National Bio-Resource Project Tropical Clawed Frog

Rearing Methods for *Xenopus tropicalis*

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In order to prevent bacterial and other types of contamination, always wash both hands thoroughly with soap and keep them clean before starting care. The optimum water temperature is 25–27°C for tadpoles and 24–26°C for frogs; therefore control of room temperature is also required to maintain water temperature. If it is not possible to control room temperature, you can use any commercially available heating system to warm the water. Aeration is not required for either tadpoles or frogs.

Below is a general description of the basic of methods. Of course local water quality, temperature, and hours of daylight may vary considerably, so you will need to develop your own specific protocol.

[Preparation]

(1) Rearing water

Attention needs to be paid to water quality when tap water is used, because chlorine contained in the water may have an adverse impact on tadpoles and frogs. To avoid such adverse impact, put tap water in a polyethylene bucket and ① leave it for 2–3 days at room temperature, or ② add one sodium thiosulfate crystal (one grain / 20 L water). For ②, the water can be used as rearing water immediately after the crystal has dissolved and disappeared. If the temperature of the water is below optimum, adjust it by adding amounts of boiling water. Pay careful attention to the temperature because both tadpoles and adults of *X. tropicalis* are extremely vulnerable to low temperatures.

(2) Tools

① Polyethylene bucket

② Rearing container (glass or plastic)

There is a great chance of contamination with chemical substances used during the manufacturing processes when new commercially available buckets and containers are used; attention needs to be paid to this issue. First fill the buckets and containers with tap water and leave them for two whole days and nights. Discard the water. Briefly wash the inner surface

of the buckets and containers with hot water, and then wash the containers and lids with tap water thoroughly before use.

③ Glass Petri dishes

Soak in tap water for a whole day and night and wash with running water before use.

④ Plastic beaker with a handle

Wash with running water before use.

⑤ Pure coarse sand, 10 kg (PL-10), GEX 50203 (GEX, Japan).

Wash thoroughly with water before use.

⑥ Sponge scrubbers (ones that do not scratch the containers)

⑦ Net screened with gauze (hand made)

⑧ Droppers or pipettes

(3) Gonadotropin

Dissolve commercially available hCG (Chorionic gonadotropin, human, SIGMA, USA) into Ringer's solution for adult frogs. Gonadotropin for animals (ASKA Pharmaceutical, Japan) and Puberogen for animals (Nippon Zenyaku Kogyo, Japan) may also be used.

(4) Feed

For frogs: Product name, “Floating feed for eels, for finishing” (Marubeni Nisshin Feed, Japan; distributor, Hiroshima Laboratory Animal Center, Japan) or salmon pellets (Oriental Kobo, Tokyo; distributor, Hiroshima Laboratory Animal Center, Japan) or crickets (IAB)

For young frogs: Feed for frogs, XL-2 (Oriental Yeast; distributor, Hiroshima Laboratory Animal Center, Japan)

For tadpoles: SERA Micron powder food (Sera Heinsberg, Germany; distributor, Hiroshima Laboratory Animal Center, Japan)

[Rearing methods]

(1) Tadpoles

① Fertilized eggs are obtained by injecting human chorionic gonadotropin (hCG) into the dorsal lymph sac. First 10–20 U of hCG is injected to adult males and females (priming), followed by 100 U 20–24 hours after the first injection (boosting). After boosting, place one male ― female pair in a container containing 25°C dechlorinated water to a depth of 5–10 cm. Fertilized eggs will be available within 4–6 hours. The use of a false bottom is recommended to allow eggs to sink beyond the reach of the adult frogs. Aggregation of eggs can be avoided by adding salt to the water at a concentration of 0.05%. After completion of egg laying, frogs are removed from the container, and change to new rearing water at this

point is recommended . If frogs do not proceed with mating behavior, this may often be initiated by injecting an additional 100 U of hCG into both male and female. Developmental stages are as described in Nieuwkoop & Faber (1956).

② When tadpoles developed from the fertilized eggs reach the end of the hatchling stage, transfer only tadpoles with normal morphology. Give a small amount of SERA Micron for food when feeding behavior is exhibited.

③ If tadpoles have a large appetite, feed them every morning and evening.

④ Water changes every other day should be fine. When changing water, use a dropper for young tadpoles or a net screened with gauze for larger tadpoles.

⑤ Size differences will gradually become obvious when tadpoles develop. Avoid overcrowded conditions in the containers. Select larger tadpoles and transfer them into separate containers (2–3 tadpoles/L). At one month after fertilization some of the larger tadpoles will have already undergone metamorphosis.

(2) Frogs

① 2 or 3 times a week should be adequate for feeding and changing water.

② Begin with 5 grains of feed per frog. Increase the amount of feed by several grains as frogs grow in size and devour all food within 30 minutes.

③ Change water 2~3 hours after each feeding. Discard old water and add new water using a beaker with handle.

Note ──

1) Container bottoms, sides, and corners tend to become dirty over time. Remove slimy accumulations with the sponge scrubber.
2) After changing water, discard any left over water remaining in the bucket and prepare new water for the next water change.
3) For frogs, two or three double handfuls of pure coarse sand at the bottom of rearing containers is helpful for water clarification.
4) Any unhealthy frogs should be immediately transferred to a separate water container with 0.3% salt added to the rearing water. In the case of tadpoles, remove and discard any sickly individuals immediately upon discovery to avoid adverse effects on healthy tadpoles.

Manipulation of *Xenopus tropicalis* eggs and embryos
──In vitro fertilization of eggs and manipulation of embryos──

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Reagents for *in vitro* fertilization and dejellying

・Crystal of sodium thiosulfate (Daito Chemical Co., Ltd., Japan)

・Human chorionic gonadotropin (Sigma)
・Ringer's solution (amphibian adults: NaCl 0.65g, KCl 0.025g, $CaCl_2$ 0.03g, $NaHCO_3$ 0.02g per 100ml distilled water)
・MS222 (Sigma)
・1×MMR (Marc' Modified Ringer's) solution : 100 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$ and 5 mM Na-HEPES, pH 7.5
・Leibovitz 15 medium (Sigma)
・Cysteine (Sigma)
・Ficoll (Sigma)
・Gentamycin (Sigma)

Maintenance equipment

・Plastic vessels
・Glass vessels
・Glass Petri dishes (20 ~25 centimeters in diameter)
・Ophthalmologists' scissors
・Forceps
・Glass Pasteur pipettes with rubber caps

When purchasing plastic or glass vessels to be used in raising frogs, tadpoles and embryos, they must be washed thoroughly with a soft sponge and soap to remove any chemical substances used in the manufacturing process adhering to inside surfaces. Endocrine disrupting substances will have detrimental effects on development and growth. Ophthalmologists' scissors, forceps, glass Pasteur pipettes and rubber caps should also be washed before each use.

・Disposable syringes (10 cc) with needles (23 Gauge) for drawing up Ringer's solution and syringes (1 cc) with needles (27 Gauge) for hypodermic injection
・9.0 cm plastic Petri dishes for use in artificial fertilization

・6.0 cm plastic Petri dishes (Falcon's cell incubation dishes) for use in dejellying

Food for raising tadpoles or frogs

・SERA Micron (Sera Heinsberg, Germany)

・Salmon pellets (Oriental Kobo, Tokyo)

・Two-spotted crickets *Gryllus bimaculatus*

Preparation for *in vitro* fertilization

X. tropicalis adults are raised in 24℃ to 26℃ chlorine (Cl)-free tap water (one crystal of sodium thiosulfate/20 L water).

Ovulation and spermiation are induced by two separate injections of human chorionic gonadotropin into the dorsal lymph sac. Adult female and male frogs initially receive 10 units of HCG in Ringer's solution, followed approximately 20 hours later by a second injection of 100 units in the same Ringer's solution. Injected frogs must be maintained in a dark quiet environment by covering trays with a black cloth. Frogs should not be kept in temperature units because electrical vibration will be detrimental to their egg laying.

Approximately 3～4 hours after the second injection, the egg mass from the uterus of the ovulating female is stripped into the 9.0 cm plastic Petri dishes and immediately mixed with a fresh sperm suspension using a wide-bore glass Pasteur pipette. The sperm suspension is prepared as follows. Males are deeply anesthetized in 0.15% MS222 in rearing water. The testes are surgically removed, and fat bodies and blood are removed by rolling on a fresh paper towel. Next the testes are placed in Leibovitz (L)15 medium and crushed into small pieces with forceps, and mobility is ascertained before use. When they are not immediately used, the testes can be stored in L15 ＋ 10% calf serum for a few days at 14℃.

Five minutes after being mixed with the sperm suspension, the eggs in the Petri dish are flooded with 0.05 × MMR. Successful fertilization is observed when eggs are oriented with the dark animal pole side up, or so called 'cortical rotation'. Eggs are maintained at 24～26℃ and observed at close intervals at the time of the first and second cleavages.

Viable hatched tadpoles at Nieuwkoop and Faber (1956) stage 35～36 are drawn into a wide-bore pipette and transferred to glass rearing vessels containing Cl-free water. Temperature is maintained at 25～27℃ throughout the larval period. Tadpoles are raised on SERA Micron, and frogs on salmon pellets or two-spotted crickets *Gryllus bimaculatus*. Food levels are adjusted to allow normal tadpole and frog development and maximum growth. Water is changed every other day.

Removing jelly coating from eggs

Immediately after the beginning of cortical rotation, eggs are transferred into the 6.0 cm Falcon's plastic Petri dishes and can be dejellied using 1.5 % cysteine in 0.1 × MMR, pH 8.0, and then rinsed carefully 3～5 times with 0.1×MMR. Following this the eggs are rinsed 3 times with 5% Ficoll and 50 mM gentamycin in 0.1× MMR and loaded into Petri dishes with bottoms coated in a layer of agarose containing the above solution to be used for transgenic or knockout microinjection.

REFERENCES

Fu, Y., Wang, Y. and Evans, S. M. (1998) Viral sequences enable efficient and tissue-specific expression of transgenic in *Xenopus*. Nature Biotechnol. 16, 253-257.

Hirsh, N., Zimmerman, L. B. and Grainger, R. M. (2002) Review. A peer reviewed forum. *Xenopus*, the next generation: *X. tropicalis* genetics and genomics. Dev. Dyn. 225, 422-432.

Kashiwagi, K., Kashiwagi, A., Kurabayashi, A., Hanada, H., Nakajima, K., Okada, M., Takase, M. and Yaoita, Y. (2010) *Xenopus tropicalis*: An ideal experimental animal in amphibian. Exp. Anim. 59, 395-405.

Kroll, K. L. and Amaya, E. (1996) Transgenic *Xenopus* embryos from sperm nuclear transplantations reveal FGF signaling requirements during gastrulation. Development 122, 3173-3183.

Murray, A. W. (1991) Cell cycle extracts. Meth. In Cell Biol. 36, 581-605.

Nieuwkoop, P. D. and Faber, J. (1956) Normal tables of *Xenopus laevis* (Dudin). North-Holland, Amsterdam.

Ogino, H., McConnell, W. B. and Grainger, R. M. (2006) High efficient transgenesis in *Xenopus tropicalis* using *I-SceI* meganuclease. Mech. Dev. 123, 103-113.

Ogino, H., McConnell, W. B. and Grainger, R. M. (2006) High-throughput transgenesis in *Xenopus* using *I-SceI*meganuclease. Nature Protocols 1, 1703-1710.

Smith, S. J., Fairclough, L., Latinkic, B. V., Sparrow, D. B. and Mohun, T. J. (2006) *Xenopus laevis* transgenesis by sperm nuclear injection. Nature Protocols 1, 2195-2203.