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Genotyping of *Munidopsis polymorpha* individuals at 8 microsatellite loci (project JaMic - report 2017.07.24.01)

A total number of 26 *Munidopsis polymorpha* samples were received at AllGenetics on 01/06/2017.

DNA isolations were carried out using the NZY Tissue gDNA Isolation kit (NZYTech) following the manufacturer's instructions, and resuspended in a final volume of 100 μL. A negative control that contained no sample was included in every isolation round to check for contamination during the experiments.

The 26 individuals (see Supplementary Table) were genotyped using 8 microsatellite loci developed by Cabezas et al. 2009 (Conservation Genetics 10:673-676) (Table 1).

The PCRs were carried out following Schuelke 2000 (Nature Biotechnology 18:233-234). Briefly, each reaction was performed with at least 3 oligonucleotides:

- A *Munidopsis polymorpha*-specific forward primer.
- A *Munidopsis polymorpha*-specific reverse primer. This reverse primer has an oligonucleotide tail at its 5' end.
- A fluorescently-labelled oligonucleotide identical to the 5' tail of the reverse primer.

The oligonucleotide tails used were the universal sequences M13 (GGA AAC AGC TAT GAC CAT) and CAG (CAG TCG GGC GTC ATC). The M13 oligonucleotide was labelled with the HEX dye. Likewise, the CAG oligonucleotide was labelled with the FAM dye.

During the first cycles of the PCR, the reverse primer with the tail is incorporated into the accumulating PCR products. When this primer is used up, the annealing temperature is lowered, so the fluorescently-labelled M13 or CAG oligonucleotide can anneal and start acting as a primer. This way, the fluorescence is incorporated into the PCR products. For detailed information about the PCR protocol, please refer to the text box at the end of the report.

All PCR rounds included a negative control to check for potential cross-contamination.

PCR products were subsequently subjected to fragment analysis.

A total number of 87 .fsa files were analysed. The loci analysed are recorded in Table 1. Alleles were called using Geneious 10.2.3. In order to avoid the generation of false variability, we did not call an allele if its pattern was unclear.

Along with this report we provide a Supplementary Table with the alleles called at each locus in each individual analysed. This file can be accessed by following the link below:

`http://services.allgenetics.eu/JaMic/XTKQ-E83D-WQKE/Supplementary_table.xlsx`

The raw .fsa files can be accessed by clicking the link below:

`http://services.allgenetics.eu/JaMic/XTKQ-E83D-WQKE/Electropherograms.zip`

Table 1: Loci analysed and multiplex setup

Locus	Forward primer	Reverse primer	Amplicon size	Motif	Multiplex	Reverse primer tail	Fluorescent label
Mp-1	TTCCACAATGAGCACTGGAC	GCATATGTGGAGCCTGGATT	350	(CTAT)16	1	CAG	6-FAM
Mp-8	AGCATCAATTCTGCCCTTTC	GATCACCCCACTTGAAGGAA	189	(TCCA)4(TCTA)9(TC)2(TATC)10	1	CAG	6-FAM
Mp-4	TGACCAAACAAATATTCCTTAGTAGG	CTCATGTGGGCCTCTGAATA	154	(GATA)13	1	M13	HEX
Mp-5	TCGTGCTTCTCTTTCAATGC	ACAATGGGAATGAGGGGAAG	296	(TATC)34	1	M13	HEX
Mp-2	GGAGAGGGAGTTATCGAGAGG	GGGGAGTCTGGAATTAAATGG	249	(CTAT)15TTAT(CTAT)6	2	CAG	6-FAM
Mp-3	CGTCTTTGGCTGCGACTAA	ACGGCGTCAGGCAATAAATA	293	(TATC)15	2	M13	HEX
Mp-6	AACCTCTCCTTGCCTTCCTT	TCGTTGGCAGAGGTAACAAAC	236	(TTTG)9	3	CAG	6-FAM
Mp-7	CTCCAGGCACAGATACTGACAC	GGTGATGACTGAACACAAGTCC	301	(TG)17	3	M13	HEX

Ready-to-use PCR protocol

PCRs were performed in a final reaction volume of 12.5 μL, containing 1 μL of DNA, 6.25 μL of the Type-it Microsatellite PCR Kit (Qiagen), 4 μL of PCR-grade water, and 1.25 μL of the primer mix (see Table 2 for details on how to set up the primer mix). The optimal PCR protocol consisted in an initial denaturation step at 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 56 °C for 90 s, 72 °C for 30 s; 8 cycles of 95 °C for 30 s, 52 °C for 90 s, 72 °C for 30 s; and a final extension step at 68 °C for 30 min. A negative control that contained no sample was included in the PCRs to check for cross contamination during the experiments.

Table 2: Primer mix setup for singleplex reactions.

Reagent	Stock concentration	Final concentration	Volume
Forward primer	100 μM	2 μM	1 μL
Reverse primer (tailed)	100 μM	0.2 μM	0.1 μL
HEX-M13 or FAM-CAG oligonucleotide	100 μM	2 μM	1 μL
PCR-grade water	-	-	Up to 50 μL

Please note that the amount of the reverse primer is 10-fold lower than that of the forward primer.

For multiplexing, please refer to the following example of a primer mix setup (Table 3). Note that, in this particular case, we are using 2 reverse primers with an M13 tail and one reverse primer with a CAG tail. Therefore, the amount of the HEX-M13 oligonucleotide must be twice that of the FAM-CAG oligonucleotide.

Table 3: Example of a primer mix setup for multiplex reactions.

Reagent	Stock concentration	Final concentration	Volume
Forward primer 1	100 μM	2 μM	1 μL
Reverse primer 1 (M13-tailed)	100 μM	0.2 μM	0.1 μL
Forward primer 2	100 μM	2 μM	1 μL
Reverse primer 2 (M13-tailed)	100 μM	0.2 μM	0.1 μL
Forward primer 3	100 μM	2 μM	1 μL
Reverse primer 3 (CAG-tailed)	100 μM	0.2 μM	0.1 μL
HEX-M13 oligonucleotide	100 μM	4 μM	2 μL
FAM-CAG oligonucleotide	100 μM	2 μM	1 μL
PCR-grade water	-	-	Up to 50 μL

Please note that different reagents may yield different results. Therefore the protocols may need some optimisation.