A fast and inexpensive high-throughput protocol for isolating high molecular weight genomic DNA from lichens

Silke Werth*, Sigrún Reynisdóttir, Hörður Gudmundsson & Ólafur S. Andrésson


Isolating high molecular weight DNA as required for genomic library preparations and other applications is a challenging task. We optimized a glass-fiber column-based, high throughput DNA isolation protocol to yield comparably large quantities of high molecular weight, pure DNA; cell lysis based on a CTAB buffer was followed by centrifugation to remove cell debris, binding to a glass-fiber membrane under high concentration of a chaotropic salt (guanidine hydrochloride), followed by two 70% ethanol washes and elution. This method was utilized successfully on several species of lichenized ascomycetes and on two non-lichenized basidiomycetes. Thus, while developed for lichens, the method is suitable for a range of fungal taxa.

Keywords: Isolation of genomic DNA, high molecular weight DNA, fungi.

Introduction

High molecular weight genomic DNA is required for restriction digests of genomic DNA, for high-throughput sequencing and other applications. Obtaining genomic DNA of high molecular weight is often a challenging task. Many lichens are small, and in small-sized organisms, low biomass limits DNA yield. Furthermore, the presence of contaminants such as polysaccharides or other bio-organic compounds may cause problems for DNA isolation and downstream applications; this is especially challenging in lichens and plants.

One method resulting in high molecular weight genomic DNA in fairly high concentration is phenol-chloroform DNA extraction (van Bürk et al. 1998). Unfortunately, this method is rather time-consuming and it is difficult to process many samples at a time, as the separation

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of aqueous and organic phases is most reliably performed by discrete pipetting. Carry-over of the organic phase or interphase material may lead to inhibition of downstream enzymatic reactions.

Several commercial suppliers provide silica-column based DNA isolation kits which allow high sample throughput. The kits designed for plants or fungi usually work for lichens, although their efficiency varies. In the ideal case, the resulting genomic DNA is clean and well suited for PCR-based applications or restriction digests. Unfortunately, most commercially available DNA isolation kits are expensive, and isolation protocols are not optimized for lichens. Moreover, the DNA is often of low molecular weight (i.e. sheared) and therefore not suitable for genomic library preparations for high-throughput sequencing, or for restriction digests as required for RAD-tag sequencing.

We therefore optimized existing DNA isolation protocols in order to obtain a protocol combining the cleanliness of column-based isolation procedures with a comparatively large yield of high molecular weight genomic DNA, while not being prohibitively expensive or time-consuming.

Materials and methods

The centrifuges used for spinning 96-well plates were either a 4-16KS (Sigma, Osterode am Harz, Germany), equipped with a Plate Rotor 2 x 96 (Qiagen, Hilden), or an Allegra 25R with rotor S5700 (Beckman Coulter, Birkerød, Denmark).

We tested various buffer chemistries. As a lysis buffer, the CTAB-based lysis buffer (2% CTAB, 100 mm Tris-HCl pH=8, 20 mm EDTA, 1.4 M NaCl) developed by Ivanova et al. (2008) was tested in combination with other binding and wash buffer systems. Multiple binding buffers were tested, including a binding buffer developed by Whitlock et al. (2008) (6 M sodium iodide, 0.2 M sodium sulphite) in combination with various lysis buffers. Moreover, we tried N3 buffers (a: 7 M Gu-HCl, 0.2 M potassium acetate, pH 4.8; b: 4.2 M guanidine hydrochloride, 0.9 M potassium acetate, pH 4.8; http://openwetware.org/wiki/Qiagen_Buffers). The binding buffer developed by Alexander et al. (2007) containing 2 M of guanidine hydrochloride dissolved in abs. ethanol was also tested. Finally, two elution buffers were tested: AE buffer (10 mm Tris-HCl, 0.5 mm EDTA; pH=9.0) and 1× TE buffer (10 mm Tris-HCl, 1 mm EDTA, pH=8.0).

We also tested two entire DNA isolation protocols: the one by Sinnemann et al. (2000) (lysis buffer: 50 mm EDTA; 100 mm NaCl; 10 mm Tris-HCl; 1% SDS; pH 8.0) with ammonium acetate (10.5 M) precipitation to remove proteins, and Qiagen’s plasmid isolation buffers P1 (resuspension buffer; 50 mm Tris-HCl, 10 mm EDTA, pH 8.0; 100 μg/ml RNaseA), P2 (lysis buffer 200 mm NaOH, 1% SDS; tested with or without NaOH) and with P3 (precipitation buffer; 3.0 M potassium acetate, pH 5.5; buffer compositions: http://people.mbi.ucla.edu/sum-chan/qiagenbuffer.html) in combination with two binding buffers (N3a: 7 M Gu-HCl, 0.2 M potassium acetate, pH 4.8; N3b: 4.2 M guanidine hydrochloride, 0.9 M potassium acetate, pH 4.8; http://openwetware.org/wiki/Qiagen_Buffers), 70% ethanol as a wash buffer and AE buffer for elution.

Finally, we tried various silica columns with our buffer system, including single tube spin columns which can be purchased separately from Omega Biotek (Ezna HP Plant DNA Mini Kit), single silica columns from the DNeasy Plant mini kit (Qiagen), 96-well plates from the DNeasy 96 Plant Mini Kit (Qiagen), 96-well plates with a smaller silica membrane diameter
(Zymo-Spin I-96 plate, # C2004), and 3 µm glass fiber plates by Macherey-Nagel (Chromafil Multi 96 GF 3 µm, Cat. Nr. 738658.M).

To visualize the molecular weight of the DNA resulting from our protocol, gel electrophoresis was performed on a 1% agarose gel in 1× TAE buffer (40 mm Tris, 20 mm acetic acid, and 1 mm EDTA) and the DNA was stained with 500 ng/ml ethidium bromide.

**Results and Discussion**

Our protocol (detailed below and in online supplementary material S1 and S2) offers multiple advantages over others for the isolation of DNA. i) The protocol results in high molecular weight genomic DNA (Fig. 1). ii) Because of the binding buffer which worked excellently in combination with the glass fibre membrane plates, the protocol results in good DNA yields even for comparatively small samples (Table 1). iii) The CTAB-based lysis buffer gives very good results with some lichens. iv) The protocol does not have many steps and thus allows rapid isolation of genomic DNA. v) Our method facilitates high throughput when used in combination with 96-well glass fibre plates.

![Fig. 1: Gel electrophoresis of genomic DNA obtained from the cyanolichen *Peltigera membranacea* with our protocol, showing a molecular weight marker (1 kb ladder, New England Biolabs), 24 samples from the lichen *Peltigera membranacea*, the molecular weight marker (1 kb ladder), and lambda DNA (50 ng, 100 ng, 200 ng and 300 ng; 48.5 kb). Upper and lower panels represent different sets of samples from two 96-well plates.](image)

The CTAB-based lysis buffer developed by Ivanova et al. (2008) worked very well for lichens. CTAB-based buffers are routinely used for isolating DNA from fungi, including lichenized species (Van Burik et al. 1998, Armaeo & Miao 1999, Cubero et al. 1999, Aras & Cansaran 2006). When we tried to isolate DNA from cyanobacterial colonies, the extractions worked better if 0.2% SDS was added to the lysis buffer and the samples were ground with sterile sand instead of a steel bead.
Werth et al.: High-throughput protocol for genomic DNA isolation

The protocol by Sinnemann et al. (2000) did not work well for the small amounts of material we intended to analyze because it was difficult to scale down (the samples turning viscous or solid upon addition of ammonium-acetate). Qiagen’s plasmid isolation buffers P1, P2 and P3 in combination with either N3a or N3b buffer did not result in high concentrations of genomic DNA for lichens, although DNA yields were slightly higher if P2 did not contain NaOH. DNA yields were very low with this method, and most of the DNA passed through the glass fibre membrane instead of being bound, as evidenced by high DNA contents of the flow-through measured with a photometer (NanoDrop 1000).

The main challenge we were faced with when optimizing the DNA isolation protocol for lichens was finding the appropriate binding buffer. Binding of the negatively charged DNA to silica membranes occurs under high concentrations of chaotropic salts (guanidine hydrochloride, guanidine thiocyanate, sodium iodide). The binding buffer developed by Alexander et al. (2007) was the key to success. It worked very well with our setup of glass fibre membranes.

### Table 1: Statistics of genomic DNA extracted with the current protocol.

The table gives the type of species (BAS, non-lichenized basidiomycete; LICH, lichen; LICOL, lichenicolous fungus), the DNA concentration, estimates of DNA purity from analysis with a spectrophotometer, DNA yield (units: ng DNA per mg lichen dryweight), and sample size (N). If more than two samples were analyzed, values represent an average (+ standard error of the mean).

<table>
<thead>
<tr>
<th>Type</th>
<th>Species</th>
<th>Conc. [ng/μl]</th>
<th>A260/A280</th>
<th>A260/A230</th>
<th>DNA yield [ng/mg]</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAS</td>
<td><em>Hydnum rufescens</em></td>
<td>26.8</td>
<td>1.24</td>
<td>0.98</td>
<td>294.8</td>
<td>1</td>
</tr>
<tr>
<td>BAS</td>
<td><em>Lepista nuda</em></td>
<td>40.4</td>
<td>1.95</td>
<td>2.31</td>
<td>444.4</td>
<td>1</td>
</tr>
<tr>
<td>LICH</td>
<td><em>Caloplaca sp.</em></td>
<td>14.5 ± 3.2</td>
<td>1.63 ± 0.06</td>
<td>1.26 ± 0.14</td>
<td>241.1 ± 53.1</td>
<td>14</td>
</tr>
<tr>
<td>LICH</td>
<td><em>Lobaria pulmonaria</em> (5 mg)</td>
<td>6.5 ± 1.0</td>
<td>—</td>
<td>—</td>
<td>286.0 ± 44.9</td>
<td>4</td>
</tr>
<tr>
<td>LICH</td>
<td><em>Peltigera aphthosa</em></td>
<td>77.9</td>
<td>1.78</td>
<td>2.02</td>
<td>249.7</td>
<td>1</td>
</tr>
<tr>
<td>LICH</td>
<td><em>Peltigera aphthosa</em> (apothecia)</td>
<td>27.4</td>
<td>1.73</td>
<td>1.31</td>
<td>168.3</td>
<td>1</td>
</tr>
<tr>
<td>LICH</td>
<td><em>Peltigera britannica</em></td>
<td>79.6</td>
<td>1.86</td>
<td>2.01</td>
<td>265.1</td>
<td>1</td>
</tr>
<tr>
<td>LICH</td>
<td><em>Peltigera islandica</em></td>
<td>30.1</td>
<td>1.86</td>
<td>2.05</td>
<td>331.1</td>
<td>2</td>
</tr>
<tr>
<td>LICH</td>
<td><em>Peltigera leucophlebia</em></td>
<td>14.5</td>
<td>1.89</td>
<td>1.39</td>
<td>159.5</td>
<td>1</td>
</tr>
<tr>
<td>LICH</td>
<td><em>Peltigera membranacea</em></td>
<td>92.2 ± 6.6</td>
<td>1.95 ± 0.01</td>
<td>1.10 ± 0.04</td>
<td>676.5 ± 48.7</td>
<td>48</td>
</tr>
<tr>
<td>LICH</td>
<td><em>Peltigera membranacea</em></td>
<td>54.9 ± 4.9</td>
<td>1.87 ± 0.01</td>
<td>1.66 ± 0.04</td>
<td>603.8 ± 54.3</td>
<td>12</td>
</tr>
<tr>
<td>LICH</td>
<td><em>Pseudephebe sp.</em></td>
<td>20.6 ± 1.2</td>
<td>—</td>
<td>—</td>
<td>111.6 ± 5.5</td>
<td>148</td>
</tr>
<tr>
<td>LICOL</td>
<td><em>Plectocarpon lichenum</em> (5 mg)</td>
<td>19.9 ± 3.8</td>
<td>—</td>
<td>—</td>
<td>877.2 ± 167.6</td>
<td>4</td>
</tr>
<tr>
<td>LICOL</td>
<td><em>Tremella lobariacearum</em> (5 mg)</td>
<td>25.2 ± 4.0</td>
<td>—</td>
<td>—</td>
<td>1111.0 ± 174.2</td>
<td>5</td>
</tr>
</tbody>
</table>

* Ca. 3 mg of material were analyzed. Species were crustose species, growing epilithic or endolithic on calcareous rocks.  
† A260/230 and A260/A280 were omitted. For the lichenicolous fungi *P. lichenum* and *T. lobariacearum*, 5 mg of gall tissue were used for DNA isolation. DNA of *P. lichenum* was slightly discolored but worked fine in quantitative Real-Time PCR, same as DNA of *T. lobariacearum* and *L. pulmonaria* (PCR efficiencies ~80%). Data: Bergmann & Werth, unpublished.  
* RNase A digest was omitted.  
° Species included *Pseudephebe minuscula* and *P. pubescens*; the amounts of starting material varied (~2.6 – 22 mg). Elution of DNA was done with 1 × 50 μl of AE buffer.
resulting in good yields of high molecular weight genomic DNA. The other binding buffers we tested did not give satisfactory results, such as that by Whitlock et al. (2008) where the DNA ended up in the flow-through when utilized in combination with various lysis buffers. Also the N3 binding buffers we tested did not result in success. Developed for plasmid isolation, the N3 buffers were most likely too acidic for our purpose, and resulted in the recovery of RNA and only little DNA.

For DNA elution, AE buffer may be replaced by 1× TE buffer; quantity and quality of the DNA eluted with TE buffer is comparable to that eluted with AE. Restriction digests performed with undiluted DNA extracts using AE elution buffer worked well (Werth et al., unpublished data).

Single columns by Qiagen and Omega Biotek resulted in high DNA yields of sufficient quality for downstream PCR-based applications (e.g. microsatellite genotyping), but this DNA was somewhat degraded (smear). The Zymo-Spin 96-well plates had a smaller diameter silica membrane. It was difficult to pass all liquid through them in the binding step, and repeated centrifugation was needed to achieve complete flow-through. DNA isolation with this type of plate worked and may be a good strategy for smaller samples if the amount of chemicals is down-scaled. The smaller diameter of the membrane should be better suited for binding small quantities of DNA. The 96-well silica membrane plates by Qiagen and 96-well glass fiber membrane plates by Macherey-Nagel exhibited the best performance of all tested devices, resulting in high molecular weight genomic DNA.

Phenol-chloroform DNA isolation methods also result in high molecular weight DNA. Their main advantages are high yield and low cost. Their main disadvantage is that often times, organic solvents are co-isolated so the DNA has to be cleaned up over a silica column prior to further enzymatic reactions. Also, the phenol-chloroform method has a considerably lower throughput (one protocol we have been using takes 4–5 hours for 16 samples). For the phenol-chloroform method, we were unable to find a way to run as many samples as 96 × 2, mainly because the separation of organic and inorganic phases is difficult to perform by multichannel pipetting. Our method has the advantage of resulting in genomic DNA which is pure enough for enzymatic reactions and has a much higher throughput (4–5 hours for 192 samples).

Using this protocol, we and our colleagues have successfully extracted DNA from several species including the cyanolichen *Peltigera membranacea* (>1000 samples isolated with this method, subsequent applications: microsatellite genotyping; RAD-tag sequencing), *Lobaria pulmonaria* (microsatellite genotyping), *Caloplaca* sp. and *Pseudephebe pubescens* (several hundred samples analyzed for each of the three species and used for PCR and Sanger sequencing). We have also successfully isolated DNA from the fungi *Hydnum rufescens* and *Lepista nuda* (Basidiomycota) employing this protocol and successfully used it for RAD-tag sequencing. Thus, the method reported here has applicability for a wide range of fungal taxa. DNA prepared in this way is suitable for downstream PCR-based applications, for restriction digests e.g. for RAD-tag sequencing, and for genomic library preparations.

**DNA isolation protocol**

The protocol below describes the DNA isolation procedure where the lichens are ground in 2×96 well collection microtubes. For a detailed protocol using single tubes and variations, see online supplementary material, Appendix S2. Dry lichens were cleaned of moss, debris and other material. Fifty mg of lichen material (about the size of a small fingernail) was torn into small pieces and added to 1.2 ml collection microtubes, racked in 96-well plate format
(Qiagen, Hilden, Germany), each with one stainless steel bead, diameter 3 mm (carbon steel polishing beads, Kugelpompel, Vienna). The samples were lyophilized overnight in a Virtis Sentry 2.0 instrument, model 2KBTES-55 (Sp Scientific, Stone Ridge, New York) or dried on the bench for 24 h. After closing the collection tubes with cap strips, the lichen samples were ground to a fine powder in a mixer mill (TissueLyser II, Qiagen, Hilden, Germany) at 30 rotations per minute for 3-5 minutes, swapping the plate orientation occasionally to guarantee even grinding. A short centrifuge spin to 4000 g was performed in order to collect any powder sticking to the caps. All pipetting steps were performed with an E1-ClipTip electronic eight-channel pipette (12.5-1250 μl, Thermo Scientific), equipped with ClipTip 1250 μl pipette tips (Thermo Scientific). Altogether 90 ml of lysis buffer were pre-heated to 65 °C and combined with 225 μl of RNase A (100 mg ml⁻¹, Qiagen, Hilden); this amount was sufficient for 2×96 samples. 400 μl of the heated lysis buffer containing RNase A were added to each collection microtube containing a ground sample. The lysis buffer followed Ivanova et al. (2008) and contained 2% CTAB, 100 mm Tris-HCl (pH=8.0), 20 mm EDTA, and 1.4 M NaCl (see online supplementary material, Appendix S3). The collection tubes were sealed with fresh cap strips, and samples were mixed vigorously by shaking up and down until the lichen powder had been completely dissolved (ca. 20 s). Subsequently, the samples were centrifuged at 6000 g for 10 minutes, with the plate centrifuge being cooled to 15–20 °C. The supernatant was transferred to new collection microtubes racked in 96 wells without disturbing the pellet. Then, 600 μl of binding buffer (2 M guanidine hydrochloride, dissolved in EtOH puriss., online supplementary material, Appendix S3) (Alexander et al. 2007) were added to the supernatant. The liquid was mixed well by repeated pipetting, taking care that the wells did not overflow when the pipet tips were inserted into the liquid. The entire volume (~1 ml) was transferred to a glass fiber membrane plate (Chromafil Multi 96 GF 3 μm, # 738658.M, Macherey-Nagel, Düren) seated on a 2.2 ml deep-well plate (# 9407615, Bartelt). The liquid was spun for 3 minutes (or longer, to ensure that the entire liquid had passed through the membrane) at 3800 g, and the flow-through was discarded. The DNA was washed by adding 500 μl of 70% ethanol to each well and spinning for 3 minutes. This step was repeated. Then, after emptying out the liquid in the deep-well plate, the plates were centrifuged for 15 minutes at 3800 g in order to remove any residual ethanol from the membranes. The glass fiber membrane plates were placed on a clean deep-well plate, and genomic DNA was eluted by adding 110 μl of AE buffer (10 mm Tris-HCl, 0.5 mm EDTA, adjusted to pH 9.0 with 1 M NaOH; composition from http://openwetware.org/wiki/Qiagen_Buffers) preheated to 65 °C, incubating for 1 minute at room temperature, and centrifugation for 1 minute at 3800 g and 20 °C. This step was repeated once. For a short protocol of the DNA extraction procedures, see online supplementary material, Appendix S1; buffer recipes, see online supplementary material, Appendix S3; list of the materials utilized, see online supplementary material, Appendix S4.

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Supplementary documents online:

Supplementary Appendix S1: Short protocol for the isolation of high molecular weight genomic DNA (96-well format).

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

Supplementary Appendix S3: Buffers used for DNA extraction

Supplementary Appendix S4: Other materials used for DNA isolation

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Supplementary Appendix S1: Short protocol for the isolation of high molecular weight genomic DNA (96-well format).

1. Grind samples with stainless steel beads in 1.2 ml collection microtubes (Qiagen) in a Tissue Lyzer II mixer mill to a fine powder.
2. Add 400 μl CTAB lysis buffer (heated to 65°C) & 1 μl RNase A (optional), mix vigorously until all powder has been dissolved.
3. Spin at 6000 g for 10 minutes at 15–20°C.
4. Pipet the supernatant into fresh collection microtubes. Then add 600 μl binding buffer.
5. Mix well. Pipet binding buffer mix (1000 μl) on the glass fibre membrane plate residing on a 2.2 ml deep-well plate.
7. Wash the membrane by adding 500 μl 70% ethanol to each well. Spin for 3 minutes*, then discard the flow-through. Repeat wash and discard the flow-through. Spin for 15 minutes* to remove residual ethanol from the membranes.
8. Place the glass fibre membrane plate on a clean deep-well plate (autoclaved). Elute genomic DNA with 110 μl AE buffer preheated to 65°C, incubate 1 minute, spin 1 min*. Repeat elution to gain a total of 220 μl of DNA.

* Spinning at 3800 g and 15–20°C. If liquid has not passed through membrane, increase centrifugation time.

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 cm² 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 lyophiliser, 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 cello tape 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.

Supplementary Appendix S3: Buffers used for DNA extraction

1. Lysis buffer (IvanoVa et al. 2008)

<table>
<thead>
<tr>
<th>Final concentration</th>
<th>Quantity for 1 litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>2% CTAB</td>
<td>20 g</td>
</tr>
<tr>
<td>100 mM Tris-HCl</td>
<td>100 ml of 1 M solution, pH=8.0</td>
</tr>
<tr>
<td>20 mM EDTA</td>
<td>5.845 g (careful: depends on molecular weight)</td>
</tr>
<tr>
<td>1.4 M NaCl</td>
<td>81.816 g</td>
</tr>
</tbody>
</table>

Heat the solution to 65°C to dissolve CTAB and NaCl. Avoid excessive shaking because it creates foam.

2. Binding buffer (Alexander et al. 2007)

<table>
<thead>
<tr>
<th>Final concentration</th>
<th>Quantity for 1 litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 M guanidine hydrochloride</td>
<td>191.06 g</td>
</tr>
</tbody>
</table>

Fill up with absolute ethanol to 1 litre, mix. It can take some time to dissolve, but it will usually dissolve overnight, or if heated to 65°C for some time. Note: Guanidine hydrochloride is a hazardous chaotropicsalt — store at room temperature, handle with gloves, dispose of separately in chemical waste. Make sure not to mix with bleach as this creates dangerous reactive compounds!

3. AE buffer (Qiagen; available at: http://openwetware.org/wiki/Qiagen_Buffers)

<table>
<thead>
<tr>
<th>Final concentration</th>
<th>Quantity for 1 litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mM Tris-HCl</td>
<td>10 ml of 1 M solution, pH=8.0</td>
</tr>
<tr>
<td>0.5 mM EDTA</td>
<td>0.14612 g (careful: depends on molecular weight)</td>
</tr>
</tbody>
</table>

Add chemicals and fill up with ddH2O to ca. 500 ml. Adjust pH to 9.0 with 1 M NaOH. Fill up to 1 litre with ddH2O and mix well.
Supplementary Appendix S4: Other materials used for DNA isolation

**Glass fiber or silica membrane plates (96 wells)**
- IVANOVA et al. (2006) report on the isolation of genomic DNA using other types of 96-well glass fiber membrane plates; we did not test these.

**Silica membrane single tubes**
- DNeasy Plant Mini kit silica columns (Qiagen)
- Ezna HP Plant DNA Mini Kit (Omega Biotek)

**Centrifuge for 96-well plates**
- Sigma 4-16KS with Qiagen rotor for 96-well plates
- Beckman Allegra X12R with plate rotor