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Primer Note

Transcriptome-facilitated development of SNPs for the Sonoran Desert rock fig, *Ficus petiolaris* (Moraceae)[1]

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- *Premise of the study:* Single-nucleotide polymorphism (SNP) primers were developed for a native North American desert fig, *Ficus petiolaris* (Moraceae), to provide markers for population genetic studies designed to quantify patterns of gene flow across a complex landscape.
- *Methods and Results:* Transcriptome sequencing and bioinformatic protocols were implemented to discover SNPs in single-copy protein-coding genes. Multiplexes of 30 nuclear and 24 organellar (chloroplast and mitochondrial) SNPs were selected for primer development and genotyping on the Sequenom MASSArray System. Of these 54 loci, 49 reliably amplified across a panel of 96 *F. petiolaris* individuals.
- *Conclusions:* This study has provided SNP primers that can be applied in future studies investigating population genetics of *F. petiolaris* and its coevolution with associated pollinating and nonpollinating fig wasps.

Key words: *Ficus petiolaris*; Moraceae; population genomics; RNA sequencing; single nucleotide polymorphism; transcriptome sequencing.

The genus *Ficus* L. (Moraceae) is a diverse (>750 species) and ecologically important lineage of tropical woody plants. Many organisms depend on figs to carry out portions of their life cycles, particularly fig wasp pollinators and parasites, which are often host fig specific. Despite substantial interest in the coevolution of figs and fig wasps (Herre et al., 2008), as nonmodel organisms genomic resources are largely lacking. Next-generation sequencing technologies have facilitated the development of genomic resources, such as single-nucleotide polymorphisms (SNPs), for nonmodel organisms. SNPs are bi-allelic markers that can yield valuable insight into ecological, genetic, and coevolutionary processes (Morin et al., 2004; Pool et al., 2010; Steiner et al., 2013).

The Sonoran Desert rock fig, *F. petiolaris* Kunth, is the only widespread, desert-adapted fig species in North America. It is also the northernmost naturally distributed *Ficus* in the New World, reaching a latitude of 31°N in the state of Sonora, Mexico. *Ficus petiolaris* supports a community of obligately associated fig wasps, including a pollinator (*Pegoscapus*) and several nonpollinators (*Aepocerus*, *Heterandrium*, *Idarnes*, and *Physothorax*). To enable ecological and evolutionary genetic studies, we sequenced the transcriptome of *F. petiolaris* to develop SNP markers optimized for high-throughput genotyping on the Sequenom MASSArray System (Agena Bioscience, San Diego, California, USA).

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METHODS AND RESULTS

RNA was extracted from nine *F. petiolaris* plants grown from seeds sampled from five populations distributed across the species' range in Baja California, Mexico (Appendix 1). Five milligrams of leaf tissue was sampled per individual, samples were pooled and homogenized in liquid nitrogen with a mortar and pestle, and RNA was extracted using the Spectrum Plant Total RNA Kit (Sigma-Aldrich, St. Louis, Missouri, USA). Extracted RNA was quantified using a NanoDrop 1000 Spectrometer (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) and then submitted to the Iowa State University (ISU) DNA Facility where it was quantified a second time using the Agilent RNA 6000 Nano Kit (Agilent Technologies, Santa Clara, California, USA). A cDNA library was prepared from the mRNA templates using the Illumina TruSeq RNA Sample Preparation Kit V2 (Illumina, San Diego, California, USA), with library construction verified using the Agilent DNA 7500 Kit (Agilent Technologies), before transcriptome sequencing at the ISU DNA Facility on an Illumina MiSeq (Illumina) with 250-cycle paired-end reads.

Illumina sequencing produced 33,294,480 reads, with an average read length of 215 bp, for a total of 7,147,200,749 bp sequenced. Low-quality reads were removed using Sickle v.1.33 (Joshi and Fass, 2011). The *F. petiolaris* transcriptome was de novo assembled using Trinity release 2013-11-10 (Grabherr et al., 2011). The final assembly contained 125,493 contigs, with a mean length of 1176 bp, mean coverage depth of 48×, N50 and N90 of 2011 and 478, respectively, and a total length of 147,624,931 bp. Reads were mapped to the assembled transcriptome using the program Bowtie2 v.2.1.0 (Langmead and Salzberg, 2012). SNP calling was performed using the Genome Analysis Toolkit (GATK) v.2.7-2 (McKenna et al., 2010). GATK input files were prepared using SAMtools v.1.1 (Li et al., 2009) and Picard v.1.97 (The Broad Institute; freely available at http://broadinstitute.github.io/picard/). GATK identified 139,254 putative SNPs, which were filtered bioinformatically using customized Python scripts. Initial SNP filtering was based on the following criteria: (1) sequence depth at the SNP position was ≥10; (2) the GATK quality score was ≥30; (3) there were no ambiguous bases, indels, or other SNPs located within 100 bp flanking the

SNP; and (4) the minor allele was represented in at least 1% of the reads (to minimize ascertainment bias). This initial filtering yielded a set of 21,228 putative SNPs.

SNPs occurring in single-copy protein-coding genes were identified as follows: (1) Primary protein transcripts for *Arabidopsis thaliana* (L.) Heynh., *Oryza sativa* L., and *Vitis vinifera* L. were obtained from the U.S. Department of Energy database (http://jgi.doe.gov). (2) Single-copy nuclear gene variants identified by Duarte et al. (2010) as shared among a diverse sampling of seed plants were retrieved from the primary protein transcripts. (3) A local BLASTX of *F. petiolaris* transcripts against the single-copy nuclear gene variant database was performed. BLAST results were filtered by *E*-value (≥1e-100), identity score (≥70%), and having hits to two or more species. This filtering yielded 3200 putative SNPs in 927 single-copy nuclear gene contigs. For contigs containing multiple SNPs, the one with the highest coverage was selected if it was also located ≥60 bp from the contig's ends and ≥20 bp from the nearest neighboring SNP.

TABLE 1. Information for the 54 SNPs validated through genotyping a panel of 96 *Ficus petiolaris* individuals.[a]

SNP ID	Multiplex[b]	Contig	Base position[c]	Major allele	Minor allele	Minor allele frequency	% Amplified	Polymorphic[d]
Fpet.01	1 (nuclear)	29,925	408	A	C	0.29	16	No (A)
Fpet.02	1 (nuclear)	22,889	893	A	G	0.13	100	Yes
Fpet.03	1 (nuclear)	27,895	714	T	C	0.14	100	Yes
Fpet.04	1 (nuclear)	24,924	2212	G	A	0.19	100	Yes
Fpet.05	1 (nuclear)	30,715	1145	C	A	0.11	99	Yes
Fpet.06	1 (nuclear)	20,750	598	T	C	0.25	99	Yes
Fpet.07	1 (nuclear)	24,920	340	T	C	0.37	100	Yes
Fpet.08	1 (nuclear)	23,050	1493	A	G	0.15	91	Yes
Fpet.09	1 (nuclear)	30,628	318	C	T	0.47	99	Yes
Fpet.10	1 (nuclear)	30,820	1147	G	A	0.40	100	Yes
Fpet.11	1 (nuclear)	18,553	748	C	T	0.38	79	Yes
Fpet.12	1 (nuclear)	18,592	469	T	A	0.11	98	Yes
Fpet.13	1 (nuclear)	24,973	636	T	C	0.20	0	N/A
Fpet.14	1 (nuclear)	28,670	1480	T	C	0.11	100	Yes
Fpet.15	1 (nuclear)	17,060	273	T	C	0.49	99	Yes
Fpet.16	1 (nuclear)	26,868	3628	C	T	0.12	100	Yes
Fpet.17	1 (nuclear)	26,617	1811	C	T	0.11	100	Yes
Fpet.18	1 (nuclear)	27,253	339	G	T	0.24	100	Yes
Fpet.19	1 (nuclear)	28,976	1599	A	G	0.18	96	Yes
Fpet.20	1 (nuclear)	22,155	293	G	C	0.28	100	Yes
Fpet.21	1 (nuclear)	23,811	845	A	G	0.12	100	No (G)
Fpet.22	1 (nuclear)	22,385	2212	T	C	0.14	0	N/A
Fpet.23	1 (nuclear)	21,647	1908	T	C	0.48	86	Yes
Fpet.24	1 (nuclear)	25,125	247	C	T	0.23	84	Yes
Fpet.25	1 (nuclear)	22,988	1450	C	T	0.27	95	Yes
Fpet.26	1 (nuclear)	28,413	935	G	A	0.32	100	Yes
Fpet.27	1 (nuclear)	28,379	559	T	C	0.27	85	Yes
Fpet.28	1 (nuclear)	21,679	382	G	A	0.44	100	Yes
Fpet.29	1 (nuclear)	22,737	3130	C	T	0.09	100	Yes
Fpet.30	1 (nuclear)	30,983	611	A	C	0.20	0	N/A
Fpet.31	2 (mtDNA)	22,102	197	C	A	0.45	99	No (C)
Fpet.32	2 (mtDNA)	30,053	546	A	G	0.16	99	No (G)
Fpet.33	2 (mtDNA)	25,896	588	A	G	0.30	100	No (G)
Fpet.34	2 (mtDNA)	25,564	1258	C	T	0.19	98	Yes
Fpet.35	2 (cpDNA)	25,544	4155	G	A	0.40	100	Yes
Fpet.36	2 (mtDNA)	23,811	1056	G	A	0.29	100	No (G)
Fpet.37	2 (mtDNA)	25,564	906	A	T	0.43	98	Yes
Fpet.38	2 (cpDNA)	28,687	1168	G	T	0.31	99	Yes
Fpet.39	2 (mtDNA)	30,053	245	T	A	0.43	98	No (T)
Fpet.40	2 (mtDNA)	25,564	501	C	T	0.40	99	Yes
Fpet.41	2 (mtDNA)	14,845	1153	G	A	0.49	100	No (G)
Fpet.42	2 (cpDNA)	30,714	1292	T	C	0.24	99	No (C)
Fpet.43	2 (cpDNA)	23,204	378	T	C	0.35	100	Yes
Fpet.44	2 (mtDNA)	19,365	1456	C	T	0.50	100	No (C)
Fpet.45	2 (cpDNA)	771	259	T	C	0.47	100	No (C)
Fpet.46	2 (mtDNA)	19,365	1577	T	C	0.28	100	No (C)
Fpet.47	2 (cpDNA)	30,714	407	T	C	0.30	100	No (C)
Fpet.48	2 (cpDNA)	15,049	2950	A	T	0.32	100	Yes
Fpet.49	2 (mtDNA)	14,845	647	G	A	0.18	99	No (G)
Fpet.50	2 (mtDNA)	25,564	681	C	G	0.40	20	No (C)
Fpet.51	2 (cpDNA)	24,260	787	A	G	0.33	100	No (G)
Fpet.52	2 (cpDNA)	25,544	288	G	T	0.38	100	Yes
Fpet.53	2 (cpDNA)	25,544	5259	C	A	0.33	95	Yes
Fpet.54	2 (mtDNA)	32,131	293	A	G	0.30	1	No

[a]Major and minor alleles and minor allele frequencies were determined from the assembled transcriptome data, whereas percentage of samples that amplified and whether the SNP was polymorphic were determined through genotyping.

[b]Sequenom multiplex number and source genome (in parentheses).

[c]Base position within the contig.

[d]Whether the SNP was polymorphic in the diversity panel (if monomorphic then the observed allele is listed in parentheses).

SNPs in organellar genomes were identified by performing tBLASTX against the mitochondrial genomes of *Malus domestica* Borkh. (GenBank no. FR714868), *V. vinifera* (FM179380), *Ricinus communis* L. (HQ874649), *Carica papaya* L. (EU431224), and *A. thaliana* (Y08501), and the chloroplast genomes of *A. thaliana* (NC_000932), *Populus trichocarpa* Torr. & A. Gray (NC_009143), and *V. vinifera* (NC_007957). The BLAST results for organellar genomes were filtered based on *E*-value (≥1e-50), identity score (≥70%), and hits to three or more mtDNA genomes, or two or more cpDNA genomes. After filtering out SNPs located near contig ends, a set of 31 putative organellar SNPs was obtained. This relatively small number of SNPs is likely due to the generally low levels of polymorphism in maternally inherited plant genomes.

The 927 nuclear and 31 organellar SNPs from *F. petiolaris* that were submitted to the Sequenom MASSArray System software for primer design had a minimum minor allele frequency of 9%, which should minimize the likelihood of calling a false SNP instead of a true SNP, particularly given the accuracy of the Illumina sequencing platform (Ross et al., 2013). The nuclear SNPs formed 31 multiplexes ranging from 27 to 30 loci, and the organellar SNPs formed two multiplexes of 24 loci and seven loci. The Sequenom software could not effectively multiplex the nuclear and organellar SNPs together, but given that genotypes were accurately scored, it is unlikely that loci in separate multiplexes would give rise to systematic bias. For genotyping, we selected two of the 33 total multiplexes: one nuclear multiplex of 30 loci, which had the highest confidence score (78.1%), and the organellar multiplex of 24 loci (confidence score 82.6%) (Table 1; Appendix 2).

SNPs were verified by genotyping 96 *F. petiolaris* individuals representing the species range in Baja California, Mexico (Appendix 1). Genomic DNA was extracted from silica-dried leaf tissue using an AutoGen Prep 740 DNA extraction robot (AutoGen, Holliston, Massachusetts, USA). DNA concentration was standardized to 20–25 ng/μL, then individuals were genotyped using the Sequenom MASSArray instrument at the ISU Genomic Technologies Facility. Of the 30 nuclear SNPs, 26 (87%) amplified successfully, of which 25 were polymorphic (Table 1). The one monomorphic SNP was likely due to poor amplification on the diversity panel (16% amplification; see Table 1). Of the 24 maternally inherited SNPs, 23 (96%) amplified successfully, of which only nine were polymorphic (Table 1). The relatively low number of polymorphic mtDNA and cpDNA SNPs may be an artifact of having a number of full siblings in our diversity panel, although further testing on additional samples is needed to verify that as the case.

CONCLUSIONS

We successfully developed primers for 49 SNPs that amplified reliably in *F. petiolaris* individuals sampled across a broad geographic range. These SNPs can be applied to future ecological, genetic, and coevolutionary studies of *F. petiolaris* and its associated pollinating and nonpollinating fig wasps.

LITERATURE CITED

Duarte, J. M., P. K. Wall, P. P. Edger, L. L. Landherr, H. Ma, J. C. Pires, J. Leebens-Mack, and C. W. dePamphilis. 2010. Identification of shared single copy nuclear genes in *Arabidopsis*, *Populus*, *Vitis* and *Oryza* and their phylogenetic utility across various taxonomic levels. *BMC Evolutionary Biology* 10: 61.

Grabherr, M. G., B. J. Haas, M. Yassour, J. Z. Levin, D. A. Thompson, I. Amit, X. Adiconis, et al. 2011. Full-length transcriptome assembly from RNA-seq data without a reference genome. *Nature Biotechnology* 29: 644–652.

Herre, E. A., K. C. Jandér, and C. A. Machado. 2008. Evolutionary ecology of figs and their associates: Recent progress and outstanding puzzles. *Annual Review of Ecology Evolution and Systematics* 39: 439–458.

Joshi, N. A., and J. N. Fass. 2011. Sickle: A sliding-window, adaptive, quality-based trimming tool for FastQ files (Version 1.33) [software]. Available at https://github.com/najoshi/sickle [accessed 29 May 2015].

Langmead, B., and S. L. Salzberg. 2012. Fast gapped-read alignment with Bowtie 2. *Nature Methods* 9: 357–359.

Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, et al. 2009. The sequence alignment/map (SAM) format and SAMtools. *Bioinformatics (Oxford, England)* 25: 2078–2079.

McKenna, A., M. Hanna, E. Banks, A. Sivachenko, K. Cibulskis, A. Kernytsky, K. Garimella, et al. 2010. The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Research* 20: 1297–1303.

Morin, P. A., G. Luikart, R. K. Wayne, and SNP Workshop Group. 2004. SNPs in ecology, evolution and conservation. *Trends in Ecology & Evolution* 19: 208–216.

Pool, J. E., I. Hellmann, J. D. Jensen, and R. Nielsen. 2010. Population genetic inference from genomic sequence variation. *Genome Research* 20: 291–300.

Ross, M. G., C. Russ, M. Costello, A. Hollinger, N. J. Lennon, R. Hegarty, C. Nusbaum, and D. B. Jaffe. 2013. Characterizing and measuring bias in sequence data. *Genome Biology* 14: R51.

Steiner, C. C., A. S. Putnam, P. E. A. Hoeck, and O. A. Ryder. 2013. Conservation genomics of threatened animal species. *Annual Review of Animal Biosciences* 1: 261–281.

Appendix 1. Source locality information for samples included in this study.

Sampling locality	Geographic coordinates	Tissue voucher no.[a]	*N*
San Bartolo, Baja California Sur, Mexico	23.736520°N, 109.843830°W	Fpet.70.08.3.A-J^{DNA}	10
		Fpet.70.38.4^{DNA}	1
		Fpet.70.56.2^{RNA}	1
		Fpet.70.56.3^{DNA}	1
Fig Canyon (San Isidro), Baja California Sur, Mexico	26.357880°N, 111.803510°W	Fpet.95.10^{DNA}	1
		Fpet.95.17B^{DNA}	1
La Paz Summit, Baja California Sur, Mexico	24.048400°N, 110.150080°W	Fpet.96.34.5^{DNA}	1
		Fpet.96.34.36^{RNA}	1
		Fpet.96.36.20^{DNA}	1
Mesa La Caguama, Baja California Sur, Mexico	27.56675°N, 113.07373°W	Fpet.112.101^{DNA}	1
		Fpet.112.102^{DNA}	1
		Fpet.112.104^{DNA}	1
Santa Agueda, Baja California Sur, Mexico	27.086955°N, 112.516378°W	Fpet.113.01.01$^{RNA, DNA}$	1
		Fpet.113.4N.17$^{RNA, DNA}$	1
Aguijito Higuera, Baja California, Mexico	29.261530°N, 114.016780°W	Fpet.158.6A.15$^{RNA, DNA}$	1
		Fpet.158.29.18.A-T^{DNA}	19
		Fpet.158.29.30^{RNA}	1
La Lagunita, Baja California Sur, Mexico	28.2172°N, 113.18943°W	Fpet.170.05.A-B^{DNA}	2
Bahia San Francisquito Rd., Baja California Sur, Mexico	28.291410°N, 113.111680°W	Fpet.172.2.11^{DNA}	1
		Fpet.172.2.23^{RNA}	1
		Fpet.172.4.13^{RNA}	1
		Fpet.172.4.18.A-Y^{DNA}	25
		Fpet.172.30.17.A-V^{DNA}	21
		Fpet.172.30.18.A-D^{DNA}	4
El Ranchito, Baja California Sur, Mexico	25.375988°N, 111.316845°W	Fpet.201.14^{DNA}	1
		Fpet.201.15^{DNA}	1

Note: *N* = number of samples.

[a]Tissue vouchers are deposited in the laboratory of J. Nason. The superscript RNA denotes samples that were used for RNA extraction and transcriptome sequencing, whereas the superscript DNA denotes samples that were used for DNA extraction and SNP genotyping.

Appendix 2. SNP primer table including the marker's ID, GenBank accession number (NCBI ss#), polymorphism type, sequence capture primers 1 and 2, Sequenom extension primer, and cellular location.

SNP ID	NCBI ss#	SNP type	Capture primer 1 (5′–3′)	Capture primer 2 (5′–3′)	Extend primer (5′–3′)	Cellular source
Fpet.01	1573990490	A/C	1-GGCGCCGGAGGGCTCCAT	2-TCCTTCAAGTCCACCATCTC	CCAACCCCCTCCACAAC	Nucleus
Fpet.02	1573990591	A/G	1-CTCCAAACTATCTTACGGTG	2-GCCAAGCAAAGCCTTTTCAC	ATGCCTTGTCAAGCATC	Nucleus
Fpet.03	1573990721	C/T	1-CACACAAAATTTGCACCCCC	2-TGTCATCCTTGCGTTGAATC	TGAATCAAAGGCTCTCC	Nucleus
Fpet.04	1573990894	A/G	1-GGAGTTGAACTAAGGGTCTG	2-TATACCCCTTCGCGCCAAAC	CCAAACCTCCATTCACTC	Nucleus
Fpet.05	1573991012	A/C	1-GGATACCCTCTTCCTTTCTC	2-TGTCGCCATTCTCAAAGAGG	TAGTACCAAAACAACGGG	Nucleus
Fpet.06	1573991131	C/T	1-GATGACTCTCGAGAAACTGC	2-CTTGTCAGCCAATTGAACTC	CCAATTGAACTCTCTTCAC	Nucleus
Fpet.07	1573991257	C/T	1-GGTTACTTGCCATCATCCAG	2-ACGGTATACCAAGCGACAAC	TTTTGCGTGACGCCACAAT	Nucleus
Fpet.08	1573991353	A/G	1-GAAGAGATTCTGGCGAAAGG	2-TTCCTCACCCTTACACCAAC	CTATTCTTCTTCTCCTCCCT	Nucleus
Fpet.09	1573991462	C/T	1-ATGAAACGCCTTGTCCAGTC	2-AGTGGCTCTGGTATTCTGTC	AGGTGAGCTGGCGCAACAG	Nucleus
Fpet.10	1573991529	A/G	1-GGGTGTATGGATAAGTTGC	2-ACGGATCACGCTTCTTTGAC	TTTTGCCAAACTGCGACCAA	Nucleus
Fpet.11	1573991680	C/T	1-AGCGTTGTTAGGATCAGGAG	2-GTGAGATGTGACAGGCTTAG	AAGGCTTTATACTCCTCGGC	Nucleus
Fpet.12	1573991771	A/T	1-AATGTTCCAACATGGCACCG	2-TAACCTGCCTGTTCTTCACG	CACTTCAACCTTGTTTCCACA	Nucleus
Fpet.13	1573991863	C/T	1-TGCTGAAGGTTTCTCTGAAC	2-TCATATCCTTGAGCTTCACC	GGTGTATTCAACCAAAGCAAC	Nucleus
Fpet.14	1573991951	C/T	1-GGCAGATCGAGTCAGTTATG	2-CAAACTGCTGTTTGAGCTCC	AAATCTCCTCTACCCTCCACTC	Nucleus
Fpet.15	1573992101	C/T	1-GAACCTACGGTGTGGTTTAC	2-GCTCCAAACGGATCTTCTTC	TTCTCTTCAAAGCAATTGTCTC	Nucleus
Fpet.16	1573992221	C/T	1-AGTACAAGTCCCCAACTGTC	2-GGCAAGATAATGGTGGATTG	ATTGCATTGAAAATATTCCTGC	Nucleus
Fpet.17	1573992381	C/T	1-GGTGGCCCTATCGGTTTAAT	2-GCTCTCCAACTCTCCATCTG	ACTATTCTCCTATTCTCAACATC	Nucleus
Fpet.18	1573992495	G/T	1-TCACACATTTTCTGATTCCG	2-CCGGGGACAACTGATAACTT	TGTACAACTGATAACTTCCAAAA	Nucleus
Fpet.19	1573992599	A/G	1-CTGTTTTTACTCCTAAGGAAG	2-ATAAAGTTTCCTTATGGGC	AGGGCCTTATGGGCAATGCTAAT	Nucleus
Fpet.20	1573992755	C/G	1-AGGGTCGGCACGTATGAATC	2-TCGTTGTCACTCATCTCTGG	GGGATGGTTAAAAGAACCAGAAG	Nucleus
Fpet.21	1573992856	A/G	1-TTCCCTAGGACTGCTATAAC	2-ATGCATGCTAATGGGGCAAG	ACACATATTTTTCGTGGTCTATAT	Nucleus
Fpet.22	1573993008	C/T	1-TTGTCTCCAATGCACCATCC	2-TGAAGACATCTGCATGAGCG	TTTCCGGGCAGCGATGATAATCCT	Nucleus
Fpet.23	1573993170	C/T	1-TGGGAGAGCAGTTATCGTTG	2-TTTCTACTGCACTGCACAGG	CGGTGGACTTGAGAGAAAGTAGAA	Nucleus
Fpet.24	1573993274	C/T	1-GATGTTGAAGTTAGCGTCCC	2-CATAGACGGTCCACTTATGC	CCCCTTTACCAAGCCAAAACGCAAT	Nucleus
Fpet.25	1573993392	C/T	1-GTTTGCCAAACTAGATGGTC	2-CTGCCGCTCATGATGTATTG	GGGACCTGGCTTATAAGAACGTATC	Nucleus
Fpet.26	1573993496	A/G	1-AAGCTCTACACCGAAGACTG	2-GATTTCCTGACACGCTTACG	AGAACCGCTTACGAATTTAACTTTCC	Nucleus
Fpet.27	1573993653	C/T	1-CCAACTCCCTCAGAGTAATC	2-CCAGCCAAGGTTCATAAAGC	GAAATAATGTTCTTAGAGGTCTGCAT	Nucleus
Fpet.28	1573993755	A/G	1-TCAGGCTGAGTTGGTTTTGG	2-CTATCGTCCAAGTAATCCCC	GAACTCACCAATCTTATTCTCTTTCCT	Nucleus
Fpet.29	1573993864	C/T	1-CCAAAGGTGACCCAAGAATC	2-CAACTTCTCTCCAAACGACC	AACCCCTCCAAACGACCAAAGCTCTTC	Nucleus
Fpet.30	1573993967	A/C	1-CTCCATATTCCATCCTCTTC	2-TGTCAAACCAGAGGGATATG	ACATTATTCAATTCCTGCTGAAATTCC	Nucleus
Fpet.31	1573994123	A/C	1-GCCTTTCTTGTACTAATACC	2-ATTCCGGTACCCCCGTGTTA	CCCCCGTGTTACTCCTT	Mitochondria
Fpet.32	1573994227	A/G	1-AGAATACGTTCTCGCATCGC	2-GAATGAAGTGGGTCAACCTC	CAACCTCTTTTTGGCTT	Mitochondria
Fpet.33	1573994336	A/G	1-GAGTTATGGCATTCAATCTC	2-CAACCATTTTTGCTCGTGCT	GCTCGTGCTAGTGCCCC	Mitochondria
Fpet.34	1573994438	C/T	1-CTTTTATCTGTTGGCTTTGG	2-CGAAAGTAGCTCTCAAGAAC	GAAAAGAAATCGCCCATT	Mitochondria
Fpet.35	1573994550	A/G	1-CTTAACAATAGGACCTGGAG	2-GCATCTAAAGCCCCCTTTAC	AGCAATAGCATGATGAAC	Chloroplast
Fpet.36	1573994673	A/G	1-TTATCCAACCCCGAGCAATC	2-GCTAAAAAAAACGCCAGTCAC	CCATACCAGCTAACGAACC	Mitochondria
Fpet.37	1573994759	A/T	1-AGCCCTTGCTCATGGTTTTG	2-TGTACCAACCCAACACACAC	CAGCACTCTCTCCCACATTT	Mitochondria
Fpet.38	1573994857	G/T	1-CCGGGTCACAATTTGTATCG	2-CGGCTCTTCGAGAATGTATC	CTAACTTTGGGAATTCCCAC	Chloroplast
Fpet.39	1573994962	A/T	1-TGACATAGCGTTCCTGATAG	2-CAAAGCAGGACTTCTTTGGC	TGGCAAAAAGAACTTGAATA	Mitochondria
Fpet.40	1573995030	C/T	1-CTCCATAAATCAAGCTCTCC	2-GCCTGGCACTAAGTGCAATG	ACCTTCCTGCTAGTATTCCTA	Mitochondria
Fpet.41	1573995130	A/G	1-ACTTTTCCGGAAAGACCACC	2-TTGGCAATCCTTGGTAGAGC	CCAGATGATTTCGTGCTGAAC	Mitochondria
Fpet.42	1573995228	C/T	1-TGAGATACAGAGGAATAAGC	2-GATGATAGTCGGCACAATTC	CCCCATGCAGCTTTAACATCTC	Chloroplast
Fpet.43	1573995397	C/T	1-GAAACTCGCCGTAAAAAAATG	2-TCAGTACAGTAGATATTCC	CCACACGTCCCTTTCTGTCTGA	Chloroplast
Fpet.44	1573995511	C/T	1-AATGATGGATTTCGCGCCAC	2-AATTGCTTTAGCGGGAGCTG	CGGTCGGTATTGGAAACGTCTT	Mitochondria
Fpet.45	1573995594	C/T	1-GATCGGTATAAACATCAAC	2-AGGTAGGATTTTTTTGGCCC	ACTTATTTGTTGAGGAGAAACT	Chloroplast
Fpet.46	1573995675	C/T	1-CGATCAGTCCAATTGAAACC	2-AGCTATTGCATTGTTTGCCC	CTTCTGCCCTAATGATGGCCTTT	Mitochondria
Fpet.47	1573995798	C/T	1-AGGGAACCTGCAAATATTGG	2-GGGTTTTTCTGGTCCAAGTG	CCAAGTGTATCTTGTTTTTACTA	Chloroplast
Fpet.48	1573995908	A/T	1-CGACAAGGAATTTCGCTACC	2-GAAGTTGGTGACCTGATGAC	GGGATGTGAACGGCGGCCGTAAC	Chloroplast
Fpet.49	1573996018	A/G	1-GGAGATTTATAGCATCATTC	2-GGTCTGGAATTAGGTGTAGC	AGTACAAGCTTATGTTTTTACGAT	Mitochondria
Fpet.50	1573996173	C/G	1-TAGGAAAGTTGTTGTAGCTG	2-TGCTGATGCAATCACCATAC	CATCACCATACTAGTACACTTAATA	Mitochondria
Fpet.51	1573996288	A/G	1-GTTTGGTGATTAAGGCGAAG	2-AAAAGGCGCTCAGCCTACAG	CCTACAGGAACTGTTTATGATATTT	Chloroplast
Fpet.52	1573996427	G/T	1-ATTCCTTAACTATTGGCGGG	2-GTTCCGGCGAACGAATAATC	ATCGTTCCGGACAACACATACAAAGA	Chloroplast
Fpet.53	1573996541	A/C	1-GGGTCCTCTATGATCGATG	2-TTGTTGCCCGGAGCAACAAG	GAGCCAGTTGGTAAGTATTAAAATCC	Chloroplast
Fpet.54	1573996656	A/G	1-ACTCGCTCTGTAGTGTTGTC	2-TGCTTTCCTAGATCTTCTCC	CTTCCTAGATCTTCTCCTTAATGTATT	Mitochondria