# A New Set of Microsatellite Primers for Coelogyne fimbriata (Orchidaceae) and Cross-Amplification in C. ovalis

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PRIMER NOTE

# A NEW SET OF MICROSATELLITE PRIMERS FOR *COELOGYNE FIMBRIATA* (ORCHIDACEAE) AND CROSS-AMPLIFICATION IN *C. OVALIS*[1]

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- *Premise of the study:* Declining orchid populations have made it necessary to prioritize the study of population structure and genetic diversity for species including *Coelogyne fimbriata* (Orchidaceae).
- *Methods and Results:* A biotin-streptavidin capture method was used to construct a microsatellite library for *C. fimbriata*. A total of 15 polymorphic nuclear microsatellite loci were isolated and characterized using 47 *C. fimbriata* individuals from two natural populations in China. The number of alleles per locus for the two populations ranged from two to 17. The observed and expected heterozygosities ranged from 0.000 to 1.000 and from 0.000 to 0.867, respectively. Among these polymorphic primers, 11 loci were also successfully amplified in *C. ovalis*, and 10 loci showed moderate to high-level polymorphism. Cross-amplification of the 15 polymorphic loci was tested in five related species: *C. cumingii*, *C. eberhardtii*, *C. mayeriana*, *C. peltastes*, and *C. velutina*.
- *Conclusions:* Fifteen microsatellites in *C. fimbriata* and 10 in *C. ovalis* have moderate to high-level genetic variation, indicating their utility in population genetic studies, thus contributing to orchid conservation.

**Key words:** *Coelogyne fimbriata*; *Coelogyne ovalis*; medicinal orchid; microsatellites; Orchidaceae; polymorphic markers.

*Coelogyne fimbriata* Lindl. (Orchidaceae), a medicinal orchid, is mainly distributed in southern China, Cambodia, northeastern Indonesia, Laos, Malaysia, Thailand, and Vietnam (Clayton and Beaman, 2002). Because southern China is the northernmost edge of its distribution region, Chinese *C. fimbriata* populations are of particular concern because populations on distribution margins are most vulnerable to disturbance (Channell and Lomolino, 2000). Furthermore, in consideration of global climate change and habitat fragmentation, it is urgent to design effective conservation strategies for endangered natural orchid populations (Swarts and Dixon, 2009). *Coelogyne fimbriata* is an epiphytic or lithophytic orchid, which requires a dormancy period in winter. This species grows on its substrate with creeping and slender rhizomes. It can reproduce both sexually via seed and vegetatively by rhizomatic growth. Usually blooming in late summer, it produces one or two flowers on a scape. The flowers exhibit a type of pollinator deception in which the flower odor mimics food for foraging female wasps (Cheng et al., 2009).

Many studies have focused on the pollination syndromes of orchids (Tang et al., 2014); however, there is a lack of genetic information documented for this species. Because genetic information is important for the conservation and sustainable utilization of orchids (Gijbels et al., 2015), we developed microsatellite markers to allow studies of the genetic diversity, genetic structure, and mating system of *C. fimbriata*. In total, 15 polymorphic microsatellite loci were isolated and characterized to study genetic variation within this species clade. These highly polymorphic loci displayed high genetic variation and extensive usability in congeneric species, and may serve as a universal tool for orchid genetic studies.

## METHODS AND RESULTS

A biotin-streptavidin capture method was employed to construct a microsatellite-enriched DNA library (Jiang et al., 2011). First, we extracted genomic DNA from silica gel–dried leaves of one *C. fimbriata* individual using a Plant Genomic DNA Extraction Kit (Tiangen, Beijing, China). The enzyme *Mse*I (New England Biolabs, Beverly, Massachusetts, USA) was used to

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digest approximately 300 ng of genomic DNA in a 25-μL reaction volume for 2 h at 37°C. Fragments 200–1000 bp in length were then ligated to an *Mse*I-adapter pair (F: 5′-TACTCAGGACTCAT-3′ and R: 5′-GACGATGAGTCCTGAG-3′). The ligation-digestion mixture was diluted with ultrapure water (1 : 4), and the diluted fragments were amplified using *Mse*I-N primer (5′-GATGAGTCCTGAGTAAN-3′) in a 25-μL PCR reaction volume at 95°C for 5 min, followed by 23 cycles of 94°C for 30 s, 53°C for 1 min, and 72°C for 1 min. Next, to obtain microsatellite-enriched DNA fragments, the PCR products were hybridized with 5′-biotinylated $(AC)_{15}$ probes. We used streptavidin-coated magnetic beads (Promega Corporation, Madison, Wisconsin, USA) to capture single-stranded DNA fragments containing microsatellites. The enriched products were amplified using *Mse*I-N primers for 28 cycles. After the PCR products were purified using a multifunctional DNA Extraction Kit (Bioteke Corporation, Beijing, China), they were ligated into *Escherichia coli* strain DH5α with the pMD19-T vector (TaKaRa Biotechnology Co., Dalian, Liaoning, China).

We randomly selected and sequenced 249 positive clones using $M13^+/M13^-$ primers on an ABI 3730 DNA Sequence Analyzer (Applied Biosystems, Foster City, California, USA). Of the 249 sequenced clones, 136 contained microsatellites. Twenty-four sequences were discarded because of short flanking regions for primer design. Finally, we designed 112 primer pairs using Premier 5.0 (PREMIER Biosoft International, Palo Alto, California, USA). We selected 28 individuals from Dawei Mountain, Yunnan Province, and 19 from Diaoluo Mountain, Hainan Province, China (Appendix 1), for PCR using these 112 primers. Of the 112 primers, 47 produced an expected band on 1% agarose gel, 40 failed to obtain amplification products, and 25 others produced multiple bands that were difficult to discriminate. To test for polymorphism, we used the M13(–21)-tailed primer method to fluorescently label alleles and PCR products, which were electrophoretically resolved using an ABI 3730 DNA Sequence Analyzer (Applied Biosystems) with an internal lane standard (GeneScan 500[−250] LIZ) (Schuelke, 2000). Microsatellite loci were amplified under the following conditions: 5 min of denaturation at 94°C; 30 cycles of 30 s at 94°C, 30 s at 53–65°C, and 30 s at 72°C; eight cycles of 30 s at 94°C, 30 s at 53°C, and 30 s at 72°C; and a final 10-min extension at 72°C. Allele binning and calling were conducted using GeneMapper 4.0 (Applied Biosystems), revealing 15 polymorphic loci.

Characteristics of the 15 polymorphic microsatellite loci developed for *C. fimbriata* are shown in Table 1. An additional 32 monomorphic loci are described in Appendix 2. Each polymorphic locus had two to 17 alleles, with a mean of 5.1. At the population level, the observed and expected heterozygosities were calculated with GenAlEx 6.5 (Peakall and Smouse, 2012) and ranged from 0.000 to 1.000 and from 0.000 to 0.867, respectively (Table 2). FSTAT 2.9.2.3 (Goudet, 1995) was used to analyze linkage disequilibrium and Hardy–Weinberg equilibrium (HWE). No significant linkage disequilibrium at any locus was detected for either population. However, significant deviation from HWE was found for most loci in the two populations (Table 2), which may be indicative of strong clonality. Signs of null alleles were detected in the loci CF1-11, CF1-51, and CF2-126 with MICRO-CHECKER 2.2.3 (van Oosterhout et al., 2004).

Two wild *C. ovalis* Lindl. populations (Appendix 1) were used to test the cross-compatibility and polymorphism of the 15 microsatellite loci. This testing was performed because *C. ovalis* and *C. fimbriata* have been visually confused as the same species, with no clear differences shown in studies of pollinaria or other morphological characters (Pelser et al., 2000). However, many researchers do recognize *C. fimbriata* and *C. ovalis* as separate species (Govaerts, 1999; Wu and Hong, 2009; George and George, 2011). In our results, 11 of 15 loci could obtain clear PCR products and 10 showed moderate to high levels of polymorphism, with the exception of one locus (CF2-147). No significant linkage disequilibrium was detected in *C. ovalis* populations; however, significant deviation from HWE was found in these populations at most loci (Table 2). Signs of null alleles were detected in the CF1-26, CF1-120, CF2-126, and CF2-172 loci. In addition, cross-amplification of 15 polymorphic loci was conducted on another five related species ($n$ = 5 for each species): *C. cumingii* Lindl., *C. eberhardtii* Gagnep., *C. mayeriana* Rchb. f., *C. peltastes* Rchb. f., and *C. velutina* de Vogel; these samples were collected from living plants at Shanghai Chenshan Botanical Garden (Appendix 1). Four to eight loci were successfully amplified in all five *Coelogyne* species (Table 3).

TABLE 1. Characteristics of 15 polymorphic microsatellite markers developed for *Coelogyne fimbriata.*

| Locus | Primer sequences (5′–3′)[a] | $T_a$ (°C) | Repeat motif | $A$ | Allele size range (bp) | GenBank accession no. |
|---|---|---|---|---|---|---|
| CF1-11 | F: <6-FAM>CAACATCCTCTCGGCATAT<br>R: GACCACACTACACCTACAC | 60 | $(GT)_9$ | 7 | 429–443 | KP676048 |
| CF1-26 | F: <6-FAM>ATAACTCACGCCCGATTC<br>R: CCTGTTGTTGCCTGCTGT | 60 | $(CT)_5$ | 3 | 210–218 | KP676049 |
| CF1-30 | F: <6-FAM>CACCTCTCCTCAATTACATCA<br>R: AGTTGGCGTAAGGCTAATG | 58 | $(ATC)_6$ | 2 | 103–109 | KP676050 |
| CF1-51 | F: <HEX>TGAGAATGTCCGTAGGTT<br>R: GGGATTGGAGTAAAGGGT | 58 | $(AG)_{14}$ | 7 | 336–362 | KP676052 |
| CF1-60 | F: <ROX>AAACCTTCGTTCGCTCCT<br>R: GTGCCTGCTAGGGTTCCA | 60 | $(TC)_5…(CT)_5…(TC)_5$ | 2 | 344–346 | KP676053 |
| CF1-120 | F: <6-FAM>GGAATCACTCTCAACTTCAC<br>R: ATCATAGGATGGACTCTGTAG | 60 | $(GT)_6$ | 5 | 362–372 | KP676054 |
| CF1-167 | F: <ROX>CAAGCAAGCACTGAGCAA<br>R: GAGACCATCACCGCATTC | 58 | $(AG)_6$ | 8 | 259–293 | KP676055 |
| CF1-229 | F: <6-FAM>AGGCTTACTCGCATACTCT<br>R: ATCTCGCTTCTGGCTTCA | 52 | $(CT)_7…(TC)_7$ | 2 | 181–187 | KP676056 |
| CF1-231 | F: <HEX>GGTGCTATGTATGTGAA<br>R: CAGACCATCAAGAAGCATA | 52 | $(AG)_{33}…(GA)_{18}$ | 2 | 285–289 | KP676057 |
| CF2-26 | F: <6-FAM>CTCCCATACCCACCATTT<br>R: ATAGCCTACCTCAAGACG | 55 | $(AG)_{16}…(AG)_6$ | 4 | 153–171 | KX237659 |
| CF2-29 | F: <ROX>TTGTAGTCTTCATCCTTT<br>R: TCTAGTCTACCCATACTT | 52 | $(TG)_5$ | 5 | 270–308 | KX237660 |
| CF2-57 | F: <HEX>TGACTTAGGACCAGGAA<br>R: GTCGCAAGCACAGATA | 55 | $(CT)_{27}$ | 17 | 173–213 | KX237661 |
| CF2-126 | F: <6-FAM>CTCCCGTGCCTATGTTTC<br>R: ATTCCGCTCTGATTTCCA | 52 | $(CT)_{20}$ | 4 | 253–259 | KX237663 |
| CF2-147 | F: <HEX>GGAGGTCTTTGATTAGAT<br>R: ATGGAGGATTATCAGTAT | 52 | $(CT)_6CA(CT)_5$ | 4 | 254–260 | KX237664 |
| CF2-172 | F: <ROX>CTTGTATTTCCCTTTCTTG<br>R: TGAGATAACTAAACCCAGA | 52 | $(CT)_9(CA)_6$ | 4 | 257–271 | KX237666 |

*Note*: $A$ = number of alleles; $T_a$ = annealing temperature.
[a]Fluorescent dyes (i.e., HEX, ROX, and 6-FAM) are presented with the forward primers.

TABLE 2. Characteristics of 15 polymorphic microsatellite loci in *Coelogyne fimbriata* and *C. ovalis* populations, respectively.[a]

| | *Coelogyne fimbriata* | | | | | | *Coelogyne ovalis* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DWS population ($n$ = 28) | | | DLS population ($n$ = 19) | | | MHX population ($n$ = 21) | | | JGX population ($n$ = 16) | | |
| Locus | $A$ | $H_o$ | $H_e$ | $A$ | $H_o$ | $H_e$ | $A$ | $H_o$ | $H_e$ | $A$ | $H_o$ | $H_e$ |
| CF1-11 | 7 | 0.259* | 0.740 | 4 | 0.500 | 0.475 | — | — | — | — | — | — |
| CF1-26 | 1 | 0.000 | 0.000 | 3 | 0.053* | 0.101 | 9 | 0.333* | 0.859 | 5 | 0.188* | 0.756 |
| CF1-30 | 2 | 1.000* | 0.500 | 2 | 0.158 | 0.229 | 1 | 0.000 | 0.000 | 2 | 0.000* | 0.469 |
| CF1-51 | 7 | 0.286* | 0.665 | 4 | 0.250 | 0.736 | — | — | — | — | — | — |
| CF1-60 | 2 | 0.643 | 0.436 | 2 | 0.688 | 0.451 | — | — | — | — | — | — |
| CF1-120 | 4 | 0.889* | 0.658 | 3 | 0.316 | 0.277 | 4 | 0.095 | 0.255 | 5 | 0.500* | 0.585 |
| CF1-167 | 7 | 0.964* | 0.766 | 5 | 0.579* | 0.672 | 1 | 0.000 | 0.000 | 4 | 0.000 | 0.516 |
| CF1-229 | 2 | 0.179 | 0.316 | 2 | 0.063 | 0.061 | — | — | — | — | — | — |
| CF1-231 | 2 | 0.680 | 0.449 | 1 | 0.000 | 0.000 | 1 | 0.000 | 0.000 | 5 | 0.200* | 0.391 |
| CF2-26 | 2 | 0.750 | 0.469 | 3 | 0.368 | 0.597 | 6 | 0.667* | 0.604 | 10 | 0.625* | 0.813 |
| CF2-29 | 3 | 0.769 | 0.500 | 4 | 0.333 | 0.474 | 4 | 0.900 | 0.546 | 3 | 0.929 | 0.554 |
| CF2-57 | 5 | 0.926* | 0.598 | 14 | 0.789* | 0.867 | 4 | 0.952* | 0.585 | 6 | 0.875* | 0.809 |
| CF2-126 | 2 | 0.111* | 0.500 | 4 | 0.474* | 0.669 | 2 | 0.000 | 0.472* | 3 | 0.333* | 0.611 |
| CF2-147 | 1 | 0.000 | 0.000 | 3 | 0.842 | 0.554 | 1 | 0.000 | 0.000 | 1 | 0.000 | 0.000 |
| CF2-172 | 2 | 0.036 | 0.035 | 3 | 0.421 | 0.639 | 10 | 0.529 | 0.877 | 7 | 0.063* | 0.510 |

*Note*: — = failed to amplify; $A$ = number of alleles; $H_e$ = expected heterozygosity based on Hardy–Weinberg equilibrium; $H_o$ = observed heterozygosity; $n$ = number of individuals genotyped.

[a]Voucher and locality information for the populations are shown in Appendix 1.

*Indicates significant deviation from Hardy–Weinberg equilibrium ($P < 0.001$).

## CONCLUSIONS

In the current study, although a majority of the developed loci showed monomorphism (68.1%), 15 polymorphic loci were identified in *C. fimbriata*. These polymorphic loci are valuable for orchid population genetic studies. For example, these markers can be used to characterize the clonal structure of *C. fimbriata* to estimate seed and pollen flow at a fine scale. Furthermore, these polymorphic loci can provide more information, such as genetic diversity indices, which are important for the conservation and management of the species.

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TABLE 3. Amplification of 15 microsatellite loci developed for *Coelogyne fimbriata* in five other *Coelogyne* species.

| Locus | *C. cumingii* ($n$ = 5) | *C. mayeriana* ($n$ = 5) | *C. eberhardtii* ($n$ = 5) | *C. peltastes* ($n$ = 5) | *C. velutina* ($n$ = 5) |
|---|---|---|---|---|---|
| CF1-11 | — | — | — | — | — |
| CF1-26 | + | + | + | + | + |
| CF1-30 | + | + | + | + | + |
| CF1-51 | — | — | — | — | — |
| CF1-60 | — | — | — | — | — |
| CF1-120 | — | + | + | + | — |
| CF1-167 | + | + | + | + | + |
| CF1-229 | — | — | — | — | — |
| CF1-231 | — | — | — | — | + |
| CF2-26 | — | + | — | + | + |
| CF2-29 | — | — | + | — | — |
| CF2-57 | — | — | + | + | + |
| CF2-126 | — | — | — | — | — |
| CF2-147 | — | — | + | — | — |
| CF2-172 | + | + | + | + | + |

*Note*: + = primer successfully amplified; — = primer failed to amplify; $n$ = number of individuals.

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Appendix 1. Locality information for the *Coelogyne fimbriata* and *C. ovalis* samples used in this study.[a]

| Species | Locality ID | Collection locality | Geographic coordinates | Collector | Collection no. | *n* |
|---|---|---|---|---|---|---|
| *Coelogyne fimbriata* Lindl. | DWS | Yunnan, China | 22.931°N, 103.685°E | Wei-Chang Huang | CS-HWC201606-2 | 28 |
| *Coelogyne fimbriata* | DLS | Hainan, China | 18.659°N, 109.916°E | Ming-Zhong Huang | CS-HMZ201610-6 | 19 |
| *Coelogyne ovalis* Lindl. | MHX | Yunnan, China | 23.051°N, 103.356°E | Wei-Chang Huang | CS-HWC201606-5 | 21 |
| *Coelogyne ovalis* | JGX | Yunnan, China | 23.523°N, 100.646°E | Wei-Chang Huang | CS-HWC201509-8 | 16 |
| *Coelogyne cumingii* Lindl.[b] | — | Taiwan | — | Wei-Chang Huang | — | 5 |
| *Coelogyne eberhardtii* Gagnep.[b] | — | Thailand | — | Wei-Chang Huang | — | 5 |
| *Coelogyne mayeriana* Rchb. f.[b] | — | Taiwan | — | Wei-Chang Huang | — | 5 |
| *Coelogyne peltastes* Rchb. f.[b] | — | Taiwan | — | Wei-Chang Huang | — | 5 |
| *Coelogyne velutina* de Vogel[b] | — | Taiwan | — | Wei-Chang Huang | — | 5 |

*Note*: — = no detailed information available; *n* = number of individuals sampled.

[a]All voucher specimens were deposited in Shanghai Chenshan Herbarium (CSH), Shanghai, China.

[b]Samples of *Coelogyne cumingii*, *C. eberhardtii*, *C. mayeriana*, *C. peltastes*, and *C. velutina* were collected from living plants at Shanghai Chenshan Botanical Garden (introduced from Taiwan and Thailand according to the record).

Appendix 2. Characteristics of 32 monomorphic microsatellite markers developed for *Coelogyne fimbriata*.

| Locus | Primer sequences (5′–3′) | $T_a$ (°C) | Repeat motif | Allele size (bp) | GenBank accession no. |
|---|---|---|---|---|---|
| CF1-33 | F: TAAGTAATTCAGCCTCCC | 55 | $(CT)_8$ | 197 | KP676050 |
| | R: CAGACCATCAAGAAGCATA | | | | |
| CF2-2 | F: CAAGTCCAAATCAGCGAAGG | 56 | $(AG)_{15}$ | 152 | KY744706 |
| | R: TCCAGAATACATCCAGGCAC | | | | |
| CF2-3 | F: GAAGAAATCAAGGACCAATG | 64 | $(TCT)_5$ | 198 | KX237656 |
| | R: TCTGAGAACGAAGGAGGC | | | | |
| CF2-8 | F: TAGGAGGTGAGGAGGAA | 56 | $(AG)_{15}$ | 402 | KX237657 |
| | R: CCAGATGCCAAGATAAA | | | | |
| CF2-15 | F: CGACTTTCTCCGGTATCTC | 56 | $(AG)_{14}$ | 248 | KY744707 |
| | R: CACTCACTCAGCCTCTTCC | | | | |
| CF2-20 | F: GGAAAATAGTAAAAGCCAT | 56 | $(TC)_{18}$ | 181 | KY744708 |
| | R: TCCCAAACTTCAAACC | | | | |
| CF2-23 | F: CTCCCCGTTGTAATCCAATCAT | 64 | $(CT)_{12}$ | 178 | KY744709 |
| | R: GTTCCTCCTTCGGCTACGTTAG | | | | |
| CF2-24 | F: ACCCTTCCTATCGCTGTATT | 62 | $(CT)_7$ | 186 | KX237658 |
| | R: CTCTTCCCACCAAGTCTTTT | | | | |
| CF2-27 | F: GAGAGTGGAGGTAGGAGAA | 62 | $(AG)_6$ | 111 | KY766112 |
| | R: GGAGGAGGCTATGGAGAA | | | | |
| CF2-59 | F: GAAGCAGAAAATAACATA | 56 | $(TC)_{22}$ | 90 | KY744710 |
| | R: TCTCACTCCACTCTATCT | | | | |
| CF2-101 | F: TGGTCAGTCGGAGGAG | 64 | $(TC)_{27}$ | 355 | KY744711 |
| | R: ATGGAGGTGGTAGTGTTGG | | | | |
| CF2-112 | F: GGGATTCGGACTGAGATT | 64 | $(GA)_{39}$ | 228 | KX237663 |
| | R: TTAGTAGGGATGCGAGGAG | | | | |
| CF2-127 | F: TCAAGTCCCATCAATC | 53 | $(CT)_{23}$ | 153 | KY744712 |
| | R: TTTAGTGCTCCACATT | | | | |
| CF2-129 | F: TTGGCATTTCGCTTCT | 59 | $(CT)_{12}…(TC)_{16}$ | 235 | KY744713 |
| | R: CGTGTCTTTGTCGGTTT | | | | |
| CF2-136 | F: TCGACCCGTAGTACGCAACA | 64 | $(TC)_8$ | 285 | KY744714 |
| | R: ATGGACACCAGGGCAAGG | | | | |
| CF2-137 | F: GGAAGGCTATGGAGAAAT | 64 | $(TC)_{10}…(CT)_7$ | 126 | KY744715 |
| | R: ATGGGATGACCAGAGGAT | | | | |
| CF2-140 | F: GAAGATGGGAAGAAAGAA | 62 | $(GA)_{10}$ | 108 | KY744716 |
| | R: TGAAAGGAGGAGTAGGAG | | | | |
| CF2-146 | F: TATGCAAATGATATGA | 53 | $(TC)_{15}…(AC)_{10}$ | 347 | KY744717 |
| | R: GAGAATGTGAGAAAGT | | | | |
| CF2-148 | F: TGAATAAGATATTCGGATCA | 56 | $(AG)_{20}$ | 227 | KY744718 |
| | R: AAATCGGTGTATGGAGAC | | | | |
| CF2-149 | F: GTCAAACAGAAAGCCAAG | 56 | $(CT)_{18}$ | 350 | KX237665 |
| | R: AAAGATCCGCTCCACTAT | | | | |
| CF2-155 | F: TCTCGTCTTTTCCTCTTACC | 56 | $(CT)_{20}$ | 104 | KY744719 |
| | R: CCATTACCTCCTCACCATAC | | | | |
| CF2-160 | F: GAATCCTCGCTCCCATTT | 53 | $(TG)_{11}…(GA)_{15}$ | 109 | KY744720 |
| | R: GTTGGTTTAGAGTTTGCAGGTA | | | | |
| CF2-171 | F: TCCTTGTTCGCGTGAAAC | 59 | $(GA)_{43}$ | 232 | KY744721 |
| | R: GAGATCCCTCGACCATAC | | | | |
| CF2-177 | F: AAGAGTTAGAAGTGGGGAGG | 59 | $(AG)_{19}AA(AG)_{15}$ | 405 | KY744722 |
| | R: GGGGAAGTGCCTTATGAT | | | | |
| CF2-192 | F: ACGGGTGAGTATCTTGGC | 64 | $(CA)_{16}$ | 274 | KY744723 |
| | R: GAGGTGGTGAACTCCATTTA | | | | |
| CF2-213 | F: ACCAATAGGAAGTGAGGAGGAA | 64 | $(CT)_{16}$ | 151 | KY744724 |
| | R: ATGGCGGAGCAAGAAAGG | | | | |
| CF2-217 | F: CTTGTTTCATAAAGCGAAGT | 64 | $(CT)_{23}…(CA)_{13}$ | 181 | KX237667 |
| | R: CTTTTATCACAGTCACCCAT | | | | |
| CF2-222 | F: TAGGGGAAACTATGGACAAA | 63 | $(CT)_{46}$ | 181 | KY744725 |
| | R: CGAGTTAGGGATTAGAGGGT | | | | |
| CF2-232 | F: AATAAGATAATGGAAGGA | 62 | $(GA)_9$ | 108 | KY744726 |
| | R: ACTCCAGTTTGTCTTTTA | | | | |
| CF2-234 | F: ATCAAAGCCTATTATTCCC | 64 | $(TC)_8$ | 292 | KY744727 |
| | R: AGATTTACCGTCGTCAGC | | | | |
| CF2-238 | F: AACGCCCACCACCAAGTA | 64 | $(GA)_{11}$ | 379 | KY744728 |
| | R: CGGCCCTATTCCCTCACA | | | | |
| CF2-240 | F: TACACGCCCTAATACCCA | 60 | $(TC)_{13}$ | 488 | KY744729 |
| | R: CGAGGAGGAGGTTGAGAT | | | | |

*Note*: $T_a$ = annealing temperature.