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# Generation of Embryonic Stem Cell-derived Transgenic Mice by using Tetraploid Complementation

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The standard protocol for the production of transgenic mouse from ES-injected embryo has to process via chimera producing and several times breeding steps, In contrast, tetraploid-ES cell complementation method allows the immediate generation of targeted murine mutants from genetically modified ES cell clones. The advantage of this advanced technique is a simple and efficient without chimeric intermediates. Recently, this method has been significantly improved through the discovery that ES cells derived from hybrid strains support the development of viable ES mice more efficiently than inbred ES cells do. Therefore, the objective of this study was to generate transgenic mice overexpressing human resistin gene by using tetraploid-ES cell complementation method. Human resistin gene was amplified from human fetal liver cDNA library by PCR and cloned into pCR□ 2.1 TOPO T-vector and constructed in pCMV-Tag4C vector. Human resistin mammalian expression plasmid was transfected into D3-GL ES cells by lipofectamine 2000, and then after 8~10 days of transfection, the human resistin-expressing cells were selected with G418. In order to produce tetraploid embryos, blastomeres of diploid embryos at the two-cell stage were fused with two times of electric pulse using 60 V 30 $\mu$sec. (fusion rate : 93.5%) and cultured upto the blastocyst stage (development rate : 94.6%). The 15~20 previously G418-selected ES cells were injected into tetraploid blastocysts, and then transferred into the uterus of E2.5d pseudopregnant recipient mice. To investigate the gestation progress, two E19.5d fetus were recovered by Casarean section and one fetus was confirmed to contain human resistin gene by genomic DNA-PCR. Therefore, this finding demonstrates that tetraploid-ES mouse technology can be considered as a useful tool to produce transgenic mouse for the rapid analysis of gene function *in vivo*.

Key words) ***Tetraploid, Human resistin, transgenic, Embryonic stem cell***