DNA Barcoding of Plants: *matK* primers for gymnosperms

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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 sequences from Genbank resulted in an alignment of 827 gymnosperm accessions representing all genera. This matrix was used for primer design. Primers were tested on 94 conifers, 16 cycads, 7 gnetophytes and *Ginkgo biloba* (representing all genera). A universal primer pair amplified the majority of samples (see Li *et al* (2011) *Journal of Systematics & Evolution* 49, 169-175), with most failures attributable to poor DNA quality validated by failure to amplify *rbcL*. Gnetophytes amplified poorly, and a clade-specific primer pair is recommended for these.

Conifers and cycads:

This primer pair routinely recovered the highest number of successful bi-directional sequences (82/95 conifers, 14/16 cycads, 3/7 gnetophytes, 1/1 *Ginkgo*).

Gym-F1A	5'-ATYGYRCTTTTATGTTTACARGC-3'
Gym-R1A	5'-TCAYCCGGARATTTTGGTTCG-3'

Gnetophytes :

This primer pair amplified all seven samples (*Ephedra* N=3. *Gnetum* N=3, *Welwitschia* N=1)

matK-GneF	5'-TCATYCRRARCTKTTVATHMG-3'
matK-GneR	5'-ATMGTACTTTTATGYTTMMARGC-3'

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 diluteExoSAP-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.

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.