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# Production of Thrombopoietin Gene Targeted Clones by Homologous Recombination at β-casein Locus of Primary Bovine Ear Skin Fibroblasts

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Research has been in progress for more than a decade to production of useful proteins by genetic modification in cattle. However, the levels of protein production in transgenic cattle have been reported very low. To enhance protein production in transgenic animal, we tried homologous recombination to donor cells for production of transgenic clone cattle through nuclear transfer procedure. Thus, we constructed the two targeting vectors of human thrombopoietin (TPO) at bovine β-casein locus using homologous recombination with 13.6 kb and 9.6 kb homology. In two targeting vectors, positive selection was through the neomycin resistance gene and negative selection was by the diphtheria toxin (DT). Gene targeting was attempted in bovine embryonic fibroblasts (bEF) and bovine ear skin fibroblasts (bESF). To determine the most appropriate concentration of neomycin for bEF and bESF, G418 resistance was confirmed by culturing the cells in various concentrations of the drug and both of the cells were optimally selected at 900 μg/ml of neomycin. The transfected bEF and bESF by the targeting vectors were colonized efficiently at the ratio of DNA to transfection reagent such as 4 μg:2 μl and 1 μg:2 μl. Comparing number of healthy clones from passage 4 to passage 8, bESF (17%) persist in culture for much longer than bEF (6%). The two gene-targeted bESF clones of 30 random-integrated clones with 9.6 kb homology length were confirmed, however, nothing was out of 72 random integration clones with 13.6 kb homology length. The DT also worked more efficiently in clones transfected with the vector of 9.6 kb homology length. Our data suggests that the choice of donor cell for long culture period should be considered to obtain targeted cell clone, and the gene-targeting frequency and the DT working efficiency are dependent on the length of target homology.

Key words) *TPO, Gene targeting, Somatic cell, Cattle*