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A non-destructive DNA sampling technique for herbarium specimens

Lara Shepherd

Abstract

A non-destructive DNA extraction method suitable for robust herbarium specimens and potentially useful for specimens with high levels of secondary compounds.

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Guidelines

Perform DNA extractions and PCR set-ups in a dedicated ancient DNA laboratory.

Protocol

Step 1

Step 1.

Prepare a Staedtler "Mars Plastic" eraser by cutting it into 7mm² pieces with a sterile razor blade.

Step 2

Step 2.

For each herbarium specimen sampled use a new piece of eraser to rub across either the leaf surface or petiole. Collect the resulting eraser fragments (also called erdu) onto paper and place into a sterile 1.5 ml microcentrifuge tube. To avoid cross-contamination, use a new paper and a new piece of eraser for each herbarium specimen and change gloves between each sample.

Step 3

Step 3.

Perform a DNA extraction on the erdu using a DNeasy blood and tissue kit (Qiagen, Valencia, CA, USA). Incubate the eraser fragments for 3 hours in Buffer ATL and proteinase-K on a heating block set at 50° C. After incubation, follow the manufacturer's instructions except use a final elution of $35~\mu$ l of Buffer AE, which can be spun through the column twice (the first elution placed back on the column and spun through a second time). Negative extraction controls containing no eraser fragments, and/or controls with eraser fragments that haven't been used for sampling should be processed in parallel with the sample extractions to monitor for reagent contamination.

Warnings

This method should only be used for robust leaves or petioles/stipes of more delicate specimens because using the eraser can cause damege to delicate leaves. I recommend trying on expendable specimens first.