Applications in Plant Sciences

PRIMER NOTE

Development and characterization of EST-SSR markers for *Carex angustisquama* (Cyperaceae), an extremophyte in solfatara fields

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PREMISE OF THE STUDY: Expressed sequence tag–simple sequence repeat (EST-SSR) markers were developed for *Carex angustisquama* (Cyperaceae) to investigate the evolutionary history of this plant that is endemic to solfatara fields in northern Japan.

METHODS AND RESULTS: Using RNA-Seq data generated by the Illumina HiSeq 2000, 20 EST-SSR markers were developed. Polymorphisms were assessed in *C. angustisquama* and the closely related species *C. doenitzii* and *C. podogyna*. In *C. angustisquama*, many loci were monomorphic within populations; the average number of alleles ranged from one to five, and levels of expected heterozygosity ranged from 0.000 to 0.580, while all markers were polymorphic in a population of *C. doenitzii*. This indicates that low genetic polymorphism of *C. angustisquama* is likely due to the species' population dynamics, rather than to null alleles at the developed markers.

CONCLUSIONS: These markers will be used to assess genetic diversity and structure and to investigate evolutionary history in future studies of *C. angustisquama* and related species.

KEY WORDS *Carex angustisquama*; *Carex doenitzii*; Cyperaceae; expressed sequence tag–simple sequence repeat (EST-SSR) markers; solfatara.

Carex L. is one of the largest and most widespread genera of the flowering plants, with approximately 2000 species (Reznicek, 1990). Most of its species are distributed in the Northern Hemisphere, especially in northern temperate and arctic regions. In addition to its global distribution, it is noteworthy that the species in the genus occur in various habitats ranging from rainforests and dry grassland to wet meadows, temperate forests, and alpine zones (Starr et al., 1999), which makes them useful models to study plant adaptation to the environment.

Carex angustisquama Franch. (Cyperaceae) is a perennial sedge that is endemic to solfatara fields in the Tohoku region of northern Japan. Solfatara fields are areas around fumaroles emitting sulfide gases containing H_2S and SO_2 even after eruption (Tsujimura, 1979; Yamamoto et al., 2018). Acidified by sulfide gases from fumaroles, the soil in solfatara fields has low pH values of 2–3 and high concentrations of sulfur and aluminum, making a harsh environment for plants to survive. *Carex angustisquama* grows close to fumaroles where no other vascular plants are able to survive (Tsujimura, 1982). Because no other closely related species in *Carex* sect. *Podogynae* Holm grow in a similar habitat, *C. angustisquama* is assumed to have adapted to solfatara fields in the process of speciation. *Carex angustisquama* also represents a disjunct geographic distribution in six main volcanic areas in the Tohoku region, which are isolated by unsuitable forested vegetation. This pattern of distribution provides an ideal model to reconstruct the historical biogeography of *C. angustisquama*.

To investigate the genetic structure and evolutionary history of *C. angustisquama*, genetic markers are needed, but there are no available markers that can be applied to this species. Expressed sequence tag–simple sequence repeat (EST-SSR) markers are widely distributed both in transcribed and nontranscribed regions (Morgante et al., 2002). EST-SSR markers are regarded as easier and less expensive markers to develop and reported to be more transferable among closely related species (Bouck and Vision, 2007). Moreover,

TABLE 1. Twenty polymorphic EST-SSR markers developed for *Carex angustisquama*.

Locus	Primer sequences (5′–3′)	Repeat motif	Allele size range (bp)	BLASTX top hit description	*E*-value	GenBank accession no.
Cang_681	F: TGTGGAATTGTGAGCGGAGCTTATTGGCCGCATGAAC R: GTTTCTTCCAACCGGATAAAGCTGCG	$(AG)_{19}$	245–251	B-box zinc finger protein 22 [*Ananas comosus*]	8.00E-77	FX986011
Cang_1267	F: TGTGGAATTGTGAGCGGTAATGTGGGTCCCGGTACTG R: GTTTCTTCGTGAAACCGAAACCTGGTC	$(AGC)_{9}$	209–221	PREDICTED: ATP-dependent helicase BRM [*Oryza brachyantha*]	0.0	FX985999
Cang_1881	F: TGTGGAATTGTGAGCGGTGTGGATGACGTGGCATTTG R: GTTTCTTTACAGCACAACATAGCCCTC	$(AT)_{11}$	316–334	Hypothetical protein GQ55_7G126900 [*Panicum hallii* var. *hallii*]	9.00E-149	FX986003
Cang_2073	F: CTATAGGGCACGCGTGGTCAGTGCAGCCGAGATTCTTG R: GTTTCTTCCCATCTCGATCCCAAATCC	$(AAG)_{10}$	466–475	Putative DEAD-box ATP-dependent RNA helicase family protein [*Zea mays*]	0.0	FX986008
Cang_3069	F: TGTGGAATTGTGAGCGGGTCTCCTCCGCCAAGTACTC R: GTTTCTTAATTGGAGGATGGCAAAGCG	$(AAG)_{10}$	398–439	Poly(A)-specific ribonuclease PARN [*Ananas comosus*]	0.0	FX986006
Cang_3862	F: CACGACGTTGTAAAACGACGATCCATCCACTCTCCCTCC R: GTTTCTTCATCCACCACGATACGCTTC	$(AG)_{17}$	173–183	Uncharacterized protein LOC100191912 [*Zea mays*]	9.00E-113	FX986009
Cang_4293	F: CTATAGGGCACGCGTGGTCGATTTCCACTGCGTGTACC R: GTTTCTTCCACTCGCAAACAACAGTCG	$(AG)_{12}$	244–250	PREDICTED: WD-40 repeat-containing protein MSI4-like [*Phoenix dactylifera*]	0.0	FX986001
Cang_4398	F: CGGAGAGCCGAGAGGTGTCCTACTAAAGTCCCTGCTGAG R: GTTTCTTGTTGGTTGAGTGAGGCTGTG	$(AG)_{12}$	147–153	PREDICTED: uncharacterized protein LOC103721079	1.00E-90	FX985994
Cang_5849	F: CACGACGTTGTAAAACGACACCCACCCATAGTTCCAGAAG R: GTTTCTTACCTATGAGTCAGCCCGAAC	$(AG)_{10}$	406–418	D-cysteine desulfhydrase 2, mitochondrial-like isoform X4	3.00E-09	FX986007
Cang_7187	F: CGGAGAGCCGAGAGGTGGCAGCGTGGGAAGGAAGAG R: GTTTCTTAAAGCGTTGGAAAGAGCGTC	$(AG)_{12}$	366–409	WD repeat-containing protein 44-like [*Ananas comosus*]	0.0	FX986004
Cang_7240	F: CACGACGTTGTAAAACGACAAAGCTTGGCAGATTCGTCG R: GTTTCTTAATGCAGGCGTCGATGTTAC	$(AGG)_{11}$	210–218	PREDICTED: protein TIC 21, chloroplastic [*Musa acuminata*]	2.00E-98	FX985998
Cang_7261	F: CACGACGTTGTAAAACGACCTTCGTTTCACCACAGCTGC R: GTTTCTTAAACCTCACCACTGCACTCG	$(AG)_{12}$	236–238	Protein ROOT PRIMORDIUM DEFECTIVE 1 [*Asparagus officinalis*]	0.0	FX986000
Cang_10657	F: CGGAGAGCCGAGAGGTGGAGGCGAATTGAGTTGCTCC R: GTTTCTTGCCAATGCCAAACTTTGAGG	$(AG)_{12}$	175–185	Probable transcription factor At5g28040 [*Ananas comosus*]	2.00E-89	FX985996
Cang_18857	F: CTATAGGGCACGCGTGGTCTCTCTCAGCTCGGACAGTG R: GTTTCTTTTCCACCGAAATCAGGGAGG	$(AG)_{12}$	398–408	PREDICTED: protein TIFY 4B-like isoform X4 [*Phoenix dactylifera*]	8.00E-58	FX986005
Cang_19507	F: TGTGGAATTGTGAGCGGCACAGTATCTTTCTCCGCCC R: GTTTCTTAGAAGTATGAGACCCGACGC	$(AG)_{19}$	201–215	PREDICTED: histone deacetylase 19-like [*Elaeis guineensis*]	0.0	FX986010
Cang_21384	F: CACGACGTTGTAAAACGACGGGTTACCGAGGCACAATTG R: GTTTCTTGATGCGACACAACTAACCCG	$(AC)_{12}$	118–136	No significant similarity found.	—	FX985993
Cang_22899	F: CTATAGGGCACGCGTGGTGGAGAGCAAATTCAGAGCGG R: GTTTCTTACAGAGAGAAGCAAGGCAGG	$(AG)_{11}$	153–157	PREDICTED: transcription factor PCL1-like [*Musa acuminata* subsp. *malaccensis*]	1.00E-74	FX985995
Cang_25819	F: CTATAGGGCACGCGTGGTGGAGTTGATGATGGGTTTAGGG R: GTTTCTTGGTCTGTGCCACTTAGTCCC	$(AG)_{17}$	287–297	No significant similarity found.	—	FX986012
Cang_46532	F: CGGAGAGCCGAGAGGTGAGCCCTAGAAACCTGACCTTG R: GTTTCTTGGACACTATGCTGTACAAGGG	$(AC)_{12}$	302–305	No significant similarity found.	—	FX986002
Cang_48335	F: TGTGGAATTGTGAGCGGAGTTGTAGGTGGTGTAGCGG R: GTTTCTTCCCTGGCACTGTTTAGCTTG	$(AG)_{10}$	185–189	No significant similarity found.	—	FX985997

they are shown to be more reliable because they have lower frequencies of null alleles than anonymous genomic SSR markers (Ellis and Burke, 2007). Therefore, we developed EST-SSR markers and examined their polymorphisms and transferability to closely related taxa.

METHODS AND RESULTS

Total RNA was extracted from *C. angustisquama* (population CA18, Appendix 1) using the Agilent Plant RNA Isolation Mini Kit (Agilent Technologies, Santa Clara, California, USA). A non-normalized cDNA library was constructed and sequenced using the Illumina HiSeq 2000 system (Illumina, San Diego, California, USA). De novo assembly of 83,484,902 cleaned 100-bp reads (DNA Data Bank of Japan [DDBJ], Bioproject PRJDB6849) using CLC Genomic Workbench version 10.1.1 software (CLC bio, Aarhus, Denmark) produced 53,628 contigs (N50: 1321 bp).

Microsatellite regions (≥8 dinucleotide repeats, ≥8 trinucleotide repeats) were searched using MSATCOMMANDER (Faircloth, 2008). We obtained 937 markers, of which 63 pairs were selected based on repeat number. For all loci, the forward primer was synthesized with one of four different M13 sequences (5′-CACGACGTTGTAAAACGAC-3′, 5′-TGTGGAATTGTGAGCGG-3′, 5′-CTATAGGGCACGCGTGGT-3′, or 5′-CGGAGAGCCGAGAGGTG-3′) and the reverse primer was tagged with a PIG-tail (5′-GTTTCTT-3′). PCR reactions were performed using a QIAGEN Multiplex PCR Kit (QIAGEN, Hilden, Germany) in a 10-μL volume containing 20–30 ng of DNA, 5 μL of 2× Multiplex PCR Master Mix, 0.01 μM of forward primer, 0.2 μM of reverse primer, and 0.1 μM of fluorescently labeled M13 primer. The PCR protocol was as follows: 95°C for 30 min; followed by 35 cycles of 94°C for 30 s, 60°C for 3 min, 72°C for 1 min; and a final extension at 68°C for 30 min. Amplified products were loaded onto an ABI 3130 autosequencer (Applied Biosystems, Foster City, California, USA) using the GeneScan 600 LIZ Size Standard (Applied Biosystems), POP7 polymer (Applied Biosystems), and 36-cm capillary array. Fragment size was determined using GeneMapper (Applied Biosystems).

For the initial PCR amplification trial, we used two individuals from population CA09 (Appendix 1). For the 32 primer pairs that showed clear peaks, two individuals from each population (CA09, CA13, CA14, and CA15; Appendix 1) were then used to check polymorphisms among populations. Using 20 primers that were polymorphic over the eight samples (details for 12 monomorphic markers are provided in Appendix 2), 24 individuals from each population (CA09, CA14, and CA15) were evaluated for within-population polymorphisms. However, because few polymorphisms were detected within each population, we examined the transferability and evaluated polymorphisms in two closely related species (*C. doenitzii* Boeckeler and *C. podogyna* Franch. & Sav.; Appendix 1) to test whether low genetic variation of *C. angustisquama* was the result of null alleles at the markers or of the species' genetic nature. GenAlEx 6.5 software (Peakall and Smouse, 2012) was used to calculate genetic diversity indices (number of alleles [*A*], observed heterozygosity [H_o], and expected heterozygosity [H_e]). FSTAT 2.9.3 software (Goudet, 1995) was used to test significance of Hardy–Weinberg equilibrium (HWE) by 1000 randomizations; the significance of the associated *P* values was adjusted by applying sequential Bonferroni correction. The test for the presence of null alleles was performed using MICRO-CHECKER version 2.2.3 (van Oosterhout et al., 2004).

For *C. angustisquama*, all primer pairs (Table 1) were polymorphic when all populations were combined; *A* ranged from two to seven, and levels of H_e and H_o ranged from 0.100 to 0.703 and 0.000 to 0.286, respectively (Table 2). For each population, *A* ranged from one to five, and levels of H_e and H_o ranged from 0.000 to 0.580 and

TABLE 2. Characteristics of the 20 polymorphic EST-SSR markers in three populations of *Carex angustisquama*.[a]

	CA09 (*N* = 24)			CA14 (*N* = 24)			CA15 (*N* = 24)			All (*N* = 72)		
Locus	***A***	H_e	H_o	***A***	H_e	H_o	***A***	H_e	H_o	***A***	H_e	H_o
Cang_681	1	0.000	0.000	1	0.000	0.000	3	0.544	0.238†	4	0.510	0.081†
Cang_1267	1	0.000	0.000	2	0.105	0.111	5	0.319	0.182	7	0.702	0.094†
Cang_1881	2	0.469	0.083†	1	0.000	0.000	1	0.000	0.000	3	0.525	0.030†
Cang_2073	1	0.000	0.000	1	0.000	0.000	2	0.466	0.217	3	0.511	0.076†
Cang_3069	2	0.249	0.292	1	0.000	0.000	3	0.232	0.174	5	0.703	0.167†
Cang_3862	1	0.000	0.000	1	0.000	0.000	1	0.000	0.000	3	0.662	0.000†
Cang_4293	1	0.000	0.000	1	0.000	0.000	1	0.000	0.000	3	0.663	0.000†
Cang_4398	1	0.000	0.000	1	0.000	0.000	1	0.000	0.000	3	0.662	0.000†
Cang_5849	2	0.249	0.292	1	0.000	0.000	1	0.000	0.000	2	0.100	0.106
Cang_7187	1	0.000	0.000	1	0.000	0.000	1	0.000	0.000	2	0.463	0.000†
Cang_7240	1	0.000	0.000	1	0.000	0.000	1	0.000	0.000	3	0.662	0.000†
Cang_7261	1	0.000	0.000	2	0.105	0.000	2	0.087	0.091	4	0.506	0.031†
Cang_10657	1	0.000	0.000	2	0.054	0.056	1	0.000	0.000	3	0.409	0.016†
Cang_18857	2	0.080	0.000	1	0.000	0.000	2	0.159	0.087	3	0.509	0.030†
Cang_19507	2	0.353	0.292	1	0.000	0.000	3	0.580	0.524	4	0.621	0.286†
Cang_21384	1	0.000	0.000	1	0.000	0.000	2	0.049	0.050	3	0.423	0.016†
Cang_22899	1	0.000	0.000	2	0.054	0.056	1	0.000	0.000	3	0.409	0.016†
Cang_25819	1	0.000	0.000	1	0.000	0.000	1	0.000	0.000	3	0.662	0.000†
Cang_46532	2	0.413	0.167†	1	0.000	0.000	1	0.000	0.000	3	0.517	0.061†
Cang_48335	2	0.041	0.042	2	0.054	0.056	3	0.275	0.188	4	0.539	0.086†
Average	1.350	0.093	0.058	1.55	0.019	0.014	1.8	0.136	0.088	3.4	0.538	0.055†

Note: *A* = number of alleles per locus; H_e = expected heterozygosity; H_o = observed heterozygosity; *N* = number of individuals genotyped.
[a]Voucher and locality information are provided in Appendix 1.
†Significant possibility of presence of null alleles (99% confidence level) detected by MICRO-CHECKER (van Oosterhout et al., 2004).

TABLE 3. Cross-amplification and genetic diversity statistics of the EST-SSR markers developed for *Carex angustisquama* in two related species.[a]

	C. doenitzii (*N* = 24)			*C. podogyna* (*N* = 16)		
Locus	*A*	H_e	H_o	*A*	H_e	H_o
Cang_681	5	0.321	0.182	2	0.444	0.333
Cang_1267	4	0.574	0.292†	2	0.305	0.250
Cang_1881	8	0.806	0.522†	1	0.000	0.000
Cang_2073	6	0.747	0.87	1	0.000	0.000
Cang_3069	7	0.694	0.609	1	0.000	0.000
Cang_3862	6	0.713	0.435†	2	0.486	0.500
Cang_4293	5	0.712	0.783	2	0.320	0.133
Cang_4398	4	0.644	0.045*†	3	0.331	0.267
Cang_5849	3	0.46	0.364	1	0.000	0.000
Cang_7187	2	0.315	0.217	1	0.000	0.000
Cang_7240	6	0.753	0.375*†	1	0.000	0.000
Cang_7261	6	0.751	0.826	1	0.000	0.000
Cang_10657	4	0.396	0.294	2	0.444	0.000
Cang_18857	5	0.694	0.739	2	0.451	0.563
Cang_19507	7	0.794	0.762	1	0.000	0.000
Cang_21384	5	0.718	0.591	2	0.117	0.125
Cang_22899	2	0.194	0.217	1	0.000	0.000
Cang_25819	5	0.688	0.571	1	0.000	0.000
Cang_46532	2	0.258	0.217	2	0.358	0.333
Cang_48335	5	0.5	0.125*†	–	–	–
Average	4.85	0.587	0.452	1.450	0.163	0.125

Note: *A* = number of alleles per locus; H_e = expected heterozygosity; H_o = observed heterozygosity; *N* = number of individuals genotyped.
[a]Voucher and locality information are provided in Appendix 1.
†Significant possibility of presence of null alleles (99% confidence level) detected by MICRO-CHECKER (van Oosterhout et al., 2004).
*Significant departures ($P < 0.01$) from Hardy–Weinberg equilibrium after Bonferroni correction.

0.000 to 0.524, respectively (Table 2). For cross-species amplification, 20 and nine primer pairs were polymorphic in *C. doenitzii* and *C. podogyna*, respectively (Table 3). Significant departures ($P < 0.01$) from HWE were detected in three loci (Cang4398, Cang7240, and Cang48335) in *C. doenitzii*, although no significant departures were detected for any of the populations or loci in both *C. angustisquama* and *C. podogyna*. Analysis with MICRO-CHECKER (at the 99% confidence level) highlighted the existence of null alleles at some loci in *C. angustisquama* and *C. doenitzii* (Tables 2, 3).

EST-SSR markers were shown to have a disadvantage of less polymorphism than genomic SSR markers (Bouck and Vision, 2007; Ellis and Burke, 2007), and we found low genetic variation in all populations of *C. angustisquama*. This may be caused by presence of null alleles. However, substantial polymorphisms were detected in *C. doenitzii*, which is the most closely related species to *C. angustisquama* (K. Nagasawa, H. Setoguchi, M. Maki, H. Goto, K. Fukushima, Y. Isagi, S. Sakaguchi, Y. Suyama, and Y. Tsunamoto, unpublished data). Moreover, in *C. angustisquama*, although most loci were homozygous within populations, these loci were fixed with different alleles for each population, which likely reflects evolutionary history rather than null alleles. Thus, we conclude that low genetic variation of *C. angustisquama* is probably caused by the species' demographic history.

CONCLUSIONS

The 20 EST-SSR markers developed for *C. angustisquama* are less polymorphic within populations. However, in intraspecific and cross-species amplification, substantial polymorphisms were detected, indicating that low genetic variation in *C. angustisquama* results from the species' demographic history, and not from the markers' characteristics. Thus these markers will be useful for investigating intraspecific relationships among *C. angustisquama* populations occurring in disjunct solfatara fields. These markers are also useful in other *Carex* species, providing novel population genetic tools in this speciose genus.

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DATA ACCESSIBILITY

Cleaned reads from the cDNA library have been deposited to the DNA Data Bank of Japan (DDBJ; Bioproject PRJDB6849). Sequence information for the developed primers has been deposited to the National Center for Biotechnology Information (NCBI); GenBank accession numbers are provided in Table 1.

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APPENDIX 1. Sample information for *Carex* species used in this study.

Species	Population	*N*	Collection locality	Geographic coordinates (Altitude, m)	Voucher specimen accession no.[a]
Carex angustisquama Franch.[b]	CA18	1	Goshogake, Senboku-shi, Akita Pref., Japan	35°21′38″N, 137°01′34″E (1002)	KYO 00023447
Carex angustisquama[c,d,e]	CA09	24	Katanuma, Osaki-shi, Miyagi Pref., Japan	38°44′02″N, 140°43′28″E (309)	KYO 00023438
Carex angustisquama[d]	CA13	2	Mt. Kurikoma, Ichinoseki-shi, Iwate Pref., Japan	38°58′47″N, 140°46′10″E (1113)	KYO 00023439
Carex angustisquama[d,e]	CA14	24	Mt. Hakkoda, Aomori-shi, Aomori Pref., Japan	40°38′56″N, 140°51′15″E (936)	KYO 00023440
Carex angustisquama[d,e]	CA15	24	Mt. Osorezan, Mutsu-shi, Aomori Pref., Japan	41°19′47″N, 141°05′10″E (216)	KYO 00023444
Carex doenitizii Boeckeler[f]	C86	24	Mt. Konsei, Nikko-shi, Gunma Pref., Japan	36°49′04″N, 139°23′37″E (2044)	KYO 00023454
Carex podogyna Franch. & Sav.[f]	C116	16	Mt. Shiogiri, Miyazu-shi, Kyoto Pref., Japan	35°39′01″N, 135°12′27″E (610)	NA

Note: N = number of individuals; NA = voucher unavailable.
[a]Vouchers are deposited at Kyoto University (KYO), Kyoto, Japan.
[b]Sample used for cDNA library construction.
[c]Sample used for initial PCR amplification trials.
[d]Samples used to check polymorphisms among populations.
[e]Samples used for detailed evaluation for polymorphisms within populations.
[f]Samples used for transferability test.

APPENDIX 2. Twelve monomorphic EST-SSR markers developed for *Carex angustisquama*.

Locus	Primer sequences (5′–3′)	Repeat motif	Allele size range (bp)	BLASTX top hit description	*E*-value
Cang_103	F: CACGACGTTGTAAAACGACGATCGGTGATTGGGCCTTTG R: GTTTCTTGCCCTGATTTCTGAACCGTG	$(AG)_{11}$	265	Glutamine synthetase root isozyme 3 [*Zea mays*]	0.0
Cang_594	F: CTATAGGGCACGCGTGGTTGCTCCAGTCCCAACCATAG R: GTTTCTTTGGGTGTGCTTCTGAGACC	$(AG)_{20}$	325	PREDICTED: calmodulin-binding transcription activator 4 isoform X1 [*Elaeis guineensis*]	0.0
Cang_1002	F: TGTGGAATTGTGAGCGGCGGTGGTTGGAATTCGAAGG R: GTTTCTTTCCAGTTCACCTCCAGCTTC	$(AG)_{11}$	434	Sulfite exporter TauE/SafE family protein 4 [*Sorghum bicolor*]	4.00E-143
Cang_1737	F: TGTGGAATTGTGAGCGGGAGAAATCAACAGAGCGGGC R: GTTTCTTAACTGCGATTGGTCCTGTTG	$(AAG)_{14}$	414	PREDICTED: eukaryotic translation initiation factor 3 subunit I-like [*Phoenix dactylifera*]	0.0
Cang_1744	F: CACGACGTTGTAAAACGACTTCCTGGATCCTTGTCGACC R: GTTTCTTGCCTACATAACCCATCGCTC	$(AG)_{20}$	276	PREDICTED: guanine nucleotide-binding protein-like NSN1 [*Elaeis guineensis*]	0.0
Cang_2515	F: TGTGGAATTGTGAGCGGACCCTAGACTCGGATCCTCC R: GTTTCTTGCCAGACTTATACTCTCCCTCG	$(AG)_{23}$	279	Carbon catabolite repressor protein 4 homolog 1-like [*Ananas comosus*]	0.00E+00
Cang_2955	F: CGGAGAGCCGAGAGGTGCTGTAACGAATCAGGTGCGG R: GTTTCTTCTCCATTACCTGCTCCCTCC	$(AAG)_{10}$	410	Threonine dehydratase biosynthetic, chloroplastic [*Ananas comosus*]	0.0
Cang_3156	F: CACGACGTTGTAAAACGACTTCAGTAGCCGAGCCTCATC R: GTTTCTTCCTCTCTTCCTGAACAAACCG	$(AG)_{12}$	413	Eukaryotic translation initiation factor 1A [*Citrus clementina*]	5.00E-81
Cang_3166	F: CACGACGTTGTAAAACGACCGCTCTTGTGCAGTTCCAAC R: GTTTCTTGGGAGAGGGATCTGAGCTTG	$(AT)_{19}$	206	PREDICTED: uncharacterized protein LOC107807406 isoform X1 [*Nicotiana tabacum*]	2.00E-177
Cang_3348	F: CTATAGGGCACGCGTGGTATTGCCTCCACAGCCTCC R: GTTTCTTAGCGGATAAGAGGAGATCGC	$(AG)_{17}$	192	PREDICTED: NADP-dependent D-sorbitol-6-phosphate dehydrogenase [*Elaeis guineensis*]	0.0
Cang_4013	F: TGTGGAATTGTGAGCGGACACGAAGCAGCTCTCTACC R: GTTTCTTATTCGCCTCTGAGTCGAGAC	$(AG)_{18}$	114	Peroxisome biogenesis protein 1 [*Ananas comosus*]	0.0
Cang_4089	F: CGGAGAGCCGAGAGGTGCACCTCCTCCTCTCTAAACCC R: GTTTCTTCTGCTCTTCTCATTGGCGTC	$(AG)_{24}$	198	Auxin response factor 18 [*Ananas comosus*]	0.0