Nanos3 Gene Targeting in Medaka ES Cells

Guijun Guan1,2, Yan Yan2, Tiansheng Chen2, Meisheng Yi3, Hong Ni2, Kiyoshi Naruse1, Yoshitaka Nagahama4,?, Yunhan Hong2,?

1. Department of Bioresource, National Institute for Basic Biology, Okazaki, Aichi 444-8585, Japan; 2. Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543; 3. School of Marine Sciences, Sun Yat-Sen University, Guangzhou, 501275, China; 4. Institution for Collaborative Relations, Matsuyama 790-8577, Ehime University, Japan.

Abstract:

Gene targeting (GT) by homologous recombination offers the best precision for genome editing in mice. nanos3 is a highly conserved gene and encodes a zinc-finger RNA binding protein essential for germ stem cell maintenance in Drosophila, zebrafish and mouse. Here we report nanos3 GT in embryonic stem (ES) cells of the fish medaka as a lower vertebrate model organism. A vector was designed for GT via homologous recombination on the basis of positive-negative selection (PNS). The ES cell line MES1 after gene transfer and PNS produced 56 colonies that were expanded into ES cell sublines. Nine sublines were GT-positive by PCR genotyping, 4 of which were homologous recombinants as revealed by Southern blot. We show that one of the 4, A15, contains a precisely targeted nanos3 allele without any random events, demonstrating the GT feasibility in medaka ES cells. Importantly, A15 retained all features of undifferentiated ES cells, including stable self-renewal, an undifferentiated phenotype, pluripotency gene expression and differentiation during chimeric embryogenesis. These results provide first evidence that the GT procedure and genuine GT on a chromosomal locus such as nanos3 do not compromise pluripotency in ES cells of a lower vertebrate.

Key Word:
ES, gene targeting, homologous recombination, nanos3, pluripotency.