

DNA extraction and telomere length determination in the coral species *Paragorgia arborea*

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DNA Extraction

High molecular weight genomic DNA of *Paragorgia arborea* was extracted using the Midi Kit for Cell and Blood Culture (Qiagen). Between 110-130 mg wet weight of tissue was homogenized in 9.5 ml G2 lysis buffer with 100 µl 20 mg/ml RNase A, and 500 µl protease (1 AU/ml, Qiagen) using Kimble hand-held homogenizer. The homogenate was further smashed through a 70 µm cell strainer. The cells were lysed for 2 h at 50°C, with occasional gentle mixing. After lysis, the sample was centrifuged for 10 minutes at 5000 x g at 4°C. The supernatant was collected and passed through the genomic tip and washed twice with 7.5 ml QC buffer. The extracted DNA was eluted using 5 ml of warm (50°C) QF buffer. 3.5 ml isopropanol and 850 µl NaAOC (0.3 M, pH 5.2) were added to the eluate and were mixed gently. The eluate was centrifuged for 30 min at 15000 x g at 4°C. The supernatant was discarded and the precipitate was suspended in 1 ml cold (4°C) 70% ethanol. This step was repeated twice. The DNA pellet was set to dry before resuspending with 100 µl TE buffer for 1 h at 50°C. The DNA concentration was measured using a Nanodrop. A 1 µl sample was collected from the surface, midpart, and bottom of the tube to check for homogeneity. The quality of the DNA was checked by performing gel electrophoresis in 1.2 % agarose gel. A sample with ~100 ng of DNA material was added with loading dye. The electrophoresis was run for 30 min at 100V in TBE 1x buffer. Only samples with high molecular weight and A260/280 ratio values between 1.7 and 2.0, and A260/230 values between 2.0 and 2.2 were used further used in telomere length assay.

Telomere length assay

We estimated individual telomere length using the telomere restriction fragment (TRF) protocol from the TeloTAGGG assay kit (Sigma Aldrich, Roche). We followed the manufacturer instructions. First, we used two restriction enzymes (*Hinf I* and *Rsa I*) to digest 1 µg DNA at 37°C for 3h, followed by a gel (0.8% agarose) electrophoresis for 3h at 50 V. After migration, DNA is denaturated by immersing the gel in a denaturation solution, neutralized and transferred on a nylon membrane (positively charged) through an overnight southern blot technic. DNA is then fixed on the membrane at 120°C for 20min. The DNA was then hybridized with a digoxigenin-labeled probe specific for telomeric sequences and incubated with antidigoxigenin-specific antibody before visualization with a Chemidoc (Bio Rad) allowing an optimal image quality. Telomere length was then determined using ImageJ software to extract telomere smear densities. Lane-specific background was subtracted from each density value and TL (mean value) was then calculated using ImageJ software in a window of 2.7–21.2 kb and extrapolating up to 150 kb to include the whole smear.

We obtained averaged TL measures from $n = 37$ samples (range: 13 – 34 kb). We used 3 samples repeated in each gel to measure intra-assay variation (CV: 5.0%) and 3 samples on both gels to obtain inter-assay repeatability (CV: 8.0%).