POLLEN PREPARATION PROCEDURE
FOR RADIOCARBON DATING

SAFETY: See associated COSSH form. Always wear PPE (lab coat, safety glasses and nitrile gloves). Inform safety officer of any spills.

10% HCl – mild burns, irritant
10% KOH – severe burns, highly flammable
3% NaOCl – mild burns, irritates respiratory system
Hydrofluoric acid (HF) – highly toxic, severe burns

First aid – run with water for ~20 minutes, seek medical help for eye incidents. For HF refer to HF safety info and COSHH form – rinse area with water and apply calcium gluconate gel until medical help arrives (DO NOT apply gel to eyes).

CHEMICALS REQUIRED:

10% HCl using Aristar quality HCl and diluting with Milli-Q water
10% KOH using Analar or Aristar KOH pellets and Milli-Q
Dilute bleach (sodium hypochlorite, NaOCl) to 3% with Milli-Q
Hydrofluoric acid (HF)

EQUIPMENT REQUIRED:

50ml centrifuge tubes
Fume cupboard
Centrifuge
150 µm mesh
Funnels
HF safety kit (gloves, apron, face shield)
Safety pack for HF
50 □m nylon mesh
Vacuum pump
Nail polish
50ml test tube racks
Water bath
Vortex /whirli mixer
Sieve holders
Teflon rods
Waste Waste bottle for HF
90 or 106□m mesh or sieves
20 □m nylon mesh
Glass viles with lids
Tissues

NOTES:

Rinse all test tubes, spatulas, sampling tubes, funnels, stirring rods, filter equipment and anything else (other than sieves) that will come into contact with the sediment with HCl followed by Milli-Q to remove any external sources of carbon.
Use aluminium foil to rest equipment on; do not use paper towels.
Subsample at least 3cm^3 of sediment, but generally do not use more than 2cm^3 per tube.
Do not add meths during this process as this introduces external sources of carbon.
This protocol is based on the Brown methodology (Brown et al., 1989), but adjusted to include an HCl rinse at the start of the prep. because I found that without this my sediments did not respond to the HF treatment.

PROCEDURE:

1. Place sample in 50ml centrifuge tube.

2. Add HCl to 2nd line of tube rack, place in 90°C hot bath for 30 minutes; centrifuge, decant and whirlimix.

3. Rinse with Milli-Q (up to 2x).

4. Set up 150□m sieves; get rid of material >150□m.

5. Add KOH to 2nd line of tube rack, place in 90°C hot bath for 20 minutes; after hot bath, dump contents of each tube into sieve; centrifuge, decant and whirlimix and repeat rinses with Milli-Q a couple of times. You want to get rid of humic acids but remember that with each rinse you will lose more pollen so do only as many rinses as necessary.

6. Rinse sediment with HCl to acidify; prepare for HF step.

7. Fill tubes with 10ml (up to first line of tube rack) of HF; place in 90°C hot bath for 60 minutes and stir (every 15 minutes) with Teflon rods.

8. When you remove the samples from the hot bath, use HCl (instead of meths) to rinse off the rods; otherwise centrifuge and decant as normal.

9. Add HCl to 2nd line of tube rack; place in 90°C hot bath for 60 minutes and stir (every 15 minutes) with Teflon rods.

10. Check sediments to make sure that most of the silica is removed.

11. Sieve using 90 or 106□m sieves; get rid of material >90 or 106□m.

12. Set up filter equipment:

13. Cut squares of 50 and 20 □m nylon mesh.

14. Set up filter and vacuum pump.

15. Add bleach to remaining sample and leave for 5 minutes; you will want to treat each sample individually since you have to clean the equipment prior to filtering each sample (which could extend the time a sample is in bleach, and destroy the pollen).

16. Sieve 50 □m: pour contents of tube over 50□m nylon mesh

17. Save the 50-90 (or 106) □m fraction; this needs to be washed with Milli-Q to remove the bleach.

18. Sieve the remaining (<50□m) material with the 20□m mesh. The Brown protocol says to re-bleach this fraction, but with my last sample I didn't find this to be necessary; decide what is best for your samples.

19. Save the 20-50□m material and wash with Milli-Q water.

20. Once both fractions (20-50 and 50-90/106 m) have been rinsed at least 2x, transfer the sample into small, glass tubes (the ones used for regular pollen preps).

21. You will likely have to centrifuge the tubes to get all of the sample into the small tubes – roll them up in tissue leaving a tail to enable their removal from the centrifuge bucket.

22. Add HCl to final sample so that it is about half HCl and half Milli-Q to prevent algal growth.

BROWN, T. A., NELSON, D. E., MATHEWES, R. W., VOGEL, J. S. & SOUTHON, J. R. (1989) Radiocarbon Dating of Pollen by Accelerator Mass-Spectrometry. *Quaternary Research,* 32**,** 205-212.