasts were co-transfected with the vectors pCMVscript, carrying the G418 gene under control of the RSV promoter and phrGFP, carrying the hrGFP gene under control of the cytomegalovirus promoter utilizing lipofectamine or lipofectine. Experiments have been carried out to utilize the transfected fibroblasts lines as a source of donor nucleus to generate transgenic animals by nuclear transfer, expressing recombinant proteins of commercial and pharmaceutical importance. Órgão Financiador : EMBRAPA