J. Ocean Univ. China (Oceanic and Coastal Sea Research)
DOI 10.1007/s11802-017-3121-2
ISSN 1672-5182, 2017 16 (1): 151-160
http://www.ouc.edu.cn/xbywb/
E-mail:xbywb@ouc.edu.cn

Development of Genomic Microsatellite Multiplex PCR Using Dye-Labeled Universal Primer and Its Validation in Pedigree Analysis of Pacific Oyster (*Crassostrea gigas*)

LIU Ting, LI Qi*, SONG Junlin, and YU Hong

Key Laboratory of Mariculture of Ministry of Education, *Ocean University of China*, *Qingdao* 266003, *P. R. China*

(Received January 28, 2016; revised July 4, 2016; accepted November 13, 2016)

Abstract There is an increasing requirement for traceability of aquaculture products, both for consumer protection and for food safety. There are high error rates in the conventional traceability systems depending on physical labels. Genetic traceability technique depending on DNA-based tracking system can overcome this problem. Genealogy information is essential for genetic traceability, and microsatellite DNA marker is a good choice for pedigree analysis. As increasing genotyping throughput of microsatellites, microsatellite multiplex PCR has become a fast and cost-effective technique. As a commercially important cultured aquatic species, Pacific oyster *Crassostrea gigas* has the highest global production. The objective of this study was to develop microsatellite multiplex PCR panels with dye-labeled universal primer for pedigree analysis in *C. gigas*, and these multiplex PCRs were validated using 12 full-sib families with known pedigrees. Here we developed six informative multiplex PCRs using 18 genomic microsatellites in *C. gigas*. Each multiplex panel contained a single universal primer M13(−21) used as a tail on each locus-specific forward primer and a single universal primer M13(−21) labeled with fluorophores. The polymorphisms of the markers were moderate, with an average of 10.3 alleles per locus and average polymorphic information content of 0.740. The observed heterozygosity per locus ranged from 0.492 to 0.822. Cervus simulations revealed that the six panels would still be of great value when massive families were analysed. Pedigree analysis of real offspring demonstrated that 100% of the offspring were unambiguously allocated to their parents when two multiplex PCRs were used. The six sets of multiplex PCRs can be an important tool for tracing cultured individuals, population genetic analysis, and selective breeding program in *C. gigas*.

Key words *Crassostrea gigas*; traceability; microsatellites; universal primer; multiplex PCR; pedigree analysis

1 Introduction

Aquaculture industry has developed rapidly in recent years. In the future, the sustainable development of aquaculture will be progressively more market driven, and will rely heavily on its capacity to meet consumers' expectations (Yue *et al*., 2012). Thus, the need for accurate product labelling to keep consumer confidence and ensure food-safety is obvious (Ogden, 2008). Traceability through physical labels is not highly reliable as physical labels can be easily changed or lost while DNA-based tracking system plays an increasingly important role for consumer protection and confidence building (Yue *et al*., 2012). As a result, the application of genetic tools in supply chain traceability provides huge potential.

The Pacific oyster *Crassostrea gigas* is a highly valued cultivated oyster species. It has been introduced for aquaculture purposes in many locations worldwide except of its natural range (Kochmann *et al*., 2012). As the quality of aquaculture products can vary greatly between farms, tracing *C. gigas* individuals to single farms is becoming increasingly important. Microsatellites, which possess the advantages of high polymorphism, codominance and conforming to Mendelian segregation, are the most popular markers in genetic studies. They have been preferred molecular markers to trace studbook information from the species down to the individual level in all types of organisms (Yu *et al*., 2014). In *C. gigas*, a large number of genomic microsatellites have been developed (Li *et al*., 2003; Sekino *et al*., 2003; Yamtich *et al*., 2005; Qi *et al*., 2009). However, most of them are usually amplified by polymerase chain reaction (PCR) as single loci, a process that is time-consuming and expensive.

Multiplex polymerase chain reaction (PCR) is a variant of PCR in which two or more loci are simultaneously amplified in the same reaction (Henegariu *et al*., 1997). To date, fourteen multiplex panels for *C. gigas* have been reported (Taris *et al*., 2005; Li *et al*., 2010; Miller *et al*., 2012; Kang *et al*., 2013; An *et al*., 2013, 2014). They all directly labeled locus-specific primers with fluorescent dyes. More cost-effective end-labeling of PCR products can be achieved through a three primer PCR approach,

* Corresponding author. Tel: 0086-532-82031622
E-mail: qili66@ouc.edu.cn

involving a fluorescently labeled universal primer in combination with modified locus-specific primers with 5' universal primer sequence tails (Blacket *et al*., 2012). Based on the capillary electrophoresis technology and three primers PCR method, we developed six multiplex PCRs from previously described genomic microsatellites of *C. gigas* for quick and economic genetic analyses, and also validated their efficiency in parentage assignment with twelve single-pair mating families. It was proved that these six multiplex PCR sets are applicable not only for tracing cultivated oyster products, but also for selective breeding program and genetic resource conservation of *C. gigas*.

2 Materials and Methods

Sexually mature one-year-old oysters were selected for artificial spawning. Twelve single-pair mating families of *C. gigas* were produced. They were used to test the resolving power of the six genomic microsatellite multiplex PCRs. Tissues from all parents were saved in pure ethanol until DNA extraction. Forty D-larvae were collected from each family randomly, preserved in 100% ethanol, and kept in the fridge until used. For broodstocks, genomic DNA was extracted from ethanol-preserved adductor muscle tissue as previously described by Li *et al*. (2006). DNA concentration and quality of each sample were evaluated by NanoDrop 2000 spectrophotometer and 1% agarose gel electrophoresis, respectively. DNA of forty D-larvae from each family was prepared by the Chelex-modification extraction method following Li *et al*. (2003).

Genomic microsatellites were selected from previously characterized microsatellite markers (Li *et al*., 2003; Sekino *et al*., 2003; Yamtich *et al*., 2005; Qi *et al*., 2009) based on their level of polymorphism, allele size range, and reliability of allele calling. Firstly, all genomic microsatellites loci were validated with ten individuals in simplex reactions as described by Schuelke (2000), but not labeled with any fluorescent dye. Then, PCR products were analyzed via 6% denaturing polyacrylamide gels using a 10 bp ladder and visualized by silver staining. If the microsatellites presented low quality profiles, such as unspecific products, poor amplification and excessive stuttering, they were excluded from further analyses. Multiplex PCRs were arranged according to the annealing temperature and size of each primer to maximize the number of loci suitable for simultaneous analysis. M13-tail with different fluorescent dyes (NED, VIC and FAM) were used so that products can be differentiated by capillary separation. The proper annealing temperature and primer concentration of each panel were then optimized using ten individuals. Multiplex PCRs were performed in 10-μL volume containing 0.25 U *Taq* DNA polymerase, 1× PCR buffer, 0.2 mmol L^{-1} dNTP mix, 2.0 mmol L^{-1} $MgCl_2$, 0.15 μmol L^{-1} forward primer, 0.15 μmol L^{-1} universal primer, 0.06 μmol L^{-1} reverse primer, and about 50 ng template DNA. The amplifications were programmed using following conditions: 3 min at 94℃; 35 cycles of 30 s at 94℃, 60 s at the optimal annealing temperature, and 75 s at 72℃; 8 cycles of 30 s at 94℃, 60 s at 53℃, 75 s at 72℃, with a final extension of 10 min at 72℃. For the subsequent genotyping step on ABI-3130 with LIZ500 as internal size standard, PCR products were diluted in pure water depending on their intensity. Fragment lengths were assessed with the GeneMapper v4.0 software.

We used Cervus 3.0 software (Kalinowski *et al*., 2007) to assign parentage. This program calculates the number of alleles (*Na*), polymorphic information content (*PIC*), the observed heterozygosity (*Ho*), expected heterozygosity (*He*) and the combined non-exclusion probability in different situations. The genotype data of all offspring were pooled together to verify the resolving power of the multiplex panels in pedigree analysis. The simulation and real parentage analysis were conducted using the allele frequencies of 504 individuals (40 larvae and 2 parents of each family) in Cervus 3.0. The goodness-of-fit for expected Mendelian segregation ratios (1:1, 1:2:1, and 1:1:1:1) was measured by chi-square analysis module of SPSS 19.0 at the 0.05 probability level.

3 Results and Discussion

Eighteen genomic microsatellite loci were selected and arranged into six multiplex PCRs that work well for *C. gigas* (Table 1). Electrophoregram of each multiplex panel showed high quality resolutions of alleles (Fig.1). Multiplex PCR is a valuable tool in various aspects such as gene deletion and mutation detection (Edwards *et al*., 1994), molecular species identification (Staudacher *et al*., 2011), as well as assessing aquaculture practices (Borrell *et al*., 2014). Instead of relying directly on labeling locus-specific primers developed in *C. gigas* (Taris *et al*., 2005; Li *et al*., 2010; Miller *et al*., 2012; Kang *et al*., 2013; An *et al*., 2013, 2014), we used universal tailed primer M13(−21) at each forward primer. Such a method allows the same level of marker multiplexing and accuracy in microsatellite genotyping attained in regular direct-labeled microsatellite fluorescent detection assays, while significantly reducing the costs (Guichoux *et al*., 2011). PCR cycling could be interrupted for the addition of the labeled primer only during the final cycles, thus minimizing unspecific amplification and competition between primers resulted in more fidelity amplification of the target regions (de Arruda *et al*., 2010). Blacket *et al*. (2012) reported a strategy in which multiple universal primers were used as tails on each locus-specific forward primer and multiple universal primers were labeled with different fluorophores. These strategies are worthy of consideration in subsequent studies on multiplex PCR to maximize locus amplification and reliability, as well as to further reduce the cost.

Genetic statistics for each locus are given in Table 2. Overall, the polymorphisms of the markers were moderate, with an average of 10.3 alleles per locus (ranged from 5 to 19) and average polymorphic information content of 0.740. The observed heterozygosity (*Ho*) and expected heterozygosity (*He*) ranged from 0.492 to 0.822 (average = 0.660) and 0.532 to 0.918 (average = 0.763), respec-

tively. There was only one locus (ucdCg-120) whose *Ho* was greater than *He*.

Table 1 Information of six multiplex PCRs

Multiplex sets	Locus	Ta (℃)	Concentration of forward primer (μmol L^{-1})	Concentration of reverse primer (μmol L^{-1})
Panel 1	ucdCg-117	58	0.06	0.15
	ucdCg-120	58	0.06	0.15
	ucdCg-198	58	0.06	0.15
	NED-M13(−21)	53	–	0.15
Panel 2	ucdCg-146	58	0.06	0.15
	Crgi3	58	0.06	0.15
	uscCgi-210	58	0.06	0.15
	FAM-M13(−21)	53	–	0.15
Panel 3	ucdCg-170	58	0.06	0.15
	ucdCg-156	58	0.06	0.15
	ucdCg-199	58	0.06	0.15
	VIC-M13(−21)	53	–	0.15
Panel 4	otgfa0_0007_B07	50	0.06	0.15
	otgfa0_0129_E11	50	0.06	0.15
	Crgi4	50	0.06	0.15
	FAM-M13(−21)	53	–	0.15
Panel 5	ucdCg-152	50	0.06	0.15
	Crgi39	50	0.06	0.15
	Crgi45	50	0.06	0.15
	VIC-M13(−21)	53	–	0.15
Panel 6	ucdCg-200	54	0.06	0.15
	otgfa0_408293	54	0.06	0.15
	otgfa0_0139_G12	54	0.06	0.15
	NED-M13(−21)	53	–	0.15

Notes: Ta, annealing temperature.

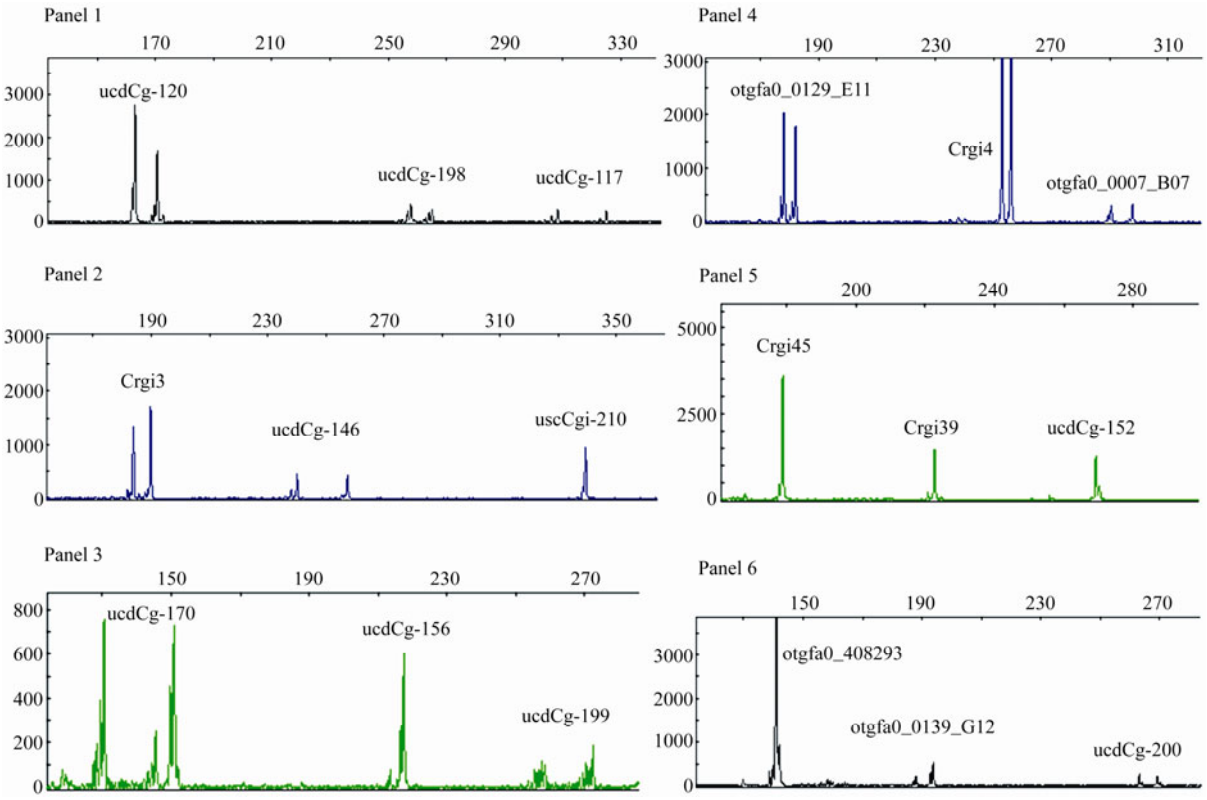

Fig.1 Electrophoregrams of six multiplex panels. Horizontal axis shows the size ranges for each locus in base pairs (bp).

Table 2 Characteristics of the six multiplex PCRs in *Crassostrea gigas*

Multiplex sets	Locus	*Na*	*PIC*	*Ho*	*He*	NE-1P	NE-2P	NE-PP	*F* (Null)
Panel 1	ucdCg-120	7	0.607	0.708	0.660	0.748	0.583	0.399	−0.0402
	ucdCg-198	12	0.869	0.729	0.822	0.390	0.241	0.090	0.0953
	ucdCg-117	19	0.912	0.768	0.918	0.283	0.165	0.044	0.0888
Panel 2	Crgi3	5	0.472	0.519	0.532	0.851	0.712	0.555	−0.0068
	ucdCg-146	17	0.877	0.822	0.887	0.366	0.224	0.076	0.0414
	uscCgi-210	9	0.796	0.703	0.818	0.525	0.351	0.168	0.0793
Panel 3	ucdCg-170	12	0.854	0.691	0.869	0.421	0.265	0.105	0.1136
	ucdCg-156	16	0.903	0.633	0.911	0.307	0.181	0.053	0.1830
	ucdCg-199	8	0.690	0.554	0.716	0.667	0.480	0.273	0.1285
Panel 4	otgfa0_0129_E11	12	0.858	0.761	0.872	0.413	0.259	0.102	0.0677
	Crgi4	6	0.589	0.492	0.642	0.767	0.604	0.423	0.1281
	otgfa0_0007_B07	10	0.716	0.662	0.751	0.632	0.455	0.261	0.0690
Panel 5	Crgi45	5	0.562	0.534	0.623	0.789	0.634	0.463	0.0720
	Crgi39	10	0.860	0.788	0.874	0.409	0.255	0.098	0.0455
	ucdCg-152	9	0.579	0.522	0.599	0.778	0.589	0.374	0.0769
Panel 6	otgfa0_408293	12	0.826	0.784	0.843	0.470	0.304	0.130	0.0384
	otgfa0_0139_G12	8	0.562	0.518	0.593	0.793	0.615	0.415	0.0594
	ucdCg-200	8	0.787	0.686	0.812	0.543	0.368	0.186	0.0879

Notes: *Na*, number of alleles; *PIC*, polymorphic information content; *Ho*, observed heterozygosity; *He*, expected heterozygosity; NE-1P, average non-exclusion probability for one candidate parent; NE-2P, average non-exclusion probability for one candidate parent given the genotype of a known parent of the opposite sex; NE-PP, average non-exclusion probability for a candidate parent pair; F (Null), frequency of null allele.

The results of the Cervus simulations revealed that only three multiplexes are required to reach 100% assignment even the number of candidate parents reached 400 (Fig.2). This is slightly higher than a previous study on great scallop (Morvezen *et al*., 2013). It is most likely due to the relative higher polymorphic information content (*PIC*) and the number of markers in our study. The number of loci was always the most important single factor in determining the accuracy of assignments (Harrison *et al*., 2013). Additionally, accurate parentage assignment depends not only on the properties of microsatellite markers, but also on the number of candidate parent pairs (Norris *et al*., 2000). Therefore, the six panels would still possess potential appliance value when a great amount of families were analysed.

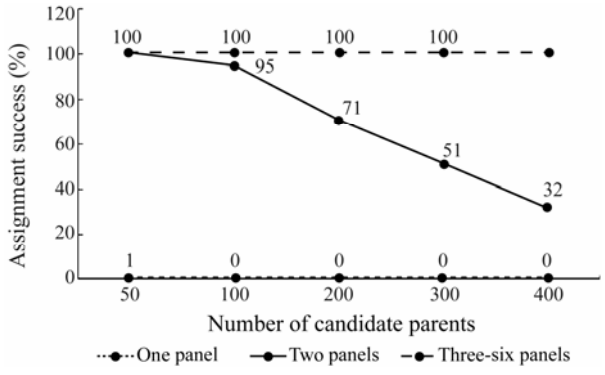

Fig.2 Assignment success rate of simulated genotype data at the 95% confidence level. Each multiplex was added in decreasing order of average polymorphic information content (*PIC*).

In practice, the actual parentage analysis was carried out with twelve single-pair mating families of *C. gigas*. The results demonstrated that 33% of all offsprings were correctly allocated to a pair of parents only based on the most informative multiplex PCR (Panel 3) with the real family data, and 100% of the offsprings were unambiguously assigned to their parents when more than two multiplex PCRs were used (Fig.3). This result is comparable with those reported for *C. gigas* (Li *et al*., 2010) and *C. farreri* (Nie *et al*., 2012) where assignment success rates of two multiplex PCRs were 97% and 100%, respectively.

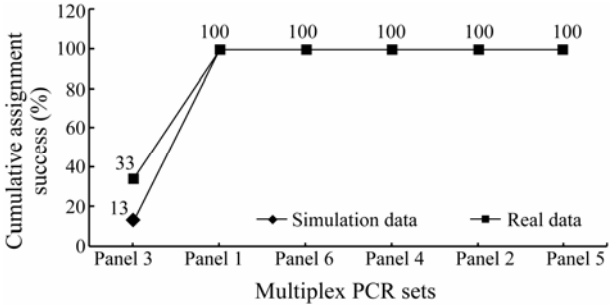

Fig.3 Cumulative assignment success rates of simulated and real genotype data at the 95% confidence level. Each multiplex was added in decreasing order of average polymorphic information content (*PIC*).

Of the 216 genotypic ratios examined (Table 3), 20 genotypic ratios (9.3%) were not compatible with Mendelian segregation after accounting for the presence of null alleles, and 5 were monomorphic (2.3%) leading to offspring identical to the parents. Null alleles in each parental genotype were inferred from offspring genotypes. Among the 18 loci studied here, 89 of the 864 parental alleles were null alleles, which was 10.3% of the total alleles (18 loci × 24 parents × 2). One common phenomenon of microsatellites in bivalves is the presence of null

alleles that fail to amplify to detected levels in the PCR assays. Microsatellites developed from *C. gigas* usually contained particularly high frequency null alleles. For instance, McGoldrick *et al*. (2000) revealed that frequencies of null alleles were above 20% of microsatellites in *C. gigas* stocks. Moreover, null alleles were detected in 46.7% of loci, accounting for 11.7% of the total alleles in a backcross family of *C. gigas* (Li *et al*., 2009). By contrast, our study showed a lower null allele frequency. A high level of sequence polymorphism in PCR primer binding sites may be responsible for the wide spread of null allele in *C. gigas* (Hedgecock *et al*., 2004). However, microsatellite loci affected by null alleles would probably not alter the overall outcome of assignment testing and could therefore be included in parentage analysis (Carlsson, 2008). The discriminatory power of a locus depends on the distribution of its alleles among the parents, but not necessarily on the presence of null alleles (Wang *et al*., 2010). Carlsson (2008) also reported that increased number of loci had more significant effect on the accuracy of assignment testing than the presence of null alleles. This information is valuable for population genetic studies of taxa that are prone to null alleles, as it may enable geneticists to utilize loci affected by null alleles (Carlsson, 2008).

In summary, we have developed and validated six genomic microsatellite multiplex panels using dye-labeled universal primer for *C. gigas*. This research provided a suit of cost-effective and accurate parentage analysis method that can be applied for tracing cultured oyster individuals, population genetic analysis, and selective breeding program in *C. gigas*.

Table 3 Segregation analysis of microsatellite alleles in 12 full-families of *C. gigas*

Family	Multiplex	Locus	Female	Male	Genotypes of progeny	Observed ratio	Expected ratio	*p* value
1	Panel 1	ucdCg-120	170/172	170/null	170/170:170/172:170/null:172/null	9:10:4:15	1:1:1:1	0.093
		ucdCg-198	234/246	234/246	234/234:234/246:246/246	6:23:11	1:2:1	0.341
		ucdCg-117	340/352	340/352	340/340:340/352:352/352	9:17:14	1:2:1	0.341
	Panel 2	Crgi3	171/183	171/183	171/171:171/183:183/183	11:17:9	1:2:1	0.795
		ucdCg-146	212/228	212/228	212/212:212/228:228/228	3:17:19	1:2:1	**0.001**
		uscCgi-210	null/346	null/342	null/null:null/342:null/346:342/346	11:8:8:12	1:1:1:1	0.727
	Panel 3	ucdCg-170	136/138	132/138	132/136:132/138:136/138:138/138	14:6:14:6	1:1:1:1	0.094
		ucdCg-156	null/null	179/null	179/null:null/null	20:18	1:1	0.746
		ucdCg-199	285/288	285/285	285/285:285/288	12:27	1:1	**0.016**
	Panel 4	otgfa0_0129_E11	179/183	179/183	179/179:179/183:183/183	8:25:6	1:2:1	0.191
		Crgi4	null/269	null/null	null/null:null/269	24:16	1:1	0.777
		otgfa0_0007_B07	297/307	297/307	297/297:297/307:307/307	10:24:5	1:2:1	0.186
	Panel 5	Cgri45	168/178	168/178	168/168:168/178:178/178	9:18:13	1:2:1	0.549
		Cgri39	218/222	218/224	218/218:218/222:218/224:222/224	10:11:10:9	1:1:1:1	0.978
		ucdCg-152	269/269	269/272	269/269:269/272	25:15	1:1	0.114
	Panel 6	otgfa0_408293	140/142	140/142	140/140:140/142:142/142	10:22:8	1:2:1	0.741
		otgfa0_0139_G12	193/195	193/193	193/193:193/195	17:23	1:1	0.343
		ucdCg-200	269/269	269/272	269/269:269/272	15:25	1:1	0.114
2	Panel 1	ucdCg-120	170/176	170/176	170/170:170/176:176/176	17:16:7	1:2:1	**0.037**
		ucdCg-198	null/252	252/261	null/252:null/261:252/252:252/261	9:10:12:9	1:1:1:1	0.896
		ucdCg-117	286/328	286/328	286/286:286/328:328/328	12:18:10	1:2:1	0.741
	Panel 2	Crgi3	171/183	183/183	171/183:183/183	20:20	1:1	1.000
		ucdCg-146	212/228	212/228	212/212:212/228:228/228	9:20:11	1:2:1	0.905
		uscCgi-210	null/null	285/null	285/null:null/null	21:19	1:1	0.752
	Panel 3	ucdCg-170	136/138	136/138	136/136:136/138:138/138	5:28:7	1:2:1	**0.037**
		ucdCg-156	167/179	167/179	167/167:167/179:179/179	7:26:7	1:2:1	0.165
		ucdCg-199	265/286	265/286	265/265:265/286:286/286	6:20:12	1:2:1	0.368
	Panel 4	otgfa0_0129_E11	173/181	173/177	173/173:173/177:173/181:177/181	12:10:10:7	1:1:1:1	0.727
		Crgi4	238/256	256/256	238/256:256/256	16:24	1:1	0.206
		otgfa0_0007_B07	269/297	297/297	269/297:297/297	22:17	1:1	0.423
	Panel 5	Cgri45	168/178	176/176	168/176:176/178	15:25	1:1	0.114
		Cgri39	270/270	250/270	250/270:270/270	16:24	1:1	0.206
		ucdCg-152	284/284	299/311	284/299:284/311	16:23	1:1	0.262
	Panel 6	otgfa0_408293	140/null	130/140	130/140:130/null:140/140:140/null	8:9:10:13	1:1:1:1	0.706
		otgfa0_0139_G12	165/195	193/195	165/193:165/195:193/195:195/195	8:14:8:10	1:1:1:1	0.494
		ucdCg-200	263/266	263/269	263/263:263/266:263/269:266/269	10:16:5:7	1:1:1:1	0.064
3	Panel 1	ucdCg-120	170/172	170/176	170/170:170/172:170/176:172/176	14:12:8:6	1:1:1:1	0.261
		ucdCg-198	234/261	234/246	234/234:234/246:234/261:246/261	8:11:7:14	1:1:1:1	0.392
		ucdCg-117	300/350	300/328	300/300:300/328:300/350:328/350	17:6:9:7	1:1:1:1	0.053
	Panel 2	Crgi3	171/183	171/183	171/171:171/183:183/183	2:19:1	1:2:1	**0.003**
		ucdCg-146	212/228	228/228	212/228:228/228	19:21	1:1	0.752
		uscCgi-210	286/342	286/342	286/286:286/342:342/342	10:21:9	1:2:1	0.928

(*to be continued*)

(*continued*)

Family	Multiplex	Locus	Female	Male	Genotypes of progeny	Observed ratio	Expected ratio	*p* value
	Panel 3	ucdCg-170	136/null	132/null	132/136:132/null:136/null:null/null	12:12:7:8	1:1:1:1	0.546
		ucdCg-156	null/null	null/215	null/null:null/215	22:18	1:1	0.527
		ucdCg-199	265/null	null/null	265/null:null/null	20:20	1:1	1.000
	Panel 4	otgfa0_0129_E11	181/null	177/null	177/181:177/null:181/null:null/null	12:10:9:9	1:1:1:1	0.896
		Crgi4	238/256	256/null	238/256:238/null:256/256:256/null	10:8:13:8	1:1:1:1	0.633
		otgfa0_0007_B07	297/297	297/297	297/297	40	1	–
	Panel 5	Cgri45	168/178	178/178	168/178:178/178	23:17	1:1	0.343
		Cgri39	222/null	218/null	218/222:218/null:222/null:null/null	11:9:11:9	1:1:1:1	0.940
		ucdCg-152	null/272	null/null	null/null:null/272	15:25	1:1	0.114
	Panel 6	otgfa0_408293	null/null	134/null	134/null:null/null	27:13	1:1	**0.027**
		otgfa0_0139_G12	193/195	193/193	193/193:193/195	23:17	1:1	0.343
		ucdCg-200	null/null	null/266	null/null:null/266	15：25	1:1	0.114
4	Panel 1	ucdCg-120	170/172	158/172	158/170:158/172:170/172:172/172	9:8:15:7	1:1:1:1	0.264
		ucdCg-198	234/246	234/246	234/234:234/246:246/246	12:19:8	1:2:1	0.655
		ucdCg-117	340/350	300/350	300/340:300/350:340/350:350/350	11:10:13:6	1:1:1:1	0.457
	Panel 2	Crgi3	169/183	169/183	169/169:169/183:183/183	7:20:13	1:2:1	0.407
		ucdCg-146	212/228	212/228	212/212:212/228:228/228	6:28:6	1:2:1	**0.041**
		uscCgi-210	342/346	342/346	342/342:342/346:346/346	7:25:8	1:2:1	0.279
	Panel 3	ucdCg-170	132/140	136/140	132/136:132/140:136/140:140/140	15:8:8:9	1:1:1:1	0.334
		ucdCg-156	165/215	167/179	165/167:165/179:167/215:179/215	9:8:10:11	1:1:1:1	0.913
		ucdCg-199	null/286	null/286	null/null:null/286:286/286	7:20:9	1:2:1	0.717
	Panel 4	otgfa0_0129_E11	179/183	179/183	179/179:179/183:183/183	13:20:7	1:2:1	0.407
		Crgi4	238/256	238/256	238/238:238/256:256/256	6:22:12	1:2:1	0.333
		otgfa0_0007_B07	299/307	299/307	299/299:299/307:307/307	6:30:4	1:2:1	**0.006**
	Panel 5	Cgri45	null/null	176/null	176/null:null/null	25:15	1:1	0.114
		Cgri39	250/256	250/250	250/250:250/256	22:18	1:1	0.527
		ucdCg-152	269/272	269/269	269/269:269/272	18:22	1:1	0.527
	Panel 6	otgfa0_408293	140/144	140/144	140/140:140/144:144/144	12:20:8	1:2:1	0.670
		otgfa0_0139_G12	193/193	169/183	169/193:183/193	20:15	1:1	0.398
		ucdCg-200	263/266	263/266	263/263:263/266:266/266	9:19:12	1:2:1	0.760
5	Panel 1	ucdCg-120	170/174	null/null	170/null:174/null	22:17	1:1	0.423
		ucdCg-198	243/249	246/258	243/246:243/258:246/249:249/258	12:6:10:10	1:1:1:1	0.572
		ucdCg-117	284/328	294/346	284/294:284/346:294/328:328/346	4:10:12:12	1:1:1:1	0.210
	Panel 2	Crgi3	171/183	171/183	171/171:171/183:183/183	5:12:23	1:2:1	**0.000**
		ucdCg-146	244/262	228/246	228/244:228/262:244/246:246/262	2:5:15:12	1:1:1:1	**0.005**
		uscCgi-210	339/345	339/339	339/339:339/345	19:20	1:1	0.873
	Panel 3	ucdCg-170	null/142	null/176	null/null:null/142:null/176:142/176	10:8:12:4	1:1:1:1	0.249
		ucdCg-156	229/229	213/229	213/229:229/229	21:18	1:1	0.631
		ucdCg-199	285/285	285/288	285/285:285/288	18:21	1:1	0.631
	Panel 4	otgfa0_0129_E11	169/null	165/177	165/169:165/null:169/177:177/null	2:13:5:20	1:1:1:1	**0.000**
		Crgi4	238/256	238/256	238/238:238/256:256/256	8:18:14	1:2:1	0.333
		otgfa0_0007_B07	299/305	293/299	293/299:293/305:299/299:299/305	18:9:8:4	1:1:1:1	**0.013**
	Panel 5	Cgri45	168/178	168/178	168/168:168/178:178/178	13:12:13	1:2:1	0.076
		Cgri39	220/224	216/222	216/220:216/224:220/222:222/224	9:9:9:4	1:1:1:1	0.490
		ucdCg-152	269/284	269/284	269/269:269/284:284/284	16:18:6	1:2:1	0.067
	Panel 6	otgfa0_408293	null/148	null/152	null/null:null/148:null/152:148/152	5:16:5:13	1:1:1:1	**0.021**
		otgfa0_0139_G12	181/193	181/193	181/181:181/193:193/193	9:19:12	1:2:1	0.760
		ucdCg-200	260/263	257/272	257/260:257/263:260/272:263/272	7:8:14:10	1:1:1:1	0.400
6	Panel 1	ucdCg-120	170/172	172/172	170/172:172/172	31:6	1:1	**0.000**
		ucdCg-198	255/267	261/264	255/261:255/264:261/267:264/267	14:6:12:8	1:1:1:1	0.261
		ucdCg-117	288/302	294/308	288/294:288/308:294/302:302/308	11:9:10:10	1:1:1:1	0.978
	Panel 2	Crgi3	171/183	171/183	171/171:171/183:183/183	10:21:9	1:2:1	0.928
		ucdCg-146	258/260	246/250	246/258:246/260:250/258:250/260	9:9:11:11	1:1:1:1	0.940
		uscCgi-210	333/342	null/null	333/null:null/342	24:16	1:1	0.206
	Panel 3	ucdCg-170	138/146	138/146	138/138:138/146:146/146	14:16:10	1:2:1	0.301
		ucdCg-156	175/185	171/185	171/175:171/185:175/185:185/185	11:9:8:11	1:1:1:1	0.875
		ucdCg-199	285/285	264/288	264/285:285/288	23:16	1:1	0.262
	Panel 4	otgfa0_0129_E11	179/183	171/179	171/179:171/183:179/179:179/183	11:6:14:9	1:1:1:1	0.334
		Crgi4	238/255	238/255	238/238:238/255:255/255	9:21:10	1:2:1	0.928
		otgfa0_0007_B07	297/299	297/299	297/297:297/299:299/299	9:19:12	1:2:1	0.760

(*to be continued*)

(*continued*)

Family	Multiplex	Locus	Female	Male	Genotypes of progeny	Observed ratio	Expected ratio	*p* value
	Panel 5	Cgri45	168/178	168/178	168/168:168/178:178/178	9:17:14	1:2:1	0.341
		Cgri39	214/214	216/222	214/216:214/222	21:19	1:1	0.752
		ucdCg-152	269/278	269/269	269/269:269/278	21:19	1:1	0.752
	Panel 6	otgfa0_408293	138/142	138/138	138/138:138/142	11:28	1:1	**0.006**
		otgfa0_0139_G12	169/173	169/169	169/169:169/173	18:22	1:1	0.527
		ucdCg-200	null/284	null/272	null/null:null/272:null/284:272/284	8:9:9:14	1:1:1:1	0.532
7	Panel 1	ucdCg-120	170/170	170/172	170/170:170/172	24:16	1:1	0.206
		ucdCg-198	243/267	264/264	243/264:264/267	20:20	1:1	1.000
		ucdCg-117	null/null	286/294	286/null:null/294	20:19	1:1	0.873
	Panel 2	Crgi3	183/185	183/183	183/183:183/185	21:19	1:1	0.752
		ucdCg-146	222/238	242/242	222/242:238/242	19:21	1:1	0.752
		uscCgi-210	327/339	339/345	327/339:327/345:339/339:339/345	7:15:8:10	1:1:1:1	0.284
	Panel 3	ucdcg-170	134/134	130/134	130/134:134/134	21:19	1:1	0.752
		ucdCg-156	165/165	165/165	165/165	40	1	–
		ucdCg-199	285/288	285/285	285/285:285/288	17:21	1:1	0.516
	Panel 4	otgfa0_0129_E11	181/183	171/179	171/171:171/183:179/181:179/183	10:10:9:11	1:1:1:1	0.978
		Crgi4	238/256	238/256	238/238:238/256:256/256	12:17:11	1:2:1	0.622
		otgfa0_0007_B07	297/null	null/null	297/null:null/null	16:24	1:1	0.206
	Panel 5	Cgri45	168/178	168/178	168/168:168/178:178/178	9:17:14	1:2:1	0.341
		Cgri39	220/224	222/224	220/222:220/224:222/224:224/224	15:9:7:9	1:1:1:1	0.308
		ucdCg-152	269/278	269/278	269/269:269/278:278/278	8:25:7	1:2:1	0.279
	Panel 6	otgfa0_408293	138/152	140/140	138/140:140/152	16:24	1:1	0.206
		otgfa0_0139_G12	181/193	193/193	181/193:193/193	14:26	1:1	0.058
		ucdCg-200	263/269	266/272	263/266:263/272:266/269:269/272	9:10:11:9	1:1:1:1	0.963
8	Panel 1	ucdCg-120	170/172	170/172	170/170:170/172:172/172	12:18:10	1:2:1	0.741
		ucdCg-198	null/null	234/264	234/null:null/264	17:23	1:1	0.343
		ucdCg-117	308/312	302/302	302/308:302/312	20:20	1:1	1.000
	Panel 2	Crgi3	171/183	171/183	171/171:171/183:183/183	4:27:9	1:2:1	**0.046**
		ucdCg-146	242/254	244/252	242/244:242/252:244/254:252/254	14:7:10:8	1:1:1:1	0.400
		uscCgi-210	342/345	333/339	333/342:333/345:339/342:339/345	9:14:9:8	1:1:1:1	0.532
	Panel 3	ucdCg-170	134/136	134/136	134/134:134/136:136/136	6:20:14	1:2:1	0.202
		ucdCg-156	175/183	183/183	175/183:183/183	23:17	1:1	0.343
		ucdCg-199	285/288	285/285	285/285:285/288	21:19	1:1	0.752
	Panel 4	otgfa0_0129_E11	165/177	167/183	165/167:165/183:167/177:177/183	10:10:10:10	1:1:1:1	1.000
		Crgi4	238/256	238/256	238/238:238/256:256/256	10:15:8	1:2:1	0.773
		otgfa0_0007_B07	295/295	297/299	295/297:295/299	14:25	1:1	0.078
	Panel 5	Cgri45	168/178	168/178	168/168:168/178:178/178	9:16:10	1:2:1	0.855
		Cgri39	220/222	222/222	220/222:222/222	25:15	1:1	0.114
		ucdCg-152	269/269	269/272	269/269:269/272	20:20	1:1	1.000
	Panel 6	otgfa0_408293	140/152	140/140	140/140:140/152	17:19	1:1	0.739
		otgfa0_0139_G12	193/193	193/195	193/193:193/195	19:17	1:1	0.739
		ucdCg-200	null/269	263/269	263/null:263/269:null/269:269/269	10:8:12:9	1:1:1:1	0.826
9	Panel 1	ucdCg-120	170/172	170/172	170/170:170/172:172/172	9:23:8	1:2:1	0.622
		ucdCg-198	261/261	261/264	261/261:261/264	17:23	1:1	0.343
		ucdCg-117	294/294	294/302	294/294:294/302	14:26	1:1	0.058
	Panel 2	Crgi3	171/183	171/183	171/171:171/183:183/183	8:23:9	1:2:1	0.622
		ucdCg-146	242/246	238/244	238/242:238/246:242/244:244/246	10:10:9:8	1:1:1:1	0.961
		uscCgi-210	339/339	333/342	333/339:339/342	23:16	1:1	0.262
	Panel 3	ucdCg-170	null/null	null/136	null/136:null/null	17:21	1:1	0.516
		ucdCg-156	183/183	183/187	183/183:183/187	17:23	1:1	0.343
		ucdCg-199	285/285	285/288	285/285:285/288	17:21	1:1	0.516
	Panel 4	otgfa0_0129_E11	null/null	null/183	null/null:null/183	16:24	1:1	0.206
		Crgi4	256/256	256/null	256/256:256/null	23:17	1:1	0.343
		otgfa0_0007_B07	299/299	297/299	297/299:299/299	20:19	1:1	0.873
	Panel 5	Cgri45	168/178	168/178	168/168:168/178:178/178	9:18:12	1:2:1	0.707
		Cgri39	216/null	220/null	216/220:216/null:220/null:null/null	8:9:14:9	1:1:1:1	0.532
		ucdCg-152	269/269	251/269	251/269:269/269	22:18	1:1	0.527
	Panel 6	otgfa0_408293	140/152	140/148	140/140:140/148:140/152:148/152	9:7:14:10	1:1:1:1	0.457
		otgfa0_0139_G12	193/195	193/195	193/193:193/195:195/195	10:20:9	1:2:1	0.962
		ucdCg-200	269/272	269/272	269/269:269/272:272/272	13:16:9	1:2:1	0.409

(*to be continued*)

(*continued*)

Family	Multiplex	Locus	Female	Male	Genotypes of progeny	Observed ratio	Expected ratio	*p* value
10	Panel 1	ucdCg-120	172/172	158/170	158/172:170/172	22:18	1:1	0.527
		ucdCg-198	null/null	243/null	243/null:null/null	20:18	1:1	0.746
		ucdCg-117	294/294	292/294	292/294:294/294	22:17	1:1	0.423
	Panel 2	Crgi3	171/183	171/183	171/171:171/183:183/183	7:20:13	1:2:1	0.407
		ucdCg-146	242/246	254/254	242/254:246/254	21:19	1:1	0.752
		uscCgi-210	339/345	339/339	339/339:339/345	17:23	1:1	0.343
	Panel 3	ucdCg-170	128/132	132/138	128/132:128/138:132/132:132/138	9:12:7:12	1:1:1:1	0.615
		ucdCg-156	181/189	189/189	181/189:189/189	26:14	1:1	0.058
		ucdCg-199	285/285	285/288	285/285:285/288	15:23	1:1	0.194
	Panel 4	otgfa0_0129_E11	171/175	175/183	171/175:171/183:175/175:175/183	15:10:8:7	1:1:1:1	0.284
		Crgi4	238/256	238/256	238/238:238/256:256/256	7:20:13	1:2:1	0.407
		otgfa0_0007_B07	293/299	null/null	293/null:299/null	21:17	1:1	0.516
	Panel 5	Cgri45	176/178	178/178	176/178:178/178	23:17	1:1	0.343
		Cgri39	null/null	222/null	222/null:null/null	18:22	1:1	0.527
		ucdCg-152	269/269	269/278	269/269:269/278	17:23	1:1	0.343
	Panel 6	otgfa0_408293	null/140	134/148	134/null:134/140:null/148:140/148	13:12:5:10	1:1:1:1	0.284
		otgfa0_0139_G12	193/193	193/195	193/193:193/195	16:24	1:1	0.206
		ucdCg-200	269/269	269/284	269/269:269/284	15:25	1:1	0.114
11	Panel 1	ucdCg-120	162/170	170/172	162/170:162/172:170/170:170/172	3:14:13:10	1:1:1:1	0.060
		ucdCg-198	255/null	243/null	243/255:243/null:255/null:null/null	9:7:8:16	1:1:1:1	0.172
		ucdCg-117	308/324	324/336	308/324:308/336:324/324:324/336	10:9:12:9	1:1:1:1	0.896
	Panel 2	Crgi3	183/185	183/189	183/183:183/185:183/189:185/189	19:8:7:6	1:1:1:1	**0.012**
		ucdCg-146	242/258	240/256	240/242:240/258:242/256:256/258	14:7:8:9	1:1:1:1	0.384
		uscCgi-210	339/345	339/339	339/339:339/345	22:18	1:1	0.527
	Panel 3	ucdCg-170	134/150	134/134	134/134:134/150	32:8	1:1	**0.000**
		ucdCg-156	189/193	185/185	185/189:185/193	18:22	1:1	0.527
		ucdCg-199	285/285	285/285	285/285	39	1	–
	Panel 4	otgfa0_0129_E11	177/181	165/175	165/177:165/181:175/177:175/181	16:8:4:9	1:1:1:1	**0.044**
		Crgi4	253/256	256/256	253/256:256/256	17:22	1:1	0.423
		otgfa0_0007_B07	289/297	295/297	289/295:289/297:295/297:297/297	14:12:2:12	1:1:1:1	**0.032**
	Panel 5	Cgri45	178/178	178/182	178/178:178/182	20:19	1:1	0.873
		Cgri39	214/216	218/220	214/218:214/220:216/218:216/220	12:7:9:12	1:1:1:1	0.615
		ucdCg-152	269/269	257/269	257/269:269/269	21:19	1:1	0.752
	Panel 6	otgfa0_408293	138/152	null/null	138/null:null/152	17:20	1:1	0.622
		otgfa0_0139_G12	193/193	187/193	187/193:193/193	20:19	1:1	0.873
		ucdCg-200	269/269	263/269	263/269:269/269	22:18	1:1	0.527
12	Panel 1	ucdCg-120	162/172	170/170	162/170:170/172	15:23	1:1	0.194
		ucdCg-198	258/264	258/264	258/258:258/264:264/264	10:17:13	1:2:1	0.509
		ucdCg-117	320/322	300/302	300/320:300/322:302/320:302/322	8:9:12:10	1:1:1:1	0.826
	Panel 2	Crgi3	183/183	183/189	183/183:183/189	20:20	1:1	1.000
		ucdCg-146	234/242	234/256	234/234:234/242:234/256:242/256	11:11:4:14	1:1:1:1	0.145
		uscCgi-210	339/345	333/339	333/339:333/345:339/339:339/345	8:10:13:9	1:1:1:1	0.706
	Panel 3	ucdCg-170	null/null	146/150	null/146:null/150	24:16	1:1	0.206
		ucdCg-156	null/null	null/171	171/null:null/null	15:25	1:1	0.114
		ucdCg-199	270/270	270/282	270/270:270/282	14:26	1:1	0.058
	Panel 4	otgfa0_0129_E11	171/177	177/177	171/177:177/177	16:24	1:1	0.206
		Crgi4	238/256	238/256	238/238:238/256:256/256	8:19:13	1:2:1	0.509
		otgfa0_0007_B07	291/299	299/299	291/299:299/299	23:16	1:1	0.262
	Panel 5	Cgri45	168/178	168/178	168/168:168/178:178/178	10:17:12	1:2:1	0.655
		Cgri39	null/null	220/222	null/220:null/222	18:22	1:1	0.527
		ucdCg-152	269/269	269/269	269/269	39	1	–
	Panel 6	otgfa0_408293	128/136	130/138	128/130:128/138:130/136:136/138	7:13:10:10	1:1:1:1	0.615
		otgfa0_0139_G12	193/193	193/193	193/193	40	1	–
		ucdCg-200	260/263	263/263	260/263:263/263	21:18	1:1	0.631

Notes: Bolded *p* values indicate genotypic ratios that are not conform to Mendelian segregation.

Acknowledgements

This study was supported by the Shandong Seed Project, the National Natural Science Foundation of China (No. 31372524), and Science and Technology Development Plan of Shandong Province, China (No. 2014GHY 115002).

References

An, H. S., Kim, W. J., Lim, H. J., Byun, S. G., Hur, Y. B., Park, J. Y., Myeong, J. I., and An, C. M., 2014. Genetic structure and diversity of *Crassostrea gigas* in Korea revealed from microsatellite markers. *Biochemical Systematics and Ecology*, **55**: 283-291.

An, H. S., Lee, J. W., Kim, W. J., Lim, H. J., Kim, E. M., Byun, S. G., Hur, Y. B., Park, J. Y., Myeong, J. I., and An, C. M., 2013. Comparative genetic diversity of wild and hatchery-produced Pacific oyster (*Crassostrea gigas*) populations in Korea using multiplex PCR assays with nine polymorphic microsatellite markers. *Genes and Genomics*, **35**: 805-815.

Blacket, M. J., Robin, C., Good, R. T., Lee, S. F., and Miller, A. D., 2012. Universal primers for fluorescent labelling of PCR fragments–An efficient and cost-effective approach to genotyping by fluorescence. *Molecular Ecology Resources*, **12**: 456-463.

Borrell, Y. J., Arias-Pérez, A., Freire, R., Valdés, A., Sánchez, J. A., Méndez, J., and Insua, A. M., 2014. Microsatellites and multiplex PCRs for assessing aquaculture practices of the grooved carpet shell *Ruditapes decussatus* in Spain. *Aquaculture*, **426**: 49-59.

Carlsson, J., 2008. Effects of microsatellite null alleles on assignment testing. *Journal of Heredity*, **99**: 616-623.

de Arruda, M. P., Gonçalves, E. C., Schneider, M. P. C., da Silva, A. L. D. C., and Morielle-Versute, E., 2010. An alternative genotyping method using dye-labeled universal primer to reduce unspecific amplifications. *Molecular Biology Reports*, **37**: 2031-2036.

Edwards, M. C., and Gibbs, R. A., 1994. Multiplex PCR: Advantages, development, and applications. *Genome Research*, **3**: S65-S75.

Guichoux, E., Lagache, L., Wagner, S., Chaumeil, P., Léger, P., Lepais, O., and Petit, R. J., 2011. Current trends in microsatellite genotyping. *Molecular Ecology Resources*, **11**: 591-611.

Harrison, H. B., Saenz-Agudelo, P., Planes, S., Jones, G. P., and Berumen, M. L., 2013. Relative accuracy of three common methods of parentage analysis in natural populations. *Molecular Ecology*, **22**: 1158-1170.

Hedgecock, D., Li, G., Hubert, S., Bucklin, K., and Ribes, V., 2004. Widespread null alleles and poor cross-species amplification of microsatellite DNA loci cloned from the Pacific oyster, *Crassostrea gigas*. *Journal of Shellfish Research*, **23**: 379-386.

Henegariu, O., Heerema, N. A., Dlouhy, S. R., Vance, G. H., and Vogt, P. H., 1997. Multiplex PCR: Critical parameters and step-by-step protocol. *Biotechniques*, **23**: 504-511.

Kalinowski, S. T., Taper, M. L., and Marshall, T. C., 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, **16**: 1099-1106.

Kang, J. H., Lim, H. J., Kang, H. S., Lee, J. M., Baby, S., and Kim, J. J., 2013. Development of genetic markers for triploid verification of the Pacific oyster, *Crassostrea gigas*. *Asian-Australasian Journal of Animal Sciences*, **26**: 916.

Kochmann, J., Carlsson, J., Crowe, T. P., and Mariani, S., 2012. Genetic evidence for the uncoupling of local aquaculture activities and a population of an invasive species-a case study of Pacific oysters (*Crassostrea gigas*). *Journal of Heredity*, **103**: 661-671.

Li, G., Hubert, S., Bucklin, K., Ribes, V., and Hedgecock, D., 2003. Characterization of 79 microsatellite DNA markers in the Pacific oyster *Crassostrea gigas*. *Molecular Ecology Notes*, **3**: 228-232.

Li, L., Guo, X., and Zhang, G., 2009. Inheritance of 15 microsatellites in the Pacific oyster *Crassostrea gigas*: Segregation and null allele identification for linkage analysis. *Chinese Journal of Oceanology and Limnology*, **27**: 74-79.

Li, Q., Park, C., and Kijima, A., 2003. Allelic transmission of microsatellites and application to kinship analysis in newly hatched Pacific abalone larvae. *Fisheries Science*, **69**: 883-889.

Li, Q., Yu, H., and Yu, R., 2006. Genetic variability assessed by microsatellites in cultured populations of the Pacific oyster (*Crassostrea gigas*) in China. *Aquaculture*, **259**: 95-102.

Li, R., Li, Q., Cornette, F., Dégremont, L., and Lapègue, S., 2010. Development of four EST-SSR multiplex PCRs in the Pacific oyster (*Crassostrea gigas*) and their validation in parentage assignment. *Aquaculture*, **310**: 234-239.

McGoldrick, D. J., Hedgecock, D., English, L. J., Baoprasertkul, P., and Ward, R. D., 2000. The transmission of microsatellite alleles in Australian and north American stocks of the Pacific oyster (*Crassostrea gigas*): Selection and null alleles. *Journal of Shellfish Research*, **19**:779-788

Miller, P. A., Elliott, N. G., Koutoulis, A., Kube, P. D., and Vaillancourt, R. E., 2012. Genetic diversity of cultured, naturalized, and native Pacific oysters, *Crassostrea gigas*, determined from multiplexed microsatellite markers. *Journal of Shellfish Research*, **31**: 611-617.

Morvezen, R., Cornette, F., Charrier, G., Guinand, B., Lapegue, S., Boudry, P., and Laroche, J., 2013. Multiplex PCR sets of novel microsatellite loci for the great scallop *Pecten maximus* and their application in parentage assignment. *Aquatic Living Resources*, **26**: 207-213.

Nie, H., Li, Q., and Kong, L., 2012. Development of four multiplex PCRs in the Zhikong scallop (*Chlamys farreri*) and their validation in parentage assignment. *Biochemical Systematics and Ecology*, **44**: 96-101.

Norris, A. T., Bradley, D. G., and Cunningham, E. P., 2000. Parentage and relatedness determination in farmed Atlantic salmon (*Salmo salar*) using microsatellite markers. *Aquaculture*, **182**: 73-83.

Ogden, R., 2008. Fisheries forensics: The use of DNA tools for improving compliance, traceability and enforcement in the fishing industry. *Fish and Fisheries*, **9**: 462-472.

Qi, H., Wu, Q., Li, L., and Zhang, G., 2009. Development and characterization of microsatellite markers for the Pacific oyster *Crassostrea gigas*. *Conservation Genetics Resources*, **1**: 451-453.

Schuelke, M., 2000. An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology*, **18**: 233-234.

Sekino, M., Hamaguchi, M., Aranishi, F., and Okoshi, K., 2003. Development of novel microsatellite DNA markers from the Pacific oyster *Crassostrea gigas*. *Marine Biotechnology*, **5**: 227-233.

Staudacher, K., Pitterl, P., Furlan, L., Cate, P. C., and Traugott, M., 2011. PCR-based species identification of *Agriotes* larvae. *Bulletin of Entomological Research*, **101**: 201-210.

Taris, N., Baron, S., Sharbel, T., Sauvage, C., and Boudry, P., 2005. A combined microsatellite multiplexing and boiling DNA extraction method for high-throughput parentage analyses in the Pacific oyster (*Crassostrea gigas*). *Aquaculture Research*, **36**: 516-518.

Wang, Y., Wang, X., Wang, A., and Guo, X., 2010. A 16-mi-

crosatellite multiplex assay for parentage assignment in the eastern oyster (*Crassostrea virginica* Gmelin). *Aquaculture*, **308**: S28-S33.

Yamtich, J., Voigt, M. L., Li, G., and Hedgecock, D., 2005. Eight microsatellite loci for the Pacific oyster *Crassostrea gigas*. *Animal Genetics*, **36**: 524-526.

Yu, G. C., Tang, Q. Z., Long, K. R., Che, T. D., Li, M. Z., and Shuai, S. R., 2014. Effectiveness of microsatellite and single nucleotide polymorphism markers for parentage analysis in European domestic pigs. *Genetics and Molecular Research*, **14**: 1362-1370.

Yue, G. H., Xia, J. H., Liu, P., Liu, F., Sun, F., and Lin, G., 2012. Tracing Asian seabass individuals to single fish farms using microsatellites. *PloS One*, **7**: e52721.

(Edited by Qiu Yantao)