SHORT COMMUNICATION

Genotyping of individual fish oocytes based on polymerase chain reaction amplification of mitochondrial DNA

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A major reason for the routine use of the polymerase chain reaction (PCR) in genetic projects is that it requires small amounts of imperfectly purified DNA for the detection of polymorphic markers. Recently, a number of protocols have been proposed that permit PCR amplification from a variety of tissues. They have been summarized briefly by Estoup, Largiader, Perrot & Chourrout (1996). Here, we propose a procedure of fast mitochondrial (mt) DNA extraction from individual salmonid eggs that allows further PCR amplification and genotype studies.

Fish eggs (in particular salmonid eggs) are the samples that require mechanical treatment before DNA extraction in order to allow contact between cytoplasm and lysis solution. Eggs of whitefish (Fig. 1A) and rainbow trout were tapwater activated (5 min), and then their chorion was pierced with a glass cannula under a stereomicroscope to allow the escape of cytoplasmic fluid (Fig. 1B). The fluid was transferred into a 0.5-mL Eppendorf tube, lysed in 3 µL of lysis buffer [200 mM KOH, 50 mM dithiothreitol (DTT)] for 30 min at 60 °C, and neutralized by 4 µL of neutralizing buffer (900 mM Tris-HCl, pH 8.3, 300 mM KCl, 200 mM HCl; modified from Li, Cui & Arnheim 1991; Fig. 1C). Before use, samples were vortexed briefly for 3 s. An aliquot of 1–3 µL (usually 2 µL) was taken as a template for a 12-µL total volume of PCR reaction.

Forty cycles of PCR (beginning with preliminary denaturation at 95 °C for 1 min) were carried out in a GeneAmp PCR system 2400 with a primer pair L-H (L: 5'-CCACTAGCTCCCCAAAGCTTA-3'; H: 5'-ACTTTCTAGGGTCCATC-3'), which spans the entire control region of both whitefish and rainbow trout mtDNA (Brzuzan 1998). The cycle consisted of 92 °C for 1 min, 50 °C for 1 min and 72 °C for 1.5 min. Positive amplifications were observed regularly and allowed further sequence analysis with restriction enzymes (Fig. 1D).

The extraction procedure presented is simple, rapid and efficient for several amplifications of mitochondrial sequences. The ability to analyse DNA sequence variation in single cells (oocytes) provides a unique tool for solving many important biological problems. For example, it permits revelation of the presence of both mutant and wild-type mtDNA (heteroplasmy) in the same fish egg, which is equal to an individual ‘fish’. Investigating mature oocytes from heteroplastic females can determine the relationship between the levels of drift occurring during oogenesis and mutation rates, thus giving an insight into the evolution of fish mtDNA (Nesbø, Arab & Jakobsen 1998). It is possible that the ‘single-cell’ PCR is applicable to the analysis of the polymorphic DNA regions in an embryo derived from an egg at the earliest stages of development. Finally, because of the type of tissue used (an egg), it
allows female individuals to be DNA typed without reducing their fitness.

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References

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