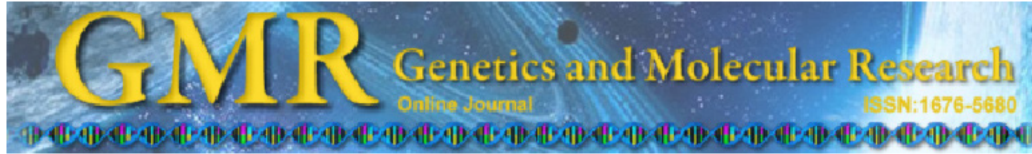

Short Communication

Universal primers to amplify the complete mitochondrial 12S rRNA gene in marine fish species

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ABSTRACT. A pair of new universal 12S mitochondrial rRNA gene primers was designed through multiple alignment analysis of the mitochondrial $tRNA^{Phe}$ and the 5’ region of 16S mitochondrial rRNA genes of different kinds of fishes. The primers were successfully used to amplify an expected product fragment of about 1.2 kb from various marine fish species, and the amplified DNA fragment was recognized to contain the complete 12S mitochondrial rRNA and $tRNA^{Val}$ genes, as well as a partial 16S mitochondrial rRNA gene of about 146 bp in length. The primers would facilitate the study of the species discrimination, population and evolution in marine fish species.

Key words: Mitochondrial DNA; 12S rRNA gene; Universal primers; Marine fish species

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Marine fishes, which include approximately 15000 species (FishBase: www.fishbase.org), are a group of dominant vertebrate species on our planet. In recent years, the diminishing fishery resources have aroused much interest in using genetic marker tools, such as allozyme analysis, restriction fragment length polymorphism, random amplified polymorphic DNA, microsatellites, mitochondrial DNA, and single nucleotide polymorphism to help establish conservation policies that can protect the local adapted fish stocks by regulating fishing activities (Cheng et al., 2012). Among these marker tools, mitochondrial DNA has been widely used because of its compact size, nearly complete maternal inheritance and fast evolutionary rate (Brown et al., 1979; Wilson et al., 1985). The 12S mitochondrial rRNA gene, located between the $tRNA^{Phe}$ and $tRNA^{Val}$ genes, is relatively conserved, evolving more slowly than the mitochondrial genome as a whole (Palumbi, 1996; Di Finizio et al., 2007). Moreover, the mutations that exist in 12S mitochondrial rRNA gene were reported to make both genes suitable for species discrimination and evolutionary and population studies of marine fishes (Balitzki-Korte et al., 2005; Cheng et al., 2005; Ren and Zhang, 2007; Cawthorn et al., 2012).

It is necessary to design universal primers to attain the complete 12S mitochondrial rRNA gene of marine fishes rapidly and on a large-scale. Although some existing universal primers of 12S mitochondrial rRNA have been used to effectively amplify 12S mitochondrial rRNA from mammals and some vertebrates effectively (Springer et al., 1995; Wang et al., 2000; Kitano et al., 2007), the amplification scope of these existent primers is relatively limited in marine fishes because of the differences in the used template gene that was used to design the universal primers. Therefore, a pair of universal 12S mitochondrial rRNA gene primers was designed based on the gene sequences of different kinds of marine fishes. It was found that the $tRNA^{Phe}$ and the 5' region of the 16S mitochondrial rRNA genes were highly conserved by aligning different kinds of fishes from GenBank. Then, a pair of primers was then designed using the conserved sequence regions. Primer informations is shown in Table 1.

Table 1. Primers used in this study.

Primer name	Sequence
Marinefish-12SrRNA-F	ACTAAAGCATAACACTGAAGAT
Marinefish-12SrRNA-R	TTCATTTCTCTTTCAGCTTTCC

To confirm the usability and robustness of the universal 12S mitochondrial rRNA gene primers, we performed polymerase chain reaction (PCR) on DNA from various samples (Table 2). DNA was extracted from the muscle of 47 fish samples using the standard phenol-chloroform procedure (Sambrook and Russell, 2001). The PCR mixture (25 µL) consisted of 1 µL of each primer, 2.5 µL of 10X *Taq* Plus polymerase buffer, 2 µL dNTPs (2.5 µM), 0.2 µL *Taq* Plus DNA polymerase (5U/µL) with proof-reading characteristic (TIANGEN), 1 µL DNA template, and 17.3 µL ddH_2O. PCR was performed on a BIO-RAD S1000. The conditions of the PCR were as follows: pre-denaturation at 95°C for 5 min; 35 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 75 s; and final extension at 72°C for 5 min. The PCR products were electrophoresed on a 2% agarose gel in TBE buffer (50 mM Tris, 1 mM EDTA, and 48.5 mM boric acid) and purified using the QIAquick PCR purification kit (Qiagen). The purified PCR products were sequenced using an ABI 3730 genetic analyzer.

The primers were successfully used to amplify an expected product fragment of about 1.2 kb from all of the 47 fishes (Figure 1). Comparing the complete mitochondrial genome of

Table 2. Species used in this study.

Number	Scientific name	Family
1	*Larimichthys polyactis*	Sciaenidae
2	*Sciaenops ocellatus*	Sciaenidae
3	*Argyrosomus argentatus*	Sciaenidae
4	*Nibea albiflora*	Sciaenidae
5	*Nibea japonica*	Sciaenidae
6	*Collichthys niveatus*	Sciaenidae
7	*Collichthys lucidus*	Sciaenidae
8	*Sebastiscus marmoratus*	Sebastidae
9	*Chrysochir aureus*	Sciaenidae
10	*Bostrychus sinensis*	Eleotridae
11	*Scartelaos viridis*	Gobiidae
12	*Glossogobius olivaceus*	Gobiidae
13	*Tridentiger barbatus*	Gobiidae
14	*Trypauchen vagina*	Gobiidae
15	*Bathygobius coalitus*	Gobiidae
16	*Odontamblyopus rubicundus*	Gobiidae
17	*Acanthogobius flavimanus*	Gobiidae
18	*Acanthogobius ommaturus*	Gobiidae
19	*Boleophthalmus Pectinirostris*	Gobiidae
20	*Chaeturichthys stigmatias*	Gobiidae
21	*Amoya chusanens*	Gobiidae
22	*Oxyurichthys formosanus*	Gobiidae
23	*Acanthogobius hasta*	Gobiidae
24	*Tridentiger bifasciatus*	Gobiidae
25	*Oxuderces dentatus*	Gobiidae
26	*Luciogobius platycephalus*	Gobiidae
27	*Harpadon nehereus*	Synodontidae
28	*Trichiurus lepturus*	Trichiuridae
29	*Mugil cephalus*	Mugilidae
30	*Siniperca chuatsi*	Percichthyidae
31	*Epinephelus awoara*	Serranidae
32	*Epinephelus areolatus*	Serranidae
33	*Paralichthys olivaceus*	Paralichthyidae
34	*Hapalogenys mucronatus*	Hapalogenyidae
35	*Hapalogenys nitens*	Hapalogenyidae
36	*Acanthopagrus latus*	Sparidae
37	*Oplegnathus fasciatus*	Oplegnathidae
38	*Parargyrops edita*	Sparidae
39	*Pleuronichthys cornutus*	Pleuronectidae
40	*Konosirus punctatus*	Clupeidae
41	*Cynoglossus trigrammus*	Cynoglossidae
42	*Psenopsis anomala*	Centrolophidae
43	*Pampus argenteus*	Stromateidae
44	*Acanthopagrus schlegelii*	Sparidae
45	*Setipinna tenuifilis*	Engraulidae
46	*Coilia mystus*	Engraulidae
47	*Carassius auratus*	Cyprinidae

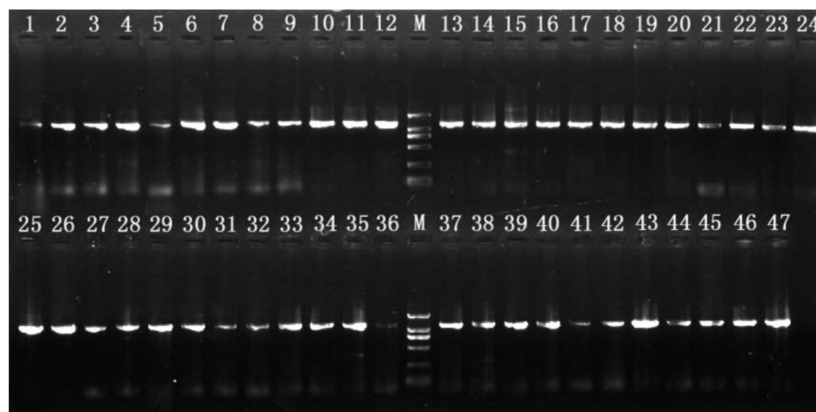

Figure 1. Images of the PCR amplicons for the 47 fish samples. *Lane M* = DL2000 DNA marker.

other published marine fishes sequences in GenBank database, the amplified DNA fragment was recognized to contain the complete 12S mitochondrial rRNA and $tRNA^{Val}$ genes, as well as a partial 16S mitochondrial rRNA gene of about 146 bp in length. Using the universal primers we have successfully amplified 12S mitochondrial rRNA gene fragments from a relatively wide variety of fish, which will facilitate future research.

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