The Fish Biology Journal MEDAKA, **10**: 33–34 (1999)

Technical Report

Chromosome Preparation from the Medaka, *Oryzias latipes*, at the Different Stages of Development

Tatiana Ladygina and Yuko Wakamatsu

The first studies concerning the chromosomes of the medaka, *Oryzias latipes*, have been done more than 70 years ago by Goodrich (1927). Detailed karyotype of the medaka has been reported by Ojima and Hitotsumachi (1969).

Here we introduce easy methods for karyotyping of the medaka both adult fish and embryos on the early stages of development. These methods were developed in our laboratory by modification of the previous ones reported by Arai (1973) and Klegerman and Bloom (1977).

I. Chromosome preparation from adult fish

Fish

A commercially available orange-red variety of the medaka is used.

Procedure

1. Put young, healthy specimens in a tank with 0.005–0.01% colchicine solution for about 7 hours at a room temperature.
2. Immediately after killing, remove the spleen and gill arches into Petri dish with 0.9% NaCl solution and rinse twice in the same solution.
3. Transfer the spleen into a watch glass with 20 μl of hypotonic KCl solution (0.075 M) and mince the tissue with thin scissors to form a cell suspension.
4. Add 200 μl of KCl solution and pipette the suspension using a Pasteur pipette. Transfer the cell suspension into a 15 ml plastic centrifuge tube.
5. Put the gill arches into the watch glass and mince with scissors.
6. After addition of 200 μl of KCl solution, gently pipette up and down to break cell clumps. Transfer the cell suspension into the same centrifuge tube.
7. Add 2 ml of KCl solution to the cell suspension and incubate at 26°C for 30 minutes.
8. Add 0.5 ml of freshly prepared ice-cold fixative (3 parts of methanol: 1 part of acetic acid) to the cell suspension and immediately centrifuge it at 1000 rpm for 6–8 minutes.
9. Remove the supernatant and resuspend the pellet in 2 ml of fresh cold fixative.
10. Fix in 2–3 changes of 2 ml cold fixative for at least 30 minutes each, and keep cell suspension at 4°C.
11. Place 3 drops of the suspension onto a clean glass slide that has been kept in cold (4°C) distilled water until use. Immediately dry the slide with flame.
12. Stain the slide in 2% Giemsa solution made up in 0.01 M phosphate buffer (pH 6.8) for 10 minutes.
13. Rinse in distilled water and air-dry.

II. Chromosome preparation from embryos of the medaka

Embryos

2–5 days embryos of the orange-red variety of the medaka are used.

Procedure

1. Using forceps, separate egg clusters into single eggs by removing attachment filaments.
2. Put eggs in Petri dish with 0.001% colchicine solution for 4–5 hours.
3. Transfer embryos into new Petri dish with the balanced solt solution (BSS) for medaka (Ando and Wakamatsu, 1995)*.
4. Remove chorions with two thin tweezers.
5. Transfer embryos into Petri dish with fresh BSS. Gently, trying not to damage embryos, remove yolks from embryos with tweezers.
6. Transfer each embryo into 1 ml micro-tube with 200 μl of KCl solution (0.075 M) for hypotonic treatment. Incubate at 26°C for 30 minutes.
7. Add 50 μl of freshly prepared ice-cold fixative (3 parts of methanol: 1 part of acetic acid) to the hypotonic solution, mix gently and remove all solution.

8. Fix the specimens in 2–3 changes in 200 μl fixative for at least 30 minutes each, and keep at 4°C.
9. Before making chromosome preparations, remove all fixative and add 80 μl of 50% acetic acid in every tube.
10. Using automatic pipette, gently pipette up and down to form cell suspension.
11. Place 40 μl of the suspension onto a dry, clean glass slide, warmed to 50–60°C. After 5 seconds withdraw the suspension from the glass slide into the pipette, leaving a ring of cells with a diameter of about 6–7 mm.
12. Repeat step 11 twice, producing 3 rings per slide.
13. Stain the slides in 2% Giemsa solution made up in 0.01 M phosphate buffer (pH 6.8) for 10 minutes.
14. Rinse in distilled water and air dry.

* ***Composition of BSS***

Solution A:	NaCl	65 g
	KCl	4 g
	$MgSO_4 \cdot 7H_2O$	2 g
	$CaCl_2 \cdot 2H_2O$	2 g
	Phenol red	5 mg
	Add distilled water to make 500 ml.	
Solution B:	5% $NaHCO_3$ in distilled water. Sterilize by filtration.	

Dilute 25 ml of solution A with 475 ml of distilled water and autoclave.
Add 1 ml of solution B to this solution.

Acknowledgments

Special thanks to Dr. H. Onozato for his kind technical advises.

References

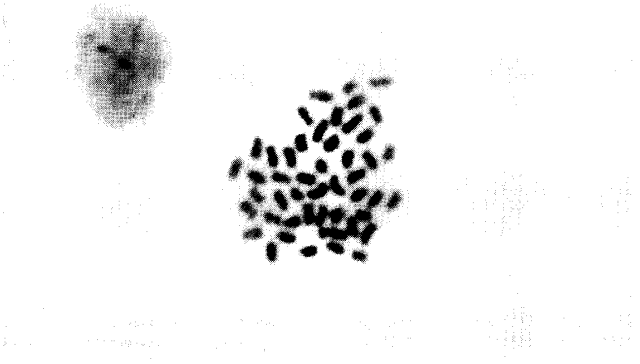
Ando, S. and Y. Wakamatsu (1995) *J. Medaka*, **7**: 65–68.

Arai, R. (1973) *Bull. Natn. Sci. Mus. Tokyo,* **16**: 173–177.

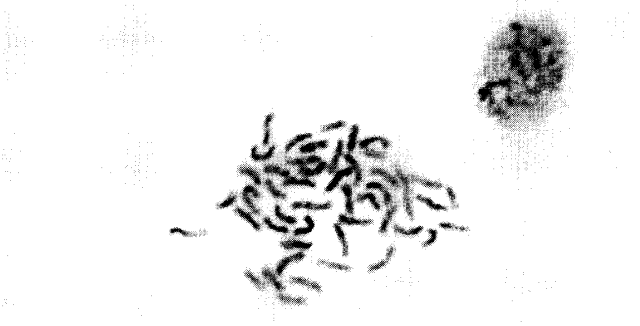
Goodrich, H.B. (1927) *J. Exp. Zool.*, **49**: 261–280.

Kligerman, A. and S. Bloom. (1977) *J. Fish Res. Board Can.,* **34**: 266–269.

Ojima, Y. and S. Hitotsumachi (1969) *Chromosome Information Service,* **10**: 15-16.



Chromosome preparation from a 2 days embryo



Chromosome preparation from an adult fish