DNA Barcoding of Plants: *matK* primers for mosses

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v.1.0 February 2012: If you use these primers please send an email to barcoding@rbge.ac.uk. This is to enable us to provide protocol updates and to solicit feedback on how well the primers perform.

Summary:

New primers to amplify the *matK* barcode in mosses were designed from *de novo* full length *matK* sequences representing 18/30 orders and 52 genera (N=66).

All full length moss *matK* sequences (except *Sphagnum*) contain adjacent A and T mononucleotide motifs in the centre of the barcode region. This is problematic in terms of sequencing, but can be alleviated by the use of Phusion *Taq* polymerase [see Fazekas *et al* (2010) *Biotechniques* 48, 277-285].

No universal primer pair could be designed. The following primer pairs each amplify between 55-65% of moss samples, but collectively amplify >80%. As there is some taxonomic bias in success rates for individual primers, primer selection as to which primers should be attempted first is guided by the sample to be analyzed. Results from laboratory trials are included at the end of this document to aid decision making (green = successfully sequenced; red = no sequence obtained; na = not available e.g. not tested).

Primer pairs:

Moss matK-P1

Moss404F: 5′-GGACTARYTATCAATCTATTCAYTC-3′

Moss1336R: 5′-TRCAAGCYAAYGTTTTAGC-3′

Moss matK-P2

Moss404F: 5′-GGACTARYTATCAATCTATTCAYTC-3′

Moss1324R: 5′-GTTTTAGCACAWGAAAATCG-3′

Moss matK-P3

Moss485F: 5′-AAATACCTYATTTTWTTCATCC-3′

Moss1336R: 5′-TRCAAGCYAAYGTTTTAGC-3′

Protocols:

PCR (final concentrations in total volume 10µl): 1x PCR buffer, 0.2mM each dNTP, 1M betaine, 0.2M trehalose, 0.5µM each primer, 0.5U Phusion *Taq* (Finnzymes) and 1ng template DNA.

PCR thermocycling parameters: 98℃ for 45 secs; 35 cycles of 98℃ for 10 secs, 58℃ for 30 secs, 72℃ for 40 secs; 72℃ for 10 mins; storage at 8℃.

PCR clean-up: add 2µl of ExoSAP-IT (diluted 1:10) to 5µl of PCR product.

PCR clean-up thermocycling parameters: 37℃ for 30 mins, followed by 80℃ for 15 mins then storage at 8℃.

Sequencing PCR (final concentrations in total volume 10ul): 1x sequencing buffer, 0.5µl BigDye, 0.32µM primer, 0.2M trehalose, 1µl template.

Sequencing thermocycling parameters: 25 cycles of 95℃ for 30 secs, 50℃ for 20 secs, 60℃ for 4 mins; storage at 8℃.

			P1	P2	P3
Takakiales	Takakiaceae	*Takakia* sp.			
Sphagnales	Sphagnaceae	*Sphagnum compactum*			
	Sphagnaceae	*Sphagnum contortum*			
	Sphagnaceae	*Sphagnum fallax*			
	Sphagnaceae	*Sphagnum girgensohnii*			
	Sphagnaceae	*Sphagnum magellanicum*			
	Sphagnaceae	*Sphagnum squarrosum*			
Andreaeales	Andreaeaceae	*Andreaea alpina*		na	
	Andreaeaceae	*Andreaea rupestris*			
Andreaeobryales	Andreaeobryaceae	*Andreaeobryum macrosporum*		na	
Oedipodiales	Oedipodiaceae	*Oedipodium griffithianum*			
Polytrichales	Polytrichaceae	*Atrichum undulatum*			
	Polytrichaceae	*Polytrichum commune*		na	
Tetraphydopsidales	Tetraphidaceae	*Tetraphis pellucida*			
Buxbaumiales	Buxbaumiaceae	*Buxbaumia punctata*			
Diphysciales	Diphysciaceae	*Diphyscium foliosum*			
Timmiales	Timmiaceae	*Timmia megapolitana*			
Gigaspermales	Gigaspermaceae	*Gigaspermum repens*		na	
Encalyptales	Encalyptaceae	*Encalypta streptocarpa*			
Funariales	Funariaceae	*Funaria hygrometrica*			
	Disceliaceae	*Discelium nudum*			
Scouleriales	Scouleriaceae	*Scouleria aquatica*			
	Drummondiaceae	*Drummondia obtusifolia*			
Bryoxiphales	Bryoxiphaceae	*Bryoxiphium norvegicum*			
Grimmiales	Grimmiaceae	*Racomitrium lanuginosum*			
	Ptychomitriaceae	*Indusiella thianshanica*			
	Seligeriaceae	*Blindia acuta*			
Archidiales	Archidiaceae	*Archidium alternifolium*			
Dicranales	Fissidentaceae	*Fissidens osmundioides*			
	Hypodontiaceae	*Hypodontium dregei*			
	Eustichiaceae	*Eustichia longirostris*			
	Ditrichaceae	*Distichum capillaceum*			
	Ditrichaceae	*Ditrichum gracile*			
	Bruchiaceae	*Bruchia bolanderi*			
	Rhachitheciaceae	*Jonesiobryum cerradensis*			
	Eropodiaceae	*Eropodium grossirete*			
	Schistostegaceae	*Schistostega pennata*			
	Rhabdoweisiaceae	*Dichodontium pellucidum*			
	Dicranaceae	*Dicranum scoparium*		na	
	Dicranaceae	*Kiaeria starkei*			
	Leucobryaceae	*Leucobryum glaucum*			
	Calymperaceae	*Calymperes palisotii*			
Pottiales	Pottiaceae	*Syntrichia intermedia*			
	Pottiaceae	*Tortella flavovirens*			
Splachnales	Splachnaceae	*Tetraplodon mnioides*			
	Meesiaceae	*Meesia uliginosa*			
Bryales	Catoscopiaceae	*Catoscopium nigritum*		na	
	Bryaceae	*Bryum alpinum*		na	
	Phyllodrepaniaceae	*Phyllodrepanium falcifolium*		na	
	Mniaceae	*Mnium stellare*		na	
	Leptostomataceae	*Leptostomum menziesii*		na	
Bartramiales	Bartramiaceae	*Breutelia chrysocoma*			

Orthotrichales	Orthotrichaceae	*Ulota bruchii*	
Hedwigiales	Hedwigiaceae	*Hedwigia stellata*	
	Rhacocarpaceae	*Rhacocarpus purpurascens*	
Rhizogoniales	Rhizogoniaceae	*Pyrrhobryum cf spiniforme*	na
	Aulacomniaceae	*Aulocomnium androgynum*	
	Orthodontiaceae	*Orthodontium lineare*	
Hypnodendrales	Racopilaceae	*Racopilum orthocarpum*	
	Hypnodendraceae	*Hypnodendron vitiense*	
Ptychomniales	Ptychomniaceae	*Garovaglia elegans*	
Hookeriales	Hypopterygiaceae	*Hypopterygium japonicum*	na
	Daltoniaceae	*Daltonia splachnoides*	na
	Schimperobryaceae	*Schimperobryum splendidissimum*	na
	Hookeriaceae	*Hookeria lucens*	na
	Pilotrichaceae	*Pilotrichella flexilis*	na
Hypnales	Rutenbergiaceae	*Neorutenbergia usagarae*	
	Fontinalaceae	*Fontinalis antipyretica*	
	Climaciaceae	*Climacium dendroides*	
	Amblystegiaceae	*Amblystegium tenax*	
	Amblystegiaceae	*Leptodictyum riparium*	
	Calliergonaceae	*Warnstorfia exannuata*	
	Helodiaceae	*Helodium blandowii*	
	Rigodiaceae	*Rigodium toxarion*	
	Leskeaceae	*Leskea polycarpa*	na
	Leskeaceae	*Ptychodium plicatum*	
	Thuidiaceae	*Thuidium tamariscinum*	
	Regmatodontaceae	*Regmatodon polycarpus*	
	Stereophyllaceae	*Pilosium chlorophyllum*	
	Brachytheciaceae	*Brachythecium rivulare*	
	Brachytheciaceae	*Cirriphyllum piliferum*	
	Meteoriaceae	*Meteorium buchanani*	
	Myriniaceae	*Myrinia pulvinata*	
	Fabroniaceae	*Fabronia pusilla*	
	Hypnaceae	*Hypnum jutlandicum*	
	Hypnaceae	*Pylaisia polyantha*	na
	Catagoniaceae	*Catagonium nitens*	
	Pterigynandraceae	*Habrodon perpusillus*	
	Hylocomiaceae	*Rhytidiadelphus triquetrus*	
	Rhytidiaceae	*Rhytidium rugosum*	
	Symphyodontaceae	*Symphyodon machristianus*	
	Plagiotheciaceae	*Plagiothecium denticulatum*	
	Entodontaceae	*Entodon concinnus*	
	Pylaisiadelphaceae	*Brotherella canadensis*	
	Sematophyllaceae	*Sematophyllum caespitosum*	
	Cryphaeaceae	*Cryphaea heteromalla*	
	Leucodontaceae	*Antitrichia curtipendula*	na
	Pterobryaceae	*Calyptothecium hookeri*	
	Phyllogoniaceae	*Phyllogonium fulgens*	
	Lepyrodontaceae	*Lepyrodon lagurus*	
	Neckeraceae	*Homalia trichomanoides*	
	Neckeraceae	*Neckera complanata*	
	Echinodiaceae	*Echinodium prolixum*	
	Leptodontaceae	*Leptodon smithii*	na
	Lembophyllaceae	*Isothecium myosuroides*	
	Myuriaceae	*Myurium hochstetteri*	
	Anomodontaceae	*Anomodon viticulosus*	