Short Communication DOI: 10.3724/SP.J.1035.2012.00365

A RAPID PCR-QUALITY DNA EXTRACTION METHOD IN FISH

LI Zhong, LIANG Hong-Wei and ZOU Gui-Wei

(*Yangtze River Fisheries Research Insititute*, *Chinese Academy of Fisheries Science*, *Wuhan* 430223, *China*)

Key words: PCR-quality DNA; Fin of fish; SSR; Mitochondrial
CLC number: Q3-3 **Document code:** A **Article ID:** 1000-3207(2012)02-0365-03

PCR has been a general preferred method for biological research in fish, and previous research have enabled us to extract and purify PCR-quality DNA templates in laboratories [1-4]. The same problem among these procedures is waiting for tissue digesting for a long time. The overabundance time spent on PCR-quality DNA extraction restricts the efficiency of PCR assay, especially in large-scale PCR amplification, such as SSR-based genetic-mapping construction [5, 6], identification of germ plasm resource[7, 8] and evolution research [9, 10], etc. In this study, a stable and rapid PCR-quality DNA extraction method was explored, using a modified alkaline lysis protocol. Extracting DNA for PCR only takes approximately 25 minutes. This stable and rapid DNA extraction method could save much laboratory time and promotes.

1 Materials and Methods

1.1 Material

Fin stored in ethanol, NaOH, Tris-HCL, β-Mercaptoethanol and polyvinyl pyrrolidone. Fish species included zebra fish, silver carp, common carp, yellow catfish, and crucian carp.

1.2 DNA extraction procedure

(1) 0.1g fin preserved in ethanol was put into a 1.5 mL microcentrifuge tube in which containing 200 μL 0.5 mol/L NaOH, 1% β-Mercaptoethanol and 0.5% polyvinyl pyrrolidone. Mix thoroughly by vortexing, and were incubated at 75℃ for 12min.

(2) 200 μL 0.5 mol/L Tris-HCL was added and mixed gentle by invert the tube for 2min to neutralize the alkaline, and were centrifuged for 10min at 13,000 rpm.

(3) 200 μL supernatant was transferred to a new microcentrifuge tube, this supernatant could be used for PCR amplification immediately, or stored at –20℃ for at least 10 days, or alternatively continued step 4 to purify the DNA as conventional protocol .

(4) Add 1/10th volume of 5 mol/L ammonium acetate and 2 volumes ethanol to the DNA sample contained in a 1.5 mL microcentrifuge tube, inverted to mix, placed the sample at –20℃ for at least 10min.

(5) 12,000 rpm in a microcentrifuge tube was centrifuged for 15 minutes at 4℃, and the supernatant was discarded.

(6) 1 mL 80% ethanol was added and the tube inverted for several times to wash the DNA pellet, and was centrifuged again for 5 minutes.

(7) Discard the supernatant, and drain inverted on a paper towel. And then placed the tube in a Savant Speed-Vac and dried the DNA pellet for about 5-10 minutes, or until dry.

(8) Add 10 μL 10:0.1 TE buffer to dissolve dried DNA for frozen storage.

1.3 The quantity and quality assay of DNA

DNA quality assay was carried out by 1.0% agarose gel electrophoresis[1], DNA concentration and purify factor A_{260}/A_{280} ratios were obtained by spectrophotometry.

1.4 PCR amplification for SSR and mitochondrial genes

PCR amplification reactions were carried out in a total volume of 20 μL containing 1.5U *Taq* DNA polymerase (TaKaRa), 2μL 10×PCR buffer (100 mM Tris-HCl, 500 mM KCl, 15mM $MgCl_2$; TaKaRa), 0.4 μL 10 mM dNTP (TaKaRa) and about 100 ng DNA template on a GeneAmp® PCR System 9700 (Applied Biosystems). The thermo-cycling conditions were as follows: predenaturing at 94℃ for 5min, followed by 32 cycles of denaturing at 94℃ for 45s, annealing at the temperature appropriate for each pair of primers listed in Tab. 1 for 45s and extending at 72℃ for 45s with a final extension at 72℃ for 10min.

Tab. 1 The primer sequences and Tm values used in this research

Primer name	Primer sequences (5′-3′)	Tm (℃)
1	F:TTTAATTCTTCTAGCTGGACACG R:CACTCCTCTTCCCTCGTAAAT	55
3	F:TGAGCMTCWAATTCMAAATA R:CGKAGRTAGAAGTAKAGGCT	55
8	F:TGYGGAGCWAATCAYAGCTT R:CTGTGGTGAGCYCAKGT	55
10	F:TTCTGAGCCTTCTAYCA R:CAAGACKGKGTGATTGGAAG	55
11	F:TTTGAYCTAGAAATYGC R:GATTATWAGKGGGAGWAGTCA	55

Received date: 2011-03-21; **Accepted date:** 2011-06-24
Foundation item: Special Fund for Agro-scientific Research in the Public Interest:200903045; Natural Science Foundation of China: 31101894
Brief introduction of author: lizhong, PhD in genetics, major in freshwater fish breeding, E-mail: lizhong@yfi.ac.cn
Corresponding author: Zou Gui-Wei, E-mail: zougw@yfi.ac.cn

Amplification products for SSR analysis were resolved via 12% nondenaturing polyacrylamide gel and visualized by silver staining. In addition, application products for mitochondrial genes were tested by 1.0% agarose gel electrophoresis and DNA ladder (Promega) was used as a reference marker for allele size determination.

2 Results and discussion

2.1 DNA quality assay

DNA in the supernatant was tested by 1.0% agarose gel electrophoresis, and the result showed that the DNA was degenerated partially (Fig. 1). In the DNA extraction procedure, high concentration of NaOH caused the linear DNA to fragmentize, though 1% β-Mercaptoethanol and 0.5% polyvinyl pyrrolidone were added to prevent DNA fragment degenerating. In step (3), A_{260}/A_{280} ratios were less than 1.7 or more than 2.0, which indicated there was either protein or RNA contamination in the DNA solution; DNA concentration varied from 4025 μg/mL to 6375 μg/mL (Tab. 2). In step (8), A_{260}/A_{280} ratios ranged from 1.81 to 1.92 (Tab. 2), indicating pure DNA. DNA quantity both in step (3) and step (8) were sufficient for PCR amplification.

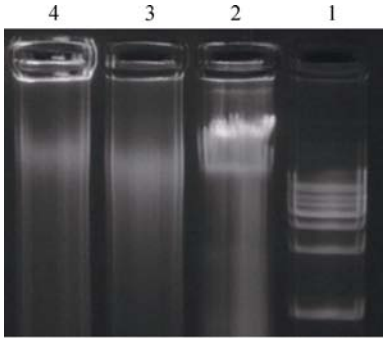

Fig. 1 DNA quality tested by 1.0% agarose gel electrophoresis
lane 1:5000 bp maker; lane 2-4: 1 μL DNA samples obtained from step (3) were loaded per lane

Tab. 2 The DNA assay of common carp

Sample	DNA parameter	DNA in step (3)	DNA in step (8)
1	A_{260}/A_{280}	2.70	1.92
	μg/mL	4965	380
2	A_{260}/A_{280}	2.46	1.81
	μg/mL	4740	490
3	A_{260}/A_{280}	1.63	1.86
	μg/mL	2680	470
4	A_{260}/A_{280}	1.58	1.85
	μg/mL	5440	675

2.2 PCR amplification for SSR and mitochondrial gene analysis.

SSR is widely used for genetic-linkage mapping construction, evolutionary research and molecular marker-assisted breeding, etc, for its stability, rapidness and accuracy. The DNA in the supernatant in this research could be used for SSR amplification (Fig. 2). To evaluate whether the DNA could be used for longer fragment PCR amplification, mitochondrial genes were amplified (Fig. 3). The largest fragment in lane 2 was approximate 1800 bp. Thus DNA in this study could be applied for mitochondrial gene amplification. As documented above, agarose gel electrophoresis appeared DNA was degenerated partially, but the partial DNA fragmentation did not bring evident negative effect to the PCR amplification.

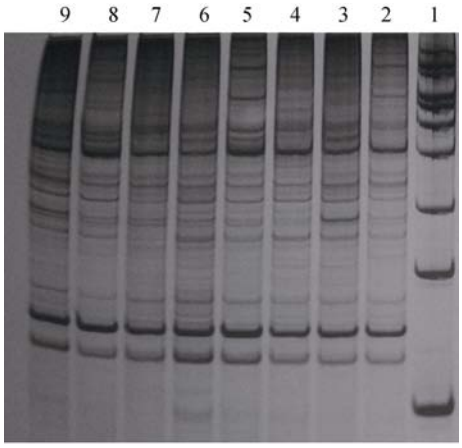

Fig. 2 PCR result of SSR for silver carp
lane 1, 1000 bp maker; lane 2-9, sample of yellow silver carp individual DNA; DNA obtain from step (3), primer name 1(see Tab. 1)

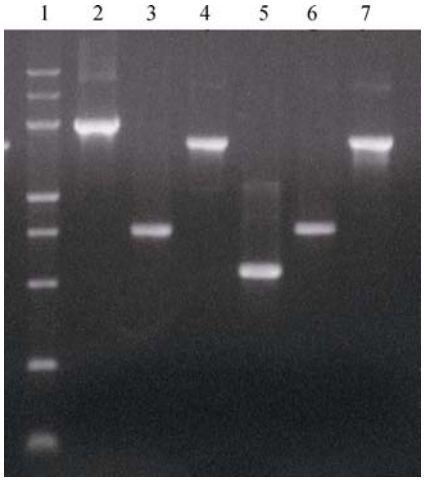

Fig. 3 PCR amplification of mitochondrial gene of common carp
lane 1, 5000 bp marker; lane 2, primer name3; lane 3, 6, primer name11; lane 4, 7, primer name8: lane 5, primer name 10; DNA obtain from step (3)

3 Conclusion

The advantages of this DNA extraction protocol were: 1, rapidness, the new method including three steps took only approximately 25 minutes to get PCR-quality DNA sample; 2, inexpensiveness, as only NaOH, Tris-HC1, β-Mercaptoethanol and polyvinyl pyrrolidone (PVP) were necessary; 3, the PCR effect was the same as other protocol; 4, purified DNA could be frozen storage for future use. The shortcoming of this method was: DNA sample from step (3) could be stored for only 10 days, and the DNA was degenerated partially.

Despite of a little disadvantage documented above, considering its rapidness and efficiency, this method could be utilized into the large-scale PCR assay research.

References:

[1] Sambrook J, Fritsch E F, Maniatis T. Molecular Cloning: a Laboratory Manual [M]. New York: Cold Spring Harbor Laboratory Press. 1989, 101—102

[2] Taggart J B, Hynes R A, Prodoh P A, *et al*. A simplified protocol for routine total DNA isolation from salmonid fishes [J]. *Journal of Fish Biology*, 1992, **40**(6): 963—965

[3] Aljanabi S M, Martinez I. Universal and rapid salt-extracted of high quality genomic DNA for PCR-based techniques [J]. *Nucleic Acids Research*, 1997, **25**(22): 4692—4693

[4] Kumar R, Singh P J, Nagpure N S, *et al*. A non-invasive technique for rapid extraction of DNA from fish scales [J]. *Indian Journal of Experimental Biology*, 2007, **45**(11): 992—997

[5] Nichol K M, Young W P, Danzmann R G. A consolidated linkage map for rainbow trout [J]. *Animal Genetics*, 2003, **34**(2): 102—115

[6] Lee B Y, Hulata G, Kocher T D. Two unlinked loci controlling the sex of blue tilapia [J]. *Heredity*, 2004, **92**(6): 543—549

[7] Aldbieser G C, Wolters W R. Definition of the USDA103 strain of channel catfish (*Ictalurus punctatus*) [J]. *Animal Genetics*, 2007, **38**(2): 180—183

[8] Lü Y P, Hu Z H, Wang C H, *et al*. SRAP and SCAR molecular marker related to body color of “Whole red” patterns of *Cyprinus carpio var. color* [J]. *Acta Hydrobiologica Sinica*, 2011, **35**(1): 45—50

[9] Maggio T, Andaloro F, Hemida F. A molecular analysis of some Eastern Atlantic grouper from the Epinephelus and Mycteroperca genus [J]. *Journal of Experiment Marine Biology and Ecology*, 2005, **321**(1): 83—92

[10] Wang L, Wang X Z, He S P. Phylogenetic relationships of seven barred species of acrossocheilus based on sequences of the mitochondrial DNA ND4 gene, with doubt on the taxonomic status of *Acrossocheilus hemispinus* [J]. *Acta Hydrobiologica Sinica*, 2010, **34**(6): 1218—1222