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—Review—
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The National BioResource Project Medaka (NBRP Medaka): An Integrated Bioresource for Biological and Biomedical Sciences

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Abstract: Medaka (*Oryzias latipes*) is a small freshwater teleost fish that serves as a model vertebrate organism in various fields of biology including development, genetics, toxicology and evolution. The recent completion of the medaka genome sequencing project has promoted the use of medaka as a comparative and complementary material for research on other vertebrates such as zebrafish, sticklebacks, mice, and humans. The Japanese government has supported the development of Medaka Bioresources since 2002. The second term of the Medaka Bioresource Project started in 2007. The National Institute for Basic Biology and Niigata University were selected as the core organizations for this project. More than 400 strains including more than 300 spontaneous and induced mutants, 8 inbred lines, 21 transgenic lines, 20 medaka-related species and 66 wild stock lines of medaka are now being provided to the scientific community and educational non-profit organizations. In addition to these live fish, NBRP Medaka is also able to provide cDNA/EST clones such as full-length cDNA and BAC/fosmid clones covering 90% of the medaka genome. All these resources can be found on the NBRP Medaka website (http://shigen.lab.nig.ac.jp/medaka/), and users can order any resource using the shopping cart system. We believe these resources will facilitate the further use of medaka and help to promote new findings for this vertebrate species.
Key words: cDNA/BAC/fosmid clones, inbred line, mutants, NBRP Medaka, wild stock

Introduction

Medaka (*Oryzias latipes*, order Beloniformes) is a small, egg-laying freshwater teleost fish that resides in the brooks and rice paddies of Japan, Korea, and China. This fish has been developed as a research material in Japan [1, 4, 11, 31, 41, 57, 58], and is now widely used in various research fields including biology, medical science, environmental science and evolution. Furthermore, large scale N-ethyl-N-nitrosourea (ENU) mutagenesis screening has identified more than 200 medaka mutants with specific defects in organogenesis [3, 33]. A draft

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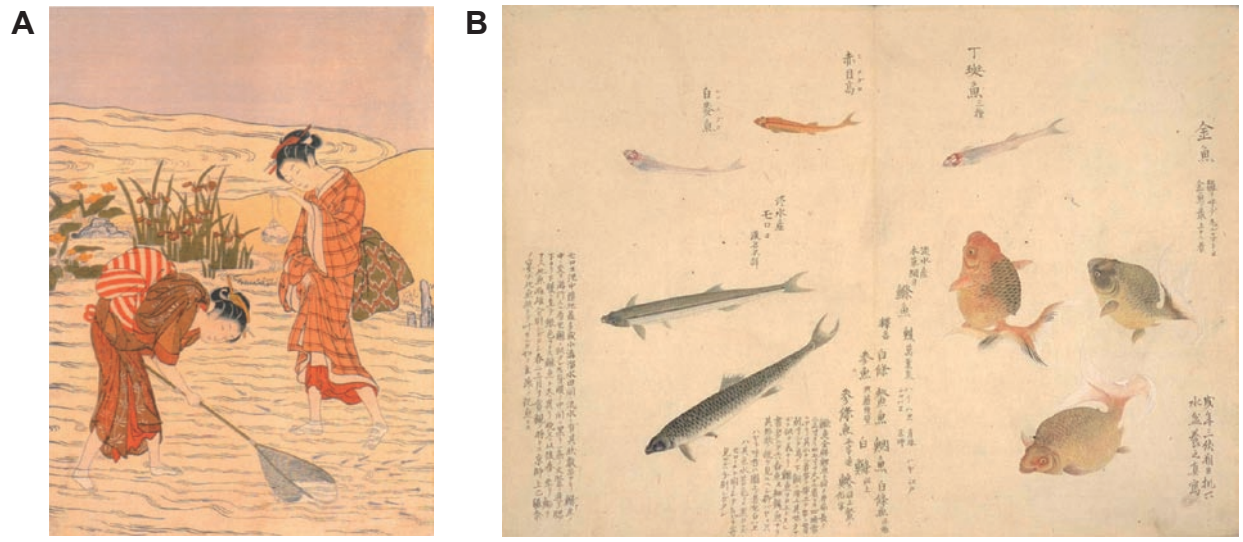

Fig. 1. Medaka description in Edo period. (A) Ukiyo-e painting entitled *Medaka sukui* (medaka scooping) by Harunobu Suzuki (1767–1768), courtesy of http://www.japanism.net/. (B) The three medaka varieties described by Baien Mouri in his fish encyclopedia (*Baien Gyofu*). White, orange-red and wild-type fish are presented (http://www.ndl.go.jp/ with permission of the National Diet Library, Japan).

level genome sequence, more than 68,000 unique cDNA/EST sequences, and BAC/fosmid clones covering 90% of the medaka genome are now available. Medaka demonstrates the largest genetic variation (an average of 4%) among the vertebrate species investigated to date [14], and these variations are clearly correlated with geographical distribution [44, 46, 47]. Furthermore, several inbred strains that represent each regional population have been established [7]. The large genetic variation is a distinctive characteristic of medaka when compared to other model fish species including zebrafish and stickleback. These features are proven to be both unique and valuable for a wide range of investigations of vertebrate animals.

Given these important characteristics, medaka was selected as one of the species to be supported by the National BioResource Project (NBRP), sponsored by the Ministry of Education, Culture, Sports, Science and Technology of Japan in 2002, and for a second term in 2007. In the second term, the National Institute for Basic Biology (Core Institute) and Niigata University were appointed to undertake the project. As a part of this project, live resources including standard strains, wild stocks, inbred strains, medaka-related species, and spontaneous and induced mutants have been collected, maintained and presented on the medaka website. In addition, genomic resources such as expressed sequence tag (EST)/cDNA /BAC/fosmid clones and hatching enzymes that are essential for manipulation of the embryos are also provided. The NBRP Medaka aims to represent a first rate biological resource with high accessibility and ease of use.

Medaka as a Model Animal to Study Biological Sciences in Vertebrates

History and characteristics of medaka

Historically, medaka was reared as an ornamental fish. Several interesting descriptions of medaka dating back to the Edo period exist. The ukiyo-e painting entitled *Medaka sukui* (medaka scooping) was first published in 1767–1768, and was followed by the release of *The Baien Gyofu* (Encyclopedia of fish) by Baien Mouri in 1835 (Figs. 1A and 1B). Baien Mouri describs three types of medaka that included the wild type, an orange-red, and a white colored fish. It is now known that the orange-red type fish contains a mutation at the *b* locus and that the white fish contains a double mutation at the *b* and *r* loci. Medaka was first academically described as *Poecilia latipes* in Siebold's *Fauna Japonica* in 1842

[50]. Following this description, medaka has been used as an experimental animal by both Japanese researchers and others throughout the world. Several important scientific achievements have been made using the medaka color mutants. First, Toyama and Ishikawa confirmed Mendel's law of inheritance in 1910 [53], while in 1921, Aida identified that the sex-linked inheritance of the *r* locus controlled the accumulation of orange pigments in xanthophores [1]. Later, Yamamoto (1953) established the d-rR strain and demonstrated artificial induction of sex reversal with estrogen and androgen in juvenile d-rR strain fish [58, 59]. The d-rR strain exhibits body-color dimorphism, with the male fish appearing orange-red in color, while females are white. These differences are due to the sex-linked inheritance of the *R* gene identified by Aida [1].

Studies of medaka sex determination and differentiation have also resulted in the identification of the primary sex-determination gene *DMY* [27, 29]. This gene was the second primary sex-determination gene to be isolated in vertebrates, and represents the functional equivalent of the *Sry* gene in mammals. The establishment of an efficient method for generating transgenic medaka [34], and the establishment of several inbred lines from genetically different natural populations [7] were also significant achievements. From around the year 2000, several important studies that established genetic/genomic resources began to be published. A large scale EST analysis was performed by Kimura *et al*. (2004) [16], with over 600,000 cDNA/EST sequences deposited in the public DNA database (DDBJ/EMBL/Genbank). In addition, up to 64,800 unique sequences (DFCI medaka gene index: http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/gimain.pl?gudb=o_latipes) have also been summarized and a genome-wide linkage map has been established [30, 32].

The medaka genome sequencing project commenced in 2002, and the draft genome sequence has now been published [14]. All of the genetic/genomic data is freely available at the UT genome browser (http://medaka.utgenome.org/), Ensembl genome browser (http://www.ensembl.org/Oryzias_latipes/Info/Index), UCSC genome browser (http://genome.ucsc.edu/cgi-bin/hgGateway?hgsid=143765092&clade=vertebrate&org=Medaka&db=0) and Medaka Map (http://medakagb.lab.nig.ac.jp/index.html) on the NBRP Medaka website. In addition to these genome resources, mutagenesis screening for the isolation of mutants exhibiting specific phenotypes during embryonic development has been conducted by several groups [3, 11, 24]. Approximately 500 mutants with specific phenotypes have been generated, and the causal genes underlying the mutations were identified [6, 25, 28, 33, 39]. Given these studies, medaka has become a representative model animal for vertebrates.

Advantages of medaka as a model fish

Medaka exhibit several advantageous characteristics as an experimental animal. As they are an egg-laying fish, embryonic development occurs externally and embryos, particularly the pigment-less mutants, are completely transparent throughout their embryonic development. Even in the adult stages, some medaka strains such as STIII [56] and Quintet (Fig. 2E) [35] remain transparent. Medaka contains four types of pigment cells termed melanophores, leucophores, xanthophores, and iridocytes. Vertebrates expressing leucophores are rare. Some medaka-related species do not contain leucophores, while other available mutant lines do not contain chromatophores or pigment granules. In addition, the two loci that control pigment cell development (the *r* locus for xanthophores and the *lf* locus for leucophores) are also sex-linked. Thus, researchers are able to distinguish the sex of embryos with or without pigment cells [1, 54].

Another advantage of using experimental medaka is that some strains demonstrate a low-temperature tolerance. Medaka is a temperate zone fish that is able to survive at 35°C in summer and 4°C in winter in the absence of any thermostatic regulation. In the laboratory, 10 to 20 eggs can be obtained every day under artificial conditions (14 h light-10 h dark at 28°C), and the generation time is approximately 2–3 months under these same conditions. The rate of embryonic development can also be controlled by the temperature of the environment. For example, up to the blastula stage, embryonic development can be arrested at 10°C and can be restarted again at 26°C.

The large genetic divergence observed among regional populations is an additional advantage which has not been identified in any other vertebrate models. To

the best of our knowledge, medaka is the most genetically divergent vertebrate (3–4% sequence divergence among regional populations) known to date [14, 44, 47]. The estimated genome size of medaka is approximately 800 million base pairs (Mbp), while that of zebrafish is 1,700 Mbp [31]. This is also advantageous for the isolation of entire genomic regions of genes with the fosmid library, which contains a relatively short insert size (approx. 36 Kbp), and for the identification of mutations in the positional cloning and TILLING (Targeting Induced Local Lesions IN Genome) library [13, 49]. The existence of numerous medaka-related species in South-East Asia and East Asia is also unique when compared to other vertebrate model systems [46]. Primers for medaka genes are able to amplify the orthologous genes present in the closely related species [43, 45]. This situation has promoted evolutionary studies that use medaka related species and medaka EST primers. Methods of sperm cryopreservation have also been established [2, 20, 36, 37]. This is important for the long term storage of mutant and transgenic lines.

The medaka egg envelope is also harder when compared to that of the zebrafish. This characteristic makes embryonic manipulations such as chimera formation difficult. This difficulty may be overcome using hatching enzymes [55, 60]. NBRP Medaka has been producing and supplying hatching enzymes since 2008. Although the hard egg envelope proves to be a disadvantage for embryonic manipulation, this feature is advantageous for the successful transportation of medaka eggs. Medaka embryos are able to survive for more than ten days in small tubes, without changing of the water. Thus, medaka embryos can be easily transported worldwide.

Resources Available from NBRP Medaka

The NBRP Medaka currently provides live resources including inbred, mutants (spontaneous and ENU induced mutants), wild-type stocks and their related strains, and transgenic fish. In addition, cDNA/EST clones including full-length cDNA and BAC/fosmid clones constructed from the Hd-rR inbred strain are also available.

Standard strains

The d-rR strain is one of the most frequently used medaka strains [58, 59]. Genetic sex is distinguished on the basis of body color in this strain. The Cab strain has been used for large scale ENU mutagenesis screening and has proven to be a healthy research strain [3, 57], perhaps even more so than the other medaka strains, such as the inbred lines. The T5 strain is useful for the construction of transgenic lines. This strain only contains xanthophores during the early stages of embryonic development [42].

Wild stock

NBRP Medaka currently provides 66 wild stocks of medaka: 55 of these stocks are from Japan, 9 are from Korea, 1 is from China and 1 from Taiwan. In addition, 20 strains of closely related species native to South-East Asia and East Asia are also available. These wild stocks are an important genetic resource as they contain various levels of genetic diversity and variation [44, 46, 47]. The medaka and related species are known to have originally resided in freshwater, but have since developed a high adaptability to salinity [10]. *Oryzias dancena* and *O. javanicus* inhabit brackish and sea-water, respectively (Figs. 2A and 2B). These species can be used in ecotoxicity tests and assessments that detect the endocrine disrupting activity of chemicals in marine waters [9, 19].

Inbred strains

More than ten inbred strains derived from genetically different wild populations have also been established at the National Institute of Radiological Sciences, Chiba, Japan since the 1970s [7]. Eight inbred strains are currently available from NBRP Medaka. Two representative inbred strains include the Hd-rR (Fig. 2C) and HNI-II (Fig. 2D) strains. The Hd-rR strain demonstrates body-color dimorphism. The female is white, while the male is orange-red. This body-color dimorphism is due to the strains derivation from the d-rR strain. The Hd-rR strain is known to belong to the southern Japanese population. Whole genome sequences of this strain are available on Ensembl, USCS, UT genome browsers, and Medaka Map. The HNI-II strain, is known to belong to the northern Japanese population, has also been used in the ge-

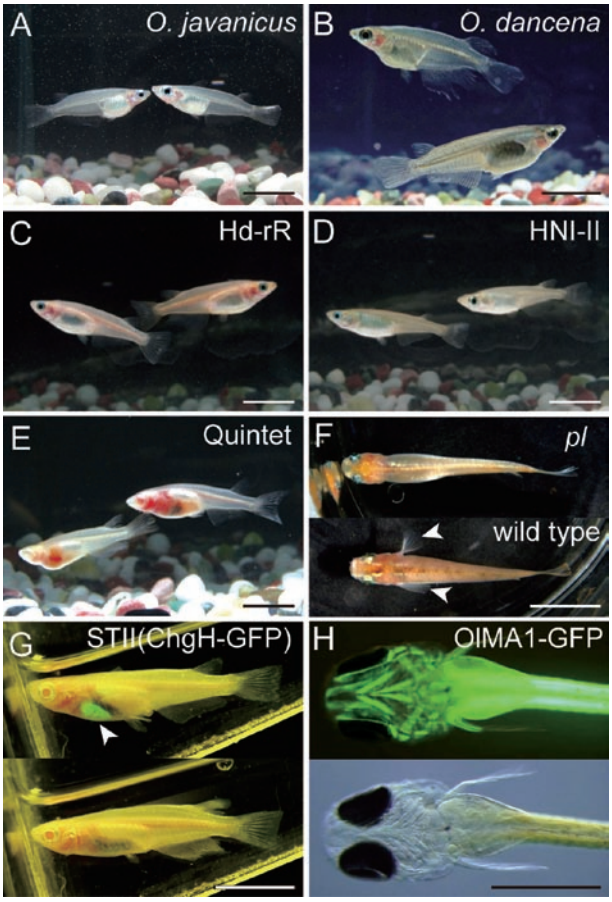

Fig. 2. Examples of medaka strains. (A, B) Medaka related species *Oryzias javanicus* (A) and *O. dancena* (B). *O. javanicus* inhabit seawater and *O. dancena* can be maintained in fresh water. (C) The Hd-rR inbred strain. This strain demonstrates body-color dimorphism which is depend on sex. The females are white (left) and the males are orange-red (right). (D) The HNI-II inbred strain. This strain demonstrates wild-type body color. The fish on the left is female and the right is male. (E) The viable translucent fish, Quintet. The fish on the left is female and the right is male. The internal organs can be observed through the skin and peritoneum. (F) The *pl* mutant does not demonstrate any pectoral fins (upper panel), while the wild-type has normal pectoral fins (lower panel, arrowheads). (G) An adult female of the STII(ChgH-GFP) transgenic strain (upper panel). Strong GFP fluorescence in the liver (arrowhead) can be observed through the peritoneum. The adult male (lower panel) does not express GFP fluorescence. The samples are illuminated with a blue excitation light and covered with yellow emission filter. (H) A fixed sample of d-rR-Tg(OlMA1-GFP) larva after hatching. Strong GFP signals can be observed in the skeletal muscles (upper panel). The GFP signal in the trunk skeletal muscle can be observed under normal light (lower panel). Ventral views are shown. Scale bars represent 1 cm in A–G; 1 mm in H.

nome sequencing project to identify genetic variations such as single nucleotide polymorphisms (SNP) and insertion/deletion polymorphisms. These studies have demonstrated that the sequence divergences between Hd-rR and HNI-II in the whole genome are 34.246/kbp (3.42%) and 18.077/kbp (1.80%) in the exonic region, and 33.984/kbp (3.39%) in the intronic region [14]. Additional inbred strains also demonstrate specific body shape and susceptibility to tumorgenesis in response to N-methyl-N'-nitro-N-nitrosoguanidine treatment [8].

Mutants

NBRP Medaka currently provides more than 300 mutant strains including 90 spontaneous mutants derived from wild populations and more than 200 ENU-induced mutants. All spontaneous mutants are homozygous viable and contain approximately 60 body-color mutants and 30 additional mutants such as fin, scale, or skeletal development defects [15, 51, 52]. By crossing the several available color mutants, viable translucent fish strains have been established. Examples of such strains include the STII, STIII [56], and Quintet [35] strains (Fig. 2E). The internal organs of these strains are easily observed directly through the skin and peritoneum throughout their life. These strains can be used for the investigation of internal organs, and are especially useful when combined with green fluorescent protein (GFP) transgenic technology (Fig. 2G).

An example of a spontaneous viable mutant with specific defects is a pectoral-finless strain (*pl*) [52]. This strain has no pectoral fins throughout its entire life (Fig. 2F). Another mutant termed *pl*-2 that demonstrates a similar phenotype, but with a different complementation group, is also available. To generate mutations that affect development, several projects for mutagenesis screening induced by the chemical mutagen ENU have been successfully performed [3, 11, 24]. NBRP Medaka currently provides over 200 different ENU mutants. Most of these mutants exhibit specific defects in development and are embryonic lethal.

Transgenics

Transgenic medaka strains are powerful tools that can be used to analyze and monitor spatio-temporal gene expression in living individuals. These strains are par-

ticularly useful when combined with fluorescent protein reporters such as GFP and DsRed. NBRP Medaka currently provides more than 20 transgenic strains. Some examples are as follows.

STII(ChgH-GFP, Fig. 2G): Choriogenins are precursor proteins present in the egg envelope that are expressed in the mature female liver following induction with estrogen. In this transgenic line, the GFP gene was introduced into the transparent see-through line STII under the control of the choriogenin H gene regulatory region [21, 40]. Estrogenic activity in the water can then be monitored as an expression of GFP in the liver of adult males or larvae. This feature can then be applied to detect the estrogenic activity of novel chemical products, and in environmental water and sewage samples.

OlMA1-GFP: This transgenic line contains a GFP gene that is regulated by the medaka skeletal muscle actin promoter (Fig. 2H) [17, 22]. This fish exhibits very strong GFP fluorescence in the skeletal muscle and can be used for developmental and anatomical research of the skeletal muscle. In addition, GFP signals in the skeletal muscle can be easily observed over several months following fixation. Fixed transgenic fish can also be shipped without special attention and in accordance with the Cartagena protocol. This is especially convenient for educational purposes in schools. In this regards, NBRP Medaka has started to provide schools with fixed embryo and adult fish samples of this line.

TG (*olvas*-GFP): Germ cells of this fish line are easily distinguished by GFP, as the medaka *vasa* (*olvas*) promoter and 3'UTR drive the GFP expression. This fish is particularly useful for studies of gonadal development [38, 48, 56]. Several additional TG lines including AD-1 (GFP expression in hemocytes) [26], cab-TG(*rag1*-EGFP: strong GFP expression in the thymus) [23], and TG(CMV-H2B-GFP: chromosomes can be observed because of histone H2B-GFP fusion-protein expression) [12] are also available.

Other materials

Hatching enzyme is required to remove the egg envelope prior to embryo manipulation procedures such as chimera formation. NBRP Medaka has provided hatching enzymes since 2008. These enzymes are provided in the form of a crude hatching solution [55, 60]. The enzyme is presently only delivered within Japan due to difficulties in transporting frozen samples overseas.

How to Obtain Resources from the NBRP Medaka

All resources provided by NBRP Medaka can be ordered via the web page (Fig. 3, http://www.shigen.nig.ac.jp/medaka/). Full-text search is also possible for all or selected resources including live resources, full-length cDNA, BAC, and fosmid clones by entering key words into a search field [Quick Search, Fig. 3(a)]. The characteristics of each resource can also be browsed in a new window [Fig. 3(b) and 3(c), right window]. Homology searches for full-length cDNA sequences and BAC/fosmid end sequences are also possible [Fig. 3(e)], and all sequence data is downloadable from the web page [Fig. 3(f)]. After locating appropriate information, each resource can be ordered by simply clicking on the shopping cart icon [Fig. 3(h)] and logging onto the website. Details for ordering are outlined in the request help function. At present, NBRP Medaka is also able to provide approximately half of the EST/cDNA clones currently deposited in public DNA data banks such as DDBJ, EMBL, and Genbank. For these orders, users can order the clones via an email that includes the accession number or clone names through "contact us" on the NBRP Medaka website [Fig. 3(g)] or through mbrc@nibb.ac.jp directly.

The NBRP Medaka web site give access to four databases: Medaka Book, experimental and breeding protocols for medaka biology [Fig. 3(i)]; Medaka Atlas, an histological atlas of nervous and vascular systems [Fig. 3(j)]; Medaka Tree, a phylogenetic tree and information regarding their collection locations of wild stocks and related species [Fig. 3(k)]; and Medaka Map, the medaka genome browser with Ensembl interface [Fig. 3(l)]. Keyword searches and blast similarity searches are also possible using Medaka Map. Medaka Map is faster than the original Ensembl genome browser, due to the relatively compact size of the data. NBRP Medaka welcomes any questions regarding the presentation of resources on the web page [Fig. 3(g)].

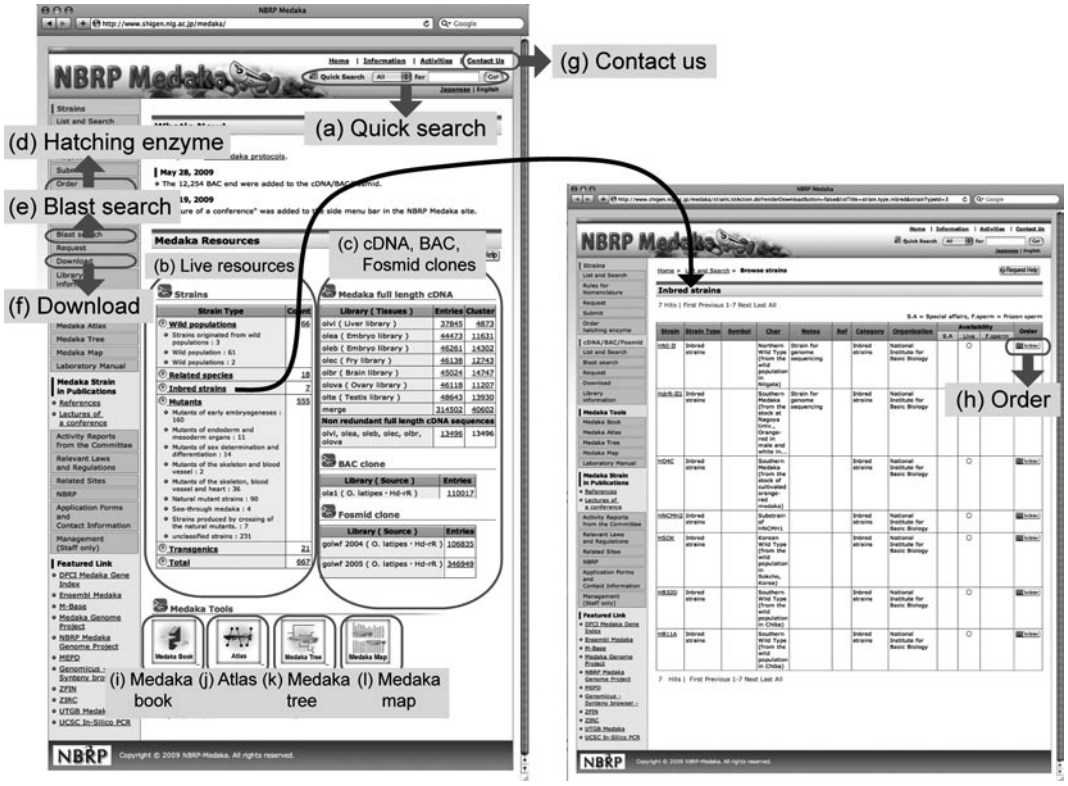

Fig. 3. NBRP Medaka web site (http://www.shigen.nig.ac.jp/medaka/). Top page of the NBRP Medaka web site (left window). Full-text search is possible of all resources by entering key words into the "Quick search" field (a). Information on live resources (b), the full-length cDNA clones, and genome DNA clones (BAC and fosmid) (c) can be browsed in a new window. The right window is an example of the information presented for inbred strains (arrow). The function for ordering hatching enzyme (d), blast search of cDNA, BAC, and fosmid sequences (e), download of the sequence data for each clone (f), and "Contact us" page (g). Required resources can be ordered by clicking the shopping cart "Order" button (h). Icons that link to four databases (i–l), protocols for medaka biology (i, Medaka Book), histology atlas of nervous and vascular system (j, Medaka Atlas), phylogenetic tree and information of collection locations (k, Medaka Tree), and medaka genome browser (l, Medaka Map).

Breeding and Maintenance of Medaka

Medaka breeding is relatively easy. In addition, breeding stocks can be easily maintained depending on the number of medaka required, as a water circulation system is not essential for breeding and medaka can be maintained outdoors. The Medaka protocol book [18] and website (Medaka Book, http://www.shigen.nig.ac.jp/medaka/medakabook/) are helpful for learning the details of medaka breeding and maintenance. The simplest way to rear medaka is by housing them in plastic containers (e.g., mouse cages) or glass aquariums (Fig. 4A). To house large numbers of medaka indoors, recirculation breeding systems are convenient in that they save space and help maintain water quality (Fig. 4B). Medaka can be bred in the same recirculation system as zebrafish. If this is the case, the rate of water flow into each tank requires only an adjustment of less than 200 ml/min, as it is difficult for medaka to swim against continuous and fast water currents. The water conditions required to successfully maintain medaka are as follows: temperature, 25–28°C; pH 6.8–7.5; conductivity, 200–450 μS/cm; ammonia, <0.2 mg NH_4^+/l; nitrite, <0.1 mg NO_2^-/l; nitrate, <20 mg NO_3^-/l; total hardness, <20–100 mg $CaCO_3$/l. Ten percent of the water in the tank also needs to be exchanged per day with de-chlorinated tap water or reverse osmosis water. When reverse osmosis water is used, conductivity should be adjusted by adding

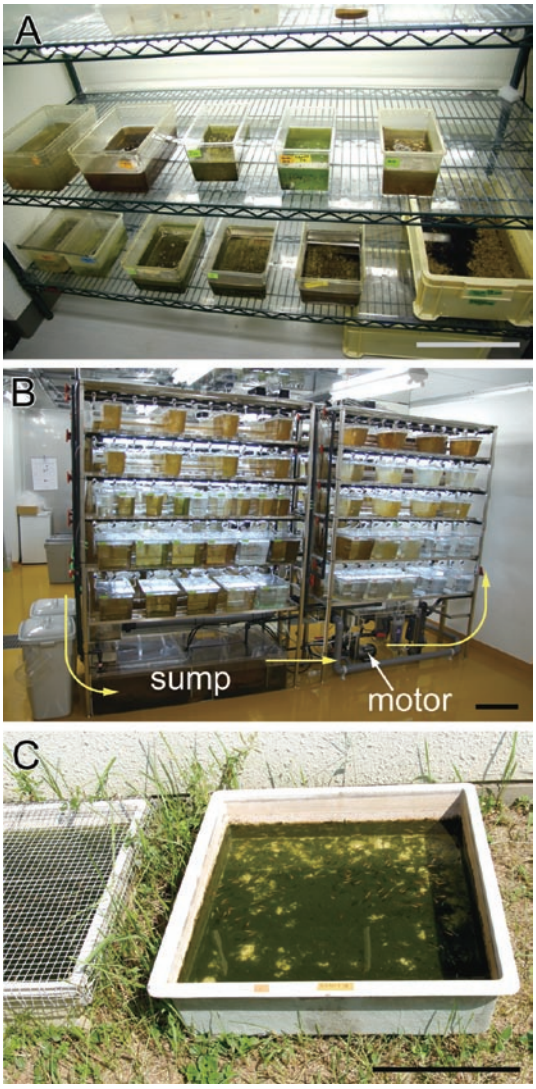

Fig. 4. Three methods of medaka maintenance. (A) Medaka can be housed in plastic containers on a steel rack. Each shelf receives light from the upper side. Microorganisms and microalgae present in the green water or dark brown algae that stick to the inside of the containers are advantageous because they stabilize water quality. (B) A multi rack re-circulating breeding system. The water circulates between the motor, fish tanks, and sump before returning to the motor. The yellow arrows show the route of water recirculation. The sump contains small balls of porous ceramic for biological filtration by nitrobacteria. (C) Medaka can also be bred outdoors in plastic containers. The containers should be covered with steel mesh lids (left container) to protect the fish from predators such as birds. The lid has been removed to show the inside of the center container in this figure. Scale bars =30 cm.

artificial sea salt. Drastic changes in water conditions should be avoided, and the fish should be housed at a light cycle of 14 h in the light and 10 h in the dark.

To collect eggs, the fish density should be maintained at 2 fish/l, and the female to male ratio should be 2:1 to 3:1 in a recirculation system. The density can be increased to three times higher when egg sampling is not required. When fish are maintained in still water, the density should be decreased to one third. It is also better to house fish with similar body size together, as adult fish tend to eat larvae and the larger fish tend to attack smaller, juvenile fish.

Brine shrimp (*Artemia*) are a good food source to rear the fish, while dry food is convenient for breeding. Some examples of dry food used in medaka breeding are as follows: TetraMin (Tetra Werke, Melle, Germany), medaka feed for adults or larvae (Medaka Honpo, Hiroshima, Japan, http://medakahonpo.com/ [in Japanese]), Otohime B1 (Marubeni Nisshin Feed, Tokyo, Japan, http://www.mn-feed.com/ [in Japanese]), Hikari Crest for Guppy (Kyorin, Hyogo, Japan, http://www.kyorin-net.co.jp/ [in Japanese]). Dry food for zebrafish can also be used to feed medaka. It may be necessary to first grind food into a fine powder so that the food is consumable by the larvae. The frequency of feeding should be 1–2 times per day. However if increasing the rate of growth or egg-laying a higher frequency is also acceptable, but supplying an excess amount of food each time should be avoided. In general, the food provided should be consumed within 10 min.

Medaka can also be housed in plastic containers outdoors throughout the year (Fig. 4C). Under these conditions, the fish will lay eggs everyday between May and August in Japan. In the winter, medaka ceases reproduction. During this time, feeding and water changes should be avoided. The book entitled “Medaka: Biology, Management and Experimental Protocols” published by Wiley-Blackwell provides protocols outlining experimental methods and basic knowledge of medaka as an experimental model system [18].

Useful Websites for Medaka Research

The following list outlines useful websites for medaka biology:

Medaka Genome Project (NIG DNA Sequencing Center) (http://dolphin.lab.nig.ac.jp/medaka/index.php)

Using this site, researchers are able to blast search the whole genome shotgun reads and assembly of the Hd-rR and HNI-II genomes. This site is also useful for searching the sequence differences between the Hd-rR and HNI-II genomes. The shotgun reads include the most fundamental sequence data, and thus, searches of the row sequence data can also be obtained on this site.

M Base (http://earth.lab.nig.ac.jp/~mbase/medaka_top.html)

This website presents a medaka genome database that focuses mainly on EST/BAC end sequences and medaka EST linkage maps.

MEPD: Medaka Expression Pattern Database (http://ani.embl.de:8080/mepd/)

MEPD outlines the expression patterns of the 1,749 medaka genes that are currently available [5].

Medaka fish homepage (http://biol1.bio.nagoya-u.ac.jp:8000/)

This site is a portal site that presents general information on medaka biology.

UCSC *in-silico* PCR (http://genome.ucsc.edu/cgi-bin/hgPcr?command=start)

Using this website, researchers are able to search any possible amplified fragments from the medaka genome using PCR primer pairs.

Genomicus synteny browser (http://www.dyogen.ens.fr/genomicus/cgi-bin/search.pl)

This browser allows researchers to navigate genomes in several dimensions including linearly along chromosome axes, transversally across different species, and chronologically along evolutionary time.

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References

1. Aida, T. 1921. On the inheritance of color in a fresh-water fish, *Aplocheilus latipes* Temmick and Schlegel, with special reference to sex-linked inheritance. *Genetics* 6: 554–573.
2. Aoki, K., Okamoto, M., Tatsumi, K., and Ishikawa, Y. 1997. Cryopreservation of medaka spermatozoa. *Zoolog. Sci.* 14: 641–644.
3. Furutani-Seiki, M., Sasado, T., Morinaga, C., Suwa, H., Niwa, K., Yoda, H., Deguchi, T., Hirose, Y., Yasuoka, A., Henrich, T., Watanabe, T., Iwanami, N., Kitagawa, D., Saito, K., Asaka, S., Osakada, M., Kunimatsu, S., Momoi, A., Elmasri, H., Winkler, C., Ramialison, M., Loosli, F., Quiring, R., Carl, M., Grabher, C., Winkler, S., Del Bene, F., Shinomiya, A., Kota, Y., Yamanaka, T., Okamoto, Y., Takahashi, K., Todo, T., Abe, K., Takahama, Y., Tanaka, M., Mitani, H., Katada, T., Nishina, H., Nakajima, N., Wittbrodt, J., and Kondoh, H. 2004. A systematic genome-wide screen for mutations affecting organogenesis in Medaka, *Oryzias latipes*. *Mech. Dev.* 121: 647–658.
4. Furutani-Seiki, M. and Wittbrodt, J. 2004. Medaka and zebrafish, an evolutionary twin study. *Mech. Dev.* 121: 629–637.
5. Henrich, T., Ramialison, M., Assouline, B., Bourrat, F., Berger, A., Himmelbauer, H., Sasaki, T., Shimizu, N., Westerfield, M., Kondoh, H., and Wittbrodt, J. 2005. MEPD: a resource for medaka gene expression patterns. *Bioinformatics* 21: 3195–3197.
6. Hibiya, K., Katsumoto, T., Kondo, T., Kitabayashi, I., and Kudo, A. 2009. Brpf1, a subunit of the MOZ histone acetyl transferase complex, maintains expression of anterior and posterior *Hox* genes for proper patterning of craniofacial and caudal skeletons. *Dev. Biol.* 329: 176–190.
7. Hyodo-Taguchi, Y. 1996. Inbred strains of the medaka, (*Oryzias latipes*). *Fish Biol. J. Medaka* 8: 11–14.
8. Hyodo-Taguchi, Y. and Matsudaira, H. 1987. Higher susceptibility to N-methyl-N'-nitro-N-nitrosoguanidine-induced tumorigenesis in an interstrain hybrid of the fish, *Oryzias latipes* (medaka). *Jpn. J. Cancer Res.* 78: 487–493.
9. Imai, S., Koyama, J., and Fujii, K. 2005. Effects of 17 beta-estradiol on the reproduction of Java-medaka (*Oryzias*

javanicus), a new test fish species. *Mar. Pollut. Bull.* 51: 708–714.

10. Inoue, K. and Takei, Y. 2003. Asian medaka fishes offer new models for studying mechanisms of seawater adaptation. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 136: 635–645.
11. Ishikawa, Y. 2000. Medakafish as a model system for vertebrate developmental genetics. *Bioessays* 22: 487–495.
12. Iwai, T., Inoue, S., Kotani, T., and Yamashita, M. 2009. Production of transgenic medaka fish carrying fluorescent nuclei and chromosomes. *Zoolog. Sci.* 26: 9–16.
13. Kamei, Y. 2009. Tilling (Gene Knockout). pp. 373–381. *In*: Medaka: Biology, Management and Experimental Protocols (Kinoshita, M., Murata, K., Naruse, K., and Tanaka, M. eds.), Wiley-Blackwell, Ames, Iowa.
14. Kasahara, M., Naruse, K., Sasaki, S., Nakatani, Y., Qu, W., Ahsan, B., Yamada, T., Nagayasu, Y., Doi, K., Kasai, Y., Jindo, T., Kobayashi, D., Shimada, A., Toyoda, A., Kuroki, Y., Fujiyama, A., Sasaki, T., Shimizu, A., Asakawa, S., Shimizu, N., Hashimoto, S., Yang, J., Lee, Y., Matsushima, K., Sugano, S., Sakaizumi, M., Narita, T., Ohishi, K., Haga, S., Ohta, F., Nomoto, H., Nogata, K., Morishita, T., Endo, T., Shin-I, T., Takeda, H., Morishita, S., and Kohara, Y. 2007. The medaka draft genome and insights into vertebrate genome evolution. *Nature* 447: 714–719.
15. Kelsh, R.N., Inoue, C., Momoi, A., Kondoh, H., Furutani-Seiki, M., Ozato, K., and Wakamatsu, Y. 2004. The Tomita collection of medaka pigmentation mutants as a resource for understanding neural crest cell development. *Mech. Dev.* 121: 841–859.
16. Kimura, T., Jindo, T., Narita, T., Naruse, K., Kobayashi, D., Shin, I.T., Kitagawa, T., Sakaguchi, T., Mitani, H., Shima, A., Kohara, Y., and Takeda, H. 2004. Large-scale isolation of ESTs from medaka embryos and its application to medaka developmental genetics. *Mech. Dev.* 121: 915–932.
17. Kinoshita, M. 2004. Transgenic medaka with brilliant fluorescence in skeletal muscle under normal light. *Fisheries Sci.* 70: 645–649.
18. Kinoshita, M., Murata, K., Naruse, K., and Tanaka, M. 2009. Medaka: Biology, Management and Experimental Protocols, Wiley-Blackwell, Ames, Iowa.
19. Koyama, J., Kawamata, M., Imai, S., Fukunaga, M., Uno, S., and Kakuno, A. 2008. Java medaka: a proposed new marine test fish for ecotoxicology. *Environ. Toxicol.* 23: 487–491.
20. Krone, A. and Wittbrodt, J. 1997. A simple and reliable protocol for cryopreservation of Medaka (*Oryzias latipes*) spermatozoa. *Fish Biol. J. Medaka* 9: 47–48.
21. Kurauchi, K., Nakaguchi, Y., Tsutsumi, M., Hori, H., Kurihara, R., Hashimoto, S., Ohnuma, R., Yamamoto, Y., Matsuoka, S., Kawai, S., Hirata, T., and Kinoshita, M. 2005. In vivo visual reporter system for detection of estrogen-like substances by transgenic medaka. *Environ. Sci. Technol.* 39: 2762–2768.
22. Kusakabe, R., Kusakabe, T., and Suzuki, N. 1999. In vivo analysis of two striated muscle actin promoters reveals combinations of multiple regulatory modules required for skeletal and cardiac muscle-specific gene expression. *Int. J. Dev. Biol.* 43: 541–554.
23. Li, J., Iwanami, N., Hoa, V.Q., Furutani-Seiki, M., and Takahama, Y. 2007. Noninvasive intravital imaging of thymocyte dynamics in medaka. *J. Immunol.* 179: 1605–1615.
24. Loosli, F., Koster, R.W., Carl, M., Kuhnlein, R., Henrich, T., Mucke, M., Krone, A., and Wittbrodt, J. 2000. A genetic screen for mutations affecting embryonic development in medaka fish (*Oryzias latipes*). *Mech. Dev.* 97: 133–139.
25. Martinez-Morales, J.R., Rembold, M., Greger, K., Simpson, J.C., Brown, K.E., Quiring, R., Pepperkok, R., Martin-Bermudo, M.D., Himmelbauer, H., and Wittbrodt, J. 2009. ojoplano-mediated basal constriction is essential for optic cup morphogenesis. *Development* 136: 2165–2175.
26. Maruyama, K., Ishikawa, Y., Yasumasu, S., and Iuchi, I. 2007. Globin gene enhancer activity of a DNase-I hypersensitive site-40 homolog in medaka, *Oryzias latipes*. *Zoolog. Sci.* 24: 997–1004.
27. Matsuda, M., Nagahama, Y., Shinomiya, A., Sato, T., Matsuda, C., Kobayashi, T., Morrey, C.E., Shibata, N., Asakawa, S., Shimizu, N., Hori, H., Hamaguchi, S., and Sakaizumi, M. 2002. *DMY* is a Y-specific DM-domain gene required for male development in the medaka fish. *Nature* 417: 559–563.
28. Morinaga, C., Saito, D., Nakamura, S., Sasaki, T., Asakawa, S., Shimizu, N., Mitani, H., Furutani-Seiki, M., Tanaka, M., and Kondoh, H. 2007. The *hotei* mutation of medaka in the anti-Müllerian hormone receptor causes the dysregulation of germ cell and sexual development. *Proc. Natl. Acad. Sci. U.S.A.* 104: 9691–9696.
29. Nanda, I., Kondo, M., Hornung, U., Asakawa, S., Winkler, C., Shimizu, A., Shan, Z., Haaf, T., Shimizu, N., Shima, A., Schmid, M., and Schartl, M. 2002. A duplicated copy of *DMRT1* in the sex-determining region of the Y chromosome of the medaka, *Oryzias latipes*. *Proc. Natl. Acad. Sci. U.S.A.* 99: 11778–11783.
30. Naruse, K., Fukamachi, S., Mitani, H., Kondo, M., Matsuoka, T., Kondo, S., Hanamura, N., Morita, Y., Hasegawa, K., Nishigaki, R., Shimada, A., Wada, H., Kusakabe, T., Suzuki, N., Kinoshita, M., Kanamori, A., Terado, T., Kimura, H., Nonaka, M., and Shima, A. 2000. A detailed linkage map of medaka, *Oryzias latipes*: comparative genomics and genome evolution. *Genetics* 154: 1773–1784.
31. Naruse, K., Hori, H., Shimizu, N., Kohara, Y., and Takeda, H. 2004. Medaka genomics: a bridge between mutant phenotype and gene function. *Mech. Dev.* 121: 619–628.
32. Naruse, K., Tanaka, M., Mita, K., Shima, A., Postlethwait, J., and Mitani, H. 2004. A medaka gene map: the trace of ancestral vertebrate proto-chromosomes revealed by comparative gene mapping. *Genome Res.* 14: 820–828.
33. Omran, H., Kobayashi, D., Olbrich, H., Tsukahara, T., Loges, N.T., Hagiwara, H., Zhang, Q., Leblond, G., O'Toole, E., Hara, C., Mizuno, H., Kawano, H., Fliegauf, M., Yagi, T., Koshida, S., Miyawaki, A., Zentgraf, H., Seithe, H., Reinhardt, R., Watanabe, Y., Kamiya, R., Mitchell, D.R., and Takeda, H. 2008. Ktu/PF13 is required for cytoplasmic pre-assembly of axonemal dyneins. *Nature* 456: 611–616.

34. Ozato, K., Kondoh, H., Inohara, H., Iwamatsu, T., Wakamatsu, Y., and Okada, T.S. 1986. Production of transgenic fish: introduction and expression of chicken delta-crystallin gene in medaka embryos. *Cell Differ.* 19: 237–244.
35. Sano, S., Takashima, S., Niwa, H., Yokoi, H., Shimada, A., Arenz, A., Wittbrodt, J., and Takeda, H. 2009. Characterization of teleost Mdga1 using a gene-trap approach in medaka (*Oryzias latipes*). *Genesis* 47: 505–513.
36. Sasado, T. 2009. Artificial insemination using frozen medaka sperm. pp. 110–116. *In*: Medaka: Biology, Management and Experimental Protocols (Kinoshita, M., Murata, K., Naruse, K., and Tanaka, M. eds.), Wiley-Blackwell, Ames, Iowa.
37. Sasado, T. 2009. Cryopreservation of medaka sperm. pp. 105–110. *In*: Medaka: Biology, Management and Experimental Protocols (Kinoshita, M., Murata, K., Naruse, K., and Tanaka, M. eds.), Wiley-Blackwell, Ames, Iowa.
38. Sasado, T., Morinaga, C., Niwa, K., Shinomiya, A., Yasuoka, A., Suwa, H., Hirose, Y., Yoda, H., Henrich, T., Deguchi, T., Iwanami, N., Watanabe, T., Kunimatsu, S., Osakada, M., Okamoto, Y., Kota, Y., Yamanaka, T., Tanaka, M., Kondoh, H., and Furutani-Seiki, M. 2004. Mutations affecting early distribution of primordial germ cells in Medaka (*Oryzias latipes*) embryo. *Mech. Dev.* 121: 817–828.
39. Sasado, T., Yasuoka, A., Abe, K., Mitani, H., Furutani-Seiki, M., Tanaka, M., and Kondoh, H. 2008. Distinct contributions of CXCR4b and CXCR7/RDC1 receptor systems in regulation of PGC migration revealed by medaka mutants *kazura* and *yanagi*. *Dev. Biol.* 320: 328–339.
40. Scholz, S., Kurauchi, K., Kinoshita, M., Oshima, Y., Ozato, K., Schirmer, K., and Wakamatsu, Y. 2005. Analysis of estrogenic effects by quantification of green fluorescent protein in juvenile fish of a transgenic medaka. *Environ. Toxicol. Chem.* 24: 2553–2561.
41. Shima, A. and Mitani, H. 2004. Medaka as a research organism: past, present and future. *Mech. Dev.* 121: 599–604.
42. Shimada, A. and Shima, A. 2004. Transgenerational genomic instability as revealed by a somatic mutation assay using the medaka fish. *Mutat. Res.* 552: 119–124.
43. Takehana, Y., Demiyah, D., Naruse, K., Hamaguchi, S., and Sakaizumi, M. 2007. Evolution of different Y chromosomes in two medaka species, *Oryzias dancena* and *O. latipes*. *Genetics* 175: 1335–1340.
44. Takehana, Y., Nagai, N., Matsuda, M., Tsuchiya, K., and Sakaizumi, M. 2003. Geographic variation and diversity of the cytochrome b gene in Japanese wild populations of Medaka, *Oryzias latipes*. *Zoolog. Sci.* 20: 1279–1291.
45. Takehana, Y., Naruse, K., Hamaguchi, S., and Sakaizumi, M. 2007. Evolution of ZZ/ZW and XX/XY sex-determination systems in the closely related medaka species, *Oryzias hubbsi* and *O. dancena*. *Chromosoma* 116: 463–470.
46. Takehana, Y., Naruse, K., and Sakaizumi, M. 2005. Molecular phylogeny of the medaka fishes genus *Oryzias* (Beloniformes: Adrianichthyidae) based on nuclear and mitochondrial DNA sequences. *Mol. Phylogenet. Evol.* 36: 417–428.
47. Takehana, Y., Uchiyama, S., Matsuda, M., Jeon, S.R., and Sakaizumi, M. 2004. Geographic variation and diversity of the cytochrome b gene in wild populations of medaka (*Oryzias latipes*) from Korea and China. *Zoolog. Sci.* 21: 483–491.
48. Tanaka, M., Kinoshita, M., Kobayashi, D., and Nagahama, Y. 2001. Establishment of medaka (*Oryzias latipes*) transgenic lines with the expression of green fluorescent protein fluorescence exclusively in germ cells: a useful model to monitor germ cells in a live vertebrate. *Proc. Natl. Acad. Sci. U.S.A.* 98: 2544–2549.
49. Taniguchi, Y., Takeda, S., Furutani-Seiki, M., Kamei, Y., Todo, T., Sasado, T., Deguchi, T., Kondoh, H., Mudde, J., Yamazoe, M., Hidaka, M., Mitani, H., Toyoda, A., Sakaki, Y., Plasterk, R.H., and Cuppen, E. 2006. Generation of medaka gene knockout models by target-selected mutagenesis. *Genome Biol.* 7: R116.
50. Temminck, C.J. and Schlegel, H. 1842. Les Poecilies. pp. 224–225. *In*: Fauna Japonica, Pisces (Siebold, P.F. ed.), Apud A. Arnz et Socios, Leiden.
51. Tomita, H. 1975. Mutant genes in the medaka. pp. 251–272. *In*: Medaka (Killifish): Biology and Strains (Yamamoto, T. ed.), Yugakusha, Tokyo.
52. Tomita, H. 1992. The lists of the mutants and strains of the medaka, common gambusia, silver crucian carp, goldfish, and golden venus fish maintained in the Laboratory of Freshwater Fish Stocks, Nagoya University. *Fish Biol. J. Medaka* 4: 45–47.
53. Toyama, K. 1916. Some examples of Mendelian characters. *Nihon Ikushugaku Kaiho* 1: 1–9 (in Japanese).
54. Wada, H., Shimada, A., Fukamachi, S., Naruse, K., and Shima, A. 1998. Sex-linked inheritance of the *lf* locus in the medaka fish (*Oryzias latipes*). *Zoolog. Sci.* 15: 123–126.
55. Wakamatsu, Y. 1997. Preparation of medaka hatching enzyme. *Fish Biol. J. Medaka* 9: 49–50.
56. Wakamatsu, Y., Pristyazhnyuk, S., Kinoshita, M., Tanaka, M., and Ozato, K. 2001. The see-through medaka: a fish model that is transparent throughout life. *Proc. Natl. Acad. Sci. U.S.A.* 98: 10046–10050.
57. Wittbrodt, J., Shima, A., and Schartl, M. 2002. Medaka—a model organism from the far East. *Nat. Rev. Genet.* 3: 53–64.
58. Yamamoto, T. 1953. Artificially induced sex-reversal in genotypic males of the medaka (*Oryzias latipes*). *J. Exp. Zool.* 123: 571–594.
59. Yamamoto, T. 1975. Control of sex differentiation. pp. 192–213. *In*: Medaka (Killifish): Biology and Strains. (Yamamoto, T. ed.), Yugakusha, Tokyo.
60. Yasumasu, S., Iuchi, I., and Yamagami, K. 1989. Purification and partial characterization of high choriolytic enzyme (HCE), a component of the hatching enzyme of the teleost, *Oryzias latipes*. *J. Biochem.* 105: 204–211.