

Benchmarks

DNA in solution were used instead of 10.75 μ L of ddH₂O and 8.5 μ L of DNA in solution. The re-amplified eluted band was electrophoresed, gel-purified and cloned into a pGEM[®]-T vector (Promega, Madison, WI, USA). We then identified clones containing recombinant plasmids, isolated plasmid DNA and used the insert of an individual clone as a hybridization probe in northern blot analysis. A single hybridizing mRNA of approximately 520 bases was identified in the lanes corresponding to the CM and, to a lesser extent, the 90-day-old WT hamster ventricles (Figure 1C).

By using the re-amplified band of interest as a hybridization probe against northern blots, we were able to isolate, clone and identify a differentially expressed cDNA despite the fact that numerous cDNA species were present in the original band obtained. The ability to isolate and clone DNA that has hybridized to specific mRNA species should increase the speed of the secondary screening following DD and should eliminate the possibility that positive bands are not discarded simply because the specific clone is overlooked in the secondary screening analysis.

REFERENCES

1. **Bauer, D., H. Muller, J. Reich, H. Riedel, V. Ahrenkeil, P. Warthoe and M. Strauss.** 1993. Identification of differentially expressed mRNA species by an improved display technique (DDRT-PCR). *Nucleic Acids Res.* 21:4272-4280.
2. **Denovan-Wright, E.M., R.A. Newton, J.N. Armstrong, J.M. Babity and H.A. Robertson.** 1998. Acute administration of cocaine, but not amphetamine, increases the level of synaptotagmin IV mRNA in the dorsal striatum of rat. *Molecular Brain Res.* 55:350-354.
3. **Liang, P. and A.B. Pardee.** 1992. Differential display of eukaryotic messenger RNA by means of polymerase chain reaction. *Science* 257:967-971.
4. **Liang, P. and A.B. Pardee.** 1997. Differential display. A general protocol. *Methods Mol. Biol.* 85:3-11.
5. **McClelland, M., F. Mathieu-Daude and J. Welsh.** 1995. RNA fingerprinting and differential display using arbitrarily primer PCR. *Trends Genet.* 11:242-246.
6. **Sambrook, J., E. Fritsch and T. Maniatis.** 1989. *Molecular Cloning: A Laboratory Manual*, 2nd ed. CSH Laboratory Press, Cold Spring Harbor, NY.
7. **Utans, U., P. Liang, L.R. Wyner, M.J. Karnovsky and M.E. Russell.** 1994. Chronic cardiac rejection: identification of five upregulated genes in transplanted hearts by differential mRNA display. *Proc. Natl. Acad. Sci. USA* 91:6463-6467.

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Simple Protocol for Extracting Nuclear DNA from Single Embryos of a Marine Snail

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The analysis of genetic polymorphisms in groups of organisms leads to an understanding of the processes that structure populations, and, increasingly, DNA polymorphisms are the subject of study. Workers may be interested in organisms as research tools for understanding either evolutionary processes, the impact of pollutants or the sustainability of harvesting regimes. Marine animals figure prominently in all of these fields, but the extraction of DNA that is suitable for polymerase chain reaction (PCR) from small marine embryos is complicated by the small quantities of tissue initially present. A recent investigation using our model organism [*Littorina saxatilis* (Olivi)] recommended the use of the relatively expensive Chelex (12). The technique we describe here extracts DNA from individual whole embryos by incubating them in a simple lysis buffer, thus allowing DNA from individual embryos to be used in both mitochondrial and nuclear investigations (nuclear investigations need a higher-quality DNA extraction product than do mitochondrial investigations). This single-step procedure is relatively inexpensive and yields DNA that is directly suitable for PCR, whereas extractions using toxic reagents (8,14) and involving several handling steps (7,16) are cumbersome when very small amounts of tissue are involved.

The embryos of the intertidal snail *Littorina saxatilis* are found in a mother's brood pouch (13). A method of extracting DNA from the adults is a phenol/chloroform-extraction (6); however, this is a difficult technique to apply when very small amounts of tissue are involved. An alternative is simply to amplify after adding an embryo to the PCR mixture, but this often fails to produce reliable amplifications (see Figure 1). In this paper, using both nuclear and mitochondrial PCR primers, we compare the success rates for two different extraction techniques: (i) directly adding whole

embryos to the PCR mixture, and (ii) adding products that are recovered from embryos using the lysis buffer.

We have used a buffer that was slightly modified from that described by Higuchi (10), which has been used for extraction of single *Caenorhabditis elegans* (1) and blood biopsies of adult *Mytilus* (a technique unsuitable for very small marine animals; Reference 5). For extraction, a single embryo (between 0.4 and 0.7 mm in diameter; Reference 11) was added to 10 μ L of extraction buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3, 2.5 mM $MgCl_2$, 0.01% gelatin, 0.9% Tween® 20 and 10 mg/mL proteinase K) in a 0.5-mL microcentrifuge tube. Mineral oil (20 μ L) was added to each tube before incubation. We used a one-step incubation/denaturation protocol, incubating the sample for 60 min at 65°C followed by denaturation for 15 min at 94°C. The extracted samples can be kept frozen at -20°C until needed for use in PCR—material stored for five months has been successfully amplified. The buffer can be made in advance and stored at -20°C indefinitely, although fresh proteinase K must be added before use.

L. saxatilis embryos develop in the maternal brood pouch from the single-cell zygote to a shell-bearing juvenile that hatches. The extraction technique has allowed successful amplifications using embryos from the early zygote stage to the early shell stage (proteinaceous shell only) and using both frozen (-70°C) and fresh embryos. Using this procedure, DNA can be extracted from large numbers of single embryos quickly and efficiently, and because only one tube is used from introduction of material until removal for

PCR, the chances of contamination or loss of material are low. The lysis procedure yields sufficient extract for multiple separate PCRs (2.5 μ L extract each). This technique is an advantage over those described by Coffroth and Mulawka (3) and Geller et al. (9), where more complicated protocols involved buffers containing hexadecyltrimethylammonium bromide (CTAB), and by Côte-Real et al. (5), which yielded sufficient extract from a single mussel larva for only one PCR.

Figure 1 shows results from use of this extraction protocol. In this trial, we amplified DNA from targets prepared using either the lysis protocol or simply from native embryos placed into the PCR mixture. We amplified products from the calmodulin region of the *Littorina* nuclear genome. The primers used were the universal CAD2 and CAD3 (4). Using the lysis protocol, 76% (of 41 amplifications) were successful vs. 28% (of 43 amplifications) when no lysis buffer was used. Only 39% (of the 31 successful amplifications) from the lysed samples yielded faint bands, indicating weak amplification; whereas, 92% (of the 12 successful amplifications) in the samples that were added directly to PCR mixtures yielded bands. In a trial where the amplification target was mitochondrial cytochrome *b*, using primers CytbFOR (5'-TTCCCGCACCTTCAAATCTT-3') and CytbREV (5'-AGGGAACCTTTTCTCCATCTCTGT-3') (C. Wilding unpublished) both lysed and non-lysed samples produced similar results with approximately 80% (of 40 amplifications) amplification, and all bands showing strong amplification.

Nuclear DNA is associated with complex protein structures (nucleosomes) (2), which have to be broken down during replication. If DNA does not dissociate from this molecule, it is unavailable to the primers in a PCR, and thus amplification does not occur. The relative ease of release of mitochondrial DNA into the solution where it is then available for PCR rests on three factors: (i) it occurs in an organelle other than the nucleus, (ii) it does not associate with nucleosomes and (iii) it has a high copy number. Extracts therefore contain higher concentrations of mitochondrial than of nuclear DNA (15). The ob-

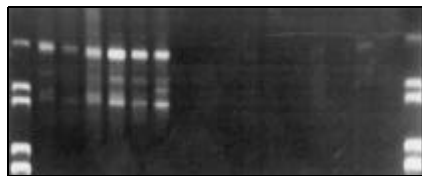


Figure 1. Genomic DNA isolated from *L. saxatilis* embryos. Lanes 1 and 18, Lambda *EcoRI-HindIII* marker DNA (Advanced Biotechnologies Ltd., Epsom, Surrey, UK); lanes 10–17, calmodulin PCR products from samples added to the PCR mixture. Lanes 2–9, calmodulin PCR products from samples that underwent the lysis protocol. Resolution on a 1% agarose gel visualized with ethidium bromide.

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served difference in efficiency of amplification between nuclear and mitochondrial targets, depending on whether lysis was used or not, is probably due to the relative ease with which quantities of DNA from rupturing mitochondria are released, as compared with the release of more sequestered DNA from the cell nucleus.

REFERENCES

1. Barstead, R.J. and R.H. Waterston. 1991. Vinculin is essential for muscle function in the nematode. *J. Cell. Biol.* 114:715-724.
2. Brown, T.A. 1998. Genetics a Molecular Approach. Chapman and Hall, London.
3. Coffroth, M.A. and J.M. Mulawka III. 1995. Identification of marine invertebrate larvae by means of PCR-RAPD species-specific markers. *Limnol. Oceanogr.* 40:181-189.
4. Côté-Real, H.B.S.M., D.R. Dixon and P.W.H. Holland. 1994. Intron-targeted PCR: a new approach to survey neutral DNA polymorphism in bivalve populations. *Mar. Biol.* 120:407-413.
5. Côté-Real, H.B.S.M., P.W.H. Holland and D.R. Dixon. 1994. Inheritance of a nuclear DNA polymorphism assayed in single bivalve larvae. *Mar. Biol.* 120:415-420.
6. Crossland, S., D. Coates, J. Grahame and P.J. Mill. 1993. Use of random amplified polymorphic DNAs (RAPDs) in separating two sibling species of *Littorina*. *Mar. Ecol. Prog. Ser.* 96:301-305.
7. Evans, B.S., R.W.G. White and R.D. Ward. 1998. Genetic identification of asteroid larvae from Tasmania, Australia, by PCR-RFLP. *Mol. Ecol.* 7:1077-1082.
8. Gasser, R.B., N.B. Chilton, H. Hoste and I. Beveridge. 1993. Rapid sequencing of rDNA from single worms and eggs of parasitic helminths. *Nucleic Acids Res.* 21:2525-2526.
9. Geller, J.B., J.T. Carlton and D.A. Powers. 1994. PCR-based detection of mtDNA haplotypes of native and invading mussels on the northeastern Pacific coast: latitudinal pattern of invasion. *Mar. Biol.* 119:243-249.
10. Higuchi, R. 1989. Simple and rapid preparation of samples for PCR, p. 31-38. In H.A. Erlich (Ed.), *PCR Technology*. Stockton Press, NY.
11. Hull, S.L., J. Grahame and P.J. Mill. 1996. Morphological divergence and evidence for reproductive isolation in *Littorina saxatilis* (Oliv.) in northeast England. *J. Mol. Stud.* 62:89-99.
12. Mikhailova, N. and K. Johannesson. 1998. A comparison of different protocols for RAPD analysis of *Littorina*. *Hydrobiologia* 378:33-42.
13. Reid, D.G. 1996. Systematics and Evolution of *Littorina*. The Ray Society, London.
14. White, L.R., B.A. McPheron and J.R. Stauffer, Jr. 1994. Identification of freshwater mussel glochidia on host fishes using restriction fragment length polymorphisms. *Mol. Ecol.* 3:183-185.
15. Wilson, A.C., R.L. Cann, S.M. Carr, M. George, U.B. Gyllenstein, K.M. Helmbjör, R.G. Higuchi, S.R. Palumbi, E.M. Prager, R.D. Sage and M. Stoneking. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. *Biol. J. Linn. Soc.* 26:375-400.
16. Wray, C.G., J.J. Lee and R. DeSalle. 1993. Extraction and enzymatic characterization of foraminiferal DNA. *Micropaleontology* 39:69-73.

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Extracting High-Quality DNA from Shed Reptile Skins: A Simplified Method

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Molecular studies involving reptiles often overlook shed skins as a source for high-quality DNA. In most cases, tissues or blood samples are preferred by researchers, but the process of sampling for these tissue types can be harmful or otherwise adversely affect the animals involved. While reptile breeders or zoological institutions are a potential source of specimens, most will likely decline requests for samples if the sampling will harm their prized animals. Generally, breeders and curators would be much more amenable to part with a shed skin—which is something they usually discard anyway.

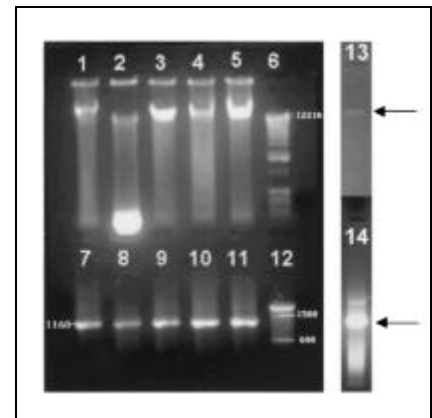


Figure 1. Agarose gel depicting the quality of the genomic DNA extractions produced by this method (lanes 1–5 and 13). Subsequent cytochrome-*b* PCRs generated from these extractions (lanes 7–11 and 14) using the LGL765 primer (5'-GAAAAACCAACGTTGTWATTC-AACT-3') of Bickham et al. (2) and the H15919 primer (5'-GACCCAKCTTTGRTTACAAG-GACAA-3') from this study are also shown. These primers produce a product that is approximately 1160 bp in length. Extractions from the different species are as follows: *L. mexicana*, lane 1; *L. g. floridana*, lane 2; *L. p. pyromelana*, lane 3; *L. alterna*, lane 4; *L. z. zonata*, lane 5. Lane 6 contains a 1-kb DNA Ladder (Life Technologies). Lanes 7–11 contain the same species (in the same order) as lanes 1–5. Lane 12 contains a 100-bp DNA ladder (Life Technologies). Lanes 13 and 14 contain extracted DNA and resulting PCR product, respectively, from the day gecko *P. madagascariensis grandis*.