DNA microarray for rapid detection of mitochondrial DNA SNPs in chum salmon

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We recently detected 20 variable nucleotide sites in about 500 bp sequence from the 5' end of the mitochondrial (mt) DNA control region of chum salmon (*Oncorhynchus keta*), which defined a total of 30 haplotypes of three genealogical clades (A, B, and C) in more than 2,100 individuals of 48 populations in the Pacific Rim (Sato et al. 2004). The observed mtDNA haplotypes are mostly associated with geographic regions, and expected to become a useful marker for the genetic stock identification (GSI). In an attempt to develop a rapid and accurate method to identify the 30 haplotypes, we applied DNA microarray hybridization technique for instantaneous detection of the observed 20 SNPs in the 5' mtDNA control region of chum salmon.

The DNA microarray method includes; 1) immobilization of synthesized 17 to 20 mer oligonucleotides containing the variable nucleotide on slide glass pre-coated with poly-carbodiimide resin, 2) two-hour hybridization with DNA microarray of biotinylated PCR fragments spanning the 5' variable portion and subsequent short washing, and 3) visualization of hybridization signals colored by conventional ABC method and comparison of scanner-taking signal image on a computer. Entire process excluding DNA extraction was completed within eight hours. The obtained DNA microarray could detect all the SNPs defining the above 30 haplotypes.

The developed DNA microarray was applied for estimating the stock composition of more than 3,200 chum salmon collected from 41 stations in the Bering Sea and/or North Pacific Ocean during research cruises of RV Kaiyo-maru September 2002 and 2003. About 1,000 samples were analyzed with DNA microarray on board ship during the cruise of 2002. Distribution of haplotypes in the Bering Sea and adjoining waters was similar in both years. The clade A and C haplotypes representing Japanese and Russian stocks were predominant in central Bering Sea, whereas, in other areas, the clade B haplotypes mostly representing North American stocks tended to predominate over the other two clades. Thus, SPAM analysis using the mtDNA microarray haplotype data demonstrated non-random distribution of chum stocks, i.e., predominance of Japanese stocks in central Bering Sea, Russian stocks in southern and western Bering Sea, and North American stocks around the Aleutian Islands.

Our DNA microarray technique will become a useful, practical post-sequencing method for chum salmon GSI on land and on board ships, and also for similar kind of studies in other animal species.