PLANT
DNA BARCODE
PROJECT

PCR and Sequencing Protocols - *mat*K

I. PCR protocol for *mat*K marker

Note: Phusion® High-Fidelity DNA polymerase was tested on a broad range of plant taxonomic groups and selected as the enzyme with the highest performance for PCR amplification of the chloroplast barcode markers.

First choice matK primers

Forward: matK-xf	TAATTTACGATCAATTCATTC	Ford et al. 2009
Reverse: matK-MALP	ACAAGAAAGTCGAAGTAT	Dunning & Savolainen, 2010

Second choice matK primers

MatK-1RKIM-f	ACCCAGTCCATCTGGAAATCTTGGTTC	Ki-Joong Kim, pers. comm.
MatK-3FKIM-r	CGTACAGTACTTTTGTGTTTACGAG	Ki-Joong Kim, pers. comm.

PCR reagents per 10 µL reaction

# of reactions	**1**	**100**
5X HF Buffer (with $MgCl_2$)	2 µL	200 µL
100% DMSO	0.3 µL	30 µL
10 mM dNTPs	0.2 µL	20 µL
10 µM Primer Forward	0.5 µL	50 µL
10 µM Primer Reverse	0.5 µL	50 µL
ddH2O	5.375 µL	537.5 µL
Phusion High Fidelity Fisher Scientific #-530 (5U/ µL)	0.125 µL	12.5 µL
Total	**9 µL**	**900 µL**
DNA template	1 µL per reaction	

Recommendation: taking into account pipette error, aliquot approximately 73 µL of the PCR cocktail in each of the upper 12 wells of the plate. Using a 12-channeled pipette, transfer approximately 8.7 µL of the PCR cocktail into each well. Add 1 µL of DNA. Centrifuge the plate before thermocycling.

PCR Thermocycling Program for *mat*K marker

First choice matK primers:
98°C for 45 seconds;
35 cycles of 98°C for 10 seconds, 54°C for 30 seconds, 72°C for 40 seconds;
final extension 72°C for 10 minutes.

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Second choice matK primers:
98°C for 45 seconds;
35 cycles of 98°C for 10 seconds, 52°C for 30 seconds, 72°C for 40 seconds;
final extension 72°C for 10 minutes.

II. Sequencing protocol for *mat*K marker

Dilute PCR product adding 40 µL of water in each well. Centrifuge the plate.

For the first choice PCR use primers:

MatK-1RKIM-f	ACCCAGTCCATCTGGAAATCTTGGTTC	Ki-Joong Kim, pers. comm.
Reverse: matK-MALP	ACAAGAAAGTCGAAGTAT	Dunning & Savolainen, 2010

For the second choice use the same primers those were used for PCR

Sequencing reagents per 10 µL reaction

# of reactions	**1**	**104**
5X Sequencing Buffer	1.875 µL	195 µL
100% DMSO	0.355 µL	37 µL
10 µM primer	1 µL	104 µL
BigDye	0.250 µL	26 µL
ddH2O	5.520 µL	574 µL
Total	**9 µL**	**936 µL**
Diluted DNA	2 µL per reaction	

Recommendation: taking into account pipette error, aliquot approximately 78 µL of the sequencing mix in each of the upper 12 wells of the plate. Using a 12-channeled pipette, transfer approximately 9.5 µL of the PCR cocktail in each well. Add 2 µL of DNA. Centrifuge the plate before thermocycling.

Sequencing Thermocycling Program for *mat*K marker

matK Forward (matK-KIM-1R-f)
94°C for 10 seconds;
35 cycles of 94°C for 20 seconds, 48°C for 20 seconds, 60°C for 4 minutes;
hold at 4°C.

matK Reverse (matK-MALP-R)
94°C for 10 seconds;
35 cycles of 94°C for 20 seconds, 50°C for 20 seconds, 60°C for 4 minutes;
hold at 4°C.

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Sequencing Cleanup

- Add Sephadex powder to the Acroprep 96 filter plate. The standard amount of powder is measured by a column loader.
- Add 300 µL of dH2O. Let Sephadex hydrate for 2 hours at room temperature or overnight at 4°C.
- Assemble the Sephadex plate onto collection plate and centrifuge at 2100 rpm for 5 minutes.
- Immediately proceed to loading sequencing product onto the Sephadex columns to avoid drying. Use a fresh plate as a collecting plate.
- Centrifuge at 2100 rpm for 5 minutes.
- Dry the cleanup product at 88°C for 20 minutes, then cover the plate with a rubber lid and place in the freezer at -20°C until it is placed in ABI capillary sequencer.
- * The front panel of the plate should be labeled by the standard order number generated by Smithsonian GeneSifter (https://smithsonian.genesifter.net/login).

References

Dunning LT, Savolainen V (2010) Broad-scale amplification of matK for DNA barcoding plants, a technical note. Botanical Journal of the Linnean Society 164: 1–9.

Ford CS, Ayres KL, Haider N, Toomey N, van-Alpen-Stohl J, et al. (2009) Selection of candidate DNA barcoding regions for use on land plants. Botanical Journal of the Linnean Society 159: 1–11.