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Isolation of Y- and X-linked SCAR markers in yellow catfish and application in the production of all-male populations

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Summary

Sex controls have been performed in some farmed fish species because of significant growth differences between females and males. In yellow catfish (*Pelteobagrus fulvidraco*), adult males are three times larger than female adults. In this study, six Y- and X-linked amplified fragment length polymorphism fragments were screened by sex-genotype pool bulked segregant analysis and individual screening. Interestingly, sequence analysis identified two pairs of allelic genes, *Pf33* and *Pf62*. Furthermore, the cloned flanking sequences revealed several Y- and X-specific polymorphisms, and four Y-linked or X-linked sequence characterized amplified region (SCAR) primer pairs were designed and converted into Y- and X-linked SCAR markers. Consequently, these markers were successfully used to identify genetic sex and YY super-males, and applied to all-male population production. Thus, we developed a novel and simple technique to help commercial production of YY super-males and all-male populations in the yellow catfish.

Keywords AFLP, all-male, *Pelteobagrus fulvidraco*, X-linked SCAR marker, Y-linked SCAR marker, YY super-male.

Field surveys and fish farming have revealed significant growth differences between females and males in many species of farmed teleost fish (Mair *et al.* 1995; Sheehan *et al.* 1999; Kocour *et al.* 2003). Yellow catfish (*Pelteobagrus fulvidraco* Richardson) exhibits a sex-dependent dimorphic growth pattern, with males growing faster and reaching a larger ultimate size than females (Park *et al.* 2004; Liu *et al.* 2007). In aquacultural practice, males grow about 30% faster than females in the first year. In the second culture year, males continue their growth up to 150–200 g, but the females remain around 50–75 g, which results in an ultimate size difference of about threefold. Therefore, the studies on sex determination mechanisms and genetic manipulation for producing all-male populations are intriguing both for theory and practice, and will be of significant benefit for yellow catfish aquaculture.

Recently, Liu *et al.* (2007) developed an YY super-male technique for producing all-male yellow catfish by integrating hormonal sex reversal and artificial gynogenesis. They demonstrated the XX/XY sex determination mechanism, generated YY super-males from XY physiological females through gynogenesis, and obtained all-male yellow catfish through the mating between the YY super-male and normal XX female. However, it is similar to the production programme of genetic all-male tilapia by YY super-males, the technological route still requires gynogenesis, hormonal sex reversal and test crosses, and thus is laborious and time consuming (Mair *et al.* 1995; Beardmore *et al.* 2001). In addition, testing cross-identification of YY super-males in yellow catfish requires killing the male fish to obtain the sperm. Therefore, an approach to accurately and rapidly identify the genetic sex of YY super-males without test crosses and without killing the fish is urgently required for the artificial sex control of this species. Previously, the isolation of sex-associated molecular markers has been attempted using different assays in African catfish (Kovacs *et al.* 2000), Nile tilapia (Lee *et al.* 2003; Ezaz *et al.* 2004), three-spined stickleback (Griffiths *et al.* 2000), and rainbow trout (Griffiths *et al.* 2000; Kovacs *et al.* 2000; Lee *et al.* 2003; Ezaz *et al.* 2004; Felip *et al.* 2005). This study aims to identify Y- and X-linked markers in yellow catfish through amplified fragment length polymorphism (AFLP) and sequence characterized amplified region (SCAR)

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approaches, and to develop a molecular marker-assisted sex control technique for mass production of all-male yellow catfish.

The gynogenetic XX, XY and YY individuals used in this study were produced as reported previously (Liu *et al.* 2007). AFLP screening and bulked segregant analysis (BSA) were performed as described (Michelmore *et al.* 1991; Vos *et al.* 1995; Ezaz *et al.* 2004). Each bulked DNA sample (XX, XY and YY bulk) was composed of eight individuals, and a single XX, XY or YY specimen was also used for the screening. A total of 256 EcoRI/MseI primer combinations were used to screen the genomic differences between these six samples. As a result, six AFLP fragments produced by five primer combinations were screened as potential Y-linked or X-linked markers. As shown in Table 1, two of them were Y-linked, and another four were X-linked markers.

Subsequently, the six Y- and X-linked AFLP fragments were cloned and sequenced as described (Li & Gui 2008), and their sequences were deposited into GenBank with accession numbers FJ560873–FJ560878. Interestingly, sequence alignment by DNAMAN software identified two pairs of alleles. *Pf63-X* and *Pf33-Y* are a pair of alleles (named *Pf33*), and *Pf62-Y* and *Pf62-X* are another pair of alleles (named *Pf62*). A 3578-bp flanking sequence of *Pf62* and a 1987-bp flanking sequence of *Pf33* were amplified from an XY male by Genome Walking Kit (Takara). Two pairs of locus-specific primers (Table S1) for *Pf33* and *Pf62* were

Table 1 Individual screening of six Y- and X-linked AFLP fragments.

Fragment name	Primer combinations	Size (bp)	Frequency			Accession number
			XX (%)	XY (%)	YY (%)	
Pf33-Y	E3-ACA/M3-CAG	225	0/8 (0)	8/8 (100)	8/8 (100)	FJ560876
Pf62-Y	E6-ACG/M2-CAC	233	0/8 (0)	8/8 (100)	8/8 (100)	FJ560874
Pf62-X	E6-ACG/M2-CAC	239	8/8 (100)	8/8 (100)	0/8 (0)	FJ560873
Pf63-X	E6-ACG/M3-CAG	225	8/8 (100)	8/8 (100)	0/8 (0)	FJ560875
Pf44-X	E4-ACT/M4-CAT	102	8/8 (100)	8/8 (100)	0/8 (0)	FJ560877
Pf117-X	E11-ATC/M7-CTG	165	8/8 (100)	8/8 (100)	0/8 (0)	FJ560878

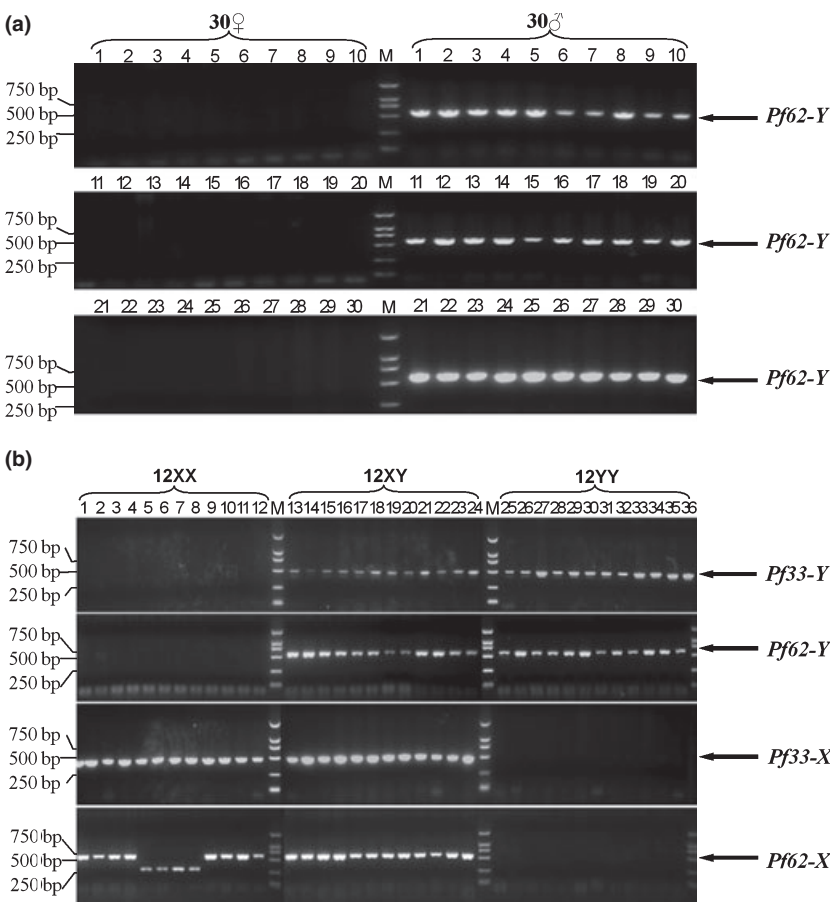

Figure 1 Test results of the Y-linked and X-linked SCAR markers (*Pf62-Y*, *Pf33-Y*, *Pf62-X*, and *Pf33-X*) in commonly propagated males and females (a) and in 12 XX, 12 XY and 12 YY gynogenetic individuals (b). The amplified Y-linked and X-linked fragments are indicated by arrows on the left. The DL2000 DNA marker sizes are shown on the right.

also designed to amplify the Y- and X-linked alleles from 8 XX, 8 XY and 8 YY genomes. Two *Pf33* alleles, *Pf33-Y* and *Pf33-X* (FJ560871; FJ560872), with respective fragment sizes of 1503 and 1502 bp and three *Pf62* alleles, *Pf62-Y*, *Pf62-X* and *Pf62-Xs* (FJ560868; FJ560869; FJ560870), with respective fragment sizes of 1382, 1415 and 1102 bp, were identified. *Pf62-X* and *Pf62-Xs* alleles are uncommonly distinguished by a 313-bp fragment insertion/deletion. According to the obtained sequences and the revealed variations (Fig. S1), we further designed two Y-linked (*Pf62-Y* and *Pf33-Y*) and two X-linked (*Pf62-X* and *Pf33-X*) SCAR primer pairs (Table S1).

To confirm the specificity of the primers, hundreds of specimens were used to test the Y- and X-linked SCAR markers (YSM and XSM). Firstly, the YSM *Pf62-Y* was amplified in 51 male and 52 female genomic specimens. As a result, a 568 bp fragment was produced from all the male specimens, whereas no amplification product was observed in any of the females (Fig. 1a). Subsequently, 12 XX, 12 XY and 12 YY specimens were used to test all four YSM and XSM. As shown in Fig. 1b, specific fragments of YSM *Pf33-Y* (462 bp) and *Pf62-Y* (568 bp) were amplified in all 12 XY and 12 YY specimens, but were absent in all the XX specimens. Similarly, specific bands of XSM *Pf33-X* (462 bp) and *Pf62-X* (598 bp or 285 bp) were amplified in all 12 XX and 12 XY specimens, but were absent in all the YY specimens. The above data indicate that the four primer pairs can be used to amplify Y-linked or X-linked bands directly from genomic DNA, and therefore, they can be easily applied to genetic sex identification.

Moreover, the YSM and XSM were used to identify genetic sex and YY super-males, and applied to the all-male population production. Firstly, the YSM *Pf62-Y* and XSM *Pf33-X* markers were used to identify genetic sex in progeny of crosses between YY physiological females × XY males. As shown in Fig. 2a, a single 568-bp fragment of YSM *Pf62-Y* was amplified in all the individuals examined, whereas a single 598-bp fragment of XSM *Pf33-X* is amplified only in some individuals. Therefore, the individuals with YSM *Pf62-Y* and XSM *Pf33-X* should be XY males, and the individuals with only YSM *Pf62-Y* should be YY super-males. After analysing a total of 114 individuals, 57 individuals (50%) were identified as YY super-males and other 57 as XY males. Additionally, we identified the genetic sex of 42 progeny collected from the cross between YY physiological females × YY super-males using the YSM *Pf33-Y* and XSM *Pf62-X*. As a result, a single 462-bp fragment of YSM *Pf62-Y* was amplified in all specimens, while the specific band of XSM *Pf62-X* was absent in all specimens, indicating that all of them were YY super-males (Fig. 2b).

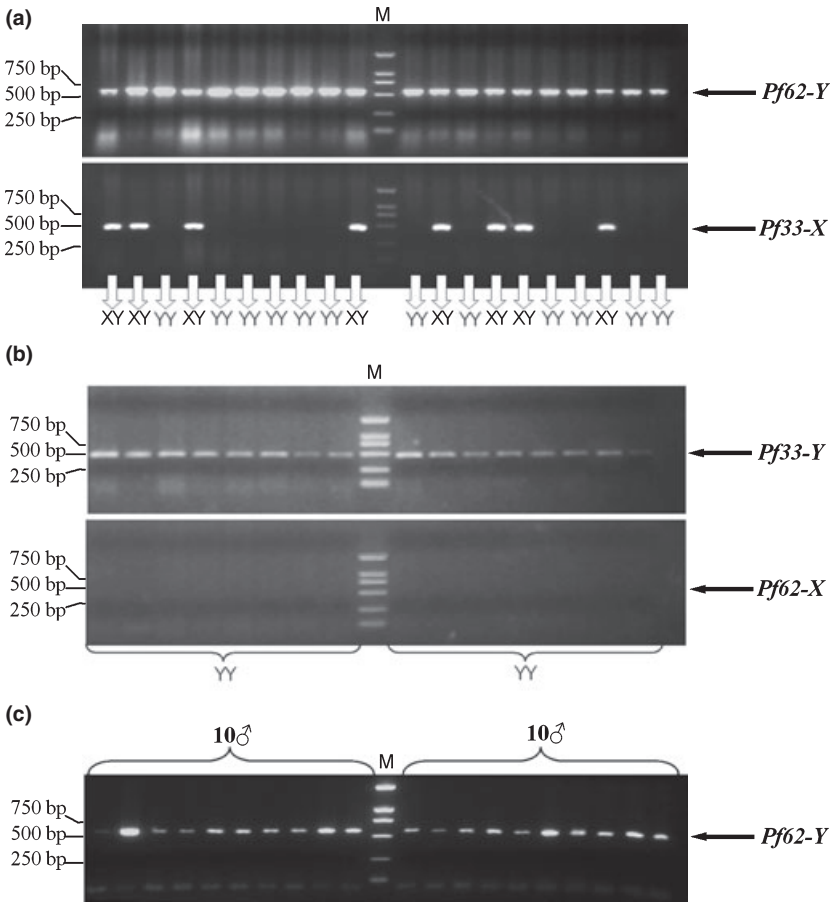

Figure 2 Application of YSM and XSM in genetic sex identification and all-male production. (a) Genetic sex identification in progeny of cross between YY physiological female and XY male using YSM *Pf62-Y* and XSM *Pf33-X*. (b) Genetic sex identification in progeny of cross between YY physiological female and YY male using YSM *Pf33-Y* and XSM *Pf62-X*. (c) All-male identification in testing progeny of YY super-male using YSM *Pf62-Y*. The amplification products are separated in 1% agarose gel, and the Y-linked and X-linked fragments are indicated by arrows on the left. The DL2000 DNA marker sizes are shown on the right.

Finally, 10 YY super-males identified from the above crosses were randomly selected to conduct test crosses with common XX females. A total of 400 progeny were randomly sampled, and all of them were confirmed to be males by an examination of the anatomy of their gonads. Furthermore, their genomic DNA was extracted from 20 random samples, and the genetic sex was identified by YSM *Pf62-Y*. As shown in Fig. 2c, a consistently genetic all-male sex was confirmed in all examined individuals.

Through the above studies, we have isolated two pairs of Y- and X-linked allele markers in yellow catfish, and thereby developed a Y- and X-linked allele marker-assisted sex control technique for mass production of all-male populations. Based on the results, we suggest that this is a novel and simple technique to help commercial production of YY super-males and all-male populations of yellow catfish. First, the XY females are identified by the YSM and XSM from sex reversal progeny through EE_2 treatment, and about 25% YY super-males can be identified from the progeny of the reversed XY female × normal XY male. Second, the YY physiological females can be obtained from the EE_2 treated YY fingerlings, and YY super-males can be produced continually from the mating of the YY physiological females × YY super-males. To enhance the genetic diversity and heterosis, YY super-males can be produced from crosses between YY physiological females × more genetically distant XY males, and then identified by YSM and XSM. Finally, thousands of YY super-males can be used for mass production of all-male progeny. In comparison with the previous approach, this novel technique can significantly enhance the yield of YY super-males without test crosses or killing of fish. Therefore, it can be used for commercial production of the all-male yellow catfish in aquacultural practice.

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Supporting information

Additional supporting information may be found in the online version of this article.

Figure S1 Allelic nucleotide sequence alignments and the primer positions of four Y-linked or X-linked SCAR primer pairs (Pf62-Y, Pf62-X, Pf33-Y and Pf33-X) in *Pf33* (a) and *Pf62* (b). The Y-specific and X-specific polymorphisms are indicated by solid background. The positions of Y-linked and X-linked SCAR primers are underlined.

Table S1 Sequences of primers.

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