Development of DNA Microarray for Rapid Detection of Mitochondrial DNA Haplotypes of Chum Salmon

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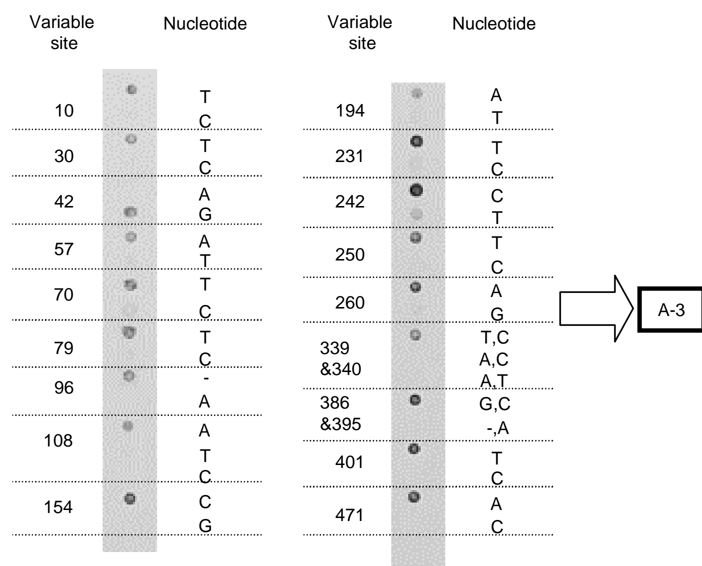
Molecular techniques to assess the genetic variation of fish populations have been suggested as a promising means of genetic stock identification (GSI) of salmon (Ferguson et al. 1995). We recently have detected a greater variation in the mitochondrial DNA (mtDNA) control region of chum salmon (*Oncorhynchus keta*) by nucleotide sequence analysis than the variation detected by the analysis of restriction fragment length polymorphisms (Sato et al. 2001, in press). Base substitutions and indels observed in 20 sites of the 5' half of the mtDNA control region defined a total of 30 haplotypes in more than 2,000 individuals from 48 populations collected from Japan, Korea, Russia, and North America, serving as a useful tool for phylogeographic analysis of Pacific Rim populations (Sato et al. 2001, in press). However, nucleotide sequence analysis requires specialized, expensive laboratory equipment and expert skill. In addition, time-consuming sequence analysis may not be suitable for salmon stock identification that will need a large number of samples. We attempted to develop a rapid and accurate method to detect mtDNA haplotypes of chum salmon for GSI using DNA microarray on a slide glass, which immobilized synthetic oligonucleotides containing the reported polymorphic nucleotide sites in the control region (Sato et al. 2001, in press). The developed DNA microarray was evaluated for determination of mtDNA haplotypes of nearly 1000 chum salmon collected during the research cruise of R/V *Kaiyo maru* of the Fisheries Agency, Japan, in the Bering Sea September 2002.

The method includes; 1) immobilization of synthesized 17 to 20 mer oligonucleotides containing the variable nucleotide on slide glass pre-coated with Poly-carbodiimide resin (CarboStation), 2) PCR amplification of the 5' variable portion with biotinylated primer from mtDNA extracted as previously described (Sato et al. 2001, in press), 3) two-hour hybridization of biotinylated PCR fragments with DNA microarray and subsequent short washing, and 4) visualization of hybridization signals colored by conventional ABC method and comparison of scanner-taking signal image on a computer. All the process of hybridization and detection was completed within eight hours.

As shown in Fig.1, the oligomer spots with intense hybridization signals were thought to contain perfectly matched sequence with a single nucleotide variation in the PCR fragments, whereas those with faint or no signals were thought to have no such sequence identity in the fragments. Therefore, an intense hybridization signal at each variable nucleotide site was taken as positive, and those positive sites were aligned to determine a corresponding

Fig.1. DNA microarray detection of polymorphic sites in the 5' half of the mtDNA control region sequence of chum salmon. The number of the variable sites indicates nucleotide position from the 5' end of the mtDNA control region. Polymorphic nucleotide in the right of each signal positive oligomer corresponds to those presented in Table 1. Alignment of the signal positive polymorphic sites indicates the haplotype to be A-3 (see also Table 1).



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haplotype (Fig. 1). Likewise, all the single nucleotide mutations defining a total of 30 haplotypes were detected by the obtained DNA microarray (Table 1). In fact, haplotype determination of about 40 chum salmon by the present DNA microarray was perfectly concordant with the results from direct sequence analysis, which further confirmed the accuracy of the DNA microarray method for detection of sequence variation.

Since the present method does not require any specialized laboratory equipment, capability of this technique to identify mtDNA haplotypes on ship for commercial fisheries was tested using 978 chum salmon collected from 17 stations in the Bering Sea (172°30'E–172°30'W 51°30'N–58°30'N) during the *Kaiyo maru* research cruise September 2002. Figure 2 shows the distribution of chum salmon mtDNA haplotypes per station that were identified immediately after catch on-board the ship. All the haplotype analysis using the DNA microarray was completed within one month. The observed distribution of mtDNA haplotypes was nonrandom, showing a predominance of groups A and C haplotypes in the central Bering Sea (180°–177°30'W 56°N–58°30'N). In other areas, group B haplotypes tended to predominate over the other two haplotype groups, although the occurrence of the haplotype B-3 was common in all the locations. These results strongly suggest the abundance of Japanese and Russian stocks in the Bering Sea, since the observed groups A and C haplotypes were specific to or predominant in Japan and Russia (Sato et al. in press). Specifically, the abundance of Japanese stocks in the central Bering Sea was inferred from the occurrence of the A-3, A-7, A-8, C-3, and C-4 haplotypes, all of which were limited to Japan (Sato et al. in press).

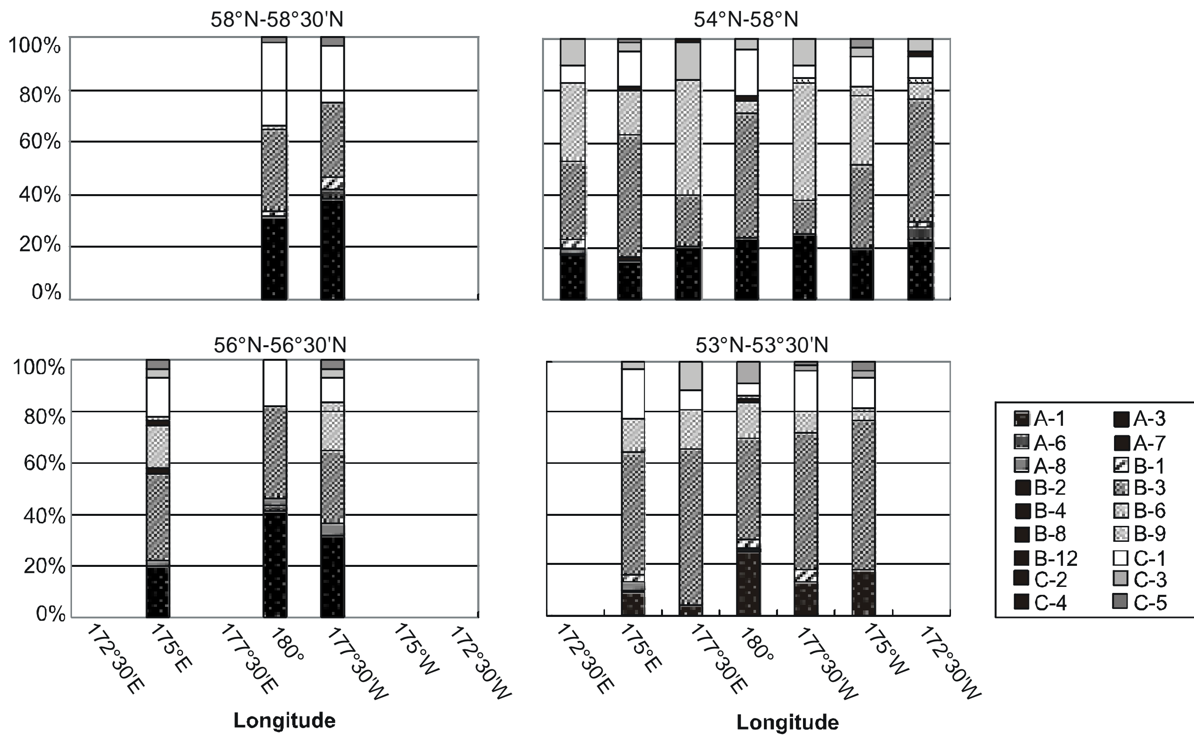
Thus, the present findings suggest that the DNA microarray method is accurate and time effective, and suitable for analyzing large numbers of samples for chum salmon GSI in the field or on-board ship.

Table 1. Thirty haplotypes defined by 20 variable nucleotide sites.

Haplotype \ Variable site	10	30	42	57	70	79	96	108	154	194	231	242	250	260	339, 340	386, 395	401	471
A-1	T	T	A	A	T	T	-	A	C	A	T	C	T	A	T,C	G,C	T	A
A-2	C	T	A	A	T	T	-	A	C	A	T	C	T	A	T,C	G,C	T	A
A-3	T	T	G	A	T	T	-	A	C	A	T	C	T	A	T,C	G,C	T	A
A-4	T	T	A	A	T	T	-	C	C	A	T	C	T	A	T,C	G,C	T	A
A-5	T	T	A	A	T	T	-	A	C	T	T	C	T	A	T,C	G,C	T	A
A-6	T	T	A	A	T	T	-	A	C	A	C	C	T	A	T,C	G,C	T	A
A-7	T	T	A	A	T	T	-	A	C	A	T	C	T	A	T,C	G,C	T	C
A-8	T	T	A	A	T	T	A	A	C	A	T	C	T	A	T,C	G,C	T	A
B-1	T	T	A	A	T	T	-	A	C	A	T	C	T	A	T,C	-,A	T	A
B-2	T	C	A	A	T	T	-	A	C	A	T	C	T	A	T,C	-,A	T	A
B-3	T	T	A	A	T	T	-	A	G	A	T	C	T	A	T,C	-,A	T	A
B-4	T	T	A	A	T	T	-	A	C	A	C	C	T	A	T,C	-,A	T	A
B-5	C	T	A	A	T	T	-	A	G	A	T	C	T	A	T,C	-,A	T	A
B-6	T	T	A	A	C	T	-	A	G	A	T	C	T	A	T,C	-,A	T	A
B-7	T	T	A	A	T	C	-	A	G	A	T	C	T	A	T,C	-,A	T	A
B-8	T	T	A	A	T	T	-	C	G	A	T	C	T	A	T,C	-,A	T	A
B-9	T	T	A	A	T	T	-	A	G	A	C	C	T	A	T,C	-,A	T	A
B-10	T	T	A	A	T	T	-	A	G	A	T	T	T	A	T,C	-,A	T	A
B-11	T	T	A	A	T	T	-	A	G	A	T	C	C	A	T,C	-,A	T	A
B-12	T	T	A	A	T	T	-	A	G	A	T	C	T	G	T,C	-,A	T	A
B-13	T	T	A	A	T	T	-	A	G	A	T	C	T	A	A,C	-,A	T	A
B-14	T	T	A	A	T	T	-	A	G	A	T	C	T	A	T,C	-,A	C	A
B-15	T	T	A	A	T	T	-	A	G	A	T	C	T	A	T,C	-,A	T	C
B-16	T	T	A	A	T	T	-	A	G	A	T	C	T	A	A,T	-,A	T	A
B-17	T	T	A	A	T	T	-	A	G	A	T	C	T	A	A,C	-,A	C	A
C-1	T	C	A	A	T	T	-	A	C	A	T	C	T	A	T,C	G,C	T	A
C-2	T	C	A	T	T	T	-	A	C	A	T	C	T	A	T,C	G,C	T	A
C-3	T	C	A	A	C	T	-	A	C	A	T	C	T	A	T,C	G,C	T	A
C-4	T	C	A	A	T	T	-	T	C	A	T	C	T	A	T,C	G,C	T	A
C-5	T	C	A	A	T	T	-	A	C	A	C	C	T	A	T,C	G,C	T	A

Number at variable site shows nucleotide positions from the 5' end of the mtDNA control region of chum salmon.

Fig.2. Distribution of chum salmon mtDNA haplotypes in 17 stations in the Bering Sea (172°30'E–172°30'W 51°30'N–58°30'N) during the Kaiyo maru research cruise September 2002. The observed distribution of mtDNA haplotypes showed a predominance of groups A and C haplotypes in the central Bering Sea (180°–177°30'W 56°N–58°30'N). In other areas, group B haplotypes tended to predominate over the other two haplotype groups, although the occurrence of the haplotype B-3 was common in all the locations.



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