Development and validation of 107 SNP markers in *Todarodes pacificus* (Ommastrephidae)

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Research Article

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Abstract

Todarodes pacificus is a commercially important squid species; however, there is little information available regarding the population genetics of this species and effective molecular markers are still unavailable. In the present study, 107 novel single nucleotide polymorphism (SNP) markers were developed using Genotyping-by-Sequencing (GBS). The observed and expected heterozygosities ranged from 0.0345 to 0.5862 and 0.0339 to 0.4994, respectively. The polymorphism information content ranged from 0.0333 to 0.3747. Among these SNPs, all loci have been confirmed to fit the Hardy-Weinberg equilibrium ($p > 0.05$). The novel SNPs developed in this study will be helpful for the genetic conservation and scientific management of *Todarodes pacificus*.

Main Text

The Japanese common squid, *Todarodes pacificus* (*T. pacificus*), is a commercially important squid species, which is mainly distributed around the Northwest Pacific Ocean (Arkhipkin et al. 2015; Sakurai 2003). The abundance of this species has fluctuated widely, exhibiting a gradual increase in recent years (Yeong and Ho 2008). It is necessary to enrich the current knowledge of *T. pacificus*, especially in terms of population genetics and diversity. To date, the majority of research on this species has focused on fisheries hydrography, stock assessment and biology (Fang and Chen 2018), with only a few studies isolating polymorphic loci using microsatellites (An et al. 2014; Iwata et al. 2008). A comprehensive understanding of *T. pacificus* population genetics remains limited, and effective molecular markers are still unavailable.

With the rapid development of NGS technologies, large numbers of sequencing-based single nucleotide polymorphisms (SNPs) have been discovered and are widely used in population genetic studies, due to their broad genome coverage and automatic detection in high throughput sequencing (Van Tassell et al. 2008; Xiao et al. 2019). Until now, there has been a gap in the application of SNP molecular markers in *T. pacificus* population genetics. Thus, it is necessary to develop a set of meaningful and readily available SNPs and apply them to the population conservation and sustainable utilization of this species. In this study, we analyzed and characterized a number of SNP markers in *T. pacificus* using Genotyping-by-Sequencing (GBS).

Muscle samples of 30 individuals were collected from three natural *T. pacificus* populations in the Sea of Japan (n=10, 36°11' N, 131°15' E) and East China Sea (n=10, 27°16' N, 132°32' E; n=10, 25°59' N, 122°28' E). Total genomic DNA was extracted using the modified phenol-chloroform method (Gu and Xu 2013) and quality assessed through 1% agarose gel electrophoresis. A GBS library was constructed for all samples and sequenced using Illumina HiSeqTM, PE150. We obtained a total of 18,838 putative SNPs by setting a criterion of <1% minor allele frequency and <10% miss rate. In order to improve reliability, SNPs with a sequencing depth greater than 10 were further filtered. One hundred and ten potential SNP loci were randomly selected and detected in 50 *T. pacificus* individuals using the multiplex PCR technique. The PCR reaction system contained 10-30 ng DNA, 1.1 µL PCR mix, 0.25 µL of each primer and RNase-free water to a final volume of 5 µL. PCR amplification conditions were as follows: 94°C for 3 min, then 40 cycles of 94°C for 30 s, annealing temperature of 56°C for 25 s, extension at 72°C for 30 s, with final extension at 72°C for 3 min. The amplification products were sequenced with the MassARRAY compact System to identify the SNP genotype according to the peak. Observed heterozygosity and expected heterozygosity were calculated using Stacks pipeline populations

program (Catchen et al. 2013). Polymorphism information content and Hardy-Weinberg equilibrium were estimated using PowerMarker software version V3.25 (Liu and Muse 2005).

Among the 110 random primer pairs, 107 SNP loci were found to be polymorphic and biallelic in 50 *T. pacificus* individuals. The summary statistics of genetic diversity are shown in Table 1. The observed heterozygosity and expected heterozygosity ranged from 0.0345 to 0.5862 and 0.0339 to 0.4994, respectively. The polymorphism information content ranged from 0.0333 to 0.3747. All loci have been confirmed to fit the Hardy-Weinberg equilibrium after Bonferroni correction ($p>0.05$). To our knowledge, this is the first study to develop SNP markers in *T. pacificus*. These novel genetic markers will expand the knowledge of this species and provide a useful exploration tool for genetic conservation and population genetics, which is beneficial for the sustainable utilization of *T. pacificus* resources.

Declarations

Conflict of interest

The authors declare that they have no conflict of interest.

Funding information

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Authors' contributions

Xiaoling Li and Xingxing Hu carried out most of the experiments; Congcong Wang designed the methods and experiments, interpreted the results and finished the discussion. Yang Liu prepared the materials and partly worked on the experiments. Huajie Lu was responsible for overall supervision, and participated in coordination. All authors read and approved the final manuscript.

Compliance with ethical standards

Experimental protocols involving live animals were approved by the Ethics Committee for the Use of Animal Subjects of Shanghai Ocean University.

References

1. An HS, Kim EM, Myeong JI, An CM (2014) Isolation and characterization of polymorphic microsatellite markers from the Korean common squid *Todarodes pacificus* (Ommastrephidae). *Genes Genomics* 36(6):727–732. <https://doi.org/10.1007/s13258-014-0206-9>
2. Arkhipkin AI, Rodhouse PGK, Pierce GJ et al (2015) World squid fisheries. *Reviews in Fisheries Science & Aquaculture* 23(2)
3. Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA (2013) Stacks: an analysis tool set for population genomics. *Mol Ecol* 22(11):3124–3140. <https://doi.org/10.1111/mec.12354>

4. Fang Z, Chen XJ (2018) Review on fishery of Japanese flying squid *Todarodes pacificus*. Marine Fisheries 40(1):102–116
5. Gu MY, Xu QH (2013) Preliminary study on improved genomic DNA extraction method from *Trematomus bernacchii*. Journal of Shanghai Ocean University 22(2):168–172
6. Liu K, Muse SV (2005) PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinformatics 21(9):2128–2129. <https://doi.org/10.1093/bioinformatics/bti282>
7. Van Tassell CP, Smith TPL, Matukumalli LK, Taylor JF, Schnabel RD, Lawley CT, Haudenschild CD, Moore SS, Warren WC, Sonstegard TS (2008) SNP discovery and allele frequency estimation by deep sequencing of reduced representation libraries. Nat Methods 5(3):247–252. <https://doi.org/10.1038/nmeth.1185>
8. Sakurai Y, Bower JR, Ikeda Y (2003) Reproductive characteristics of the ommastrephid squid *Todarodes pacificus*. Report of the working group on modern approaches to assess maturity and fecundity of warm- and cold-water fish and squids, 105–115
9. Xiao M, Hu Q, Zhao Y, Bao F (2019) Development of SNP markers in *Leiocassis longirostris* Günther using high-throughput sequencing. Conservation Genetics Resources. <https://doi.org/10.1007/s12686-019-01084-0>
10. Iwata Y, Lian CL, Sakurai Y (2008) Permanent genetic resources: Development of microsatellite markers in the Japanese common squid *Todarodes pacificus* (Ommastrephidae). Molecular Ecology Resources 8(2). <https://doi.org/10.1111/j.1471-8286.2007.01995.x>
11. Yeong G, Ho CK (2008) Fluctuations in abundance of common squid, *Todarodes pacificus* in the Far East. J Environ Biol 29(4):449–452

Tables

Table 1 Characterization of the 107 SNP markers in *Todarodes pacificus*

Primer ID	Primer sequences (5'-3')	SNP type	Size (bp)	SNP position	PIC	H_o	H_e	P_{HWE}
TpSNP 1	F: ACGTTGGATGCACAAAGACTGATATTCGCC R: ACGTTGGATGTCTGCATAGCTGAAAGGGTG EXT: caTGAAAGGGTGCATTTTGAT	T/A	104	54	0.2278	0.1724	0.2622	0.0652
TpSNP 2	F: ACGTTGGATGTCTGACGCATGTTTCATCCG R: ACGTTGGATGTTGGCCCATTCTCATTACCC EXT: ccacCTTTCGCTTTCAAAGCTCTGA	A/C	99	49	0.2278	0.1724	0.2622	0.0652
TpSNP 3	F: ACGTTGGATGTCTTAGAATGGTGTAATCCC R: ACGTTGGATGCCAGTCTCTAGTTCAGTTGG EXT: CAGTTGGTACAGATGAGT	G/T	110	56	0.3747	0.3448	0.4994	0.0956
TpSNP 4	F: ACGTTGGATGTGGATAAAGGTGCCAACTGC R: ACGTTGGATGTATTCTTTTGTCCCACCCTG EXT: AGGTGTGGCAGTGGA	A/T	92	55	0.2447	0.2069	0.2854	0.1386
TpSNP 5	F: ACGTTGGATGAAGTCAGCTCCTCTCATGTG R: ACGTTGGATGAAGTGCAAACGAAGCGGAAG EXT: ggacCGAAGCGGAAGGTTTATAAAGGG	A/G	96	45	0.2447	0.3448	0.2854	0.2619
TpSNP 6	F: ACGTTGGATGCATTCGTCACTAGCTATGTC R: ACGTTGGATGTATTTTCGGTGTCCGGCATGTC EXT: ggtTGAAGATCTGGAATTCTACTATTTA	T/C	106	61	0.2278	0.3103	0.2622	0.3226
TpSNP 7	F: ACGTTGGATGACGCATTCGCGCAATCAGG R: ACGTTGGATGTCCTTCAATTCCCCTTCCTC EXT: CCTTCCTCCACATCTAC	C/G	93	77	0.2278	0.3103	0.2622	0.3226
TpSNP 8	F: ACGTTGGATGTGACCTCTCTCTAAAAAGTC R: ACGTTGGATGCCATTCAATTAGAAAGCGTC EXT: cccacTTAGAAAGCGTCAATCTGAAC	T/C	101	57	0.3738	0.5862	0.4976	0.3377
TpSNP 9	F: ACGTTGGATGAGTGATCAAATTACCGCTG R: ACGTTGGATGGCCAAGTTGCAGGTAGATAG EXT: cACAAATGCTTCTTTACAAAAGGA	G/A	107	46	0.2095	0.2759	0.2378	0.3889
TpSNP 10	F: ACGTTGGATGTAGCTGGATACTTTCCGGAC R: ACGTTGGATGAGTAACTGCCCACTCATTTCG EXT: aactGGTAGACATGTCATGGAGC	T/C	111	41	0.2095	0.2759	0.2378	0.3889
TpSNP 11	F: ACGTTGGATGGCGGTCATGGTATTGTTTAG R: ACGTTGGATGTCCCCCTCTCTAATAACTCC EXT: ccctgACGAAAATAACGACGACCATTGA	G/A	112	73	0.2743	0.2759	0.3282	0.3906
TpSNP 12	F: ACGTTGGATGCAAACCTAGCCAGACAATGGG R: ACGTTGGATGGTAAAAAGAAGAAATCACAC EXT: TCACACAATATATATGACACTATCTCTT	C/T	102	65	0.2743	0.2759	0.3282	0.3906
TpSNP 13	F: ACGTTGGATGTGACATGTACCAACAGGAGG R: ACGTTGGATGTTTGCCATCACTGGTGATAG EXT: AGCGGAGATCCCATAG	G/A	83	59	0.1897	0.2414	0.2123	0.4598
TpSNP 14	F: ACGTTGGATGGAGCTGGTTAGGAAATTTTGC R: ACGTTGGATGGGAATCTTGGACTACATGAG EXT: tGACTACATGAGAATACCAAG	T/G	117	52	0.3364	0.4828	0.4281	0.4914
TpSNP 15	F: ACGTTGGATGCGAGATTTAGCCTGAAAAG R: ACGTTGGATGCTGTTTCACATGTCAACGTC EXT: attaACATGTCAACGTCTTCATAC	T/C	91	44	0.3364	0.4828	0.4281	0.4914
TpSNP	F:	G/T	98	78	0.1683	0.2069	0.1855	0.5344

16	ACGTTGGATGCTTTGTGAGCATTTTCCTAG R: ACGTTGGATGATGTGGCGACGTACATCAAC EXT: CAACAGGAGGCAGAAT							
TpSNP 17	F: ACGTTGGATGGCTATGCTGTCTAAAACCTC R: ACGTTGGATGGGTTGAGAGAAAAATGTCAG EXT: ttAGAGAAAAATGTCAGCTGGAT	G/A	116	46	0.3747	0.5517	0.4994	0.5727
TpSNP 18	F: ACGTTGGATGGGAAAGGGAGAATGGGAGTG R: ACGTTGGATGAGAGACATTGCAAGCACTCC EXT: CTCCCCCTCCCTCATT	G/T	91	68	0.1451	0.1724	0.1576	0.6114
TpSNP 19	F: ACGTTGGATGCTGTCCGACATGAAATTGCC R: ACGTTGGATGAACTCCATTTTCTGCTTTG EXT: tgcACTCCATTTTCTGCTTTGGAATTTG	T/C	89	54	0.1451	0.1724	0.1576	0.6114
TpSNP 20	F: ACGTTGGATGCGAAAAATATTAGTCTGAATATG R: ACGTTGGATGTGTGGAGAAATGACAAGGCG EXT: ttggaGGAGACCAAAGTGGAAGGAAAT	T/C	102	41	0.2873	0.3793	0.3478	0.6256
TpSNP 21	F: ACGTTGGATGCAGTTGTACTAACTGTACGC R: ACGTTGGATGATTTTCCACGAGTCAGTCAG EXT: ggGAGTCAGTCAGGAAAGT	C/A	92	65	0.2873	0.3793	0.3478	0.6256
TpSNP 22	F: ACGTTGGATGCTGACAGACTTGTTTCTCTTG R: ACGTTGGATGTAGTGACGAAAAGACAAAGG EXT: ggaggAAAGACAAAGGCAGCAA	G/A	95	76	0.3702	0.4483	0.4905	0.6430
TpSNP 23	F: ACGTTGGATGTCACGAACAACTTTGAAAGC R: ACGTTGGATGTGCGACGCTTAGATCTGTAAC EXT: aacacACTAGTAACGAAAACGTGTTTATA	C/T	98	50	0.3285	0.3793	0.4144	0.6485
TpSNP 24	F: ACGTTGGATGTATTCTCCTGATGAGTCGTG R: ACGTTGGATGAATAAGGGATGCAGTCACCG EXT: TCGCCTTCCATCCTA	T/A	99	53	0.2278	0.2414	0.2622	0.6691
TpSNP 25	F: ACGTTGGATGAGTGAGGCCAAGTTTCACAG R: ACGTTGGATGTGCTGAACCCAAATGACTCG EXT: gCCGGTTGGGTAGAGT	C/A	96	67	0.2278	0.2414	0.2622	0.6691
TpSNP 26	F: ACGTTGGATGGCCACAAGGTTGGGAACCAT R: ACGTTGGATGCTGGAGTACTTGGATCCTTG EXT: CCCATCTTTTGAAACCTG	A/G	91	45	0.1202	0.1379	0.1284	0.6900
TpSNP 27	F: ACGTTGGATGTAGCAAGAACAAAGCAGACC R: ACGTTGGATGTCCTGAGAACTTATGTGTC EXT: CGTTGAATGACAACAAAACCTG	T/C	103	67	0.1202	0.1379	0.1284	0.6900
TpSNP 28	F: ACGTTGGATGTATGACGTCCAATTTGCGCTG R: ACGTTGGATGACAACAGAAGGGTTCTTCTC EXT: agCATTAGTGTTACTGGTATATAATTTG	G/A	100	68	0.1202	0.1379	0.1284	0.6900
TpSNP 29	F: ACGTTGGATGGTGTCCAGGCAATAAACGAG R: ACGTTGGATGTTTGTGCTGCTGCGTATATC EXT: CCGTGATTGAGTCCG	A/T	107	71	0.1202	0.1379	0.1284	0.6900
TpSNP 30	F: ACGTTGGATGTCTTTAGATTCTGTATGC R: ACGTTGGATGTCCAGCATCATAAACCTGAC EXT: ATGAACCAATGGAATGTG	G/A	98	61	0.1202	0.1379	0.1284	0.6900
TpSNP	F:	T/C	99	42	0.1202	0.1379	0.1284	0.6900

31	ACGTTGGATGGACATGCTCTGCTCTCCAAG R: ACGTTGGATGGTGAAGCTAATTTACGCCATC EXT: accccTCACCTGAGATATTAGTGAT							
TpSNP32	F: ACGTTGGATGACTCAGATGTTTAGGGTGGC R: ACGTTGGATGATGATGCGGATGAAAGCCTG EXT: ggGAAAATGTCATATAAGACTCAAAG	G/A	111	60	0.1202	0.1379	0.1284	0.6900
TpSNP33	F: ACGTTGGATGCAGTTCCTTCATCAGCTTCG R: ACGTTGGATGACCAGAGAAGATTCCACCAC EXT: cCCACCCCCACCAGCGGC	C/T	105	59	0.1202	0.1379	0.1284	0.6900
TpSNP34	F: ACGTTGGATGGGTTTTGTGGATTATGCCTG R: ACGTTGGATGCGAAAGTGGTGATATAATAC EXT: ccccACAATGTTTTCATTTTGAAAGA	G/A	124	44	0.3498	0.4828	0.4518	0.7125
TpSNP35	F: ACGTTGGATGACAGCTTTAGAAGGAATAGG R: ACGTTGGATGGCATTGGTATATGCTCTAAG EXT: TGCATTCTTGTTTTCATGTATA	G/A	100	46	0.2992	0.3448	0.3662	0.7530
TpSNP36	F: ACGTTGGATGTCCTTGGTAATCCGTGCAAC R: ACGTTGGATGTGAGCTATCAACAGACAGAC EXT: cccaaTCAAACAAGAAATTCATGCCG	G/A	100	67	0.0933	0.1035	0.0981	0.7690
TpSNP37	F: ACGTTGGATGCTAGCGATTCTGATAGCGTC R: ACGTTGGATGGGTGATGAAACCAAGTGTAG EXT: GTGTAGTTATGATGTTTTTCAG	A/G	100	54	0.0933	0.1035	0.0981	0.7690
TpSNP38	F: ACGTTGGATGTTACCTCAAACCTCCCCTG R: ACGTTGGATGACCATCACTACGTGCAAGAG EXT: ggagTGGCAGTATTTGTGTGG	G/A	104	54	0.0933	0.1035	0.0981	0.7690
TpSNP39	F: ACGTTGGATGCAACCGTATGACAAAATAGC R: ACGTTGGATGGTCTGCTGCTTTTTGTATGCC EXT: ctcCACCTTTATTGTTTCGGAC	G/A	103	60	0.0933	0.1035	0.0981	0.7690
TpSNP40	F: ACGTTGGATGACGCATTGTCCAGACACAAG R: ACGTTGGATGTGGAGAGGAGATTTCTGGAC EXT: ACATTACTGACCAGACG	T/C	101	40	0.0933	0.1035	0.0981	0.7690
TpSNP41	F: ACGTTGGATGTTAGAATTGAGCCCTCTGCG R: ACGTTGGATGGGAGAATTCAAGTCGTTGGTC EXT: TACACAACCTACAAAGTGATACA	A/G	112	72	0.0933	0.1035	0.0981	0.7690
TpSNP42	F: ACGTTGGATGAGATGGACTGAAGGATTTGC R: ACGTTGGATGTCTGTTTGATGAACACTGAG EXT: GAACACTGAGTTTGCATC	G/A	112	50	0.0933	0.1035	0.0981	0.7690
TpSNP43	F: ACGTTGGATGGACACAAGGTCAATTTGGTG R: ACGTTGGATGCCAATCTTTTAGGTGTTTC EXT: TTAGGTGTTTCTACCCC	C/A	96	41	0.3738	0.5172	0.4976	0.8319
TpSNP44	F: ACGTTGGATGATCAGCAGAGCTTACACTGG R: ACGTTGGATGTAGCGCAACCTTCATGGATG EXT: cTGCTCCTTTTCAAATATTTTTC	T/C	124	68	0.0644	0.0690	0.0666	0.8475
TpSNP45	F: ACGTTGGATGGCTGGCAACGAAATGAGAAG R: ACGTTGGATGTATCAGAAGATCCGTCAAGC EXT: TAGCTGTAATGTTCTATTTGAATT	A/G	117	46	0.0644	0.0690	0.0666	0.8475

TpSNP 46	F: ACGTTGGATGAGAATTACAGACAGCGCGAG R: ACGTTGGATGCTTCACGTCATACGCGTTAC EXT: TCGCAACACACCCGA	C/T	94	40	0.0644	0.0690	0.0666	0.8475
TpSNP 47	F: ACGTTGGATGACAGATGCTACACCTTGCAC R: ACGTTGGATGCATAAGACCAGAACCTGAAC EXT: CAGAACCTGAACACCTCT	G/T	92	48	0.0644	0.0690	0.0666	0.8475
TpSNP48	F: ACGTTGGATGGAATATAATGGGCCATGTGC R: ACGTTGGATGTGTACTTGATAACAACCCAC EXT: ccccATAACAACCCACAGGAAT	A/G	105	41	0.0644	0.0690	0.0666	0.8475
TpSNP 49	F: ACGTTGGATGACTGCGGCAGTGTTACAAAG R: ACGTTGGATGCCTGATCGCACTGCAGTTTA EXT: AGTTTATATATTCTACATGAAGAAATAT	G/A	124	70	0.0644	0.0690	0.0666	0.8475
TpSNP 50	F: ACGTTGGATGGTGGAAAGTTGGTTTTGGGC R: ACGTTGGATGACCAGTGCTGAATGGTAGTG EXT: cttctTGGTAGTGACCAACAATCAG	T/C	91	55	0.0644	0.0690	0.0666	0.8475
TpSNP 51	F: ACGTTGGATGCGAGGCCAGTAAAGTTGTAA R: ACGTTGGATGGCCTTCACAATCAAGTCTGG EXT: TTGTTCCACTGATCAGTGAC	A/T	118	54	0.0644	0.0690	0.0666	0.8475
TpSNP 52	F: ACGTTGGATGTTGTATAGAGCATAATGTG R: ACGTTGGATGCACAAACATAGCTCACGGTC EXT: cCACGGTCTCTCATTTTAC	T/C	83	43	0.0644	0.0690	0.0666	0.8475
TpSNP 53	F: ACGTTGGATGCTCTATTGGTAAACTGGGTG R: ACGTTGGATGCTTTGTTCATAGGGTGGAGC EXT: caGTGGAGCTATGTTCTGT	T/C	105	43	0.0644	0.0690	0.0666	0.8475
TpSNP 54	F: ACGTTGGATGAGTAACTGGGCTTCCATCTC R: ACGTTGGATGCAAAAAGTGA CTCCACAGGC EXT: tcttCACCAGTGTTTCCCC	A/T	93	46	0.0644	0.0690	0.0666	0.8475
TpSNP 55	F: ACGTTGGATGAGGAGACGAACAACAGATTG R: ACGTTGGATGATGTTACGGCTTCCTCTTCG EXT: TTCGTTATTTTTCTTTACTGGC	T/G	102	73	0.3197	0.4138	0.3995	0.8475
TpSNP 56	F: ACGTTGGATGGAGTCTAGCCTAGAAATGTC R: ACGTTGGATGCCAATCTGGATTAGGTCAGT EXT: AGTTGAATTGGTAATGTGAAACTA	C/T	107	62	0.2447	0.2759	0.2854	0.8575
TpSNP 57	F: ACGTTGGATGCCAACGACATAAGCCATTCC R: ACGTTGGATGTACTACTGCTGGCCCTTGTAT EXT: TGTTGCTGGCCCTCT	T/C	104	71	0.2447	0.2759	0.2854	0.8575
TpSNP58	F: ACGTTGGATGGGCTAGAAAATAGCTACAAG R: ACGTTGGATGTCTAGACGGTGAAAATAGGC EXT: GTGAAAATAGGCGACTAAAA	G/A	104	63	0.3364	0.4138	0.4281	0.8575
TpSNP 59	F: ACGTTGGATGGTAGATGATATTACAGAATAC R: ACGTTGGATGTAGCTGTGCCTTCACAAATC EXT: gAAATCAATAATATAATTGATGTTTCATG	A/G	105	50	0.0333	0.0345	0.0339	0.9247
TpSNP 60	F: ACGTTGGATGGAGAAATTGACTGGTAAAC R: ACGTTGGATGGTAGCAATCCCAGCAGATAC EXT: CCCAGCAGATACTGCAAT	G/A	101	60	0.0333	0.0345	0.0339	0.9247

TpSNP 61	F: ACGTTGGATGCTTCAATGTTGATAAGCAGG R: ACGTTGGATGTTCCCGTACCGGCTTATTTT EXT: gtcTCAGCATCCATCGGA	A/G	93	62	0.0333	0.0345	0.0339	0.9247
TpSNP 62	F: ACGTTGGATGACTGGCATAGATTTCTACCG R: ACGTTGGATGACCGACTCGGACAGCAATAT EXT: gagATCTTCACGATTCAATTTGT	G/A	110	52	0.0333	0.0345	0.0339	0.9247
TpSNP 63	F: ACGTTGGATGTTTACATGCACCCATCCCTC R: ACGTTGGATGTATTACAGATGGTGCCGTCC EXT: gggagCTCTGGGAAAGGTTAGTTTA	C/T	110	61	0.0333	0.0345	0.0339	0.9247
TpSNP 64	F: ACGTTGGATGGGCCTCTGTGCTATTCTATG R: ACGTTGGATGTGCGTAATACCAGTGATGAC EXT: TGACATATGGCTGCG	G/A	105	54	0.0333	0.0345	0.0339	0.9247
TpSNP 65	F: ACGTTGGATGCATTTGGCCTTGAGACACAC R: ACGTTGGATGTTTCCTGGTGTCTACTGAG EXT: TGAGCTATGCTTCAATGTC	C/T	100	72	0.0333	0.0345	0.0339	0.9247
TpSNP 66	F: ACGTTGGATGCCCTCCTTGAGATGGATATG R: ACGTTGGATGTGTGGCTTTGGAACAGTTTC EXT: CGACTCCTCGTTCAACA	T/C	116	69	0.0333	0.0345	0.0339	0.9247
TpSNP 67	F: ACGTTGGATGGCGATCAATGCTCAAAAGCG R: ACGTTGGATGATCGTCATCACTTTTGTTC EXT: AATTCTTCTTACTCAACTTCCTC	T/G	104	64	0.0333	0.0345	0.0339	0.9247
TpSNP 68	F: ACGTTGGATGCTTTGTTTGTGCAGCCGATG R: ACGTTGGATGTACTAGCAAGGAGACTCCTC EXT: ccccCCGTACGCTGAGTAGAC	T/C	117	63	0.0333	0.0345	0.0339	0.9247
TpSNP 69	F: ACGTTGGATGCATGTCAACCTAGAAACACC R: ACGTTGGATGACATAGATATAGCACTTTT EXT: gATAGCACTTTCTAAAATGTACTG	G/A	91	69	0.0333	0.0345	0.0339	0.9247
TpSNP 70	F: ACGTTGGATGTTGAAAGTGGCTGCGTCATC R: ACGTTGGATGAGCAAGAAAGGCAAAATGGAG EXT: ATGGAGTTATTGCCCCAAAT	C/G	99	80	0.0333	0.0345	0.0339	0.9247
TpSNP 71	F: ACGTTGGATGGGCAACTATCGCTCCATTAC R: ACGTTGGATGCGAGAATGTTTGTTAGTCGG EXT: AGTATTTTGTGAAGAGTTTATATATG	T/G	105	54	0.0333	0.0345	0.0339	0.9247
TpSNP72	F: ACGTTGGATGCCCTAGTCATTGAGTAAAATC R: ACGTTGGATGTGTACCATGAAGTATGTGTG EXT: AGTATGTGTGATGAACTTGA	A/G	82	47	0.0333	0.0345	0.0339	0.9247
TpSNP 73	F: ACGTTGGATGTTCTCAACTGGGAGATCGTC R: ACGTTGGATGGGAAAGGAGTGAAGAAGAGG EXT: agGATGAGGAAGATTATGAGGA	C/T	101	41	0.0333	0.0345	0.0339	0.9247
TpSNP 74	F: ACGTTGGATGCTGTGAAAAGAGCGTCTTCC R: ACGTTGGATGCAGATTGGCAGTAATAAGCTC EXT: GGCAGTAATAAGCTCTAAACAAAAC	G/A	95	61	0.0333	0.0345	0.0339	0.9247
TpSNP 75	F: ACGTTGGATGGATGTGACGTTGGTTGTCAG R: ACGTTGGATGGGCCAACGATTTTCGTTGATG	C/G	98	53	0.0333	0.0345	0.0339	0.9247

TpSNP 76	EXT: tctcgTGGCCGCCGTCAATTA F: ACGTTGGATGATTCAATTGTGGTGTGGAGGG R: ACGTTGGATGTAGCACCTTGAATAAGACCC EXT: ccatACCCAATAAATCCGAATCTCTG	T/A	104	79	0.0333	0.0345	0.0339	0.9247
TpSNP 77	F: ACGTTGGATGCCCAGCAACATCTCCAATT R: ACGTTGGATGGGAATAAAGTAACAAACACCG EXT: ggacAACACCGAAGTAGGGT	T/G	101	70	0.0333	0.0345	0.0339	0.9247
TpSNP 78	F: ACGTTGGATGGTATGCACGCATATACACTC R: ACGTTGGATGATACACACAACATATGTGTGG EXT: aaaaAACTATGTGTGGGGATGT	T/C	94	68	0.0333	0.0345	0.0339	0.9247
TpSNP 79	F: ACGTTGGATGAACGACTGTCCTTGTCTCGG R: ACGTTGGATGGCAAGACGCGTATATTTGAG EXT: CCCTCTTCGTAATCTATGTAA	A/G	118	57	0.0333	0.0345	0.0339	0.9247
TpSNP 80	F: ACGTTGGATGGTGGGATTTGGGATTTACGC R: ACGTTGGATGACCGCTGTCACTCTATATCC EXT: atagATCCTATTCTCCATCCTCTTATTA	G/A	110	80	0.0333	0.0345	0.0339	0.9247
TpSNP 81	F: ACGTTGGATGAGATGGACAGACATCTGGTG R: ACGTTGGATGTATCTAGGGTGGAAATTCAGG EXT: GTAATATTAGCTTATGTTATAAAAAAGTG	G/A	126	70	0.0333	0.0345	0.0339	0.9247
TpSNP 82	F: ACGTTGGATGATCCATTACAGAAATGAAC R: ACGTTGGATGTTACTTTGAAATGGCAAGC EXT: ggggATGGCAAGCTAACATGTC	T/G	90	47	0.0333	0.0345	0.0339	0.9247
TpSNP 83	F: ACGTTGGATGGTGTGGTTCCAAGTTTTCCC R: ACGTTGGATGCAGATGTCGCATGAATATGG EXT: gtgtTTTCACTCCGGTGTGTGCTCGCA	G/A	107	75	0.0333	0.0345	0.0339	0.9247
TpSNP 84	F: ACGTTGGATGCAAAATACAGAATTGCATAGC R: ACGTTGGATGTGTCTCCAATGTTGCACTCG EXT: tCACAAAGTCAGCACACTAT	G/T	106	51	0.0333	0.0345	0.0339	0.9247
TpSNP 85	F: ACGTTGGATGGTCGCCTTTTATACTACACAG R: ACGTTGGATGAGGTGTTCTCCTAAGAGGTG EXT: AGGTGAATGTTACTGCTA	A/T	103	73	0.0333	0.0345	0.0339	0.9247
TpSNP 86	F: ACGTTGGATGTTCTTCTTCTCCTCAGCTC R: ACGTTGGATGTAAGACAGAGCACACACAGG EXT: agcaTCCCTCGGGTCTTGA	C/G	101	41	0.0333	0.0345	0.0339	0.9247
TpSNP 87	F: ACGTTGGATGCGTTCAACGAAATTGCAATC R: ACGTTGGATGACTGGCAGAGTTAGAAGTGG EXT: tcctATGATGCAATCCTAATCTTTACA	C/T	114	53	0.0333	0.0345	0.0339	0.9247
TpSNP 88	F: ACGTTGGATGGATTAGTCCCAAAAGGTCAG R: ACGTTGGATGAGGAATTAGGCAAAGGCCAG EXT: GGCCAGCAATCTCAAGA	C/A	98	62	0.0333	0.0345	0.0339	0.9247
TpSNP 89	F: ACGTTGGATGGGTTTCTCAACTAAGCCTG R: ACGTTGGATGTGACACATCATAGCCAGACC EXT: ctACCGATCCAAAGAAACACAAA	G/A	102	50	0.0333	0.0345	0.0339	0.9247
TpSNP 90	F: ACGTTGGATGTCTTGACATCTGACGATCGG R: ACGTTGGATGTGTTGAAGTCATCTGTCTGC EXT: gagTCTGTCTGCAAGCTTGTTTC	A/G	80	55	0.0333	0.0345	0.0339	0.9247

TpSNP 91	F: ACGTTGGATGGATTATGGATTATCTCCTC R: ACGTTGGATGACTGAACTACTTCACGGGAG EXT: TGGACAGCTTTTATAGCTTG	C/T	88	56	0.0333	0.0345	0.0339	0.9247
TpSNP 92	F: ACGTTGGATGCGATTTTGCCTGAAGCAGT R: ACGTTGGATGTATGACGCAGGGCAATTCAG EXT: CAATTCAGATATATGCTTTATCTATG	A/T	114	56	0.0333	0.0345	0.0339	0.9247
TpSNP 93	F: ACGTTGGATGCTCTCGTCTGTAAGATCTGC R: ACGTTGGATGAGTCGGGTTTTCTGGATATG EXT: AAAGAGGGTAGACCAAAC	C/T	87	41	0.0333	0.0345	0.0339	0.9247
TpSNP 94	F: ACGTTGGATGGAGGAGCATTGCGTCAAAAT R: ACGTTGGATGCGACAAAATGCAGTAAAAG EXT: TTTTCTTTCATTTCACTATTTTATTAG	C/T	133	77	0.0333	0.0345	0.0339	0.9247
TpSNP 95	F: ACGTTGGATGACGCGTACGTCTTCTGTTTC R: ACGTTGGATGTTTGACACGGGATATATGGG EXT: tatgGTGATAATAATTTACATCTGTC	A/T	124	71	0.0333	0.0345	0.0339	0.9247
TpSNP 96	F: ACGTTGGATGGTTCTAGTGACACTAGTGCC R: ACGTTGGATGCAAGTCTCTCTTCCATGCAC EXT: CTGAGTGAAGGGCAA	C/T	100	47	0.0333	0.0345	0.0339	0.9247
TpSNP 97	F: ACGTTGGATGTTCTTGGCTGCCATGTATCC R: ACGTTGGATGTGGTCTGCCAATATGGAACG EXT: cATGGAACGTATTATGAAGGC	C/A	100	62	0.0333	0.0345	0.0339	0.9247
TpSNP 98	F: ACGTTGGATGGTTTTTGTGAACTTTACAG R: ACGTTGGATGTGTTGCCTTCAAGACAATGC EXT: GATTGCTTCTCTGACCT	A/G	107	47	0.0333	0.0345	0.0339	0.9247
TpSNP 99	F: ACGTTGGATGCCAGTTCTGGTAGTGTTGTC R: ACGTTGGATGCTCTTGTGTTGATTACCTCC EXT: tTTGATTACCTCCATCACCA	T/C	104	44	0.0333	0.0345	0.0339	0.9247
TpSNP 100	F: ACGTTGGATGGTTGATTCCTCACAACGAAG R: ACGTTGGATGGCCGCACTATTACTTCAATC EXT: gaCTGAACACTAATTTAGAAAATGT	T/A	94	42	0.0333	0.0345	0.0339	0.9247
TpSNP 101	F: ACGTTGGATGCGCCTATCGGTGACATTTAG R: ACGTTGGATGCTGAATAGTCCGTATTTAG EXT: caccAGTCCGTATTTAGAGACTC	T/G	93	55	0.0333	0.0345	0.0339	0.9247
TpSNP 102	F: ACGTTGGATGGGCTGGAAATTGCTAACTGC R: ACGTTGGATGACCATGAAGTATGTGTGATG EXT: TGTGTGATGAACTTGATATAAGTGA	G/T	116	68	0.0333	0.0345	0.0339	0.9247
TpSNP 103	F: ACGTTGGATGTGCATTGAAGATGAGGCAGG R: ACGTTGGATGGGATGATCAATATTTTAGC EXT: cctcTTTTTAGCAATCTGCAACAT	A/G	80	80	0.0333	0.0345	0.0339	0.9247
TpSNP 104	F: ACGTTGGATGGTCTCTGATGTAAGGCCAAG R: ACGTTGGATGCTAGCAGATGCCACTTGATG EXT: aagcTGCCACTTGATGGATCTTCTA	G/A	86	56	0.0333	0.0345	0.0339	0.9247
TpSNP 105	F: ACGTTGGATGCATACAAAACAGACTCAAGC R: ACGTTGGATGCTTAGTGGTGCCACTGTTAG EXT: ACTGTTAGTCTCATTTTGACCATA	G/A	99	63	0.3435	0.4483	0.4406	0.9247
TpSNP 106	F: ACGTTGGATGGAACAGGCTGTCCAATTGAG	C/T	99	74	0.2601	0.3103	0.3074	0.9585

TpSNP 107	R:								
	ACGTTGGATGTTGGAGACATATTGCCAGCG								
	EXT: actGCGTTGTTCTATTCAGG								
	F:	G/C	89	77	0.3675	0.4828	0.4851	0.9789	
	ACGTTGGATGGCCATCAGATATGCCATCAA								
	R:								
	ACGTTGGATGTCTGCTCATTTGCATATAGG								
	EXT: TGCATATAGGTATCTTAGTAAAGGAT								

Primers for each locus including a forward primer (F), a reverse primer (R) and an extension primer (EXT), *Ho* observed heterozygosity, *He* expected heterozygosity, *P_{HWE}* the *P* value of the departures from Hardy-Weinberg equilibrium.