Appendix S2. PCR and sequencing protocols used for the assembly of the DNA barcode library of Canadian vascular plants. (A) PCR and sequencing protocols for *rbcL* and ITS2. (B) PCR and sequencing protocols for *matK*.

Part A.PCR protocols for *rbcL* and ITS2 with Platinum DNA Polymerase (Invitrogen, Carlsbad, California, USA). Protocols previously published (Kuzmina and Ivanova, 2011; Fazekas et al., 2012).

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| --- | --- | --- |
| Primer | Primer sequence 5′–3′ | Reference |
| rbcLa-F (forward) | ATGTCACCACAAACAGAGACTAAAGC | Levin et al., 2003 |
| rbcLa-R (reverse) | GTAAAATCAAGTCCACCRCG | Kress et al., 2009 |
| ITS-S2F (forward) | ATGCGATACTTGGTGTGAAT | Chen et al., 2010 |
| ITS4 (reverse) | TCCTCCGCTTATTGATATGC | White et al., 1990 |

PCR cocktail per reaction: 10% trehalose 6.25 μL, ddH2O 2 μL, 10× buffer 1.25 μL, 50 mM MgCl2 0.625 μL, 10 μM primer (forward) 0.125 μL, 10 μM primer (reverse) 0.125 μL, 10 mM dNTPs 0.0625 μL, Platinum DNA Polymerase (5 U/μL) 0.06 μL. Total: 10.5 μL

DNA template: 2 µL

*rbcL*: 94°C for 4 min; 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1 min; final extension 72°C for 10 min; hold at 4°C

ITS2: 94°C for 5 min; 35 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 45 s; final extension 72°C for 10 min; hold at 4°C

Sequencing (with the same primers): 96°C for 2 min; 30 cycles of 96°C for 30 s, 55°C for 15 s, 60°C for 4 min; hold at 4°C

Part B. PCR protocol for *matK* with Phusion High-Fidelity DNA Polymerase (Thermo Fisher, Waltham, Massachusetts, USA).

|  |  |  |
| --- | --- | --- |
| Primer | Primer sequence 5′–3′ | Reference |
| matK-xf (choice I) (forward) | TAATTTACGATCAATTCATTC | Ford et al., 2009 |
| matK-MALP (choice I) (reverse) | ACAAGAAAGTCGAAGTAT | Dunning and Savolainen, 2010 |
| matK-1RKIM-f (choice II) (forward) | ACCCAGTCCATCTGGAAATCTTGGTTC | Ki-Joong Kim, pers. comm. |
| matK-3FKIM-r (choice II) (reverse) | CGTACAGTACTTTTGGTGTTTACGAG | Ki-Joong Kim, pers. comm. |

PCR cocktail per reaction: 5× High-Fidelity buffer (with MgCl2) 2 µL; 100% DMSO 0.3 µL; 10 µM primer (forward) 0.5 µL; 10 µM primer (reverse) 0.5 µL; 10 mM dNTPs 0.2 µL; ddH2O 5.375 µL; Phusion High-Fidelity DNA Polymerase F530 (5 U/µL) 0.125 µL. Total: 9 µL

DNA template: 1 µL

*matK*: 98°C for 45 s; 35 cycles of 98°C for 10 s, 54°C for 30 s, 72°C for 40 s; final extension 72°C for 10 min; hold at 4°C

Sequencing (for choice I and II use matK-1RKIM-f and matK-MALP primers): 96°C for 2 min; 30 cycles of 96°C for 30 s, 50°C for 15 s, 60°C for 4 min; hold at 4°C

**LITERATURE CITED**

Chen, S., H. Yao, J. Han, C. Liu, J. Song, L. Shi, Y. Zhu, et al. 2010. Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. *PLoS ONE*. 5: e8613. doi:101371/journalone0008613.

Dunning, L. T., and V. Savolainen. 2010. Broad-scale amplification of *matK* for DNA barcoding plants, a technical note. *Botanical Journal of the Linnean Society* 164: 1–9. doi:10.1111/j.1095-8339.2010.01071.x.

Fazekas, A. J., M. L. Kuzmina, S. G. Newmaster, and P. M. Hollingsworth. 2012. DNA barcoding methods for land plants. *In* W. J. Kress and D. L. Erickson [eds.], Methods in molecular biology, vol. 858: DNA barcodes: Methods and protocols, 223–252. Springer, New York, New York, USA.

Ford, C. S., K. L. Ayres, N. Toomey, N. Haider, J. Van Alphen Stahl, L. J. Kelly, N. Wilkstöm, et al. 2009. Selection of candidate coding DNA barcoding regions for use on land plants. *Botanical Journal of the Linnean Society* 159: 1–11. doi:10.1111/j.1095-8339.2008.00938.x.

Kress, W. J., D. L. Erickson, F. A. Jones, N. G. Swenson, R. Perez, O. Sanjur, and E. Bermingham. 2009. Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. *Proceedings of the National Academy of Sciences of the United States of America* 106: 18621–18626. doi/10.1073/pnas.0909820106.

Kuzmina, M., and N. Ivanova. 2011. PCR amplification for plants and fungi. Available at website <http://ccdb.ca/site/wp-content/uploads/2016/09/CCDB_Amplification-Plants.pdf> [accessed 14 November 2017].

Levin, R. A., W. L. Wagner, P. C. Hoch, M. Nepokroeff, J. C. Pires, E. A. Zimmer, and K. J. Sytsma. 2003. Family-level relationships of Onagraceae based on chloroplast *rbcL* and *ndhF* data. *American Journal of Botany* 90: 107–115. doi: 10.3732/ajb.90.1.107.

White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White [eds.], PCR protocols: A guide to methods and applications, 315–322. Academic Press, New York, New York, USA.