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Extract-N-Amp Equivalent DNA Extraction Protocol V.3

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

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Abstract

This extraction protocol uses an Extract-N-Amp equivalent solution to quickly and cheaply perform DNA extractions. This protocol has been widely used for dried specimens of macrofungi, but will work for other organismal groups as well. This protocol is an amalgamation of two different protocols that are currently in use by academic labs in the United States.

This protocol is easily scalable for larger batch sizes. The standard protocol assumes 100mL batches. This is enough extraction solution for 5,000 specimens. The primary thing needed to scale up are larger sizes of the sterile vacuum filter flasks. They come in each size you may need - 250mL, 500mL, or 1000mL.

For larger batch considerations:
100mL is enough for ~5,000 extractions
250mL is enough for ~12,500 extractions
500mL is enough for ~25,000 extractions
1000mL is enough for ~50,000 extractions

Materials

Extraction Solution (ES)
1 M Tris stock (pH 8.0-9.0) (**teknova**) 1000mL $66.00 (enough for 50,000 specimens)
KCl (**Amazon** [Lab Grade or Reagent Grade]) 1 lb $20.00
EDTA (**IBI Sci**) 1L $60.49
Molecular Water (**IBI Scientific**) 1L $45.00
1 M NaOH (**Amazon**) 1L $20.00

Equipment & Consumables
50mL Sterile Tubes (**Amazon**) - 50 tubes - $20.00
250mL, 500mL or 1000mL Vacuum Flasks - Sterile 0.22um PES Disposable (**Ebay**) - $50.00
Vacuum Pump (**Amazon**) - $160.00

Up front equipment costs - $440

~$586 for first 50,000 extractions. $0.0117/extraction

Ongoing costs are primarily the Tris stock and Vacuum flasks.

Create the Extraction Solution

1 In a flowhood or other clean environment, add 70 mL of molecular water. A sterile 50mL tube can be used for these measurements.

For larger batches:
175mL for 500mL of end product in a 250mL flask.
350mL for 500mL of end product in a 500mL flask.
700mL for 1000mL of end product in a 1000mL flask.

2 Add 10 mL of 1 M Tris stock pH 8.0-8.5 into a 100mL Erlenmeyer flask.

For larger batches:
25mL for 250mL of end product in a 250mL flask
50mL for 500mL of end product in a 500mL flask.
100mL for 1000mL of end product in a 1000mL flask.

3 Add 1.86 g of KCl. Swirl to dissolve.

For larger batches:
4.65g for 500mL of end product in a 250mL flask.
9.3g for 500mL of end product in a 500mL flask.
18.6g for 1000mL of end product in a 1000mL flask.

4 Add 2 mL of 0.5M EDTA.

For larger batches:
5mL for 500mL of end product in a 250mL flask.
10mL for 500mL of end product in a 500mL flask.
20mL for 1000mL of end product in a 1000mL flask.

5 Add 1 M NaOH starting with 10 mL and then adding 1 mL at a time until the pH is above 9. The goal is to push just past the buffering range of Tris and end up with a pH between 9 and 9.5. This can be measured with pH paper or a pH meter. You do not want to add too much NaOH.

For reference, it takes us 13 mL of NaOH to push past pH 9 making 500mL of end product.

6 Top up your final solution to 100, 500, or 1000mL total volume (depending on how much you are making), using molecular grade water.

7 Filter sterilize your solution with the vaccum flask and pump. Put your final solution into sterile 50mL tubes. Store in 50mL tubes at 4 °C for up to 1 year.

Optional: Use low TE (10 mM tris + 0.1mM EDTA) to dilute extracts.

8 Add 100 mL DNA grade 1 M Tris buffer pH 8.0-8.5 to a very clean 1 L vessel using a clean 50 mL tube to measure (you can use the one that you used previously for measuring tris).

9 Add 200 µL 0.5 M EDTA using a pipette with sterile, filtered tips.

10 If prepared outside of a laminar flow cabinet or if using non-sterile reagents, filter sterilize using a 0.22 micron vacuum filter. Put your final solution into sterile 50mL tubes.

Use this solution to dilute your raw DNA extracts. A standard dilution is 1:10, however experiment with less or more to optimize your protocol.

Extraction Procedure

11 Put a small amount of tissue into one cell of an 8-strip tube. Maintain a spreadsheet of which specimens are going into which tube.

12 Add 20uL of Extraction Solution (ES) into each tube.

13 Optional: Particularly for older or recalcitrant tissue, add a 1.5mm stainless steel ball bearing to each tube and then vortex them for about 5 minutes. We have a BioSpec Mini-BeadBeater 96 and/or a Qiagen Tissue Lyser III for this purpose. Spin down.

14 Incubate the tubes for 10 minutes at 95 °C.

15 Vortex the tubes for 00:00:05 and spin them down for 00:00:05. We typically do this on a PCR rack and vortex 6 or 12 strips at a time, and spin down two plates at a time.

16 Your extract is now ready to use 1uL of the final solution as the template directly into a PCR reaction. Store at -20 °C .