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1 **Development of a set of SNP markers for population genetics of the red gorgonian**
2 **(*Paramuricea clavata*), an emblematic species of the Mediterranean coralligenous**

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11

12 **Abstract**

13 Transcriptome sequencing was used for the development of single nucleotide polymorphisms
14 (SNP) for the red gorgonian (*Paramuricea clavata*). A total of 20,736 SNPs were identified,
15 and 1,718 had a coverage of over 100 reads. Of the 480 SNPs tested, 347 SNPs were
16 successfully genotyped at 95 samples from the NW Mediterranean using a MassARRAY
17 System. This set of markers will be of great value for population genetics and phylogeography.

18

19 **Keywords**

20 *Paramuricea clavata*, Single nucleotide polymorphism, MassARRAY

21

22 **Introduction**

23 The gorgonian *Paramuricea clavata* is one of the most emblematic and conspicuous
24 coralligenous species in the Mediterranean Sea. A species of great interest for conservation
25 given its role in the persistence and stability of several associated species (Ballesteros 2006).

26 However, given its slow dynamics, *P. clavata* is particularly vulnerable to disturbances. The
27 red gorgonian has suffered the most drastic reductions in density and biomass over a broad
28 spatial scale, as a consequence of sea temperature anomalies in the NW Mediterranean (Cupido
29 et al. 2009; Santangelo et al. 2015). It is, therefore, essential to provide an understanding of
30 the natural genetic variability of *P. clavata* populations, as well as their ability to withstand
31 perturbations.

32 The genetic structure of *P. clavata* populations has usually been studied with the use of a few
33 microsatellite markers, sometimes resulting in conflicting results (Pilczynska et al. 2016;
34 Padrón et al. 2018), and thus motivating the development of SNP markers for the species. Here,
35 we describe a set of 118 SNP markers developed for population genetics of *P. clavata*, that
36 were analyzed using iPLEX Gold technology on a MassARRAY System (Agena Bioscience,
37 San Diego, USA).

38

39 **Material and Methods**

40 *Transcriptome sequencing*

41 Total RNA from ten individuals was used to sequence the transcriptome of *P. clavata*, using
42 paired-end Illumina HiSeq 3000. RNAseq was performed at the GeT-PlaGe core facility, INRA
43 Toulouse. RNA-seq libraries were prepared according to Illumina's protocols using the
44 Illumina TruSeq Stranded mRNA sample prep kit to analyze mRNA. Briefly, mRNA was
45 selected using poly-T beads. Then, RNA were fragmented to generate double stranded cDNA
46 and adaptators were ligated to be sequenced. 11 cycles of PCR were applied to amplify
47 libraries. Library quality was assessed using a Fragment Analyser and libraries were quantified
48 by QPCR using the Kapa Library Quantification Kit. RNA-seq experiments have been
49 performed on an Illumina HiSeq3000 using a paired-end read length of 2x150 pb with the
50 Illumina HiSeq3000 sequencing kits.

51

52 *Marker development and screening*

53 The quality of the raw Illumina sequence reads was initially assessed using FastQC
54 v0.10.1 (Andrews 2010). Based on the analysis report, Trimmomatic v0.32 was used to remove
55 all the low quality reads with a Phred score below 20, as well as the Illumina adapters (Bolger
56 et al. 2014). FastQC was performed again to verify the integrity of the remaining raw Illumina
57 sequence reads. Given the lack of a reference genome for the species, high-quality reads were
58 then used for the *de novo* transcriptome assembly, using Trinity with its default *k-mer* value of
59 25 (Grabherr et al. 2011). DiscoSnp++ v2.2.10 (Uricaru et al. 2015) was then used with default
60 parameters to call SNPs using the assembled transcriptome as a reference.

61 A total of 1,718 SNPs with good coverage (>100 reads) were identified from the
62 transcriptome of *P. clavata* and submitted for assay design using the MassARRAY Assay
63 Designer version 4.0.0.2 (Agena Biosciences). Genomic DNA was extracted from 95 colonies
64 of the red gorgonian *P. clavata* sampled at different locations within the Ligurian Sea (Table
65 1). Twelve multiplexes of 40 SNPs, for a total of 480 SNPs, were designed for the genotyping
66 which was performed on the 95 samples using the iPLEX Gold chemistry following Gabriel *et*
67 *al.* (2009) on a MassARRAY System (Agena Biosciences). Allele calling was carried out with
68 Typer Viewer v.4.0.24.71 (Agena Bioscience). We filtered out all monomorphic SNPs, loci
69 with weak or ambiguous signal (i.e., displaying more than three clusters of genotypes or
70 unclear cluster delimitation) and loci with too much missing data. The genetic parameters,
71 including observed heterozygosity, expected heterozygosity, and the Hardy–Weinberg
72 equilibrium (HWE), were calculated using GenoDive 2.0b23 (Meirmans and van Tienderen,
73 2004).

74

75 **Results**

76 A total of 347 SNPs were successfully amplified, of which 303 loci were polymorphic,
77 resulting in a detectable rate of 87.32 %. The observed heterozygosity ranged from 0 to 1, while
78 the expected heterozygosity varied from 0.007 to 0.5. 204 loci were significantly departed from
79 HWE ($p < 0.01$) after Bonferroni's correction (Supplementary Material). In summary, this is
80 the first report of SNP markers in *P. clavata* developed using transcriptome and MassARRAY
81 analysis. These SNPs would provide an invaluable resource for conservation genetics.

82

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87 Roca and B Hesse, for their technical assistance for field sampling. RNA extractions were
88 performed using instruments and facilities of the Marine Biodiversity and Biotechnology
89 (Bio2Mar) platform of the OOB. SNP genotyping were performed at the Genome
90 Transcriptome Facility of Bordeaux (grants from ANR-10-EQPX-16) with the help of Adline
91 Delcamp and Christophe Boury.

92

93 **References**

94 Andrews S (2010) FastQC: a quality control tool for high throughput sequence data. Available
95 online at: <http://www.bioinformatics.babraham.ac.uk/projects/fastqc>
96 Ballesteros E (2006) Mediterranean coralligenous assemblages: a synthesis of present
97 knowledge. *Oceanography and Marine Biology* 44:1–74.
98 Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: A flexible trimmer for Illumina
99 Sequence Data. *Bioinformatics* 30(15): 2114–2120. 10.1093/bioinformatics/btu170

100 Cupido R, Cocito S, Barsanti M, Sgorbini S, Peirano A, and Santangelo G (2009) Unexpected
101 long-term population dynamics in a canopy-forming gorgonian coral following mass mortality.
102 *Marine Ecology Progress Series* 394, 195–200.

103 Gabriel S, Ziaugra L, Tabbaa D (2009) SNP genotyping using Sequenom MassARRAY iPLEX
104 platform. *Curr Protoc Hum Genet*, Chapter 2: Unit 2.12.

105 Grabherr M, Haas B, Yassour M, Levin J, Thompson D, Amit I, Adiconis X, Fan L,
106 Raychowdhury R, Zeng Q, Chen Z, Mauceli E, Hacohen N, Gnirke A, Rhind N, di Palma F,
107 Birren BW, Nusbaum C, Lindblad-Toh K, Friedman N, Regev A (2011) Full-length
108 transcriptome assembly from RNA-seq data without a reference genome. *Nat Biotechnol*. 2011
109 May 15;29(7):644-52. doi: 10.1038/nbt.1883. [PubMed PMID: 21572440](#).

110 Meirmans P, and van Tienderen P (2004) GENOTYPE and GENODIVE: two programs for
111 the analysis of genetic diversity of asexual organisms. *Molecular Ecology Resources* 4(4), 792–
112 794.

113 Padrón M, Costantini F, Bramanti L, Guizien K, and Abbiati M (2018) Genetic connectivity
114 supports recovery of gorgonian populations affected by climate change. *Aquatic Conservation*
115 DOI: 10.1002/aqc.2912.

116 Pilczynska J, Cocito S, Boavida J, Serrão E, and Queiroga H (2016) Genetic diversity and local
117 connectivity in the Mediterranean red gorgonian coral after mass mortality events. *PLoS ONE*
118 11(3), 1–16.

119 Santangelo G, Cupido R, Cocito S, Bramanti L, Priori C, Erra F, and Iannelli M (2015) Effects
120 of increased mortality on gorgonian corals (Cnidaria, Octocorallia): different demographic
121 features may lead affected populations to unexpected recovery and new equilibrium points.
122 *Hydrobiologia* 1–17

123 Uricaru R, Rizk G, Lacroix V, Quillery E, Plantard O, Chikhi R, Lemaitre C, Peterlongo

124 P (2014) Reference-free detection of isolated SNPs. *Nucleic Acids*

125 Research. [doi:10.1093/nar/gku1187](https://doi.org/10.1093/nar/gku1187)

126

Supplementary Material – Characteristics of 347 SNP markers in *Paramuricea clavata*

SNP ID	Alleles	Primer F	Primer R	Ho	He
SNP_10270	C/ A	CGCTGGCGGAAGTATTTTAC	GTCCTTGGAAACAATCCCTG	0.279	0.242
SNP_10524	G/ A	CGATGGACGAAATGTTGAGC	TCGATGGAGAATTCGAACGG	0.176	0.151
SNP_10540	C/ T	TGTCTTCGTCCTTCTCATGC	GCTCTCGAACAAGAAGAAGC	0.422	0.423
SNP_10599	A/ G	GTCTGAGGATGCTAGACTAC	TTGCCAGCGAGATCTCTATC	0.149	0.127
SNP_10670	T/ C	AGACGGCTGTGTTTGTGTTG	TGTGGCACAGAACAATGACG	0.279	0.242
SNP_10819	C/ T	TCAGCGATATTTCCCTGCAC	GACCAGGATTTACACTGTTG	0.341	0.370
SNP_10870	G/ C	ACATTAGTCCACCACTCAAC	TTGACATGCCATACTTCGCC	0.153	0.164
SNP_11020	C/ T	GCAGTTTTCGGCACAACATC	TTTTCGACATGACTGTTGGC	0.262	0.226
SNP_11054	G/ C	ATTTCTGTTTCGGCGAGGTC	TATCGCTTCAGGGTCTACAG	0.426	0.443
SNP_1123	C/ T	TGGGAAATTTCTTACGGCTC	CTTTTCAGTCCCAGAAGACG	0.000	0.000
SNP_11288	C/ T	ATGCACTTGCCTGAATGCTG	ACCACCACCAAAGAGGAAAC	0.390	0.420
SNP_11320	T/ C	GTCTCGTTATCGTATCGACC	CCCTGTGGAAGAATATGACG	0.417	0.418
SNP_11339	C/ T	GGGACCAGAACAATTTAGAG	GTGTCTTGGTCGTAATAAGG	0.477	0.420
SNP_11364	C/ G	CTGATTGGTCTCAGCTTCTC	TGATTTGGCCAACAACGTG	0.353	0.341
SNP_11394	A/ G	TTCTCGGATCGTCCAGTCAG	ACATCAAACCACGATCCGC	0.050	0.069
SNP_1146	G/ A	ATGTGCGCCTCGATAGTAAC	TAGAATTGGATGGCAAGACG	0.013	0.012
SNP_11478	G/ A	TGATCCCGAAACCAAGAAAC	CATATATCTAGCAGTACCCG	0.189	0.422
SNP_11506	G/ C	GAACAAGTTGGGCACTTCTC	CTGACACACAGATTAAGAGG	0.145	0.292
SNP_11564	A/ G	ACGGTGCTAAAGTTGATCGC	GACATCCACAGCAAAAGCAC	0.450	0.421
SNP_11582	C/ T	GATCCACCTCCGAAATCATC	CCCTGGTGACGTATTCTATC	0.000	0.000
SNP_11616	G/ A	CTTTCTCTGTCTTGCCTGTC	TCCGTAGGAGGTGGTAAATC	0.453	0.420
SNP_11619	G/ C	AATGTCATGCTATCCAGCCG	AGAATGGCGAAGTTTGGCAC	0.300	0.300
SNP_11704	T/ C	TCGGCTGAACAGAAAGAATC	CTTGCTTCATCACGATTGAC	0.393	0.375
SNP_11770	A/ G	ACACATCAGCTGGAACCATC	AAGATACTGTGGTGTCTTGC	0.457	0.446
SNP_11882	G/ A	ATCGACGAATGTGCTGAGAG	GCCAGGTTTGCATACACATT	0.482	0.426
SNP_12083	T/ C	GCTCGAAGATCAGCAGTTTC	GGTTTGATGGCATCCACAAG	0.018	0.017
SNP_12101	G/ A	TGAAGGCTTCTTGTAGCTCG	TTGCCAGTTGCAATACAGCG	0.602	0.480
SNP_12120	G/ A	AATTCTGCGACTGGAACGTG	CTTTGCCTCGGTAACAGAAC	0.433	0.384
SNP_12259	G/ A	AAGCTCTCAGACTGCGATG	GCTCTTCCTGTAATCGTTTC	0.025	0.032
SNP_1229	A/ G	ACCTTTGCGGCCTTCTTGTGTC	TTTGCCAGACATCTACACAG	0.163	0.199
SNP_12309	C/ T	AGCGCCAAGTTATTTAAAG	CACGCTGATGAAAAATCACG	0.274	0.235
SNP_12330	C/ T	GAAATGAAAAATACAGAAGG	CGTATTGCATTGAAGCGTCC	0.380	0.356
SNP_12339	A/ T	GACTCCACAAAAGTTCAAGAC	ATCACTCCCTCTAGACGTTT	0.303	0.313
SNP_1235	C/ T	CTACCTCTTCGGCCATTTTC	TGTTTGAGTGGAGACTGTTT	0.346	0.377
SNP_12396	C/ T	CAAACCTGCGGAAACCGAAG	GCGTTGTTTAGCTTCTCGG	0.496	0.432
SNP_12408	C/ T	GGACCAAGCATTTGTTTCAGG	AATTCCTTTGCCAGTCGCTC	0.171	0.184
SNP_12410	G/ A	ATACACGCGAGTTGATCCAC	GACATGTGGAGTTTTGGATG	0.344	0.331
SNP_12451	G/ C	ATGCTTGTGGCGTAGTTTCG	GAGAGTGTGGCTGAAGATG	0.074	0.068
SNP_1253	C/ T	AAAGAGCGAGAGGAAATGGC	TGATCCAAAGTTCTCCCGAC	0.350	0.352
SNP_12548	A/ G	GTCAAATGATCACACTATCC	GTGTGCCAAAGTTTTCTG	0.000	0.000
SNP_1256	C/ T	ACATCCCTATTACCTCCGAC	TTGGTACTGACGTCGTTTGC	0.415	0.332
SNP_12634	T/ C	ACCATGTAGACACCCCAAAG	CTGAACACAATTTGGCTCAC	0.065	0.117
SNP_12675	G/ A	TGGAAACTCAAGCGGGAAC	GTATCGTCTAGCGATCACAC	0.364	0.345

SNP ID	Alleles	Primer F	Primer R	Ho	He
SNP_12676	A/ G	CTTCTGTCAACTTGAACGCC	TTCAGAGTGCTTGCCTGAAG	0.046	0.071
SNP_12753	T/ C	ATTAAGTGCAGCAACGAGG	TAGGCAGCAATTGTCATTCG	0.146	0.121
SNP_1285	C/ A	TCGTTGTGACTGTGAGCTAC	GCTGATCTCAATATGAACCC	0.237	0.267
SNP_12854	A/ T	CATGACGGAGCCTATTGATG	TCTGGCAACTCCAAACCTTC	0.318	0.303
SNP_12925	A/ G	GTCTGTTCTCTTCTCCATC	AAACAACGTGCTACCCAGAG	0.099	0.113
SNP_12958	G/ A	ACCTAACTCGCTTTTCGTGG	TGACGTCAAGGAATTCTCGG	0.393	0.351
SNP_13079	C/ T	ACCTTGTTGCAAAGTCTGCC	AGGAATATGCAGCGAGGATG	0.250	0.254
SNP_13227	G/ A	CATCAAGCAGAACTTCCAGC	CCGAGCTGAACATACAGTTG	0.169	0.153
SNP_13306	C/ T	TCCTGAGCCGTGAAACATTC	AAGGTGAGTACACACTTCCC	0.408	0.405
SNP_1332	A/ T	GTTTATCGACATGGCCTTG	CGTCCAAACATTTAGCGCTC	0.381	0.431
SNP_13353	C/ T	ATATCAACGCATCCAGGTGC	TCACTCTCCTGTGAATCAGC	0.323	0.343
SNP_13401	G/ A	TTCCTTCGCAAGGCTTGTTG	AAGGCTTCGAATCGCACATC	0.000	0.408
SNP_13505	C/ T	ACAAACGGGTCTGTGTTTC	GGGAGAGTATGTAGAAGTTG	0.400	0.357
SNP_13634	G/ A	AAGACATGGTCGAAGCGATG	CATTCTGTAGGCAACCGAC	0.019	0.038
SNP_13693	C/ T	CCTTTTGACTTTACATCTTCC	GAGCGAACGAGTTGAAAAGG	0.108	0.100
SNP_13760	C/ T	CCAGTCAGATTTTGAAGGCG	GTCGTTGGCTAGATCTAAGG	0.101	0.195
SNP_13806	C/ A	CTTTCAGTGTGATTGTTTC	GGCAAGAAACATGAAGACGC	0.000	0.234
SNP_13855	A/ G	AATTCCTGTTTCATCTCGCC	GACATTGTTTTTGAATGG	0.354	0.376
SNP_1388	T/ C	GCCACGTTGAGTAGATTTCAG	CTGCAGACTGTCTTTGTTTCG	0.084	0.067
SNP_13901	C/ A	CCAGTACAACGCCCTTAATC	GATTGTTGAGGCTGGATTGG	0.211	0.229
SNP_13908	C/ A	ATCGCCAAAGTTTGCAAAG	GCGAGAAACGCAAGTTCATC	0.000	0.105
SNP_13932	G/ T	GGCTGTTTGCCACAAGAATC	TTACAGGCTCGTGCTTGCTC	0.284	0.330
SNP_13981	G/ A	GCTATGGATGGTACAGAAGG	AACTGGGATGGTATTGGTC	0.304	0.273
SNP_13993	C/ T	CATGTACTCGTGCTTTCTC	CGTTGACGAACTTCGAAAGG	0.195	0.194
SNP_14046	C/ T	GACGTTTCCTTTCTCTGCTG	TATGGAATTGGAAGTCCACG	0.681	0.407
SNP_14275	T/ C	TGTACTACTAGCGGCTGCC	ACCACTACTGCAACTACAGG	0.031	0.027
SNP_14312	C/ A	TAAGCTTGTCAGCAGTAGG	GGCAGTGTACAGCTATACC	0.323	0.309
SNP_14315	G/ T	ATTTGACGTCCAGAGACAG	CCTCTCCGGTCTTATTGATG	0.000	0.000
SNP_1446	T/ A	AGTCACATAAAGCACGTGCC	GCTGGACTTAAGAGCAACTG	0.446	0.394
SNP_14492	T/ C	GTGCTAACATCTTCCCAAGG	GATCCGCATGGATAAAGTTG	0.480	0.415
SNP_14496	G/ A	TAAACTCTGCCTACGACTG	TTCCGCCACAGATATCAAC	0.173	0.204
SNP_14523	T/ C	GGCAAAGTTTGACCGAAATG	ATATCTTCGGGACGACGAAC	0.345	0.363
SNP_14565	G/ A	TCACATGGTGGATGAGTTGG	AGTGCCTCCCCATTCTTTTC	0.270	0.279
SNP_14570	T/ A	CAATTGTCAAAGGAGGGTC	TCACCTCGTGAATCCTCCTC	0.113	0.098
SNP_14598	A/ G	CAATGCGGAGGAGTGATGAA	AACCTCCATCTGTCCATCAC	0.393	0.378
SNP_14698	C/ T	GAGCAAGTTTGCTTTTAGATG	AGAACCAAACAGGCAAAGC	0.423	0.434
SNP_14739	A/ G	AAATCGGAACCGAATTGGGC	GCTATGAAACAGGTTGCAGG	0.036	0.031
SNP_14774	G/ C	GAAAGTACTGACTGCATGCC	CAGCAACAATGTTGGATCTT	0.307	0.412
SNP_14809	T/ A	AGGCAACATCGAAGGTCTAC	TTACAGCCGATTCAACGAG	0.491	0.435
SNP_14896	T/ C	TGGGCATGTAGTAATCCTTG	AACTGAACCTTTGCCAGCTC	0.221	0.191
SNP_14929	G/ T	GAGTCAAACCATTGTTGACC	TGGTCAACCGCAACAACAG	0.343	0.414
SNP_15089	T/ C	GCTGAACAAAATTCCTCGG	GCTATAAACCTTTCTTTCAC	0.208	0.176
SNP_15124	C/ G	GGCCCTTTTCCAATTTCGTT	TCTAGCACTTAGCGACTGCC	0.161	0.164
SNP_15221	T/ C	CCGAGAAAAATGATGTCGTTG	CGACAGATATTGCAGTAAAG	0.431	0.394
SNP_15231	G/ A	TTCTGCAGAGAAAGCCTACC	TGGCTGGTTCAAACACGAG	0.069	0.064

SNP ID	Alleles	Primer F	Primer R	Ho	He
SNP_15393	G/ T	CCAAAAACGCAGAGATATCC	TGTGCTTCTTGAAAAATCTC	0.457	0.402
SNP_15479	G/ A	CTAAAGGGCAGTAAACCATC	GGGTCGGTCATGTAGAAATC	0.096	0.087
SNP_15519	T/ C	TTGCAGATCTTGTTGTTGGC	GTACTIONTGCCAAGCGTTCTG	0.364	0.346
SNP_15610	A/ G	ACGACTGTGTGGAAGAAGTGTG	CATCGCTGTACCAAATGGTGTG	0.272	0.262
SNP_15651	T/ C	TGGTAAATCAGTCGTCTTGG	GCTTCTTCAACAACCTTTGAG	0.423	0.397
SNP_15809	T/ A	ATCACTCTGTTCTTCCGTTG	AGGCCAAGTTGACGAAGATG	0.312	0.324
SNP_15907	G/ A	AACTGCTCTTCTGAACATGG	TTGAACCTGTCATGATGAAC	0.203	0.184
SNP_15911	C/ T	GGCAAATTTTCATTGTCGGC	TGCAAATGCTCAGTTGAGGG	0.371	0.424
SNP_16021	A/ G	GGCCACCTCTAAGAACAAAC	GGTTGGATCGTTTCTAGAAC	0.203	0.227
SNP_16079	G/ A	AAGTGCCCGTTTCTTCGATAG	CGACCAGTTGTTTTACCCAC	0.087	0.078
SNP_16208	G/ A	GTACCCTTCGACGATTTCTC	TTGGCAAGGAGAAGCTTGAC	0.000	0.000
SNP_16249	G/ A	TGTGTCCGTCGTTTTGTTCC	TGGATAGATGCAGGCAGTAG	0.361	0.346
SNP_16318	C/ T	TGGACCACATGATTCACACG	GAGCATTCCCATGGTTAGAG	0.296	0.387
SNP_16335	T/ C	TTGGGAGATTGCCGGTACAC	CCTACTTGTGTGCTTTTAGC	0.100	0.083
SNP_16476	C/ T	AGCCACCTTAACTCCTCAAC	GCTGGCCCTTTTTGTATTGG	0.333	0.390
SNP_16583	G/ T	CACTGCTTGCCTTGAACATC	ATCACTTGCAGATCTGCAGG	0.070	0.059
SNP_16598	G/ A	CGTCATGCATGTGAAACTTG	TGGTGAAGACGATGATGGAC	0.499	0.451
SNP_16611	C/ G	TGGTGCCTGTTACAACGTG	TGAGACTCTCTTTAGTGC	0.362	0.326
SNP_16676	T/ C	AGCATGGTCTATAGGTGGAG	TAAAACCAGCTGCTCCCAAG	0.406	0.382
SNP_16687	G/ A	CCTTTCCTTCTTCTCAACC	CTCACAGGCAAATAGGCTGG	0.306	0.394
SNP_16955	G/ A	CAAAATTCGACCACTCTGGC	TGCTCGTTCCTATCGTTAC	0.358	0.352
SNP_17055	C/ T	AGCAAGTCGGCAAGTAAACC	CCAGTATAGTTACCCAAACC	0.392	0.410
SNP_17060	G/ T	TCAGCTCCAATGGTTCCAAC	TTAGCCATAATGTTGTTTCGC	0.236	0.187
SNP_17162	G/ A	CTGGGAAGTATCGCCTAAAC	TTGGAAGGCGTTTCGAAGAC	0.442	0.378
SNP_17172	G/ A	GCATTTGGTCACCTTAGCAG	TACCGAGGCGAAAATCTGTC	0.358	0.334
SNP_17179	G/ A	ATTTGATTTACTGGACGAGG	CTCTCCTTGCAAATTA AAAAAG	0.251	0.202
SNP_17184	A/ G	CAAAGTACACGGCAGGATTG	TGATCGTGAAATGTCTGGAG	0.207	0.294
SNP_17190	T/ C	GGCACTGCATCAAAATCTTC	CGGACATTGAACGAGAGAAG	0.183	0.154
SNP_1722	A/ G	GTGATGTGATTATGCTTTCGG	AATCGTGAACACTTGGAGGC	0.226	0.216
SNP_17351	C/ T	CCATATCGGCATCATACTTC	GAACGCGAAAAGAGGAAGAG	0.288	0.309
SNP_17412	G/ C	TAGCTTTAGTCTGTGCAGCG	CTTCAAAGGTATTCGACCCG	0.396	0.300
SNP_17451	T/ A	TGATCTTGCAGACAGTCACTC	ATCAACGCCTTCCAAGTTCC	0.219	0.242
SNP_1746	T/ A	TTTTCTGGATCGATGTCGTC	TTGAAGGCGTTATTGCCTCC	0.223	0.420
SNP_17519	T/ A	ATGCAGCACATTGTTCCCTCC	TCAGATGACGTCACATCCTC	0.347	0.308
SNP_17549	G/ A	TCGGAGACACAGCTTACAAC	GACTTAGCTTCTGTTTTTCGG	0.279	0.362
SNP_17570	C/ T	CGCAACACCATAACCTTTCC	CGGCAGTGGAAAACAGAGTT	0.286	0.212
SNP_17745	A/ G	TGTTCCGTCGCAAAGCTTTC	TTCATGTTTGATTACGCCTC	0.138	0.119
SNP_17771	T/ C	CATCCTTCGTCGTCTTTTCC	TAGGTCACTATCACACGCTG	0.279	0.244
SNP_17782	C/ T	TCGCAAGCGTTGGAGTATTG	TTGTCTCCCTGCTTGGAAAG	0.325	0.284
SNP_17853	A/ G	TGAAGGATCGATCCCAGTTG	TGAAGGCTGTGGATGGACTC	0.208	0.340
SNP_17854	T/ A	GTGGATTGATCCTGATGAAG	GGTCTCCAATTCGCCATAT	0.521	0.455
SNP_17907	C/ T	CCTCTCATGCGCAGAACTTC	TGGCTATCCCATGCAACAAC	0.401	0.409
SNP_17944	G/ A	GATCTCCCAACTTTCTGACG	ATATTTGGAGGCTAACGCGG	0.270	0.244
SNP_17967	G/ A	CACCCTGGATTCTTTCATC	CGAGTATGTGAGGAAATTGC	0.229	0.205
SNP_1804	A/ T	ACCCTGTTGCTTTGGGTATC	GAAACGACCTTTGTGCTTGG	0.052	0.064

SNP ID	Alleles	Primer F	Primer R	Ho	He
SNP_18058	C/ A	TCTGAACCCCTCCTCTGAATC	GGAACAAGAAATTGAAGACG	0.071	0.065
SNP_18067	T/ C	CAAGCATCCAGAATACTGCG	ACAATACGTGATACCCCTGAG	0.700	0.415
SNP_18076	A/ G	GTAAGAAAGGTTACCATGGC	TTGACAATTTCGCTGAGCTG	0.188	0.158
SNP_18088	G/ A	GATCTTCTTTCATGTAGAG	GGTGGATCTTTCCTGTTGC	0.411	0.412
SNP_18374	T/ C	CGGAATGCTGCTTGAATCTG	GAAATTTGCCGCCACTACTG	0.051	0.043
SNP_18402	C/ A	AGCATGCCATCTCAACAACC	GTTGGAGTACTTCTGCTTGG	0.319	0.364
SNP_18404	A/ G	TGCCACACGAGTTGTGATG	GCGACACGTTTTTACGATCC	0.093	0.075
SNP_18524	T/ C	TGGCTAAAGCTGCAAAAGCG	TTGTCGCCACATGGTATGAG	0.152	0.154
SNP_1860	G/ A	ATCGGAATTCCTTCTCAATC	AAGGCGACGATAAGTCTCGG	0.000	0.083
SNP_18639	G/ A	TAGACGAATGAGGTTCTGCG	ATAGAGGAGGACAAAGGTGG	0.411	0.414
SNP_18674	T/ A	TGATCTTCTTTCCTTTTG	ACTGAAGAACTCGATGAGGG	0.117	0.280
SNP_18715	A/ G	GAAACAGCGCCTTGAAGAAC	AGTGGTTGTCATGGTGACAG	0.608	0.481
SNP_18797	T/ C	AACCAACCAGCGAACTTCAC	TCTTCGCTGGTCTTCAATC	0.031	0.036
SNP_18805	T/ C	AGGTTCAATATGGTGGACGG	GTCAGTAAACCAGACATGGG	0.404	0.445
SNP_18834	A/ T	GCAAGAAAAAGTCGCCAAG	CCTCTTTTCGTTGTCGATAG	0.938	0.490
SNP_18847	G/ A	GACAAAATGCTCGACATGCC	CAATTTGTGGAGCTTGCAGG	0.445	0.397
SNP_18991	C/ T	GATACTTTCAGATCCCCAGG	AGAATCCACCCTGTGAGAAC	0.494	0.405
SNP_1900	C/ T	GAAGGACAGCGCTCATGAAG	TCTCTTGGTTTTGTTTCCCG	0.433	0.424
SNP_19078	G/ A	GGCTCAACTCCAAGATCTTC	CTCGTCTAACACGGGATATG	0.505	0.380
SNP_19104	C/ T	AACGTTTTAGTCCCTCGATAG	CTTAGTGATCACATTTGAC	0.087	0.112
SNP_19116	T/ C	ATTTGAGCGTGTGTTGAGGAC	TATGTGATATGGGTCGGGAG	0.319	0.332
SNP_19125	G/ A	AAGATGATACACCTGACGCC	TTCTCCACAATGTCGCTTCC	0.117	0.104
SNP_19126	A/ G	AAAATCTCCCCTCCTCCTTC	GCTACTGACGTCAGCATTTG	0.237	0.223
SNP_19147	C/ T	GACATCATAGCCGTGATTGG	ATGCCAGGATGATGGCTAAC	0.367	0.402
SNP_19267	C/ T	CTCCCTCTACTGTTGTTTAC	GGGTGATATCTGGTAGAACG	0.341	0.354
SNP_19277	C/ T	ATGATGTGTTGAGATGCGCC	TCCGAGTTTATGCACACATC	0.120	0.118
SNP_19327	A/ G	CGGAAGAGACGCTGTTATTG	CCAGCCAAAGTACAAGAGAG	0.303	0.299
SNP_1954	T/ C	TACAGAGTCGTATTCGTTGC	ATCCTCAAATGCCTTAAAC	0.486	0.374
SNP_1960	C/ T	GAGATCTCTGATCAGACGAC	CGTCTCAGTATGCCATACAG	0.219	0.208
SNP_19609	C/ T	AGAAGCTTCTCAAGCTGATG	TTTTGGAGTGCTGCTTGGTC	0.197	0.170
SNP_19646	C/ T	GAAGAGATCGCCATTGACAG	AGCAGAAGCGATGCTAAATG	0.356	0.367
SNP_19749	G/ A	CCAGATTCAGCTTCTCCAAC	CAACGGAGAGTAAAGAGAGC	0.000	0.000
SNP_19926	A/ G	ACATGCAACACTGTTTCAGG	TGCAGGAGATTTTGGATGAC	0.287	0.327
SNP_19945	C/ T	CACACTCTTGTTGAAATTGG	AGTTCCTCTTGCTAAAGAC	0.232	0.419
SNP_19956	A/ G	CCGTGAGTTTTTGC GTTTCG	CTTCACATCAGCTCGAGTTC	0.178	0.335
SNP_2007	G/ A	GGCTTGAAACCAATACCTCG	CGGCAAATGCAGAAAGTACC	0.418	0.345
SNP_20077	C/ T	AACAATCCACCATCGATACC	GTTTAATGAAAAGCCACGCC	0.608	0.481
SNP_20191	C/ T	CTGGCATTCTGAGTTAATGG	TCAGACCTCTTCACAGAGAC	0.411	0.327
SNP_20242	G/ T	GTCAAAGTAGCAACTGCGTG	TACTACTGCCATTTCAAGTGGG	0.351	0.373
SNP_20340	A/ G	TTCCCCCAGCGTTAACAAC	TCTTCGACGCTAACTATGAC	0.141	0.113
SNP_2041	G/ C	TAATGCGCCTGGTTTTCTC	TGTTGACGTCCTGGTTTAC	0.055	0.085
SNP_20462	A/ G	GTTGTACTCCAATGGTTGTG	GGAACATATCGGTTTCAATGG	0.424	0.383
SNP_20742	C/ T	TCCCCCATCTGATATCTCTC	TGACAGTGACGTAGTTCTGG	0.117	0.208
SNP_20778	C/ T	ATGCATCCCAGACATGTTCC	AAGTCGTAATGCGAGTTGGG	0.485	0.392
SNP_20807	C/ T	TCTTGTAGAACCACGTCGAG	ATACAACAACGCCTGGGAAC	0.213	0.191

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SNP_20813	G/ T	AGCTGTTGATATCCTAGCTG	AGATAACACCTCGCTCTCTC	0.000	0.000
SNP_20823	A/ G	ATGCCACGTCGTACGATTTT	TCTGTTCCATTAACAGGAGG	0.489	0.412
SNP_20856	T/ C	CCTCATTGTTTTATCCGCTG	CGTTTATGTCTTCCCTCACC	0.321	0.260
SNP_20911	A/ G	TTCGTCGGAGAGCATCTTTG	GCTGGCTGTGGTTTTTATGG	0.413	0.439
SNP_2093	C/ G	GTGAAGACACTGACTGACTC	TGGTGTTCCTCAGTGATG	0.102	0.111
SNP_21016	G/ A	TCATCATGGCCATCTTCACC	ATGTGGTCCATGCGTGTTTC	0.423	0.397
SNP_21049	G/ A	TCAGCCAAGAAATCAACGGG	GCCAACATTATCTCAAGGTC	0.436	0.437
SNP_21091	G/ A	ATCCAGTTGCTTCTTTGTCG	TCTACCATCTTCTGACGGAG	0.246	0.255
SNP_21257	G/ A	CTCTTCGACAAATACGTACC	GTCTGGTATAGGTGTGATCT	0.450	0.368
SNP_21270	G/ A	ATCAATACCAGCAACCAAGG	GGCGTAAGGCAGTAATTGTG	0.392	0.370
SNP_21417	G/ A	TAGTACCATATGCTCTTCTG	ATGTACGATGCCGATGAGAC	0.164	0.357
SNP_21481	C/ T	AATACACAAGATCGCTGAGG	AGTGGCATTCTTCTCCACAGG	0.282	0.274
SNP_21512	C/ A	TCAGTACTGCGGAATGAAGG	ACTGCTATAGGCCCTTTCAC	0.500	0.454
SNP_21554	G/ A	TCAACCTGGGTATGGACAAC	TGTCCATGAGCCATGTGTTG	0.621	0.480
SNP_21565	G/ A	TGTTACAATCCCACGACGAG	TATCTTCTTCGTCGTCTGGG	0.119	0.176
SNP_2181	C/ G	AGACCATCCGTCGCCATTC	CGTCATACTCTGCAATCCTC	0.144	0.144
SNP_21828	C/ T	CCTTGTCTGTACTGCAATC	TTAGATGTCTGACTGTTTCAGG	0.320	0.320
SNP_21872	A/ G	AAGGCCAAACGATGATGAGG	TTGTGACGACTCAGCTAGAC	0.431	0.431
SNP_21884	C/ G	AATTCCTCCGCATGAGTTC	TAGTCAAGGAAGCCAAGAGG	0.095	0.083
SNP_21937	C/ T	TTCTGAAAGACCAGGTGCTC	TGTGCCAAGCGTTGCTTTTC	0.180	0.177
SNP_21973	C/ T	GAAAGGCGTTCCTTAGCTTG	TGTGAGACGTTTCATCTGCTC	0.000	0.000
SNP_21974	G/ C	TATGAAGGAGAGCACGGAAC	GATAAAGTGCAGAATCGCTC	0.238	0.234
SNP_21976	C/ T	TCTCTTCTCGTAGTCTCAGC	AATGATGCCTCCGGAGAAAG	0.150	0.140
SNP_22047	C/ T	TATCCTCGTTCATCCTAGGC	TCGATGCGGTTGGAAGTAAC	0.479	0.448
SNP_22128	T/ C	TAGAGATATCTTTGTAGCG	GTGATGAAAGAACACGAAAG	0.366	0.347
SNP_22185	C/ A	CGCTTGAGTTTTTTGTAACGG	CTTTCAGATGCTACCCTCAC	0.432	0.356
SNP_22686	C/ A	TTTCATCATGGGTGCACCAC	AGACAGGGCAAAGAACGAG	0.494	0.420
SNP_22699	T/ C	AAGGCGTACAGGGATCAAAG	TGCGCCAGCTTCTCCTTGAT	0.175	0.148
SNP_22752	G/ A	GTCAGGTTGAGAGGAATTTG	TCGATTCTTTGTTTCACGAC	0.000	0.000
SNP_22796	C/ T	CGTAATTTATCAGGCCTTCC	GATGGCAGATACCATGACAG	0.123	0.111
SNP_22990	C/ T	AGCAGAATTAATGGCTGCC	AACGATGGCGTGATAGGATG	0.328	0.346
SNP_23047	G/ A	TAAAATTGTCCAGCATCGGC	GGAGCTTTAGGATGGGTTAG	0.418	0.411
SNP_23189	G/ A	CCCAAGGGTTGTAACCTTC	ATTACGAGCGAAGGACACAG	0.000	0.226
SNP_23198	G/ A	CGTGTTGCAGAAGCTTTTCG	GACCCACAAAATGTCAAAGC	0.432	0.396
SNP_23219	G/ T	CTGTATGCTCAGTATCCTCC	CGAAAGAGACAACTTTTCGC	0.148	0.317
SNP_23318	G/ A	AGCCCACGATACAAAAGCTG	GACGCCTTCATGGTCGAAAT	0.402	0.463
SNP_23352	G/ T	GAACCAGCTAACGTTAACGG	AAATCCAGAACACCGCTCAG	0.404	0.423
SNP_23390	A/ T	CATGTCAGCCAAAATTAAGG	TGGCAGAGATTGGTTCAAGC	0.011	0.284
SNP_23478	C/ T	CATCGAGGCGAGACATAAAG	CTCCGCCAATGAGATTGTTT	0.127	0.139
SNP_23488	G/ A	AGACAATCTGTCAAGCTGCG	ACAGAATGACGAGTACTCGG	0.000	0.000
SNP_2351	G/ A	CCTCGTGAATATTGTACCCC	GGGTAACCTTGGCATCAGGG	0.101	0.234
SNP_23693	G/ A	CTGGACTTCCTTGGTTTGTG	GAGTGATCGTATGTCTGCTG	0.383	0.383
SNP_23709	T/ C	TGAGGGATCGATTCGGAAC	TCATTTACGCGTGCGCAAC	0.026	0.045
SNP_23723	A/ G	CTGTAAAACACACAGCCAGC	TGTTGTGACTCGGAGTGAAC	0.400	0.361
SNP_23794	G/ A	TGAAAGTGCCAGTGAATGTC	CAGCTGCATATAAGCGAAGG	0.382	0.454

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SNP_23796	G/ A	TCAAAAACGTCTGGTTACCC	ATGCCGACTGTATGTGTTGC	0.218	0.193
SNP_238	C/ A	GTAATCCTTCAAGTACAACCTG	GGACGAGAATGGTGAAGAAC	0.279	0.252
SNP_23817	G/ A	CACAAGTTCCTAATCCTATC	CCACCAGCCAGGATATTTTG	0.240	0.318
SNP_23887	G/ C	TCCTTGAGACGATGAACTAC	GTGATGGTCGATAACCATGGG	0.324	0.396
SNP_24069	C/ A	AGTGTGTACTACCTGCCAAG	CCTTTGCCATGATTTGGAGG	0.460	0.448
SNP_24148	A/ T	TGACATCCTAGACACAGCAG	AAGAATCCTTCGCCACTACG	0.301	0.274
SNP_24220	G/ T	AGGCACCATTTGGTCAATCG	GTGTTCTTGAGTTCGTTCTG	0.272	0.317
SNP_24236	T/ C	GAGAAACGTACACAGCTCAC	TGTGGAATACTCTGGGTGTC	0.459	0.442
SNP_24295	C/ A	ATTGCGTACGCAATCTGGTC	TCTGAGACGTCTGTTGGTTC	0.366	0.337
SNP_2436	G/ A	GTGATGTCACCATTTCTCC	GACAACGCTAAAATCCGACC	0.321	0.325
SNP_24363	G/ A	TCGCTTCACTCACAGTTGAC	GCCATTGGCTGATTGCATTG	0.354	0.427
SNP_24376	G/ T	CGATCAATTTTCATGATCAC	AGAGCGAAACGAGAAGTGTG	0.203	0.171
SNP_24405	T/ C	TTGGTGGCCGGATAAAATAC	GGTTTATAATCTGAACGAGAC	0.276	0.366
SNP_24424	A/ G	GGTATTTGTGGATCGTGTGC	GATGTATCATCAATACGAC	0.232	0.244
SNP_24577	T/ C	TGAGGCTAGCGTAGAGAATG	GTTCTCCGTTCTTCTGTTGC	0.299	0.293
SNP_24681	T/ C	GGATCTTGTGCGTTGTTTCC	GTGTGAACAACACGCGAGAC	0.030	0.028
SNP_24682	T/ A	TTTTTCCAAAGCAGCGCACG	GTGTGACCATTTGAGGTTTC	0.512	0.416
SNP_24774	G/ A	CAAGAGGTGTTGAAAACCTGC	TGAGCTTGTGCTTATCGAG	0.302	0.259
SNP_24818	G/ T	CCGTCTGTGATGGAGATAAC	CTGCCTTGCCGTTATTGAG	0.268	0.275
SNP_24849	T/ C	ACTTCCTTTGCACGTCTTCC	TCATGTTATCCAGGAGGGAC	0.157	0.158
SNP_24998	G/ A	AATGAGGTTGTTCACTTCGC	CAATTTCAAGACGATCAGGG	0.211	0.355
SNP_25046	G/ A	AGCTAATCCTTTTGGTGGCG	GGCTGTTTAGCTTCAACACC	0.152	0.124
SNP_25227	A/ G	GGTTCATGTAAACAGTGCCG	GAACGTAATAATGGTCCTCGC	0.198	0.335
SNP_2687	G/ A	AATGCTCGACACAACCTCTGC	CTATAAAGCCAGGATGGAGG	0.007	0.007
SNP_2764	C/ T	TACGGCCAGTACTGTACAAC	CCTTGCCAGTGTGATCTTG	0.061	0.065
SNP_281	G/ C	TGCAAGTAATATTTTCGGCAG	TCGAAAAAGATGTTTCGTACC	0.314	0.271
SNP_2969	C/ T	GCACTTCCACCAAGAGATAC	ACGCAGGCATTTTATGTGAC	0.267	0.361
SNP_2972	G/ A	AGAAGCGATCATGCACGACC	TTCAGAGGTCACCTGGTTTG	0.249	0.382
SNP_3125	G/ A	CGAACTAAACGCTGGAATGG	TGCCCTTCGAGAAATTCGTC	0.090	0.077
SNP_3144	G/ A	CTTCATTTGGTTCATCAGGG	GGCTTATACATGCACATCAG	0.306	0.312
SNP_3161	A/ G	CTTGTACGGTCATGGTTTGC	AAGCTTCCCATGCCCTCTTC	0.347	0.362
SNP_3212	C/ T	AAGATCGTGACAACGATGCC	CTCGCTCTTCCATTCTCTG	0.383	0.403
SNP_3217	T/ A	AGGTGTAATATCCACGCGTC	ACAGATCCTCTGGTTGCCAC	0.125	0.063
SNP_3297	A/ T	TTCATAGCAACACCACGGAC	CCAATGTTGAAAGCTGGTCG	0.305	0.288
SNP_3398	A/ T	CGGCTTTTCCTTTGGTTTCG	CAAGGAGGATGAAAACAAGG	0.272	0.269
SNP_372	G/ A	GAAAGGATATAGCAAAACACG	CAAATGTCCTTTGGACGCTC	0.094	0.277
SNP_3894	G/ A	AGAATTACCTGCTGAGGGAG	TCACGTGCTTCTTCGCATTG	0.000	0.000
SNP_3959	G/ C	TCAACTGGCGATCACAGAAC	GGCAGAAACCCGAGAAAATG	0.150	0.201
SNP_4056	C/ T	CCCAAGCTAAGTACGCATTG	AGTCGACTCGCCATCTTATG	0.147	0.153
SNP_4082	T/ A	TTGAGGGGAATGGTGCAAGG	TCACCTTCTGCATTGTAACG	0.113	0.109
SNP_4122	A/ T	AAACACGGCTTGCCAATGAG	GTCGTTTTGTCAACACCTCC	0.213	0.222
SNP_4165	G/ T	ACTTCATTTTCTCCGCGAAC	AACTCCGCCTCAGGATAAAG	0.470	0.453
SNP_4295	C/ T	AGCTCCTGTTTCTTTTGTGTC	AGAGTCTTCTTCTTCTCGC	0.025	0.023
SNP_4365	A/ G	GAGAGAGTGGTCATTTTCGAG	GCATGCTCATCTTCACGTTG	0.000	0.000
SNP_4510	T/ C	TTCGTACCAAGTAATCCTGC	AGGTCTTGGCATCGTCTGTG	0.869	0.479

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SNP_4525	G/ A	TTCAAACAGCACATCAGCAC	GGTTGAGTTGGCCAAAATCC	0.350	0.338
SNP_454	G/ A	TACAAACACCAGAAGCTTTC	TGGGAATGAGAAGACAGAAC	0.053	0.050
SNP_4552	C/ A	CAGGTGGATGTGTTTCAACG	CGGAAGCCATTCTGTTGAAG	0.233	0.325
SNP_4564	C/ T	CTGCTCATATCTGCGTCAAG	GTCCAAGTACCTGACTTCTC	0.000	0.063
SNP_4603	A/ T	TGGTAAATATTCAACGCAGG	GTTGTACAGGCTTGTCGTTT	0.132	0.220
SNP_4631	G/ A	AAGAAACGCCGGGCTATCAG	TTTGAACGATTCGTCGTCC	0.139	0.150
SNP_4660	G/ A	GCGAAAACGTTACCGTCAA	CTGGCTTTCCAGAAATCTTC	0.000	0.000
SNP_4717	G/ A	CCACTTGGCAAGATGATAGG	AAGATGTTTTTCAGTGCCGCC	0.137	0.147
SNP_473	C/ T	CCCCTACCATAGTATCTCC	TGAGGCATCCCTCCTTTTTG	0.212	0.202
SNP_4871	C/ T	CTCTTGTGGAATCACTGACC	CCTTCACTTGTGACATGCTG	0.293	0.223
SNP_5076	A/ G	TTCCTGAGCACCTTTGAAGC	TATCGATGCGCTCACTCCAC	0.295	0.263
SNP_5097	C/ T	TGTCGAAATCTATGGCTGGG	AATTGGAGGAGATCTTCCAC	0.000	0.174
SNP_5108	C/ T	AACGATCTGCAACATGGGAG	TTCAGATAGTCAGCCATGCG	0.357	0.341
SNP_5278	G/ C	TAGGCACGGTGTGTTTGTGAG	AAGCGCCTTCAAAGTTGTCC	0.404	0.442
SNP_5299	A/ G	CAACGTGAACGTGTCCTTAG	TTCGAGAGCGCGGATTGAAC	0.468	0.443
SNP_5313	G/ T	ATCAGATGTCAGACAGGACG	TTCCAAGAGGAACAAGTGGC	0.021	0.271
SNP_5355	C/ T	GAAGTACCTAAGGGACATGC	GCTTCCTCGTCGCTTAATTC	0.480	0.457
SNP_536	C/ A	CAAACCTGCGTCACGACATC	CTCTTGAGGACTGCTTGAAC	0.475	0.378
SNP_5397	G/ A	AACTTCATCGCATCACGGAC	GTTCTTGCCTGGTGGTTTAG	0.000	0.000
SNP_5421	A/ T	AATAGAAGGAGACCAAGGCG	AGTATTCCTCTTGCTCAGCG	0.000	0.094
SNP_5528	T/ A	TTACGATGCCGAACCTCGTTG	AAATAAAAATCCCAGGAGGC	0.107	0.156
SNP_5554	C/ G	ACGTAACGTTGCCATGGTTG	GACAGTTGAACCGAAAAGAC	0.502	0.433
SNP_5581	G/ T	GTTTCGGAACAATTAAGGAC	GTTTCGGTGACATTAAGTGG	0.321	0.283
SNP_5625	T/ C	TGTTTGATTGAGCTCTGGCG	TATGCAAGAGCTGCTGCCGA	0.235	0.240
SNP_5644	C/ G	AACACGCGGGATATAGTAGG	TTGCCTAACCACGTGTTTCG	0.124	0.130
SNP_5678	C/ T	CAGGAAAGAGATACAGTGAC	CGGTCCATCCTTTTCCAATC	0.418	0.334
SNP_5714	C/ T	GCACCAGAACACGTAATTCC	CGGGATCAAGGATAAACAGG	0.066	0.063
SNP_5803	C/ T	ATTATAGTTGGAGAAACCG	ACGACACCACTCTTCACTTG	0.198	0.191
SNP_5894	G/ C	CTCGGAACTTGAAGAGATCA	GTCTTAGCGGTTCTCTTTAC	0.159	0.149
SNP_5899	G/ A	CACAACAACGACTTTCGTGC	AGAGTCTGCCTACCACAAAG	0.000	0.000
SNP_5902	T/ A	TTCTCTTGGTCCTGGAGAAC	ACATAAACAGAGAGAAGCAG	0.068	0.057
SNP_5931	A/ G	GTAAGTTCACGAATCCTGCG	TCTTGGCAACAAGAAGTCTAG	0.419	0.419
SNP_6245	G/ T	CTGCAAGGCTTCCATAAAAG	TGACCAAGGCTGATCTTGTC	0.284	0.362
SNP_6308	G/ A	TCCCTTGTCAGTAAAGCTGG	CGTTCCTGTTTCGCTTTTCG	0.396	0.386
SNP_6476	C/ T	AAAATCAGCACTGCTTAGCG	TGTATGCCAACGACAAGCTG	0.044	0.041
SNP_6505	C/ A	CCTCGTCAAGAAGTGTATGG	GGAGAAGTCTCATAGAAGG	0.313	0.284
SNP_6509	G/ A	GTTAATGAATCAAAAATGGC	ACTCCAATGACTCTGCAAC	0.180	0.352
SNP_6711	C/ T	GAACCTCATCTCGAATGCGTC	CAATCGTCGAGTTTTGAAGG	0.426	0.407
SNP_6751	G/ A	GAGTTTGTGAGAATTGATCC	CACATCAGGGAGTTTAAATTG	0.408	0.377
SNP_6827	T/ C	CATTCACAGGCTTGCTTCTC	CCTGCAATATGTTTGTGATG	0.289	0.255
SNP_6963	A/ T	GTACATGGAAACGTGAGAGC	GGCTGTTTCGTTACGATATCT	0.185	0.374
SNP_7006	G/ A	GAAATTCTGAAACCAGGCCG	GGTATCAAGTATGACCCAAG	1.000	0.500
SNP_7201	C/ T	AGAATTGCAGAGAATGCGAC	GTCGTCCTTCAATACACTTC	0.400	0.350
SNP_7263	G/ A	TTCAGAAGGTGAAGTGGTCG	CTAACAGCCATTTCTCGACC	0.246	0.201
SNP_7387	C/ T	TCTGGGAATGTCTAACACGC	ATGGGTAACACACGCGATTC	0.324	0.288

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SNP_7482	C/ A	CGGCTCTAACGTGATTCAG	TCATTACTCCATCTTTGCCG	0.428	0.402
SNP_7541	T/ C	CTCCAGCCATGTTTAGTTGC	TCAAGCAACTGTCACAGTGG	0.028	0.026
SNP_7562	C/ T	TATGCACTTCCTCGGGTAAC	GTCAAGCTACCACAGATGAG	0.000	0.000
SNP_7579	G/ A	CTCTCTCATTTTCTTGCCCTC	TAAAGAGCTAGCAGAGAAGG	0.349	0.396
SNP_7614	C/ A	TCACTTATCACCTGGTCGAG	GAAGATTATCTCTGAAGCGG	0.280	0.235
SNP_7619	T/ C	AACAGTACAAGGTCGTTGGG	GGTGCAAAGATGTTTCATACG	0.352	0.261
SNP_7661	T/ C	AAATGCAGCTCGACATCGAC	TAGTAATCCCAGCCCATGTC	0.339	0.334
SNP_7739	C/ G	TAAGACTGCATCAACCGCTG	ACAACACGTGCAACCTTGTC	0.319	0.308
SNP_7760	G/ T	ACTCCACAATATCGCTGCAC	ATGAAAAGCCAACGCCAAG	0.279	0.221
SNP_7783	T/ C	TGACGATTCCAGCTTCACAG	GGAGTGGTTCTTTTGGAGAC	0.000	0.000
SNP_7893	G/ C	CCACCGTACGCACCATAATC	AGGTGGATGGGGCTTTAATG	0.431	0.380
SNP_7976	T/ C	ATCTCAGGCTGAGGAAGATG	GTTCTGAATGGCTCTACCAC	0.141	0.120
SNP_799	C/ T	AATGCTCTCGTACCTTCTAC	GCCAAATGCAATGCAAAAAC	0.000	0.000
SNP_8002	C/ T	GCAGCAATGTCCATTGACTG	TTCTTGTTGTGTCGAAGACG	0.283	0.223
SNP_8041	A/ T	ATGTTACAAGATTGGCCTC	CTTTCCTCCTCCACCAAATC	0.196	0.428
SNP_8129	C/ A	CCACTACACTCGACATCATC	CCTGAGGGTACAAGTCCAAG	0.865	0.482
SNP_8159	G/ A	TCCGTCATGTAGAGAACCTC	GAGTCCAAAGATCAAGTTGC	0.000	0.000
SNP_8219	C/ T	TGATGTCAGCAGTTGATGCC	CCGTTTGTATGAGCTTGCTG	0.300	0.405
SNP_8320	T/ C	GGGTCGTTGACAATTCATC	AAATCAGAAGACGTTCCCGC	0.304	0.301
SNP_8327	C/ T	GGCAGTGACAATTTCTGCTT	GGTAAATGGTGAAATCCCGC	0.161	0.155
SNP_8381	G/ A	CATGCTCCTCATCATTCTCC	TGTTTCAGCGGTCAGGTTAAG	0.154	0.144
SNP_8421	T/ C	CTTGGTGATATTCTCATGGG	TACACAGGGTACAGATAGGG	0.025	0.024
SNP_8440	G/ A	AAGTTGCCAGAGAGCGAAAG	TAAGTTCAACATCGCATGCC	0.415	0.324
SNP_8543	A/ G	TGCTCTTAACTTCTGGTCTG	CCAAGCTGTTTCGTGATTTG	0.338	0.356
SNP_8671	C/ T	CCAGAACTTTGGCGTGCTTG	GATCATAAGCACGTGATTGG	0.426	0.372
SNP_881	C/ T	AGTAGTTCATCGTCTCAAGG	GACAGGTCTCCATATCTGTG	0.362	0.315
SNP_9014	C/ T	GACGTTTTTGGTTGCGTCTG	AAAGTTCCTGTGGTTGGTGG	0.269	0.243
SNP_9146	C/ G	GGGCTACACTATTTTCATTGC	AACAGCCCTATTTTCGATGAC	0.244	0.291
SNP_9347	T/ C	CATCAAAGAAGTGCTTGCTG	GGCAATAAGTCAGGCAGAAG	0.429	0.391
SNP_9393	C/ T	TGGTGGTTACCCAATTGCTG	CCTTGGCTACTGTTGTACTG	0.198	0.250
SNP_9431	T/ C	TTGCCAAAGATAAAGGCGAC	GTGTTTCCAAAAGGACCTCG	0.363	0.342
SNP_9910	T/ C	AATCAGAAGCGCTCTTACC	CACAGAAGTCGTGAAGTTCG	0.225	0.218
SNP_9933	C/ T	GATTGGGACTTTTTGCCTGC	GCATCATCCACCCAATCATC	0.000	0.000
SNP_9998	C/ T	CTGCTTCCAAGCCATTTAAG	GAAAATTGCCAATCTCAACGG	0.000	0.000

Table 1.- Location and number of individuals (n) of *Paramuricea clavata* used for SNP selection using MassArray

Location	Site	Latitude	Longitude	n
Bastia	Lavasina	42.7552	9.4783	10
	Roche Mimosa 1	42.7072	9.4605	10
	Roche Mimosa 2	42.7078	9.461	10
La Spezia	Tinetto Parete	44.0236	9.8505	10
	Tinetto Secca	44.0226	9.8513	10
	Punta Tino	44.0293	9.8472	10
Punta Mesco	Punta Mesco 1	44.1326	9.6345	10
	Punta Mesco 2	44.1324	9.6349	10
Toulon		43.0438	6.0610	15