Deteksi Molekuler Tumbuhan Langka menggunakan Marka *rpoC1* secara *In Silico*

diajukan untuk memenuhi sebagian syarat untuk memperoleh gelar Sarjana Sains
Program Studi Biologi

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Oleh
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Sebuah Skripsi yang diajukan untuk memenuhi salah satu syarat memperoleh gelar Sarjana Sains pada Fakultas Pendidikan Matematika dan Ilmu Pengetahuan Alam

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LEMBAR PERNYATAAN

Dengan ini saya menyatakan bahwa skripsi yang berjudul, **"Deteksi Molekuler Tumbuhan Langka menggunakan Marka *rpoC1* secara *In Silico*"** *ini beserta seluruh isinya adalah benar-benar karya saya sendiri, dan saya tidak melakukan penjiplakan atau pengutipan dengan cara-cara yang tidak sesuai dengan etika ilmu yang berlaku dalam masyarakat keilmuan. Atas pernyataan tersebut, saya siap menanggung risiko yang dijatuhkan kepada saya apabila di kemudian hari ditemukan adanya pelanggaran etika keilmuan dalam karya ini, atau ada klaim dari pihak lain terhadap keaslian karya saya.*

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ABSTRAK

Penelitian ini bertujuan untuk mengembangkan primer diagnostik berbasis gen kloroplas *rpoC1* secara *in silico* untuk identifikasi spesies tumbuhan langka. Metode yang digunakan meliputi pengambilan data sekuen DNA dari GenBank, penyejajaran sekuen ganda (*multiple sequence alignment*) menggunakan ClustalX, pembuatan konsensus sekuen dengan BioEdit, dan perancangan primer menggunakan perangkat lunak FastPCR. Sebanyak 182 kandidat primer telah dihasilkan, kemudian diseleksi menjadi 17 primer terbaik berdasarkan kriteria panjang, kandungan GC, dan suhu leleh (Tm). Uji *in silico* PCR terhadap 25 sekuen spesies tumbuhan langka yang digunakan dalam proses desain menunjukkan bahwa satu pasangan primer terbaik (1:F_1035-1055 dan 1:R_1041-1061) mampu memberikan hasil amplifikasi positif sebesar 84-88%. Namun, pada uji efektivitas terhadap 10 sekuen spesies tumbuhan langka tambahan dan 10 sekuen spesies non-langka, tidak diperoleh hasil amplifikasi positif. Hal ini menunjukkan bahwa efektivitas penanda gen *rpoC1* dalam mendeteksi tumbuhan langka melalui primer diagnostik secara *in silico* belum dapat digeneralisasi terhadap spesies di luar dataset awal, sehingga memerlukan pengembangan lebih lanjut.

Kata kunci: DNA *barcoding*, *in silico* PCR, konservasi tumbuhan, primer *rpoC1*, tumbuhan langka.

ABSTRACT

This study aims to develop diagnostic primers based on the chloroplast rpoC1 gene in silico for the identification of rare plant species. The methods used include retrieval of DNA sequence data from GenBank, multiple sequence alignment using ClustalX, sequence consensus generation with BioEdit, and primer design using FastPCR software. A total of 182 primer candidates were generated, then selected into 17 best primers based on the criteria of length, GC content, and melting temperature (Tm). In silico PCR tests on 25 rare plant species sequences used in the design process showed that one of the best primer pairs (1:F_1035-1055 and 1:R_1041-1061) was able to provide positive amplification results of 84-88%. However, in the effectiveness test against 10 additional rare plant species sequences and 10 non-rare species sequences, no positive amplification results were obtained. This suggests that the effectiveness of the rpoC1 gene marker in detecting rare plants through in silico diagnostic primers cannot be generalized to species outside the initial dataset, thus requiring further development.

Keywords: *DNA barcoding, in silico PCR, rare plants, rpoC1 primer, plant conservation.*

DAFTAR ISI

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DAFTAR PUSTAKA

Afgan, E., Baker, D., Batut, B., van den Beek, M., Bouvier, D., Čech, M., Chilton, J., Clements, D., Coraor, N., Grüning, B. A., Guerler, A., Hillman-Jackson, J., Hiltemann, S., Jalili, V., Rasche, H., Soranzo, N., Goecks, J., Taylor, J., Nekrutenko, A., & Blankenberg, D. (2018). The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. *Nucleic Acids Research*, *46*(W1), W537–W544. https://doi.org/10.1093/nar/gky379

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, *25*(17), 3389–3402. https://doi.org/10.1093/nar/25.17.3389

Angermueller, C., Pärnamaa, T., Parts, L., & Stegle, O. (2016). Deep learning for computational biology. *Molecular Systems Biology*, *12*(7), 878. https://doi.org/https://doi.org/10.15252/msb.20156651

Arief, A. (2001). *Hutan dan Kehutanan*. Yogyakarta: Kanisius.

Arvidsson, S., Kwasniewski, M., Riaño-Pachón, D. M., & Mueller-Roeber, B. (2008). QuantPrime – a flexible tool for reliable high-throughput primer design for quantitative PCR. *BMC Bioinformatics*, *9*(1), 465. https://doi.org/10.1186/1471-2105-9-465

Baker, D., & Sali, A. (2001). Protein Structure Prediction and Structural Genomics. *Science*, *294*(5540), 93–96. https://doi.org/10.1126/science.1065659

Bartlett, J. M. S., & Stirling, D. (2003). A short history of the polymerase chain reaction. *Methods in Molecular Biology (Clifton, N.J.)*, *226*, 3–6. https://doi.org/10.1385/1-59259-384-4:3

Bendich, A. J. (2004). Circular Chloroplast Chromosomes: The Grand Illusion. *The Plant Cell*, *16*(7), 1661–1666. https://doi.org/10.1105/tpc.160771

Benita, Y., Oosting, R. S., Lok, M. C., Wise, M. J., & Humphery-Smith, I. (2003). Regionalized GC content of template DNA as a predictor of PCR success. *Nucleic Acids Research*, *31*(16), e99–e99. https://doi.org/10.1093/nar/gng101

Benson, D. A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., & Sayers, E. W. (2013). GenBank. *Nucleic Acids Research*, *41*(D1), D36–D42. https://doi.org/10.1093/nar/gks1195

Bikandi, J., Millán, R. S., Rementeria, A., & Garaizar, J. (2004). In silico analysis of complete bacterial genomes: PCR, AFLP–PCR and endonuclease restriction. *Bioinformatics*, *20*(5), 798–799. https://doi.org/10.1093/bioinformatics/btg491

Birky, C. W. (1995). Uniparental inheritance of mitochondrial and chloroplast genes: mechanisms and evolution. *Proceedings of the National Academy of Sciences*, *92*(25), 11331–11338. https://doi.org/10.1073/pnas.92.25.11331

Boutros, P. C., & Okey, A. B. (2005). Unsupervised pattern recognition: An introduction to the whys and wherefores of clustering microarray data. *Briefings in Bioinformatics*, *6*(4), 331–343. https://doi.org/10.1093/bib/6.4.331

Bray, C. M., & West, C. E. (2005). DNA repair mechanisms in plants: crucial sensors and effectors for the maintenance of genome integrity. *New Phytologist*, *168*(3), 511–528. https://doi.org/https://doi.org/10.1111/j.1469-8137.2005.01548.x

Bridson, D., & Forman, L. (2010). *The Herbarium Handbook. Royal Botanic Gardens, Kew*.

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L., Vandesompele, J., & Wittwer, C. T. (2009). The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. *Clinical Chemistry*, *55*(4), 611–622. https://doi.org/10.1373/clinchem.2008.112797

Bustin, S. A., Mueller, R., & Nolan, T. (2020). Parameters for Successful PCR Primer Design. In R. Biassoni & A. Raso (Eds.), *Quantitative Real-Time PCR: Methods and Protocols* (pp. 5–22). Springer New York. https://doi.org/10.1007/978-1-4939-9833-3_2

Bzymek, M., Thayer, N. H., Oh, S. D., Kleckner, N., & Hunter, N. (2010). Double Holliday junctions are intermediates of DNA break repair. *Nature*, *464*(7290), 937–941. https://doi.org/10.1038/nature08868

Campbell, N. A., Reece, J. B., Urry, L. A., Cain, M. L., Wasserman, S. A., Minorsky, P. V, & Jackson, R. B. (2008). Biologi edisi kedelapan jilid 2. *Jakarta: Erlangga*.

Cao, Y., Wang, L., Xu, K., Kou, C., Zhang, Y., Wei, G., He, J., Wang, Y., & Zhao, L. (2005). Information theory-based algorithm for in silico prediction of PCR products with whole genomic sequences as templates. *BMC Bioinformatics*, *6*(1), 190. https://doi.org/10.1186/1471-2105-6-190

CBOL Plant Working Group, Hollingsworth, P. M., Forrest, L. L., Spouge, J. L., Hajibabaei, M., Ratnasingham, S., van der Bank, M., Chase, M. W., Cowan, R. S., Erickson, D. L., Fazekas, A. J., Graham, S. W., James, K. E., Kim, K.-J., Kress, W. J., Schneider, H., van AlphenStahl, J., Barrett, S. C. H., van den Berg, C., … Little, D. P. (2009). A DNA barcode for land plants. *Proceedings of the National Academy of Sciences*, *106*(31), 12794–12797. https://doi.org/10.1073/pnas.0905845106

Chase, M. W., Cowan, R. S., Hollingsworth, P. M., van den Berg, C., Madriñán, S., Petersen, G., Seberg, O., Jørgsensen, T., Cameron, K. M., Carine, M., Pedersen, N., Hedderson, T. A. J., Conrad, F., Salazar, G. A., Richardson, J. E., Hollingsworth, M. L., Barraclough, T. G., Kelly, L., & Wilkinson, M. (2007). A Proposal for a Standardised Protocol to Barcode All Land Plants. *Taxon*, *56*(2), 295–299. http://www.jstor.org/stable/25065788

Chase, M. W., Salamin, N., Wilkinson, M., Dunwell, J. M., Kesanakurthi, R. P., Haidar, N., & Savolainen, V. (2005). Land plants and DNA barcodes: short-term and long-term goals. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *360*(1462), 1889–1895. https://doi.org/10.1098/rstb.2005.1720

Chen, S., Yao, H., Han, J., Liu, C., Song, J., Shi, L., Zhu, Y., Ma, X., Gao, T., Pang, X., Luo, K., Li, Y., Li, X., Jia, X., Lin, Y., & Leon, C. (2010). Validation of the ITS2 Region as a Novel DNA Barcode for Identifying Medicinal Plant

Species. *PLOS ONE*, *5*(1), e8613-. https://doi.org/10.1371/journal.pone.0008613

Chen, S.-L., Yu, H., Luo, H.-M., Wu, Q., Li, C.-F., & Steinmetz, A. (2016). Conservation and sustainable use of medicinal plants: problems, progress, and prospects. *Chinese Medicine*, *11*(1), 37. https://doi.org/10.1186/s13020-016-0108-7

Chen, W., Feng, P., Yang, H., Ding, H., Lin, H., & Chou, K.-C. (2017). iRNA-AI: identifying the adenosine to inosine editing sites in RNA sequences. *Oncotarget*, *8*(3), 4208–4217. https://doi.org/10.18632/oncotarget.13758

China Plant BOL Group 1, Li, D.-Z., Gao, L.-M., Li, H.-T., Wang, H., Ge, X.-J., Liu, J.-Q., Chen, Z.-D., Zhou, S.-L., Chen, S.-L., Yang, J.-B., Fu, C.-X., Zeng, C.-X., Yan, H.-F., Zhu, Y.-J., Sun, Y.-S., Chen, S.-Y., Zhao, L., Wang, K., … Duan, G.-W. (2011). Comparative analysis of a large dataset indicates that internal transcribed spacer (ITS) should be incorporated into the core barcode for seed plants. *Proceedings of the National Academy of Sciences*, *108*(49), 19641–19646. https://doi.org/10.1073/pnas.1104551108

Christenhusz, M. J. M., & Byng, J. W. (2016). The number of known plants species in the world and its annual increase. In *Phytotaxa* (Vol. 261, Issue 3, pp. 201–217). Magnolia Press. https://doi.org/10.11646/phytotaxa.261.3.1

Daniell, H., Lin, C. S., Yu, M., & Chang, W. J. (2016). Chloroplast genomes: Diversity, evolution, and applications in genetic engineering. *Genome Biology*, *17*(1), 1–29. https://doi.org/10.1186/s13059-016-1004-2

de Vere, N., Rich, T. C. G., Ford, C. R., Trinder, S. A., Long, C., Moore, C. W., Satterthwaite, D., Davies, H., Allainguillaume, J., Ronca, S., Tatarinova, T., Garbett, H., Walker, K., & Wilkinson, M. J. (2012). DNA Barcoding the Native Flowering Plants and Conifers of Wales. *PLOS ONE*, *7*(6), e37945-. https://doi.org/10.1371/journal.pone.0037945

Dieffenbach, C. W., Lowe, T. M., & Dveksler, G. S. (1993). General concepts for PCR primer design. *PCR Methods and Applications*, *3*(3), S30-7. https://doi.org/10.1101/gr.3.3.s30

Dodsworth, S. (2015). Genome skimming for next-generation biodiversity analysis. *Trends in Plant Science*. https://doi.org/10.1016/j.tplants.2015.06.012

Dong, W., Liu, J., Yu, J., Wang, L., & Zhou, S. (2012). Highly Variable Chloroplast Markers for Evaluating Plant Phylogeny at Low Taxonomic Levels and for DNA Barcoding. *PLOS ONE*, *7*(4), e35071-. https://doi.org/10.1371/journal.pone.0035071

Drouin, G., Daoud, H., & Xia, J. (2008). Relative rates of synonymous substitutions in the mitochondrial, chloroplast and nuclear genomes of seed plants. *Molecular Phylogenetics and Evolution*, *49*(3), 827–831. https://doi.org/10.1016/j.ympev.2008.09.009

Dunham, I., Kundaje, A., Aldred, S. F., Collins, P. J., Davis, C. A., Doyle, F., Epstein, C. B., Frietze, S., Harrow, J., Kaul, R., Khatun, J., Lajoie, B. R., Landt, S. G., Lee, B.-K., Pauli, F., Rosenbloom, K. R., Sabo, P., Safi, A., Sanyal, A., … HudsonAlpha Institute UC Irvine Stanford group (data production and analysis), C. (2012). An integrated encyclopedia of DNA elements in the human genome. *Nature*, *489*(7414), 57–74. https://doi.org/10.1038/nature11247

Engelmann, F. (2011). Use of biotechnologies for the conservation of plant biodiversity. *In Vitro Cellular & Developmental Biology - Plant*, *47*(1), 5–16. https://doi.org/10.1007/s11627-010-9327-2

FAO, & UNEP. (2020). The State of the World's Forests 2020. In *The State of the World's Forests 2020*. FAO and UNEP. https://doi.org/10.4060/ca8642en

Fernald, G. H., Capriotti, E., Daneshjou, R., Karczewski, K. J., & Altman, R. B. (2011). Bioinformatics challenges for personalized medicine. *Bioinformatics*, *27*(13), 1741–1748. https://doi.org/10.1093/bioinformatics/btr295

Ford, C. S., Ayres, K. L., Toomey, N., Haider, N., Van Alphen Stahl, J., Kelly, L. J., Wikström, N., Hollingsworth, P. M., Duff, R. J., Hoot, S. B., Cowan, R. S., Chase, M. W., & Wilkinson, M. J. (2009). Selection of candidate coding DNA barcoding regions for use on land plants. *Botanical Journal of the Linnean Society. Linnean Society of London*, *159*(1), 1–11. https://doi.org/10.1111/j.1095-8339.2008.00938.x

Frankham, R., Ballou, J. D., & Briscoe, D. A. (2010). *Introduction to conservation genetics*. CUP.

Frankham, R., Bradshaw, C. J. A., & Brook, B. W. (2014). Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. *Biological Conservation*, *170*, 56–63. https://doi.org/https://doi.org/10.1016/j.biocon.2013.12.036

Furukawa, T., Curtis, M. J., Tominey, C. M., Duong, Y. H., Wilcox, B. W. L., Aggoune, D., Hays, J. B., & Britt, A. B. (2010). A shared DNA-damage-response pathway for induction of stem-cell death by UVB and by gamma irradiation. *DNA Repair*, *9*(9), 940–948. https://doi.org/10.1016/j.dnarep.2010.06.006

Galimberti, A., De Mattia, F., Losa, A., Bruni, I., Federici, S., Casiraghi, M., Martellos, S., & Labra, M. (2013). DNA barcoding as a new tool for food traceability. *Food Research International*, *50*(1), 55–63. https://doi.org/https://doi.org/10.1016/j.foodres.2012.09.036

Gehlenborg, N., O'Donoghue, S. I., Baliga, N. S., Goesmann, A., Hibbs, M. A., Kitano, H., Kohlbacher, O., Neuweger, H., Schneider, R., Tenenbaum, D., & Gavin, A.-C. (2010). Visualization of omics data for systems biology. *Nature Methods*, *7*(3), S56–S68. https://doi.org/10.1038/nmeth.1436

Goecks, J., Nekrutenko, A., Taylor, J., Afgan, E., Ananda, G., Baker, D., Blankenberg, D., Chakrabarty, R., Coraor, N., Von Kuster, G., Lazarus, R., Li, K., Taylor, J., & Vincent, K. (2010). Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. *Genome Biology*, *11*(8). https://doi.org/10.1186/gb-2010-11-8-r86

Gualberto, J. M., & Newton, K. J. (2017). Plant Mitochondrial Genomes: Dynamics and Mechanisms of Mutation. *Annual Review of Plant Biology*, *68*(Volume 68, 2017), 225–252. https://doi.org/10.1146/annurev-arplant-043015-112232

Hadrys, H., Balick, M., & Schierwater, B. (1992). Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. In *Molecular Ecology* (Vol. 1, Issue 1, pp. 55–63). https://doi.org/10.1111/j.1365-294X.1992.tb00155.x

Hajibabaei, M., Singer, G. A. C., Hebert, P. D. N., & Hickey, D. A. (2007). DNA barcoding: how it complements taxonomy, molecular phylogenetics and

population genetics. *Trends in Genetics*, *23*(4), 167–172. https://doi.org/10.1016/j.tig.2007.02.001

Hall, T. A. (1999). BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 95–98.

Handoyo, D., & Rudiretna, A. (2000). POLYMERASE CHAIN REACTION (PCR) [General Principles and Implementation of Polymerase Chain Reaction]. In *Unitas* (Vol. 9, Issue 1).

Hardison, R. C. (2003). Comparative genomics. *PLoS Biology*, *1*(2), e58.

Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1512), 313–321. https://doi.org/10.1098/rspb.2002.2218

Henegariu, O., N.A., H., S.R., D., G.H., V., & and Vogt, P. H. (1997). Multiplex PCR: Critical Parameters and Step-by-Step Protocol. *BioTechniques*, *23*(3), 504–511. https://doi.org/10.2144/97233rr01

Henriques, A., Cereija, T., Machado, A., & Cerca, N. (2012). In silico vs in vitro analysis of primer specificity for the detection of Gardnerella vaginalis, Atopobium vaginae and Lactobacillus spp. *BMC Research Notes*, *5*(1), 637. https://doi.org/10.1186/1756-0500-5-637

Hoffmann, A. A., & Sgrò, C. M. (2011). Climate change and evolutionary adaptation. *Nature*, *470*(7335), 479–485. https://doi.org/10.1038/nature09670

Hollingsworth, P. M. (2011). Refining the DNA barcode for land plants. *Proceedings of the National Academy of Sciences*, *108*(49), 19451–19452. https://doi.org/10.1073/pnas.1116812108

Hollingsworth, P. M., Graham, S. W., & Little, D. P. (2011). Choosing and Using a Plant DNA Barcode. *PLOS ONE*, *6*(5), e19254-. https://doi.org/10.1371/journal.pone.0019254

Hollingsworth, P. M., Li, D.-Z., van der Bank, M., & Twyford, A. D. (2016). Telling plant species apart with DNA: from barcodes to genomes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1702), 20150338. https://doi.org/10.1098/rstb.2015.0338

Indrawan, M., Primack, R. B., & Supriatna, J. (2007). *Biologi Konservasi: Edisi Revisi*. Yayasan Pustaka Obor Indonesia.

Innis, M. A., Gelfand, D. H., Sninsky, J. J., & White, T. J. (1990). Optimization of PCRs. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR Protocols: A Guide to Methods and Applications* (pp. 3–12). Academic Press.

IUCN. (2024). *The IUCN Red List of Threatened Species*. https://www.google.com/url?sa=t&source=web&rct=j&opi=89978449&url=https://www.iucnredlist.org/&ved=2ahUKEwjIkOS9lfqIAxWkzjgGHVt_ElcQFnoECCIQAQ&usg=AOvVaw3NU2Qu5n3T_b9qB486nLdk

IUCN Standards and Petitions Committee. (2024). *Guidelines for Using the IUCN Red List Categories and Criteria.* John Wiley & Sons, Ltd. https://doi.org/https://doi.org/10.1111/cobi.14227

Jansen, R. K., & Ruhlman, T. A. (2012). Plastid Genomes of Seed Plants. In R. Bock & V. Knoop (Eds.), *Genomics of Chloroplasts and Mitochondria* (pp. 103–126). Springer Netherlands. https://doi.org/10.1007/978-94-007-2920-9_5

Ji, Y., Ashton, L., Pedley, S. M., Edwards, D. P., Tang, Y., Nakamura, A., Kitching, R., Dolman, P. M., Woodcock, P., Edwards, F. A., Larsen, T. H., Hsu, W. W., Benedick, S., Hamer, K. C., Wilcove, D. S., Bruce, C., Wang, X., Levi, T., Lott, M., … Yu, D. W. (2013). Reliable, verifiable and efficient monitoring of biodiversity via metabarcoding. *Ecology Letters*, *16*(10), 1245–1257. https://doi.org/https://doi.org/10.1111/ele.12162

Kaeppler, S. M., Kaeppler, H. F., & Rhee, Y. (2000). Epigenetic aspects of somaclonal variation in plants. *Plant Molecular Biology*, *43*(2), 179–188. https://doi.org/10.1023/A:1006423110134

Kalendar, R., Khassenov, B., Ramankulov, Y., Samuilova, O., & Ivanov, K. I. (2017). FastPCR: An in silico tool for fast primer and probe design and advanced sequence analysis. *Genomics*, *109*(3), 312–319. https://doi.org/https://doi.org/10.1016/j.ygeno.2017.05.005

Kalendar, R., Lee, D., & Schulman, A. H. (2011). Java web tools for PCR, in silico PCR, and oligonucleotide assembly and analysis. *Genomics*, *98*(2), 137–144. https://doi.org/https://doi.org/10.1016/j.ygeno.2011.04.009

Kalendar, R., Shevtsov, A., Otarbay, Z., & Ismailova, A. (2024). In silico PCR analysis: a comprehensive bioinformatics tool for enhancing nucleic acid amplification assays. *Frontiers in Bioinformatics*, *Volume 4-2024*. https://www.frontiersin.org/journals/bioinformatics/articles/10.3389/fbinf.2024.1464197

Kazama, Y., Hirano, T., Nishihara, K., Ohbu, S., Shirakawa, Y., & Abe, T. (2013). Effect of high-LET Fe-ion beam irradiation on mutation induction in *Arabidopsis thaliana*. *Genes & Genetic Systems*, *88*(3), 189–197. https://doi.org/10.1266/ggs.88.189

Kent, W. J., Sugnet, C. W., Furey, T. S., Roskin, K. M., Pringle, T. H., Zahler, A. M., & Haussler, D. (2002). The human genome browser at UCSC. *Genome Research*, *12*(6), 996–1006. https://doi.org/10.1101/gr.229102. Article published online before print in May 2002

Kim, J., Kim, M. S., Koh, A. Y., Xie, Y., & Zhan, X. (2016). FMAP: Functional Mapping and Analysis Pipeline for metagenomics and metatranscriptomics studies. *BMC Bioinformatics*, *17*(1), 420. https://doi.org/10.1186/s12859-016-1278-0

Kimura, S., Tahira, Y., Ishibashi, T., Mori, Y., Mori, T., Hashimoto, J., & Sakaguchi, K. (2004). DNA repair in higher plants; photoreactivation is the major DNA repair pathway in non-proliferating cells while excision repair (nucleotide excision repair and base excision repair) is active in proliferating cells. *Nucleic Acids Research*, *32*(9), 2760–2767. https://doi.org/10.1093/nar/gkh591

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glöckner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research*, *41*(1), e1–e1. https://doi.org/10.1093/nar/gks808

Kress, W. J., & Erickson, D. L. (2007). A Two-Locus Global DNA Barcode for Land Plants: The Coding rbcL Gene Complements the Non-Coding trnH-psbA Spacer Region. *PLOS ONE*, *2*(6), e508-. https://doi.org/10.1371/journal.pone.0000508

Kress, W. J., & Erickson, D. L. (2008). DNA barcodes: Genes, genomics, and bioinformatics. *Proceedings of the National Academy of Sciences*, *105*(8), 2761–2762. https://doi.org/10.1073/pnas.0800476105

Kress, W. J., García-Robledo, C., Uriarte, M., & Erickson, D. L. (2015). DNA barcodes for ecology, evolution, and conservation. *Trends in Ecology & Evolution*, *30*(1), 25–35. https://doi.org/10.1016/j.tree.2014.10.008

Kress, W. J., Wurdack, K. J., Zimmer, E. A., Weigt, L. A., & Janzen, D. H. (2005). Use of DNA barcodes to identify flowering plants. *Proceedings of the National Academy of Sciences*, *102*(23), 8369–8374. https://doi.org/10.1073/pnas.0503123102

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, *33*(7), 1870–1874. https://doi.org/10.1093/MOLBEV/MSW054

Kwok, S., Kellogg, D. E., McKinney, N., Spasic, D., Goda, L., Levenson, C., & Sninsky, J. J. (1990). Effects of primer-template mismatches on the polymerase chain reaction: Human immunodeficiency virus type 1 model studies. *Nucleic Acids Research*, *18*(4), 999–1005. https://doi.org/10.1093/nar/18.4.999

Lahaye, R., van der Bank, M., Bogarin, D., Warner, J., Pupulin, F., Gigot, G., Maurin, O., Duthoit, S., Barraclough, T. G., & Savolainen, V. (2008). DNA barcoding the floras of biodiversity hotspots. *Proceedings of the National Academy of Sciences*, *105*(8), 2923–2928. https://doi.org/10.1073/pnas.0709936105

Levinson, G., & Gutman, G. A. (1987). Slipped-strand mispairing: a major mechanism for DNA sequence evolution. *Molecular Biology and Evolution*, *4*(3), 203–221. https://doi.org/10.1093/oxfordjournals.molbev.a040442

Li, X., Yang, Y., Henry, R. J., Rossetto, M., Wang, Y., & Chen, S. (2015). Plant DNA barcoding: from gene to genome. *Biological Reviews*, *90*(1), 157–166. https://doi.org/https://doi.org/10.1111/brv.12104

Libbrecht, M. W., & Noble, W. S. (2015). Machine learning applications in genetics and genomics. *Nature Reviews Genetics*, *16*(6), 321–332. https://doi.org/10.1038/nrg3920

Linhart, C., & Shamir, R. (2005). The Degenerate Primer Design Problem: Theory and Applications. *Journal of Computational Biology*, *12*(4), 431–456. https://doi.org/10.1089/cmb.2005.12.431

Lisch, D. (2009). Epigenetic Regulation of Transposable Elements in Plants. *Annual Review of Plant Biology, 60*(Volume 60, 2009), 43–66. https://doi.org/10.1146/annurev.arplant.59.032607.092744

Logacheva, M. D., Penin, A. A., Samigullin, T. H., Vallejo-Roman, C. M., & Antonov, A. S. (2007). Phylogeny of flowering plants by the chloroplast genome sequences: in search of a "lucky gene." *Biochemistry (Moscow)*, *72*(12), 1324–1330. https://doi.org/10.1134/S0006297907120061

Luscombe, N. M., Greenbaum, D., & Gerstein, M. (2001). What is bioinformatics? A proposed definition and overview of the field. *Methods of Information in Medicine*, *40*(04), 346–358.

Mammadov, J., Aggarwal, R., Buyyarapu, R., & Kumpatla, S. (2012). SNP Markers and Their Impact on Plant Breeding. *International Journal of Plant Genomics*, *2012*(1), 728398. https://doi.org/https://doi.org/10.1155/2012/728398

Mardis, E. R. (2008). Next-Generation DNA Sequencing Methods. *Annual Review of Genomics and Human Genetics, 9*(Volume 9, 2008), 387–402. https://doi.org/10.1146/annurev.genom.9.081307.164359

Meijer, W. (1997). Rafflesiaceae. *Flora Malesiana-Series 1, Spermatophyta*, *13*(1), 1–42.

Meyer, C. P., & Paulay, G. (2005). DNA Barcoding: Error Rates Based on Comprehensive Sampling. *PLOS Biology*, *3*(12), e422-. https://doi.org/10.1371/journal.pbio.0030422

Miller, G. A. D., Suzuki, N., Ciftci-Yilmaz, S., & Mittler, R. O. N. (2010). Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant, Cell & Environment*, *33*(4), 453–467. https://doi.org/https://doi.org/10.1111/j.1365-3040.2009.02041.x

Mitsuhashi, M. (1996). Technical report: Part 2. Basic requirements for designing optimal PCR primers. *Journal of Clinical Laboratory Analysis*, *10*(5), 285–

293. https://doi.org/10.1002/(SICI)1098-2825(1996)10:5<285::AID-JCLA9>3.0.CO;2-7

Mittler, R. (2017). ROS Are Good. *Trends in Plant Science*, *22*(1), 11–19. https://doi.org/10.1016/j.tplants.2016.08.002

Nei, M., & Kumar, S. (2000). *Molecular Evolution and Phylogenetics*. Oxford University Press.

Newmaster, S. G., Fazekas, A. J., & Ragupathy, S. (2006). DNA barcoding in land plants: evaluation of rbcL in a multigene tiered approach. *Canadian Journal of Botany*, *84*(3), 335–341. https://doi.org/10.1139/b06-047

Nic Lughadha, E., Bachman, S. P., Leão, T. C. C., Forest, F., Halley, J. M., Moat, J., Acedo, C., Bacon, K. L., Brewer, R. F. A., Gâteblé, G., Gonçalves, S. C., Govaerts, R., Hollingsworth, P. M., Krisai-Greilhuber, I., de Lirio, E. J., Moore, P. G. P., Negrão, R., Onana, J. M., Rajaovelona, L. R., … Walker, B. E. (2020). Extinction risk and threats to plants and fungi. *PLANTS, PEOPLE, PLANET*, *2*(5), 389–408. https://doi.org/https://doi.org/10.1002/ppp3.10146

Nugent, C. M., Elliott, T. A., Ratnasingham, S., & Adamowicz, S. J. (2020). coil: an R package for cytochrome c oxidase I (COI) DNA barcode data cleaning, translation, and error evaluation. *Genome*, *63*(6), 291–305. https://doi.org/10.1139/gen-2019-0206

Obradovic, J., Jurisic, V., Tosic, N., Mrdjanovic, J., Perin, B., Pavlovic, S., & Djordjevic, N. (2013). Optimization of PCR conditions for amplification of GC-Rich EGFR promoter sequence. *Journal of Clinical Laboratory Analysis*, *27*(6), 487–493. https://doi.org/10.1002/jcla.21632

Owczarzy, R., Tataurov, A. V, Wu, Y., Manthey, J. A., McQuisten, K. A., Almabrazi, H. G., Pedersen, K. F., Lin, Y., Garretson, J., McEntaggart, N. O., Sailor, C. A., Dawson, R. B., & Peek, A. S. (2008). IDT SciTools: a suite for analysis and design of nucleic acid oligomers. *Nucleic Acids Research*, *36*(suppl_2), W163–W169. https://doi.org/10.1093/nar/gkn198

Panjkovich, A., & Melo, F. (2005). Comparison of different melting temperature calculation methods for short DNA sequences. *Bioinformatics*, *21*(6), 711–722. https://doi.org/10.1093/bioinformatics/bti066

Parks, M., Cronn, R., & Liston, A. (2009). Increasing phylogenetic resolution at low taxonomic levels using massively parallel sequencing of chloroplast genomes. *BMC Biology*, *7*(1), 84. https://doi.org/10.1186/1741-7007-7-84

Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT–PCR. *Nucleic Acids Research*, *29*(9), e45–e45. https://doi.org/10.1093/nar/29.9.e45

Phillips, J. D., Gillis, D. J., & Hanner, R. H. (2022). Lack of Statistical Rigor in DNA Barcoding Likely Invalidates the Presence of a True Species' Barcode Gap. *Frontiers in Ecology and Evolution*, *Volume 10-2022*. https://www.frontiersin.org/journals/ecology-and-evolution/articles/10.3389/fevo.2022.859099

Plant, C. B. O. L. (2009). Group W. A DNA barcode for land plants. *Proc Natl Acad Sci US A*, *106*(12794), 7.

Pomerantz, A., Peñafiel, N., Arteaga, A., Bustamante, L., Pichardo, F., Coloma, L. A., Barrio-Amorós, C. L., Salazar-Valenzuela, D., & Prost, S. (2018). Real-time DNA barcoding in a rainforest using nanopore sequencing: opportunities for rapid biodiversity assessments and local capacity building. *GigaScience*, *7*(4), giy033. https://doi.org/10.1093/gigascience/giy033

Prance, G. T. (1997). The conservation of botanical diversity. In N. Maxted, B. V Ford-Lloyd, & J. G. Hawkes (Eds.), *Plant Genetic Conservation: The in situ approach* (pp. 3–14). Springer Netherlands. https://doi.org/10.1007/978-94-009-1437-7_1

Primack, R. B. (2006). *Essentials of conservation biology* (Vol. 23). Sinauer Associates Sunderland.

Primack, R. B., Indrawan, M., & Supriatna, J. (2012). *Biologi Konservasi*. Jakarta : Yayasan Pustaka Obor Indonesia.

Rachlin, J., Ding, C., Cantor, C., & Kasif, S. (2005). Computational tradeoffs in multiplex PCR assay design for SNP genotyping. *BMC Genomics*, *6*(1), 102. https://doi.org/10.1186/1471-2164-6-102

Ratnasingham, S., & Hebert, P. D. N. (2007). bold: The Barcode of Life Data System (http://www.barcodinglife.org). *Molecular Ecology Notes*, *7*(3), 355–364. https://doi.org/https://doi.org/10.1111/j.1471-8286.2007.01678.x

Ririe, K. M., Rasmussen, R. P., & Wittwer, C. T. (1997). Product Differentiation by Analysis of DNA Melting Curves during the Polymerase Chain Reaction. *Analytical Biochemistry*, *245*(2), 154–160. https://doi.org/https://doi.org/10.1006/abio.1996.9916

Rodríguez, A., Rodríguez, M., Córdoba, J. J., & Andrade, M. J. (2015). Design of primers and probes for quantitative real-time PCR methods. *Methods in Molecular Biology (Clifton, N.J.)*, *1275*, 31–56. https://doi.org/10.1007/978-1-4939-2365-6_3

Rotmistrovsky, K., Jang, W., & Schuler, G. D. (2004). A web server for performing electronic PCR. *Nucleic Acids Research*, *32*(suppl_2), W108–W112. https://doi.org/10.1093/nar/gkh450

Ruiz-Villalba, A., van Pelt-Verkuil, E., Gunst, Q. D., Ruijter, J. M., & van den Hoff, M. J. B. (2017). Amplification of nonspecific products in quantitative polymerase chain reactions (qPCR). *Biomolecular Detection and Quantification*, *14*, 7–18. https://doi.org/https://doi.org/10.1016/j.bdq.2017.10.001

Rychlik, W. (1995). Selection of primers for polymerase chain reaction. *Molecular Biotechnology*, *3*(2), 129–134. https://doi.org/10.1007/BF02789108

Rychlik, W., Spencer, W. J., & Rhoads, R. E. (1990). Optimization of the annealing temperature for DNA amplification in vitro ; *Nucleic Acids Research*, *18*(21), 6409–6412. https://doi.org/10.1093/nar/18.21.6409

Sa, B. (2009). The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry*, *55*, 4611–4622.

Sachse, K. (2004). Specificity and performance of PCR detection assays for microbial pathogens. *Molecular Biotechnology*, *26*(1), 61–80. https://doi.org/10.1385/MB:26:1:61

Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B., & Erlich, H. A. (1988). Primer-Directed Enzymatic Amplification of DNA with a Thermostable DNA Polymerase. *Science*, *239*(4839), 487–491. https://doi.org/10.1126/science.2448875

SantaLucia, J. (1998). A unified view of polymer, dumbbell, and oligonucleotide DNA nearest-neighbor thermodynamics. *Proceedings of the National Academy of Sciences*, *95*(4), 1460–1465. https://doi.org/10.1073/pnas.95.4.1460

Särkinen, T., Pennington, R. T., Lavin, M., Simon, M. F., & Hughes, C. E. (2012). Evolutionary islands in the Andes: persistence and isolation explain high endemism in Andean dry tropical forests. *Journal of Biogeography*, *39*(5), 884–900. https://doi.org/10.1111/j.1365-2699.2011.02644.x

Schuler, G. D. (n.d.). *Sequence Mapping by Electronic PCR*.

Shaw, J., Shafer, H., Leonard, R., Kovach, M., Schorr, M., & Morris, A. (2014). Chloroplast DNA sequence utility for the lowest phylogenetic and phylogeographic inferences in angiosperms: The tortoise and the hare IV. *American Journal of Botany*, *101*, 1987–2004. https://doi.org/10.3732/ajb.1400398

Sodhi, N. S., Koh, L. P., Brook, B. W., & Ng, P. K. L. (2004). Southeast Asian biodiversity: an impending disaster. *Trends in Ecology & Evolution*, *19*(12), 654–660. https://doi.org/10.1016/j.tree.2004.09.006

Sodhi, N. S., Posa, M. R. C., Lee, T. M., Bickford, D., Koh, L. P., & Brook, B. W. (2010). The state and conservation of Southeast Asian biodiversity. *Biodiversity and Conservation*, *19*(2), 317–328. https://doi.org/10.1007/s10531-009-9607-5

Spielman, D., Brook, B. W., & Frankham, R. (2004). Most species are not driven to extinction before genetic factors impact them. *Proceedings of the National Academy of Sciences*, *101*(42), 15261–15264. https://doi.org/10.1073/pnas.0403809101

Sucher, N. J., & Carles, M. C. (2008). Genome-based approaches to the authentication of medicinal plants. *Planta Medica*, *74*(6), 603–623. https://doi.org/10.1055/s-2008-1074517

Sugiyono. (2005). *Metode Penelitian Kuantitatif Kualitatif*. Alfabeta. http://www.pendidikanekonomi.com/2015/03/pengertian-fungsi-dan-tujuan-

Sun, J., Liu, B., Rustiami, H., Xiao, H., Shen, X., & Ma, K. (2024). Mapping Asia Plants: Plant Diversity and a Checklist of Vascular Plants in Indonesia. *Plants (Basel, Switzerland)*, *13*(16). https://doi.org/10.3390/plants13162281

Sutarno, S., & Setyawan, A. D. (2015). Indonesia's biodiversity: the loss and management efforts to ensure the sovereignty of the nation. *Prosiding Seminar Nasional Masyarakat Biodiversitas Indonesia*, *1*, 1–13.

TA, H. (1999). *BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser* (Vol. 41, p. 95). https://cir.nii.ac.jp/crid/1370285712524628736.bib?lang=en

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., & Willerslev, E. (2012). Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology*, *21*(8), 2045–2050. https://doi.org/https://doi.org/10.1111/j.1365-294X.2012.05470.x

Thomas, C. D., Cameron, A., Green, R. E., Bakkenes, M., Beaumont, L. J., Collingham, Y. C., Erasmus, B. F. N., De Siqueira, M. F., Grainger, A., & Hannah, L. (2004). Extinction risk from climate change. *Nature*, *427*(6970), 145–148.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., & Higgins, D. G. (1997). The CLUSTAL_X Windows Interface: Flexible Strategies for Multiple Sequence Alignment Aided by Quality Analysis Tools. *Nucleic Acids Research*, *25*(24), 4876–4882. https://doi.org/10.1093/nar/25.24.4876

Thomsen, P. F., & Willerslev, E. (2015). Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity. *Biological Conservation*, *183*, 4–18. https://doi.org/https://doi.org/10.1016/j.biocon.2014.11.019

Thornton, B., & Basu, C. (2011). Real-time PCR (qPCR) primer design using free online software. *Biochemistry and Molecular Biology Education : A Bimonthly Publication of the International Union of Biochemistry and Molecular Biology*, *39*(2), 145–154. https://doi.org/10.1002/bmb.20461

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., & Rozen, S. G. (2012). Primer3—new capabilities and interfaces. *Nucleic Acids Research*, *40*(15), e115–e115. https://doi.org/10.1093/nar/gks596

Urban, M. C. (2015). Accelerating extinction risk from climate change. *Science*, *348*(6234), 571–573. https://doi.org/10.1126/science.aaa4984

Urban, M. C., Bocedi, G., Hendry, A. P., Mihoub, J.-B., Pe'er, G., Singer, A., Bridle, J. R., Crozier, L. G., De Meester, L., Godsoe, W., Gonzalez, A., Hellmann, J. J., Holt, R. D., Huth, A., Johst, K., Krug, C. B., Leadley, P. W., Palmer, S. C. F., Pantel, J. H., … Travis, J. M. J. (2016). Improving the forecast for biodiversity under climate change. *Science*, *353*(6304), aad8466. https://doi.org/10.1126/science.aad8466

van Steenis, C., Hamzah, A., Toha, M., & Kartawinata, J. A. (2006). *Flora pegunungan Jawa: dilengkapi 57 gambar berwarna menampilkan 456 spesies tumbuhan berbunga asli pegunungan Jawa, yang dilukis dalam ukuran sebenarnya berdasarkan spesimen hidup*. Pusat Penelitian Biologi, Lembaga Ilmu Pengetahuan Indonesia (LIPI).

Varshney, R. K., Nayak, S. N., May, G. D., & Jackson, S. A. (2009). Next-generation sequencing technologies and their implications for crop genetics and breeding. *Trends in Biotechnology*, *27*(9), 522–530. https://doi.org/10.1016/j.tibtech.2009.05.006

Vijayan, K., & Tsou, C. H. (2010). DNA barcoding in plants: Taxonomy in a new perspective. *Current Science*, *99*, 1530–1541.

Walker, S. E., & Lorsch, J. (2013). Chapter Nineteen - RNA Purification – Precipitation Methods. In J. Lorsch (Ed.), *Methods in Enzymology* (Vol. 530, pp. 337–343). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-420037-1.00019-1

Wang, Z., Gerstein, M., & Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. *Nature Reviews Genetics*, *10*(1), 57–63. https://doi.org/10.1038/nrg2484

Waterworth, W. M., Drury, G. E., Bray, C. M., & West, C. E. (2011). Repairing breaks in the plant genome: the importance of keeping it together. *New Phytologist*, *192*(4), 805–822. https://doi.org/https://doi.org/10.1111/j.1469-8137.2011.03926.x

Weitschek, E., Van Velzen, R., Felici, G., & Bertolazzi, P. (2013). BLOG 2.0: a software system for character-based species classification with DNA Barcode

sequences. What it does, how to use it. *Molecular Ecology Resources*, *13*(6), 1043–1046. https://doi.org/https://doi.org/10.1111/1755-0998.12073

White, B. (2005). Polymerase Chain Reaction (PCR): Design and Optimization of Reactions. In *Methods Mol. Biol.* (Vol. 226). https://doi.org/10.1038/npg.els.0005340

Whitten, T., Widyantoro, A., Soeriaatmadja, R. E., S.N., K., Utama, T. B., & Afiff, S. A. (1999). *Ekologi Jawa dan Bali*. Jakarta : Prenhallindo.

Wicke, S., Schneeweiss, G. M., dePamphilis, C. W., Müller, K. F., & Quandt, D. (2011). The evolution of the plastid chromosome in land plants: gene content, gene order, gene function. *Plant Molecular Biology*, *76*(3), 273–297. https://doi.org/10.1007/s11103-011-9762-4

Widjaja, E., Rahayuningsih, Y., Setijo Rahajoe, J., Ubaidillah, R., Maryanto, I., Walujo, E., & Semiadi, G. (2014). *Kekinian Keanekaragaman Hayati Indonesia 2014*.

Wiriadinata, H. (2010). *Keanekaragaman Tumbuhan Langka dan Endemik di Indonesia*. Jakarta: LIPI Press.

Wolfe, K. H., Li, W. H., & Sharp, P. M. (1987). Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. *Proceedings of the National Academy of Sciences*, *84*(24), 9054–9058. https://doi.org/10.1073/pnas.84.24.9054

Xiang, X.-G., Hu, H., Wang, W., & Jin, X.-H. (2011). DNA barcoding of the recently evolved genus Holcoglossum (Orchidaceae: Aeridinae): A test of DNA barcode candidates. *Molecular Ecology Resources*, *11*, 1012–1021. https://doi.org/10.1111/j.1755-0998.2011.03044.x

Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., & Madden, T. L. (2012). Primer-BLAST: A tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics*, *13*(1), 134. https://doi.org/10.1186/1471-2105-13-134

Zhang, H., Lang, Z., & Zhu, J.-K. (2018). Dynamics and function of DNA methylation in plants. *Nature Reviews Molecular Cell Biology*, *19*(8), 489–506. https://doi.org/10.1038/s41580-018-0016-z

Zhu, Y., Strassmann, J. E., & Queller, D. C. (2000). Insertions, substitutions, and the origin of microsatellites. *Genetical Research*, *76*(3), 227–236. https://doi.org/10.1017/s001667230000478x