JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

Vol. 42, No. 3
June, 2011

# Development and Characterization of 68 Expressed Sequence Tag Derived Simple Sequence Repeat Markers in the Pacific Oyster, *Crassostrea gigas*

Jie Bai, Qi Li[1], Rihao Cong, Wenjing Sun, Jun Liu, and Yanwei Feng

*Fisheries College, Ocean University of China, Qingdao 266003, China*

The Pacific oyster, *Crassotrea gigas*, which was widely distributed along the seashore of China, Korea, and Japan, has been introduced into many countries and become one of the most commercially important species. It has had the highest worldwide production of any cultured aquatic species since 1993; in 2006, world production of this species was 4.6 million metric tons (FAO 2008). Still, Pacific oysters are in an early stage of domestication. Genetic studies, which offer great potential to detect associations between allelic forms of a gene and phenotypes, will accelerate the development of the oyster industry.

For a long time, simple sequence repeats (SSRs) has been widely used for genetic studies (Liu and Cordes 2004). To date, about 214 SSRs were developed in *C. gigas* (Magoulas et al. 1998; Huvet et al. 2000; McGoldrick et al. 2000; Li et al. 2003; Sekino et al. 2003; Yu and Li 2007, 2008; Wang et al. 2008; Qiu et al. 2009a, 2009b; Li et al. 2009; Sauvage et al. 2009), including 119 genomic SSRs and 95 expressed sequence tag derived SSRs (EST-SSRs). These SSR markers provide sufficient resource in this species to evaluate wild and cultured genetic resources, but are still deficient for SSR-based mapping studies, which is necessary for the identification and mapping of quantitative trait loci, marker-assisted selection, positional cloning of genes, and contig assembly (Hubert and Hedgecock 2004). Besides, screening for many more loci will also open new possibilities to implement new approaches such as genome scans and population genomics, which require hundreds of markers to cover the entire genome of a species under study (Wenne et al. 2007; Hauser and Seeb 2008).

As the isolation and characterization of SSR markers via traditional methods (i.e., the screening of size-fractionated genomic DNA libraries) are costly and time consuming (Squirrell et al. 2003), identification of EST-SSRs has been extensively used as an alternative strategy. ESTs are part of expressed genes, and the EST-SSRs can be considered as Type I markers and used to map genes of known functions, which are more valuable for comparative gene mapping. Moreover, rapid increase in the availability of EST collection provides abundant resources for large amount of SSR markers design.

In this study, we developed and characterized a set of 68 new EST-SSRs for *C. gigas*. Mendelian segregations were tested for 32 of the markers that were polymorphic in the family studied.

## Materials and Methods

A total of 23,816 *C. gigas* ESTs from Genebank (July 8, 2008) were downloaded and assembled within contigs by DNASTAR Lasergene 7.0 program (DNASTAR Inc., Madison, WI, USA), which made it more convenient to identify SSRs with a bioinformatics pipeline. The SSRHUNTER program (Li and Wan 2005) was used to search for SSRs within this unigene set. The parameters were set for detection of di-, tri- and tetranucleotide motifs with a minimum of five repeats, respectively. ESTs containing SSRs were annotated using BLASTX and BLASTN tool (http://www.ncbi.nlm.nih.gov/BLAST). Sequence homology was accepted

[1] Corresponding author.

based on a cut-off $E$ value of $1.0 \times 10^{-8}$. Primers flanking EST-SSRs units were designed using the Primer Premier 5.0 program (http://www.premierbiosoft.com/).

To screen for polymorphic EST-SSR markers, 30 wild individuals of *C. gigas* were collected from Weihai, Shandong province, China. Genomic DNA was extracted from adductor muscle by standard proteinase K digestion, phenol–chloroform extraction, and DNA precipitation. Polymerase chain reactions (PCRs) were performed in a total of 10 μL volumes containing 0.25 U *Taq* DNA polymerase (Takara Bio Inc., Otsu, Shiga, Japan), 1× PCR buffer, 0.2 mM dNTP mix, 1 μM of each primer set, 1.5 mM $MgCl_2$, and about 100 ng template DNA. PCR was performed on a GeneAmp 9700 PCR System (Applied Biosystems, Foster City, CA, USA) as follows: 3 min at 94 C, 35 cycles of 1 min denaturation at 94 C, 1 min annealing (annealing temperatures for different primer pairs are described in Table 1) and 1 min extension at 72 C, and an additional 5 min extension at 72 C at the end of the 35 cycles. Allele information associated with each polymorphism was screened on 6% denatured polyacrylamide gels and visualized by silver staining (Bassam et al. 1991). A 10-bp DNA ladder (Invitrogen Co., Carlsbad, CA, USA) was used as a reference marker for allele size determination. The number of alleles, the levels of expected, observed heterozygosity ($H_E$, $H_O$), and tests of deviation from Hardy–Weinberg equilibrium (HWE) in EST-SSRs markers were calculated using GENEPOP 4.0 (Rousset 2008). Significant levels were calculated per locus using sequential Bonferroni correction.

To verify the Mendelian inheritance, all polymorphic markers were tested in a full-sib family with two parents and sixty 1-yr-old progeny. Chi-square test was used to measure the goodness-of-fit for expected Mendelian segregation ratios.

## Results and Discussion

Sequence assembly generated 14,883 potential unigenes that contain contigs and singletons from all EST sequences, 778 of the unigenes were found containing SSRs fragments with a proportion of 3.27%. The distribution of the microsatellite unit size proportion was as follows: dinucleotide repeats were the most abundant within *C. gigas* ESTs, accounting for 632 loci (81.2%); tri and tetranucleotide repeats were found in 130 loci (16.7%) and 16 loci (2.1%), respectively. Two hundred of the 778 loci with good and sufficient flanking sequences were selected for primers design and optimization.

Of the 200 primer pairs, 108 (54%) produced single band of the expected size, comprising 68 primer pairs with polymorphic profiles and 40 primer pairs with monomorphic profiles (Table A1, Appendix). Twenty-six primer pairs generated unexpected large size, which was not suitable for genotyping, and 66 primer pairs failed to amplify any product or amplified noisy bands. All the polymorphic loci are different from the published EST-SSRs (Yu and Li 2007, 2008; Wang et al. 2008; Li et al. 2009; Sauvage et al. 2009; Qiu et al. 2009a, 2009b). The presence of introns may be the main reason resulting in failure of PCR amplification. It is not uncommon to find intron as EST is short subsequence of transcribed cDNA. The introns may exist in PCR products, causing it too large to be amplified, or in primer sequences, causing it unable to bind to the DNA template (Wang et al. 2009). The 68 EST sequences were BLAST searched against GenBank. Fifteen ESTs had homology to known genes and predicted proteins from other organisms.

The observed number of alleles ranged from 3 to 13 with an average of 4.9 alleles per locus (Table 1). Observed heterozygosities varied from 0.0333 to 0.9667, whereas expected heterozygosities varied from 0.0655 to 0.9149. Twenty-two of the loci deviated significantly from HWE after a Bonferroni correction. Several factors including lack of random mating, sampling effect, and null alleles may explain the problem (Sekino et al. 2003). Significant linkage disequilibrium was detected among 12 loci (CgF23, CgF62, CgF78, CgF114, CgF142, CgF147, CgL14, CgL15, CgL26, CgL33, CgL40, and CgL51; $P < 0.01$) before sequential Bonferroni correction for

TABLE 1. *Characterization of 68 polymorphic EST-derived SSRs for* C. gigas.

| Locus | Accession no. | Repeat sequence | Primer sequences (5′ –3′) | Size range (bp) | $T_a$ (C) | $N_a$ | *P* value | $H_O$ | $H_E$ | Homology | Transferability | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | *C. angulata* | *C. ariakensis* |
| CgF11 | CU685132 | $(AT)_5$ | F: TTTGTTATTTGTGACGAAGAC<br>R: TAGGATGTTGGATATTTTGC | 162–168 | 52.0 | 4 | 0.033 | 0.9000 | 0.6780 | Unknown | — | — |
| CgF12 | AM865183 | $(TTG)_7$ | F: GTGTTTCAATGCAGTCCTTTC<br>R: TATCTCACGGTTTCCTGTTCT | 144–160 | 56.0 | 9 | 0.000* | 0.0690 | 0.8336 | Unknown | 2 | 2 |
| CgF15 | AM865584 | $(CT)_5$ | F: TACATTGCGTTGACTGCG<br>R: TCTGGCTTTTAGGATATAGGAC | 112–120 | 55.0 | 5 | 0.312 | 0.5455 | 0.6277 | Unknown | 2 | — |
| CgF22 | AM865298 | $(AT)_7$ | F: GGTACGGAATCCAGAAAA<br>R: GAAAATACACCCAGTAAACAG | 140–146 | 53.4 | 4 | 0.076 | 0.6364 | 0.6753 | Unknown | — | — |
| CgF23 | AM863169 | $(TC)_5$ | F: CATTGATACTCGAAAGCGTG<br>R: CAAAGAGCGGAATCTAAGCA | 170–227 | 56.0 | 4 | 0.000* | 0.0333 | 0.3226 | Unknown | 1 | 1 |
| CgF31 | AM860954 | $(TG)_5$ | F: AATACGGCGATAACAAGGG<br>R: CACAATACCGCTCATCCAAA | 231–235 | 55.6 | 3 | 0.100 | 0.3636 | 0.5368 | Unknown | 2 | 2 |
| CgF36 | AM869532 | $(TA)_5$ | F: TTCTTCCAAAGGATGAACAGTC<br>R: AAACAGGATGGGGCAACG | 160–166 | 52.0 | 4 | 0.000* | 0.4667 | 0.6525 | Unknown | 2 | 3 |
| CgF44 | AM865858 | $(CT)_5$ | F: ATGTCATCTAACGCTGTTTATTG<br>R: GTTTTTGGCGGAAATCTGTA | 134–146 | 54.0 | 5 | 0.000* | 0.2759 | 0.5572 | DHFR-TS | — | — |
| CgF49 | AM859353 | $(TG)_5$ | F: AGTGGAAAACCAGGTAGAAC<br>R: CAGAGCACGCAGGATAAT | 235–239 | 55.4 | 3 | 0.001 | 0.2000 | 0.4994 | Unknown | 2 | — |
| CgF58 | AM858426 | $(CA)_5$ | F: TATTTGTCGTCTGCTTCTCC<br>R: AATCGTTTGTAATGTCCACC | 155–159 | 54.7 | 3 | 0.002 | 0.2000 | 0.4723 | MGC69529 protein | 3 | 2 |
| CgF62 | CU683493 | $(AG)_5$ | F: GCAACAGTAAATGGGATAATAGA<br>R: CAATGAACATCTAAACTGGCTT | 185–205 | 54.5 | 4 | 0.701 | 0.5517 | 0.5330 | Unknown | — | 2 |
| CgF63 | AM856593 | $(AT)_5$ | F: ATTTCACCGCTGTCTTCAT<br>R: AAGTCAGAATGCGTGTTTTAG | 173–179 | 53.7 | 3 | 0.051 | 0.6364 | 0.5411 | Small ubiquitin-related modifier 2 precursor | 2 | 2 |
| CgF73 | AM857677 | $(AT)_5$ | F: CGTTTTGATGGTGGAGAA<br>R: GATGGTAGTGGGACTGGTG | 202–208 | 56.2 | 4 | 0.117 | 0.3636 | 0.5584 | Heat shock protein family B, member 11 | 2 | 2 |
| CgF78 | AM868167 | $(AC)_6$ | F: TTCGCCAATGACGGTTTT<br>R: GCTTCAAAGGATTCAACAGAG | 253–277 | 54.5 | 3 | 0.631 | 0.5172 | 0.4241 | Unknown | — | — |
| CgF80 | EW777716 | $(AG)_7$ | F: CAAGTGAGGGAGTTTTCATT<br>R: GCACCGCAGTAGTCGTAA | 131–135 | 55.5 | 3 | 0.072 | 0.5357 | 0.5578 | Unknown | 3 | 2 |

TABLE 1. *Continued*

| Locus | Accession no. | Repeat sequence | Primer sequences (5′–3′) | Size range (bp) | $T_a$ (C) | $N_a$ | *P* value | $H_O$ | $H_E$ | Homology | Transferability | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | *C. angulata* | *C. ariakensis* |
| CgF81 | AM862918 | $(TGA)_5$ | F: GGGATAGTAGGAGGTTCGC<br>R: AGGGGTGTTCTCTCTCTTTGT | 210–214 | 59.0 | 3 | 0.028 | 0.3333 | 0.5384 | Unknown | — | 3 |
| CgF85 | AM859465 | $(GA)_7(AG)_8$ | F: GCAGTCAATGTTACTCCCTT<br>R: TTCATCATTCCACCCCTC | 192–212 | 55.0 | 11 | 0.000* | 0.1600 | 0.8939 | Unknown | 2 | 1 |
| CgF95 | AM859904 | $(TA)_7$ | F: TCATTTGTTGTTGGAGGGG<br>R: ATCTGGAGGGCTGCTTCA | 289–293 | 56.4 | 3 | 1.000 | 0.3636 | 0.3247 | Unknown | — | 1 |
| CgF103 | AM861608 | $(TA)_5$ | F: ACATTACTTGCGGGGACA<br>R: TGAAACGCTGACAGAGGTG | 122–128 | 56.3 | 4 | 1.000 | 0.3667 | 0.3260 | Unknown | 2 | 3 |
| CgF114 | AM854045 | $(TG)_5$ | F: ATAAAGTTCCCATATCAAAGAAA<br>R: TCAGCACTACAAGGGCAGA | 191–215 | 54.5 | 13 | 0.006 | 0.6897 | 0.7979 | Unknown | 3 | 3 |
| CgF116 | AM854019 | $(GT)_5$ | F: ATCTGATTGGTGCCATAGTTG<br>R: TGGAGGGAGTTGTCATTGTG | 202–206 | 56.9 | 3 | 0.703 | 0.6364 | 0.5065 | Unknown | 2 | 2 |
| CgF121 | AM853915 | $(TGC)_6N(TGC)_6$ | F: CACCAAGGACCATCTGTTC<br>R: AGTCCATTCTGAAGTCCAAAG | 271–279 | 58.0 | 3 | 0.000* | 0.2759 | 0.4017 | Unknown | — | 3 |
| CgF132 | AM857248 | $(AC)_5(CT)_6$ | F: CACTCCGTTGTTGGTAAA<br>R: CGGTGTAAAATGACTCCC | 165–171 | 53.9 | 4 | 0.257 | 0.3636 | 0.5022 | Unknown | 1 | 2 |
| CgF141 | AM857850 | $(AG)_5$ | F: CCACCAGAAAAGTAGTCCCC<br>R: CATTCATCAACCACATTCCCTA | 143–147 | 58.1 | 3 | 1.000 | 0.5455 | 0.4545 | Unknown | — | — |
| CgF142 | AM857834 | $(TA)_5$ | F: GACCAGACAATGCCAAAAG<br>R: CTCCAAAGTCAACATACACCTC | 124–146 | 57.0 | 10 | 0.000* | 0.3077 | 0.8884 | Unknown | 2 | 4 |
| CgF147 | AM857105 | $(AT)_5$ | F: ATGGTCATTGAAGGTCGCTC<br>R: GAAAGAATCGGGCTTGTGAA | 220–240 | 57.0 | 10 | 0.000* | 0.4333 | 0.7955 | D-amino acid oxidase | — | 2 |
| CgF149 | AM855942 | $(AGAC)_5$ | F: ACGCATCCTCCTTATCCT<br>R: TCTTTCTTTCCGTCTTCTTAT | 155–171 | 53.6 | 5 | 0.017 | 0.5333 | 0.5870 | Unknown | 2 | 3 |
| CgF152 | AM855877 | $(AG)_5$ | F: TTTTCACTATGCGAGGGTA<br>R: CGCTGGTGTCGTTTTATC | 181–185 | 54.0 | 3 | 0.000* | 0.0385 | 0.3401 | Unknown | — | — |
| CgF154 | AM856294 | $(AT)_5$ | F: GCAACAGCCAATAATGAACAA<br>R: CAAACAAACTAAATAAGTCCCAAA | 272–278 | 53.8 | 4 | 1.000 | 0.9000 | 0.6503 | Unknown | 1 | 2 |
| CgF157 | AM856195 | $(AAC)_7$ | F: CCAATTTCTAAACCCGATGAT<br>R: ATAGTCCGCCGATTCCCT | 223–233 | 56.0 | 6 | 0.198 | 0.6667 | 0.6658 | Unknown | 2 | — |
| CgL6 | CU681600 | $(TG)_5$ | F: GACGATGCCATTGAAGAT<br>R: TGGTGTTGGGGTCTGTAT | 196–208 | 53.8 | 7 | 0.006 | 0.5667 | 0.8226 | Unknown | 2 | 2 |

TABLE 1. *Continued*

| Locus | Accession no. | Repeat sequence | Primer sequences (5′–3′) | Size range (bp) | $T_a$ (C) | $N_a$ | $P$ value | $H_O$ | $H_E$ | Homology | Transferability | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | *C. angulata* | *C. ariakensis* |
| CgL10 | AM861996 | $(GT)_5$ | F: GCTGTGATTCTGGCATTT<br>R: AGTGGAGTCCCTTCCTTTA | 279–285 | 54.1 | 4 | 1.000 | 0.9667 | 0.6068 | Eukaryotic translation initiation factor | 1 | 1 |
| CgL11 | AM862282 | $(TA)_5$ | F: ATGCCCATAAAACAAGAAA<br>R: GCAACAGCAATCCCTAAA | 214–226 | 51.0 | 7 | 0.000* | 0.5000 | 0.7559 | Unknown | 2 | 2 |
| CgL12 | AM859900 | $(TA)_7$ | F: TCTTTCTCCCCGTCTTTT<br>R: AGCAATACTTACAATCCAATCT | 181–191 | 54.0 | 7 | 0.000* | 0.6667 | 0.7288 | Unknown | — | — |
| CgL13 | CU683312 | $(GA)_5$ | F: TCCCACATTCCATTCCAG<br>R: TTCGTAAAGTTTTCCGTCCT | 339–347 | 54.3 | 5 | 0.754 | 0.4667 | 0.4503 | Unknown | — | — |
| CgL14 | CU684615 | $(AT)_5$ | F: ATTAGGAAAGGGAAACCA<br>R: TCTGAAATAACGGAAGCA | 160–168 | 50.5 | 3 | 0.000* | 0.8667 | 0.5492 | Unknown | 2 | 1 |
| CgL15 | AM859269 | $(TG)_6$ | F: ATGTATCGCAATGCTCCT<br>R: CAACTTGTAACCCATAAACC | 199–205 | 53.2 | 4 | 0.040 | 0.2667 | 0.5435 | Unknown | 2 | 1 |
| CgL17 | AM855244 | $(AT)_6$ | F: GCATTGGACCGAGTGATT<br>R: ATTGTAGCGAGGTATTTGTTG | 390–394 | 54.6 | 3 | 1.000 | 0.5333 | 0.4249 | Unknown | 3 | 2 |
| CgL18 | AM860081 | $(AT)_6$ | F: GGTTGTGATTTTAGCCCA<br>R: GCGTCCTAATAAATACCAGTT | 110–120 | 53.4 | 6 | 0.001 | 0.2667 | 0.4734 | Unknown | — | — |
| CgL21 | CU681592 | $(AC)_5A(AC)_5$ | F: TGGCTGTCAATCAAATAAAT<br>R: CTCCTGCTACTGAGTCTTCC | 154–158 | 54.7 | 3 | 0.020 | 0.4333 | 0.6266 | Unknown | — | 3 |
| CgL23 | CU684689 | $(AC)_5$ | F: AAACTATGGGTAGGGGAGG<br>R: CGTGGAGAAAGGGAAACT | 316–330 | 57.5 | 3 | 0.764 | 0.4286 | 0.3604 | Unknown | 2 | 1 |
| CgL26 | AM857700 | $(TG)_5$ | F: TGATGATTATTCCTGGTCG<br>R: ATAGAGTAAAGTCAATGTCGC | 343–351 | 52.0 | 5 | 0.717 | 0.7241 | 0.6636 | Unknown | 1 | — |
| CgL27 | EE677439 | (TA)5 | F: TACAAGGTGGAATGAGAAAGA<br>R: GAGGCTGACAATAACAATAAGA | 311–315 | 54.3 | 3 | 0.133 | 0.4545 | 0.4978 | Unknown | — | — |
| CgL29 | AM853963 | $(TA)_6$ | F: AGGGTTGAGCAAGCAAAT<br>R: GCCGATAACACTGGAAGC | 361–365 | 55.0 | 3 | 0.307 | 0.7273 | 0.5152 | Unknown | — | 3 |
| CgL30 | AM863302 | $(TCC)_5$ | F: CCCAGAATACGGACAGCAG<br>R: TTATCGGTCGGGAACAGG | 324–330 | 58.5 | 3 | 0.578 | 0.6667 | 0.6672 | ETS-family transcription factor | — | 1 |
| CgL33 | EW778247 | $(TA)_5$ | F: GGAAAGCCAACTGACATAG<br>R: CTCCAAATCTGAACGAAAA | 195–203 | 55.0 | 5 | 0.000* | 0.0667 | 0.5446 | Unknown | 2 | 2 |

TABLE 1. *Continued*

| Locus | Accession no. | Repeat sequence | Primer sequences (5′–3′) | Size range (bp) | $T_a$ (C) | $N_a$ | *P* value | $H_O$ | $H_E$ | Homology | Transferability | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | *C. angulata* | *C. ariakensis* |
| CgL35 | EW778969 | $(TA)_5$ | F: ATTTTCCTGCCTACCAGC<br>R: GCAAAGTCATTTTCCACC | 164–174 | 55.0 | 5 | 0.000* | 0.2500 | 0.7367 | Unknown | — | — |
| CgL40 | EW777557 | $(TC)_{13}C(CT)_7$ | F: CAAATAATGGCGATAAAGG<br>R: TTGATTTCCCAAAACTGC | 207–219 | 52.5 | 7 | 0.000* | 0.8621 | 0.8125 | Unknown | 2 | 2 |
| CgL43 | EW778377 | $(CT)_{24}(CA)_8$ | F: ACTGATGCCGACTTCCTC<br>R: ACCGCTAAATACATACAAACTG | 181–227 | 55.0 | 14 | 0.000* | 0.5000 | 0.9149 | Unknown | — | 2 |
| CgL44 | EW779405 | $(CAT)_5$ | F: AAACCCATTACCACAGCC<br>R: GAAGCATAGACAAGAAAGACG | 245–257 | 55.5 | 5 | 1.000 | 0.5455 | 0.4719 | cAMP responsive element binding protein | — | — |
| CgL45 | EW779289 | $(TGGA)_6$ | F: TCAATGAAGCGACACCTG<br>R: AAGTCAGCGATTATTTACACC | 175–197 | 54.6 | 4 | 0.000* | 0.2333 | 0.6175 | Unknown | — | — |
| CgL46 | EW779136 | $(AG)_{14}$ | F: TGTCATTCCCATTTGCTT<br>R: AGAGTCTTTGTCTGCTTTCC | 230–242 | 53.1 | 7 | 0.111 | 0.6667 | 0.8057 | Unknown | — | — |
| CgL50 | CU684921 | $(AAG)_{14}(GAT)_5$ | F: TGTCCTTTACCTTCCTCCTC<br>R: TTTCGTGGTCCCTTTTGT | 219–231 | 55.3 | 5 | 0.597 | 0.6364 | 0.6883 | Hypothetical protein | — | — |
| CgL51 | CU684705 | $(GA)_7$ | F: GCTTCAGCACAGAATCGC<br>R: AAAGGAGGACAGGGGACT | 149–159 | 57.0 | 6 | 0.770 | 0.6333 | 0.5492 | Unknown | 2 | 3 |
| CgL52 | CU684064 | $(GA)_5$ | F: CGTATCCCCGAGTTTGCT<br>R: GAGCCCGTTCATTCCTTG | 153–159 | 57.0 | 4 | 0.000* | 0.2000 | 0.6653 | FAT tumor suppressor homolog 4 precursor | 2 | 1 |
| CgL54 | CU682812 | $(CG)_5$ | F: TCATTGGCAACATGGTTA<br>R: TGATAGAGGAAAGACGGATA | 210–218 | 52.0 | 4 | 0.000* | 0.1111 | 0.5332 | GTPase RHO1 | 1 | 1 |
| CgL58 | CU682999 | $(ACT)_5$ | F: GAGAACTTGCCTCTGAATC<br>R: TGCCTGGTAAAAGTGAGC | 201–267 | 55.0 | 7 | 0.000* | 0.4828 | 0.7744 | Unknown | 1 | 1 |
| CgL60 | CU683624 | $(AT)_5$ | F: TGATAAACGGCATCAACCT<br>R: TCCGCTTCCTTTTCCATA | 175–183 | 54.0 | 5 | 0.000* | 0.2333 | 0.6435 | Unknown | — | — |
| CgL61 | CU682210 | $(CAC)_5$ | F: CGACACCTGCCATGACCA<br>R: ATAATCCCATCCTGACCGT | 178–183 | 57.5 | 3 | 1.000 | 0.0667 | 0.0655 | Unknown | 3 | 3 |
| CgL64 | CU681748 | $(GTA)_6$ | F: TCTGGCTGAAACAGGTAAA<br>R: TCCGCTAGAACATAGGAATA | 283–298 | 53.5 | 6 | 1.000 | 0.4333 | 0.3774 | Rhodopsin kinase | 1 | 2 |
| CgL70 | AM868574 | $(GT)_5$ | F: TTCCAAGTTCTTAGCCACA<br>R: AACCCCGTCCAAGTAGTC | 202–208 | 55.0 | 4 | 0.016 | 0.3793 | 0.5729 | DNA ligase I | 3 | 3 |

TABLE 1. *Continued*

| Locus | Accession no. | Repeat sequence | Primer sequences (5′–3′) | Size range (bp) | $T_a$ (C) | $N_a$ | *P* value | $H_O$ | $H_E$ | Homology | Transferability | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | *C. angulata* | *C. ariakensis* |
| CgL73 | AM868143 | $(GA)_5N(ATG)_6$ | F: TTTCGTTTTCGTTGCTTG<br>R: TTCATTGTCGTCTGCTGGT | 273–277 | 53.0 | 3 | 0.392 | 0.3000 | 0.3203 | Unknown | — | 1 |
| CgL76 | AM866440 | $(TG)_5$ | F: CCCGCTATTTCATCATCT<br>R: ACACAATTTGCACTCTGGT | 274–284 | 53.0 | 6 | 0.021 | 0.6667 | 0.6486 | Unknown | — | — |
| CgL78 | AM865011 | $(AT)_5$ | F: CGAAATCTATGAGAACAGGTA<br>R: AGGTTTTATGAAAGGCAGA | 112–118 | 52.6 | 4 | 0.211 | 0.6333 | 0.6593 | Reverse transcriptase | 2 | — |
| CgL80 | AM867943 | $(AG)_{16}$ | F: TGTCGTTTACACCCTCGTT<br>R: CTGTCTTGTTGAAGCCCAT | 331–341 | 55.4 | 6 | 0.521 | 0.5455 | 0.7229 | Unknown | 4 | 2 |
| CgL91 | AM862985 | $(AG)_5$ | F: GGACTGGTGGCAGAAGATG<br>R: CGATAGCAGGGAGGATGAT | 140–146 | 58.5 | 4 | 0.572 | 0.4483 | 0.4259 | Unknown | 3 | 3 |
| CgL93 | AM863848 | $(TG)_5$ | F: TACCCCGACGGTTACTCC<br>R: GCCTTCGTGATGTTTCCTG | 131–137 | 58.6 | 3 | 0.604 | 0.4545 | 0.4502 | Hypothetical protein | — | — |
| CgL95 | AM863517 | $(TG)_5$ | F: CAAATACGCCACCTCAAT<br>R: GCATCCGTGTTATCCAAC | 235–247 | 53.9 | 7 | 0.000* | 0.3333 | 0.8407 | Unknown | 2 | 1 |

EST-SSR = expressed sequence tag derived simple sequence repeat; $T_a$ = annealing temperature; $N_a$ = number of alleles; $H_O$ = observed heterozygosity; $H_E$ = expected heterozygosity.

*Indicates significant departure ($P < 0.05$) from expected Hardy–Weinberg equilibrium conditions after correction for multiple tests ($k = 68$).

TABLE 2. *Segregation analysis of 32* C. gigas *EST-SSRs in a full-sib family.*

| Locus | Sire | Dam | Genotypes of progeny | Expected ratio | Observed ratio | *P* value |
|---|---|---|---|---|---|---|
| CgF12 | BN | AN | AB:AN:BN:NN | 1:1:1:1 | 26:12:20:2 | 0.0000* |
| CgF23 | AA | BN | AB:AN | 1:1 | 23:32 | 0.2249 |
| CgF31 | BB | AB | AB:BB | 1:1 | 42:18 | 0.0019 |
| CgF49 | AB | AB | AA:AB:BB | 1:2:1 | 0:60:0 | 0.0000* |
| CgF62 | AA | AB | AA:AB | 1:1 | 29:28 | 0.8946 |
| CgF78 | AB | AA | AB:AA | 1:1 | 30:32 | 0.7995 |
| CgF81 | AB | BB | AB:BB | 1:1 | 20:35 | 0.0431 |
| CgF85 | AB | AC | AA:AB:AC:BC | 1:1:1:1 | 7:20:16:17 | 0.0993 |
| CgF121 | AB | AB | AA:AB:BB | 1:2:1 | 0:60:0 | 0.0000* |
| CgF141 | AN | BN | AN:BN:AB:NN | 1:1:1:1 | 19:8:29:4 | 0.0000* |
| CgF142 | AN | BN | AN:BN:AB:NN | 1:1:1:1 | 14:20:26:0 | 0.0000* |
| CgF147 | AA | AB | AA:AB | 1:1 | 24:36 | 0.1213 |
| CgF149 | AB | AB | AA:AB:BB | 1:2:1 | 15:34:11 | 0.4493 |
| CgL11 | AB | AB | AA:AB:BB | 1:2:1 | 11:34:15 | 0.4493 |
| CgL15 | AB | AA | AB:AA | 1:1 | 20:40 | 0.0098 |
| CgL17 | BB | AB | AB:BB | 1:1 | 26:34 | 0.3017 |
| CgL18 | AB | BN | AB:AN:BB+BN | 1:1:2 | 15:15:30 | 1.0000 |
| CgL21 | AB | AB | AA:AB:BB | 1:2:1 | 0:60:0 | 0.0000* |
| CgL30 | AN | AN | AA+AN:NN | 3:1 | 54:6 | 0.0073 |
| CgL33 | AB | AB | AA:AB:BB | 1:2:1 | 3:41:17 | 0.0011 |
| CgL35 | AB | BB | AB:BB | 1:1 | 41:19 | 0.0045 |
| CgL40 | BN | AN | AN:BN:AB:NN | 1:1:1:1 | 14:21:18:7 | 0.0620 |
| CgL43 | AC | BC | AB:AC:BC:CC | 1:1:1:1 | 23:17:15:5 | 0.0107 |
| CgL45 | AN | AN | AA+AN:NN | 3:1 | 35:25 | 0.0029 |
| CgL46 | AN | BN | AN:BN:AB:NN | 1:1:1:1 | 8:11:6:35 | 0.0000* |
| CgL58 | AB | AB | AA:AB:BB | 1:2:1 | 0:60:0 | 0.0000* |
| CgL60 | BN | AB | AB:BB+BN:AN | 1:2:1 | 21:37:3 | 0.0016 |
| CgL61 | AB | AB | AA:AB:BB | 1:2:1 | 0:60:0 | 0.0000* |
| CgL78 | AB | AA | AB:AA | 1:1 | 35:25 | 0.1967 |
| CgL91 | AB | AB | AA:AB:BB | 1:2:1 | 0:30:0 | 0.0000* |
| CgL93 | AB | AB | AA:AB:BB | 1:2:1 | 8:40:12 | 0.0273 |
| CgL95 | AB | BN | AB:AN:BB+BN | 1:1:2 | 31:9:20 | 0.0000* |

*Significant deviation ($P < 0.05$) from expected Mendelian ratios after Bonferroni correction ($k = 32$). *N* represents inferred null alleles.

multiple tests (Rice 1989), however, only two pairwise combinations of four loci (CgF114 and CgF147, CgL40, and CgL51) were significant after correction. Cross-species amplification was examined in two other *Crassostrea* species, including *C. angulata* and *Crassotrea ariakensis*. *C. angulata* was collected from Fujian province in China, whereas *C. ariakensis* was sampled from Shandong province in China. Six individuals of each species were used for the examination of the transferability of the 68 EST-SSRs. Fifty primer sets amplified successfully at least one species, with 40 *C. angulata* and 45 *C. ariakensis*.

All the 68 polymorphic EST-SSRs were analyzed in one *C. gigas* family with 60 progeny produced by single-pair mating in 2008. Thirty-six loci were monomorphic (AA × AA genotype) and thus resulted in offspring identical to the parents. The remaining 32 loci were polymorphic and segregated in the family. Genotypic frequencies in the parents and offspring at each of 32 loci were shown in Table 2. Eleven loci showed unexpected progeny phenotypes that were best explained by null alleles (16.2%). The frequency of null alleles is lower compared to genomic SSRs in the *C. gigas* (McGoldrick et al. 2000; Li et al. 2003). Null alleles are in high frequency in SSRs because of the extremely high level of polymorphism likely located just in flanking region to which PCR primers are designed to anneal (Pemberton

et al. 1995). Presence of null alleles may lead to compound results in population genetic studies, but they would be useful for mapping studies and pedigree analysis when controlled crosses were performed (Li and Kijima 2005). In this study, 32.4% of the 68 loci significantly deviating from HWE might result from the existence of null alleles.

Although null alleles were accounted for, 11 loci showed significant deviation from Mendelian ratios after Bonferroni correction. Similar phenomenon was reported in this species in previous literature (McGoldrick et al. 2000). Zygotic viability selection appears to result in departures from Mendelian ratios. Launey and Hedgecock (2001) demonstrated experimentally that high genetic load with resulting strong zygotic selection during the larval stage was the cause of segregation distortion in the Pacific oyster. By genotyping progeny at 6 h after fertilization and then 2–3 mo later, they confirmed that segregation distortion was minimal at the early zygote stage and increased during development, supporting the theory that some of the microsatellite alleles were selected against because of their linkage to highly deleterious fitness gene alleles (Reece et al. 2004).

In conclusion, 68 new SSRs were successfully developed from EST in the Pacific oyster. Although some of the EST-SSRs have low levels of polymorphism, most of them are moderately polymorphic and segregate in Mendelian ratios. They should be useful for genome mapping and population genetics studies.

## Acknowledgments

This study was supported by the grants from National High Technology Research and Development Program (2006AA10A409), 973 Program (2010CB126406) and Chinese Ministry of Education (707041).

## Appendix

TABLE A1. *Primer sequences, repeat types and annealing temperatures for the EST-SSRs generating monomorphic profiles and larger than expected PCR products.*

| Locus | Accession no. | Repeat sequence | Primer sequences (5′–3′) | $T_a$ (C) | Amplification |
|---|---|---|---|---|---|
| CgF1 | AM855712 | $(AC)_5$ | F: TTTCGTATTCGGGGTGTA<br>R: CCTTTTCTTCTTTCGCTTT | 51.9 | Monomorphic |
| CgF10 | AM865741 | $(TG)_5$ | F: CTTGTGGCTGTCGTGTCTC<br>R: CCTTTGGTCAGTTGAAGTATCT | 58.0 | Monomorphic |
| CgF16 | AM865592 | $(GA)_5$ | F: CAGAGGCATTATTCTTGGAC<br>R: CTTTGTGGGTGTATGAGGTG | 56.8 | Monomorphic |
| CgF18 | AM867733 | $(AT)_5$ | F: CGCCAGGTTTTAATGTGATA<br>R: GCCAGTGAAGAACGGGAC | 56.6 | Monomorphic |
| CgF19 | AM869565 | $(AG)_6$ | F: TTTTTGATGATGTTAGTGATTGC<br>R: GGTAAGGTGGGCTGGTCT | 56.3 | Monomorphic |
| CgF20 | EW779017 | $(GA)_5$ | F: GAGCAAGAGGAAGCAGAGA<br>R: CTTTGAGCTAAGTCGGTGTC | 57.7 | Monomorphic |
| CgF21 | EE677618 | $(TA)_6$ | F: TGTGAGGAGGTATGTGGACTG<br>R: TATCTTTGGCGACTTTCTTTT | 56.1 | Monomorphic |
| CgF28 | AM863328 | $(TA)_5$ | F: CTAAACAGTCGGAAAGTCGT<br>R: TGATTACCACAGCAACCAT | 54.5 | Monomorphic |
| CgF38 | EW779419 | $(TA)_7$ | F: TGATTTGCTTGAAGGTTTA<br>R: ACATTTTGCCATCCAGTT | 49.7 | Monomorphic |
| CgF43 | AM863801 | $(TG)_5$ | F: GAAACCGTGGAAGTGTAGG<br>R: ACAGGTATTTGGTGAGTTGAA | 55.9 | Monomorphic |
| CgF48 | AM857705 | $(TA)_5$ | F: TACAATGTTTTAGCACCCAA<br>R: ATGAAGTGACTGTGATAGAAAGA | 53.2 | Monomorphic |
| CgF51 | AM853440 | $(AT)_5$ | F: TAAGAGCATTTATCAGTTTCACC<br>R: AAACATACTCAAGCAATAGGAAG | 54.8 | Monomorphic |

TABLE A1. *Continued*

| Locus | Accession no. | Repeat sequence | Primer sequences (5′–3′) | $T_a$ (C) | Amplification |
|---|---|---|---|---|---|
| CgF53 | AM858587 | $(AT)_5$ | F: AACTACATTCCAATACAGCAA<br>R: AGTCTCCGTAAACACTCAAAC | 54.1 | Monomorphic |
| CgF66 | AM861741 | $(AT)_5$ | F: AGAATGACCGAAGCAAAA<br>R: TTCCCCATAATCTAACGAG | 51.9 | Monomorphic |
| CgF68 | AM858504 | $(TA)_5$ | F: ATGGATACGGATACAAGAAGGA<br>R: AAATGAGGCATCACAAAATAAA | 53.6 | Monomorphic |
| CgF74 | AM857638 | $(TG)_5$ | F: AAATGTTGTCAAGGGGTAGTA<br>R: TTCAGCATAGATAGATTAGTTCAG | 54.6 | Monomorphic |
| CgF87 | AM865354 | $(AT)_5$ | F: AAAACTTCGCTGTCTCCG<br>R: TAGCATTCAGTAGGTGGGC | 56.3 | Monomorphic |
| CgF88 | AM859618 | $(AG)_6(AG)_5$ | F: CCTCCATCTAAAGTGAGCAAA<br>R: CTGTCTGTCTAAGTCCCTGAA | 57.0 | Monomorphic |
| CgF90 | AM869426 | $(AG)_7(AG)_{10}$ | F: GTCAGGAAAAGAAACCAACA<br>R: AAACGCAATCCGTATCAAC | 53.5 | Monomorphic |
| CgF92 | EW778818 | $(AAG)_{11}(GAT)_5$ | F: CCTTTACCTTCCTGCTCG<br>R: TCATTTCGTGGTCCCTTT | 55.0 | Monomorphic |
| CgF93 | CU685019 | $(CT)_5$ | F: TTTTACTGATGGCTGGGAC<br>R: CCGCTTTATTATTGAAACTGA | 53.8 | Monomorphic |
| CgF98 | AM860611 | $(GT)_5$ | F: CAAGTTGTGAGTGCGTGTAT<br>R: CGTATGTATGATTCGTGGC | 55.6 | Monomorphic |
| CgF99 | CU684663 | $(TA)_5$ | F: ACTTTTGAGGGTGGACTTG<br>R: GCCTAATGCTGGTTCTATCT | 55.6 | Monomorphic |
| CgF101 | AM857068 | $(GA)_{27}$ | F: ACATAGGGGACCAACAAAACC<br>R: ATTGAACATTTTAGCAAGCACTG | 56.4 | Monomorphic |
| CgF106 | AM861541 | $(TA)_5$ | F: CCAGCAACACTGATGAGGA<br>R: CTTCTTACTGACACCAAACCC | 57.8 | Monomorphic |
| CgL4 | AM857547 | $(GA)_5$ | F: GTGATGTGGGAGGTGGAT<br>R: CCTGCTCGTGGTAGATTTT | 56.3 | Monomorphic |
| CgL7 | AM866818 | $(GAT)_5N(GAT)_5$ | F: AGCCCAGACAGCAACAGC<br>R: CGTAATCCCCAGGGTCATA | 58.6 | Monomorphic |
| CgL8 | AM860740 | $(TG)_6$ | F: ATGTAAGCCCCTTCCTATC<br>R: ATTCTCAAGCCAAACAGATAC | 54.7 | Monomorphic |
| CgL16 | AM864202 | $(TG)_5$ | F: TGGCGTCTACAAGGAGGT<br>R: CTGTCCACTCGCTCAGATT | 57.4 | Monomorphic |
| CgL42 | EW779482 | $(AT)_6$ | F: GCGTGTCGTCGGTGTAGT<br>R: GCAACCAAGCAGTAGCCT | 58.4 | Monomorphic |
| CgL48 | EW778589 | $(AG)_6N(TAC)_6$ | F: TTTGGACCCCATACAGAA<br>R: CGTTACCACCAGACAGGA | 55.0 | Monomorphic |
| CgL71 | AM866066 | $(GT)_5$ | F: ATGAATGCGGGAAGGTGT<br>R: GTAGCCATAGTGTTCTGGTCTG | 57.5 | Monomorphic |
| CgL77 | AM867538 | $(TA)_5$ | F: TGCTGATGGCTGTAGTGAT<br>R: CCGACCGTAATTTTCTCAA | 54.3 | Monomorphic |
| CgL79 | AM867965 | $(TGA)_5$ | F: AAAACTGGCGATTCTAAAC<br>R: CGATGACAGGATTGATGC | 53.1 | Monomorphic |
| CgL81 | AM868226 | $(AT)_5$ | F: GGCAGATTTACTACGGCTT<br>R: CGTCAGACGTTCAGATTTTAT | 54.8 | Monomorphic |
| CgL83 | AM864076 | $(TTA)_5$ | F: TTGTTCCAGCAAGTCATT<br>R: CTGGGAAAGTTGTTCTCAT | 51.9 | Monomorphic |
| CgL84 | AM862311 | $(TTA)_5$ | F: AGCAGCAGATAAGGGTGG<br>R: CTTTTCAGTTCCTGTTTTGG | 55.5 | Monomorphic |
| CgL88 | AM859001 | $(AG)_5$ | F: CCCCAGATTATGGACTTACT<br>R: TGATGTTATCGTGGACTATGT | 54.9 | Monomorphic |
| CgL89 | AM859691 | $(AT)_5$ | F: AGTGCTGCCGATGTATTT<br>R: TTCTGCTGTACGCTTTTCT | 53.0 | Monomorphic |

TABLE A1. *Continued*

| Locus | Accession no. | Repeat sequence | Primer sequences (5′–3′) | $T_a$ (C) | Amplification |
|---|---|---|---|---|---|
| CgL94 | AM863555 | $(TA)_5$ | F: TAATGGGAAAACCCGAAAC<br>R: GACCCCTAACCCTGGAACT | 56.5 | Monomorphic |
| CgF16 | AM865592 | $(GA)_5$ | F: CAGAGGCATTATTCTTGGAC<br>R: CTTTGTGGGTGTATGAGGTG | 56.5 | Unexpected |
| CgF39 | EW778358 | $(AG)_5$ | F: ATACCATCCATACAACCGA<br>R: ACTACAAAATGCTCCTCACA | 54.5 | Unexpected |
| CgF61 | AM856912 | $(GA)_5$ | F: ACCAAGCCCTACAGTGAG<br>R: TCTTCGGTTTTGTTCCAG | 55.0 | Unexpected |
| CgF115 | AM854041 | $(GT)_5$ | F: ACACTTCAATCCAACTCGTCA<br>R: GGCTGCTCCTCCAATAACA | 56.5 | Unexpected |
| CgF117 | AM858298 | $(TAA)_5$ | F: TCACGATGGCTGAAAACTC<br>R: TTTACCCTTTCTTTCTGATGG | 54.5 | Unexpected |
| CgF131 | AM855797 | $(AG)_5$ | F: CAGAGCCAGTTCCGTAGTGT<br>R: CTTCCAGATCCTTGTTGAGC | 59.0 | Unexpected |
| CgF135 | AM865095 | $(AG)_5$ | F: TTGGATTGATAAATGTCGG<br>R: AGTAGATGGGGACTTTGATGT | 53.5 | Unexpected |
| CgF136 | AM860899 | $(GT)_5$ | F: AAAACAAATATGGCGACG<br>R: GAACAAGGGCTGCTGAAC | 52.5 | Unexpected |
| CgL1 | AM866018 | $(GA)_5$ | F: GAAACCAAGACAATACCGTG<br>R: CCCGACCCCTAACTCCTA | 54.0 | Unexpected |
| CgL5 | AM858438 | $(AC)_5$ | F: TAAAGACATACGGAGACACAACA<br>R: GGACCAGAAACGATGAAACA | 55.5 | Unexpected |
| CgL9 | AM858527 | $(GA)_6$ | F: GTCAGGAGTCGGCAATCA<br>R: CTGTCTCGGCTGTCTTCAA | 54.0 | Unexpected |
| CgL19 | AM855565 | $(AC)_5$ | F: GCTTCCGCATAACAACTG<br>R: CTCGCTTCATTTGGTCTC | 51.0 | Unexpected |
| CgL22 | AM859098 | $(GAA)_5(GAT)_5$ | F: TGAGGATGCTAAACCCAG<br>R: TGCCATTGTTGATTTGACT | 51.0 | Unexpected |
| CgL34 | EW778058 | $(AAC)_6$ | F: GGCGTAACTGTTGGTGGA<br>R: CTGACCGCTTAGTCGTGC | 54.0 | Unexpected |
| CgL36 | EW778928 | $(GGC)_9$ | F: CGGTGACAATCAAAACAGG<br>R: TTTCCAGCATTGGTAAGAGTA | 53.5 | Unexpected |
| CgL37 | EW779216 | $(AGG)_5$ | F: GTCCCTATCTCCCTCGTCT<br>R: TCCAGCCATCCTGTTGAA | 53.5 | Unexpected |
| CgL47 | EW779077 | $(GA)_5$ | F: GAGTATGGCTTGCCTGTC<br>R: GGCGATTCCTCTTGTAGTA | 50.0 | Unexpected |
| CgL56 | CU683135 | $(GCA)_5$ | F: GAAAACGGCATCAACTACA<br>R: CCTTCTCGTGGAGACCTG | 52.0 | Unexpected |
| CgL65 | AM869154 | $(AG)_5$ | F: ATTCGGTATTCGGGTGAC<br>R: CTGGGATTTTCTTCCTGTTT | 53.0 | Unexpected |
| CgL67 | AM868743 | $(GAG)_5$ | F: TTGGAGGACCGAAGTAAG<br>R: CTGTTGGTGGTTGTAGGAG | 53.0 | Unexpected |
| CgL72 | AM866491 | $(GAT)_6$ | F: CACAAGTGGCTCTGAACAA<br>R: CTTTTGAATCTGTATGGGTTT | 51.5 | Unexpected |
| CgL75 | AM864601 | $(AAG)_5$ | F: AAATGGGAAACCAGCAAG<br>R: CATCTATTATCTCCTCCAGAGTG | 53.0 | Unexpected |
| CgL86 | AM858654 | $(GA)_5N(CAT)_5$ | F: AAATTCTACCCTTGCTTCA<br>R: TTTTGTCTTTGGTCCTCTT | 49.5 | Unexpected |
| CgL87 | AM859055 | $(TC)_5$ | F: GTGCTCTTCGGTGATGGA<br>R: TTGTGGCTTAATGGTGGAT | 53.5 | Unexpected |
| CgL90 | AM862244 | $(ATG)_5$ | F: TTTTAGGCGGAGATAGTGA<br>R: CAATTTGGAGCAGCAGATA | 52.0 | Unexpected |
| CgL92 | AM863863 | $(TG)_5$ | F: ACTTGCGTGCGAGTTCTGT<br>R: CGGCATTGTTTCCCCTCT | 57.0 | Unexpected |

EST = expressed sequence tag derived simple sequence repeat; PCR = polymerase chain reaction.

## Literature Cited


**Bassam, B. J., G. Caetano-Anollés, and P. M. Gresshoff.** 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. Analytical Biochemistry 196: 80–83.

**FAO (Food and Agriculture Organization).** 2008. World aquaculture production of fish, crustaceans, molluscs, etc., by principal species. Accessed October 20, 2009, at ftp://ftp.fao.org/fi/stat/summary/default.htm.

**Hauser, L. and J. E. Seeb.** 2008. Advances in molecular technology and their impact on fisheries genetics. Fish and Fisheries 9:473–486.

**Hubert, S. and D. Hedgecock.** 2004. Linkage maps of microsatellite DNA markers for the Pacific oyster *Crassostrea gigas*. Genetics 168:351–362.

**Huvet, A., P. Boudry, M. Ohresser, C. Delsert, and F. Bonhomme.** 2000. Variable microsatellites in the Pacific oyster *Crassostrea gigas* and other cupped oyster species. Animal Genetics 31:71–72.

**Launey, S. and D. Hedgecock.** 2001. High genetic load in the Pacific oyster *Crassostrea gigas*. Genetics 159:255–265.

**Li, Q. and A. Kijima.** 2005. Segregation of microsatellite alleles in gynogenetic diploid Pacific abalone (*Haliotis discus hannai*). Marine Biotechnology 7:669–676.

**Li, Q. and J. M. Wan.** 2005. SSRHUNTER: development of a local searching software for SSR sites. Hereditas 27:808–810.

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

**Li, Q., S. K. Liu, and L. F. Kong.** 2009. Microsatellites within genes and ESTs of the Pacific oyster *Crassostrea gigas* and their transferability in five other *Crassostrea* species. Electronic Journal of Biotechnology 12:3.

**Liu, Z. J. and J. F. Cordes.** 2004. DNA marker technologies and their applications in aquaculture genetics. Aquaculture 238:1–37.

**Magoulas, A., B. Ghjetvaj, V. Terzoglou, and E. Zouros.** 1998. Three polymorphic microsatellites in the Japanese oyster, *Crassostrea gigas* (Thunberg). Animal Genetics 29:69–70.

**McGoldrick, D. J., D. Hedgecock, L. J. English, P. Baoprasertkul, and R. D. Ward.** 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.

**Pemberton, J. M., J. Slate, D. R. Bancroft, and J. A. Barrett.** 1995. Nonamplifying alleles at microsatellite loci: a caution for parentage and population studies. Molecular Ecology 4:249–252.

**Qiu, X. M., S. Z. Liu, X. L. Wang, and X. Y. Meng.** 2009a. Eight SSR loci from oyster *Crassostrea gigas* EST database and cross-species amplification in *C. plicatula*. Conservation Genetics 10:1013–1015.

**Qiu, X. M., X. Li, S. Z. Liu, X. L. Wang, and X. Y. Meng.** 2009b. Eleven polymorphic simple sequence repeat markers from expressed sequence tags of Pacific oyster *Crassostrea gigas* EST database. Conservation Genetics 10:1773–1775.

**Reece, K. S., W. L. Ribeiro, P. M. Gaffney, R. B. Carnegie, and S. K. Allen.** 2004. Microsatellite marker development and analysis in the eastern oyster (*Crassostrea virginica*): Confirmation of null alleles and non-Mendelian segregation ratios. Journal of Heredity 95:346–352.

**Rice, W. R.** 1989. Analyzing tables of statistical tests. Evolution 43:223–225.

**Rousset, F.** 2008. GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resource 8:103–106.

**Sauvage, C., P. Boudry, and S. Lapègue.** 2009. Identification and characterization of 18 novel polymorphic microsatellite makers derived from expressed sequence tags in the Pacific oyster *Crassostrea gigas*. Molecular Ecology Resource 9:853–855.

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

**Squirrell, J., P. M. Hollingsworth, M. Woodhead, J. Russell, A. J. Lowe, M. Gibby, and W. Powell.** 2003. How much effort is required to isolate nuclear microsatellites from plants? Molecular Ecology 12: 1339–1348.

**Wang, Y., R. Ren, and Z. Yu.** 2008. Bioinformatic mining of EST-SSR loci in the Pacific oyster, *Crassostrea gigas*. Animal genetics 39:287–289.

**Wang, Y. P., Y. H. Shi, and X. M. Guo.** 2009. Identification and characterization of 66 EST-SSR markers in the eastern oyster *Crassostrea virginica* (Gmelin). Journal of Shellfish Research 28:227–234.

**Wenne, R., P. Boudry, J. Hemmer-Hansen, K. P. Lubieniecki, A. Was, and A. Kause.** 2007. What role for genomics in fisheries management and aquaculture? Aquatic Living Resources 20:241–255.

**Yu, H. and Q. Li.** 2007. EST-SSR markers from the Pacific oyster, *Crassostrea gigas*. Molecular Ecology Notes 7:860–862.

**Yu, H. and Q. Li.** 2008. Exploiting EST databases for the development and characterization of EST-SSRs in the Pacific oyster (*Crassostrea gigas*). Journal of Heredity 99:208–214.