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## Microsatellites within genes and ESTs of the Pacific oyster *Crassostrea* gigas and their transferability in five other *Crassostrea* species

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Keywords: indwelling medical devices, infections, surface attachment.

Abbreviations: AFLP: amplified fragment length polymorphism

EST: expressed sequence tags H<sub>e</sub>: expected heterozygosity H<sub>o</sub>: observed heterozygosity HWE: Hardy-Weinberg equilibrium PCR: polymerase chain reaction SSR: simple sequence repeat

We developed 15 novel polymorphic microsatellites for the Pacific oyster Crassostrea gigas by screening genes and expressed sequence tags (ESTs) found in GenBank. The number of alleles per locus ranged from two to 24 with an average of 8.7, and the values of observed heterozygosity  $(H_0)$  and expected heterozygosity  $(H_e)$ ranged from 0.026 to 0.750 and from 0.120 to 0.947, respectively. No significant pairwise linkage disequilibrium was detected among loci and eight loci Hardy-Weinberg equilibrium. conformed to Transferability of the markers was examined on five other Crassostrea species and all the markers were amplified successfully in at least one species. These new microsatellites should be useful for population genetics, parentage analysis and genome mapping studies of C. gigas and closely related species. The nine markers identified from known genes are expected to be especially valuable for comparative mapping as type I markers.

in Japan, China and Korea, has become a worldwide aquaculture species because of its fast growth rate, high disease resistance and adaptability to different environments. 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 (Food and Agriculture Organization, 2008). Still, oysters are in an early stage of domestication. To improve the increasingly valuable Pacific oyster industry, genetic improvement programs have been initiated in several countries (Langdon et al. 2003), and to date two genetic linkage maps that would facilitate marker-assisted selection, quantitative trait locus (QTL) mapping, and functional genomic research have been developed using amplified fragment length polymorphism (AFLP) (Li and Guo, 2004), and microsatellite markers (Hubert and Hedgecock, 2004). However, the AFLP maps are poorly transferable, and the microsatellite map is only moderately. There is a great need for developing codominant markers in the Pacific oyster.

Microsatellites, or simple sequence repeats (SSRs), are

The Pacific oyster, Crassostrea gigas, naturally distributed

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Locus	Repeats	Primer sequence (5'-3')	Τ.	N. (size range, bp)	Gene identity	Location in the gene	$H_*$	Н.	Р	Accession no.
CGG001	(AT)6	F: GTGCCTCCTCTATGTGGG	60	3(320-340)	Alpha amylase A	1st intron	0.0.50	0.441	0.000*	AF320688
		R: GGATCGCTGTTTCACTCG								
CGG002	(AG)s	F: CAAACAAGGTATCATCCG	54	16 (204-244)	SS ribosome RNA	NTS	0.256	0.888	0.000*	AY765366
		R: AATAATAGCAGCTTCCATC								
CGG003	(AG) <sub>le</sub>	F: GGAGGACAAAGAGGAGGT	58	9(405-413)	Metallothionein	lst intron	0.525	0.812	0.001	AJ242657
		R: CCAGTCCCGCATGTCTAG								
CGG004	(GA)12	F: GAGTATTGAGGAGACGGAC	56	13 (631-639)	Activin-like type 1 æceptor	lst intron	0.105	0.903	*000.0	AJ309316
		R: GACAGTTCATCAGGTGCTTC								
CGG00S	(GA)26	F: AGCACGGCAGAGGATAGG	56	19 (361-415)	Chitinase-like protein l	3rd intron	0.180	0.939	*000.0	AJ971241
		R: ACTATTCGGCGGGTCTCA								
CG G006	(AG)18	F: AGCCTTGAATAATCGGTC	56	7(430-448)	Metallothionein 2	lst intron	0.750	0.594	0.374	AJ297818
		R: ATTTACACCCGTCACCAC								
CGG007	(GA)22	F: ATTGTTTCTGTCTTCGTC	50	3 (345-349)	LPS-binding protein	3UTR	0.026	0.285	0.000*	AY165040
		R: TCGTACAAGAATAAAGGAT								
CGG008	(AG)20	F: TCTCCTCTACCCCGACAG	56	24 (176-254)	Iron regulatory protein l-like protein	lst intron	0.650	0.947	0.000*	AJ579915
		R: GTGATGAACAAACCACCAAC								
CGG009	(AT)s	F: CCTTAACTGCACTGGTCCC	60	2(154-160)	Heat shock protein 68	3UTR	0.083	0.178	0.021	AB122062
		R: CGTGCAATGATAAATGATGC								
CGE027	(AG)s	F: GCCGCCTTTCAGACTTTC	54	8(278-298)	Unknown	Unknown	0.675	0.761	0.274	ES789161
		R: GATGGGACAAACAACGACA								
CGE028	(CT)s	F: CATCTTGTTGTAACCCACG	54	3(246-254)	Unknown	Unknown	0.125	0.120	1.000	DV736587
		R: GCTTCCCTTTAATGATTCTC								
CGE030	(AC)s	F: TGCTGCCTGTGGAATGTT	52	2(193-195)	Unknown	Unknown	0375	0.392	1.000	BQ427330
		R: TTGTGTTGAGTCGCAAGAGT								
CGE031	(CT)s	F: GATCATTGAAAGAAAGTC	48	3(116-124)	Unknown	Unknown	0359	0.403	0.295	BQ426470
		R: CATIGTIGAATAGGTIGT								
CGE032	(AG);	F: TCGTTGAAGGTGACAAGT	55	15(140-172)	Unknown	Unknown	0.667	0.899	0.000*	CX068987
		R: AACCGAACCATTTACATC								
CGE033	(TA)s	F: AGTGCCTTCCTATTGTTG	55	4(174-182)	Unknown	Unknown	0.263	0.411	0.411	BQ426509
		R: GGCGATGATAATGATGAC								

#### Table 1. Characterization of 15 novel polymorphic microsatellites within genes and ESTs for the Pacific oyster Crassostrea gigas.

To, optimal annealing temperature (°C); No, number of alleles; Ho, observed heterozygosity; Ho, expected heterozygosity; P, exact P value for Hardy-Weinberg equilibrium test; \*, statistically significant after Bonferroni conection (P < 0.01/1.5).

extremely useful markers for genetic linkage mapping because of their high polymorphism, abundance, codominance and small length, which facilitates genotyping using polymerase chain reaction (PCR) (Liu et al.1999; Wang et al. 2007). For C. gigas, numerous microsatellites have been developed recently (Li et al. 2003). But, the pace of development has been limited by the time-consuming and labor intensive requirement to construct, enrich and sequence genomic libraries (Edwards et al. 1996). Recently, identification of microsatellites from expressed sequences has been extensively used as an alternative strategy. In addition to requiring less time and money to develop, expressed sequence tags (EST)-derived microsatellites have a number of intrinsic advantages. They tend to be more widely transferable between species, and even genera (Bouck and Vision, 2007). More importantly, because they represent genes, they serve as type I markers, which are more valuable for comparative gene mapping (Liu et al. 1999). To date, a large number of EST-SSRs have been successfully developed from public sequence databases in several aquatic animals, including fish (Serapion et al. 2004), shrimp (Perez et al. 2005), sea urchin (Kong and Li,

2008) and molluscs such as the Eastern oyster (Wang and Guo, 2007) and Pacific oyster (Yu and Li, 2007; Yu and Li, 2008; Wang et al. 2008; Sauvage et al. 2009). Although detection of microsatellites within ESTs generates potential type I markers (Serapion et al. 2004), usually only a small proportion of EST-SSRs shows high identity to previously annotated genes in aquatic species. For example, 16 of 65 (24.6%) showed high identity to annotated genes and was confirmed as type I markers in Atlantic salmon, and similar results have been reported for Eastern oyster (32.1%) and Pacific oyster (37.5%) (Ng et al. 2005; Wang and Guo, 2007; Yu and Li, 2008). In contrast, microsatellites identified from known genes are all type I markers. The identification of specific SSRs in known genes permits not only the location of the genes in linkage maps, but also the unraveling of the biological significance of SSR distribution, expansion, and contraction on the function of the genes themselves (Li et al. 2004). In the present study we report the identification of microsatellites within all known genes and ESTs from C. gigas, as well as their characterization and cross-species amplification in five other commercially important Crassostrea species.

## MATERIALS AND METHODS

All the Pacific oyster gene sequences and 1168 EST sequences were downloaded from GenBank (NCBI, http://www.ncbi.nlm.nih.gov/sites/entrez). The SSRHUNTER program (Li and Wan, 2005) was used to search for SSRs. The criteria used in SSRHUNTER to identify SSRs were as follows: five repeats for di-, tri- and tetranucleotide repeats. EST sequences containing microsatellites were assembled using SeqMan II sequence assembly software (DNASTAR Inc. Madison, USA) for clustering analysis. Sequences containing SSRs were annotated using BLAST software. Primers flanking microsatellites were designed using the Primer Premier 5.0 program.

To evaluate polymorphism, 40 individuals of *C. gigas* collected from coastal waters in Hiroshima, Japan, were used. Genomic DNA was extracted from adductor muscle by standard proteinase K digestion, phenol-chloroform extraction, and DNA precipitation. PCRs were performed in 10- $\mu$ L volumes containing 0.25 U *Taq* DNA polymerase (Takara Inc.), 1 x PCR buffer, 0.2 mM dNTP mix, 1  $\mu$ M of each primer set, 1.5 mM MgCl<sub>2</sub> and about 100 ng template DNA. PCR was performed on a GeneAmp 9700 PCR System (Applied Biosystems) as follows: 3 min at 94°C; 35 cycles of 1 min at 94°C, annealing (Table 1, annealing temperatures) for 1 min, 72°C for 1 min per cycle; followed by 5 min at 72°C. Amplification products were resolved via

6% denaturing polyacrylamide gel, and visualized by silver-staining. A 10-bp DNA ladder (Invitrogen Inc.) was used as a reference marker for allele size determination. The number of alleles ( $N_a$ ), expected heterozygosity ( $H_e$ ) and observed heterozygosity ( $H_o$ ) were calculated using Microsatellite Analyser software (Dieringer and Schlötterer, 2003). Tests for linkage disequilibrium and deviations from Hardy-Weinberg equilibrium (HWE) were performed using the GENEPOP program. Significant levels were calculated per locus using sequential Bonferroni method (Rice, 1989).

### **RESULTS AND DISCUSSION**

A total of 327 gene sequences and 1168 EST sequences were screened for microsatellite repeats, from which 21 gene sequences and 37 EST sequences harbored microsatellite-type repeats. From the sequences containing microsatellites, 41 primer pairs were designed for microsatellite marker optimization. Of the 41 primer pairs, 12 were not easily amplified, 14 were monomorphic (Appendix A) and 15 produced polymorphic profiles of the expected size. All these loci are different from the published EST-SSRs (Yu and Li, 2007; Yu and Li, 2008; Wang et al. 2008; Sauvage et al. 2009). Characterizations of these polymorphic loci are summarized in Table 1. The numbers of alleles ranged from 2 to 24 with an average of 8.7 alleles per locus. The observed and expected heterozygosities ranged from 0.026 to 0.750 and from

T	C. plicatula	C. hongkongensis	C. ariakensis	C. nippona	C. sikamea	
Locus	( <i>n</i> = 30)	( <i>n</i> = 30)	(n = 30)	(n = 30)	( <i>n</i> = 30)	
CGG001	5 (300-316)	—	—	—	6 (310-332)	
CGG002	7 (206-238)	6 (214-236)	7 (216-236)	5 (210-226)	5 (216-234)	
CGG003	7 (405-421)	1 (330)	1 (330)	1 (310)	5 (407-421)	
CGG004	—	6 (631-651)	3(641-651)	3 (639-643)	4 (628-641)	
CGG005	8 (381-399)	6 (379-397)	5 (381-399)	8 (381-397)	3 (387-395)	
CGG006	3 (436-446)	—	—	1 (436)	4 (434-446)	
CGG007	3 (174-180)	3 (184-188)	1 (186)	1 (186)	1 (216)	
CGG008	14 (190-240)	2 (162-172)	1 (172)	2 (330-336)	7 (196-236)	
CGG009	4 (150-160)	—	—	—	—	
CGE027	5 (282-294)	3 (288-298)	2 (294-298)	3 (288-298)	2 (276-282)	
CGE028	1 (248)	4 (246-254)	3 (248-254)	2 (248-254)	2 (248-254)	
CGE030	1 (195)	2 (195-197)	2(195-197)	1 (195)	2 (189-195)	
CGE031	3 (116-120)	2 (118-122)	3(116-122)	2 (116-118)	5 (114-124)	
CGE032	6 (154-168)	3 (150-156)	_	2 (156-158)	4 (166-174)	
CGE033	3 (178-182)	8 (174-194)	4(176-184)	_	3 (184-190)	

Table 2. Cross-species amplification of 15 microsatellites from *C. gigas* in five other Crassostrea species including *C. plicatula*, *C. hongkongensis*, *C. ariakensis*, *C. nippona* and *C. sikamea*.

Numbers of alleles and allele size range (in parentheses); n, number of samples.

0.120 to 0.947, respectively. No significant pairwise linkage disequilibrium was found among loci. Seven of the 15 microsatellites significantly deviated from the HWE after correction for multiple tests, and all of them showed heterozygote deficiency. We used MICRO-CHECKER (Van Oosterhout et al. 2004) to estimate the most probable cause for departures from HWE. The MICRO-CHECKER analysis suggested there were no indications for scoring error due to stuttering or for large allele dropout. All the seven loci were prone to null alleles (P < 0.01). In fact, null alleles are very common in the Pacific oyster (Li et al. 2003; Hedgecock et al. 2004); due to extremely high levels of DNA sequence variation.

Among the 15 polymorphic markers, nine were identified from known genes, and the other six from ESTs. GenBank (BLASTX) searches indicated that the six SSR-bearing ESTs had no significant matches to genes of known functions at E values less than 10<sup>-4</sup>. The nine microsatellites derived from known genes are all type I markers. Of them, six were located in introns, two (CGG007 and CGG009) in the 3' untranslated region (3'UTR), and one (CGG002) in the non-transcribed spacer (NTS) of 5S ribosome RNA gene (Table 1). Goldstein and Schlötterer (1999) suggest that null alleles in EST-derived SSRs are usually lower than in genomic SSRs due to lower mutation at spicing sites. The fact that all the microsatellites from known genes are located at non-coding sequences might be responsible for high frequency of null alleles (7 of 15 or 46.7%) detected in this study.

Cross-species amplification was examined in five other species, including C. Crassostrea plicatula, С. hongkongensis, C. ariakensis, C. nippona and C. sikamea, which were collected from their typical habitats. C. plicatula and C. hongkongensis were collected from Fujian and Guangxi provinces in China, respectively; C. ariakensis and C. nippona were sampled from the Sea of Ariake and Okatsu Bay, Japan; C. sikamea which originated from Japan were collected from Newport, Oregon, USA. Thirty individuals of each species were used for the examination of the transferability of genic microsatellites. All the primer sets amplified successfully at least one species, with C. plicatula and C. sikamea sharing 14 primer pairs, C. hongkongensis and C. nippona 12, and C. ariakensis 11, indicating the high rate of transferability across Crassostrea species (Table 2).

In summary, these genic SSR markers showed adequate level of polymorphism and high rate of transportability. Thus, they can be used as molecular markers for population genetics, pedigree analysis, and genome mapping studies not only in the Pacific oyster but also in related species. The ones located in genes, as type I markers, are expected to be especially useful for mapping these genes in linkage maps and comparative mapping.

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# APPENDIX

Appendix A. Primer sequences, repeat types and annealing temperatures for 26 unamplified and monomorphic microsatellite loci in this study.

Cgiv12  (TAG)  R. GGGACAATGCCAGTCACTAAA  Unamphifed  AB26    Cgiv12  (TAG)  F. TGGTGCCGTTGGATTTGA  S4  Unamphifed  AB26    CgET1  (CAG)  F. TGCTGCCCATTCAGA  S4  Monemorphic  ES78    CgET2  (TG)  F. TTCTCCAGGATAACGACC  S4  Monemorphic  ES78    CgET3  (GA)  F. TCTCTGGAGTAACGACC  S4  Unamphifed  ES78    CgET3  (GA)  F. GACTCAAGGTGCCCAACG  S8  Monemorphic  ES78    CgET4  (CA)  F. CACAAACAATCAGGGAAAGG  S4  Monemorphic  ES78    CgET5  (GTA)  F. GACTCAGGATAACGACAGG  S6  Monemorphic  ES78    CgET6  (CCG)  F. AAGTGGTGCAGAAGACAGG  S6  Monemorphic  ES78    CgET6  (GTA)  F. AGGTGCGGAAGAGAAC  S2  Unamphifed  ES78    CgET6  (GT)  F. GGGACGGGAAAGAAAAC  S2  Unamphifed  ES78    CgET7  (TO)  F. GGGACGGGAAAGAAAAC  S2  Unamphifed  ES78    CgET12  (GT)  F. GGGACGGGAAAGAAAAC  S2	Locus	Repeats	Primer sequence (5'-3')	Τ	Amplification	Accession no.
Cgiy12  (TAG), R: TGAGGCTTGCCCATTGAGA  S4  Unamplified  AB26    CgET1  (CAG), R: CTGTATTCATTTGGTTGCCTA  S4  Monomorphic  ES78    CgET2  (TG), R: CTGTATTCATTTGGTTGCCTA  S4  Unamplified  ES78    CgET3  (GA), F: GCAAACATCAGGCGCAGG  S4  Monomorphic  ES78    CgET4  (CA), F: CACAAACTCAGGCCAGG  S4  Monomorphic  ES78    CgET4  (CA), F: CACAAACTCGGCCAAGG  S4  Monomorphic  ES78    CgET4  (CA), F: CACAAACATCGGCCACGCAT  C  C  ES78    CgET5  (GTA), F: CACAAACATCGGCACCACGAC  S6  Monomorphic  ES78    CgET6  (CCG), F: AGACGATAACGACCACGAC  S6  Monomorphic  ES78    CgET6  (CGB), F: AGGTGGAGGGAAAGAAAAC  S2  Unamplified  ES78    CgET9  (TC), F: GGGGAGGGGAAAGAAAAC  S2  Unamplified  ES78    CgET10  (ATG), F: GGGAGGGGAAAGAAAAC  S2  Unamplified  ES78    CgET12  (GT), F: GGGAGGGGAAGGAATGA  S2  Unamplified  ES78    CgET12  (GT), F: GGGAGGGGAAGGAATGAA  S2	Cgjyll	(AG)s	F: CACGAAGGGACCCAAAGA	56	Unamplified	AY551097
R. TCAGGCTTCCCCATTCAGA    C      CgET1    (CAG);    F: GTCCCCATTCACTTACCTCAA    54    Monomorphic    ES78      CgET2    (TG):    F: TTTTCTCACGGATACCGACC    54    Unamphified    ES78      CgET3    (GA);    F: GACTCAGGCGATG    C    C      CgET3    (GA);    F: GACTCAGGTCGCCACG    58    Monomorphic    ES78      CgET4    (CA);    F: CACAACAGCGGTAAGG    54    Monomorphic    ES78      CgET4    (CA);    F: CACAACAGCGGTAAGG    54    Monomorphic    ES78      CgET5    (GTA);    F: AGGTGGCAGGAACACCCT    C    C    C    ES78      CgET6    (CCG);    F: AAGTTGGCATCACGGGAAAGAACC    52    Unamphified    ES78      CgET8    (TC);    F: GGGGGGGAAGAAAAC    52    Unamphified    ES78      CgET10    (ATG);    F: GTGCACGGGAAAGAAAAC    52    Unamphified    ES78      CgET10    (ATG);    F: GTGCCCACGGAAAGAAAAAC    52    Unamphified    ES78      CgET10    (ATG);			R: GGG ACAATGCCAG TCACTAAA			
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Cgjy12	(TAG),	F: TGGTGCCGTTGGATTTGA	54	Unamplified	AB262086
CERT    R: CTG TATTCATTTCGTTCCCTA      CgET2    (TG)    F: TTTTCTCACGGATACCACC    S4    Unamplified    ES78      CgET3    (GA)    F: GAACAATCAGCGCACG    S8    Monomorphic    ES78      CgET4    (CA)    F: GACAACATCGGCAAACG    S8    Monomorphic    ES78      CgET4    (CA)    F: GACAACATCGGCAAAGG    S4    Monomorphic    ES78      CgET5    (GTA)    F: GACGAACATCGGCAAAGG    S4    Monomorphic    ES78      CgET6    (CCG)    F: AGACGATAGCACACCACGAC    S6    Monomorphic    ES78      CgET6    (CGG)    F: AGGGACGGCAACGACCACCACCAC    S6    Monomorphic    ES78      CgET8    (GT)    F: GGCACTGGCAACGAACCACCACCACCACCACCACCACCACCACCAC			R: TCACGCTTCCCCATTCAGA			
$\begin{array}{c c} C_{g}ET2 & (TG)_{b} & F: TTTCTCACGGATAACGACC & S4 & Unamplified & ES78 \\ \hline R: GCAAACAATCAGGCGATG & \\ \hline R: CCAAGCAATCAGGCGATG & \\ \hline C_{g}ET3 & (GA)_{s} & F: GACTAAGGTCCCAAGG & S8 & Monomorphic & ES78 \\ \hline R: TTCTCTG AGGCTCTGTTCCAT & \\ \hline C_{g}ET4 & (CA)_{s} & F: AGACGATAACGACCGACG & S6 & Monomorphic & ES78 \\ \hline R: GTTCCCAGGATATTGTTTTC & \\ \hline C_{g}ET5 & (GTA)_{k} & F: AGACGATAACGACCACGAC & S6 & Monomorphic & ES78 \\ \hline R: GTTCCCAGGATAGTTGTCAC & \\ \hline C_{g}ET6 & (CG)_{s} & F: AGATGGTGGAGGAGAGCAG & S6 & Monomorphic & ES78 \\ \hline R: AAGGTCCGATATTGGAAGGAGCAG & \\ \hline C_{g}ET8 & (GT)_{s} & F: GGGGAGGGAAAGAAAAC & S2 & Unamplified & ES78 \\ \hline R: CGACTTGACACGGGAATGA & \\ \hline C_{g}ET9 & (TC)_{s} & F: GGGGAGGGAAAGAAAAC & S2 & Unamplified & ES78 \\ \hline R: CGACTTGACACGGGAATGA & \\ \hline C_{g}ET10 & (ATO)_{s} & F: GGGCATGGATAGCACCT & \\ \hline C_{g}ET12 & (GT)_{s} & F: GAGCTGAATGTGTAAGGACCT & \\ \hline C_{g}ET12 & (GT)_{s} & F: GAGCTGAATGTGTATCTTT & S6 & Monomorphic & DV73 \\ \hline R: TCGGTTGATACTGGATAGCACCT & \\ \hline C_{g}ET16 & (AT)_{s} & F: GACTTCACCAGGATAAACG & S2 & Monomorphic & DV73 \\ \hline R: TCGGTTGATATTGTCCC & \\ \hline C_{g}ET16 & (AT)_{s} & F: GACTTCACCAAGAATACG & S2 & Monomorphic & DV73 \\ \hline R: TCGGTTGATATTGTCCC & \\ \hline C_{g}ET17 & (GT)_{s} & F: GACTTCACCAAGAATACG & S2 & Monomorphic & CX06 \\ \hline R: TCGATTGCAATCTGTTACTCCC & \\ \hline C_{g}ET17 & (GT)_{s} & F: GACTTCACCAAGGGCTGAGAAC & \\ \hline C_{g}ET19 & (AC)_{s} & F: CCACTAGCACCACC & S6 & Monomorphic & CX06 \\ \hline R: TCGACTTCACGAGTTACACCCCC & S6 & Monomorphic & CX06 \\ \hline R: CACATAGACGGGCTGAGAACC & \\ \hline R: CACATAGACGGGCTGAGAACC & \\ \hline R: CACATAGACGGGCTGAGATTCCTT & \\ \hline C_{g}ET20 & (TA)_{s} & F: CCACTCTGAGTTTCCTT & S8 & Unamplified & BQ42 \\ \hline R: AATGGCGTGACTTCCTT & \\ \hline C_{g}ET24 & (CA)_{s} & F: CAGTCTGCTGTGGGCTAGAGTT & \\ \hline C_{g}ET24 & (CA)_{s} & F: CAGACTTGATGTGAGTTCCT & \\ \hline C_{g}ET24 & (CA)_{s} & F: CAGACTTGCATTAGGGCTATA & \\ \hline C_{g}ET27 & (TG)_{s} & F: CTAGCTCTGGGTGAGAGTT & \\ \hline C_{g}ET28 & (AG)_{s} & F: CTGGTATGGGTGAGCATTA & \\ \hline C_{g}ET28 & (AG)_{s} & F: CTGGTATGCAGCACTT & \\ \hline C_{g}ET29 & (GA)_{s} & F: CAGCTTGGTGGTGGCT & \\ \hline C_{g}ET30$	CgET1	(CAG) <sub>5</sub>	F: GTCCCCATCATTACCTCAA	54	Monomorphic	ES789767
R: GCAAACAATCAGGCGATG      CgET3    (GA)s    F: GACTCAAGGTCGCCAACG    S8    Monomorphic    ES78      CgET4    (CA)s    F: CACTAAGGTCGCCAACG    S8    Monomorphic    ES78      CgET5    (GTA)s    F: CACAAACATCGGTAAAGG    S4    Monomorphic    ES78      CgET5    (GTA)s    F: AGACGATAGGACCACCAC    S6    Monomorphic    ES78      CgET6    (CCG)s    F: AAGGTCGAGGAGAGCAG    S6    Monomorphic    ES78      CgET8    (GTb)s    F: GGGGGAGGGAAAGAAAAC    S2    Unamphified    ES78      CgET9    (TC)s    F: GGGGGGGGAAAGAAAAC    S2    Unamphified    ES78      CgET10    (ATG)s    F: GTGCTACGGGAATGA    C    ES78      CgET10    (ATG)s    F: GTGCTACGGAATGAA    C      CgET10    (ATG)s    F: GTGCTACGGATAGAACCT    S2    Unamphified    ES78      CgET16    (ATG)s    F: GTGCTACGGATAGAACCT    S2    Unamphified    ES78      CgET17    (GTb_s    F: CGACTTGACAGGATAGAACT    S2    Unamphifie			R: CTG TATTCATTTCGTTCCCTA			
CgET3  (GA)s  F. GACTCAAGGTCGCCAACG  S8  Monomorphic  ES78    CgET4  (CA)s  F. CACAAACTCGGTAAAGG  S4  Moromorphic  ES78    CgET5  (GTA)k  F. AGACGATAACGACCACGAC  S6  Monomorphic  ES78    CgET5  (GTA)k  F. AGACGATCACCACCT  C  C    CgET6  (CCG)s  F. AAGGTCGGAAGGAGACAG  S6  Monomorphic  ES78    CgET6  (CCG)s  F. AAGGTCGGATAACGACCCT  C  C  C  CgET6  (CCG)s  F. AAGGTCGGAAAGAAAAC  S2  Unamphified  ES78    CgET6  (GT)s  F. GGGGAGGGGAAAGAAAAC  S2  Unamphified  ES78    CgET9  (TC)s  F. GGGGAGGGAAAGAAAAC  S2  Unamphified  ES78    CgET10  (ATG)s  F. GTCTACGGATTCAGGATTCATGA  C  C  C  C    CgET110  (ATG)s  F. TGCTACGGATAGCACCT  C	CgET2	(TG)s	F: TTTCTCACGGATAACGACC	54	Unamplified	ES789749
R: TCTCTG AGCCTCTGTTCCAT      CgET4    (CA)s    F: CACAAACATCGGTAAAGG    54    Monomorphic    ES78      R: GTTOCCAGG ATAGTTCTTC    C    C    CETT    GTTACCAGGATAACGACCACGAC    56    Monomorphic    ES78      CgET5    (GTA)k    F: AGACTATGGCATCCACCCT    C    C      CgET6    (CCG)s    F: AGACTGGTGAGGGAAGCACCACCCC    56    Monomorphic    ES78      CgET8    (GT)s    F: GGGGAGGGGAAAGAAAC    52    Unamplified    ES78      CgET9    (TC)s    F: GGGAGGGGAAAGAAAAC    52    Unamplified    ES78      CgET10    (ATG)s    F: GTTCTACGGATTGACAGGGAATGA    54    Unamplified    ES78      CgET12    (GT)s    F: AGGACTGGATAGGACT    54    Unamplified    ES78      CgET16    (ATG)s    F: AGGACTGTGATAGGACT    54    Unamplified    ES78      CgET17    (GT)s    F: CCATAGCAGGTCAGTCTTGT    56    Monomorphic    DV73      CgET14    (ATG)s    F: AGGACTGCAGTCTTGTT    58    Unamplified    C260			R: GCAAACAATCAGGCGATG			
CgET4  (CA);  F: CACAAACATCGGTAAAGG  54  Monomorphic  ES78    CgET5  (GTA);  F: AGACGATTACGACCACCACCAC  56  Monomorphic  ES78    CgET6  (CCG);  F: AAGTTGGGGGGGAGCACCACC  56  Monomorphic  ES78    CgET6  (CCG);  F: AAGTTGGGGGGGAGCACC  56  Monomorphic  ES78    CgET6  (CCG);  F: AAGTGCGGAAGAGAACC  52  Unamplified  ES78    CgET9  (TC);  F: GGGCAGGGGAAGGAAAAC  52  Unamplified  ES78    CgET10  (ATG);  F: GTCCTACGGATTCAAGGGAATGA  0  0  0    CgET10  (ATG);  F: GGCAGGGGAAGGAAAAC  52  Unamplified  ES78    CgET10  (ATG);  F: GTCCTACGGATTCAAGCT  0  0  0  0    CgET112  (GT);  F: GGACTTCACGATTCAGGATTCTGTG  56  Monomorphic  DV73    R: TCACTATCGGTTAACTTTTCTCC  0  R: TCACTATCGGTTCTTGT  58  Unamplified  CE66    CgET117  (GT);  F: CACGCTTCGGTGTGTTCTT  58  Unamplified  DV73  0  0  0	CgET3	(GA) <sub>s</sub>	F: GACTCAAGGTCGCCAACG	58	Monomorphic	ES789722
R:GTTGCCAGGATAGTCTTC  ES78    CgETS  (GTA)  F:AGACGATAAGGACCACGAC  56  Monomorphic  ES78    CgET6  (CCG)  F:AAGTGGCATCCACCCT  ES78    CgET8  (GT)  F:GGGAGGGGAAGGAGGGACG  56  Monomorphic  ES78    CgET8  (GT)  F:GGGCAGGGGAAGGAGAGAC  52  Unamplified  ES78    CgET9  (TC)  F:GGGGAGGGGAAGGAAGAAAC  52  Unamplified  ES78    CgET9  (TC)  F:GGGGAGGGGAAGGAAGAAAC  52  Unamplified  ES78    CgET10  (ATG)  F:GGCTAGGGATCAAGGGAATGA  ES78  ES78  ES78    CgET12  (GT)  F:GGGAGGGGAAGGAATGAAC  52  Unamplified  ES78    CgET12  (GT)  F:GGCATCAGCTGAGTTCTTGT  54  Unamplified  ES78    CgET12  (GT)  F:AGGACGGTCAGTCATTGTT  54  Monomorphic  DV73    CgET12  (GT)  F:AGGACGGTCAGAATACGCC  C  C  C  C    CgET14  (AT)  F:CCACATAGACGGCTTAGCAC  C  C  C  C  ES78  C  C			R: TCTCTGAGCCTCTGTTCCAT			
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	CgET4	(CA)s	F: CACAAACATCGGTAAAGG	54	Monomorphic	ES789663
C CgET6R: GACTATGGCATCCACCCTC C CgET6CgET6(CCG)sF: AAGTTGGTGGAGGAGCAG56MonomorphicF: GGGGAGGGCAAAGAAAGC52CgET8(GT)sF: GGGGAGGGAAAGAAAAC52CgET9(TC)sF: GGGGAGGGAAAGAAAAAC52CgET10(ATG)sF: GTCTTGACACGGGAATGACgET10(ATG)sF: GTGCTACGGATCAAGAGCACCTCgET112(GT)sF: GGGGAGGGCAAGTAAGCACCTCgET12(GT)sF: GGGGAGGGCAGTTGATGCACCGCgET16(AT)sF: GGGGAGGGTCAGTTCTTGTCgET16(AT)sF: GGCTCACGAGATCAAGGACCTCgET17(GT)sF: CTCCCTTCGGTGTTCCTTCgET18(AGA)sF: ACACTACGGGTAAGCACCCgET19(AC)sF: CTCCCTTCGGTGTTCCTTCgET18(AGA)sF: ACATGCAACGGCTCAGCACCgET19(AC)sF: CACATAGACGGCTGAGAACCgET20(TA)sF: TCCATTCTCGTGATTTCTGTGCgET23(AG)aF: CACATAGACGGACTCCTGTCgET24(CA)sF: CAGACTTAGCAACTTCCTGCgET25(TC)sF: CAGACTTAGCAACTTCCTGTCgET24(CA)sF: CAGACTTAGCAACTTCCTGCgET24(CA)sF: CAGACTTAGCAACTTCCTGCgET25(TC)sF: CAGACTTAGGCAGGCTAGCgET26(TC)sF: CAGACTTACGACCCACCCCCgET27(TG)sF: CAGACTTACGACCACCCCCgET28(AG)sF: CAGACTTACGACCACCCCCCgET29(GAA)sF: AGTGCTGAGCTCCTCgET29(GA)sF: AGAGGCGCAACCACCCCCCgET29(GA)sF: AGACGCACCACCCC			R: GTTGCCAGGATAGTTCTTC			
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	CgETS	(GTA) <sub>6</sub>	F: AGACGATAACGACCACGAC	56	Monomorphic	ES789514
C CgET8R: AAGGTCCGATATTTGAAGCS2CgET8(GT)sF: GGGGAGGGAAAGAAAACS2CgET9(TC)sF: GGGGAGGGAAAGAAAACS2CgET10(ATG)sF: TGTCTAGGGATCAAGGGAATGACgET10(ATG)sF: TGTCTAGGGATCAAGGGAATGACgET112(GT)sF: AGACGTGGATGGATGGAAGGAAAGAATACCgET12(GT)sF: AGACGTCACGTGCTTGTTGTCgET16(ATb)sF: GACTTCACCAAGAATACGCgET16(ATb)sF: GACTTCACCAAGAAATACGCgET16(ATb)sF: GACTTCACCAAGAAATACGCgET17(GT)sF: CTGCCTCGGTGTTCCTTCgET18(AGA)sF: ACATGCAACGACCACCgET19(ACb)sF: CACATGCAACAACCCCCCgET19(ACb)sF: ACATGCAACTACAAAACCCCCCgET19(ACb)sF: ACATGCAACTACAAAACCCCCCgET20(TAb)sF: TCCATCTCCAGGTTTCCTGTCgET23(AGb)sF: TCCATCTCCAGGTTCCTGTCgET24(CAb)sF: CAGATTGACTGAGTGATTCCgET24(CAb)sF: CAGATTGACGAGCAGCTCgET25(TC)sF: CAGACTACCAACCCACCTCgET24(CAb)sF: CAGACTACCAACCCACCTCgET25(TC)sF: CAGACTACCAACCCACCTCgET27(TG)sF: CAGACTACCAACCCACCTCgET28(AG)sF: CAGACTACCAACCCACCTCgET29(GA)sF: CAGACTACCAACCCACCTCgET29(GA)sF: AGAGCTACCAACCCACCTCgET29(GA)sF: AGAGCGCAGGTGAGGTCgET29(GA)sF: AGAGCGCACATTAGGGGAGGTAGTGCgET29(GA)sF: AGAGCGCACATTAGGGGAGGTAGTG			R: GACTATGGCATCCACCCT			
$\begin{array}{c c} C_{g}ET8 & (GT)_{8} & F: GGGGAGGGAAAGAAAAC & S2 Unamplified ES78 \\ \hline R: CGACTTGACACGGGAATGA & \\ \hline C_{g}ET9 & (TC)_{5} & F: GGGGAGGGGAAGAAAAC & S2 Unamplified ES78 \\ \hline R: CGACTTGACACGGGAATGA & \\ \hline C_{g}ET10 & (ATG)_{8} & F: TGTCCTACGGATTCAAGATT & S4 Unamplified ES78 \\ \hline R: CCATAGACTGGATAGCACCT & \\ \hline C_{g}ET12 & (GT)_{5} & F: AGGACGGTCAGTTCTTGT & S6 Monomorphic DV73 \\ \hline R: TTGGTGTAATGTTGTCCC & \\ \hline C_{g}ET16 & (AT)_{5} & F: GACTTCACCAAGAATACG & S2 Monomorphic CX06 \\ \hline R: TCACTATCGGTTACAGCAC & \\ \hline C_{g}ET17 & (GT)_{5} & F: CTGCCTTCGGTGTTCCTT & S8 Unamplified CB61 \\ \hline R: CCACTAGGGTGAGTGCCC & \\ \hline C_{g}ET18 & (AGA)_{5} & F: CTGCTTCGGTGATCCT & S8 Unamplified & CB61 \\ \hline R: CCACTAGCAGGGCTGAGAAC & \\ \hline C_{g}ET18 & (AGA)_{5} & F: CTGCTTCGGTGATTTCGTG & S2 Unamplified & BQ42 \\ \hline R: AGTGCGTGACTTCCTGT & \\ \hline C_{g}ET20 & (TA)_{5} & F: CAGTCTGGTGATTTCGTG & S2 Unamplified & BQ42 \\ \hline R: AGTGGCGTGACTTCCTGT & \\ \hline C_{g}ET23 & (AG)_{6} & F: CAGTCTGGTGATTTCGTG & S2 Unamplified & BQ42 \\ \hline R: CAGCACATATTTGGTGG & S2 Unamplified & BQ42 \\ \hline R: CAGCACATATTTGGTGG & S2 Unamplified & BQ42 \\ \hline R: CAGGACACATTTGTGTGG & S2 Unamplified & BQ42 \\ \hline R: CAGGACACATTTGTGTG & S4 Monomorphic & CX73 \\ \hline R: CAGGACTACCAACCCACC & S6 Unamplified & BQ42 \\ \hline R: CAGGACTATCAACGACGCAGT & \\ \hline C_{g}ET24 & (CA)_{5} & F: CAGTCTGGTGATTCCTGT & \\ \hline C_{g}ET25 & (TC)_{5} & F: CAGTACTACAACGCACGT & \\ \hline C_{g}ET27 & (TG)_{6} & F: CAGACTTACCAACCCACCT & S6 Unamplified & BQ42 \\ \hline R: CAGGACTATCAACAACGCAAGT & \\ \hline C_{g}ET28 & (AG)_{5} & F: GAGTATGAGCAGTTAGGGCAGAGT & \\ \hline C_{g}ET28 & (AG)_{5} & F: GAGTATGAGCAATGGGGGTGACGATT & \\ \hline C_{g}ET28 & (AG)_{5} & F: GAGTATGAGCAGTAGGGTAGG & \\ \hline C_{g}ET29 & (GAA)_{5} & F: AGACTGCTGATGGGGTGACGATTA & \\ \hline C_{g}ET29 & (GAA)_{5} & F: AGACTGCTGATGGGGTGAGGGTAGTG & \\ \hline C_{g}ET29 & (GAA)_{5} & F: AGACTGCTGATGGGGTTAT & \\ \hline C_{g}ET30 & (AG)_{5} & F: AGACTGCTGATGGGTTAT & \\ \hline C_{g}ET30 & (AG)_{5} & F: AGACTGCTGATGGGTTAT & \\ \hline C_{g}ET30 & (AG)_{5} & F: ATGGACTATCAAACAGGAGT & \\ \hline C_{g}ET31 & (CT)_{6} & F: TTGGACTATCACATCCCA & \\ \hline C & \\ \hline C_{g}ET31 & (CT)_{6}$	CgET6	(CCG)s	F: AAGTTGGTGGAGGAGCAG	56	Monomorphic	ES789479
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$			R: AAGGTCCGATATTTGAAGC			
$\begin{array}{c c} C_gET9 & (TC)_s & F: GGGGAGGGAAAGAAAAC & 52 & Unamplified & ES78 \\ \hline R: CGACTTGACACGGGAATGA & \\ \hline C_gET10 & (ATG)_s & F: TG TCTAACGATCAAGATT & 54 & Unamplified & ES78 \\ \hline C_gET12 & (GT)_s & F: AGGACGGTCAGTTCTGT & 56 & Monomorphic & DV73 \\ \hline R: TTGGTGTAATGTTGTCCC & \\ \hline C_gET16 & (AT)_s & F: GACTTCACCAAGAAATACG & 52 & Monomorphic & CX06 \\ \hline R: TCACTATCGGTTACAGCAC & \\ \hline C_gET17 & (GT)_s & F: GACTTCACCAAGAAATACG & 52 & Monomorphic & CX06 \\ \hline R: CCACATAGACGGGCTCAGTCTT & 58 & Unamplified & CB61 \\ \hline C_gET18 & (AGA)_s & F: ACATGCAACTACAACCCC & 56 & Monomorphic & AJ56 \\ \hline R: CACATAGACGGGGCTCAGTGTT & 58 & Unamplified & CB61 \\ \hline R: CACATAGACGGGGCTCAGCAC & \\ \hline C_gET18 & (AGA)_s & F: ACATGCAACTACAACACCCC & 56 & Monomorphic & AJ56 \\ \hline R: TGGATGCCCAATCACAAGG & \\ \hline C_gET19 & (AC)_s & F: TCAGTCTGTGGTATTCGTG & 52 & Unamplified & BQ42 \\ \hline R: AGTGGCGTGACTTCCTGT & \\ \hline C_gET20 & (TA)_s & F: CTAGTCTGTGATTTGTGG & 52 & Unamplified & BQ42 \\ \hline R: GAGCACATATTTGAGTTC & 54 & Monomorphic & CX73 \\ \hline R: CAAGATTGAGCAAGCTACAACCCCT & 56 & Unamplified & BQ42 \\ \hline C_gET24 & (CA)_s & F: CAGATTGAGCAAGGTTA & \\ \hline C_gET25 & (TC)_s & F: CCAACTACCAACCCACCT & 56 & Unamplified & BQ42 \\ \hline R: CAACGATGAGCAACTACCAACCCACCT & 56 & Unamplified & BQ42 \\ \hline C_gET25 & (TC)_s & F: CCAACTACAACAGCGAGTT & \\ \hline C_gET27 & (TG)_s & F: CTGACTACCAACACCCACCT & 56 & Unamplified & BQ42 \\ \hline R: CAACGATGAGCAATTAGCGCAAGT & \\ \hline C_gET28 & (AG)_s & F: GAGTATGAGGGAGAT & 55 & Monomorphic & BQ42 \\ \hline R: CTGTGAACAACGGGCACATTAGGG & \\ \hline C_gET28 & (AG)_s & F: ATACCGTGATGGC & \\ \hline C_gET29 & (GAA)_s & F: AGATGTGATGGGTTTAT & 55 & Monomorphic & BQ42 \\ \hline R: CTGTGTATGAGCAATTGCGCATTA & \\ \hline C_gET30 & (AG)_s & F: ATACCATTGCTGATGGGTTTAT & \\ \hline C_gET30 & (AG)_s & F: ATACCATTGAGGAGGTTTA & \\ \hline C_gET31 & (CT)_e & F: TTGGACTATCACAACCGAT & 55 & Monomorphic & CK17 \\ \hline R: TCAACTATCATACAACGGAT & 55 & Monomorphic & CK17 \\ \hline C_gET31 & (CT)_e & F: TTGGACTATCACATCTCA & 58 & Monomorphic & DV73 \\ \hline \end{array}$	CgET8	(G T)s		52	Unamplified	ES789233
C  R: CGACTTGACACGGGAATGA    CgET10  (ATG)s  F: TG TCCTACGGATTCAAGATT    Status  R: CCATAGACTGGATAGCACCT    CgET12  (GT)s  F: AGGACGGCTAGCTAGCTACCAC    CgET16  (AT)s  F: GACTTCACCAAGAATACG    CgET16  (AT)s  F: GACTTCACCAAGAATACG    CgET16  (AT)s  F: GACTTCACCAAGAATACG    CgET17  (GT)s  F: CTGCCTTCGGTGTTCCTT    Status  R: TCACTATGGGCTACAGCAC  C    CgET17  (GT)s  F: CTGCCTTCGGTGTTCCTT    Status  R: CACATAGAACGGCTCAGAAC  C    CgET18  (AGA)s  F: ACATGCAACTACAACCCCC  56    Monomorphic  AJS6  R: GAGTGGCGTGACTTCCTGT  S2    Unamplified  BQ42  R: AGTGGCGTGACATTCACAAG  C    CgET19  (AC)s  F: TCCATCTCAAGTTCGTG  52  Unamplified  BQ42    CgET20  (TA)s  F: TCCATCTCAAGTTGAGGTTA  C  C  C  C    CgET23  (AG)a  F: CAGACTACCAACCACCT  54  Monomorphic  C  C    CgET24  (CA)s  F: CAGACTACCAACCACCT  56 <t< td=""><td></td><td></td><td>R: CGACTTGACACGGGAATGA</td><td></td><td></td><td></td></t<>			R: CGACTTGACACGGGAATGA			
$\begin{array}{c c} C_gET10 & (ATG)_b & F: TG TCCTACGGATTCAAGATT & S4 & Unamplified & ES78 \\ \hline & R: CCATAGACTGGATAGCACCT & & & & \\ \hline & R: CCATAGACTGGATAGCACCT & & & & \\ \hline & R: CGATGGATAGGTCGTTGTC & S6 & Monomorphic & DV73 \\ \hline & R: TTGGTGTAATGTTGTCCC & & & & \\ \hline & C_gET16 & (AT)_b & F: GACTTCACCAAGAAATACG & S2 & Monomorphic & CX06 \\ \hline & R: TCACTATCGGTTACAGCAC & & & \\ \hline & R: TCACTATCGGTTACAGCAC & & & \\ \hline & R: CACATAGACGGGCTGAGAAC & & & \\ \hline & R: CACATAGACGGGCTGAGAAC & & & \\ \hline & R: CACATAGACGGGCTGAGAAC & & & \\ \hline & R: CACATGCAATCACAACCCCC & S6 & Monomorphic & AJ56 \\ \hline & R: TGGATGCCAATCACAACCCCC & S6 & Monomorphic & BQ42 \\ \hline & R: GGATGCCGATTCGTGTGTGTGTGT & & \\ \hline & C_gET19 & (AC)_s & F: TCCATCTTCAAGTTCGG & S2 & Unamplified & BQ42 \\ \hline & R: AGTGGCGTGACTTCCTGT & & \\ \hline & C_gET20 & (TA)_s & F: TTCCATCTTCAAGTTCGG & S2 & Unamplified & BQ42 \\ \hline & R: GAGCACATATTTGGTGTATA & & \\ \hline & C_gET23 & (AG)_e & F: CAGATTGATGAGAGATTA & & \\ \hline & C_gET24 & (CA)_s & F: CAGACTACCAACCCACCT & S6 & Unamplified & BQ42 \\ \hline & R: CAAGATTGAGCAGAGTTA & & \\ \hline & C_gET25 & (TC)_s & F: TCAACTACAAAATGCTCCTC & 46 & Unamplified & BQ42 \\ \hline & R: ATACGGTGGTGACGACTTC & 54 & Monomorphic & CX73 \\ \hline & C_gET24 & (CA)_s & F: CAGACTACCAACCACCT & S6 & Unamplified & BQ42 \\ \hline & R: CAAGATTGAGCAAGATTA & & \\ \hline & C_gET24 & (CA)_s & F: CAGACTACCAACCGACCT & S6 & Unamplified & BQ42 \\ \hline & R: ATACGGTGGTGACGATTA & & \\ \hline & C_gET28 & (AG)_s & F: AGATGACAATAGCGCAAGT & & \\ \hline & R: ATACGGTGGTGACGATTA & & \\ \hline & C_gET28 & (AG)_s & F: GAGTATGAGGCAGCAGGAG & S4 & Monomorphic & BQ42 \\ \hline & R: GTTGAAACAGAGGCAGCAGCAGG & S4 & Monomorphic & BQ42 \\ \hline & R: GTATCGTTGCTTGCTTGCTC & & \\ \hline & R: GTATCGTTGCATCGAGGGTAGTG & & \\ \hline & C_gET29 & (AG)_s & F: AGATGCTGATGGGTTAT & S5 & Monomorphic & BQ42 \\ \hline & R: GTATCGTTGCATTGCTTTC & & \\ \hline & C_gET30 & (AG)_s & F: ATACGATCAAACGGAT & S5 & Monomorphic & CK17 \\ \hline & R: TCAACTACAAAATGCTCCT & & \\ \hline & R: TCAACTACAAAATGCTCCT & & \\ \hline & R: TCAACTACAAAATGCTCCT & & \\ \hline & C_gET31 & (CT)_e & F: TTGGACTTCACATCACATCCA & S8 & Monomorphic & DV73 \\ \hline & C_gET31 & (CT)_e & F: TTGGACTATCACATCCAATCCAA$	CgET9	(TC),	F: GGGGAGGGGAAAGAAAAC	52	Unamplified	ES789175
$\begin{array}{c c} C_gET10 & (ATG)_b & F: TG TCCTACGGATTCAAGATT & S4 & Unamplified & ES78 \\ \hline & R: CCATAGACTGGATAGCACCT & & & & \\ \hline & R: CCATAGACTGGATAGCACCT & & & & \\ \hline & R: CGATGGATAGGTCGTTGTC & S6 & Monomorphic & DV73 \\ \hline & R: TTGGTGTAATGTTGTCCC & & & & \\ \hline & C_gET16 & (AT)_b & F: GACTTCACCAAGAAATACG & S2 & Monomorphic & CX06 \\ \hline & R: TCACTATCGGTTACAGCAC & & & \\ \hline & R: TCACTATCGGTTACAGCAC & & & \\ \hline & R: CACATAGACGGGCTGAGAAC & & & \\ \hline & R: CACATAGACGGGCTGAGAAC & & & \\ \hline & R: CACATAGACGGGCTGAGAAC & & & \\ \hline & R: CACATGCAATCACAACCCCC & S6 & Monomorphic & AJ56 \\ \hline & R: TGGATGCCAATCACAACCCCC & S6 & Monomorphic & BQ42 \\ \hline & R: GGATGCCGATTCGTGTGTGTGTGT & & \\ \hline & C_gET19 & (AC)_s & F: TCCATCTTCAAGTTCGG & S2 & Unamplified & BQ42 \\ \hline & R: AGTGGCGTGACTTCCTGT & & \\ \hline & C_gET20 & (TA)_s & F: TTCCATCTTCAAGTTCGG & S2 & Unamplified & BQ42 \\ \hline & R: GAGCACATATTTGGTGTATA & & \\ \hline & C_gET23 & (AG)_e & F: CAGATTGATGAGAGATTA & & \\ \hline & C_gET24 & (CA)_s & F: CAGACTACCAACCCACCT & S6 & Unamplified & BQ42 \\ \hline & R: CAAGATTGAGCAGAGTTA & & \\ \hline & C_gET25 & (TC)_s & F: TCAACTACAAAATGCTCCTC & 46 & Unamplified & BQ42 \\ \hline & R: ATACGGTGGTGACGACTTC & 54 & Monomorphic & CX73 \\ \hline & C_gET24 & (CA)_s & F: CAGACTACCAACCACCT & S6 & Unamplified & BQ42 \\ \hline & R: CAAGATTGAGCAAGATTA & & \\ \hline & C_gET24 & (CA)_s & F: CAGACTACCAACCGACCT & S6 & Unamplified & BQ42 \\ \hline & R: ATACGGTGGTGACGATTA & & \\ \hline & C_gET28 & (AG)_s & F: AGATGACAATAGCGCAAGT & & \\ \hline & R: ATACGGTGGTGACGATTA & & \\ \hline & C_gET28 & (AG)_s & F: GAGTATGAGGCAGCAGGAG & S4 & Monomorphic & BQ42 \\ \hline & R: GTTGAAACAGAGGCAGCAGCAGG & S4 & Monomorphic & BQ42 \\ \hline & R: GTATCGTTGCTTGCTTGCTC & & \\ \hline & R: GTATCGTTGCATCGAGGGTAGTG & & \\ \hline & C_gET29 & (AG)_s & F: AGATGCTGATGGGTTAT & S5 & Monomorphic & BQ42 \\ \hline & R: GTATCGTTGCATTGCTTTC & & \\ \hline & C_gET30 & (AG)_s & F: ATACGATCAAACGGAT & S5 & Monomorphic & CK17 \\ \hline & R: TCAACTACAAAATGCTCCT & & \\ \hline & R: TCAACTACAAAATGCTCCT & & \\ \hline & R: TCAACTACAAAATGCTCCT & & \\ \hline & C_gET31 & (CT)_e & F: TTGGACTTCACATCACATCCA & S8 & Monomorphic & DV73 \\ \hline & C_gET31 & (CT)_e & F: TTGGACTATCACATCCAATCCAA$			R: CGACTTGACACGGGAATGA		_	
R: CCATAGACTGGATAGCACCTMonomorphicDV73CgET12(GT)gF: AGGACGGTCAGTTCTTGTS6MonomorphicDV73CgET16(AT)gF: GACTTCACCAAGAAATACGS2MonomorphicCX06CgET17(GT)gF: CTGCCTTCGGTGTTCCTTS8UnamplifiedCB61CgET17(GT)gF: ACATAGAGGGGCTGAGAACCCCgET18(AGA)gF: ACATGCAACTACAACCCCCS6MonomorphicAJ56CgET19(AC)sF: TCGGTGTGGTGATTCCTGS2UnamplifiedBQ42CgET20(TA)gF: TCCAGTTGGTGATTCCTGTCCCgET23(AG)gF: CAGATTGATGAGTTGGTGATTCCCgET24(CA)gF: CAGACTACCAACCACCACCTS6MonomorphicCX73CgET25(TC)gF: CAGACTACCAACCACCACCTS6UnamplifiedBQ42CgET24(CA)gF: CAGACTACCAACCACCTS6UnamplifiedBQ42CgET25(TC)gF: CAGACTACCAACCACCTS6UnamplifiedBQ42CgET27(TG)gF: CAGACTACCAACCACCTS6UnamplifiedBQ42CgET27(TG)gF: CAGACTACCAACCACCTS6UnamplifiedBQ42CgET27(TG)gF: CAGACTACCAACCACCTS6UnamplifiedBQ42CgET27(TG)gF: CAGACTACCAACCACCTS6UnamplifiedBQ42CgET27(TG)gF: CAGACTACCAACCACCTS6UnamplifiedBQ42CgET27(TG)gF: GAGTGGTGGTGGGGGAGGGGGGGGGGGGGGGGGGGGGG	CgET10	(ATG)s		54	Unamplified	ES789168
R: TTGGTGTAATGTTGTCCC  Image: Constraint of the second			R: CCATAGACTGGATAGCACCT		-	
R: TTGGTGTAATGTTGTCCC    CgET16  (AT)s  F: GACTTCACCAAGAAATACG  52  Monomorphic  CX06    CgET17  (GT)s  F: CTGCCTTCGGTGTCCTT  58  Unamplified  CB61    CgET18  (AGA)s  F: ACATAGACGGGCTGAGAAC	CgET12	(GT)s	F: AGGACGGTCAGTTCTTGT	56	Monomorphic	DV736792
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$			R: TTGGTGTAATGTTGTCCC			
$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	CgET16	(AT)s		52	Monomorphic	CX068904
R: CACATAGACGGGCTGAGAAC  CgET18  (AGA) <sub>5</sub> F: ACATGCAACTACAACCCCC  S6  Monomorphic  AJS6    CgET18  (AGA) <sub>5</sub> F: ACATGCAACTACAACCCCC  S6  Monomorphic  AJS6    CgET19  (AC) <sub>5</sub> F: TCAGTCTGGTGATTTCGTG  S2  Unamplified  BQ42    R: AGTGGCGTGACTTCCTGT  C  C  CgET20  (TA) <sub>5</sub> F: TTCCATCTTCAAGTTCGG  S2  Unamplified  BQ42    CgET23  (AG) <sub>6</sub> F: CAGACTATTTTGCGTA  C  C  CZ73    CgET23  (AG) <sub>6</sub> F: CAGACTATTTGAGTC  S4  Monomorphic  CX73    CgET24  (CA) <sub>5</sub> F: CAGACTACCACCACCT  S6  Unamplified  BQ42    R: CCACAGAATGACGCAAGGTA  C  C  CZ73  C  CZ212  (CA) <sub>5</sub> F: CAGACTACCAACCCACCT  S6  Unamplified  BQ42    R: CCACAGAATGACGCAGCAGTA  C  C  CZ73  C  CZ214  CAGACTACCAACCAACCCACCT  S6  Unamplified  BQ42    R: CCACAGAATGACGCAAGTA  S  Monomorphic  BQ42  C  C  CZ217  (TG) <sub>5</sub> F: CAGACTACCAACGCACCT  S6			R: TCACTATCGG TTACAGCAC		-	
R: CAC ATAGACG GGCTG AGAACAlsoCgET18(AGA)sF: AC ATGC AACTAC AACCCCC56MonomorphicAJ56CgET19(AC)sF: TC AGTCTGGTGATTTCGTG52UnamplifiedBQ42CgET20(TA)sF: TTCC ATCTTC AAGTTCGG52UnamplifiedBQ42CgET23(AG)sF: TTCC ATCTTC AAGTTCGG52UnamplifiedBQ42CgET23(AG)sF: CAGATTTGATTGTGGTTACCCgET24(CA)sF: CAGACTACCAACCCACCT54MonomorphicCX73CgET25(TC)sF: CCAGACTACCAACCCACCT56UnamplifiedBQ42CgET25(TC)sF: CCAGACTACCAACCCACCT56UnamplifiedBQ42CgET27(TG)sF: CCACAGAATGACGCACCT56UnamplifiedBQ42CgET25(TC)sF: TCAACTACAAAATGCTCCTC46UnamplifiedBQ42CgET27(TG)sF: GTTGAGACATTAGGGAGAT55MonomorphicBQ42CgET28(AG)sF: GAGATTGAGGCAGCAGCAGTA55MonomorphicBQ42CgET29(GAA)sF: AGATGCTGATGGGTTTAT55MonomorphicCK17CgET30(AG)sF: ATACCATTCATCACACTTTTCCCK17CgET31(CT)6F: TTGGACTATCACATCCACATCCA58MonomorphicDV73	CgET17	(G T),		58	Unamplified	CB617346
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$						
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	CgET18	(AGA) <sub>5</sub>		56	Monomorphic	AJ565768
$\begin{array}{c cccc} C_{g} ET19 & (AC)_{s} & F: TCAGTCTGGTGATTTCGTG & S2 & Unamplified & BQ42 \\ & R: AGTGGCGTGACTTCCTGT & & & \\ C_{g} ET20 & (TA)_{s} & F: TTCCATCTTCAAGTTCGG & S2 & Unamplified & BQ42 \\ & R: GAGCACATATTTTGCGTTA & & & \\ C_{g} ET23 & (AG)_{6} & F: CAGATTGATGTAGTTC & S4 & Monomorphic & CX73 \\ & R: CAAGATTGAGCAGAGTTA & & & \\ C_{g} ET24 & (CA)_{s} & F: CAGACTACCAACCCACCT & S6 & Unamplified & BQ42 \\ & R: CCACAGAATGACGCAAGT & & & \\ C_{g} ET25 & (TC)_{s} & F: TCAACTACAAAATGCTCCTC & 46 & Unamplified & BQ42 \\ & R: ATACGGTGGTGACGATTA & & & \\ C_{g} ET27 & (TG)_{s} & F: GTTGAGACATTAGGGAGAT & S5 & Monomorphic & BQ42 \\ & R: ATACGGTGGTGAGGAGAT & S5 & Monomorphic & BQ42 \\ & R: GTGTGTAAACAGAGGGAGAGG & 54 & Monomorphic & BQ42 \\ & R: GTATCGTTTGCTTTGTGC & & \\ C_{g} ET29 & (GAA)_{s} & F: AGATGCTGATGGGTTTAT & S5 & Monomorphic & CK17 \\ & R: CCCTG TTTCACTCTTTC & & \\ C_{g} ET30 & (AG)_{s} & F: ATACCATTCATACAAACGGAT & S5 & Monomorphic & CK17 \\ & R: TTCAACTACAAAATGCTCCT & & \\ C_{g} ET31 & (CT)_{6} & F: ATACCATTCATACAAACGGAT & S8 & Monomorphic & DV73 \\ \end{array}$						
R: AGTGGCG TGACTTCCTG TImage: Constraint of the system of the	CgET19	(AC)s	F: TCAGTCTGGTGATTTCGTG	52	Unamplified	BQ427090
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$						· · · · · · · · · · · · · · · · · · ·
R: GAGCACATATTTTGCGTTA  CgET23    CgET23  (AG)6  F: CAGATTGAGTGTGTGTAGTTC  54  Monomorphic  CX73    R: CAAGATTGAGCAGAGGTTA  CZGET24  (CA)5  F: CAGACTACCAACCCACCT  56  Unamplified  BQ42    CgET25  (TC)5  F: TCAACTACAAAATGCTCCTC  46  Unamplified  BQ42    CgET27  (TG)5  F: GTTGAGCACATTAGGGAGAT  55  Monomorphic  BQ42    R: ATACGGTGGTGAGCATTA  C  C  E  E  E  E    CgET27  (TG)5  F: GTTGAGACATTAGGGAGAT  S5  Monomorphic  EQ42    R: ATACGGTGGTGAGGGTAGGGGTAGTG  E <td< td=""><td>CgET20</td><td>(TA),</td><td></td><td>52</td><td>Unamplified</td><td>BQ426771</td></td<>	CgET20	(TA),		52	Unamplified	BQ426771
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$						
R: CAAG ATTGAGCAGAGTTA  CgET24    CgET24  (CA) <sub>5</sub> F: CAGACTACCAACCCACCT  56  Unamplified  BQ42    CgET25  (TC) <sub>5</sub> F: TCAACTACAAAATGCTCCTC  46  Unamplified  BQ42    CgET27  (TG) <sub>5</sub> F: TCAACTACAAAATGCTCCTC  46  Unamplified  BQ42    CgET27  (TG) <sub>5</sub> F: GTTGAGACATTAGGGAGAT  55  Monomorphic  BQ42    CgET28  (AG) <sub>5</sub> F: GAGTATGAGGCAGCAGCAGGG  54  Monomorphic  BQ42    CgET29  (GAA) <sub>5</sub> F: AGATGCTGATGGGTTTAT  55  Monomorphic  BQ42    CgET30  (AG) <sub>5</sub> F: ATACCGTTCATCATTCTTTC  CgET31  (CT) <sub>6</sub> F: TTGGACTATCACAACGGAT  58  Monomorphic  CK17	CgET23	(AG)6		54	Monomorphic	CX739659
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$			R: CAAGATTGAGCAGAGTTA		-	
R: CCACAGAA TGACGCAAGT  CgET25    CgET25  (TC) <sub>5</sub> F: TCAACTACAAAATGCTCCTC  46  Unamplified  BQ42    R: ATACGGTGG TGACGATTA  CgET27  (TG) <sub>5</sub> F: GTTGAGACA TTAGGGAGAT  55  Monomorphic  BQ42    CgET28  (AG) <sub>5</sub> F: GAGTATGAGGCAGCAGCAGGG  54  Monomorphic  BQ42    R: GTATCGTTTGCTTTGTGC  C  C  C  C  CK17    R: GTATCGTTGCTTGCTTGTGC  C  C  CK17  C  CK17    R: CCCTG TTTCACTGA TGGGTTTAT  S5  Monomorphic  CK17    R: CCCTG TTTCACTGTTTCC  C  C  CK17    CgET30  (AG) <sub>5</sub> F: ATACCATTCATACAACCGAT  S5  Monomorphic  CK17    CgET31  (CT) <sub>6</sub> F: TTGGACTATCACATCCA  S8  Monomorphic  DV73	CgET24	(CA)s		56	Unamplified	BQ427097
R: ATACGGTGGTGACGATTA  CgET27    CgET27  (TG)s  F: GTTGAGACATTAGGGAGAT  SS    Monomorphic  BQ42    CgET28  (AG)s  F: GAGTATGAGGCAGCAGAGG  S4    Monomorphic  BQ42    CgET28  (AG)s  F: GAGTATGAGGCAGCAGAG  S4    Monomorphic  BQ42    R: GTATCGTTTGCTTTGTGC  CgET29    (GAA)s  F: AGATGCTGATGGGTTTAT  S5    R: CCCTG TTTCACTCTTTC  CgET30  (AG)s  F: ATACCATTCATACAACCGAT    S5  Monomorphic  CK17    R: TTCAACTACAAAATGCTCCT  CgET31  (CT)s  F: TTGGACTATCACATCCAA						
R: ATACGGTGGTGACGATTA  CgET27    CgET27  (TG)s  F: GTTGAGACATTAGGGAGAT  SS    Monomorphic  BQ42    CgET28  (AG)s  F: GAGTATGAGGCAGCAGAGG  S4    Monomorphic  BQ42    CgET28  (AG)s  F: GAGTATGAGGCAGCAGAG  S4    Monomorphic  BQ42    R: GTATCGTTTGCTTTGTGC  CgET29    (GAA)s  F: AGATGCTGATGGGTTTAT  S5    R: CCCTG TTTCACTCTTTC  CgET30  (AG)s  F: ATACCATTCATACAACCGAT    S5  Monomorphic  CK17    R: TTCAACTACAAAATGCTCCT  CgET31  (CT)s  F: TTGGACTATCACATCCAA	CgET25	(TC)s		46	Unamplified	BQ426985
R: TGTGTAAACAGAGGGTAGTG  CgET28  (AG) <sub>5</sub> F: GAGTATGAGGCAGCAGAG  54  Monomorphic  BQ42    CgET29  (GAA) <sub>5</sub> F: AG ATGCTGATGGCTTGGTGC  C  C    CgET29  (GAA) <sub>5</sub> F: AG ATGCTGA TGGGGTTTAT  SS  Monomorphic  CK17    CgET30  (AG) <sub>5</sub> F: ATACCATTCATCCTTTTC  C  C  CK17    CgET30  (AG) <sub>5</sub> F: ATACCATTCATACAACCGAT  SS  Monomorphic  CK17    CgET31  (CT) <sub>6</sub> F: TTGGACTATCACATCCA  S8  Monomorphic  DV73						
R: TGTGTAAACAGAGGGTAGTG  CgET28  (AG) <sub>5</sub> F: GAGTATGAGGCAGCAGAG  54  Monomorphic  BQ42    CgET29  (GAA) <sub>5</sub> F: AG ATGCTGATGGCTTGGTGC  C  C    CgET29  (GAA) <sub>5</sub> F: AG ATGCTGA TGGGGTTTAT  SS  Monomorphic  CK17    CgET30  (AG) <sub>5</sub> F: ATACCATTCATCCTTTTC  C  C  CK17    CgET30  (AG) <sub>5</sub> F: ATACCATTCATACAACCGAT  SS  Monomorphic  CK17    CgET31  (CT) <sub>6</sub> F: TTGGACTATCACATCCA  S8  Monomorphic  DV73	CgET27	(TG)s	F: GTTGAGACA TTAGGGAGAT	55	Monomorphic	BQ426493
CgET28  (AG) <sub>5</sub> F: GAGTATGAGGCAGCAGAG  54  Monomorphic  BQ42    CgET29  (GAA) <sub>5</sub> F: AGATGCTGATGGGTTTGTGC						
R: GTATCGTTTGCTTTGTGC    Monomorphic    CK17      CgET29    (GAA)s    F: AGATGCTGATGGGTTTAT    SS    Monomorphic    CK17      R: CCCTGTTTCACTCTTTC    C    C    C    CK17    CK17      CgET30    (AG)s    F: ATACCATTCATACAACCGAT    SS    Monomorphic    CK17      R: TTCAACTACAAAAATGCTCCT    C    C    CK17    CK17      CgET31    (CT)s    F: TTGGACTATCACAATGCTCA    S8    Monomorphic    DV73	CgET28	(AG),		54	Monomorphic	BQ426346
CgET29  (GAA)s  F: AGATGCTGATGGGTTTAT  SS  Monomorphic  CK17    R: CCCTGTTTCACTCTTTC  CgET30  (AG)s  F: ATACCATTCATACAACCGAT  SS  Monomorphic  CK17    R: TTCAACTACAAAATGCTCCT  R: TTCAACTACAAAATGCTCCT  CgET31  (CT)s  F: TTGGACTATCACATCTCA  S8  Monomorphic  DV73						
R: CCCTG TTTCACTCTTTTC      CgET30    (AG) <sub>5</sub> F: ATACCATTCATACAACCGAT    SS    Monomorphic    CK17      R: TTCAACTACAAAATGCTCCT    CgET31    (CT) <sub>6</sub> F: TTGGACTATCACATCTCA    S8    Monomorphic    DV73	CgET29	(GAA)s		55	Monomorphic	CK172355
CgET30    (AG) <sub>5</sub> F: ATACCATTCATACAACCGAT    SS    Monomorphic    CK17      R: TTCAACTACAAAATGCTCCT </td <td></td> <td>,<i>,</i>,</td> <td></td> <td></td> <td></td> <td></td>		, <i>,</i> ,				
R: TTCAACTACAAAATGCTCCT    CgET31    (CT)6    F: TTGGACTATCACATCTCA    58    Monomorphic    DV73	CgET30	(AG),		55	Monomorphic	СК172332
CgET31 (CT)6 F: TTGGACTATCACATCTCA 58 Monomorphic DV73						
	CgET31	(CT)s		58	Monomorphic	DV736587
R: ACAGTITATTACCTACTICTA		<u>,/-</u>				
	CgET32	(CAA) <sub>6</sub>		55	Unamplified	DV736339
R: CGTGATGAACAATAGCGAAA		<u></u>				2

 $T_{a}$ , optimal annealing temperature (°C).