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# Analysis of Transgenic Mice Produced by Testis-Mediated Gene Transfer

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Recently, research techniques have been innovated in animal reproduction field, and as a result of such innovation, transgenic (including gene knock out) mice have been produced. The most widely used method to produce transgenic mice is the direct microinjection of foreign DNA into pronuclei of fertilized eggs. It was reported that sperm-mediated gene transfer was possible to producce TG mice (Lavitrano M. et al., 1989). They reported that mouse spermatozoa were able to capture foreign DNA molecules simply by incubating spermatozoa in a medium containing DNA for 15-30 min. They also showed that transgenic mice could be obtained using these spermatozoa for IVF and ET. This method was simple and rapid compared to the microinjection of foreign DNA into pronuclei, therefore, there were many attempts to repeat their result. However, unfortunately many researchers failed to follow their methods with some exceptions (Brinster et al., 1989).

Sperm-mediated gene transfer has been drawing attention to capitalize on an easier approach to obtain transgenic mice since then. Before Lavitrano's report, there were reports on the ability of animal spermatozoa to capture foreign DNA (Horan et al., 1991) and DNA binding molecules on sperm surface (Wu et al., 1990; Zani et al., 1995). Furthermore, foreign DNA were also introduced into bovine spermatozoa by electroporation (Gagne et al., 1991) and into mouse or avian spermatozoa by using liposome vesicles (Bachiller et al., 1991). Recently, a new method for producing transgenic mice by direct injection of exogenous DNA into testes of male mice has been attempted (Sato et al., 1994; Ogawa et al., 1995). This direct injection of foreign DNA/liposome complex into a testis is expected to be a simple and convenient tools for production of transgenic animals.

In this experiment, we tried to produce transgenic mice by mating untreated female mice with male mice that DNA/liposome complex was injected into their testes and the transgenecity of following generation was examined. The pBluescript II KS+ plasmid

that was linearized by EcoR1 was mixed with liposome solution (Lipofect Amine Reagent; GIBCO-BRL, Gaithersburg, MD, USA) and 20 ml of this DNA/liposome (10 mg/ml DNA) complex was directly injected once into each testis of adult C57BL/6N or ICR strain male mice. One week after the injection, all male mice were mated with normal ICR female mice and F1 progeny were collected after term.

Three weeks after birth, the genomic DNA of F1 progeny was extracted from their tail and examined by southern blot analysis. Transgenic F1 progeny were mated again and the transgenecity to following generation was examined. In 4 replicates, total 38 F1 progeny were born and 5 mice out of 38 were confirmed their transgenesis. These 5 male mice were mated with normal ICR females and 2 of 5 male mice had F2 progeny with exogenous DNA.

Now we are confirming the localization of exogenous DNA in chromosomes by FISH and analyzing a fragmentation of DNA in details. Furthermore, we are trying to produce new transgenic mice by this method using new constructed expression vector that has a marker gene.

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