The Fish Biology Journal MEDAKA, **10**: 31–32 (1999)

Technical Report

Identification of genetic sex of the medaka, *Oryzias latipes*, by PCR

Ai Shinomiya[1], Masaru Matsuda[2], Satoshi Hamaguchi[1], and Mitsuru Sakaizumi[1]

1) Graduate School of Science and Technology, Niigata University, Ikarashi, Niigata 950-2181, Japan.
2) Laboratory of Reproductive Biology, Department of Developmental Biology, National Institute for Basic Biology, Okazaki 444-8585, Japan.

In the medaka, *Oryzias latipes*, the mechanism of sex determination (XX/XY) has been revealed by genetic crosses using a particular pigment gene (Aida, 1921; Yamamoto, 1955, 1958). In the d-rR strain, in which the allele *R* of the *r* locus is located on the Y chromosome, the female results in a white body color and the male results in a orange-red body color, but these body colors cannot be distinguished at embryonic stages. The second sex-linked coloration locus *lf* (leucophore free) has also been demonstrated (Wada *et al.*, 1998). In the Qurt strain, where the Y chromosome carries the wild type *lf* allele, the male always has leucophores, but the female does not. This can be observed at the second day after fertilization. However, with these pigment-gene markers, YY males, which are produced by sex-reversed XY females, cannot be distinguished from XY males, and the genetic sex of embryos before the second day after fertilization cannot be identified. YY male is indispensable to obtain all XY-male progeny, and the identification of the genetic sex of embryos at early stages is necessary to make the chimeras that consists of XX and XY cells.

Recently, a sex-linked DNA marker was isolated using inbred strains of medaka (Matsuda *et al.*, 1997). A congenic strain, Hd-rR.Y^{HNI}, was established, in which only the sex-determining region from HNI strain is introduced to the genetic background of the Hd-rR strain. Using differences between the genomes of the Hd-rR.Y^{HNI} and the Hd-rR strains, a sex-linked clone (pHO5.5) was isolated from a random genomic library constructed from the HO5 strain. A progeny analysis demonstrated perfect linkage between the pHO5.5-related sequence and the sex, indicating that the pHO5.5-related sequence is located on the sex chromosomes of medaka. We designated it as *S*ex-*L*inked *1* (*SL1*). Based on the sequence of *SL1*, a set of primers for polymerase chain reaction (PCR) was designed. Using these primers, we could identify the genetic sex of the fish. It is now possible to distinguish the sex chromosome composition (XX/XY/YY) of the medaka after the blastula stage with this method.

Applicable fish strains

The genetic sex of Hd-rR inbred strain and the Hd-rR.Y^{HNI} congenic strain can be identified by PCR. The genetic sex of the HNI strain can be determined by southern blotting (refer to Matsuda *et al.*, 1997).

Preparation of template DNA for PCR

Adult fish:

1. Anesthetize the fish with 0.05%–0.1% ethylene glycol monophenyl ether (EGME).
2. Cut a part of the caudal fin with a razor (approximately 1 × 1 mm), transfer the fin to 1.5 ml tube. Return the fish to an aquarium.
3. Add 100 μl of lysis buffer (100 mM NaCl, 1% SDS, 100 mM EDTA, 50 mM Tris; pH 8.0), incubate with 1 μl of proteinase K (10 mg/ml) at 55°C for 60 min.
4. Add one volume of phenol/chloroform, mix with a Vortex mixer for 1 min, spin at 15,000 rpm for 5 min and transfer the supernatant to new tube.
5. Add one tenth volume of 3M sodium acetate and one volume of isopropyl alcohol, precipitate at –20°C for 20 min.
6. Spin at 15,000 rpm for 10 min, discard the supernatant.
7. Wash the pellet with 70% ethanol and air dry.
8. Resolve the DNA in 50 μl TE buffer (1 mM EDTA, 10 mM Tris; pH 8.0).

Fry and the embryo at later stage:

1. Cut off a part of the specimen with a razor and transfer the part to 1.5 ml tube. For embryos, remove the chorion with hatching enzyme solution (Ando and Wakamatsu, 1995) before cutting.

2. Add 150 μl of lysis buffer, incubate with 1.5 μl of proteinase K at 55°C for 3 hr.
3. Extract DNA according to the above protocol, with one extra phenol/chloroform extraction.

Embryo at blastula stage:

1. Remove the chorion with hatching enzyme solution, transfer the embryo to the balanced salt solution for medaka eggs (Iwamatsu, 1983) in a petri dish.
2. Discard the yolk by pricking with fine forceps under a stereoscopic microscope, transfer to 1.5 ml tube by micropipete.
3. Add 15 μl of lysis buffer-SDS (–) (100 mM NaCl, 100 mM EDTA, 50 mM Tris; pH 8.0), incubate with 0.15 μl of proteinase K at 55°C for 60 min.
4. Boil for 5 min.

Procedure of PCR analysis

SL-1 fragments are amplified using the PCR primer set, pHO5.5-F (5′-CCTGCAATGGGAAA-TTATTCTGCTC-3′) and pHO5.5-RV (5′-CTTT-TGTGTCTTTGGTTATGAAACGATG-3′). The reaction mixture for PCR contains Ex Taq Buffer (TaKaRa), 0.2 mM dNTPs, 0.2 μM each of the primer, 1 μl of template DNA, and 0.6 units of Ex Taq polymerase (TaKaRa) in a final volume of 25 μl. The reaction proceeds as follows: 95°C for 2 min, followed by 30 cycles of 95°C for 90 sec, 55°C for 2 min, 72°C for 2 min, and finally an additional 72°C for 2 min. For blastula embryos, use 2 μl of the template DNA and modify the repeat program of the PCR into 95°C for 30 sec, 55°C for 1 min, 72°C for 1 min. The PCR products are separated by electrophoresis in a 7.5% polyacrylamide gel (Fig. 1). For the Hd-rR.Y^{HNI} strain, it is possible to separate the products with 1.0% agarose gel.

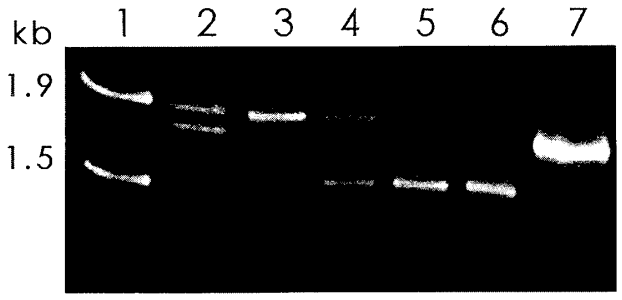

Figure 1. Detection of genetic sex and strain differences by PCR. Polyacrylamide gel electrophoresis of the products of PCR using pHO5.5 primers. Lane 1, DNA size markers; lane 2, Hd-rR male, lane 3, Hd-rR female; lane 4, Hd-rR.Y^{HNI} male; lane 5, HNI male; lane 6, HNI female; lane 7, pHO5.5. Sizes are given in kb.

References

Aida, T. (1921) *Genetics*, **6**: 554–573.

Ando, S. and Y. Wakamatsu (1995) *Fish Biol. J. Medaka*, **7**: 65–68.

Iwamatsu, T. (1983) *J. Exp. Zool.*, **228**: 83–89.

Matsuda, M., T. Kusama, T. Oshiro, Y. Kurihara, S. Hamaguchi, and M. Sakaizumi (1997) *Genes Genet. Syst.*, **72**: 263–268.

Wada, H. A. Shimada, S. Fukamachi, K. Naruse and A. Shima (1998) *Zool. Sci.*, **15**: 123–126.

Yamamoto, T. (1955) *Genetics*, **40**: 406–419.

Yamamoto, T. (1958) *J. Exp. Zool.*, **137**: 163–179.