Fungal DNA Workshop

Paul F. Hamlyn

(Note: this is an overview of the workshop and not intended as a protocol for carrying out DNA extraction and purification)

Brian Douglas who has been involved with the Lost and Found Fungi project at Kew kindly agreed to organise a practical fungal DNA workshop at the Risley Moss Visitor Centre for members of the NWFG in August 2019. Because it was a hands-on event numbers had to be limited for practical purposes. We were introduced to the techniques for the extraction and purification of fungal DNA to get it into a suitable form for amplification and subsequent examination using gel electrophoresis.

Although none of the reagents are noted to be toxic some may be an irritant and we were advised to use gloves at all times. As with any work involving DNA amplification extreme care is necessary to avoid unwanted contamination. Pipette tips need to be changed every time they are used for a new sample or if they touch anything outside the intended tube by accident. Tubes and tips should not be reused but disposed of carefully.

DNA extraction involves taking a very small amount of fungal tissue from a fresh or dried specimen and grinding the tissue in lysis buffer until broken up into small fragments using a sterile pestle. The tube containing the homogenised sample is then centrifuged leaving the cellular debris at the bottom of the tube and a clear solution at the top.

To purify the extracted DNA prior to amplification a dipstick made from filter paper is placed in the clear solution carefully avoiding the solid debris. Some of the DNA will stick to the filter paper. The dipstick is then placed in a tube containing a wash solution to remove impurities that could inhibit the DNA amplification process while leaving sufficient DNA attached to the filter paper. Finally the dipstick is placed in a tube containing the PCR mix where a small amount of purified DNA will be released into the solution. Only an extremely tiny amount of DNA is required.

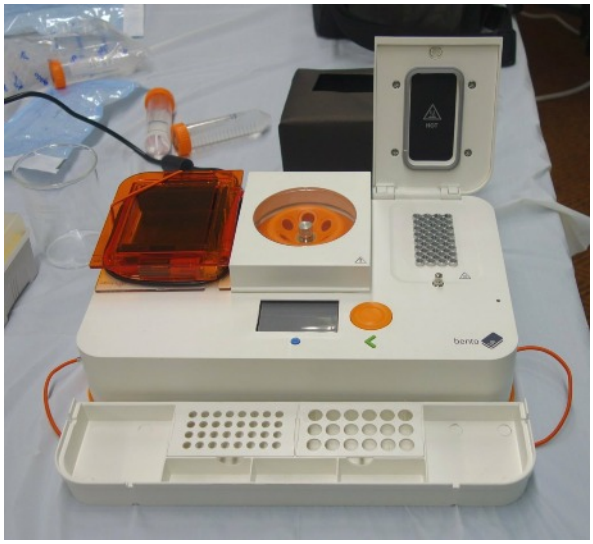
There was insufficient time to carry out DNA amplification using the thermocycler (this step is known as PCR or the Polymerase Chain Reaction) however samples prepared by members of the Group were taken away by Brian for subsequent processing. Brian also demonstrated preparing and running an agarose gel using the electrophoresis tank. Gel electrophoresis is used to visualise the DNA after amplification (i.e. the PCR products) to verify that the amplification has worked and the products are going to be suitable for sending away to be sequenced. I include a photograph of a gel that I prepared many years ago not using fungal DNA but it illustrates what to expect.

We finished with a brief discussion on where to send the amplified DNA extracts for sequencing, how to assess sequence quality and then search for similar

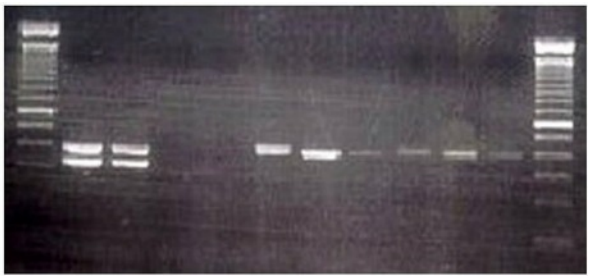
sequences in public databases. However, we could probably do with another workshop to cover these aspects in much more detail.

Members of the Group extracting DNA from a small piece of fungal tissue

Brian Douglas demonstrates the loading of an agarose gel prior to electrophoresis



Bento Lab a portable mini-lab (left) incorporating a thermocycler, centrifuge, gel electrophoresis tank and visualizer all in one package.



DNA amplification products visualised on an agarose gel (above)

The 600 base pair band has increased intensity to serve as a reference point. The PCR products are in the lanes in between and are about 400 base pairs in size. Double bands are generally indicative of contamination. The two blank lanes are controls not containing any extracted and purified DNA to check that the reagents have not become contaminated.

A.G.M.

Saturday 29 February 2020, 10.30 a.m., at the Risley Moss Visitor Centre