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Microsatellite-centromere mapping in the Japanese eel (*Anguilla japonica*) by half-tetrad analysis using induced triploid families

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Abstract

Genetic improvement of the Japanese eel (*Anguilla japonica*) can be achieved by artificially controlling its life cycle using recent advances in reproductive biology. In this study, we developed 43 microsatellite loci to confirm Mendelian inheritance at 10 of them as well at 16 previous reported in two full-sib families produced by artificial insemination. In order to establish a base for aquaculture genetics of this species in the near future, these microsatellite loci were mapped in relation to the centromere by half-tetrad analysis using four artificially induced triploid families. The second division segregation frequency (y) of the microsatellite loci ranged from 0.008 to 0.968 (mean ± SD = 0.645 ± 0.298). These results suggest the presence of strong chiasma interference in the eel. Significant differences were observed for the map distances of microsatellite loci between the two isolation procedures. Microsatellites isolated using the enrichment procedure were mapped to various sites starting from the centromere to the telomere, whereas those from the conventional size-selected library showed a tendency to be distributed in the telomeric region.

Keywords: *Anguilla japonica*, gene-centromere mapping, half-tetrad analysis, interference, microsatellite, triploid

1. Introduction

The Japanese eel (*Anguilla japonica*) is one of the most important species in aquaculture because of its high economic value, particularly in East Asia. Recently, we succeeded in obtaining artificially matured gametes by using hormonal treatment (Ohta et al., 1997). Viable leptocephali (Tanaka et al., 2001) and glass eels (Tanaka, 2003) have been successfully produced by the development of appropriate rearing techniques.

Genetic improvement will gain importance in eel aquaculture when mass production of artificially propagated glass eels is realized. Genetic mapping is one of the most effective approaches for understanding the genome of the target species. In aquacultural organisms, linkage mapping and its application to the effective selection of desirable traits by using comparative syntenic information of other species is a more practical approach than determining whole genome sequences for each species.

Microsatellite loci are useful markers for genetic mapping (see Reviews, Chamvers and MacAvoy, 2000). In the genus *Anguilla*, several authors have reported a number of microsatellite markers that have been mainly used for population studies (Daemen et al., 1997; Ishikawa et al., 2001; Tseng et al., 2001; Wirth and Bernatchez, 2001); however, the existent number of markers is still too small to construct a linkage map.

Recent advances in the artificial induction of maturation of the Japanese eel have made it possible to produce progeny for genetic studies by artificial fertilization of mature gametes in the laboratory. This enables the use of chromosome manipulation techniques on eel zygotes. In our previous study, we successfully induced triploid individuals for the first time in the Japanese eel by the heat shock treatment, which inhibited the second polar body release after normal fertilization (Nomura et al., 2003). The production of triploid families enables the application of half-tetrad analysis for

genetic mapping. Half-tetrad analysis can be performed if two of the four strands from a single meiosis can be recovered (Zhao and Speed, 1998). In fish species, triploids or gynogenetic diploids produced by the inhibition of the second polar body release provide a means to analyze meiosis II (MII) half-tetrads (Zhao and Speed, 1998). The

5   recombination rate between the gene or marker and the centromere can be estimated from the frequency of recombinant heterozygous genotype in the half-tetrad progeny of the heterozygous mother. The proportion of heterozygous progeny is a measure of the frequency of second division segregation (y). Thus, the gene-centromere distance can be estimated by using an appropriate map function. The G-C map provides genetic

10   information, such as the distribution of marker or gene loci along the chromosome, and it is the key to evaluating the success of the meiotic gynogenesis and the mitotic gynogenesis, which results from polar body inhibition and cleavage inhibition, respectively.

     In this paper, we developed 43 microsatellite loci and, mapped 10 of them as well

15   as 16 previously reported loci relative to the centromere of the eel chromosomes, by using four triploid families produced by inhibition of the second polar body release.

2. Materials and methods

Development of microsatellite markers

Microsatellite array of the Japanese eel was isolated using two methods: one

5   from the partial genomic libraries that were selected for obtaining small insert size DNA

and the other from the microsatellite (MS)-enriched genomic libraries.

The first procedure was performed in a manner similar to that used for the loach

Misgurnus anguillicaudatus (Teleostei: Cobitidae)(Morishima et al., 2001) with the

following modifications. The genomic DNA that was isolated from the blood of a

10   cultured eel was digested with *Hae*III; the digested fragments were then electrophoresed

on a 0.8% agarose gel in order to select fragments with sizes ranging between 300 and

600 bp. These size-selected fragments were ligated into the *Srf*I site of pPCR-Script™

Amp SK (+) vector, and transformed by using PCR-Script™ Amp Cloning kit according

to the manufacturer's protocol (Stratagene, La Jolla, CA, USA).

15   The second method of microsatellite isolation was performed by using FIASCO

(Fast Isolation by AFLP of Sequences Containing repeats) as described by Zane et al.

(2002) along with some modifications. Briefly, the genomic DNA isolated from the

blood of a cultured eel was digested with *Mse*I and ligated to *Mse*I AFLP adapters

(5´-TACTCAGGACTCAT-3´/5´-GACGATGAGTCCTGAG-3´), or digested with

20   *Hae*III and ligated to *Hae*III (blunt end) adapters (5´-

CTCTTGCTTACGCGTGGACTA-3´/5´-pTAGTCCACGCGTAAGCAAGAGCACA-3

´; Edwards et al., 1996). The digestion-ligation mixture was used as template for PCR,

which was performed using an *Mse*I AFLP adapter-specific primer

(5´-GATGAGTCCTGAGTAAN-3´) or a *Hae*III adapter-specific primer (5´-

25   CTCTTGCTTACGCGTGGACTA-3´). The DNA was then hybridized with a

biotinylated $(AC)_{17}$ probe and captured by streptavidin-coated beads. The

beads-probe-DNA complex was separated from the hybridization buffer by using a magnetic field. Nonspecific DNA was removed by three nonstringency washes and three stringency washes. The DNA was separated from the beads-probe complex by a denaturation step and used as template for PCR, which was performed using each adapter-specific primer. The PCR products were ligated to the pDrive Cloning Vector, and transformed by QIAGEN PCR Cloning kit following the manufacturer's protocol (QIAGEN, Hilden, Germany).

The resulting genomic libraries were screened using an alkaline phosphatase-labeled $(GT)_{10}$ probe. The positive colonies were suspended in distilled water and boiled for 10 min. The solution was used as a template for PCR employing T3 and T7 primers. The PCR products were purified and sequenced on an ABI prism 373A autosequencer using an FS cycle sequencing kit (Applied Biosystems, Foster City, CA, USA).

Forward and reverse primers were designed based on the unique flanking regions of each microsatellite repeat. In particular, the forward primers of each microsatellite locus isolated from the MS-enriched libraries were designed to include the 15-bp M13 sequence (5´-AGTCACGACGTTGTA-3´) at their 5´ end for the M13-tailed primer method described by Zhou et al. (2002). Newly developed microsatellite loci were serially named as *Ajp-1*, *Ajp-2*, and *Ajp-N*. Sequence data were deposited in DDBJ under the following accession numbers **AB194929-AB194936**, **AB233944-AB233978**.

Experimental diploid and triploid families

Cultured eels purchased from a commercial farm were acclimated to seawater at the National Research Institute of Aquaculture, Mie, Japan. Hormonal treatment was carried out for artificial maturation, as described previously (Ohta et al., 1996; Kagawa

et al., 1997). Four females (1-4) were repeatedly injected with salmon pituitary extract, followed by injection with 17α, 20β-dihydroxy-4-pregnen-3-one (SIGMA, St. Louis, MO, USA). Similarly, four males (1-4) were repeatedly injected with human chorionic gonadotropin (Teikoku hormone MFG Co. Tokyo, Japan). The gametes were obtained

5   by gently stripping ovulating females and mature males. Thirty to fifty grams of the eggs were inseminated with 10 ml of prediluted milt (Ohta et al., 1996) and then divided into two groups; control groups to generate normal diploid families for testing Mendelian inheritance, and heat shock groups to generate triploid families for the half-tetrad analysis. Each group was stocked in a glass container containing filled with

10   500 ml of filtered seawater (pore size of filter, 0.2 μm) containing 100,000 IU/l of penicillin G potassium (Banyu Pharmaceutical, Tokyo, Japan) and 0.1 g/l of streptomycin sulfate (Meiji Seika, Tokyo, Japan). The water temperature was maintained at 23°C ± 1.0°C throughout the embryogenesis except for the period of heat shock treatment. The eggs of heat shock groups were immersed in the seawater kept at

15   37°C for 3 min, starting at 10 min after fertilization (m.a.f.) for families 1 and 2, and the eggs were treated from 5 to 8, 7 to 10, 10 to 13 m.a.f. for families 3 and 4. Damaged eggs and embryos, which were clouded and never hatched, were removed with a glass pipette in order to prevent a decline in the quality of seawater.

20   *Ploidy determination and DNA extraction*

The larvae were sampled from each group at two days post hatching (d.p.h), fixed with 70% ethanol, and stored at –20°C until analysis. A small piece of each larva was used to assess of the ploidy level by flow cytometry (FCM) using the PA type flow cytometer (Partec, Münster, Germany) as described previously (Nomura et al., 2003).

25   The residue body of each larva was used for DNA extraction using Wizard SV Genomic DNA Purification System (Promega, Madison, WI, USA).

Microsatellite genotyping

Genotyping was carried out by the following two methods.

(1) The normal PCR were performed in a reaction mixture (10 μl) containing 50 ng template DNA, 40 μM dNTPs, 0.75 pmol of each primer, and 0.1 U of ExTaq polymerase (TaKaRa, Tokyo, Japan) under the following conditions: 30 cycles of denaturation for 1 min at 93°C, annealing for 30 s at 55°C-60°C, and extension for 30 s at 72°C. The PCR products were electrophoresed on 15% nondenatured polyacrylamide gels and visualized by SYBR Green I (TaKaRa, Tokyo, Japan) using UV transillumination. The microsatellite alleles were designated according to their molecular sizes (base pairs), which were estimated from a 20-bp DNA ladder (TaKaRa, Tokyo, Japan).

(2) The M13-tailed primer method and automated collection method were carried out as described previously by Zhou et al. (2002) with the following modifications. PCR was performed in a reaction mixture (10 μl) containing 50 ng template DNA, 40 μM dNTPs, 0.3 pmol M13-tailed forward primer, 3.0 pmol reverse primer, 3.0 pmol fluorescently labeled M13-tailed primer (5´-6FAM or PET or NED or VIC-CCCAGTCACGACGTTGTA-3´), and 0.025 U of TaKaRa Taq polymerase (TaKaRa, Tokyo, Japan) under the following conditions: denaturation for 1 min at 94°C, 30-40 cycles of denaturation for 15 s at 94°C, annealing for 15 s at 56°C, and extension for 30 s at 72°C, followed by final extension for 1 h at 72°C. Typical combinations of markers for capillary electrophoresis were prepared by combining PCR products for markers having alleles with a difference of at least 100 bp in size and different fluorescent labels. One microliter of each PCR product was added to 10 μl of HiDi formamide and 0.1 μl of ROX standard (Gene Scan 500 LIZ Size standard, Applied Biosystems, Tokyo, Japan) for genotyping and electrophoresis on an ABI PRISM 3130

Genetic Analyzer (Applied Biosystems, Tokyo, Japan). The genescan output files were
analyzed using Gene Mapper 3.7 software (Applied Biosystems, Tokyo, Japan).

Microsatellite-centromere (M-C) mapping using triploids

5   Microsatellite genotypes were screened in all four-parent pairs used for
triploidization. The M-C mapping by half-tetrad analysis can be carried out only at the
locus for which the maternal genotype is heterozygous and the paternal alleles can be
distinguished from the maternal alleles. The M-C recombination rate (second meiotic
division segregation frequency = *y*) was estimated from the frequency of recombinant
10   heterozygotes in triploid progeny at the locus that is genetically heterozygous in the
mother eel. Assuming complete chiasma interference, the map distance in centimorgans
(cM) will equal 100 (*y*/2).

3. Results

Microsatellite marker

Fifty microsatellite loci were isolated from the size-selected genomic library comprising approximately 4,300 clones (positive clone percent = 1.16%). Also, 72 microsatellite loci were isolated from the MS-enriched library comprising approximately 450 clones (positive clone percent = 16.00%). These clones were sequenced, and 28 primer sets (*Ajp-1~28*) selected from the size-selected library and 50 primer sets (*Ajp-29~78*) selected from the MS-enriched library were designed to amplify specific microsatellite loci. The remaining loci were not used for reasons such as proximity to the cloning site, degenerate repetitive sequences in one of the flanking regions, extremely small or extremely large microsatellites, and/or an unreadable sequence of the repeat region. Of the 28 loci selected from the size-selected library of the 50 loci selected from the MS-enriched library, 8 and 35 loci, respectively, yielded reproducible PCR products that corresponded to a single locus of the expected size (see Appendix A). Furthermore, cross-amplifications were obtained using the 7 primer sets from *A. anguilla* and *A. rostrata*. New primers were designed for loci that produced a smear or nonspecific amplification and tested again for amplification under various PCR conditions; however, the results were unsatisfactory and these loci were not used for further analysis. Sequences of primers and annealing temperatures of 68 microsatellite loci that used in this study are listed in Appendix A.

Triploidization

The rates of triploidy ranged from 0% to 5% in the control groups. The triploidy rates in families 1 and 2, which were subjected to heat shock starting at 10 m.a.f., were

6.9% and 6.2%, respectively; and families 3 and 4, which were subjected to heat shock starting at 5, 7, and 10 m.a.f., had triploid rates of 35.8% and 30.5%, respectively.

Families and microsatellite loci screening for M-C mapping

Of the 68 loci screened for the half-tetrad analysis, 26 loci in four families were heterozygous in the female parent and the male parent had alleles that could be distinguished from the maternal alleles. Of these loci, 18 (*Ajp-33, 45, 48, 49, 55, 58, 70, 76, 77, AjTR-5, 12, 22, 23, 24, 42, 43, 44*, and *Ang075*) were in family 1, 7 (*Ajp-7, AjTR-5, 12, 15, 22, 23*, and *Ang114*) were family 2, 12 (*Ajp-33, 49, 55, 58, 76, 77, AjTR-12, 23, 24, 44, 48*, and *Ang101*) were in family 3, and 14 *(Ajp-7, 33, 45, 48, 49, 55, 70, AjTR-11, 15, 17, 22, 43, 44*, and *45*) were in family 4.

Mendelian segregation

Genotypes of the 25 of the 26 loci examined in one or two full-sib families (family 1 and/or 2) showed Mendelian segregation and are suitable as genetic markers (Table 1). The exception, *Ajp-45* in family 1, showed significant segregation distortion (Chi-square test, $P < 0.005$).

At some microsatellite loci, inconsistent genotype segregation was observed between parents and offspring in Family 1 and/or 2. For example, the observed parental genotypes of *AjTR-48* in Family 2 were *122/122* in females and *132/126* in males. Although, the genotype is frequencies of offspring would be expected to be equal for *122/132* and *122/126*, individuals with only one allele, either *132* or *126,* were observed. The genotypes observed in the offspring -- *122/132, 122/126, null/132,* and *null/126 --* can be explained in the female genotype was *122/null*. Similar distortions observed at 5 other loci; *AjTR-11* in Family 1, *AjTR-44* in Family 2, *Ang075* in Family 2, and *Ang101* in Families 1 and 2, can also be explained by the occurrence of a null allele.

Microsatellite-centromere recombination

The microsatellite-centromere recombination frequencies (second division

segregation frequencies = y) for the 26 loci in one, two, or three triploid families are

5 shown in Table 2. The frequency of y ranged from 0.008 at *Ajp-55* to 0.968 at *AjTR-12*.

The mean y value was 0.645 for the 26 loci and the value of y at *AjTR-12* was

approximately equal to one. This result indicates the presence of complete or near

complete chiasma interference in the eel. Because the distribution of y values were

uneven and biased toward a high value, we compared the distributions of y between two

10 groups of loci, *Ajp-33~70* (7 loci) and *AjTR-5~48* (13 loci), which developed by

different isolation procedures. The distribution of y differed significantly between

Ajp-33~70 obtained from an MS-enriched library (*Mse*I-digested inserts) and

AjTR-5~48 obtained from a size-selected library (300-600bp *Alu*I- and *Hae*III-digested

inserts)(Fig. 1, Mann-Whitney test, $P < 0.01$).

15 Two non-recombinant maternal homozygotes occurred at almost equal

frequencies. In most cases, the segregation of the paternal loci was in good agreement

with the expected Mendelian segregation ratio. However, five paternal loci, namely,

Ajp-45 in triploid family 1, *Ajp-49* in triploid family 3, *AjTR-22* in triploid family 4,

AjTR-23 in triploid family 2, and *AjTR-44* in triploid family 3 showed significant

20 segregation distortion ($P < 0.05$).

4. **Discussion**

Isolation efficiency of microsatellite loci

5 The percentage of clones containing dinucleotide repeats in the size-selected libraries was 1.16%. Previous studies of Japanese eel had success rates of 1.76% (Ishikawa et al., 2001) and 3.82% (Tseng et al., 2001). The mean frequency observed in 16 species of fish was 3.1% (Zane et al., 2002). In contrast, the percentage of positive clones in the MS-enriched libraries was 16.0%. The isolation frequency increased by approximately 10 fold as compared to that of the size-selected method; however, this

10 frequency was quite low as compared to that (50%-95%) reported by Zane et al. (2002). The MS-enriched method is more efficient than the conventional size-selected procedure for isolating microsatellite loci because the costs and times required to build libraries is similar.

15 *Inheritances of microsatellite loci*

Inheritances of the 26 microsatellite loci, which were mapped in relation to the centromere, were examined by using two full-sib families. All loci showed Mendelian inheritance except *Ajp-45*, which also had significant segregation distortion for both full-sib family 1 and triploid family 1, which originated in the same parents.

20 Segregation distortion was not observed at *Ajp-45* in the triploid family 4, which was the product of different parents. These results suggest that the segregation distortion observed at *Ajp-45* is not a locus-specific phenomenon but one that depends on the families involved. Possibly, *Ajp-45* is linked to the trait locus that affects survival of larvae. It is not likely that this segregation distortion generally affects the value of

25 *Ajp-45* as a genetic marker.

In the analysis of triploid families, significant distortion was observed only in the

segregation of paternally derived alleles in the five loci. We have not determined the reason behind this interesting phenomenon.

Null alleles in microsatellite loci

5

We inferred the presence of null alleles from analyses of the full-sib families. Null alleles at microsatellite loci were reviewed in detail by Dakin and Avise (2004). The null alleles detected in the present study are probably a result of "poor primer annealing due to nucleotide sequence divergence," which is one of the three factors

10 resulting in a null allele as classified by Dakin and Avise (2004). Such types of null alleles have also been reported in other fish species (Jones et al., 1998; Holm et al., 2001). These null alleles were confirmed by the fact that the allelic segregation law is contradicted in the full-sib families. If recognition of the existence of a null allele in the pedigree analysis is possible, such an allele can be treated as a null allele analogous to

15 the "*O*" allele of the human ABO blood group system. In this study, the allelic segregation at the loci in which null alleles were assumed, followed the Mendelian laws of inheritance, and segregation distortion was not observed. However, when these markers are used for population genetics studies, it is necessary to apply a model that considers the existence of null alleles in order to prevent bias caused by typing errors

20 due to the null alleles.

Distribution of y values and chiasma interference

In this study, the mean of *y* values at 26 microsatellite loci was 0.645, and the

25 distribution of *y* was uneven and biased toward a high value. Distributions of *y* biased toward a high value have also been reported at 34 microsatellite loci in the pink salmon

(*Oncorhynchus gorbuscha*), 101 anonymous DNA loci flanked by paired interspersed nuclear elements (PINEs) (Lidner et al., 2000), 37 allozyme loci (Matsuoka et al., 2004), and at 10 microsatellite loci in the zebrafish (*Danio rerio*) (Kauffman et al., 1995). In contrast, a relatively even distribution of *y* has been reported at 168 amplified fragment

5   length polymorphisms (AFLPs) in the pink salmon (Lidner et al., 2000), at 25 allozyme loci in the raibow trout (*Oncorhynchus mykiss*) (Allendorf et al., 1986), and at 15 microsatellite loci in the loach (*Misgurnus anguillicaudatus*) (Morishima et al., 2001).

The cause of the biased distribution of *y* toward the telomeric region observed in the present study may be related to: (1) distribution of the recombination spot along the

10   chromosome arm, (2) distribution of the isolated microsatellite loci in the genome, and (3) the relationship between the strength of the chiasma interference and the chromosome length.

Generally, the frequencies and distributions of the recombination are not random. Recombination decreases in the neighborhood of the telomeric region and centromeric

15   heterochromatin, while it does not occur in the centromeric heterochromatin (Hawley and Walker, 2003). Consequently, the distribution of *y* does not reflect the precise physical location of the marker loci, even if the distribution of marker loci is physically uniform.

It is likely that the bias of the genome fragments that are generated during the

20   isolation procedure would influence the distribution of *y* rather than the actual distribution of the loci in the whole genome. The composition of the cloned fragments is probably more related to the processes used for genome fragmentation than to the ligation into the cloning vector. The distribution of *y* of microsatellite was different between the two types of isolation procedures (Fig. 1). These differences may be

25   attributed to the differences in the recognition sites of the restriction enzymes used for the fragmentation of the genome. The recognition site for *Mse*I, which was used for the

isolation of *Ajp-33~70*, is TTAA and is biased toward A and T. On the other hand, the recognition sites for *Alu*I and *Hae*III, which were used for the isolation of *AjTR-5~48*, are AGCT and GGCC respectively, and are biased toward G and C. The recognition sites for the restriction enzyme are probably more abundant in the AT-rich region for *Mse*I and in the GC-rich region for *Alu*I and *Hae*III. Viñas et al. (1994) observed a difference in the G-band patterns of the chromosomes when *Mse*I and *Hae*III were used in the European eel (*Anguilla anguilla*). This result clearly suggests that the distributions of the recognition sites for these restriction enzymes may be different in the eel genome. Lidner et al. (2000) reported that in the pink salmon, the distribution of *y* at AFLP loci was different from the microsatellite loci and PINEs. Their results suggest that the distribution of *y* among different types of markers may depend on the restriction enzyme used. Hence, the choice of procedure used for isolating microsatellite loci is important. If uniformly distributed markers are to be produced, the genome should be randomly cut using a sonication technique, etc.

In meiosis, a recombination event interferes with additional; recombination in the adjacent area of the same chromosome. Chiasma interference normally decreases with increased distance and finally disappears. However, when an extremely strong chiasma interference exists generally within on organism or on a specific segment of a chromosome, recombination may occur only once per bivalent chromosome. In this study, the *y* values of several loci exceeded 0.667 and the maximum *y* value was 0.968. From these results, we can conclude: (1) there is a strong chiasma interference and (2) recombination does not occur between sister chromosomes or the chiasma interference does not interfere with the recombination between the homologous chromosomes even if recombination between sister chromosomes occurs.

The value of *y* exceeding 0.667 has also been reported in other fish species (Thorgaard et al., 1983; Streisinger et al., 1986; Arai et al., 1991; Kauffman et al., 1995;

Lindner et al., 2000; Morishima et al., 2001; Matsuoka et al., 2004); and this phenomenon appears to be common in fishes. Here, the *y* value is estimated under the following conditions: (1) the existence of strong chiasma interference, (2) a uniform physical distribution of markers, and (3) an even distribution of recombination spots on

5   a chromosome. The *y* value linearly increases with the G-C distance near the centromeric region and exceeds 0.667 due to the influence of the chiasma interference. Subsequently, the *y* value stabilizes at 0.667. Therefore, the distribution of *y* is biased toward the telomeric region.

10   *Perspective of aquaculture genetics in the eel*

       In this study, by using four induced triploid families, we mapped 26 microsatellite loci in relation to the centromere of the eel chromosomes. The loci included ones that we developed as well as those reported by other researchers. In the future, we intend to

15   prepare a high-resolution linkage map with the location information of the centromere by using these microsatellite loci. Such integration of a gene-centromere map and linkage map will satisfy an essential requisite not only in elucidating syntenies among different species but also in identifying commercially important quantitative traits in the aquaculture species. To apply marker-assisted selection (MAS) to improvement of eel

20   strains genetically for aquaculture, further genetic mapping must be conducted and new chromosome manipulation techniques must be developed.

Acknowledgements

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Figure caption

Figure 1. Relationship between isolation procedure of microsatellite loci and

5   distribution of second division segregation frequency (y) of each microsatellite loci.

Distribution of y were significantly different between *Ajp-33~70* originated from a

MS-enriched library (*Mse*I-digested inserts) and *AjTR-5~48* originated from a

size-selected library (300-600 bp *Alu*I-, *Hae*III-digested inserts) (Mann-Whitney test, *P*

< 0.01). Bar (-) indicates the mean of y value.

10

Table 1.
The genotypic segregation in 26 microsatellite loci in two full-sib families of the Japanese eel.

Locus	Family	Female	Male	Progeny Observed (Expected)				Total	d.f.	χ^2
Ajp-7	1	146/140	140/140	146/140 20 (19)	140/140 18 (19)			38	1	0.105
	2	146/140	152/148	146/152 8 (9.25)	146/148 8 (9.25)	140/152 11 (9.25)	140/148 11 (9.25)	37	3	1.000
Ajp-33	1	135/131	127/119	135/127 11(10.75)	135/119 15(10.75)	131/127 5(10.75)	131/119 12(10.75)	43	3	4.907
Ajp-45	1	163/157	184/142	163/184 3(11)	163/142 10(11)	157/184 11(11)	157/142 20(11)	44	3	13.273**
Ajp-48	1	198/183	181/141	198/181 8(10.25)	198/141 7(10.25)	183/181 16(10.25)	183/141 10(10.25)	41	3	4.756
Ajp-49	1	226/222	216/212	226/216 12(10.75)	226/212 12(10.75)	222/216 9(10.75)	222/212 10(10.75)	43	3	0.673
Ajp-55	1	160/158	156/156	160/156 16(22)	158/156 28(22)			44	1	3.273
Ajp-58	1	148/138	144/142	148/144 12(10.75)	148/142 12(10.75)	138/144 7(10.75)	138/142 12(10.75)	43	1	1.744
Ajp-70	1	114/106	132/108	114/132 11(9.75)	114/108 7(9.75)	106/132 9(9.75)	106/108 12(9.75)	39	3	1.513
Ajp-76	1	154/146	148/144	154/148 10(8.5)	154/144 6(8.5)	146/148 13(8.5)	146/144 5(8.5)	34	3	4.824
Ajp-77	1	186/172	180/162	186/180 7(9.75)	186/162 9(9.75)	172/180 12(9.75)	172/162 11(9.75)	39	3	1.513
AjTR-5	1	194/170	189/175	194/189 13 (9.5)	194/175 10 (9.5)	170/189 9 (9.5)	170/175 6 (9.5)	38	3	2.632
	2	198/178	194/194	198/194 18 (19)	178/194 20 (19)			38	1	0.105
AjTR-11	1	109/null	109/105	109/109 or 109/null 21 (18.5)	109/105 12 (9.25)	null/105 4 (9.25)		37	2	4.135
	2	117/89	117/101	117/117 16 (9.5)	117/101 6 (9.5)	89/117 8 (9.5)	89/101 8 (9.5)	38	3	6.211
AjTR-12	1	137/133	175/139	137/175 16 (9.25)	137/139 6 (9.25)	133/175 6 (9.25)	133/139 9 (9.25)	37	3	7.216
	2	145/143	163/133	145/163 7 (9.5)	145/133 11 (9.5)	143/163 10 (9.5)	143/133 10 (9.5)	38	3	0.947
AjTR-15	1	145/127	159/null	145/159 8 (9.5)	145/null 11 (9.5)	127/159 7 (9.5)	127/null 12 (9.5)	38	3	1.789
	2	177/155	133/133	177/133 22 (19)	155/133 16 (19)			38	1	0.947
AjTR-17	1	89/67	89/85	89/89 8 (9.25)	89/85 4 (9.25)	67/89 15 (9.25)	67/85 10 (9.25)	37	3	6.784
	2	95/91	91/97	95/91 10 (9.5)	95/97 9 (9.5)	91/91 7 (9.5)	91/97 12 (9.5)	38	3	1.368
AjTR-22	1	102/94	98/88	102/98 12 (9.5)	102/88 9 (9.5)	94/98 6 (9.5)	94/88 11 (9.5)	38	3	2.211
	2	100/98	108/92	100/108 10 (9.5)	100/92 10 (9.5)	98/108 4 (9.5)	98/92 14 (9.5)	38	3	5.368
AjTR-23	1	121/105	101/101	121/101 18 (18.5)	105/101 19 (18.5)			37	1	0.027
	2	113/115	127/107	113/127 11 (9.5)	113/107 10 (9.5)	115/127 10 (9.5)	115/107 7 (9.5)	38	3	0.947
AjTR-24	1	213/171	142/124	213/142 12 (9.5)	213/124 13 (9.5)	171/142 5 (9.5)	171/124 8 (9.5)	38	3	4.316
	2	181/133	133/139	181/133 7 (9.25)	181/139 12 (9.25)	133/133 11 (9.25)	133/139 7 (9.25)	37	3	2.243
AjTR-42	1	133/125	135/129	133/135 7 (9.5)	133/129 12 (9.5)	125/135 7 (9.5)	125/129 12 (9.5)	38	3	2.632
	2	109/107	107/105	109/107 8 (9.5)	109/105 11 (9.5)	107/107 9 (9.5)	107/105 10 (9.5)	38	3	0.526
AjTR-43	1	182/166	178/172	182/178 8 (9.25)	182/172 10 (9.25)	166/178 7 (9.25)	166/172 12 (9.25)	37	3	1.595
	2	188/182	182/182	188/182 18 (18)	182/182 18 (18)			36	1	0.000
AjTR-44	1	122/106	154/138	122/154 11 (9)	122/138 11 (9)	106/154 7 (9)	106/138 7 (9)	36	3	1.778
	2	114/null	150/106	114/150 11 (9.25)	114/106 6 (9.25)	null/150 11 (9.25)	null/106 9 (9.25)	37	3	1.811
AjTR-45	1	137/131	131/135	137/131 10 (9.5)	137/135 11 (9.5)	131/131 9 (9.5)	131/135 8 (9.5)	38	3	0.526
AjTR-48	1	134/114	134/114	134/134 13 (9.25)	134/114 13 (18.5)	114/114 11 (9.25)		37	2	3.486
	2	122/null	132/126	122/132 13 (9.5)	122/126 4 (9.5)	null/132 10 (9.5)	null/126 11 (9.5)	38	3	4.737
Ang075	1	149/111	117/105	149/117 11 (9.25)	149/105 11 (9.25)	111/117 5 (9.25)	111/105 10 (9.25)	37	3	2.676
	2	133/null	125/117	133/125 13 (9.5)	133/117 11 (9.5)	null/125 9 (9.5)	null/117 5 (9.5)	38	3	3.684
Ang101	1	141/null	167/141	141/167 10 (9.5)	141/141 or null/141 14 (19)	null/167 14 (9.5)		38	2	3.474
	2	147/139	147/null	147/147 or 147/null 19 (19)	147/139 11 (9.5)	139/null 8 (9.5)		38	2	0.474
Ang114	1	111/107	115/103	111/115 12 (9.5)	111/103 9 (9.5)	107/115 8 (9.5)	107/103 9 (9.5)	38	3	0.947
	2	110/98	116/108	110/116 12 (9.25)	110/108 6 (9.25)	98/116 11 (9.25)	98/108 8 (9.25)	37	3	2.459

** $P < 0.005$

Table 2.
Microsatellite-centromere recombination frequencies (*y*) and map distances (cM) of 26 microsatellite loci examined in four triploid families.

| Locus | Family | Parental genotype (bp) | | Genotypes of triploid larvae | | | | | | Total | Recombination frequency | Microsatellite-Centromere | χ^2 | |
		Female (a/b)	Male (c/d)	a/b/c	a/b/d	a/a/c	a/a/d	b/b/c	b/b/d		(y)	distance (cM)	a/a:b/b= 1:1	c:d =1:1
Ajp-7	2	146/140	152/158	7	9	1	1	1	2	21	0.762	38.1	0.200	0.429
	4	148/142	154/144	20	20	1	3	2	1	47	0.894	44.7	0.143	0.021
											0.828	**41.4**		
Ajp-33	1	135/131	127/119	2	0	5	8	9	5	29	0.069	3.4	0.037	0.310
	3	133/131	127/119	3	2	5	9	8	8	35	0.143	7.1	0.133	0.257
	4	129/125	127/119	0	1	6	9	15	12	43	0.023	1.2	3.429	0.023
											0.078	**3.9**		
Ajp-45	1	163/157	184/142	1	4	1	7	3	6	22	0.227	11.4	0.059	6.545⁻
	4	170/142	188/148	3	1	5	13	11	10	43	0.093	4.7	0.231	0.581
											0.160	**8.0**		
Ajp-48	1	198/183	181/141	7	9	1	0	1	2	20	0.800	40.0	1.000	0.200
	4	194/183	185/185	32	-	4	-	4	-	40	0.800	40.0	0.000	-
											0.800	**40.0**		
Ajp-49	1	226/222	216/212	4	5	5	3	3	5	25	0.360	18.0	0.000	0.040
	3	231/218	224/211	4	9	6	8	2	8	37	0.351	17.6	0.667	4.568⁻
	4	218/206	224/211	5	10	9	3	9	7	43	0.349	17.4	0.571	0.209
											0.353	**17.7**		
Ajp-55	1	160/158	156/156	0	-	11	-	11	-	22	0.000	0.0	0.000	-
	3	165/158	167/149	0	1	10	10	13	6	40	0.025	1.3	0.026	0.900
	4	175/165	167/149	0	0	12	11	13	8	44	0.000	0.0	0.091	0.818
											0.008	**0.4**		
Ajp-58	1	148/138	144/142	15	5	1	4	2	2	29	0.690	34.5	0.111	1.690
	3	158/131	148/144	7	13	7	2	6	5	40	0.500	25.0	0.200	0.000
											0.595	**29.7**		
Ajp-70	1	114/106	132/108	3	1	5	5	6	7	27	0.148	7.4	0.391	0.037
	4	122/102	112/100	2	3	7	7	10	7	36	0.139	6.9	0.290	0.111
											0.144	**7.2**		
Ajp-76	1	154/146	148/144	6	3	3	3	3	2	20	0.450	22.5	0.091	0.800
	3	158/148	146/144	1	2	4	1	4	3	15	0.200	10.0	0.333	0.600
											0.325	**16.3**		
Ajp-77	1	186/172	180/162	10	11	1	2	0	1	25	0.840	42.0	1.000	0.360
	3	180/176	186/182	18	12	0	1	0	0	31	0.968	48.4	1.000	0.806
											0.904	**45.2**		
AjTR-5	1	178/160	174/164	14	10	2	4	0	3	33	0.727	36.4	1.000	0.030
	2	203/177	193/193	17	-	2	-	2	-	21	0.810	40.5	0.000	-
											0.768	**38.4**		
AjTR-11	4	119/111	103/100	5	13	0	0	2	1	21	**0.857**	**42.9**	3.000	2.333
AjTR-12	1	137/133	175/139	10	22	2	0	0	0	34	0.939	47.0	2.000	2.941
	2	145/141	163/133	7	14	0	0	0	0	21	1.000	50.0	-	2.333
	3	162/148	176/140	27	26	1	0	0	1	55	0.964	48.2	0.000	0.018
											0.968	**48.4**		
AjTR-15	2	177/155	133/133	18	0	1	0	1	0	20	0.900	45.0	0.000	-
	4	136/126	147/133	10	12	9	3	5	3	42	0.524	26.2	0.800	0.857
											0.712	**35.6**		
AjTR-17	4	95/93	86/84	27	18	0	0	2	1	48	**0.938**	**46.9**	3.000	2.083
AjTR-22	1	102/94	98/88	18	13	1	1	1	0	34	0.912	45.6	0.333	1.059
	2	101/93	110/98	8	8	2	0	1	2	21	0.762	38.1	0.200	0.048
	4	108/106	104/100	13	21	2	4	0	7	47	0.723	36.2	0.077	6.149⁻
											0.799	**40.0**		
AjTR-23	1	125/109	107/107	34	-	0	-	0	-	34	1.000	50.0	0.000	-
	2	113/107	129/115	14	3	3	0	1	0	21	0.810	40.5	1.000	10.714⁻⁻
	3	102/98	112/112	51	-	1	-	2	-	54	0.944	47.2	-	-
											0.918	**45.9**		
AjTR-24	1	202/162	136/120	11	13	2	3	0	4	33	0.706	35.3	0.111	1.485
	3	173/131	203/127	24	19	2	4	2	3	54	0.796	39.8	0.091	0.074
											0.751	**37.5**		
AjTR-42	1	110/104	114/106	15	16	1	2	1	0	35	**0.886**	**44.3**	1.000	0.029
AjTR-43	1	182/166	178/172	16	16	0	0	0	2	34	0.941	47.1	2.000	0.118
	4	221/181	193/183	19	19	3	2	0	4	47	0.826	41.3	0.111	0.191
											0.884	**44.2**		
AjTR-44	1	120/106	148/136	16	16	2	0	0	0	34	0.941	47.1	2.000	0.118
	3	125/103	115/101	14	27	1	5	4	3	54	0.759	38.0	0.077	4.741⁻
	4	125/95	113/99	21	20	2	3	2	0	48	0.854	42.7	1.286	0.083
											0.851	**42.6**		
AjTR-45	4	131/121	144/138	9	4	10	11	6	6	46	**0.283**	**14.1**	2.455	0.348
AjTR-48	3	134/130	140/126	18	25	3	1	2	6	55	**0.782**	**39.1**	1.333	1.473
Ang075	1	150/122	127/113	14	10	1	2	4	2	33	**0.727**	**36.4**	1.000	0.758
Ang101	3	151/143	149/133	26	17	4	3	3	2	55	**0.782**	**39.1**	0.333	2.200
Ang114	2	122/100	126/108	8	6	0	1	5	1	21	**0.667**	**33.3**	3.571	1.190
										Mean	**0.645**	**32.2**		

* *P* < 0.05, **P < 0.005
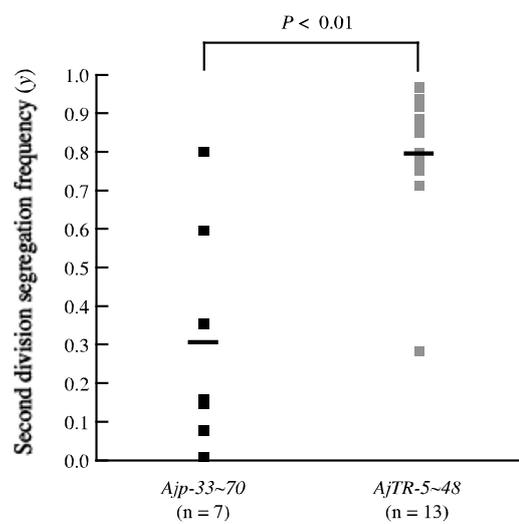
Fig. 1. Nomura et al.

Appendix A.
Microsatellite loci developed in Anguilla species, showing core sequences, sequences of primer sets for amplification, annealing temperatures, references, accession numbers in DDBJ, restriction enzyme for fragmentation, type of genomic library for isolation, and the manner of genotyping.

Locus	Species	Primer sets Forward (5'-3') Reverse (5'-3')	Core sequence	Annealing temperature (°C)	Reference	DDBJ accession number	Isolation procedure Fragmentation	Library	Genotyping
Ajp-1	A.Japonica	AATCCATACACATGTACACATG TAAACAGTGACAGATAACTAGG	$(CA)_6AA(CA)_8$	60	Present study	AB194929	HaeIII	Size-selected	Nomal PCR and PAGE
Ajp-4	A.Japonica	ACACGGATACTGTTTTCCACAA ATTCCACCACAGTTGTGCAT	$(GT)_{20}$	52	Present study	AB194930	HaeIII	Size-selected	Nomal PCR and PAGE
Ajp-5	A.Japonica	GACCCTAAGGGACCTGTTTT CAAGCCGAAGCCTCTGAACG	$(CA)_4(CG)_2$	60	Present study	AB194931	HaeIII	Size-selected	Nomal PCR and PAGE
Ajp-7	A.Japonica	TCCTGAACCGAATGCTCTAT ATATTCACACCCTGCCGAAT	$(GT)_7GA(GT)_4C(TG)_{10}$	60	Present study	AB194932	HaeIII	Size-selected	Nomal PCR and PAGE
Ajp-11	A.Japonica	GCACTGAATACACGCACAAT ATTAGGATCTGATCCTGCTG	$(CA)_7$	55	Present study	AB194933	HaeIII	Size-selected	Nomal PCR and PAGE
Ajp-15	A.Japonica	CCTCGGTCAATTCTTAACAGTA GCACATTGATAGGCATCAGA	$(CA)_5$	55	Present study	AB194934	HaeIII	Size-selected	Nomal PCR and PAGE
Ajp-16	A.Japonica	GCGAGACACCAACAGAGAAA ATGACGAAGCTGAAGTCTGG	$(GA)_4GG(GA)_{12}$	52	Present study	AB194935	HaeIII	Size-selected	Nomal PCR and PAGE
Ajp-22	A.Japonica	TTCCCTCTCCACACCTCTGT CTCACCCCCAGATTCCCATC	$(TG)_{25}$	50	Present study	AB194936	HaeIII	Size-selected	Nomal PCR and PAGE
Ajp-30	A.Japonica	AGTCACGACGTTGTATGTGCTTATGAATGCTGCCA TGATGATGCAAAATGCAGCC	$(GT)_5TT(GT)_8$	56	Present study	AB233944	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-32	A.Japonica	AGTCACGACGTTGTATGACAATATACGTTGGGATGC AATGTGTGTATGCGTGTGCGT	$(AC)_5A_5(AC)_{19}$	56	Present study	AB233945	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-33	A.Japonica	AGTCACGACGTTGTAACTTCCTTCTATGGTGAGCGT TGGCATTGTTCACACGATTG	$(CA)_{10}$	56	Present study	AB233946	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-34	A.Japonica	AGTCACGACGTTGTACCTTTCCTAGTGGGACAAACA CCAGTGCGAGACACACAAAGA	$(CT)_7(GTCT)_3$	56	Present study	AB233947	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-35	A.Japonica	AGTCACGACGTTGTACATGCACAGAGTCTTACATGCA CACATTTTCTGAGTTTAGGTG	$(CA)_{36}GA(CA)_9$	56	Present study	AB233948	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-38	A.Japonica	AGTCACGACGTTGTACCTAAACACACACACACAAAGA TGTAGCGCGAATAAAAACGGT	$(CA)_5CCTG(CA)_8$	56	Present study	AB233949	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-39	A.Japonica	AGTCACGACGTTGTAGCATGGCTCATAGACAGGTCA CCCGATCTGAATCCAATTGA	$(AC)_7AT(AC)_{11}$	56	Present study	AB233950	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-41	A.Japonica	AGTCACGACGTTGTAGCCTGCTGATACACATAACGA TGGGAACATATAGCGTGTGTG	$(AC)_{13}$	56	Present study	AB233951	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-42	A.Japonica	AGTCACGACGTTGTATGGCATTCATTCTGAAAGCAC GGATCTTCAGATTGCGTATATA	$(CA)_{16}$	56	Present study	AB233952	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-43	A.Japonica	AGTCACGACGTTGTAGCTTCATGGCTTTATGGAGA AGCGAAGACAAGCACAACAA	$(GT)_6(GCGT)_2(GT)_5$	56	Present study	AB233953	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-44	A.Japonica	AGTCACGACGTTGTAAAGGGAGGCATAGAGCTTTCA ATGAAGTGTGTGTGTCTCAGGT	$(CA)_9$	56	Present study	AB233954	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-45	A.Japonica	AGTCACGACGTTGTAAATGAGTCCCTGTTTGGCTCT TGAAGCAGCTCCAAGTGTTT	$(AC)_4CT(CA)_{15}(CA)_6$	56	Present study	AB233955	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-47	A.Japonica	AGTCACGACGTTGTATTGTTTAGCTTCATGGGCTGG CCGACTCATTTTGCGTGTAT	$(CA)_8CG(CA)_8$	56	Present study	AB233956	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-48	A.Japonica	AGTCACGACGTTGTATGGAAGGGCAGACAAGTAGAGA TGACCTGAGACAGCATAAGTG	$(AC)_4GC(AC)_6$	56	Present study	AB233957	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-49	A.Japonica	AGTCACGACGTTGTAATTCAGCATTAGGGGTGTGG ATTTCAGGCGGTATTCAGGA	$(GT)_{11}$	56	Present study	AB233958	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-50	A.Japonica	AGTCACGACGTTGTATTCCTCAGTCACAGACAGGGA ATGACCGCTGAAAAGGACAGT	$(GT)_6GAGTGTGA(GT)_9$	56	Present study	AB233959	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-51	A.Japonica	AGTCACGACGTTGTATGCATGTGTGTTTGAGTGCA TGTAGCGATCGGTCAATCAA	$(GT)_{14}$	56	Present study	AB233960	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-52	A.Japonica	AGTCACGACGTTGTACCTTTGGTTTTTCATTCACA TGGAACCTGAATTGTTGGGA	$(CA)_{11}$	56	Present study	AB233961	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-53	A.Japonica	AGTCACGACGTTGTATGATTTACGTGCGTGCTTG AGAATGAGAGGGGAGGACAGA	$(GT)_6GC(GT)_6GC(GC)_3$	56	Present study	AB233962	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-54	A.Japonica	AGTCACGACGTTGTACCTCACACATTCTGAATGGAA TGCATGTGTATGTCTGTGGAA	$(CA)_4CG(CA)_{20}$	56	Present study	AB233963	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-55	A.Japonica	AGTCACGACGTTGTATGGAGCACCTCAACTGTTATT ATTTGTGAGCAAGGCTTGCA	$(GT)_{16}$	56	Present study	AB233964	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-56	A.Japonica	AGTCACGACGTTGTACAACCCTAAGCTTACAGTCAATTT CCTGATGTGTATGCATGATGTG	$(AT)_8AC(AT)_8(AC)_{17}$	56	Present study	AB233965	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-57	A.Japonica	AGTCACGACGTTGTATCCTCCCAGTTCTTTCATCTT TGAAATGCTGAGCAGTGTGTG	$(CA)_{14}$	56	Present study	AB233966	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-58	A.Japonica	AGTCACGACGTTGTAAAAACCGGATTTGCTAGCCA GGCAGCGTTCTAAGAATCAA	$(CA)_{17}$	56	Present study	AB233967	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-59	A.Japonica	AGTCACGACGTTGTATTTCCCTGAATCCCTTCTGA CGCATGTGTGGGTGCTTACA	$(CA)_{18}CGCA(CG)_2(CA)_8$	56	Present study	AB233968	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-61	A.Japonica	AGTCACGACGTTGTAAGAGGGATTTTTACACTGCCA ATGCTTGATTTGAGGTAGCCT	$(CA)_{16}$	56	Present study	AB233969	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-63	A.Japonica	AGTCACGACGTTGTATTGAGCACCTGCACACACCTA ATGCAGCATGCGTATACACA	$(AC)_{11}GT(AC)_7$	56	Present study	AB233970	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-66	A.Japonica	AGTCACGACGTTGTATGCACACACAATCTCCTTCTC TCAGCAGAAGGGACACTGCA	$(CA)_{14}CG(CA)_3$	56	Present study	AB233971	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-67	A.Japonica	AGTCACGACGTTGTATATTGGGAAAGAGACGCAGA TTCAAAGCTCTATGCCAACG	$(GT)_5CG(GT)_8$	56	Present study	AB233972	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-68	A.Japonica	AGTCACGACGTTGTAATTCTGCTGCATTAGCCGTGT ACTTCCTGCACAGACTCAGCG	$(GT)_{14}$	56	Present study	AB233973	MseI	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-69	A.Japonica	AGTCACGACGTTGTATTTTTCGAGCAGGGACACAA TGAAGGCGGGGAATGTCT	$(CA)_6G(CA)_5$	56	Present study	AB233974	MseI	MS-enrichment	Tailed-primer PCR and automated collection

Appendix A.

Microsatellite loci developed in Anguilla species, showing core sequences, sequences of primer sets for amplification, annealing temperatures, references, accession numbers in DDBJ, restriction enzyme for fragmentation, type of genomic library for isola

Locus	Species	Primer sets Forward (5'-3') Reverse (5'-3')	Core sequence	Annealing temperature (°C)	Reference	DDBJ accession number	Isolation procedure Fragmentation	Library	Genotyping
Ajp-70	A.Japonica	AGTCACGACGTTGTATACACGTGTGCTCACACAGG TCTCTGGGCAAGCTCAACAA	$(CA)_{14}$	56	Present study	AB233975	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-76	A.Japonica	AGTCACGACGTTGTAAGCGGGCGTTCACACTTCTG GTCCGGTCTTCATGCACACCT	$(GT)_{10}$	56	Present study	AB233976	*Hae*III	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-77	A.Japonica	AGTCACGACGTTGTACCAGGCTGATCAAAGATGG GTCCAGTACAACACAGTCAG	$(CA)_{10}$	56	Present study	AB233977	*Hae*III	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-78	A.Japonica	AGTCACGACGTTGTATCAAGCTCACCAGTTTGGACT ACCTCCCAAGGACAAGAATTT	$(CA)_{11}$	56	Present study	AB233978	*Hae*III	MS-enrichment	Tailed-primer PCR and automated collection
AjTR-5	A.Japonica	GGAGCAGTATGGAATAACATGA GTATTTACATAGGGGATGACCA	$(CA)_{18}TA(CA)_{5}$	60	Isikawa *et al.* (2001)	AB051082	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-11	A.Japonica	GGAAGTTGCTGACTTTTAGA GGCAGTTCTATGTGTGCTTA	$(CTCA)_{11}$	55	Isikawa *et al.* (2001)	AB051083	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-12	A.Japonica	AACGTTAGTCCCTAGGTTCC TATATGTTCAGTGGAGGGAG	$(GA)_{13}$	55	Isikawa *et al.* (2001)	AB051084	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-15	A.Japonica	GCCATATGATCGAACAGATG CGGATGGGGAAAAACTTCAT	$(AC)_{5}GTG(CA)_{12}AA(AC)_{7}$	55	Isikawa *et al.* (2001)	AB051085	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-17	A.Japonica	CAGTTATGCACTCACGCTAA AGGTATGTTGGAGAGTAAGG	$(CA)_{10}$	55	Isikawa *et al.* (2001)	AB051086	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-22	A.Japonica	CTCAACTTCACCGGCTTTTC TTACCTGTCATTCCAATGGA	$(TC)_{7}(AC)_{15}$	52	Isikawa *et al.* (2001)	AB051087	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-23	A.Japonica	GCAGCATCGGTGTATCTTTC AGTGTTCAGTTGGCTACTAC	$(TC)_{11}(AC)_{11}$	60	Isikawa *et al.* (2001)	AB051088	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-24	A.Japonica	CAACATACACCAATACCAGC GATCCCTCTGAATGATATGG	$(AC)_{13}$	60	Isikawa *et al.* (2001)	AB051089	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-25	A.Japonica	CGAGTGAGAACAAACACCAA TGTAACATAAAGGTGACCGG	$(CA)_{7}AAGG(CA)_{9}$	55	Isikawa *et al.* (2001)	AB051090	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-26	A.Japonica	TCTTTGTGAAGTGTGCATGA TTGTGAGGACAAATGATGGC	$(CA)_{5}CC(CA)_{15}$	55	Isikawa *et al.* (2001)	AB051091	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-27	A.Japonica	CGCCTGAATTCCAACTCTTG GGTCCTTTGGCATTCTTACG	$(TC)_{6}(CT)_{3}$	60	Isikawa *et al.* (2001)	AB051092	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-37	A.Japonica	CTAGATTTACTGCTCAGGGA AAATTCAATTGTGCCCTCCG	$(TG)_{14}$	60	Isikawa *et al.* (2001)	AB051094	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-42	A.Japonica	TGAAGCAGAACTGTCATGCTAT TATAAACTAGCCTACTAACTGT	$(TG)_{12}$	55	Isikawa *et al.* (2001)	AB051097	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-43	A.Japonica	CCAGGAATAAGCATCTGACACA CCGGATCCTGTGGTGTATTG	$(TTTA)_{10}$	55	Isikawa *et al.* (2001)	AB051098	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-44	A.Japonica	TGACTGATTGAAGTGGTATGTG GCTTATTCCTGGGAAGATCT	$(CT)_{14}$	55	Isikawa *et al.* (2001)	AB051099	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-45	A.Japonica	ACTCTAATGGGAGCCACATT TAGAGTGAGGACAGTAGAGG	$(CT)_{7}T(TC)_{2}C(CT)_{6}$	55	Isikawa *et al.* (2001)	AB051100	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-48	A.Japonica	ATTATAACCCCTGCATGGCA GAAGATAAGACCTGGTCGCA	$(TG)_{7}TTA(GT)_{6}$	60	Isikawa *et al.* (2001)	AB051101	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AJMS-3	A.Japonica	GGTATGAATGCAGGCGTTTATG CTGGAGATCAAATCGGTTGC	$(GT)_{10}$	52	Tseng *et al.* (2001)	AJ297601	*Alu*I, *Hae*III, and *Rsa*I	Size-selected	Nomal PCR and PAGE
Aan05	A. Anguilla	GCTTGTATGCATATGTATGTTCATGC CTAGTAGACTGCTTCAGGCCATGCTG	$(TG)_{18}$	63	Daemen *et al.* (1997)	AY028638	*Hae*III, *Pvu*II and *Eco*RV	Size-selected	Nomal PCR and PAGE
Ang101	A. Anguilla	ACAATCGGGTACCACAGTAA ATGGAGCCACTCAATGAAGA	$(CA)_{17}$	60	Wirth and Bernatchez (2001)	AF237900	No description	No description	Nomal PCR and PAGE
Ang151	A. Anguilla	GATGTTGGTTTGGTCTGTCG TAGCATGCCTAGAACTGGAC	$(TG)_{16}$	60	Wirth and Bernatchez (2001)	AF237901	No description	No description	Nomal PCR and PAGE
Ang114	A. Anguilla	TCCTGTGAATCCAACAGGTG CTGGAGCAGTCACACCAATT	$(CT)_{27}$	60	Wirth and Bernatchez (2001)	AF237902	No description	No description	Nomal PCR and PAGE
Ang075	A. Anguilla	TATCAGGAACTCGATACGCC ACGCATCCACCAGCCCTTGC	$(GA)_{15}$	60	Wirth and Bernatchez (2001)	AF237903	No description	No description	Nomal PCR and PAGE
Aro054	A. Rostrata	CTCAACTCCAGCACACTGGA ACAAAATAGCTCCGTAACAC	$(CA)_{14}$	64	Wirth and Bernatchez (2001)	AF237896	No description	No description	Nomal PCR and PAGE
Aro063	A. Rostrata	CAGATACCTTGACAACGGC TCAAGAGCTTCCTGACCCTC	$(GA)_{12}$	62	Wirth and Bernatchez (2001)	AF237899	No description	No description	Nomal PCR and PAGE