Manual dissection of the Schistosoma mansoni head and back end for transcriptomic analysis

PLOS One

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Working

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ABSTRACT

Total genomic DNA was extracted from leaf tissue to amplify and sequence individually five specific target loci from nuclear and chloroplast genome.

EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Ley AC, Nissen J, Wölk A, Röser M (2018) Glacial refugia and speciation in a group of wind-pollinated and -dispersed, endemic Alpine species of *Helictotrichon* (Poaceae). PLoS ONE 13(10): e0205354. doi: 10.1371/journal.pone.0205354

PROTOCOL STATUS

Working

1 Total genomic DNA was extracted from leaf tissue using the NucleoSpin®Plant-Kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions.

2 Three chloroplast and one nuclear marker were amplified.

DNA-Region	Primer name	Sequence (5' →3')	Reference
Chloroplast:			
rps16	*rps*F	GTG GTA GAA AGC AAC GTG CGA CTT	[45]
	*rps*R2	TCG GGA TCG AAC ATC AAT TGC AAT	[45]
*rpL*32-*trnL* (UAG)	*rpL*32-F	CAG TTC CAA AAA AAC GTA CTT C	[46]
	trnL (UAG)	CTG CTT CCT AAG AGC AGC GT	[46]
*ycf*3In1	*ycf*3In1-F	TGA CAG ATC ACG GCC ATA TT	[47]
	*ycf*3In1-R	TTA YAG AGA TGG TGC GAT TT	[47]
*ycf*3In2	*ycf*3In2-F	GCY TGT TTC CAA TAC TCA GCA	[47]
	*ycf*3In2-R	ATG GCC GTG ATC TGT CAT TA	[47]
Nuclear:			

At103	*At103*-F	CTT CAA GCC MAA GTT CAT CTT CTA	[48]
	At103-R	TTG GCA ATC ATT GAG GTA CAT NGT MAC ATA	[48]

3 Amplification of the target loci were was conducted in a Mastercycler (Eppendorf, Hamburg, Germany). Each 20 µl volume contains 2.00 µl 10× PCR buffer (without MgCl2), 1.00 µl DMSO, 0.20 µl 100 mM dNTPs, 1.4 µl 50 mM MgCl2, 0.20 µl 0.05 mM each forward and reverse primers, 0.20 µl Taq DNA polymerase (BioTherm DNA polymerase 5 u/µl from GeneCraft, Lüdinghausen, Germany), 1 to 2 µl genomic DNA extract and filled up with H2O. Amplification cycles were as follows: one cycle of 3 min at 94°C, 35 cycles of 30 s at 94°C, 30 s at 50°C, 1.5 min at 72°C with a final extension period of 10 min at 72°C.

4 PCR products were sequenced by LGC Genomics (Berlin, Germany).

5 Sequences were edited and then aligned manually (after preliminary automatic alignment in MUSCLE [23]) with the program Geneious 4.8.3 (http://www.geneious.com [24]).