

Development of DNA - free sediment for ecological assays with genomic endpoints. Kay T.Ho
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Recent advances in genomics are currently being exploited to discern ecological changes that have conventionally been measured using laborious counting techniques. For example, next generation sequencing technologies can be used to create DNA libraries from benthic community assemblages living under disparate ecological conditions. The content of the libraries can then be compared, and differences in taxon composition attributed to the ecological factor(s) in question. One challenge in this method is to obtain DNA-free sediment that serves as a control in field and laboratory experiments with genomic endpoints. We tested long-term sediment storage and autoclaving as methods of eliminating DNA in sediments. Our treatments were field-collected sediments that had been stored at 4°C in the dark for 5, 12 and 18 years, and then the same sediments were autoclaved and tested at zero, one and two weeks post autoclave. In addition, a freshly collected sediment sample was included as a positive control. DNA was extracted from all samples in triplicate using a modified method of Power Soil DNA Isolation KitTM from MoBio. DNA content of each extract was evaluated fluorometrically (nanodrop) and via agarose gel electrophoresis. Results of both the nanodrop assay and electrophoresis indicated sediments stored for prolonged periods of time (up to 18 years) still contained quantifiable concentrations of DNA, whereas subsamples of those same sediments contained no/negligible amounts of DNA after being autoclaved. Neither the storage time (5, 12, 18 years), or holding sediments for up to two weeks after autoclaving affected the DNA concentration. To ensure that extracts obtained from stored, autoclaved sediments are sufficiently 'clean' enough for use in future ecological genomics studies, they will be subjected to PCR analysis.

Purpose

This research chronicles the methods used to develop DNA –free sediments necessary for use in benthic community studies with genomic endpoints.

Key words

benthic communities; sediments; DNA-free; genomic endpoints