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Gene Targeting in Mouse Embryos Mediated by recA and Modified Single-Stranded Oligonucleotides

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Gene targeting is an in situ manipulation of endogenous gene with precise manner by the introduction of exogenous DNA. The process of gene targeting involves a homologous recombination reaction between the targeted genomic sequence and an exogenous targeting vector. In elucidating the function of many genes, gene targeting has become the most important method of choice. Conventional gene targeting has been achieved through a use of embryonic stem cells. However, such procedure is often long, tedious and expensive and has been limited only in the mouse due to a lack of usable embryonic stem cells in other species. This study was carried out to develop a much simplified procedure of gene targeting using *E. coli* recombinase recA and modified single-stranded oligonucleotides. The new procedure was attempted to modify X-linked hypoxanthine phosphoribosyltransferase (HPRT) gene. Single-stranded oligonucleotide to target an exon 3 of HPRT was 74 bases in length and included three phosphorothioate linkages at each termini (also known as S-oligo) to be resistant against exonucleases when introduced into zygotes. The oligonucleotide sequence was homologous to the target gene except a single nucleotide that induces a mismatch between the introduced oligonucleotide and endogenous HPRT gene. Although the exact mechanism is yet unknown, endogenous repairing of such mismatch would give rise to the conversion of TAT to TAG stop codon thereby losing the function of the target gene. Prior to an introduction into zygotes, modified single-stranded oligonucleotides were preincubated with recA recombinase to enhance the homologous recombination. The recA-oligonucleotide complex was microinjected into the pronucleus of zygote. Individual microinjected embryos developed to the blastocyst stage were analyzed for the expected nucleotide conversion using PCR and subsequent sequencing. The conversion of TAT to TAG stop codon was confirmed in two embryos among forty tested blastocysts, so that the frequency of gene targeting was approximately 5%. The result suggests that the gene targeting was feasible by relatively easier and direct method. Subsequent transfer of gene-targeted embryos to recipients to obtain transgenic mice missing the function of HPRT gene is underway. Further technical refinement and enhancement of homologous recombination frequency will be required for the practical use of this new approach for gene targeting in mice.

Key words : ***Gene targeting, RecA, S-oligo, Pronuclear microinjection***