DNA Barcoding of Plants: *matK* primers for ferns and allies

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Funded by The Gordon & Betty Moore Foundation

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:

Collation of published and unpublished sequences of ferns and allies resulted in an alignment of N=159, representing all 14 orders and 47/48 families *sensu* Christenhusz *et al* (2011: *Phytotaxa* 19, 7-54). This matrix was used for primer design. Several universal primers were tested, but yielded unacceptably low numbers of amplicons across a diverse sample set (all 14 orders, 45/48 families, N=94). Therefore, primers were designed for specific lineages.

Polypodiales:

This clade represents ca. 80% of extant fern lineages.

1st round:

This primer pair routinely recovered the highest number of successful bi-directional sequences (43/57; 75%) in a sample representing 23/28 families. Representatives of all sub-familial clades of the large families Pteridaceae, Dryopteridaceae and Polypodiaceae were included.

PolypodF1	5′-ATTTYTGGARGAYAGAYTDCC-3′
PolypodR1	5′-CGTRGTATATATCTCRATYTACGC-3′

2nd round:

This primer added 3 sequences to those achieved in the 1st round, giving a total of 46/57 (81%):

PolypodF2	5′-AATTTCRCARTCYAYYCATTC-3′
PolypodR1	5′-CGTRGTATATATCTCRATYTACGC-3′

Despite the small sample size, there appears to be some taxonomic bias in samples that were difficult to amplify. These include early-diverging families such as Saccolomataceae, Cystodiaceae and Lindsaeaceae, and also the large and diverse Pteridaceae. Primers for these lineages are given here, but have not been tested in the laboratory.

Early-diverging Polypodiales:

SCL-F	5′-GCCGACGAATTCAAGATG-3′
SCL-R	5′-GCTAATTTMSTAASWGGACGTCC-3′

Pteridaceae:

PTE-F	5′-ACTYYAATTCGATTGTTTCG-3′
PTE-R	5′-AARGAAAAVACTTGCCAAAG-3′

Cyatheales:

This lineage is the second most speciose in ferns. The test sample (N=8) represented 6/8 families: no samples were available for Loxomataceae or Metaxyaceae, but the primers match sequences of these taxa from which the primers were designed.

This primer pair amplified and sequenced all eight samples from Cyatheales tested.

TreeFernF	5′-CACATMTHTCAAGRTTGGYTCTC-3′
TreeFernR	5′-ATATCTYAATCTACGCAAYCC-3′

Primers for early diverging lineages:

These lineage-specific primers have not been tested in the laboratory.

Lycopodiales + Isoetales:

LYC-F	5′-CTTATACGAATTTTTCGTCGACG-3′
LYC-R	5′-TTTTYGCACATGAAAATCG-3′

Selaginellales:

SEL-F	5′-TAGRTACCGAATTATCTTACTC-3′
SEL-R	5′-TATGTGTTGCATTCGGTAC-3′

Equisetales:

EQU-F	5′-GAATCTTTTATTCGAATTCTTCG-3′
EQU-R	5′-GTCGTACTTTTATGTTTACGAGC-3′

Ophioglossales:

OPH-F	5′-TMTTATTCGAMTTCYTCGTCG-3′
OPH-R	5′-TTGTACTTTTRTGTTTGCAAGC-3′

Marattiales + Pasilotales:

MAR-F	5′-RTTCGAATYTTTCGTCGAC-3′
MAR-R	5′-TCTTATGTTTACAAGCCAACG-3′

Osmundales:

OSM-F	5′-GAATCCAGGATGCTTCG-3′
OSM-R	5′-ATAATCCATCTCTATCTATTGATCC-3′

Hymenophyllales:

HYM-F	5′-CRKCRAATCAAGGATGC-3′
HYM-R	5′-CATYATCYGTTAACGTAGTCC-3′

Gleicheniales:

GLE-F	5′-GCTTYRATTCGAATSTTTCG-3′
GLE-R	5′-TCTGTHAAAGTAGTCCRAGC-3′

Schizaeales:

SCH-F	5′-RAYTCGAAYRTTTCGTCG-3′
SCH-R	5′-TYGTASTTTTATGTTTACAAGC-3′

Salviniales:

SAL-F	5′-TTTRATTCGAATGTTTCGTAG-3′
SAL-R	5′-KGATTTACWAACAGGATGTCC-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). Template: 1ng.

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.

Technical notes:

A range of different PCR additives, component concentrations and thermocycling conditions were tested. The inclusion of 1M betaine and 0.2M trehalose gave increased amplification success compared to all other additives assayed. Dilution of DNA template can also improve amplification: these tests diluted all samples to ca 1ng per 10µl reaction.

Amplicon clean-up protocols tested did not differ significantly, therefore dilute ExoSAP-IT was used for economic reasons. Lack of an amplicon clean-up step resulted in poor quality unreadable sequences in many cases.

Sequencing reactions routinely included 0.2M trehalose as this increased read length.