Electronic Journal of Biotechnology 25 (2017) 70–74

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

Research article

Development of SSR marker by RNA-seq and its application in genotyping pearl sac in pearl oyster *Pinctada fucata martensii*

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ARTICLE INFO

Article history:
Received 23 July 2016
Accepted 16 November 2016
Available online 2 December 2016

Keywords:
Pinctada fucata martensii
SSR development
Pearl sac
Donor oyster
Host oyster
Genotyping

ABSTRACT

Background: Pearl oyster *Pinctada fucata martensii* is cultured for producing round nucleated pearls. Pearl production involves a surgical operation where a mantle tissue graft from a donor oyster and a round nucleus are implanted in the gonad of a host oyster. Whether the mantle graft implanted in the gonad of a host oyster contributes to the formation of a pearl sac that secretes pearl nacre to form a pearl should be determined. In April 2012, two full-sib families were separately used as donor and host oysters for a nucleus insertion operation. The pearl sac was sampled from the host oysters at day 60 after nucleus operation. A large number of simple sequence repeat (SSR) markers were developed using Illumina HiSeq™ 2000 platform. The two full-sib families were also used to mine diagnostic SSR markers for genotyping donor oyster, host oyster, and pearl sac.
Results: A total of 3168 microsatellite loci were identified in 39,078 unigenes, and 1977 SSR primers were designed by Primer 3.0. Forty-seven SSR primers were validated, and the rate of successful amplification was 72.3%. Two diagnostic SSR primers could successfully genotype pearl sac, donor oyster, and host oyster. Donor and host oysters were both homogenous, and the alleles in pearl sac were identical to those in donor and host oysters.
Conclusions: The present results confirmed that the mantle graft implanted in the gonad of host oyster contributed to the formation of the pearl sac in pearl oyster *P. fucata martensii*.

1. Introduction

Pearl oyster *Pinctada fucata martensii* is an important economical shellfish species in southern China. This species is cultured to produce round nucleated pearls. Pearl production is a complex process in which a mantle graft prepared from a donor oyster is implanted into the gonad of a host oyster together with a small inorganic nucleus. A common concern during this process is the fate of the mantle graft implanted in the gonad of the host oyster. Earlier histological studies could not clearly verify graft success and the persistence of cells from the donor oyster [1]. More recently, several molecular techniques have been applied to investigate the formation of pearl sac in pearl oyster *Pinctada maxima* and *Pinctada margaritifera* [2,3,4,5] Pearl sac formation in *P. fucata martensii* might be similar to that in *P. maxima* and *P. margaritifera*. However, the mechanism for pearl sac formation should be determined, and suggestions for breeding program design in *P. fucata martensii* are needed.

Many different molecular DNA markers have been developed for pearl oyster *P. fucata martensii*. These markers include sequence-related amplified polymorphism [6], simple sequence repeat (SSR) [7,8,9], amplified fragment-length polymorphism [10,11], and single nucleotide polymorphism [12]. SSRs, also known as microsatellites, are short tandem repeats of 1–6 or 2–8 nucleotides. SSRs are widely and abundantly dispersed in most nuclear eukaryotic genomes. Compared with restriction fragment length polymorphism, random amplified polymorphic DNA, and other molecular markers, SSR markers have the advantages of codominant inheritance, highly polymorphic loci, rich information content, good transferability between species, easy visualization, and stability [13]. Thus, SSR markers can be applied for various purposes such as genetic diversity detection, gene mapping, and marker-assisted selection.

SSR markers can be generally developed from genomic DNA through the construction of a genomic library and isotope or through the sequencing of candidate clones; these methods make the process time-consuming, costly, and labor intensive [14]. With the large number of expressed sequence tags (ESTs) in the public domain, the development of EST-derived SSRs is an efficient and cost-effective option. Next-generation sequencing (NGS) technologies using various platforms, such as Roche 454, Illumina HiSeq, and Applied Biosystems

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Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

http://dx.doi.org/10.1016/j.ejbt.2016.11.004

Table 1
Summary of EST-SSR search results.

Total number of examined sequences	39,078
Total size of examined sequences (bp)	15,452,096
Total number of identified SSRs	3168
Number of SSR-containing sequences	2701
Number of sequences containing more than one SSR	324
Number of SSRs present in compound formation	323
Mono-nucleotide	16.54%
Di-nucleotide	12.03%
Tri-nucleotide	30.84%
Tetra-nucleotide	30.74%
Penta-nucleotide	5.68%
Hexa-nucleotide	4.17%

SOLiD, are valuable in producing millions or billions of sequence reads at a lower cost than traditional Sanger sequencing [15]. NGS is a quick, efficient, and cost-effective technique for the development of molecular markers for nonmodel organisms. Several studies have already reported a number of SSR marker developments using NGS in pearl oyster species [8,9,12,13].

In the present paper, we reported the development of microsatellite markers (EST-SSR) using NGS Illumina platform. The developed SSR markers were used to detect whether the mantle grafts implanted in the gonad of a host oyster contribute to the formation of pearl sac in *P. fucata martensii*. This study aimed to develop EST-SSRs and increase the number of validated and polymorphic markers. More importantly, we aimed to determine the contribution of the implanted mantle graft to the formation of the pearl sac in the species.

2. Material and methods

2.1. Experimental animals

In March 2010, 36 full-sib families were established by selecting mature breeders from the third-generation line selected for fast growth in the base population [16]. In April 2012, two full-sib families with fast growth were selected and used for nucleus insertion operation. The family with high height growth rate was used as donor oyster, and the other one with high shell width growth rate was used as host oyster. Thirty mantle grafts were obtained from an individual donor oyster. A mantle graft was implanted into an individual host oyster together with a nucleus. A total of 10 donor oysters and 300 host oysters were used in the experiment. After the surgical operation, the 300 host oysters were placed separately in 10 pocket nets and cultured in the sea. The host oysters were checked every 7 d, and dead oysters were removed from the nets.

The mantle grafts of donor oysters were sampled and preserved in 70% ethanol. Host oysters were dissected on day 60 after the surgical operation. In addition, the pearl sac and adductor muscle of host oysters were sampled and preserved in 70% ethanol. Owing to the differences in survival rate and retention rate of host oysters implanted with mantle grafts from different donor oysters, 90 host oysters and 5 donor oysters were used for genotyping.

Twenty animals were sampled from each of the two full-sib families and then used for SSR validation. The mantle tissue of each sample was dissected and separately preserved in 70% ethanol.

2.2. SSR marker identification and primer design

A mantle tissue transcriptome dataset was obtained using Illumina/Hiseq-2000 RNA-seq. The sequences have been deposited in the NCBI Sequence Read Archive under the accession number of SRP081263 and were subjected to microsatellite maker screen using MISA (http://pgrc.ipk-gatersleben.de/misa/), with a repeat threshold of 12 mono-; six di-; four tri-; and three tetra-, penta-, or hexa-nucleotide repeats. Primer pairs were designed through pearl scripts, which allowed the interaction with Primer3 [17]. The presence of at least 50-bp sequence on both sides of the microsatellite repeats was considered sufficient for primer design using Primer 3.0 and potentially amplifiable loci.

2.3. DNA extraction, PCR amplification, and genotyping

Total DNA was extracted using a Universal Genomic DNA Mini Isolation Kit. Genomic DNA was assessed by gel electrophoresis using 1% agarose gel.

The designed SSR primer pairs were validated in the two full-sib families. A total of 47 SSR primers were validated. PCR amplification for validation and genotyping was performed in a 25-μL reaction containing 20 ng of genomic DNA, 250 nmol/L of each primer, 200 nmol/L dNTPs, and 1 U of Taq polymerase. The PCR protocol consisted of a 5-min denaturing step at 95°C, followed by 32 cycles consisting of denaturation at 95°C for 30 s; annealing at 55°C, 56°C, or 60°C for 30 s; and extension at 72°C for 1 min. The final PCR cycle was followed by a 10-min extension at 72°C. The resultant PCR products were tested by 8% polyacrylamide gel electrophoresis with a 20-bp DNA ladder. Following electrophoresis, gels were stained with silver and imaged using a Gel Doc™ XR+ system.

3. Results

3.1. Primer design and validation

Using MISA software, we identified 3168 potential EST-SSRs from 39,078 sequences. Among these sequences, 324 sequences contained more than one EST-SSRs, and 323 EST-SSRs were present in

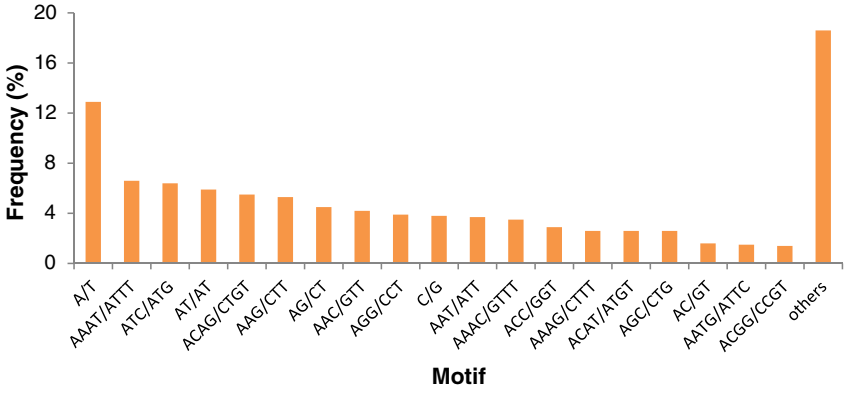

Fig. 1. Frequency distribution of EST-SSRs based on motif sequence types. Among the EST-SSRs searched, 150 motif sequence types were identified. The A/T mono-nucleotide repeat motif was the most abundant motif detected in our EST-SSRs.

Table 2
Characteristics and success of the polymorphic microsatellites in two full-sib families of pearl oyster *P. fucata martensii*.

Index	Motif and repeat number	Forward primer	Reverse primer	Polymorphism	Number of alleles
1P-1	(G)12	TTTTATTATACTCCCGCAAACAAA	CACTGCAGTTGTGCTCCTGT	N	2
1P-2	(A)13	TTTGTTCCATTGTCATTTGAGTG	CACCCTTTCTTGTTTTCTGTGA	P	2
1P-3	(G)12	TTTGTGACCATCTTCCCCTT	ATTAAGACGTTACGCGTGGG	N	1
1P-4	(A)13	TTTGTCTCACCAACAAAGTGCT	TTGCATGAACTCTACTCAGAGC	P	3
1P-5	(T)12	TTTGTACGGAGTTATGCCCC	TGTCTTGCATGTCACTGAAATATG	N	2
1P-6	(A)12	TTTGCACAAAAGTTAGGGGG	TTCATCTAAAGCAGTATTTTGGGA	N	1
1P-7	(A)12	TTTCTGCGCTGCAACTGTAT	CACATCCTCGAAAAATGGGT	N	1
1P-8	(G)12	TTTCGATATCCATCCCATCC	GATTTACCCAAAATGCTGCG	F	3
1P-9	(A)14	TTTATGGTGCTGTCGTTGGA	AGGATCCAATCGTCTTTGTCA	F	-
1P-10	(T)14	TTTAAATAAGCCGCTTTGCC	TGAGAAGCGTTGCCATGATA	N	2
1P-11	(T)12	CATCAAGTTTTGGATTCCCG	CCGACCACAAACTTTGAAGAA	P	2
1P-12	(T)13	TTGGGGTGGGATTTATGAGA	CAAGGTAAGAACCCCAACCC	P	2
2P-1	(AT)7	TTTTTCTCACATCCTTGTATCTCAA	ATTTCCCGGGTTTTTACGAC	P	3
2P-2	(TA)6	TTTTGTGTTGTTTGGAAGCG	TGCAGTACTAACAGTGTGTTAATTGAT	F	-
2P-3	(AT)6	TTTTGATTTATTCGACATCAGCA	CGATTCCAAAGATGCTCACA	P	2
2P-4	(TA)7	TTTGGGGTAATCGGTGTTGT	CGATCATGAATCACAGAAATGAA	N	2
2P-5	(CT)7	TTTGAAATAAGGTTGTTTGAATGAA	TGCAAGTCTTTTCATGCGTC	N	2
2P-6	(GT)6	AACCATCTAAACTTGCGTCTGA	TTGCCAGCAATCTCAGTGTC	P	2
2P-7	(CT)6	TTTCAAAAATGCTTTCCGCT	AAAGTGAATCATCCGTCATGG	P	3
2P-8	(TA)9	TTTAGTGTCGTGGTCGCTTG	TTCAGTACTGGTGGGAGAGGA	F	-
2P-9	(AT)7	TTGTTGTTGCCACCACCTTA	GCGAGATGAATACTTTGTTTGG	F	-
2P-10	(TA)7	TTGTTAATATATTTCTAAGTGCCGGT	TTGAAAAGTGGTGGGTGACA	F	-
2P-11	(AT)7	TTGGTGGTAACGCCTCGTAT	TGATTCGAAGATTGTGCTGC	P	4
2P-12	(TC)15	TTGCGGTCTTCAAACATTCA	AGGCTGTTGAGAATGTCGCT	P	3
3P-1	(AAG)4	TTTTGGTTTGATCGAAGACG	CCTCCTTGAACCTGGAAACA	P	3
3P-2	(TAT)4	TTTTGGGTCCATTTGTCTGA	TTAGGGGTTACCATGCGTGT	F	-
3P-3	(GGT)4	TTTTCGTTGGGATTACGAGC	CCTGTGTACTGCTGCTCCAA	F	-
3P-4	(TAA)4	TTTTCCCCTTTGTTCAACTG	CTTTTGCAAAGTGCTCAAACA	P	3
3P-5	(ATA)5	TTTTAGAAAGAAGGCAAATGGA	AAACTGTCGGCTTGACAACAC	F	-
3P-6	(TGT)4	TTTGTAGAGGAGGTGGTGGC	GCTTCATCCCACCAAACTGT	P	3
3P-7	(CTA)4	TTTGTACATTTTGCCCCTCC	GTCAGCCGAACAACACAGAA	F	-
3P-8	(ATC)4	TTTGGTTGTCGGTAGACGTG	TGGATCAATACATCCTTTTCCA	P	2
3P-9	(ATA)7	TTTGCTGAGAGATGCGTAAAA	ATCTTAGGGGGTCTGATGGC	F	-
3P-10	(ATC)4	TTTCTTATGCCACAGTCCCC	CCTCTCCGAATAAGCCATCA	P	3
3P-11	(AAT)4	TTTCCGTGACTACTCGGGTC	GTACATAAAGGGCGGTGCAT	N	2
4P-1	(TGTT)3	TTTCACTTCAGAATTGGAGTACTTG	GTGCCCTGTGGTATCCAGTT	F	-
4P-2	(TGTC)4	TTTATCTGCCGGAATCTTGC	ATGGCACTTTTAGGACAAACA	P	3
4P-3	(AGCC)3	TTTAGATCGGGACGTTCAGG	TGAACACGATGAAAGCAAGG	P	2
4P-4	(ACAT)3	TTTACAAGGGATGAGGTGGG	TGAACCAACAAATCTGAGACAG	F	-
4P-5	(CATA)3	TTGTTTCCTCGTATCAAGAACTTTT	TTTCCATCATTGGTGCACAT	N	2
4P-6	(AAGA)3	TTGTTGAATTCTTTGGCTTCC	GGGTATACGCCCATGTAAGG	N	1
4P-7	(TCAT)3	TTGTTCCGAAAGATAGTAGACAAA	TGCTGTAAGTTTGCCAATGAA	P	3
4P-8	(TTTC)3	TTGTTATCTGCATTCTGCGG	TTGCTATTTAAGAAATTGGACGTG	P	3
4P-9	(TGTC)3	TTGTGTTGTCTTTTTGTCTGTCC	CAATCATACGGCACACGCTA	P	2
4P-10	(AAAT)3	TTGTGTAAGGCATGCTACTGAGA	TCTCATATACATTAACCGCATTGAA	N	2
4P-11	(ATAA)3	TTGTGCAATTCTGTTTTGAGAAA	CAACAGACGGATTCCTCCTT	P	3
CP-1	(TC)7tt(TC)8	GCTTCATCATTCAGCAACAGA	GATGCCTTAAGATGCGATCC	P	3

P, N, and F separately represent polymorphism, monopolymorphism, and amplifying failure, respectively.

compound form. On average, one EST-SSR was found in every 4.88 kb of the unigene, and the frequency of EST-SSRs was 6.91%. The most abundant types of repeat motifs were tri-nucleotide (30.84%) and tetra-nucleotide (30.74%), followed by mono-nucleotide (16.54%), di-nucleotide (12.03%), penta-nucleotide (5.68%), and hexa-nucleotide (4.17%) (Table 1).

The frequencies of EST-SSRs with different numbers of repeat units were calculated. Among the detected SSRs, approximately 150 motif types were identified. The frequency of the 150 motif types is illustrated in Fig. 1. Among these types, A/T (12.8%) was dominant, followed by AAAT/ATTT (6.6%), ATC/ATG (6.4%), AT/AT (5.9%), ACAG/CTGT (5.5%), AAG/CTT (5.3%), AG/CT (4.5%), and AAC/GTT (4.2%).

A total of 1977 SSR primers were successfully designed using Primer 3.0. To determine the successful amplification proportion of these SSRs, we randomly selected 47 primer pairs for validation in the two full-sib families. Approximately 72.3% (34/47) of these pairs were successfully amplified in the SSR primers, and two diagnostic SSR primers were obtained for genotyping. The characteristics and success of these polymorphic SSRs are listed in Table 2.

3.2. PCR amplification and genotyping

The characteristics of the two diagnostic primers are listed in Table 3. The two SSR primers (1P-11 and 2P-6) could successfully genotype the donor oyster, host oyster, and pearl sac. For example, at 1P-11 locus, genotypic differences were evident among donor oyster, pearl sac, and host oyster. The genotypes of donor and host oysters were both homogeneously produced bands of 130/130 and 142/142, respectively, whereas those of pearl sac were both homogenous (142/142) and heterogeneous (130/142) (Fig. 2). The percentage of

Table 3
Primer sequence, annealing temperature, and allele size of two diagnostic SSR primers for genotyping.

Loci	Primer sequence	Annealing temperature (°C)	Allele size (bp)
1P-11	F:CATCAAGTTTTGGATTCCCG R:CCGACCACAAACTTTGAAGAA	55	130, 142
2P-6	F:AACCATCTAAACTTGCGTCTGA R:TTGCCAGCAATCTCAGTGTC	56	122, 132

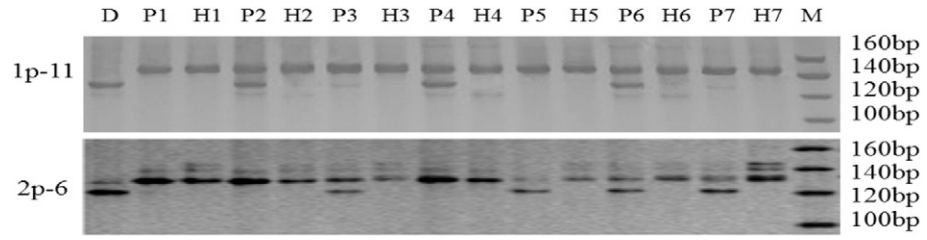

Fig. 2. Amplified profiles at 1p-11 and 2P-6 loci in donor oyster (D), pearl sac (P), and host oyster (H) in pearl oyster *P. fucata martensii*.

heterogeneous genotype in the pearl sac was 70.0% (63/90) (Table 4). At 2P-6 locus, the genotypes of donor and host oyster both homogeneously produced bands of 122/122 and 132/132, respectively (Fig. 2). Approximately, 73.3% (66/90) of pearl sac was heterogeneous and produced bands (122/132) (Table 4). Heterogeneous genotypes in pearl sac displayed two alleles separately from donor and host oysters, which confirmed that the pearl sac contained cells originating both from the mantle graft (donor oyster) and host oyster.

4. Discussion

In the present study, we conducted a general screening of pearl oyster transcripts for the presence of microsatellites. We also analyzed the distribution and frequency of these markers. A total of 3168 SSRs in 39,078 unigenes were obtained. The frequency (6.91%) of SSRs detected in this study was higher than 1.53% detected in *P. maxima* [13] and 3.10% detected in hard clam *Meretrix meretrix* [18] and lower than 10.22% detected in clam *Paphia textile* [15]. Tri-nucleotide repeat motifs were the most frequent repeat type found in the unigenes and represented 30.84% of identified SSR loci; this type was followed by tetra-nucleotide (30.74%), mononucleotide (16.54%), di-nucleotide (12.03%), penta-nucleotide (5.68%), and hexa-nucleotide (4.17%) repeat units. The repeat motifs were evidently different from those reported in other shellfish species. The frequencies, types, and distributions of the potential SSRs are significantly different in the published studies, which may be caused by the various SSR search tools and criteria used. When mining SSR markers from an EST dataset, the SSR frequency is dependent on the following factors. First, the parameters used in mining microsatellites, such as the repeat length threshold and number of repeat unit, affect the results dramatically. Second, the genome structure or composition may also partially account for the EST-SSR frequency. Third, different software types used in detecting SSRs may also affect the frequency. Some SSR identification tools, such as Sputnik, can determine imperfect SSR, whereas others (such as SSRIT and MISA) can only identify perfect SSRs [19].

To determine the polymorphism level among our set of new SSR markers, we validated 47 primers in two full-sib families. Among the 47 primer pairs randomly selected for PCR validation, 34 pairs produced clear bands. The PCR success rate (72.3%) was comparable with the results obtained in other shellfish species. For example, success rates were 65% in the clam *M. meretrix* [18], 50% in the clam *Mercenaria mercenaria* [20], 80.7% in the oyster *Crassostrea virginica* [21], and 36% in freshwater mussel *Villosa lienosa* [22]. Therefore, the EST-SSRs identified in this study will provide a valuable resource for further functional gene analyses, genetic map construction, and quantitative trait loci mapping in the species.

Table 4
Genotype number of donor oyster, pearl sac, and host oyster of pearl oyster *P. fucata martensii* at the two diagnostic SSR loci.

	Donor oyster	Pearl sac	Host oyster
1P-11	130/130(1)	130/142(16); 142/142(4)	142/142(20)
	130/130(1)	130/142(15); 142/142(8)	142/142(23)
	130/130(1)	130/142(12); 142/142(4)	142/142(16)
	130/130(1)	130/142(13); 142/142(5)	142/142(18)
	130/130(1)	130/142(7); 142/142(6)	142/142(13)
2P-6	122/122(1)	122/132(15); 132/132(5)	132/132(20)
	122/122(1)	122/132(16); 132/132(7)	132/132(23)
	122/122(1)	122/132(13); 132/132(3)	132/132(16)
	122/122(1)	122/132(14); 132/132(4)	132/132(18)
	122/122(1)	122/132(8); 132/132(5)	132/132(13)

Pearl production involves a surgical operation, where a mantle graft from a donor oyster and a nucleus are implanted into the gonad of a host oyster. A pearl sac is gradually formed around the nucleus after the surgical operation. Early studies based on histological observation showed that the inner epidermis and mesodermal layers of the mantle graft degenerated after the surgical operation and only the outer epidermal layer remained [23,24]. The pearl sac might be formed of the epithelial cells of the mantle epidermis growing around the nucleus to encase it completely. At approximately 40 d after the surgical operation, the nacreous layers begins to form [25]. At 4 d after implantation, the epidermal cells in the outer epithelium of the graft begin to proliferate, emigrate around the nucleus, and form the pearl sac [26]. Our previous studies also showed that the epithelial cells of pearl sac may originate from the outer epithelial cells of the mantle graft implanted in host oyster [27]. The abovementioned studies based on traditional techniques showed that pearl sac may be formed of the epithelial cells of the mantle epidermis growing around the nucleus to encase it completely.

Recently, molecular evidence has confirmed that the mantle graft implanted in host oyster contributes to pearl sac formation in the pearl oyster [2,3,4,5,28,29]. In pearl oyster *P. margaritifera*, for example, Arnaud-Haond et al. [2] found that the pearl sac cells contain genome from the donor oyster at 18 months after nucleus insertion using SSR markers [2]. McGinty et al. [4] designed a xenograft experiment by sampling pearl oysters *P. maxima* and *P. margaritifera* as host and donor oysters, respectively. They found that donor oyster cells were transcriptionally active in the expression of N44 and N66 biomineralization genes. They also concluded that the donor oyster was an important contributor to biomineralization in pearl culture [4]. McGinty et al. [5] developed species diagnostic single nucleotide polymorphisms using high-throughput mRNA sequencing derived from allografted *P. maxima* and *P. margaritifera* pearl sacs to detect putative biomineralization genes expressed in pearl sac tissue. They found that donor graft cells played a leading role during pearl formation, and the receptor also expressed a few genes in pearl sac formation [5]. In the present study, the alleles at the locus that are separately specific for donor and host oysters co-existed in the pearl sac. Our studies also confirmed the contribution of the donor oyster during pearl sac formation in pearl oyster *P. fucata martensii*.

Pearl sac is composed of one layer of epithelium cells that secretes nacre to form pearl. When pearl sac is sampled, it is inevitable to sample the tissues surrounding to pearl sac. Genomic DNA of pearl sac is a mixture of donor and host oyster DNAs. Owing to the high sensitivity of PCR, it is very probable that an admixture of host and donor genotypes was amplified from the pearl sac. For example, at locus 2p-6, the genotype of donor oyster is homogenous (122/122) and the genotype of host oyster is homogenous (132/132). Approximately 73.3% of pearl sac was heterogeneous (132/122). Similar studies have

been reported by Arnaud-Haond et al. [2], who genotyped muscle (host oyster) and pearl sac of pearl oyster *P. margaritifera* using three SSR pairs. They found a high number (approximately 65%) of differences between the muscle and corresponding pearl sac genotypes [2]. In the present studies, the differences among the genomes of donor oyster, host oyster and pearl sac demonstrated that mantle graft implanted in host oyster participated in pearl sac formation.

The pearl quality of pearl oyster *P. fucata martensii* in China has evidently decreased in recent years. This deterioration is dominantly caused by genetic factors. Identification of the contribution of the donor oyster to pearl sac formation can help to improve pearl quality through genetic breeding programs. Pearl quality is correlated with five variations, namely, size, shape, color, luster, and surface complexion; among these variations, color is the most subjective indicator for measuring pearl quality [3]. Several studies also showed that the shell nacre color of the donor oyster affected the overall color of the pearl produced [3,30]. In Japanese pearl oyster *P. fucata martensii*, Wada and Komaru [30] reported that the frequency of yellow pearls was significantly lower in the group produced by grafting mantle tissue from white-colored line than that from brown-colored line. They concluded that pearl color was determined by the genetic characteristics of the donor oysters in Japanese pearl oyster [30]. In our breeding programs, four types of shell color strains of *P. fucata martensii* were established in the prismatic layer of the base population after three successive selections for shell color [31]. We also conducted two experiments to investigate the effects of colored strains on pearl quality. Our results showed that the black-colored strain with rapid growth and white-colored ones were preferable for host and donor oysters, respectively, to produce high-value pearls [32].

In summary, the large number of assembled unigenes (39,078) and detected EST-SSRs (1977) derived from pearl oyster *P. fucata martensii* transcriptome suggested that Illumina paired-end sequencing was a cost-effective approach for molecular marker development in *P. fucata martensii*. Pearl sac formation is a complex cooperation between donor and host oysters. Our studies also confirmed that the mantle graft implanted in the host oyster contributed to pearl sac formation in *P. fucata martensii*.

Financial support

The study was financially supported by the National Natural Science Foundation of China (31372526), the Administration of Ocean and Fisheries of Guangdong Province (Z2014011 and A201308A10) and "Haizhifan-qihang" Project of Guangdong Ocean University.

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