DNA Barcoding of Plants: *matK* primers for liverworts

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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:

de novo sequencing of full length plastid *matK* combined with sequences from Genbank resulted in an alignment of 54 liverwort taxa representing 13/15 orders. This matrix was used for primer design. Primers were tested on 94 liverwort species encompassing all 15 orders and 74/81 families. These primers did not amplify all samples. We therefore adopted a 2-phase approach. The first is amplification with the best performing primer pair, followed by using one of four clade-specific primer pairs.

1st round:

Primer combination LivF1A+LivR1A amplified ca. 70% of tested liverwort taxa.

matK-LivF1A: 5′-TYCATCCWGAAATTTTGATTCG-3′

matK-LivR1A: 5′-ATAGTACTTTTRTGTTTACATGC-3′

2nd round:

Four pairs of clade-specific primers (clades *sensu* Forrest *et al* 2006 *Bryologist* 109, 303-334) amplified the majority of samples not successful in the first round PCR. We did not have success with these primers in a cocktail.

Complex thalloid liverworts:

LivCTH-442F: 5′-ATACCTTAYTTTTTTCAYCC-3′

LivCTH-1171R:5′-CATTATCTGDTAATGTTGTCC-3′

Simple thalloid liverworts clade 1:

LivSTH1-442F: 5′-RTACCYYAYTBYKTYCAYCC-3′

LivSTH1-1171R:5′-CRTTATCVGYCAATGTYGTCC-3′

Simple thalloid liverworts clade 2:

LivSTH2-442F: 5′-ATACCHYATTSSBTTCATCC-3′

LivSTH2-1171R:5′-CRTTATCGGTCAADGTTGTCC-3′

Leafy liverworts:

LivLFY-442F: 5′-ATACCWTATTCTTTYCAYCC-3′

LivLFY-1171R:5′-CATDATCTGYSAAIGTTGTCC-3′

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 liverwort taxa to improve the sequence resources from which primers can be designed.