Discovery of EST-Derived Microsatellite Primers in the Legume Lens culinaris (Fabaceae)

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PRIMER NOTE

DISCOVERY OF EST-DERIVED MICROSATELLITE PRIMERS IN THE LEGUME *LENS CULINARIS* (FABACEAE)[1]

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- *Premise of the study:* We developed microsatellite markers in the legume *Lens culinaris* from publicly available databases to enrich the limited marker resource available for the crop.
- *Methods and Results:* Eighty-two primer sets were identified using expressed sequence sets of *L. culinaris* available in the National Center for Biotechnology Information (NCBI) database and were characterized in six species of *Lens*. Among them, 20 simple sequence repeat (SSR) primers produced no amplification product, 43 produced monomorphic products, and 19 were polymorphic. The primers amplified mono-, di-, tri-, tetra-, penta-, and hexanucleotide repeats with one to four alleles. These SSR loci successfully amplified in five related wild species, with a total of 61 primer pairs in *L. nigricans* and *L. odemensis* (98.39%), 59 in *L. tomentosus* (95.1%), and 60 in *L. ervoides* and *L. orientalis* (96.7%), respectively.
- *Conclusions:* The microsatellite markers discovered in this study will be useful in genetic mapping, marker-assisted breeding, and characterization of germplasm.

Key words: EST-SSRs; Fabaceae; *Lens culinaris*; microsatellites.

Lentil (*Lens culinaris* Medik. subsp. *culinaris*) is a self-pollinated crop ($2n = 2x = 14$) belonging to the Vicieae tribe in the Fabaceae family. Lentil is a rich source of protein and micronutrients and is grown mainly in the Indian subcontinent, Middle East, North Africa, southern Europe, North and South America, Australia, and West Asia. Lentil has varied uses for consumption as a main dish, salads, or infant foods, and mixed with cereals to make bread and cakes. Although lentil is a highly nutritious food legume, its conservation and breeding potential is largely limited by a lack of molecular markers available for the crop.

Microsatellites are well-known genetic markers because of their codominant inheritance, polymorphism, and abundant coverage. They have become the markers of choice for many crops for studying genetic relatedness, diversity analysis, and constructing framework genetic maps. A limited number of microsatellite markers (approx. 100) have been published in *L. culinaris* to date. Hamwieh et al. (2005) developed 35 simple sequence repeat (SSR) markers, but these have reported no amplification or limited polymorphism, creating a major bottleneck to gene tagging and mapping studies in this crop. Kaur et al. (2011) developed 51 SSRs in *L. culinaris*, but these are less polymorphic and not sufficient to be used for genetic studies or for marker-assisted selection. An effective strategy for enrichment of microsatellite markers is the screening of expressed sequence tags, thereby reducing the time and cost for microsatellite development. The objective of this study was to develop new microsatellite markers using this strategy and characterize them in 18 *L. culinaris* accessions. The amplification success of these markers was also investigated in five wild *Lens* Mill. species (*L. nigricans* (M. Bieb.) Godron, *L. odemensis* Ladiz., *L. tomentosus* Ladiz., *L. ervoides* (Brign.) Grande, *L. orientalis* Popow) for potential genetic application or improvement of cultivated lentil (*L. culinaris*).

METHODS AND RESULTS

A total of 9513 *L. culinaris* expressed sequence tags (ESTs) were downloaded from the dbEST/GenBank database as of 15 January 2012 (http://www.ncbi.nlm.nih.gov). The ESTs were trimmed for poly(A) tails, and vector sequence contamination were removed using the SeqClean program (Masoudi-Nejad et al., 2006). The trimmed EST sequences were assembled into unigenes with the Cap3 program (Huang and Madan, 1999) to reduce redundancy. The unigenes containing 951 contigs and 3092 singletons produced a total of 251 putative SSRs using the software Troll (Martins et al., 2009). The fragments with inappropriate flanking sequences or with less than 500 bp were excluded and 82 SSRs were designed using Primer3 software (Rozen and Skaletsky, 2000).

The newly identified microsatellite markers were screened on 32 individuals representing six *Lens* species including 18 accessions from cultivated species and 14 wild accessions comprising five species. Voucher specimens were sourced from the International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria, and the lentil breeding program at the Indian Agricultural Research Institute (IARI), New Delhi, India (Appendix 1). The genomic DNA from each individual was isolated using the cetyltrimethylammonium bromide (CTAB) method as described by Murray and Thompson (1980). PCR mixtures of 20 µL consisted of 2.0 µL 10× buffer (100 mM Tris-HCl, 500 mM KCl, 15 mM $MgCl_2$, 0.01% gelatin), 200 µM each dNTP, 0.5 µM each of forward and reverse primers, 1 U *Taq* DNA polymerase (PCR reagents and primers procured from Sigma-Aldrich, St. Louis, Missouri, USA), and ~40 ng DNA and were performed in a Veriti Thermal Cycler (Applied Biosystems, Life Technologies, Singapore). The PCR protocol consisted of one denaturation cycle at 94°C for 4 min followed by 30 cycles of 94°C for 1 min, annealing at 59–62°C (depending upon the primer) for 30 s, extension at 72°C for 1 min, and a final extension at 72°C for 8 min. The amplification fragments were separated on 3% MetaPhor Agarose gels (Lonza, Rockland, Maine, USA) and

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visualized by ethidium bromide staining. The band size was obtained in comparison to a 100-bp DNA ladder (MBI, Fermentas, Vilnius, Lithuania). Among these 82 SSRs, 62 amplified successfully in most of the species, providing an amplification success rate of 75.6%, and 19 of them showed more than two clear scorable bands in the *Lens* species (Table 1). Primer sequences and related information for 43 monomorphic EST-SSR primers are available as Appendix 2. The conservation of SSRs across species has been validated by sequencing one amplicon from each species. The EST-SSR markers amplified one to four alleles among the six species. The expected heterozygosity was determined on the basis of the number of genotypes amplified per species and ranged from 0 to 0.875 (Table 2). The putative functions of SSR-associated unigenes were determined by using BLASTX (Altschul et al., 1997) against the nonredundant GenBank database.

CONCLUSIONS

The EST-SSR markers identified and characterized in this study have enriched the limited microsatellite marker resources in *Lens* species. The markers developed will be helpful in saturating *Lens* genetic maps and for tagging and mapping of genes and quantitative trait loci associated with important traits to be further used in marker-assisted breeding for enhancing productivity and quality. These markers would also be helpful in studying genetic diversity and detecting interspecies polymorphisms for marker-based introgression of genes from related species.

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Table 1. Characteristics of the 19 polymorphic EST-SSRs identified in *Lens culinaris.*

No.	EST-SSR locus	GenBank EST no. (Probe DB_id)	Primer sequences (5′–3′)	Repeat motif	T_a (°C)	Allele size range (bp)	Putative function (organism)	BLASTX *E*-value
1	PLC5	GT626272 (16537804)	F: CATTGCAGCTTATTCTCACAGC R: TGACCCATCCTCATCCTTAAAT	$(CAATGG)_5$	60	320–360	Auxin-induced protein 5NG4-like (*Glycine max*)	2E-41
2	PLC10	GT62175 (16581945)	F: TGCAACAAAGGACACTAGAGGTT R: ATTTCTTTCTCCCTAACCAGCC	$(AT)_6$	59	279–328	Predicted aspartic proteinase nepenthesin-1 like protein (*Glycine max*)	1E-157
3	PLC16	GT627608 (16581946)	F: CGTTTGATCTTCTAAGCCCCTA R: AAGGGAAAGGATGTTTGACTTG	$(T)_{10}$	59	255–270	Uncharacterized protein (*Glycine max*)	2E-10
4	PLC17	GT624932 (16581947)	F: AAGCTGAAGGAAATCAAAGTGG R: TCAACACACTCCATGTTTAGAGC	$(TCTTT)_3$	59	315–335	Peptidyl prolyl cis-trans isomerase (*Arabidopsis thaliana*)	1E-118
5	PLC21	GT626993 (16581949)	F: AACTCGCATCCTCTTCACAACT R: GGACCTTTCCCTTGTAGTCACC	$(TTC)_6$	60	264–286	Glutathione peroxidase (*Medicago truncatula*)	5E-112
6	PLC22	GT626865 (16581950)	F: TACACTGAAGGAGATGCACTGG R: TAACAACAAAACACAGCTTCGC	$(T)_{11}$	60	279–290	DNA-directed RNA polymerase I, II, and III subunits (*Medicago truncatula)*	1E-43
7	PLC30	GT625366 (16581951)	F: TTGGTCAGGTTCTCAATCCTCT R: ACGGATGAACGCTTGTAAAGAA	$(T)_{10}$	61	243–257	Uncharacterized protein (*Glycine max*)	3E-39
8	PLC35	GT619232 (16581952)	F: TTGCTTCCTCCTCTTCTCACTC R: AGCCTCAGTACCCTCCTCTTTT	$(T)_{10}$	60	260–277	GDP-L-galactose phosphorylase-1 like (*Glycine max*)	7E-141
9	PLC38	GT626497 (16581953)	F: CCTGGAGAAGTCTGTGGAAGAT R: AGCTCTAGCATTTTGCATGTGA	$(TTTGT)_3$	59	309–334	LEA protein (*Medicago truncatula*)	4E-65
10	PLC39	GT624018 (16581954)	F: CAGAGAAATCCCCTGCTGAG R: CATGATTCCCATAGCCTTGC	$(AAG)_5$	62	158–178	5′-adenylylsulfate reductase 3 (*Glycine max*)	1E-177
11	PLC42	GT626865 (16581955)	F: AACCAATCATGGCTTCTGCT R: TTTCACCGTCTTTATGAACCA	$(GA)_8$	60	183–210	BZIP transcription factor ATB2 (*Medicago truncatula*)	6E-78
12	PLC46	GT624901 (16581956)	F: CAAACTGGAAGATGCTGCTG R: TGACCCATCCTCATCCTTAAA	$(CAATGG)_3$	61	192–220	Auxin-induced protein 5NG4-like (*Medicago truncatula*)	2E-71
13	PLC51	GT624642 (16581957)	F: CCATGATGAGCCTTGAATGA R: TCTTCAATCTCCAGGAACACTTT	$(GAA)_{10}$	62	125–143	Peroxidase (*Medicago truncatula*)	0.0
14	PLC60	GT624076 (16581958)	F: TGCTTGGACCCTAAATTTGC R: AAGAAAAGGGCAACCACTGA	$(TA)_6$	60	130–145	Cysteine proteinase inhibitor (*Medicago truncatula*)	4E-131
15	PLC63	GT619353 (16581959)	F: TTGATGGCTATGGGAGTGGT R: TGGTCCCAACAAAATACCAA	$(TTA)_8$	60	175–189	Early nodulin-like protein (*Medicago truncatula*)	1E-51
16	PLC70	GT618700 (16581960)	F: CATCTCTTCGTGGCGTAAT R: AGCAAACAACAGCACACATA	$(GTT)_9$	60	179–195	Albumin-2 (*Pisum sativum*)	6E-159
17	PLC74	GT624794 (16581961)	F: GATTTACCGATGGATCTTCA R: CTAAGGGAGAGAAAGAAAAGG	$(TTA)_6$	61	168–191	Xylose isomerase (*Medicago truncatula*)	5E-68
18	PLC81	GT621832 (16581962)	F: GGGTAGAGTATTATTGAAGGTGG R: AGAATCGCTAGTTTAGAGCAAG	$(TA)_6$	61	182–209	Chromo-domain–containing protein LHP1 (*Medicago truncatula*)	8E-34
19	PLC82	GT621329 (16581963)	F: CACCAATCTTCACTTCACTTTC R: CAAGTACAAGGACTGACTAGGG	$(GAA)_4$	60	178–200	Legumin protein (garden pea)	5E-121

Note: T_a = annealing temperature.

Table 2. Total number of alleles (A) and expected heterozygosity (H_e) of EST-SSRs in six *Lens* species.

	L. culinaris Medik. (n = 18)		*L. nigricans* (M. Bieb.) Godron (n = 3)		*L. odemensis* Ladiz. (n = 3)		*L. tomentus* Ladiz. (n = 2)		*L. ervoides* (Brign.) Grande (n = 4)		*L. orientalis* Popow (n = 2)	
EST-SSR locus	A	H_e	A	H_e	A	H_e	A	H_e	A	H_e	A	H_e
PLC5	3	0.494	2	0.444	1	0.000	2	0.500	2	0.688	2	0.500
PLC10	3	0.586	2	0.444	2	0.444	1	0.000	2	0.375	1	0.000
PLC16	4	0.799	2	0.444	1	0.000	2	0.500	2	0.500	1	0.000
PLC17	2	0.198	2	0.444	2	0.444	1	0.000	2	0.375	2	0.500
PLC21	4	0.722	1	0.000	2	0.444	1	0.000	2	0.375	1	0.000
PLC22	3	0.667	2	0.444	2	0.444	2	0.500	3	0.625	2	0.500
PLC30	3	0.648	2	0.444	1	0.000	1	0.000	2	0.500	1	0.000
PLC35	4	0.667	2	0.444	1	0.000	2	0.500	2	0.375	2	0.500
PLC38	4	0.722	2	0.444	2	0.444	1	0.000	2	0.375	2	0.500
PLC39	3	0.537	2	0.444	3	0.667	1	0.500	2	0.500	2	0.500
PLC42	3	0.648	2	0.444	1	0.000	1	0.000	2	0.375	2	0.500
PLC46	3	0.046	3	0.667	4	0.222	2	0.500	3	0.438	1	0.000
PLC51	3	0.278	2	0.444	3	0.667	2	0.500	2	0.375	2	0.500
PLC60	4	0.747	2	0.444	2	0.444	1	0.000	2	0.625	1	0.000
PLC63	4	0.623	3	0.667	1	0.000	2	0.500	3	0.375	1	0.000
PLC70	—	—	2	0.444	1	0.000	—	—	2	0.875	1	0.000
PLC74	4	0.747	3	0.667	1	0.000	2	0.500	3	0.625	2	0.500
PLC81	4	0.386	2	0.778	3	0.667	1	0.000	1	0.000	1	0.000
PLC82	4	0.574	2	0.444	2	0.444	1	0.000	3	0.625	1	0.000

Note: — = no amplification; n = number of accessions used for each species.

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Appendix 1. Voucher information for *Lens* species used for the EST-SSR polymorphism study.

Species	Country of origin/source	Voucher accession no.	Specimens collected/maintained
Lens culinaris Medik.	Turkey	IG-70208, IG-70211, IG-112, IG-115, IG-12	ICARDA, Aleppo, Syria
	Ethiopia	IG-208, IG-69502, IG-69513, IG-69517, IG-69522, IG-70174	ICARDA, Aleppo, Syria
	India	L4149, PL08, L9-12, L830, L4603	NBPGR and IARI, New Delhi, India
	Syria	FLIP2004-7L, ILL4605	ICARDA, Aleppo, Syria
L. nigricans (M. Bieb.) Godron	Syria	ILWL-111, ILWL-22, ILWL-445	ICARDA, Aleppo, Syria
L. odemensis Ladiz.	Syria	ILWL-254, ILWL-35, ILWL-153	ICARDA, Aleppo, Syria
L. tomentosus Ladiz.	Syria	ILWL-91, ILWL-93	ICARDA, Aleppo, Syria
L. ervoides (Brign.) Grande	Syria	ILWL-126, ILWL-206, ILWL-139, ILWL-393	ICARDA, Aleppo, Syria
L. orientalis Popow	Syria	ILWL-7, ILWL-81	ICARDA, Aleppo, Syria

Note: IARI = Indian Agricultural Research Institute; ICARDA = International Center for Agricultural Research in Dry Areas; NBPGR = National Bureau of Plant Genetic Resources.

Appendix 2. Primer sequences and characteristics of the 43 monomorphic EST-SSR markers in *Lens*.[a]

No.	EST-SSR locus	Primer sequences (5′–3′)	Repeat motif	T_a (°C)	Allele size (bp)	Putative function (organism)	BLASTX *E*-value
1	PLC2	F: TTGACTGTTCTGGCGTTTTCTA	$(T)_{19}$	56	330	Peptidyl-prolyl cis-trans isomerase (*Medicago truncatula*)	5E-101
		R: TGCACCATCTTTTGCCTACATA					
2	PLC4	F: CCTATCGGGAAACTACATGGAA	$(GGCAGC)_3$	58	359	Calnexin-like protein (*Zea mays*)	1E-157
		R: TCTGCATTGGTCTTCTTCTCAA					
3	PLC6	F: ATCAAGTTAGGGACGATTGGAA	$(GTA)_6$	56	185	Hypothetical protein MTR_5g092090 (*Medicago truncatula*)	2E-65
		R: TGGTTGTAGTCTTTTAGGGTTTGC					
4	PLC7	F: GCTTTTATGATCTTCTCGTGGT	$(GAAT)_4$	56	184	Chitinase domain-containing protein 1-like (*Glycine max*)	6E-17
		R: CGAGGATTACTTTTCAATGGTC					
5	PLC8	F: CTCCTTCCATTTCTCTTTCTGC	$(TTC)_6$	58	158	Uncharacterized protein (*Glycine max*)	1E-108
		R: TCCTGAACGACACCAACACTAC					
6	PLC9	F: ATGTGGATACGTCAGAAACCCT	$(TATCTA)_4$	56	348	Glycinin subunit G7 (*Glycine max*)	8E-56
		R: TCGAGAACTGGGAGAGTCAAAT					
7	PLC11	F: GTTTGTTTGGTTTGACTGGGAT	$(TTA)_6$	58	185	Histone H1 (*Pisum sativum*)	1E-35
		R: TTAGGAACGGTGTCGAGTACAA					
8	PLC12	F: GGAAGCAAGATGGAAGAAGTTG	$(T)_{11}$	60	146	Heat-shock protein (*Medicago truncatula*)	2E-49
		R: GCGCCATTAGTGCAGAGTAAAT					
9	PLC13	F: TCACCATTTTGGGTTATCTTCC	$(T)_{13}$	56	211	Hydrophobic protein LTI6B-like (*Cicer arietinum*)	1E-17
		R: AGCTTCACACTATCAATTCCACAC					
10	PLC14	F: TCTGGAAGAGGGTTTGTACCAT	$(T)_{12}$	56	210	Uncharacterized protein (*Cicer arietinum*)	1E-66
		R: GCAGTTAGATCACAGCTACCAAAA					
11	PLC15	F: CCAGTAAAAGAGCTTGCATTCC	$(A)_{10}$	58	345	Vicilin precursor (*Vicia faba*)	1E-179
		R: AGAAAAGAGTTGCAGAGAAGCG					
12	PLC18	F: GGACCATCAACTAGCACATGAC	$(GAA)_6$	56	382	Peroxidase (*Medicago truncatula*)	1E-91
		R: TCACATCATCAACATGCTCAAC					
13	PLC25	F: GTTGCAGAAAATGTAACTGCGT	$(A)_{12}$	56	396	Uncharacterized mRNA (*Glycine max*)	8E-44
		R: ACAATGAGAGGCCAGTGCTTA					
14	PLC28	F: CAAGGTTGGAAAAGACAAGAGG	$(A)_{18}$	60	398	60S ribosomal protein L36 (*Medicago truncatula*)	2E-68
		R: TTTGGAGCTAGACTTCGCATTT					
15	PLC40	F: CAACTCGCATCCTCTTCACA	$(TTC)_6$	60	163	Glutathione peroxidase (*Medicago truncatula*)	2E-108
		R: CAAAGGGGTTGGAGTCGTAA					
16	PLC41	F: TTTGTTGATGTTGTTGGCGT	$(T)_{12}$	60	164	Arabinogalactan peptide 16-like (*Cicer arietinum*)	1E-12
		R: CTCCTCCGCGTTCTACAAAC					
17	PLC48	F: TGTGGTACATGCACACCAAAT	$(ACC)_5$	58	168	Proline-rich protein (*Medicago truncatula*)	1E-27
		R: GGTGGTAGCAGTGGTGGAGT					
18	PLC49	F: TTGTTTTGAGAACCTTCCCC	$(T)_{28}$	58	200	Hypothetical protein PRUPE_ppa010183mg (*Prunus persica*)	3E-35
		R: TTTTGCAAGGGTATTTCTTTTTG					
19	PLC50	F: CGATTGGTCTTATATGGTTCTG	$(ATGTA)_4$	60	172	Peptide transporter PTR3-A (*Medicago truncatula*)	9E-41
		R: AAGCTACCTGCATACTTGGTC					
20	PLC52	F: CGTTTGATCTTCTAAGCCCC	$(T)_{10}$	58	198	Uncharacterized protein (*Glycine max*)	3E-10
		R: TCGGCACATTGTTGAAAAGA					
21	PLC53	F: TCGTGATAAAAACGGGGAAG	$(GAA)_5$	56	200	BRI1-KD interacting protein (*Medicago truncatula*)	2E-94
		R: TATCTTTGCCACTGCCTCCT					
22	PLC54	F: GTAAACGAAGCTCAGAGCCG	$(GGA)_5$	56	200	Glycine-rich RNA-binding protein (*Medicago truncatula*)	3E-48
		R: CATATCCACGATCCCTGCTT					

Appendix 2. Continued.

No.	EST-SSR locus	Primer sequences (5′–3′)	Repeat motif	T_a (°C)	Allele size (bp)	Putative function (organism)	BLASTX *E*-value
23	PLC55	F: AGACACCGGCATCAAATCAT	$(A)_{10}$	60	173	Acylamino-acid-releasing enzyme (*Medicago truncatula*)	3E-111
		R: CATATTCAAATATTCAGTGTCATGTTC					
24	PLC57	F: GGAAGTGATTGTGGTTTTTAATCA	$(A)_{17}$	60	182	WD repeat-containing protein 26-like (*Glycine max*)	8E-45
		R: ATTGCTCATTCCCACCAAAG					
25	PLC58	F: TGGAAGAAAGAGAAGGGCAA	$(T)_{12}$	60	138	Putative zinc finger protein (*Arabidopsis thaliana*)	1E-102
		R: CACAGCTACCAAAAATCAGTTCC					
26	PLC59	F: TTGTTTAGCTGGTGTGGTTTTC	$(A)_{18}$	56	180	F-box protein SKP2B-like (*Glycine max*)	1E-46
		R: CTACAGCACGTTTGCAAGGA					
27	PLC61	F: ACTAGGAAAGGAAAACGGCG	$(TC)_{26}$	56	145	No significant similarity	—
		R: GAGTGACACGTGAATGGTGG					
28	PLC62	F: GCAAAGAACAAGAATAACGTGG	$(AAAC)_4$	56	126	Beta-1,3-galactosyltransferase 2-like isoform 1 (*Glycine max*)	1E-91
		R: CAAACCGAAGAATAAGAGAGGG					
29	PLC64	F: CAAACTCTTCACCGACACGC	$(TCTTC)_5$	60	181	Bcr-associated protein (BAP) putative (*Ricinus communis*)	4E-83
		R: AACGAGGGTTAGGATGAGAAGC					
30	PLC65	F: TGTTGCAATGCTTTTAGCCT	$(A)_{11}$	56	165	40S ribosomal protein SA (*Medicago truncatula*)	3E-110
		R: CAGAAGCTTTTCGGTGTTCC					
31	PLC66	F: ATTTGGAGCAAAGATGCAGG	$(A)_{10}$	56	200	D-tyrosyl-tRNA^{Tyr} deacylase-like (*Glycine max*)	1E-69
		R: GGATCGACCTCCAATCAAGA					
32	PLC67	F: GCATAATCAGTTTGTTTTTGCG	$(A)_{23}$	58	190	Cyclin-dependent kinases regulatory subunit 1-like (*Cicer arietinum*)	3E-45
		R: TTCTGCAAAAGCTTCTGGGT					
33	PLC68	F: AAAAAGAGGCCATCATGTTCA	$(A)_{18}$	56	156	Ferritin (*Pisum sativum*)	1E-48
		R: CAGCAGTGACGGCAATTTTA					
34	PLC69	F: CGCTCTACCAACAGCATAA	$(CT)_{19}$	56	195	No significant similarity	—
		R: GAGGTCTCTTTTGTTCTTCACT					
34	PLC71	F: AGTGAGCAAGGAATAAAACG	$(AG)_{38}$	58	276	Legumin J acidic chain (*Pisum sativum*)	0.0
		R: GAGTAGCAAGGAAAGTGAAAAC					
36	PLC72	F: TATGATGAAAGCCAGGACA	$(TAT)_8$	58	142	Aminocyclopropane-1-carboxylate oxidase (*Pisum sativum*)	9E-180
		R: GACTGCACAATCTTAAACACC					
37	PLC73	F: GAAAGGAAAGGTTTTAGCTG	$(AG)_{13}$	60	198	40S ribosomal protein S18 (*Medicago truncatula*)	8E-91
		R: CTTTGATTGAGGTAAGAGCA					
38	PLC75	F: TCGTTCCATATCTGTGTTCA	$(AATC)_3$	56	195	Xylose isomerase (*Medicago truncatula*)	5E-68
		R: GTAGCGAGATTCATACCTATCC					
39	PLC76	F: AGGAAGGTGGAGTTACGG	$(CT)_{52}$	56	160	Cyclin-like F-box (*Medicago truncatula*)	6E-164
		R: AAACCTAGAAGTAAAGGGGAAG					
40	PLC77	F: GGAAAGAGCCAAGAAGTTG	$(CAATGG)_5$	56	230	Auxin-induced protein 5NG4-like (*Glycine max*)	8E-70
		R: ACCCATCCTCATCCTTAAAT					
41	PLC78	F: CTATGACTGCTCAAACTCAAGA	$(GAT)_6$	56	150	Nascent polypeptide-associated complex subunit alpha-like (*Medicago truncatula*)	1E-115
		R: CCTTCTACATCATCATCTTCCT					
42	PLC79	F: AATTTCTGGTGTTTCTGGTG	$(GAT)_7$	58	165	Translational elongation factor 1 subunit beta (*Pisum sativum*)	2E-94
		R: TCTTCTCTTCCTCAGTCTCTTC					
43	PLC80	F: GCTAACAAACAACACCATGA	$(GAA)_{10}$	58	150	Peroxidase (*Arabidopsis thaliana*)	3E-176
		R: GCATCTAAGTTCTTCAATCTCC					

Note: T_a = annealing temperature.

[a] Only polymorphic primers were submitted to GenBank, therefore GenBank IDs for monomorphic markers are not available.