P0319

Conditions for Selection of Targeted Colonies in the Primary Cells

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The random insertion of useful gene in genome has been a common method to produce transgenic animals. This method is inefficient for induction of high levels gene expression in transgenic animals. To improve this limit, we tried to develop the system which target the gene at the specific genomic region. Thus, in our experiment, the vector system to target the human thrombopoietin (TPO) gene was developed. Targeting vector including TPO, neo and DT genes was transfected into bovine embryonic fibroblasts (bEF) or bovine ear skin fibroblasts (bESF). First of all, we determined concentration of the geneticin (G418) for selection of transfected cell lines. Our results showed that 1200 and 900 μg/ml of G418 were the most proper for selection of transfected bEF and bESF cells. In this study, lipofectamine was used as a transfection reagent. Thus, the proper ratio of DNA:lipofectamine for transfection was also required to elevate targeting efficiency in primary mammalian cells. Our result indicates that the most proper ratios of DNA:lipofectamine were 4:2 and 1:2 in bEF and bESF cells. According to the optimized these conditions, single colonies were picked following transfection and were analyzed by PCR. More than 90% of the single colonies have TPO gene. However, there were no colonies with targeted TPO at the specific genomic region. Therefore, further experiments to select the specifically targeted colonies and to find more efficient methods such as reducing selection time and shortening a size of TPO gene are required.

Key words) ***Transfection, TPO, Primary cells***