May 06, 2022

Pre-extraction sample processing for CALIBER project

DOI
dx.doi.org/10.17504/protocols.io.n92ldz7xov5b/v1

James JN Kitson[1]

[1]Newcastle University

Network Ecology Group

James JN Kitson
Newcastle University

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.n92ldz7xov5b/v1

Protocol Citation: James JN Kitson 2022. Pre-extraction sample processing for CALIBER project. **protocols.io https://dx.doi.org/10.17504/protocols.io.n92ldz7xov5b/v1**

Protocol status: Working
We use this protocol and it's working

Created: May 06, 2022

Last Modified: May 06, 2022

Protocol Integer ID: 62111

Keywords: samples during the caliber project, extraction sample processing, caliber project, collected sample, sample, extraction, further processing, document

Funders Acknowledgements:
BBSRC
Grant ID: BB/T010851/1

Abstract

This is a document that outlines the steps needed to prepare field-collected samples during the CALIBER project for archiving and further processing.

Materials

Plastics

- 2ml screwcap tubes
- 5ml fliptop tubes
- 15ml screwcap tubes

Tools

- Scalpel handle and disposible blades e.g. Swann Morton #23 blades
- fine forceps
- premade labels for plant grinding tubes
- premade labels for plant drying envelopes
- premade labels for insect storage tubes

Troubleshooting

Safety warnings

This procedure involves the use of scalpels and sharp entomological forceps, ensure you are familiar with the relevant risk assessment for dissection in your organisation.

Setup

1 Source steel beads (ball bearings) for tissue grinding (Tungsten beads are not usually necessary). We use hardened **carbon steel** or **stainless steel bearings from simplybearings.co.uk**. This protocol requires two beads per sample tube.

2 Beads are usually shipped coated in manufacturing oil (especially the carbon steel beads). To remove this, place beads in a borosilicate glass beaker or Duran bottle with the pouring lip and lid removed then bake for at least 12 hours at 250 ^{O}C.

Figure 1: Depending on baking time, carbon steel beads will change colour, this is normal.

3 Prepare 5 ml screwcap collection tubes (**e.g. Starlab #E1450-1100**) containing three 3 mm hardened steel beads in batches of 96 tubes.

Figure 2: Plant grinding tubes ready for plant material.

Insect sample processing

4 Pick out a frozen plant sample bag from the freezer and note the sample name.

Figure 3: Typical sample bags with sample names highlighted.

5 Using clean forceps, pick off all insects and place in a screwcap tube of the appropriate size (2ml/5ml/15ml) then fill the tube with a solution of 95% Ethanol and 5% Glycerol.

6 Label the tube with the corresponding tube label.

Figure 4: Insect tube labels.

Figure 5: Labelled tube with insects ready for storage.

7 Store at -20 ^{O}C until transported to SASA for qPCR analysis.

Figure 6: Box of insect tubes for longer term storage.

Archive plant sample processing

8 Write the sample name on a manilla sample drying envelope and find the corresponding dried sample label.

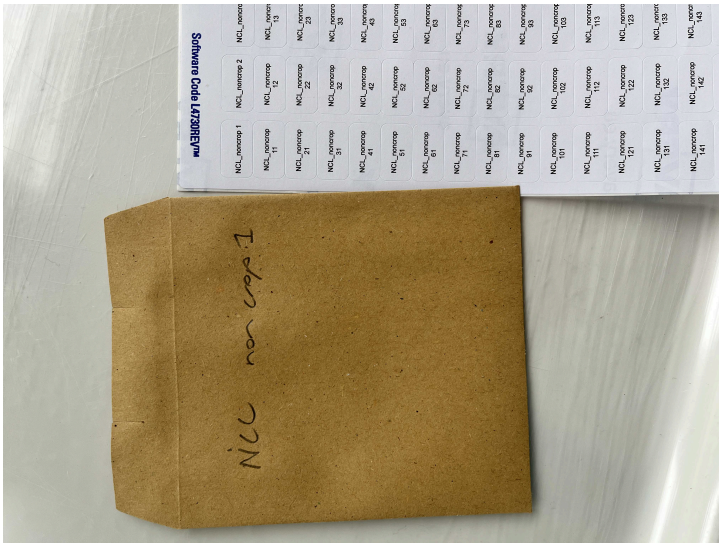

Figure 7: plant drying envelope and dried sample labels that are used to seal the flap.

9 Into this envelope, place approximately 2-5 g of leaf and chopped stem material.

10 Seal with the dried sample label and place in an oven overnight at 35-40 ^{o}C with a tray of silica gel in the bottom.

Figure 8: Plant sample drying over silica in the oven.

11 The archive sample is ready for long term storage at room temperature.

DNA extraction plant sample processing

12 Using a clean scalpel, slice and place ~ 50 mg of plant material into the pre-prepared collection tubes containing the hardened steel ball bearings (from step 3).

13 Label the tube with the corresponding tube label.

Figure 9: Plant grinding tube with label, sample and ball bearings ready for storage.

14 Store at -20 ^{O}C until ready to proceed with DNA extraction.

Cleanup and reset

15 The remaining plant material and packaging can be disposed of in a clinical waste bin.

16 Wash forceps and scalpel in 1x chemgene.

17 Rinse forceps and scalpel in molecular grade water.

18 Rinse forceps in 100% Isopropanol and leave to dry.

19 Wipe down dissection area with blue roll and 1x chemgene.

20 Proceed with the next sample.