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SOMATIC CELL NUCLEAR TRANSFER IN ZEBRAFISH

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The first and only report of successful cloned zebrafish was published in 2002. Since then, there have been no other reports on the use of somatic cell nuclear transfer (SCNT) in this species. This is due to, in part, a lack of characterization of the technical and biological parameters necessary for a successful implementation of the procedure. Here, we report a reliable and reproducible protocol for zebrafish SCNT that addresses concerns of egg's enucleation and parthenogenetic activation. For the confirmation of the origin of the cloned animals, we used phenotypic traits, as well as DNA fingerprint markers. A homozygous mutant *golden* strain (albino) [Zebrafish International Resource Center, ZIRC] was used as donor nuclei source and eggs from a transgenic *H2Az-GFP* strain with AB background [ZIRC] were used as recipients for the entire study to facilitate phenotypic screening of cloned fish. Our approach employed non-activated recipient eggs, laser targeting ablation of the metaphase plate of the egg, and micropylar nuclear transferring. Recipient eggs were collected and held in vitro in Chinook salmon ovarian fluid until micromanipulation was completed. While keeping the chorion intact, the fluorescence DNA stained metaphase plate of the egg was ablated using a laser XYClone module [Hamilton Thorne Biosciences, Inc.]. The donor nucleus was then transferred through a micropyle, the natural sperm entry site in the chorion. Reconstructed embryos were activated in egg water and allowed to develop in embryo water. We tested donor cells derived from either cultured adult fin cells or fresh isolated cells from tail part of an embryo at 15-20 somites (18-20 hours post fertilization). The developmental rate of cloned embryos from cleavage to complete gastrula stage was comparable in both donor cell sources. Two to six percent of cloned hatch fry (3 day old) were obtained from donor cells derived from adult fin and embryonic tail clip, respectively. Two percent of reconstructed embryos from embryo-derived cells developed to adult fish (3 months), but none of the cloned fish derived from donor nuclei of adult fin cells did so. Of eleven single nucleotide polymorphism markers tested, we found a complete matched genetic identity between the donor cells and the cloned fish, no match with the recipient egg donor was observed. All of the cloned fish were *golden* phenotype. Cloned fish that reached their reproductive maturity were fertile and produced *golden* offspring. Ongoing research is focused on determining the most suitable type of donor nucleus for SCNT taking into consideration its epigenetic state prior to the procedure. Subsequent studies with cell lines that have undergone homologous recombination are warranted.