Rainforest XPRIZE Species Identification Through DNA Barcoding

Overview:

This memo seeks to answer whether it is viable to DNA barcode up to hundreds of samples (insects and plants) in 48 hours. It will outline the general process of state-of-the-art portable species DNA barcoding using the nanopore sequencer and miniPCR. Research suggests that although it is certainly viable to barcode >100 samples in 48 hrs, cost may be a factor of consideration. Because nanopore sequencing is not yet a high-throughput method (meaning not able to sequence many samples at once), the ability to run processes in parallel will be crucial in expediting DNA barcoding.

DNA Barcoding Process Flow:

After obtaining a sample of interest, DNA is first extracted. DNA extraction is usually done through the addition of chemicals (Gupta, 2019). Depending on the method used, extraction could be done in <15 min (Zymo, 2021). After the DNA is extracted, the barcode is then copied and amplified through polymerase chain reaction (PCR). This step usually involves a thermocycler, so the miniPCR is used here for portability. The PCR cycle should take <40 min. Alternatively, rapid barcoding and novel methods could be implemented here to pool and tag multiple sequences together in order to improve efficiency (Oxford Nanopore, 2021). After amplification, the sample is taken for sequencing in the nanopore sequencer. Although the nanopore sequencing method is error prone, previous trials have shown that it is a viable method of documenting the genetic data of rare species in the field (Hayden, 2015; Pomerantz, 2018; DeSalle, 2019). Nanopore sequencing takes ~10 min (Oxford Nanopore, 2021). However, a rate limiting step occurs here as each nanopore sequencer can only read one sample at once. In addition, flow cells in the sequencer are replaced after ~5 runs (Oxford Nanopore, 2021). Therefore, cost could be another factor of consideration. After the sequence is obtained, the sample is documented and the DNA barcoding process is complete.

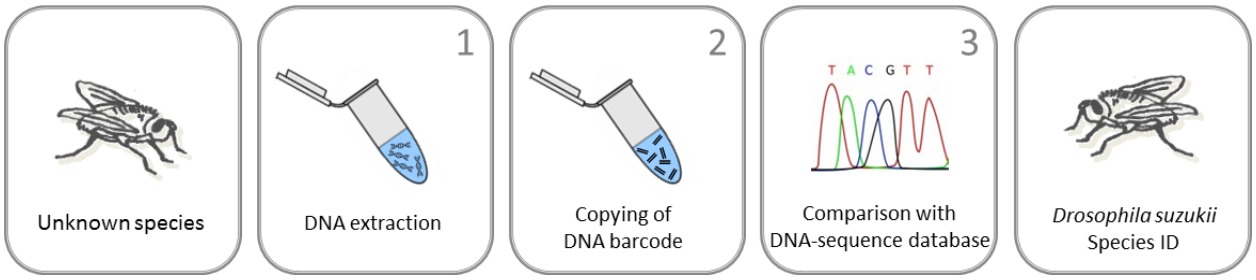


Figure 1: Workflow of DNA Barcoding (image taken from Sinsoma)

Discussion:

In short, it is certainly viable to barcode hundreds of samples in 48 hours. DNA extraction could be easily carried out in a short period of time using the right commercial reagents. The PCR step, although long, should also be relatively simple to carry out, especially as multiple samples can be run jointly in the miniPCR. The most tedious part of the barcoding project will be sequencing. Because the sequencer only takes in one sample at a time, large scale barcoding would be problematic as processing time and cost of flow cells would add up. For example, barcoding 100 samples would mean 100 runs of the sequencer. With two sequencers, the

number can be cut down to 50. However, with each additional sequencer, cost will also increase dramatically. Although this is certainly troubling, the inefficiencies also reveal that there is room for innovation. Workflows could be developed to improve the current method of portable DNA barcoding in the field. More specifically, targeting efficient methods of pooling and tagging samples seem to hold tremendous potential in increasing sequencing capabilities.

References:

Check Hayden E. Pint-sized DNA sequencer impresses first users. Nature. 2015 May 7;521(7550):15-6. doi: 10.1038/521015a. PMID: 25951262.

DeSalle, R. and P. Goldstein. “Review and Interpretation of Trends in DNA Barcoding.” Frontiers in Ecology and Evolution 7 (2019): n. pag.

Gupta, Nalini. “DNA Extraction and Polymerase Chain Reaction.” Journal of cytology vol. 36,2 (2019): 116-117. doi:10.4103/JOC.JOC_110_18

Oxford Nanopore Technologies.
https://nanoporetech.com/applications/dna-nanopore-sequencing

Oxford Nanopore Technologies.
https://store.nanoporetech.com/us/rapid-barcoding-kit.html

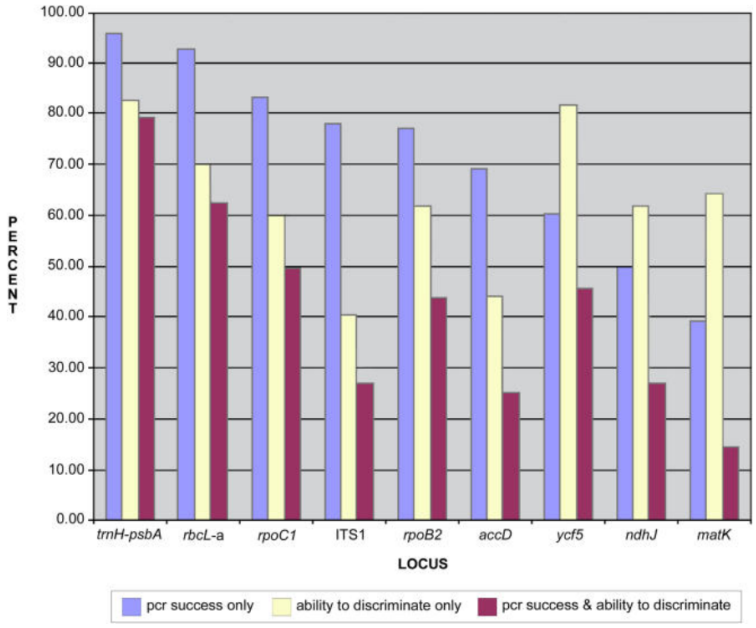
Pomerantz A, Peñafiel N, Arteaga A, Bustamante L, Pichardo F, Coloma LA, Barrio-Amorós CL, Salazar-Valenzuela D, Prost S. Real-time DNA barcoding in a rainforest using nanopore sequencing: opportunities for rapid biodiversity assessments and local capacity building. Gigascience. 2018 Apr 1;7(4):giy033. doi: 10.1093/gigascience/giy033. PMID: 29617771; PMCID: PMC5905381.

Appendices:

*Plant Papers:
Joe Parker, Andrew J. Helmstetter, Dion Devey, Tim Wilkinson, and Alexander S. T. Papadopulos. Field-based species identification of closely-related plants using real-time nanopore sequencing
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5566789/

Plant DNA Barcoding:
https://link-springer-com.proxy.lib.duke.edu/protocol/10.1007%2F978-1-4939-1966-6_8

Better Plant DNA Barcoding:
https://www-ncbi-nlm-nih-gov.proxy.lib.duke.edu/pmc/articles/PMC1876818/



Zymo Research.
"https://www.zymoresearch.com/collections/quick-dna-tissue-insect-kits/products/quick-dna-tissue-insect-miniprep-kit"