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## ISOLATION AND CHARACTERIZATION OF NOVEL EST-DERIVED GENIC MARKERS IN *PISUM SATIVUM* (FABACEAE)<sup>1</sup>

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- **Premise of the study:** Novel markers were developed for pea (*Pisum sativum*) from pea expressed sequence tags (ESTs) having significant homology to *Medicago truncatula* gene sequences to investigate genetic diversity, linkage mapping, and cross-species transferability.
- **Methods and Results:** Seventy-seven EST-derived genic markers were developed through comparative mapping between *M. truncatula* and *P. sativum* in which 75 markers produced PCR products and 33 were polymorphic among 16 pea genotypes.
- **Conclusions:** The novel markers described here will be useful for future genetic studies of *P. sativum*; their amplification in lentil (*Lens culinaris*) demonstrates their potential for use in closely related species.

**Key words:** comparative mapping; expressed sequence tags; lentil; marker-assisted selection; pea; synteny.

Pea (*Pisum sativum* L.) is an important grain legume grown in temperate regions of the world because its seeds are a cheap and rich source of protein and contribute to the nutritional quality of human and animal diets. Marker-assisted selection (MAS) for agronomic traits such as yield, quality, and tolerance to abiotic and biotic stresses is not widely applied in pea due to unavailability of a reference pea genome and the limited number of molecular markers for tagging of agronomically important genes in pea improvement programs (Jain et al., 2012; Smykal et al., 2012). Pea expressed sequence tag (EST) sequences ([http://www.ncbi.nlm.nih.gov/dbEST/dbEST\\_summary.html](http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html)) are valuable tools for developing breeder-friendly markers from coding regions of genes and have been used in the past to develop a modest number of simple sequence repeat (SSR) markers in pea (Xu et al., 2012; Mishra et al., 2012; DeCaire et al., 2012; Zhuang et al., 2013). Genomic resources of the sequenced model legume *Medicago truncatula* Gaertn. (<http://gbrowse.jcvi.org/cgi-bin/gbrowse/medicago/>) also offer a wealth of information for developing EST-derived genic markers in closely related species using a comparative genomics approach (Smykal et al., 2012). Genic markers developed in this study using the conserved sequences between the two legumes are valuable because they can add density to gene-rich linkage maps of pea, establish macro- or microsynteny between *M. truncatula* and pea, and have higher chances of transferability between closely related species. This information can help in identifying markers that are tightly linked to the genes of interest or candidate gene/quantitative trait locus for agronomic traits.

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Investigation of conserved regions in different studies has provided strong evidence for sequence correlations between *M. truncatula* and pea (Choi et al., 2004a; Aubert et al., 2006; Bordat et al., 2011). This information can be used to develop genic markers based on sequence homology between the related species. Choi et al. (2004b) developed EST-based intron-targeted primers after aligning *M. truncatula* ESTs with the homologous genomic sequences of *Arabidopsis* (DC.) Heynh. and used them to construct a genetic map of *M. truncatula*. The basic assumption for this strategy is that introns or noncoding regions contain more DNA polymorphism than exons or coding regions (Brauner et al., 2002). A similar strategy—one that allows amplification of genomic DNA fragments covering two or more exons and bracketing polymorphic intron regions between those exons—was used in this study to develop pea EST-derived genic markers. Markers developed in this study are also available as cross-species markers within the legume family.

### METHODS AND RESULTS

Primers were designed from pea EST sequences having significant similarity (score  $\geq 100$ ;  $E$ -value  $\leq e^{-50}$ ) using the BLASTn search with *M. truncatula* gene calls from the contig assembly (Mt3.0) of *M. truncatula*. Approximately 1200 *M. truncatula* gene calls were searched for presence of introns. One or more introns were present in 510 of the 1200 *M. truncatula* gene calls and were aligned with the available pea ESTs ( $n = 18576$ ) in the database. Seventy-seven primers were designed from the pea ESTs having well-conserved sequences with *M. truncatula* gene calls spanning one or more introns. Primers were designed by importing sequences into Primer-BLAST ([www.ncbi.nlm.nih.gov/tools/primer-blast/](http://www.ncbi.nlm.nih.gov/tools/primer-blast/)) and selecting primers 18–24 bp long with annealing temperatures of 55–65°C. New primers were designed to amplify fragments from 150 to 1200 bp.

Genomic DNA of 16 pea genotypes including widely grown cultivars and plant introduction lines (i.e., Shawnee, Melrose, Medora, Lifter, Radley, PI 179449, Green Arrow, Frolic, A778-26-6, Sparkle, J173, Bohatyr, ICI12043, PI 240515, PI 103709, PI 169603) was extracted from leaf tissue using a modified cetyltrimethylammonium bromide (CTAB) extraction protocol (Rogers and Bendich, 1985). PCR amplifications were performed in 25- $\mu$ L reaction mixtures with 50 ng of template DNA, 0.2  $\mu$ M of each forward and reverse primers, 200  $\mu$ M

TABLE 1. Specific primer sequences and characteristics of 75 EST-derived genic markers developed in *Pisum sativum*.

Locus	Primer sequences (5'–3')	Product size (bp)	T <sub>a</sub> (°C)	<i>M. truncatula</i> gene call number	Pea EST
Mt5_001*	F: AGGAAAATCCAGAAGTGTGCTCCCC R: GCAAGAACATTGGCGCTTCCCC	510–540	62	Medtr5g008110.1	gbEX568712.11
Mt5_002	F: GGCAGAGACGGTTGGAAAGCC R: GAGGGAGCAAAAGTGGAGCTATCGG	310–1200	60	Medtr5g007580.1	gbICD860473.11
Mt5_003*	F: GTGGATGCCATGTTGGGAAGGT R: CCTAACATGTCTCAAACACCAGCA	350	62	Medtr5g011160.1	gbEX569130.11
Mt5_004*	F: TGTTCACGTGCATATTAGTGGAGGC R: TTGGGGCAGTTTCAAATCAGAGTGGG	810–1200	62	Medtr5g011250.2	gbICD861142.11
Mt5_005*	F: TGGACCGAATGAGCGAGCCG R: CCAATTACTTGGCTCCATCGTCGC	300	62	Medtr5g012870.1	gbIGH720478.11
Mt5_006*	F: CAAGTTGAAGTGTGGTTTCAGAATCCG R: GAACCACCACCTTCTGCCACGC	380	62	Medtr5g013110.1	gbIFG530896.11
Mt5_007	F: AATCTGAAACTGACAGTGAAGAGTCCG R: ACCATAAAGCATCTCTGTGCGG	505	58	Medtr5g013750.2	gbIFG530508.11
Mt5_008*	F: AAGAAACACAGCGCACCGGC R: ATGGCAGCCAAGCCCAATGCC	340–1200	62	Medtr5g016230.1	gbIFG536800.11
Mt5_010	F: TGCTTTGTTCAACACTTCTGGATGGT R: GCAGCGCAATCATGGTAATGGAG	320	62	Medtr5g016380.1	gbICD858783.11
Mt5_012*	F: GGTTGATCCGGAGATTCTCGACGC R: AGGAGTATTGGCCAGAACACGGG	1200	62	Medtr5g016490.1	gbIFG537114.11
Mt5_013	F: AGTCGTGTGTACTCATTCATCCGC R: TGTTACTTTCAGAACGATGGAAACCG	250	62	Medtr5g018040.1	gbIFG536363.11
Mt5_015	F: CGTTGGAGAAGATGTTCTGTTTGGG R: CCATCCGCATAATAGCCCCACC	330–450	62	Medtr5g019760.1	gbIFG535260.11
Mt5_017	F: CCATGGCCCATCAATTGCTGATGC R: ACTGATATGGTGGATGAGTTGCTTGC	350	62	Medtr5g021320.1	gbICD859147.11
Mt5_018*	F: TCCACATAATGCCAGCAAAATCCC R: GCCGACGTTGTTGCCACCG	200	62	Medtr5g021730.1	gbIFG530030.11
Mt5_019	F: TGCCCATTTGGTTTTCCCTGCGG R: CATGATCGAAGATGATTGCGCCG	1200	62	Medtr5g022640.1	gbICD861082.11
Mt5_020*	F: CACCCGAAGAGACTGCGAAAGCG R: TCTGGACTGTGCTTTTTGTACTGCC	520	62	Medtr5g024350.1	gbIFG530254.11
Mt5_021	F: GGAGATGCATFGGAGCCGGG R: CGAGTTCCTTTCCAATGAGTTTCTCCC	510	62	Medtr5g027470.1	gbICD859365.11
Mt5_022	F: GGCAGTGAATAGTGGAAAGAGAGTCCG R: TGGTGTTTGGAAAGTACAGTGAACCC	510	62	Medtr5g032270.1	gbIFG534942.11
Mt5_023	F: AGTGGATGAAGCGGGGTGCC R: CACCCTTTTACCAGCACGGC	150	62	Medtr5g034530.1	gbIFG536062.11
Mt5_024*	F: AAAGCTCCTGTTCTGTCCCG R: TCACCTCACATCCTTCTCCAATGGG	300–420	62	Medtr5g036270.1	gbIFG530106.11
Mt5_025*	F: ACACAGGAAGACGCGATTCTGCC R: CGTGATGCTTTGTAAGAAGGGCGC	720	62	Medtr5g036610.1	gbIFG535769.11
Mt5_026	F: ACTCTTAGTGTGGATTGGAGGGC R: ACGACTTCTCAAAGCCATCCGC	390	62	Medtr5g038320.1	gbIFG530798.11
Mt5_027*	F: GCCATGTGATGTTTTGGGTTGCC R: TGAGCAGCAAAATGCCTCAGCCC	600–1200	58	Medtr5g038460.1	gbIFG536762.11
Mt5_028	F: GGCTCCATCTCCCGCATCCG R: CTCCTCAAAGGTACATCAGCTCGC	980	58	Medtr5g039270.1	gbIFG535137.11
Mt5_029	F: TCCACAACCCCAACAACAACAACA R: ACTTTGTGTCCATGCTTTTGACACC	280	60	Medtr5g044680.1	gbIFG533184.11
Mt5_030	F: CATGGTGCACACCTCCACGC R: TTTCCGTGCTTCAGCAGCCG	490–550	60	Medtr5g045820.1	gbIFG533235.11
Mt5_031	F: TTCTTTCCGGAGGGAACACGC R: GGCTTGAGTCAGCACACTTGCG	520	60	Medtr5g046470.1	gbEX569990.11
Mt5_033*	F: AGTTGGGTGAGGAATTGCAGGC R: TGGAGCTTATAGTGAAGATTTGCCGC	420–430	62	Medtr5g048930.1	emblAM161971.11
Mt5_034*	F: ACATGAATCTTGACATCGTCACCAGG R: ACTGTCATAGATACTCTTTGCAAGCCG	480	60	Medtr5g049600.1	gbIGH720878.11
Mt5_036	F: FCATGAGTCTTTGTCTCAGAGGCCG R: TCTAGCCGCAACTTTTCTGAATTTGCC	510	60	Medtr5g065000.1	gbIFG530443.11
Mt5_037*	F: AGTCCTGATCTGTCTTAGGTGTGCC R: GCAAAGCCTTCTCCATTACTGAGGG	550	62	Medtr5g065120.1	gbIFG533265.11
Mt5_038	F: GATGTTGCAACTGGTTATGGTGTGGG R: ACTGAGGAACAAACCCGATTGGCC	510	61	Medtr5g066790.1	gbIFG529092.11
Mt5_039	F: TGCAGACGATGTGTTACCACCGG R: CCATGCCAGTTCTCAGTCGTGGA	430	61	Medtr5g067140.1	gbIFG533231.11
Mt5_041	F: TTATGGCTGTGGAAGACACCGG R: GCCTTGTGATAATGCATCCTCAGCC	290	62	Medtr5g068460.1	gbIFG531379.11

TABLE 1. Continued.

Locus	Primer sequences (5′–3′)	Product size (bp)	T <sub>a</sub> (°C)	<i>M. truncatula</i> gene call number	Pea EST
Mt5_042*	F: AACTTGCTCTGGTGCATGGGC R: AACTTCCTGGCTCGAGCACTCCG	320	62	Medtr5g068500.1	gbIFG530312.11
Mt5_043*	F: TCCAAGAACCACCAACCCTGCA R: TCCAGATCCTCCTGTACAGCCAGA	400	58	Medtr5g069000.1	gbIFG534946.11
Mt5_044	F: TGGCTGAGAAAAGTACCCTGGG R: AAACCTGGCATGAAAGAGTAACCG	660	62	Medtr5g069480.1	gbIFG535471.11
Mt5_045	F: TGGTTCTCATGTCTGGTGGGCC R: CCCTATGCGGGTTTGGACGC	395	62	Medtr5g071720.1	gbIFG530120.11
Mt5_046*	F: TCAGTTCTTCTATGCAATAGAGCGGC R: AGCCTCAAACAAGCCCTTGCC	380	62	Medtr5g072140.1	gbIFG536413.11
Mt5_047	F: GCACTTGAATCCGCGGAACGC R: TGTGCTTTGCTCCTTGTGGCC	490	62	Medtr5g072570.1	gbIFG530391.11
Mt5_048*	F: CATTTGCGGTCTGGCCCCG R: TGTTTTGTTGTCAGTCCATGAATTGGC	630	62	Medtr5g072790.1	gbIFG536675.11
Mt5_049	F: ACAAGATCAGCACCATTGAGGGCC R: TCGCTCAATAATCTGTGCAACCCC	210	62	Medtr5g072900.1	gbIFG535776.11
Mt5_050*	F: CGGACAGAAGGAAGAAAGCAGAGGC R: GAGAAGTTCAGAGCAAGACCAAGCC	800	60	Medtr5g073680.1	gbEX568722.11
Mt5_051*	F: ACTGAGCTGCCTCCAACCTCACCC R: GTGCTATCCTTGTATGACTCCTCTCCC	480–500	58	Medtr5g073770.1	gbEX568722.11
Mt5_052	F: CTGGATATAGTGCCGCATCGCC R: GAAGTGCACATAAACCTTCCAAGGC	355	58	Medtr5g075640.1	gbICD860246.11
Mt5_053*	F: GCTTTTGATGTTGATGATGTGGACCC R: CCTTAGCTCTTCGAGTGCCTCGG	550	58	Medtr5g077400.1	gbEX569929.11
Mt5_054	F: CTCAAATGACTGACATCTTCGAGGGC R: CCACGTCGAAGGCTTTCACCTGC	540	58	Medtr5g077950.1	gbIFG531468.11
Mt5_055*	F: CACCTTGTGCTGTAATAACCAAAAGCC R: CTGTCAAGTTTCTAAGGGTTCTCTCCG	450	58	Medtr5g079090.1	gbICD858894.11
Mt5_057	F: AACCCCGAAAGGCACATCGG R: ACATTTGCAAGTTTTTCCGCCCGG	290	60	Medtr5g079650.1	gbIGH719720.11
Mt5_058*	F: GGATACTATTTTCGAGGGATCTGTGGC R: CGATTTGCAACGCCTGGCCG	550	58	Medtr5g080340.1	gbICD858878.11
Mt5_059*	F: TGGCAGCCTCTATACTACGCGC R: CGGTAGTCCTCGAGTTTGTGCCC	700	58	Medtr5g080730.1	gbIFG531745.11
Mt5_060	F: CCATCTCCTCCTCACC CGG R: GATAACCACGCGCTTCAGCCC	490	62	Medtr5g080900.1	gbIFG533819.11
Mt5_061	F: AAGAAGCTGTGTTGGACTCTCAGAGGG R: CTTACAGATCCTTGATTTGTCAACCCC	495	62	Medtr5g081470.1	gbIFG538061.11
Mt5_064	F: GCCACAGCAGCTCGTATTCTGT R: TGCTGTTCTTGCATCTCTTCTCCC	610–1200	58	Medtr5g082780.1	gbIGH720629.11
Mt5_065*	F: GGATCGTCAGGTTTGGGGTCCG R: CCACCCAAACATCAACAGCAACGG	150–350	58	Medtr5g083280.1	gbIGH719482.11
Mt5_066*	F: ACAACACCCGAACGCTGTGCC R: CCTCGGCTGTCCACTCCTCCC	8200	58	Medtr5g083430.1	gbEX571173.11
Mt5_067	F: GCGCTCCCTTGACATTTGCGG R: GAGATTTGCACCAAGTATTTCAGCC	520	55	Medtr5g084140.1	gbIFG534893.11
Mt5_068	F: GTTGTCATTGTTGTTATGCCACGCC R: CTGAACCTCCATGCTGCTGTAGGG	290	55	Medtr5g084410.1	gbIFG533023.11
Mt5_069*	F: AAGCCTCAGCTCTCAACATTTAAGGC R: TAGCATCTTCATCAAACCCGCGG	320	58	Medtr5g084550.1	gbIFG529821.11
Mt5_070*	F: CGCTCCTCGTTGCGATACCGG R: AGTTCAACATGCTGCACCCGGG	700	58	Medtr5g084740.1	gbIGH720486.11
Mt5_071*	F: CCGGCTCATTGATGATATGGTGGC R: TGTTTTGCTGGTTTCTCCACCC	400	58	Medtr5g084890.1	gbICD860585.11
Mt5_072*	F: TCTCACATCTGGCATGGCTGGC R: GCCACCCAAACAAGTAATGGCG	200	58	Medtr5g085020.1	gbICD860768.11
Mt5_073	F: ACACGTGGAATGGATGTTGAAGGG R: AAGAACCACCCCTCAGCCTTTCGC	1200	55	Medtr5g085470.1	gbIFG533378.11
Mt5_074	F: TGCACAGCAGTTGCCAGAGGA R: CTTGTTCCACTCATCCGAGCTTCA	800	55	Medtr5g085560.1	gbIFG537000.11
Mt5_075*	F: CAGAGAACAAGCAAGAAAAGGGGG R: ACCGGTCATCCACCTCCCGC	750–800	58	Medtr5g085630.1	gbIFG534721.11
Ps4_001	F: TTGGCCAAACTGCTTGTCAAACCTGG R: GCCTTGGGGGCATCATTAAACATCATCC	363	59	Medtr8g008440	FG531483
Ps4_003	F: TCCCGGTACATGGAGCTCTAGTTG R: AGGCTGCAAGAGAAAATTCTTGGTCC	568	51	Medtr8g008880	FG537838
Ps4_004	F: TTCGGGTTACAACCCCTTTCACGG R: GCGCGCCATGACTATAGCAGC	684	55	Medtr8g011640	FG530764
Ps4_005	F: TTCTGGATTTGACCAAGAAGCGGC R: TGAAAATCTCCGAACCGGGAACAC	628	58	Medtr8g011640	FG530764

TABLE 1. Continued.

Locus		Primer sequences (5'–3')	Product size (bp)	$T_a$ (°C)	<i>M. truncatula</i> gene call number	Pea EST
Ps4_006	F:	TGTCCCAAACCTACTTCGCTCCG	220	61	Medtr8g015460	FG538362
	R:	TGGCCGTCAACTTTCTATTACCCG				
Ps4_007	F:	GAACCCAATCAAGTGTGTGTACGC	275	59	Medtr8g021260	FG530143
	R:	TCATCACGTACAATCACTGACATACGC				
Ps4_009	F:	AGGTGGCAGGCTCGAATCGG	601	59	Medtr8g024670	FG533947
	R:	AGGTGTCGACGTACTCCCGC				
Ps4_010	F:	GCACACGATGATGTGGATGGAGAATGC	210	58	Medtr8g026430	FG529623
	R:	TGTCACAGCCGAAGTGAGCCC				
Ps4_012	F:	AGCTGGATGGGTTTGTATGCCCC	425	56	Medtr8g027050	EX570946
	R:	ATCCAATCGCCAGGCCGC				
Mt8_002*	F:	GTGCTTCTACAAGATCATATTGGCCGC	300	61	Medtr8g008860	FG537838
	R:	GCTTGCAACTGATACTCTTGGACCG				

Note:  $T_a$  = annealing temperature.

\*Polymorphic EST-derived genic markers.

dNTPs, 2.5 mM MgCl<sub>2</sub>, 1× PCR buffer, and 0.5 U *Taq* DNA polymerase in a Veriti 96-Well Fast Thermal Cycler (Applied Biosystems, Carlsbad, California, USA). The PCR profile included an initial denaturation at 95°C for 3 min followed by 35 cycles of 95°C for 1 min, 51–62°C for 50 s (according to the primer's annealing temperature), 72°C for 1 min, and a final extension at 72°C for 10 min. Length polymorphism was viewed with ethidium bromide in 8% polyacrylamide gels run in a Mega-Gel high-throughput electrophoresis system for 5 h at 250 V (C.B.S. Scientific, San Diego, California, USA). If length polymorphism was not detected, PCR products were digested with restriction enzymes (New England BioLabs, Ipswich, Massachusetts, USA) to generate cleaved amplified polymorphic sequence (CAPS) markers and separated on 2% agarose to detect polymorphism. Amplified fragments were run with a 25/100-bp DNA ladder (Bioneer, Alameda, California, USA) and analyzed for fragment size using AlphaView Stand Alone analysis software version 3.4 (ProteinSimple, Santa Clara, California, USA). Each EST-derived genic marker was considered polymorphic when the PCR band pattern of one of the 16 pea genotypes was different from the others with regard to size or CAPS polymorphism (Appendix S1). Different polymorphic fragments for a particular locus were considered as different alleles. Seventy-five primer pairs resulted in successful PCR amplification in which 66% (42 primer pairs) were monomorphic and 44% (33 primer pairs) were polymorphic among the 16 pea genotypes, which are parents of several pea mapping populations being used to map different disease resistance loci. The segregation analysis using these polymorphic markers has been conducted in a large number of mapping populations developed from crossing of these genotypes as parents (data not shown). All the primers generated a clear fragment pattern, with PCR products ranging in size from 150 to 1200 bp with two to three alleles per marker. Table 1 summarizes the forward and reverse primer sequence, size range of the original fragment (bp), annealing temperature, *M. truncatula* gene call, and the equivalent pea EST GenBank accession number. These EST-derived genic markers are codominant, highly reproducible, and easy to score. PCR products among the 16 pea genotypes were analyzed for allele number, observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ) or gene diversity, and polymorphic information content (PIC) using PowerMarker version 3.25 (Liu and Muse, 2005) (Table 2).  $H_o$  and  $H_e$  values ranged from 0.0000 to 0.0625 and from 0.0377 to 0.6391, respectively. The PIC ranged from 0.0370 to 0.5659 with an average of 0.2708. Twenty-four EST-derived genic markers were tested in two lentil (*Lens culinaris* Medik.) genotypes, and PCR amplification of 12 markers determined the transferability of these markers in related genera (Appendix S2). This lends support from other studies on transferability of cross-species markers based on conserved sequences (Phan et al., 2006). Cross-species transferability of EST-derived genic markers is due to the conserved nature of primers picked up from coding sequences. More detailed polymorphism analysis and linkage analysis using mapping populations will establish connections between the genetic and genomic information of the closely related species.

## CONCLUSIONS

The current study identifies and characterizes new EST-derived genic markers based on comparative mapping between pea and *M. truncatula*. Thirty-three polymorphic and 42 monomorphic primer sequences were described in this study. These EST-derived

genic markers were mined from conserved *M. truncatula* gene sequences; therefore, they can be used to anchor genomic regions between pea and *M. truncatula* and possibly among other members of the legume family. These markers show polymorphism among 16 pea genotypes that include parents of several pea mapping populations being used to map different disease resistance loci. These molecular markers will be useful to develop gene-rich linkage maps and to tag genes for agronomically important traits. In addition, amplification of these markers in lentil demonstrates the transferability of these markers across related species.

TABLE 2. Results of 33 polymorphic EST-derived genic loci screened in 16 genotypes of *Pisum sativum*.

Locus	A	$H_e$	$H_o$	PIC
Mt5_01	2	0.4800	0.0000	0.3648
Mt5_03	3	0.3507	0.0000	0.3222
Mt5_04	3	0.6391	0.0000	0.5659
Mt5_05	2	0.2041	0.0000	0.1833
Mt5_06	2	0.4032	0.0000	0.3219
Mt5_08	2	0.4234	0.0000	0.3338
Mt5_12	2	0.2604	0.0000	0.2265
Mt5_18	2	0.0377	0.0385	0.0370
Mt5_20	2	0.4527	0.0000	0.3502
Mt5_24	3	0.3225	0.0000	0.2896
Mt5_25	2	0.4872	0.0400	0.3685
Mt5_27	2	0.3200	0.0000	0.2688
Mt5_33	2	0.1528	0.0000	0.1411
Mt5_34	2	0.2188	0.0000	0.1948
Mt5_37	2	0.3750	0.0000	0.3047
Mt5_42	2	0.0605	0.0625	0.0587
Mt5_43	2	0.4992	0.0000	0.3746
Mt5_46	2	0.4800	0.0000	0.3648
Mt5_48	2	0.3935	0.0000	0.3161
Mt5_50	2	0.4970	0.0000	0.3735
Mt5_51	3	0.5408	0.0000	0.4529
Mt5_53	2	0.2604	0.0000	0.2265
Mt5_55	2	0.0740	0.0000	0.0712
Mt5_58	2	0.0740	0.0000	0.0712
Mt5_59	3	0.5910	0.0385	0.5252
Mt5_65	2	0.4800	0.0000	0.3648
Mt5_66	2	0.3107	0.0000	0.2624
Mt5_69	2	0.0740	0.0000	0.0712
Mt5_70	2	0.1420	0.0000	0.1319
Mt5_71	2	0.4970	0.0000	0.3735
Mt5_72	2	0.1420	0.0000	0.1319
Mt5_75	2	0.4734	0.0000	0.3613
Mt8_002	2	0.1420	0.0000	0.1319

Note: A = number of alleles;  $H_e$  = expected heterozygosity;  $H_o$  = observed heterozygosity; PIC = polymorphic information content.



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