# 구두발표-2

## Transgenic fish and animal cloning

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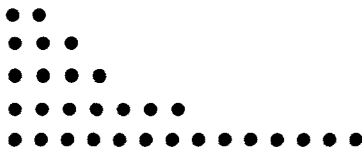
### Introduction

Zebrafish has become an important organism for the study of vertebrate development due to its accessibility to forward genetics, embryonic manipulation and transgenic analysis. Methods are available to perform large-scale mutagenesis screens, allowing identification of key regulatory genes in development. Nevertheless, to fully realize the potential of this organism, tools of reverse genetics are needed. Once the scheduled zebrafish genome project is complete, targeted genetic manipulations will become even more desirable for zebrafish. Production of cloned animal using cultured somatic cells offers the possibility of targeted genetic manipulations. After the birth of Dolly from differentiated cells, a number of successful animal cloning experiments using somatic cells have been achieved, including recent reports producing "gene-knockout"sheep and pigs by nuclear transfer from genetically manipulated somatic cells. We have recently shown that fertile transgenic zebrafish can be produced by nuclear transfer (NT) using long-term cultured somatic cells. To utilize this technique for future characterization of novel genes and gene functions in zebrafish, we performed genetic manipulation on the cultured cells using the gene-trap strategy.

### Meterials and Methods

Eggs of good quality are slightly granular and yellowish in color. The eggs were immediately placed in Holtfreter's solution and dechorionated with pronase. These eggs can be used as recipients up to 1 hour after



activation. Cells were kept on ice in DMEM until nuclear transfer. Normally, the cells will be used for nuclear transfer less than 60 minutes after preparation. To remove the egg pronuclei, recipient eggs were placed in a drop of Hank's solution containing 1.5% BSA. Each dechorionated egg was oriented using the holding/injection needle to determine the position of pronucleus. The nucleus was sucked out from the egg with a fine glass needle by aspirating a small amount of cytoplasm just underneath the polar body. Nuclear transplants were cultured in Holtfreter's solution at 28玫 until hatching. Hatched embryos were then placed into fish water and reared to the adult stage.

**Results and Discussion**

The donor nuclei, modified by retroviral insertions expressing the green fluorescent protein (GFP), were transplanted into manually enucleated eggs. Overall, a 2% success rate was achieved. These nuclear transplants produced fertile and diploid offspring and their F1/F2 progeny continued to express GFP in a pattern identical to that of the founder fish. We have also infected the long-term cultured cells using a GFP gene-trap virus carrying a splicing acceptor and obtained positive trap events. For SA/GFP-TP, approximately 0.2% cells of the neo-resistant population appeared positive for GFP expression, indicating generation of a GFP fusion protein (or proteins) by splicing. For Neo-TP. Molecular analysis indicates that gene-trap approaches can be used to identify expressed genes in cultured zebrafish cells(data not shown). Their competency for production of viable nuclear transplants is currently being tested. If successful, zebrafish will provide all of the comparable genetic tools offered by mammalian systems.