WERTH, S., REYNISDÓTTIR, S., GUÐMUNDSSON, H. & ANDRÉSSON, Ó. S. 2016. A fast and inexpensive high-throughput protocol for isolating high molecular weight genomic DNA from lichens. – Herzogia **29**: 610–616.

Supplementary Appendix S2: Long protocol, detailing several variants of the DNA extraction method.

Variants are given for a) grinding in 96-well plates, b) grinding in single tubes. All centrifugation steps at 15°C–25°C.

1. Weigh out 50 mg of an air-dried lichen thallus, or a thallus area of 0.5–2 cm^2 in *Peltigera membranacea* or *Lobaria pulmonaria*. Rip into several small pieces so it grinds easily. Put into a) a racked 1.2 ml collection microtube (Qiagen) or b) into a 2 ml screw-lid tube (Sarstedt, # 72.694). Add a 3 mm stainless steel bead.

2. Lyophilize overnight (with caps open), or freeze for 30 min at -80°C prior to grinding, or leave as is, if the sample is quite dry.

3. Close all tubes and grind samples using a mixer mill (models, see Appendix 4). This may require several rounds of shaking. The sample has to be ground into a fine, flour-like powder. When using the TissueLyzer II, it is best to grind at 30 rotations per minute for 1.5 minutes and then rotate the tubes and grind another 1.5 minutes, so that all samples get evenly ground. For optimal results, it is imperative that the sample is very finely ground.

   Troubleshooting: If the grinding does not work properly, freeze dry lichens in opened a) collection microtubes or b) single tubes e.g. in a lyophilisator, freeze the lichen for 30 minutes at -80°C, or freeze the lichen by adding very carefully a small volume of liquid N and wait for the N to evaporate. Then, repeat the bead grinding. (*One can also wet-grind by adding 50 µl of grinding buffer from ALEXANDER et al. (2007) which does not foam. Grind maximum 2-3 minutes total in the liquid. Work quickly after adding liquid due to danger of sample degradation.*). NB. CTAB buffer precipitates in cold conditions. Our lysis buffer (Appendix 3) is therefore not ideal for use with samples ground in mortar with liquid nitrogen. For that purpose, it is better to use an alternative, e.g. 250 mMTris-HCl (pH=8.5), 250 mMNaCl, 25 mM EDTA, 0.5% SDS.

4. a) Add 400 µl of CTAB lysis buffer (65°C) and 1 µl RNase A (100mg/ml) to the lichen powder, close all tubes, and shake vigorously for 15–30 s, until the powder has dissolved completely. (*RNase A and CTAB buffer can be premixed. Left-over should be discarded.* a) *For 2 x 96 samples, combine 90 ml lysis buffer heated to 65°C with 225 µl RNase A, concentrated at 100 mg/ml*). For a), put a cover on top of the collection microtubes before shaking. For b), shake while holding a rack on top of the tubes so they do not fall out during shaking. We add the lysis buffer with a) a multichannel pipette to the collection microtubes, or b) an expandable multichannel pipette to the single tubes (see Appendix 4), filling 800 µl and dispensing 400 µl so that two rows of tubes receive lysis buffer with each pipet fill.

   Troubleshooting: If the powder does not dissolve completely, it is possible to b) slide the bottom of the tubes over the top of an empty rack, which will shake them to dissolve the powder more easily. Dissolving is usually only problematic if tubes contain too much

material. Add more lysis buffer if the material is still not dissolved, and shake vigorously until dissolved.

5. Spin at **a)** 3800–6000 g or **b)** maximum speed for 10–20 minutes.

   Note: The CTAB in the lysis buffer precipitates if the centrifuge is cooled below room temperature.

6. **a)** Multi-pipet the supernatant into a new set of racked collection microtubes (Qiagen). **b)** When using single tubes, single-pipet into racked collection microtubes in this step, paying attention to each one to avoid the pellet. Usually, the recovery is around 300-400 µl. Make sure not to transfer parts of the pelleted cell debris, nor any junk floating on the surface.

   Note: Label each row of the racked collection microtubes with a row number and each plate rack with a plate number. Before adding the liquid, it is best to remove an empty collection microtuberow from its rack and place it into an empty rack in which the pipeting step is performed. After adding the supernatant, put the microtube row back into the original rack in the lane and correct orientation. This procedure helps to avoid sample cross-contaminations. **b)** Since single-tube racks are usually not see-through, we do not multi-pipet in this step because we would inevitably transfer cellular debris.

7. Pull out the first row of collection microtubes containing the supernatant from their rack, and place into an empty rack. Multipipet 600 µl binding buffer into the microtubes (at least 1.5 volumes of the lysate volume – e.g. for 400 µl lysate, use 600 µl binding buffer). Mix by pipetting 1000 µl volume out and back into the collection microtubes, repeat mixing once. Add the samples into the first row of a labelled silica membrane plate which is placed on a 2.2 ml deep-well plate (materials, see Appendix 4). Seal the filled row on the silica membrane with cellotape to avoid double pipetting and cross contaminations. Then, proceed with the remaining rows in the same way.

   Note 1: It is possible to fill the collection microtubes with 600 µl of binding buffer prior to adding the supernatant (step 6). In this case, be especially careful to mix the samples very well before applying the liquid onto the silica membrane.

   Note 2: Save both pipetting steps as separate programs on the electronic pipette, e.g. P0 = fill 600 µl, P1 = fill 1000 µl. Make sure to keep tubes in orientation to avoid a mix-up of samples. Be careful while pipetting liquid from and into the collection microtubes, as there is the danger of overflow if the entire volume is in the tube and the pipet tips are too far down.

8. Centrifuge silica plates residing on deep-well plates at 3800 g for 3 minutes, or until all liquid has passed through the membranes. (Might take up to 10 minutes). Discard the flow-through. Do not increase the centrifuge speed for this step if it can be avoided.

9. Add 500 µl 70% ethanol to each well of the silica plate to wash the membrane. Spin at 3800 g for 3 minutes, then discard the flow-through. Repeat the washing step and discard the flow-through. Centrifuge plates for 15 minutes to remove all residual ethanol from the membranes.

10. Place the membranes onto clean (autoclaved) deep-well plates in the correct orientation. Elute DNA by multi-pipetting 110 µl AE buffer, preheated to 65°C, onto each

membrane, incubating for 1 minute, and centrifuging for 1 minute. Repeat elution step.
Note: If a concentrated DNA extract is desired, elute twice with 50–70 µl AE buffer.