**A homemade DNA extraction and PCR purification kit**

Two DNA extraction methods were used: initially, DNA extractions were conducted according to Summerfield (2003), who used the method described in Cubero *et al.* (1999) with slight modifications. Modifications of this method to include the use of silica membrane spin columns resulted in a quick, robust, low cost method, equivalent to commercial DNA extraction kits.

This method included the first steps from Summerfield (2003) and Cubero *et al.* (1999), which includes sample preparation, cell disruption with liquid N2, cell lysis in CTAB *Extraction Buffer* followed by only one chloroform extraction.

The subsequent steps are based on the approach of Ye *et al.* (2004), who have used a commercial PCR purification kit to purify raw genomic DNA extracts, however in place of a commercial kit the present study used separately purchased low-cost silica membrane spin columns (*EconoSpin® All-In-One Mini Spin Columns*, Epoch Life Sciences Inc.) and homemade reaction buffers, based on recipes provided by the column manufacturer.

*EconoSpin®* columns and homemade buffers (*PEX* binding buffer, *WS* wash buffer, *EB* elution buffer) were used according to the instruction manual of the *PureLink®* PCR Purification Kit (Invitrogen, Life TechnologiesTM). Raw genomic DNA extract was used instead of un-purified PCR product, *PEX* binding buffer replaced *B2* buffer, *WS* wash buffer replaced *W1* buffer, *EB* elution buffer (or 1 x *TE* buffer) replaced *E1* buffer. The only modification compared to the *PureLink®* manual, is the use of 5 volumes of *PEX* binding buffer instead of 4 volumes of *B2*, as this ratio has been suggested in the *PEX* recipe provided by Epoch Life Sciences.

The same homemade buffers and *EconoSpin®* columns were later used for purification of PCR products, as this is the purpose for which the manufacturer sells the columns and provides the buffer recipes.

**Composition of reagents for DNA extraction**

*Extraction Buffer* 100 mM Tris-HCl, pH 8.0; 1 M NaCl; 20 mM EDTA; 1% CTAB (w/v).

*PEX* binding buffer 5.5 M guanidine hydrochloride (GuHCl); 20 mM Tris-HCl, pH 6.6 (25°C) (eventually an improved recipe with 25% v/v isopropanol was used).

*WS* wash buffer 10 mM Tris-HCl, pH 7.5 (25°C) with 80% ethanol [final

 concentration after addition of ethanol].

*EB* elution buffer 10 mM Tris-HCl, pH 8.5 (25°C).

1x *TE* buffer 10 mM Tris-HCl, pH 8.0; 1mM EDTA.

**DNA Extraction Procedure Part I – DNA raw extracts**

*Sample preparation*. Thallus fragments (dry weight 1-50 mg) were thoroughly cleaned by manual removal of adhering dirt, moss, etc. under a dissecting microscope using super-fine tweezers and rinsing in de-ionised H2O.

*Tissue disruption.* Cleaned and air-dried thallus fragments were ground up to a fine powder inside a 1.5 ml microcentrifuge tube using a micro-pestle and liquid N2.

*Cell lysis.* 500 μl of *Extraction Buffer* and approximately 5 mg (a spatula tip) of polyvinylpolypyrrolidone (PVPP) powder were added to the tube. The tube was briefly mixed by vortexing and incubated in a heat block at 70°C for 30 minutes. (Old or smaller samples were incubated for 1 hour to increase the yield.)

*Chloroform extraction.* Under a fume hood an equal volume of chloroform (i.e. 500 μl) was added, and thoroughly mixed by manual shaking. Tubes were centrifuged at maximum speed (> 10000 g) for 5 minutes at room temperature in a bench top centrifuge to separate phases.

The upper aqueous phase (DNA raw extract) was carefully transferred into a new tube, without touching the phase boundary. Usually it was possible to recover 400 μl of the originally 500 μl aqueous phase. The interphase cell debris and lower chloroform phase were discarded.

**DNA Extraction Procedure Part II – Spin-column purification of DNA raw extracts (and PCR products)**

*Binding*. 5 volumes *PEX* binding buffer were added to the DNA raw extract (or PCR product) and gently mixed by slowly pipetting up and down several times. The mixture was transferred to an *EconoSpin®* column and centrifuged for 1 minute at maximum speed; the flow through was discarded. (For 400 μl of raw DNA extract, the volume was split into two tubes with 200 μl each before adding *PEX*, and the column was refilled and centrifuged several times).

*Wash*. 650 μl *WS* wash buffer were added to the column, and it was centrifuged for 1 minute at maximum speed. After discarding the flow through, the column was dried by centrifuging again for 3 minutes.

*Elution*. The column was placed in a new 1.5 ml microcentrifuge tube, and 50 μl *EB* elution buffer (or alternatively 1x *TE*) were added to the centre of the column. The column was incubated at room temperature for 2 minutes, and then centrifuged for 2 minutes at maximum speed.

The yield was measured using a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific).

**References**

**Cubero, O. F., Crespo, A., Fatehi, J. & Bridge, P. D. (1999)** DNA extraction and PCR amplification method suitable for fresh, herbarium-stored, lichenized, and other fungi. *Plant Systematics and Evolution* 216(3-4): 243-249.

**Summerfield, T. C. (2003)** Investigation of symbiont specifity in cyanolichens and differential gene expression in symbiotic Nostoc strain. A thesis submitted for the degree Doctor of Philosophy, University of Otago, Dunedin, New Zealand, i-xiv, 1-151 pp.

**Ye, J., Ji, A., Parra, E. J., Zheng, X., Jiang, C., Zhao, X., Hu, L. & Tu, Z. (2004)** A Simple and Efficient Method for Extracting DNA From Old and Burned Bone. *Journal of Forensic Sciences* 49(4): 1-6.

**Table 5**. Voucher details of specimens used in this study. The column “Lane” refers to the lane of the same number in Fig. 5, showing results of the mating type screening. The column “OTA…” gives the herbarium accession of the voucher specimens held in the Otago Regional Herbarium (OTA).

|  |  |  |
| --- | --- | --- |
| **Lane** | **OTA …** | **Provenance, collection date** |
| 01 | 063988 | Swampy Summit, Dunedin, NZ, 45°48'03.7"S 170°28'57.5"E, ~720 m, 07.09.2012 |
| 02 | 063971 | Campbell Island, NZ, 52.54371°S 169.15179°E, 23.11.2012 |
| 03 | 062510 | Mt. Cargill area, near Dunedin, NZ, 45°48'40.2"S 170°33'57.8"E, 12.05.2013 |
| 04 | 064242 | Heaphy Track, near Perry Saddle Hut, NZ, 40°53'59.2"S 172°24'20.4"E, 01.01.2014 |
| 05 | 064241 | Heaphy Track, near James Mackay Hut, NZ, 40°53'11.8"S 172°13'01.8"E, 04.01.2014 |
| 06 | 063992 | old Huon Track, Tahune Forest Reserve, Tasmania, 43°06'S 146°42' E, 09.01.2013  |
| 07 | 062511 | near Ajax Hill, Catlins, NZ, 46°26'00"S 169°19'04"E, 14.12.2012 |
| 08 | 062512 | Ajax Hill bog, Catlins, NZ, 46°25'49.6"S 169°17'43.9"E, 14.12.2012 |
| 09 | none | Coalburn catchment, Southland, NZ, 46°10'42.6"S 166°42'10.8"E, 26.03.2013. Voucher held by Department of Conservation as part of TIER1 biodiversity monitoring program: plot G171, specimen IDs NV201300029 and NV201300189. |
| 10 | 064240 | Croesus Track, near Greymouth, NZ, 42°20'05"S 171°23'48"E, 07.01.2014 |
| 11 | 064245 | Silver Peaks North of Dunedin,NZ, 45°44'32.1"S 170°27'04.6"E, 15.09.2013 |
| 12 | 064246 | Silver Peaks near Dunedin, NZ, 45°44'48.0"S 170°27'27.7"E, 15.09.2013 |
| 13 | 069657 | Swampy Summit N of Dunedin, 45°47'45.3"S 170°28'53.7"E, 21.10.2014 |
| 14 | 064307 | Swampy Summit N of Dunedin, 45°47'45.2"S 170°28'51.7"E, 21.10.2014 |
| 15 | 069658 | Swampy Summit N of Dunedin, 45°47'44.9"S 170°28'52.4"E, 21.10.2014 |
| 16 | 069659 | Swampy Summit N of Dunedin, 45°47'45.8"S 170°28'56.2"E, 21.10.2014 |
| 17 | 069660 | Swampy Summit N of Dunedin, 45°47'46.9"S 170°29'00.5"E, 21.10.2014 |
| 18 | 069661 | Swampy Summit N of Dunedin, 45°47'55.4"S 170°28'55.9"E, 05.08.2014 |
| 19 | 069662 | Swampy Summit N of Dunedin, 45°47'46.7"S 170°28'59.7"E, 05.08.2014 |
| 20 | 069663 | Swampy Summit N of Dunedin, 45°47'45.8"S 170°28'59.7"E, 05.08.2014 |
| 21 | 069664 | Swampy Summit N of Dunedin, 45°47'45.3"S 170°28'53.7"E, 05.08.2014 |
| 22 | 069665 | Swampy Summit N of Dunedin, 45°47'45.5"S 170°28'51.2"E, 05.08.2014 |
| 23 | 069666 | Swampy Summit N of Dunedin, 45°47'44.9"S 170°28'52.4"E, 05.08.2014 |
| 24 | 069908 | Swampy Summit N of Dunedin, 45°47'42.3"S 170°28'50.1"E, 05.08.2014 |
| 25 | 069667 | Swampy Summit N of Dunedin, 45°47'45.4"S 170°28'53.6"E, 05.08.2014 |
| 26 | 069668 | Swampy Summit N of Dunedin, 45°47'46.5"S 170°28'56.3"E, 05.08.2014 |
| 27 | 069669 | Swampy Summit N of Dunedin, 45°47'46.5"S 170°28'59.2"E, 05.08.2014 |
| 28 | 069670 | Swampy Summit N of Dunedin, 45°47'50.4"S 170°29'00.2"E, 05.08.2014 |