

## PE-4

# DNA Profiles Analysed by Polymerase Chain Reaction-Random Amplified Polymorphic DNAs in Shortnecked Clam (*Ruditapes philippinarum*) Populations

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## ABSTRACT

Out of 20 primers, 6 generated a total of 1,111 major and minor RAPD bands, producing approximately 4.2 average polymorphic bands per primer in shortnecked clam (*Ruditapes philippinarum*) population from Anmyeondo. The bandsharing value altered from 0.15 to 0.74, with the average of 0.51, as calculated by bandsharing analysis. The RAPD profiles obtained with DNAs of two populations from Anmyeondo and Seocheon, respectively, were considerably different (0.20 and 0.51, respectively). The varying degrees of difference among populations may also be of relevance to the restricted hybridization of wild bivalve. Besides gene mapping and breeding applications, PCR-RAPD system could be very useful for the rapid certification and quality control of seed production and for every projects based on PCR amplification of specific bivalve DNA fragments.

## INTRODUCTION

As in bivalve species, the wild shortnecked clam, population/density of this mollusks is decreased significantly owing mainly to imprudent tidal land reclamation project and reckless development during the last four decades. In spite of its economic and scientific importance, little information is available on the phylogenetic relationships among a few of wild shortnecked clam populations in Korea. Thus, the applications of RAPD to aquaculture had been to identify genetic similarity and diversity between a few of fish species and mollusks apart from geographic sites (Klinbunga et al., 2000). In the present work, this research was made by PCR-RAPD using two decades of random primers and also by BS analysis in order to identify genetic differences within and between populations in wild shortnecked clam (*Ruditapes philippinarum*).

## MATERIALS AND METHODS

### **Muscle collection, Sources of genomic DNA, primer, amplification and data analysis**

Muscle samples collected from shortnecked clam (*Ruditapes philippinarum*) were refrigerated at -70°C until use. Samples of sliced muscle were placed into 10 ml test tubes, to which an 4 volumes of lysis buffer I was added. In order to achieve good results, DNA extraction should be performed according to the general separation and extraction procedures. Purity was estimated by calculating the ratios of the absorbance measured at 260~280 nm. The final

concentration was estimated by agarose electrophoresis and EtBr staining. Bandsharing of DNA products was quantified using the formula of Jeffreys and Morton (1987): If the comparison between the three lanes, the formula would be:  $BS = 3(Nabc)/(Na+Nb+Nc)$  etc. Only bands which were readily visible were scored.

## RESULTS AND DISCUSSION

Six primers yielded a total of 533 polymorphic bands, of which 278 bands in shortnecked clam from Anmyeondo and 255 in that from Seocheon (Table 1). The values in shortnecked clam population from Seocheon altered from 0.15 to 0.74 as calculated by bandsharing analysis, as summarized in Table 1. The average level of bandsharing value was approximately  $0.20 \pm 0.05$  in shortnecked clam population from Anmyeondo. However, the average level of BS value obtained in shortnecked clam population from Seocheon was  $0.51 \pm 0.08$  which was higher than that in that from Anmyeondo. BS value generated by primer OPA-08 was lower than any other primers in shortnecked clam population from Seocheon, which was 0.15. In the long run, besides gene mapping and breeding applications, PCR-RAPD system could be very useful for the rapid certification and quality control of seed production and for all projects based on PCR amplification of specific bivalve/fish DNA fragments (Tassanakajon et al., 1998).

Table 1. Number and bandsharing of polymorphic products generated by 6 random primers of shortnecked clam (*R. philippinarum*) from two sites (11 bivalves/primer). \* Standard error

Primer	Number of polymorphic products		Average number of polymorphic products		BS values	
	Anmyeondo	Seocheon	Anmyeondo	Seocheon	Anmyeondo	Seocheon
OPA-08						
OPA-09	23	27	2.1	2.5	0.14	0.15
OPA-11	66	55	6.0	5.0	0.26	0.66
OPA-16	61	54	5.5	4.9	0.10	0.48
OPA-18	52	30	4.7	2.7	0.42	0.74
OPA-20	63	63	5.7	5.7	0.11	0.44
Subtotal	278	255	25.2	23.2	1.18	3.05
Total	533		48.4		$0.20 \pm 0.05^*$	$0.51 \pm 0.08^*$
Average	46.3	42.5	4.2	3.9		

## REFERENCES

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