DNA Barcoding of Plants: *matK* primers for hornworts

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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:

Following *de novo* sequencing of three full length plastid *matK* from hornworts in addition to a single accession from GenBank, consensus primer sequences were designed and tested on a sample (N=16) representing six genera (*Anthoceros*, *Dendroceros*, *Megaceros*, *Nothoceros*, *Phaeomegaceros*, *Phaeoceros*) from three families. The best primer pair amplified 14/16 samples (88%). Of the two failures, one could not be amplified for *rbcL*, and the other was difficult to amplify for *rbcL*. Other members of the same genus sequenced without many problems.

HORN-F1	5′-GCAAGAACGTTTCTTATATCC-3′
HORN-R2	5′-TTTRGCACATGAAAATCGAAG-3′

Laboratory procedures and protocols:

PCR (final concentrations in total volume 10µl): 1x PCR buffer, 0.2mM each dNTP, 2.5mM $MgCl^2$, 1M betaine, 0.2M trehalose, 0.5µM each primer, 0.5U Platinum *Taq* (Invitrogen) and 1ng template DNA.

PCR thermocycling parameters: 94°C for 4 mins; 10 cycles of 94°C for 30 secs, 52°C for 30 secs, 72°C for 1 min; 25 cycles of 88°C for 30 secs, 48°C for 30 secs, 72°C for 1 min; 72°C for 10 mins; storage at 8°C.

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

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

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°C for 30 secs, 50°C for 20 secs, 60°C for 4 mins; storage at 8°C.

Note:
Researchers are encouraged to generate full length plastid *matK* sequences for additional hornwort taxa under study, as to add these to the sequence data generated here will potentially lead to improved universal *matK* primer design in hornworts. This specifically applies to rare or geographically restricted taxa in the families not covered here.