Agricultural Sciences, 2014, 5, 1097-1099
Published Online October 2014 in SciRes. http://www.scirp.org/journal/as
http://dx.doi.org/10.4236/as.2014.512119

DNA Extraction from Formalin Fixed *Coilia macrognathos* Fin Tissues

Xiping Yuan*, Hong Li, Chongrui Wang, Bo Hong

Fishery Environmental Monitoring Station, Fisheries Research Institute of Hunan Province, Changsha, China
Email: *kerryuan@163.com

Received 10 August 2014; revised 12 September 2014; accepted 13 October 2014

Abstract

Approximately 200 mg of fin tissue from each specimen was cut into pieces and treated with a gradient of ethanol (65%, 70%, 75%, 80%, 85%, 90%, 95% and 100%) and then DNA was extracted from formalin fish fin tissues. Agarose gel visualization of the DNA confirmed DNA extracted. MtDNA cyt *b* gene banding pattern of samples with agarose gel was clearly visible and could be used to be sequenced. In terms of DNA purity, the 260/280 ratio of 87.5% samples ranged between 1.8 and 2.0, indicating that DNA of the majority of the samples was pure. The developed DNA extraction procedure from formalin fish fin tissues by pretreatment with a gradient of ethanol system will be a useful tool to study the genetic structure and phylogenesis of endangered fish, by specimens preserved formalin-fixed in museum and herbarium.

Keywords

Formalin, Fish Fin, Mitochondria DNA

1. Introduction

Formalin can cross-link with the biological macromolecules such as proteins and DNA to produce a stable, closed cross-networking, so that it can maintain a good shape for organism and is widely used for long-term preservation of specimens. Formalin-fixed samples contain DNA, but the classical method was very difficult to extract genomic DNA and DNA fragment degraded seriously [1] [2]. With the birth and development of PCR, it is possible to extract and amplify DNA directly from DNA from formalin fish fin tissues. In this study, the developed DNA extraction method from formalin fish (*Coilia macrognathos*) fin tissues by pretreatment with a gradient of ethanol system is used to study the genetic structure and phylogenesis of endangered fish, using the specimens in museum and herbarium.

*Corresponding author.

How to cite this paper: Yuan, X.P., Li, H., Wang, C.R. and Hong, B. (2014) DNA Extraction from Formalin Fixed *Coilia macrognathos* Fin Tissues. *Agricultural Sciences*, **5**, 1097-1099. http://dx.doi.org/10.4236/as.2014.512119

2. Material and Method

Six specimens of *Coilia macrognathos* were conserved in Fishery Research Institute of Hunan Province for 35 years. Approximately 200 mg of fin tissue from each specimen was cut into pieces and treated with a gradient of ethanol (65%, 70%, 75%, 80%, 85%, 90%, 95% and 100%), each for 20 minutes, centrifuged 3000 g × 15 minutes to gradually remove the formalin. Then they were put into the dryer for overnight. The fin tissue was subsequently digested with 500 μL cell lysate (10 M NaCl, 500 mM Trisbase, 20 mM EDTA and 0.5% SDS, pH 8.0) and 20 μl protenase K (20 mg/ml). The samples were incubated at 55˚C overnight, after which 500 μL of 4.5 M NaCl and 300 μL chloroform were added to each sample. Samples were vortexed for 1 min at maximum speed, and tubes were centrifuged for 10 min at 10,000 *g*. The supernatant was transferred to fresh tubes. An equal volume of isopropanol was added to each sample, mixed well, and samples were incubated at –20˚C for 30 min. Samples were then centrifuged for 20 min, 4˚C, at 10,000 *g*. The deposit was washed with 70% ethanol, dried and finally were dissolved in 500 μL dd H_2O.

The quantity and quality of DNA was measured using a UV-2450 Spectrophotometer (Shimaseiki, JAPAN). Additionally, DNA quality was visually checked on 1.0% agarose gel after staining with ethidium bromide. Furthermore, PCR amplification for mtDNA cyt *b* gene was carried out with a 50 μL reaction volume with a final concentration of 1× *Taq* polymerase buffer and 0.6 U of *Taq* polymerase, 1.5 mM $MgCl_2$, 50 mM of each dNTP and 2.5 pM of each primer. Primers used in the experiment were as follows: L14322 (forward) 5'-CGCCTGTTTATCAAAAACAT-3'; H15576 (reverse): 5'-GCGCTAGGGAGGAATTTAACCTCC-3' for the cyt *b* gene [3]. The thermal profile for hot-start polymerase chain reaction (PCR) included an initial denaturation 94˚ for 5 min, followed by 35 cycles of 30 s at 94˚C, 30 s at 54˚C, 60 s at 72˚C and a final cycle of 10 min at 72˚C. Amplicons were purified with QIAquick kit (QIAGEN) following the manufacturer's instructions. Both strands were sequenced using an ABI 3730XL Genetic analyser (Applied Biosystems, Inc.).

3. Result and Discussion

The results show that DNA can be extracted from formalin fish fin tissues by pretreatment with a gradient of ethanol. Agarose gel visualization of the DNA confirmed this. Genomic dna molecular weight, extracted from *Tenualosa reevesii* formalin-fixed specimens, ranged from 500 to 1800 bp, with that of *Psephuyrus gladius* ranging from 600 to 2000 bp; that of *Lipotes vexillifer* ranging from 200 to 2000 bp [4] [5]. The results suggested there were more consistent with this experimental result. MtDNA cyt b gene banding pattern of samples with agarose gel were clearly visible (**Figure 1**) and could be used to be sequenced. In terms of DNA purity, the 260/280 ratio of 87.5% samples ranged between 1.8 and 2.0, indicating that DNA of the majority of the samples was pure [6].

4. Conclusion

In summary, the developed DNA extraction from formalin fish fin tissues by the pretreatment with a gradient of ethanol system can be a useful tool to study the genetic structure and phylogenesis of endangered fish, using formalin-fixed preserved specimens from museums and herbarium.

Figure 1. The 1140 base pair fragment of the mitochondrial cyt b gene from DNA extracted from formalin fixed fish fin tissues; Lane 1 indicates the molecular weight size marker 500 bp ladder; Lanes 2 - 7 indicates fish specimen 1 - 6; Lane 8: negative control.

Acknowledgements

This study was supported by the project of Fishery Resources and Environment Key Field Station of Upper-middle Reaches of Yangtze River (YWTZ1003), of Key Laboratory of Freshwater Ecology and Aquaculture of Ministry of Agriculture (2010FEA03017) and of Key Laboratory of Freshwater Biodiversity Conservation and Utilization, Ministry of Agriculture (LFBCU0715).

References

[1] Jackson V (1978) Studies on Histone Organization in the Nucleosome Using Formaldehyde as a Reversible Cross-Linking Agent. *Cell*, **15**, 945-954. http://dx.doi.org/10.1016/0092-8674(78)90278-7

[2] Koshiba, M., Ogawa, K., Hamazaki, S. (1993) The Effect of Formalin Fixation on DNA and the Extraction of High Molecular Weight DNA from Fixed and Embedded Tissues. *Pathology Research and Practice*, **189**, 66-72. http://dx.doi.org/10.1016/S0344-0338(11)80118-4

[3] Palumbi, S.R. and Metz, E.C. (1991) Strong Reproductive Isolation between Closely Related Tropical Sea Urchins (genus *Echinometra*). *Molecular Biology and Evolution*, **8**, 227-239.

[4] Xu, L.S., Zhang, Z.B., Song, M.J., Chao, X.P., Wang, F.S. and Zhang, C.G. (2002) A Method of Extracting Genomic DNA from Animal Specimen Preserved in Formalin. *Acta Zoologica Sinica*, **48**, 264-269.

[5] Yang, Z., Zhu, B. and Chang, J.B. (2008) DNA Extraction Formalin-Fixed Tissue of *Psephurus gladius* and the Applicability of Microsatellite Developed from Its Related Species. *Journal of Lake Sciences*, **20**, 242-250.

[6] Henry, R.J. (1997) Practical Applications of Plant Molecular Biology. 1st Edition, Chapman and Hall, London. http://dx.doi.org/10.1007/978-1-4899-3037-8