

The reproduction of Asian arowana: Analysis by polymorphic DNA markers

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Asian arowana or dragonfish (*Scleropages formosus*, Osteoglossidae) is an ancient teleost with highly unusual characteristics. Its approach to reproduction is atypical for a large bodied fish: it lays very few eggs of a huge size (1 cm in diameter). Following fertilization the embryos/larvae are nurtured in the mouth of one of the parents during their early development. Although Asian arowana is one of the most expensive species in the ornamental trade, traditional practices employed in its aquaculture do not allow the farmers to establish selective breeding projects due to lack of essential information on parentage. Currently only one of the parents (assumed to be the male) can be identified as the offspring are harvested from his mouth. The other parent cannot be identified from among a dozen potential parents present in the pond. Using over a dozen microsatellite markers previously developed in our laboratory we genotyped all brooders and more than 120 batches of their offspring to create a genotype database. Analysis of the microsatellite-based genotypes allowed us to verify the known parent and identify his partner in all batches of offspring tested. Suspected paternal mouthbrooding of this species was also confirmed by comparative analysis of several mtDNA genes.

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Cloning and expression analysis of an inducible Hsp70 gene from Pacific abalone (*Haliotis discus hannai*)

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Heat shock protein 70, the primary member of HSPs that are responsive of thermal stress, is found in all multicellular organisms and functions mostly as molecular chaperon. The inducible heat shock protein 70 (*HSP70*) cDNA was cloned from Pacific abalone *Haliotis discus hannai* using rapid amplification of

cDNA ends (RACE), which was highly homologous to other *HSP70* genes. The full-length cDNA of the Pacific abalone *HSP70* was 2631 bp, consisting of a 5'-terminal untranslated region (UTR) of 90 bp, a 3'-terminal UTR of 573 bp with a canonical polyadenylation signal sequence AATAAA and a poly (A) tail, and an open reading frame of 1968 bp. The *HSP70* cDNA encoded a polypeptide of 655 amino acids with the ATPase domain of 382 amino acids, the substrate peptide binding domain of 161 amino acids and the C-terminus domain of 112 amino acids. The temporal expression of *HSP70* was measured by semi-quantitative RT-PCR after heat shock and bacterial challenge. Challenge of Pacific abalone with heat shock or the pathogenic bacteria, *Vibrio anguillarum*, resulted in a dramatic increase in the expression of *HSP70* mRNA levels in muscle, followed by a recovery to normal level after 96 h. Unlike the muscle, the levels *HSP70* expression in gill reached the top at 12 h and maintained a relatively high level compared with the control after thermal and bacterial treatment. The upregulated mRNA expression of *HSP70* in the abalone following heat shock and infection response indicates that the *HSP70* gene is inducible and involved in the immune response.

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pbS-Sfi-CAT, a better delivery vector for experimental DNA vaccination of Atlantic salmon (*Salmo salar*)

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Successful protection of trout against VHV and IHNV by DNA vaccination has heightened interest in this technique for other finfish diseases. Moreover, in recent times there has also been a keen interest in the use of expression library immunization (ELI). ELI has the potential to provide both a means of identifying and ultimately delivering DNA based vaccines, particularly for diseases for which there are currently no effective vaccines. In order to streamline this process, new delivery vectors capable of maximizing expression of foreign genes in fish as well as facilitating in vitro