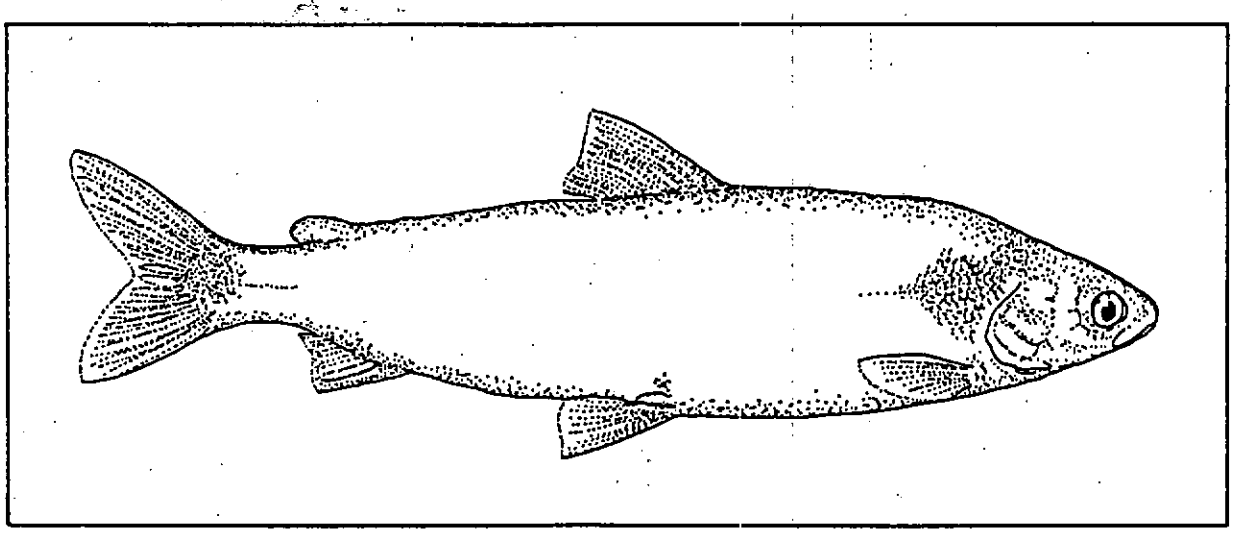
OCS Study
MMS 92-0039

GENETIC STUDIES OF THE ARCTIC CISCO (Coregonus autumnalis) IN ALASKA — MULTIPLE STOCKS?



U.S. Department of the Interior
Minerals Management Service
Alaska OCS Region

OCS Study
MMS 92-0039

GENETIC STUDIES OF THE ARCTIC CISCO (Coregonus autumnalis) IN ALASKA — MULTIPLE STOCKS?

Prepared by

LGL Ecological Research Associates, Inc.

1410 Cavitt Street
Bryan, Texas 77801

Funded by

U. S. DEPARTMENT OF INTERIOR

Minerals Management Service and
Alaska Fish and Wildlife Research Center
U. S. Fish and Wildlife Service, Region 8

and

BP EXPLORATION (ALASKA) INC.

U.S. Department of the Interior
Minerals Management Service
Alaska OCS Region

July 1992

The statements and conclusions contained in this report are those of the authors and do not necessarily reflect the view of the U.S. Department of the Interior, nor does mention of trade names or commercial products constitute endorsement or recommendation for use by the Federal Government.

CONTENTS

Page

EXECUTIVE SUMMARY 1

MANUSCRIPT 1 BIOGEOGRAPHY, PHYLOGENY, AND RELATIVE RATES OF EVOLUTION OF MITOCHONDRIAL DNA IN ARCTIC AND BERING CISCOES FROM THE WESTERN ARCTIC OF NORTH AMERICA. John W. Bickham, John C. Patton, Benny J. Gallaway, and Juan Carlos Morales 7

MANUSCRIPT 2 IDENTIFICATION OF ARCTIC AND BERING CISCOES IN THE COLVILLE RIVER FISHERY. John W. Bickham, John C. Patton, Shamone Minzenmayer, L. L. Moulton, and Benny J. Gallaway 49

MANUSCRIPT 3 GENETIC STOCK ASSESSMENT OF ARCTIC CISCO (COREGONUS AUTUMNALIS) FROM THE BEAUFORT SEA USING NUCLEOTIDE SEQUENCES OF THE MITOCHONDRIAL CONTROL REGION. John W. Bickham, John C. Patton, and Benny J. Gallaway 63

APPENDICES 78

APPENDIX I Table of Restriction Site Analysis 79
APPENDIX II Table for 1992 Restriction Site Analyses for Colville, Sagavanirktok, Peel, Arctic Red Rivers and Prudhoe Bay Samples 81
APPENDIX III Chromatograms of Different Sequence Haplotypes 91
APPENDIX IV Sequence of Arctic Cisco Haplotypes 109
APPENDIX V Sequence Alignments of A Haplotypes 115
APPENDIX VI Sequence Alignments of C Haplotypes 126
APPENDIX VII Table of Composite Haplotypes 132
APPENDIX VIII Tables of Scorings of Individuals for Composite Haplotytpes 134
APPENDIX IX Table of Composite Haplotype Distributions by Locality 145
APPENDIX X Nuclear DNA Investigations 147

EXECUTIVE SUMMARY

EXECUTIVE SUMMARY

In spring of 1991, genetic studies of the Arctic cisco (Coregonus autumnalis) were jointly-funded by the U. S. Department of Interior (Minerals Management Service and U. S. Fish and Wildlife Service, Region 8) and BP Exploration (Alaska) Inc. The overall goals of these investigations were to 1) determine whether the Arctic cisco in Alaska were representatives of a source population associated with a specific drainage, such as the Peel River, or a random composite of several or all of the spawning populations of this species represented in the Mackenzie River and 2) determine if molecular genetic data could be used to estimate the proportion of the source population(s) of Canadian Arctic cisco that used Alaska for rearing. Additional questions to be addressed were the distinctiveness of Bering cisco (Coregonus laurettae) as compared to Arctic cisco, and whether Bering cisco in the Alaskan fishery were being confused with Arctic cisco.

The proposed study focused on restriction-enzyme assays of six regions of the mitochondrial DNA (mtDNA) molecule as the primary means to meet study objectives, and also included a small-scale pilot investigation to experimentally determine the utility of using nuclear DNA to better define the genetic diversity of the target species. Subsequent to receipt of the Contract, DNA sequencing capabilities added to our laboratory were used to conduct a mtDNA sequence analysis of key samples in the control region of the molecule. The findings of this investigation were used to evaluate the results yielded by the restriction-enzyme assay approach.

The results of the study have been prepared in manuscript format and will be or have been submitted to scientific journals for possible publication. This

report, submitted simultaneously to each of the program sponsors, is comprised of the subject manuscripts, with each presented in the style required by the target journal, and a data appendix.

The first manuscript, "**Biogeography, Phylogeny, and Relative Rates of Evolution of Mitochondrial DNA in Arctic and Bering Ciscoes from the Western Arctic of North America**", has been submitted for publication in the journal Evolution. This manuscript describes an extensive study of variation of mitochondrial DNA from these two species using restriction-enzyme assays performed on PCR amplified DNA. The major contributions of this manuscript include 1) clarification of the systematic status and relationship of these two closely related species; 2) documentation of the remarkably different amounts of variation expressed by these two species; 3) documentation that different regions of the mitochondrial DNA molecule evolve at different rates in closely related species; 4) a method for the identification of these two species using mitochondrial DNA; and 5) a map of the distribution of mitochondrial DNA haplotypes along the Beaufort Sea coast and throughout the Mackenzie River drainage.

The second manuscript, "**Identification of Arctic and Bering Ciscoes in the Colville River Fishery**", has been submitted for publication in the American Fisheries Society Symposium on Arctic Fish Biology. This paper describes an experiment to test the ability of the fishery biologists, who are conducting studies on Arctic cisco population dynamics at the Colville River, to accurately differentiate Arctic from Bering ciscoes in the field. This is important because the ranges of the two species overlap in this locality and, in some years, the numbers of Bering ciscoes could be great enough to cause misinterpretation of the population data if many Bering ciscoes were misidentified as Arctic ciscoes. The

results showed that the field biologists accurately identified all but two of the fish (N = 69) in the study. However, it was concluded that two possibly misidentified specimens were, in fact, of hybrid ancestry. Thus, this study confirms the accuracy of the field biologists and documents the introgression of mitochondrial DNA from the Bering cisco into the Arctic cisco.

The third manuscript, "**Genetic Stock Assessment of Arctic Cisco (Coregonus autumnalis) from the Beaufort Sea using Nucleotide Sequences of the Mitochondrial Control Region**", is being prepared for submission to the journal Science and focuses on the usefulness of mitochondrial DNA sequence data for genetic stock identification of Arctic ciscoes. This manuscript has not yet been submitted for publication, but will be in August or September of 1992. This study reports nucleotide sequence data for 326 fish from four coastal sites and four Mackenzie River spawning sites. These fish were also studied for restriction-site variation and reported in the first manuscript of this report. We did not report all of the sequence data available in this paper because some localities (Point Barrow and Kaktovik) had sample sizes that were too small to be of value. We also did not use the Liard River spawning sample because previous genome-size studies showed conclusively that the majority of the animals from this stock stayed in the Mackenzie Bay. The results of this study showed: 1) multiple stocks occur in the Mackenzie River population of Arctic cisco; 2) the stocks only weakly differentiated genetically; and 3) the Peel River stock, and to a lesser extent the Arctic Red River stock, predominates in Alaskan waters. Thus, it is the Peel River stock that should be most closely monitored for potential effects of causeways on fish survival and migration.

One objective of the study that is not directly addressed in any of the three manuscripts is the determination of whether or not significant year-to-year

variation occurs in the samples from coastal sites and spawning sites. We analyzed samples from the Sagavanirktok River, Peel River, and Arctic Red River collected in 1985 and 1988, then compared their haplotype frequencies using the Chi-square exact test. The probabilities of identity for each comparison (1985 x 1988 for each locality) were P = 0.423, 0.817, and 0.111, respectively. It is apparent from this analysis that, although considerable variation in haplotype frequency occurs among years (especially in the two spawning sites), none of the comparisons were significant at either $\alpha = 0.05$ or 0.10. Therefore, the samples from different years were combined in the stock assessment manuscript.

The final part of this report consists of the appendices. The first nine of these present the mtDNA data on which the three papers included in this report were based. Thus, restriction haplotypes (and their identifications) are presented for all localities as are the nucleotide sequences. The mtDNA appendices only include data from the 1991-1992 study and not the restriction-site data from the 1990-1991 study. The appendices are particularly important as supportive material for the third manuscript. They also afford reviewers sufficient information to evaluate the conclusions of all the report in a way not possible from the summary data in the manuscripts because the manuscripts were designed to be published in peer-reviewed journals. The last appendix summarizes the results of our pilot studies of nuclear DNA.

To what extent were the overall goals of the project achieved? Arctic cisco from Canada using Alaska appear to be predominantly of Peel River origin, not a random mixture of spawning populations. Molecular genetics data do not provide a basis for determining the proportions of the stock(s) involved, <u>but</u> have provided a basis for determining where to look — tributaries of the lower

Mackenzie River Basin. The source population(s) there would need to be examined for an Alaskan mark, which would not be genetic in nature (e.g., isotope ratios, parasites).

MANUSCRIPT 1

BIOGEOGRAPHY, PHYLOGENY, AND RELATIVE RATES OF EVOLUTION OF MITOCHONDRIAL DNA IN ARCTIC AND BERING CISCOES FROM THE WESTERN ARCTIC OF NORTH AMERICA

John W. Bickham, John C. Patton, Benny J. Gallaway,
and Juan Carlos Morales

Submitted To Evolution

BIOGEOGRAPHY, PHYLOGENY, AND RELATIVE RATES OF EVOLUTION OF MITOCHONDRIAL DNA IN ARCTIC AND BERING CISCOES FROM THE WESTERN ARCTIC OF NORTH AMERICA

John W. Bickham,[1,2] John C. Patton,[1] Benny J. Gallaway,[1]
and Juan Carlos Morales[2]

[1]LGL Ecological Genetics, 1410 Cavitt St., Bryan, Texas 77801

[2]Department of Wildlife and Fisheries Sciences, Texas A&M University
College Station, Texas 77843

Abstract.--Restriction-endonuclease analyses of mitochondrial DNA (mtDNA) was used to investigate evolutionary and biogeographic relationships of ciscoes from the western Arctic of Canada and Alaska. A total of 488 fish was examined from spawning runs of Arctic ciscoes (*Coregonus autumnalis*) in five tributaries of the Mackenzie River, one spawning run of Bering ciscoes (*C. laurettae*) from the Yukon River, and seven coastal sites from the Beaufort Sea which represent mixed populations. Our methodology employed the amplification of approximately 2-kb sized regions of the mtDNA molecule using the Polymerase Chain Reaction (PCR). Six regions were investigated as to their usefulness in differentiating haplotypes in a subset of samples. The most useful regions (ORF1, ORF2, ORF5-6) were used for all fish. Each region was analyzed using 20 restriction enzymes with four to six-base recognition sequences for the majority of samples. Haplotypes were determined by a new procedure called the shared-fragment pattern method. Extensive nucleotide divergence was noted between haplotypes characteristic of Arctic and Bering ciscoes which is consistent with the latter

having descended from a population inhabiting the Beringian Refugium and the former from a population inhabiting the Mississippian Refugium or Siberia during the Pleistocene. Samples taken from coastal sites in the Beaufort Sea represent a mixture of Arctic and Bering cisco haplotypes with the frequency of the latter being highest at Point Barrow and decreasing toward the east. The presence of fish with Bering cisco haplotypes in the spawning runs of Arctic cisco is evidence of genetic introgression and is consistent with reported patterns of allozyme variation. Nucleon diversity is much greater in Bering ciscoes than in Arctic ciscoes presumably as a result of the Yukon River being unglaciated whereas the Mackenzie River was covered with ice during the Wisconsin glaciation. Both cluster analyses of the proportions of shared fragment patterns and nucleon diversity indices (estimated separately for three different regions of the mitochondrial DNA molecule) show evidence of an increased evolutionary rate in Arctic ciscoes relative to Bering ciscoes for the ORF5-6 region. This study illustrates the advantages of using a PCR based restriction-enzyme analysis for investigating genetic variation in natural populations. Data from large sample sizes can be generated quickly, relatively cheaply, with excellent resolution, and the investigation can focus on the most appropriate regions of the molecule to answer the question at hand.

Key words.--Salmonidae, Coregoninae, mitochondrial DNA, glacial refugia, haplotype diversity, polymerase chain reaction.

The Arctic cisco is an anadromous coregonine fish that is limited in distribution in North America to the Mackenzie River system for spawning habitat and to the Beaufort Sea coast from Point Barrow in Alaska to Victoria Island in the Canadian Northwest Territories for feeding and overwintering habitat (Craig, 1989). The closely related Bering cisco spawns primarily in the Yukon River system and its coastal distribution reportedly overlaps that of the Arctic cisco from Point Barrow eastward to the Colville River in Alaska (Craig, 1989; Edge, 1991). The systematic status of these taxa is uncertain. Presently they are considered distinct species that can be differentiated by gill-raker counts, although these overlap (Dymond, 1943; McPhail, 1966; Dillinger, 1989). However, no fixed differences in allozymes were found between them although there were strong frequency differences at two loci (Bickham et al., 1989; Morales et al., in press). Thus, it is possible that these taxa are conspecific or, as presently viewed, sibling species.

It is likely that Pleistocene glacial events played a key role in the differentiation and present distributions of Arctic and Bering ciscoes. Much of this region, including nearly all of the Mackenzie River system, was glaciated. Thus, the Arctic cisco population of the Mackenzie River probably originated as a founder event less than 10,000 years ago (McPhail and Lindsey, 1970; Lindsey and McPhail, 1986). On the other hand, the Yukon River system was not glaciated and thus the population of Bering ciscoes that spawns in that river is likely much older than the population of Arctic ciscoes of the Mackenzie River. The origin of the Mackenzie River population is unknown. Bickham et al. (1989) hypothesized that the Mackenzie was colonized by a few founders from the Yukon River, based on the fact that allozyme alleles were shared between Arctic and Bering ciscoes and that an

absence of restriction-site variation in mitochondrial DNA was observed in a sample of Arctic ciscoes, indicative of a severe population bottleneck. On the other hand, Bernatchez and Dodson (1991) demonstrated that the Mackenzie River population of lake whitefish (Coregonus clupeaformis) was derived from a Mississippian, rather than a Beringian Refugium, which casts some doubt on the hypothesis of a Yukon origin for Arctic ciscoes.

The use of restriction endonucleases to analyze variation in mitochondrial DNA is a particularly valuable technique for the determination of evolutionary relationships and geographic variation within species and among closely related species (Avise et al., 1979, 1987, 1988; Avise, 1986; Brown, 1983; Carr et al., 1986; Honeycutt and Wheeler, 1990; Lamb et al., 1989; Moritz et al., 1987; Riddle and Honeycutt, 1990). This results from the molecule being maternally inherited with a presumed lack of selection. Thus, lineage survival tends to be stochastic and the rapid evolutionary rate that characterizes the molecule produces monophyletic lineages that, in the absence of gene flow, are geographically sequestered. Patterns of mitochondrial DNA variation often are useful to identify vicariant events that have inhibited gene flow in the past (Riddle and Honeycutt, 1990). Moreover, the molecule is a sensitive indicator of population bottlenecks (Wilson et al., 1985).

This study investigates patterns of mitochondrial DNA variation within and among spawning-site populations of ciscoes from five tributaries of the Mackenzie River system, one spawning run from the Yukon River, and ostensibly mixed-population aggregations from seven sites along the Beaufort Sea coast of Alaska and Canada (Fig. 1). We attempt to determine the taxonomic and evolutionary relationships of these taxa as reflected in the

phylogeny of maternal lineages, the possible origin of the Mackenzie River population, the taxonomic makeup of coastal aggregations, and the relative nucleotide diversities and rates of evloution in particular regions of the mitochondrial DNA of the two species. In addition, we present a PCR-based approach to doing restriction-enzyme analyses that holds great promise in evolutionary and population genetic studies.

MATERIALS AND METHODS

Sample Collection

A total of 488 ciscoes was collected from 13 sample localities in 1985, 1988, and 1991 (Fig. 1, Table 1). Specimens collected from coastal sites in the Beaufort Sea were mostly adults or subadults. Specimens collected from spawning runs in the Mackenzie and Yukon River systems were adults. Some specimens were frozen whole and shipped on dry ice to our laboratories at Texas A&M University and LGL Ecological Genetics. Others specimens were biopsied in the field with tissues being frozen in liquid nitrogen. Various tissues were used in this study including heart, skeletal muscle, liver, kidney, and spleen. Voucher specimens are deposited in the Texas Cooperative Wildlife Collection, Texas A&M University.

DNA Isolation and Amplification

Mitochondrial DNA was purified from some samples using CsCl-propidium iodide gradient centrifugation (Carr and Griffith, 1987). However, from most samples total genomic DNA was isolated. Solid tissue (20-40 mg) was placed in a 1.5 ml microcentrifuge tube and ground with a pestle. Tissue was digested with proteinase K (50 µL), 50 µL of 20% SDS, and 450 µL STE for one hour at 55°C. The tissue was then treated with NaCl (150 µL of 5M solution) and shaken vigorously. Methylene chloride (25-50 µL) was then added, the tube shaken again, and then set in an ice bath for one hour. After centrifugation the pellet was removed and 500 µL methylene chloride was added and the tube shaken. The solution was centrifuged again and the pellet discarded. DNA was precipitated by adding 0.5 x volume isopropyl alcohol. The alcohol was then removed and 500 µL 70% ethanol was added and then

removed to rinse the button of DNA. The DNA lastly was resuspended in 200 μL of TE buffer.

Amplification of specific regions of the mitochondrial DNA was accomplished with the Polymerase Chain Reaction (PCR) using Taq DNA polymerase and an automated thermal cycler (Perkin-Elmer Cetus). Double-stranded amplifications were performed in 25-μL (0.1 M Tris pH 8.5, 10 mM 2-mercaptoethanol, 0.025 M $MgCl_2$, 0.5 M KCl, 1 mg/ml bovine serum albumin, 2mM dTTP, dATP, dCTP, and dGTP, 1 unit Amplitaq polymerase [Perkin-Elmer Cetus], and 5 μL of a 10 μM solution of each primer). The amplification cycle was 95°C for 45 s, 50°C for 30 s, and 70°C for 150 s. Amplifications were run for a total of 32 cycles.

Six primer sets (LGL Ecological Genetics, Inc.) were used in this study and their positions on the mitochondrial DNA molecule are illustrated in Fig. 2. The first primer set (ORF1) amplifies a region of approximately 2 kb of the 16S ribosomal gene and the Open Reading Frame 1 gene. The second primer set (ORF2) amplifies a region approximately 2.2 kb in the Open Reading Frame 2 and Cytochrome Oxydase I genes. The third primer set (ORF3-4) amplifies a region approximately 2.3 kb of Open Reading Frames 3, 4L, and 4. The fourth primer set (ORF5-6) amplifies a region approximately 2.4 kb of Open Reading Frames 5 and 6. The fifth primer set (CytB) amplifies a region approximately 1.4 kb of the Cytochrome B gene. The sixth primer set (12S-16S) amplifies a region approximately 1.7 kb of the 12S and 16S ribosomal genes.

Restriction Analyses and Electrophoresis

A total of twenty restriction enzymes were used to cut each of the six amplified regions. About 25 restriction enzymes were tested for each region to select the 20 that were most useful. Because some enzymes did not cut within a particular region, the same 20 enzymes were not used for each region (Table 2).

Fragments of digested DNA were separated by electrophoresis using 2% agarose gels, stained with ethidium bromide, and photographed with polaroid film on a UV light box (Fig. 3). Lambda phage DNA digested with HindIII was used as a size standard in each gel. Fragment patterns were identified by examination of the gels and photographs. The common pattern observed in Arctic ciscoes was designated A for all enzymes from all regions. The common pattern for Bering ciscoes, if different from that of the Arctic ciscoes, was designated B. Haplotypes were identified as the composite fragment patterns for all enzymes from all six regions (Table 2).

In this study, restriction sites were not mapped nor were the number of shared fragments determined for pairs of haplotypes. Rather, the different fragment patterns for each restriction enzyme were identified for each region of the mitochondrial DNA and analyses were performed under the assumption that any two patterns produced by a particular enzyme differed by a minimum of one mutation. This is clearly an underestimate of divergence in many cases but the fact that so many fragment patterns were produced (20 enzymes for each of three regions) resulted in a sampling of the genetic diversity adequate for the purposes of this study. Although increased resolution could be obtained by a site-map or shared-fragment analysis,

considerably more effort and expense would be incurred. Therefore, this study presents a new method for analyzing data produced by restriction enzymes--the shared-fragment pattern method. We recommend this method be used in population studies where the use of large numbers of restriction enzymes and multiple regions of DNA amplified by PCR are combined.

Phenetic Relationships and Nucleon Diversity Estimates

To obtain a measure of the relationships among all haplotypes identified in the ORF1, ORF2, and ORF5-6 regions (analyzed both separately and simultaneously), a simple matching coefficient was utilized (Rohlf, 1988). For each pair-wise comparison the coefficient was obtained by dividing the number of matches, that is, the same fragment pattern per restriction enzyme, by the total number of characters (in this case 20 for single regions or 60 for all three regions). This results in an estimate of the proportion of the shared haplotypes, and ranges from one when all the haplotypes for all enzymes are the same for two individuals to zero where two individuals differ for each enzyme. A symmetric similarity matrix was constructed, and the phenetic relationships among all the haplotypes was depicted in a dendrogram produced by the unweighted pair-group method using arithmetic means (UPGMA; Rohlf, 1988). This procedure was done using the 264 individuals for which a complete data set was obtained from 20 enzymes for each of three fragments (ORF1, ORF2, ORF5-6). It was first done combining the three regions surveyed into a single matrix, and then for each gene region separately.

Genotypic diversity was quantified using the nucleon diversity index (h) using equation 7 of Nei and Tajima (1981). Total nucleon diversity was

estimated for each of the major haplotype lineages (Arctic and Bering ciscoes) using the 264 individuals from which a complete data set was obtained. Nucleon diversities were estimated for all Beaufort Sea and Mackenzie River drainage samples using all 488 individuals analyzed and based upon the grouped haplotypes (Table 1).

RESULTS

No variation was observed in an initial survey of 10 Arctic and 2 Bering ciscoes cut with 20 enzymes for the 12-16S region. Therefore, this region was not utilized for the remainder of the fish. The ORF1, ORF2, and ORF5-6 regions each were cut with 20 enzymes for 264 ciscoes representing all sample localities. There were two major haplotype lineages identified: one was characteristic of Arctic ciscoes and the other characteristic of Bering ciscoes (Fig. 4). A total of 35 distinct haplotypes comprised the Arctic cisco lineage, represented by 234 individuals, and a total of 27 distinct haplotypes comprised the Bering cisco lineage represented by 30 individuals (Table 2, Fig. 4). Only two haplotypes in the Bering cisco lineage were found in more than one individual. The Arctic cisco lineage was dominated by a common (AC-01) haplotype found in 164 individuals. Only three other haplotypes were found in more than two individuals.

An analysis of 32 Arctic and one Bering ciscoes was performed for the ORF3-4 and CytB gene regions (Table 3). The single Bering cisco was well differentiated in both of these regions. For the CytB region, 30 Arctic ciscoes had the common haplotype and 2 individuals had unique haplotypes. In the ORF3-4 region, 24 Arctic ciscoes had the common haplotype, 3 individuals had unique haplotypes and 5 individuals had a low-frequency haplotype. When the data from the two regions were combined, there were 8 haplotypes identified among all these individuals (Table 3). However, because these regions provided little added resolution they were not used in the population and phenetic analyses.

Cluster analysis was used to estimate genetic relationships among the 35 Arctic cisco and 27 Bering cisco haplotypes identified in the analysis of the ORF1, ORF2, and ORF5-6 regions. Within the Arctic ciscos, three major lineages (A-C) are apparent (Fig. 4). The most divergent is the C lineage which includes haplotypes 33-35. For purposes of estimating the divergence of populations these three are combined into a single grouped haplotype C1. The A lineage includes haplotypes 1-20 which were combined into three grouped haplotypes: A1 consists of haplotypes 1-8 and 11-16, A2 consists of haplotypes 9-10, and A3 consists of haplotypes 17-20. The B lineage (haplotypes 21-32) comprises four grouped haplotypes: B1 consists of haplotypes 21-22, B2 consists of haplotypes 23-24, B3 consists of haplotypes 25-28, B4 consists of haplotypes 29-32. The Bering cisco haplotypes were all included in a single grouped haplotype because nearly every haplotype consists of only a single individual. Grouped haplotypes were identified in order to allow larger population sample sizes to be assayed with fewer restriction analyses.

The ORF1 fragment from an additional 224 ciscoes was examined using three enzymes (DdeI, HaeIII, and HinfI). These enzymes were found in the previous data set to be sufficient to identify all fish as to belonging to Bering or Arctic cisco lineages and, among fish of the Arctic cisco lineage, as belonging to the A, B or C lineages. Thirty-four of these same fish were further examined with five to seven restriction enzymes applied to the ORF5-6 region (DdeI, Sau96, DraI, SspI, TaqI, Bsp1286I) which allowed each fish to be categorized according to the grouped haplotypes defined above.

The number of nucleotides sampled was estimated for each of the six regions of the mitochondrial DNA examined in this study. For each

restriction enzyme, Nei's (1987) value of r, which is the number of nucleotides in the recognition sequence (Nei, 1987, Table 5.7), was determined as was the minimum number of sites (s) present in all individuals. Thus, the number of nucleotides sampled was r x s for each enzyme. These values were summed for all enzymes for each fragment. The estimated number of nucloetides sampled for each fragment was: ORF1 (n = 289), ORF2 (n = 243), ORF3-4 (n = 170), ORF5-6 (n = 314), CytB (n = 108), 12S-16S (n = 172). The percentage of the total number of nucleotides sampled in each fragment was: ORF1 (14.5%), ORF2 (11%), ORF3-4 (7.4%), ORF5-6 (13%), CytB (7.7%), 12S-16S (10%). The maximum number of nucleotides sampled in the study (including all six regions) was 1,296 which represents 10.8% of the 12 kb amplified by the six primer sets and 8.1% of the entire mitochondrial DNA genome (estimated to be 16.5 kb). However, most of the analyses in this study are based upon the ORF1, ORF2, and ORF5-6 gene regions for which 846 nucleotides were sampled per individual. This represents 12.8% of the 6.6 kb contained in these three regions or 5.1% of the entire mitochondrial DNA genome.

DISCUSSION

Previous studies of genetic variation of Arctic and Bering ciscoes showed an absence of variation in mitochondrial DNA from a small sample of Arctic ciscoes and normal levels of nuclear gene variability in both species as measured by protein electrophoresis (Bickham et al., 1989; Morales et al., in press). Bickham et al. (1989) hypothesized that the absence of restriction-site variation in Arctic ciscoes was an indication of a previous population bottleneck. This bottleneck was a result of the Mackenzie River cisco population having been initiated by a small number of founders after the receeding of glacial ice that covered most of the Mackenzie system until the end of the Wisconsin (approximately 10,000 years ago). Further, Bickham et al. (1989) hypothesized that these founders were derived from the Bering cisco population of the Yukon River system because the absence of fixed allelic differences between these populations in allozyme loci was indicative of a close relationship, and because the Yukon River population is geographically the closest source of anadromous ciscoes of this group. Implicit in this hypothesis is the assumption that Arctic and Bering ciscoes do not represent distinct species. Finally, they predicted that variation in mitochondrial DNA of the Yukon River population of Bering ciscoes should be greater than that found in Arctic ciscoes. This is because the Yukon system was unglaciated, and thus the Bering cisco population is potentially much older than the Arctic cisco population.

The patterns of variation of mitochondrial DNA revealed in this study confirms the prediction of Bickham et al. (1989) that Bering ciscoes have higher levels of variability than Arctic ciscoes. Overall nucleon diversity indices (Nei and Tajima, 1981) were $\underline{h}$ = 0.9908 for Bering ciscoes and $\underline{h}$ =

0.5046 for Arctic ciscoes. This is reflected in the ratio of haplotypes per number of individuals sampled: 27 Bering cisco haplotypes found in 30 individuals and 35 Arctic cisco haplotypes found in 234 individuals. It thus appears that the Yukon system has allowed for the preservation of a high level of genetic variability in anadromous fishes. If this is found to be the case in other species that spawn in the Yukon system such as broad whitefish (Coregonus nasus), least cisco (C. sardinella), and several species of salmon, this could be an important consideration in the conservation and management of Alaskan and Canadian fisheries stocks. For example, Brown et al. (1992) found that the level of variation in mitochondrial DNA in anadromous white sturgeon (Accipenser transmontanus) surprisingly was lower in the Columbia River population than in the Fraser River population even though the former river was unglaciated and the latter covered by ice during the Wisconsin. Brown et al. (1992) attributed this result to a severe population bottleneck caused by human intervention including overfishing and habitat destruction. The conservation and management of anadromous fish stocks inhabiting galcial refugia such as the Yukon and Columbia Rivers should receive high priority.

The hypothesis that the Arctic cisco population of the Mackenzie River system was derived from the Bering cisco population of the Yukon River system (Bickham et al., 1989) is not supported by the results of this study. The mitochondrial DNAs that characterize these taxa are distinctly different (Fig. 4) which is consistent with the present classification of these forms as distinct species (Craig, 1989; McPhail, 1966). Although it is possible that an unknown population of Bering ciscoes in the Yukon River system has mitochondrial DNA like that of the Arctic cisco, it seems more likely that the Mackenzie

River population was colonized by fish from the Mississippian refugium to the south as suggested by Dillinger (1989) or from Asia. The closest population of Arctic ciscoes outside of the Mackenzie River system is found in western Siberia. Dispersal from that population, or from a population that might have existed in the presently submerged Beringian land bridge, is a possibility. The fact that the mitochondrial DNA haplotype that is characteristic of Arctic ciscoes is distinctly different from the haplotype characteristic of what is probably the most closely related species (Bering ciscoes) means that this molecule should be potentially useful in elucidating the systematic relationships and origin of the North American populations of these species.

Any study that addresses the systematic status of Arctic and Bering ciscoes must consider the possibility of hybridization between these taxa. In this study animals from the coastal sites were identified as Arctic or Bering ciscoes based only on their mitochondrial DNA. Thus, no direct test was done regarding the identification of potential hybrids because nuclear genes were not examined. However, the fact that four specimens with Bering cisco mitochondrial DNA were taken in spawning runs of Arctic ciscoes (Table 1) indicate hybridization likely occurs (or has occurred) between these taxa. Moreover, an absence of fixed allelic differences between the two species (Bickham et al., 1989; Morales et al., in press) is consistent with the hybridization hypothesis.

The distribution of mitochondrial DNA haplotypes along the Beaufort Sea coast of Alaska and Canada indicates that coastal aggregations include both Arctic and Bering ciscoes. The relative frequencies of the two species changes dramatically between the Colville and Sagavanirktok River deltas of

Alaska and in general there is a trend to reduce the proportion of Bering ciscoes from west to east. The westernmost locality, Point Barrow (Fig. 1), is the only coastal locality from which our sample was primarily composed of Bering ciscoes (0.73). The Colville River, the next locality to the east, had the next highest proportion of Bering ciscoes (0.26). Proportions of Bering ciscoes in our samples from localities farther to the east were: Sagavanirktok River (0.04), Kaktovik (0.22), Stokes Point (0.04), Atkinson Point (0.08). It should be noted that although Kaktovik had a relatively high frequency of Bering ciscoes this was probably an artifact of small sample size (N = 9; Table 1).

Overall, the frequency of Bering ciscoes in coastal sites east of the Colville River is 0.056. This is similar to, and not significantly different from ($P < 0.05$) the frequency of Bering cisco haplotypes observed in the five spawning rivers of the Mackenzie River system (0.02). Thus, although the Beaufort Sea coastal aggregations are a mixture of the two species, Bering ciscoes are not found in appreciable numbers east of the Colville River. The possibility exists, moreover, that some if not all of the individuals with Bering cisco haplotypes east of the Colville River and in the Mackenzie River tributaries are of hybrid ancestry.

The methods used in this study, which included the amplification of specific regions of the mitochondrial DNA and subsequent restriction analysis, allow for relative rates of evolution and gene order to be examined in ways not possible in previous studies of mitochondrial DNA. We used six primer sets that amplified approximately 12 kb of the molecule. In all cases, amplification products of the expected size were obtained which indicated that no gene rearrangements have occurred in the evolution of these two species to disrupt the spatial relationships of these primers. Such

mitochondrial DNA (Carr and Griffith, 1992 [illegible]

The use of PCR [illegible]

purified mitochondrial DNA [illegible]

methodology [illegible]

nucleotide [illegible] DNA and then [illegible]

for [illegible] the [illegible] study [illegible]

that allow for [illegible] of

mammals (J. C. Patton, unpublished [illegible]

sometimes has proven necessary for their application in ongoing studies of

We conclude that Pleistocene glacial events have

fish, reptiles, and birds in our lab. Nonetheless, primers are now available to

influenced the variability of mitochondrial DNA in Arctic [illegible] ciscoes

amplify virtually all parts of the mitochondrial DNA in vertebrates. Morales

Moreover, the high degree of divergence between these [illegible]

(1992) has developed procedures to quickly and accurately map restriction

conclude that they are distinct biological species—no [illegible]

sites directly from PCR products which will allow for the increased

evidence presented here for hybridization. The level of [illegible]

resolution necessary for phylogenetic analyses. We thus conclude that the

relatively low, with only 2% of the Arctic ciscoes [illegible] ans

methodology presented in this study has great potential for evolutionary,

having Bering cisco haplotypes. Mitochondrial DNA [illegible]

phylogenetic, and population genetic studies of vertebrates and represents a

of past hybridization events and thus introgression of [illegible]

significant technical advance over traditional assays.

should not by itself be taken as sufficient evidence to [illegible]

are conspecific. White-tailed and mule deer, for example [illegible] d

areas of sympatry and ecological distinction but these species

hybridize and exchange mitochondrial DNA haplotypes ([illegible] 1987;

Carr and Hughes, in press; Carr et al., 1986; Derr et al., 1991; C[illegible]

1991).

The methodology employed in this study combines PCR [illegible] of specific DNA regions of the mitochondrial DNA with restriction [illegible]. This methodology is relatively inexpensive and generates [illegible] much more rapidly than the traditional [illegible] of purified

ACKNOWLEDGMENTS

We thank the many people who have aided us in obtaining specimens of ciscoes from Canada and Alaska. Many of the specimens used in this study were also examined in previous studies of allozymes and flow cytometry. Additional specimens not used in those studies were provided by L. Moulton, C. George, R. Fechhelm, W. Griffiths, and S. Lockwood. We thank the Canadian Department of Fisheries and Oceans for granting collecting permits. This study was jointly funded by BP Exploration (Alaska) Inc., the Minerals Management Service, and the U.S. Fish and Wildlife Service, Region 8.

LITERATURE CITED

AVISE, J. C. 1986. Mitochondrial DNA and the evolutionary genetics of higher animals. Phil. Trans. R. Soc. London, Ser. B 312:325-342.

AVISE, J. C., J. ARNOLD, R. M. BALL, E. BERMINGHAM, T. LAMB, J. E. NEIGEL, C. A. REEB, N. C. SAUNDERS. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Ann. Rev. Ecol. Syst. 18:489-522.

AVISE, J. C., B. W. BOWEN, T. LAMB, A. B. MEYLAN, AND E. BERMINGHAM. 1992. Mitochondrial DNA evolution at a turtle's pace: evidence for low genetic variability and reduced microevolutionary rate in the Testudines. Mol. Biol. Evol. 9:457-473.

AVISE, J. C., C. GIBLIN-DAVIDSON, J. LAERM, J. C. PATTON, AND R. A. LANSMAN. 1979. Mitochondrial DNA clones and matriarchal phylogeny within and among geographic populations of the pocket gopher, Geomys pinetis. Proc. Natl. Acad. Sci. USA 76:6694-6698.

BALLINGER, S. W., L. H. BLANKENSHIP, J. W. BICKHAM, AND S. M. CARR. 1992. Allozyme and mitochondrial DNA analysis off a hybrid zone between white-tailed and mule deer (Odocoileus) in West Texas. Biochem. Genet. 30:1-11.

BERNATCHEZ, L., AND J. J. DODSON. 1991. Phylogeographic structure in mitochondrial DNA of the lake whitefish (Coregonus cluepeaformis) and its relation to Pleistocene glaciations. Evolution 45:1016-1035.

BICKHAM, J. W., S. M. CARR, B. G. HANKS, D. W. BURTON, AND B. J. GALLAWAY. 1989. Genetic analysis of population variation in the Arctic Cisco using electrophoretic, flow cytometric, and mitochondrial DNA restriction analyses. Biol. Pap. Univ. Alaska 24:112-122.

BROWN, W. M. 1983. Evolution of animal mitochondrial DNA, pp. 62-88. In M. Nei and R. K. Koehn (eds.), Evolution of Genes and Proteins. Sinauer Associates, Sunderland, MA.

BROWN, W. M., M. GEORGE, JR., AND A. C. WILSON. 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. USA 76:1967-1971.

CARR, S. M., S. W. BALLINGER, J. N. DERR, L. H. BLANKENSHIP, and J. W. BICKHAM. 1986. Mitochondrial DNA analysis of hybridization between sympatric white-tailed and mule deer in west Texas. Proc. Natl. Acad. Sci. U.S.A. 83: 9576-9580.

CARR, S. M., AND O. M. GRIFFITH. 1987. Rapid isolation and animal mitochondrial DNA in a small fixed-angle rotor at ultrahigh speed. Biochem. Genet. 25:385-390.

CARR, S. M., AND G. A. HUGHES. In Press. The direction of hybridization between species of North American deer (Odocoileus) as inferred from mitochondrial cytochrome B sequences. J. Mammal.

CRAIG, P. C. 1989. An introduction to anadromous fishes in the Alaskan Arctic. Biol. Pap. Univ. Alaska 24:27-54.

CRONIN, M. A., M. E. NELSON, AND D. F. PAC. 1991. Spatial heterogeneity of mitochondrial DNA and allozymes among populations of white-tailed deer and mule deer. J. Hered. 82:118-127.

CRONIN, M. A., E. R. VYSE, AND D. G. CAMERON. 1988. Genetic relationships between mule deer and white-tailed deer in Montana. J. Wildl. Manage. 52:320-328.

DERR, J. N., D. W. HALE, D. L. ELLSWORTH, AND J. W. BICKHAM. 1991. Fertility in an F_1 male hybrid of white-tailed deer (Odocoileus virginianus) X mule deer (O. hemionus). J. Reprod. Fert. 93:111-117.

DILLINGER, R. E. 1989. An analysis of the taxonomic status of the Coregonus autumnalis species complex in North America, and an investigation of the life histories of whitefishes and ciscoes (Pisces; Coregoninae) in North America and Eurasia. Ph.D. Diss. Memorial U. of Newfoundland, St. John's.

DYMOND, J. R. 1943. The coregonine fishes of northwestern Canada. Trans. Roy Canad. Inst. 24:171-232.

EDGE, T. 1991. Status of the Bering cisco, Coregonus laurettae, in Canada. Canad. Field-Natur. 105:169-172.

FERL, R. J., C. J. NAIRN, J. SHE, E. WAKELAND, AND E. ALMIRA. 1991. The application of automated DNA sequence analysis to phylogenetic studies, pp. 45-58. In M. M. Miyamoto and J. Cracraft (eds.). Phylogenetic analysis of DNA sequences. Oxford Univ. Press, N.Y.

GOLD, J. R., AND L. R. RICHARDSON. 1991. Genetic studies of marine fishes. IV. An analysis of population structure in the red drum (Sciaenops ocellatus) using mitochondrial DNA. Fish. Res. 12:213-241.

HONEYCUTT, R. L., AND W. C. WHEELER. 1990. Mitochondrial DNA: variation in humans and higher primates, pp. 91-129. In S. K. Dutta and W. P. Winter (eds.). DNA systematics. Volume II: Human and higher primates. CRC Press, Inc., Boca Raton, FL.

KIMURA, M. 1979. The neutral theory of evolution. Sci. Am. 241:98-126.

LAMB, T., J. C. AVISE, AND J. W. GIBBONS. 1989. Phylogeographic patterns in mitochondrial DNA of the desert tortoise (Xerobates agassisi), and evolutionary relationships among the North American gopher tortoises. Evolution 43:76-87.

LINDSEY, C. C., AND J. D. McPHAIL. 1986. Zoogeography of fishes of the Yukon and Mackenzie basins, pp. 639-674. In C. H. Hocutt and E. O. Wiley (eds.). Zoogeography of North American Freshwater Fishes. John Wiley and Sons, N.Y.

LOCKWOOD, S. F., and J. W. BICKHAM. 1991. Genetic stock assessment of spawning Arctic cisco (Coregonus autumnalis) populations by flow cytometric determination of DNA content. Cytometry 12:260-267.

——. In Press. Geographic trends in DNA content in Beaufort Sea coastal populations of Arctic cisco (Coregonus autumnalis) identified by flow cytometry. Trans. Amer. Fish. Soc. (in press).

McPHAIL, J. D. 1966. The Coregonus autumnalis complex in Alaska and northwestern Canada. J. Fish. Res. Board Can. 23:141-148.

McPHAIL, J. D., AND C. C. LINDSEY. 1970. Freshwater fishes of northwestern Canada and Alaska. Fish. Res. Board Can. Bull. 173:381 pp.

MORALES, J. C. 1992. Molecular systematics of the tree bats of the genus Lasiurus in North, Central, and South America. Ph.D. dissert., Texas A&M University, College Station, Texas.

MORALES, J. C., J. W. BICKHAM, J. N. DERR, and B. J. GALLAWAY. In Press. Genetic analysis of population structure in Arctic cisco (Coregonus autumnalis) from the Beaufort Sea. Copeia.

MORITZ, C., T. E. DOWLING, AND W. M. BROWN. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. Ann. Rev. Ecol. Syst. 18:269--292.

NEI, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

NEI, M., AND F. TAJIMA. 1981. DNA polymorphism detectable by restriction endonucleases. Genetics 97:145-163.

RIDDLE, B. R., AND R. L. HONEYCUTT. 1990. Historical biogeography in North American arid regions: an approach using mitochondrial-DNA phylogeny in grasshopper mice (Genus Onychomys). Evolution 44:1-15.

ROHLF, F. J. 1988. NTSYS-pc. Numerical taxonomy and multivariate analysis system. Version 1.50. Exter Software, Setauket, New York (on disk).

SHIELDS, G. F., AND A. C. WILSON. Calibration of mitochondrial DNA evoltuion in geese. J. Mol. Evol. 24:212-217

WILSON, A. C., ET AL. 1985. Mitochondrial DNA and two perspecitives on evolutionary genetics. Biol. J. Linnean Soc. 26:385-400.

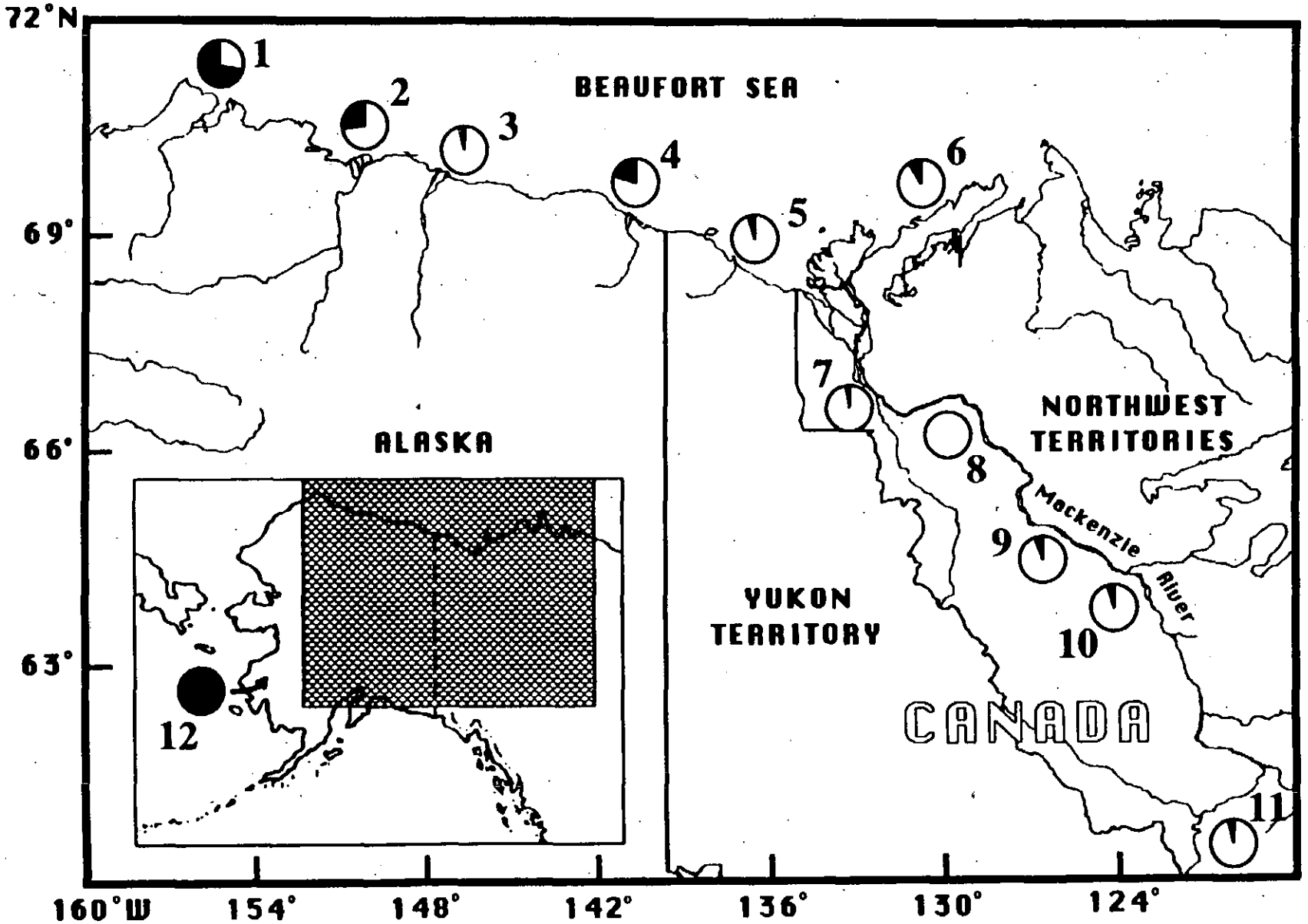
FIG. 1. Sample localities of Arctic and Bering ciscoes used in this study. Locality names are given in Table 1. Localities 1-6 represent coastal aggregations, 7-11 are Arctic cisco spawning streams, and 12 represents a spawning run of Bering ciscoes.

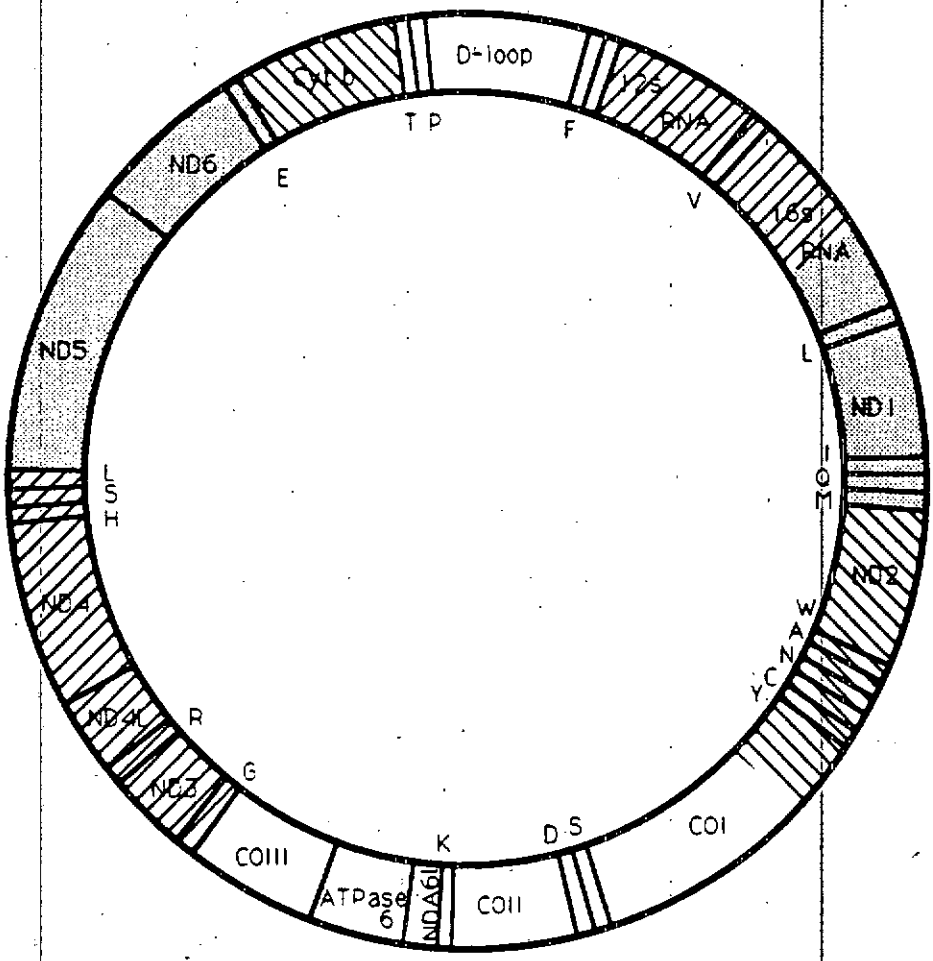
FIG. 2. Diagrammatic representation of the generalized vertebrate mitochondrial DNA molecule. The cross hatched and stippled areas represent the six regions that were amplified and amplified cut with restriction enzymes in this study.

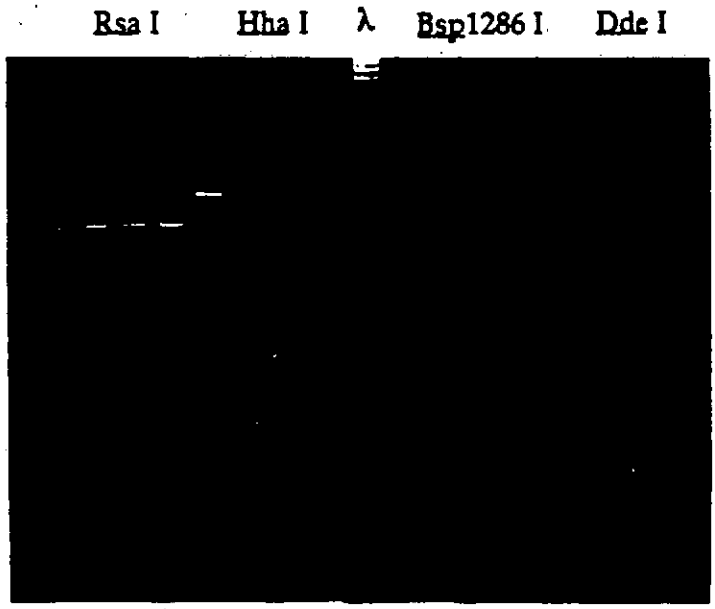
FIG. 3. Photograph of a gel illustrating restriction-site variation in four individuals of Arctic cisco. DNA in lanes 1-4 are cut with RsaI which produces a four-fragment (three-cut) phenotype shared by all four individuals. DNA in lanes 5-8 are cut with HhaI which typically (haplotype A) produces a five-fragment phenotype (lanes 6-8). The individual in lane 5 has a D haplotype with four fragments resulting from the presumed loss of the restriction site that produces the two largest fragments in haplotype A. Lane 9 contains a size standard (Lambda DNA cut with Hind III). DNA in lanes 10-13 were cut with Bsp1286 I which typically produces an eight-fragment phenotype (haplotype A: lanes 11-13). Lane 10 contains a C haplotype in which the fourth largest fragment of haplotype A is cut. Only one of the resulting fragments (the fifth largest in haplotype C) is visible on this gel. DNA in lanes 14-17 are cut with DdeI which typically produces a seven-fragment phenotype (haplotype A: lane 17). Lanes 14 and 16 illustrate the B haplotype and lane 15 is the C haplotype.

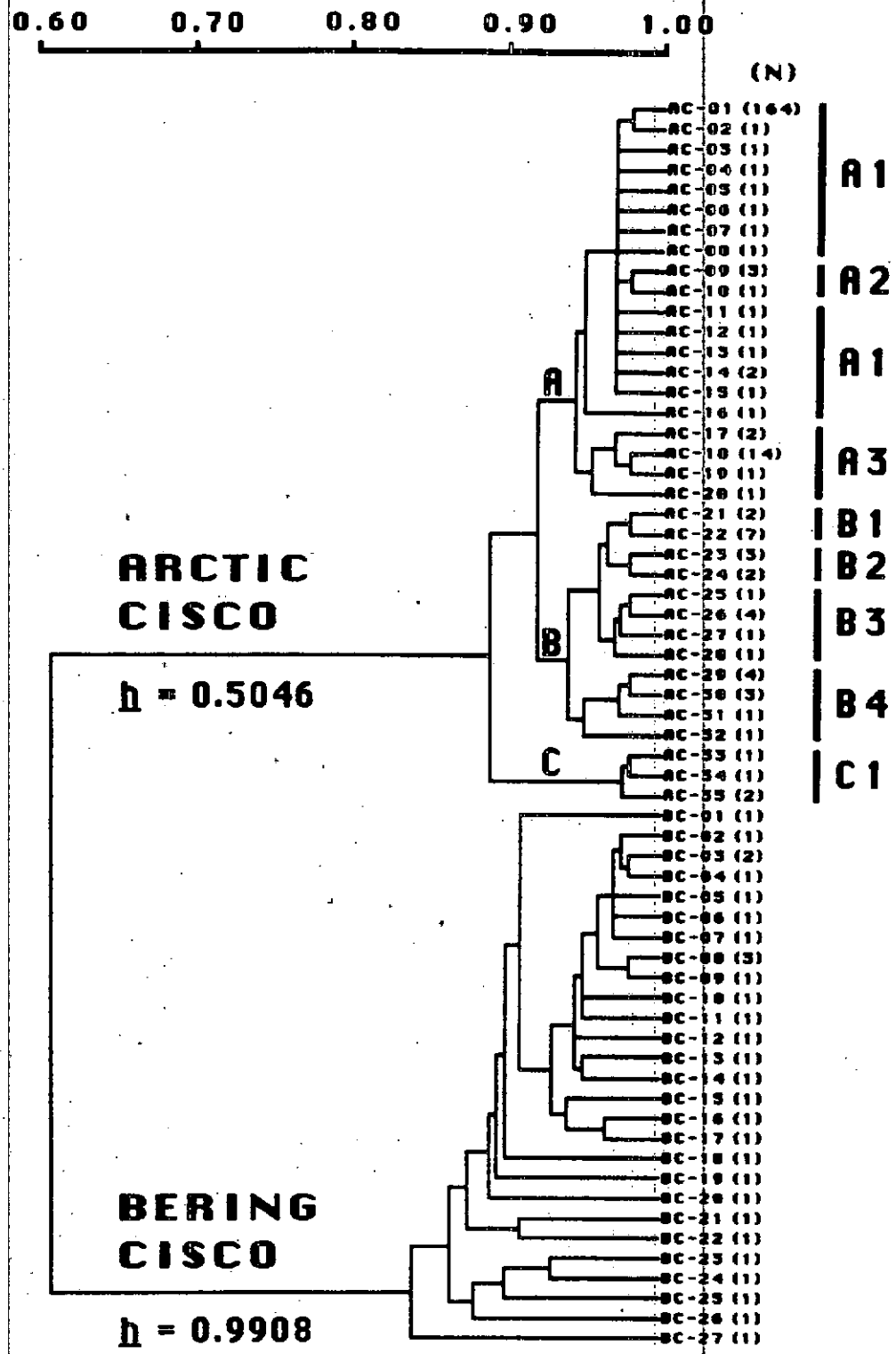
FIG. 4. Cluster analysis (UPGMA) of 264 individuals of Arctic and Bering ciscoes. This analysis combined the data from the ORF1, ORF2, and ORF5-6 regions which produced 62 haplotypes. Nucleon diversity estimates (h) are given for both Arctic and Bering cisco maternal lineages. Also denoted are the three major lineages of Arctic cisco (A-C), the grouped haplotypes (A1-A3, B1-B4, C1) listed in Table 1; and sample sizes (N) for each of the 62 haplotypes.

FIG. 5. Cluster analyses and nucleon diversity estimates (h) for 264 Arctic (A. C.) and Bering (B. C.) ciscos. Different levels of relative diversity are revealed in the three regions.


72°N
69°
66°
63°
160°W
154°
148°
142°
136°
130°
124°
BEAUFORT SEA
ALASKA
YUKON TERRITORY
NORTHWEST TERRITORIES
Mackenzie River
CANADA
1
2
3
4
5
6
7
8
9
10
11
12


D-loop
Cyt b
ND6
ND5
ND4
ND4L
ND3
COIII
ATPase 6
ND6L
COII
COI
ND2
ND1
16S RNA
12S RNA
T P
E
F
V
L
M
W
N
A
Y
C
R
G
K
D
S
H
I


Rsa I
Hha I
λ
Bsp1286 I
Dde I
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17


0.60
0.70
0.80
0.90
1.00
(N)
A1
A2
A1
A3
B1
B2
B3
B4
C1
A
B
C
ARCTIC CISCO
h = 0.5046
BERING CISCO
h = 0.9908

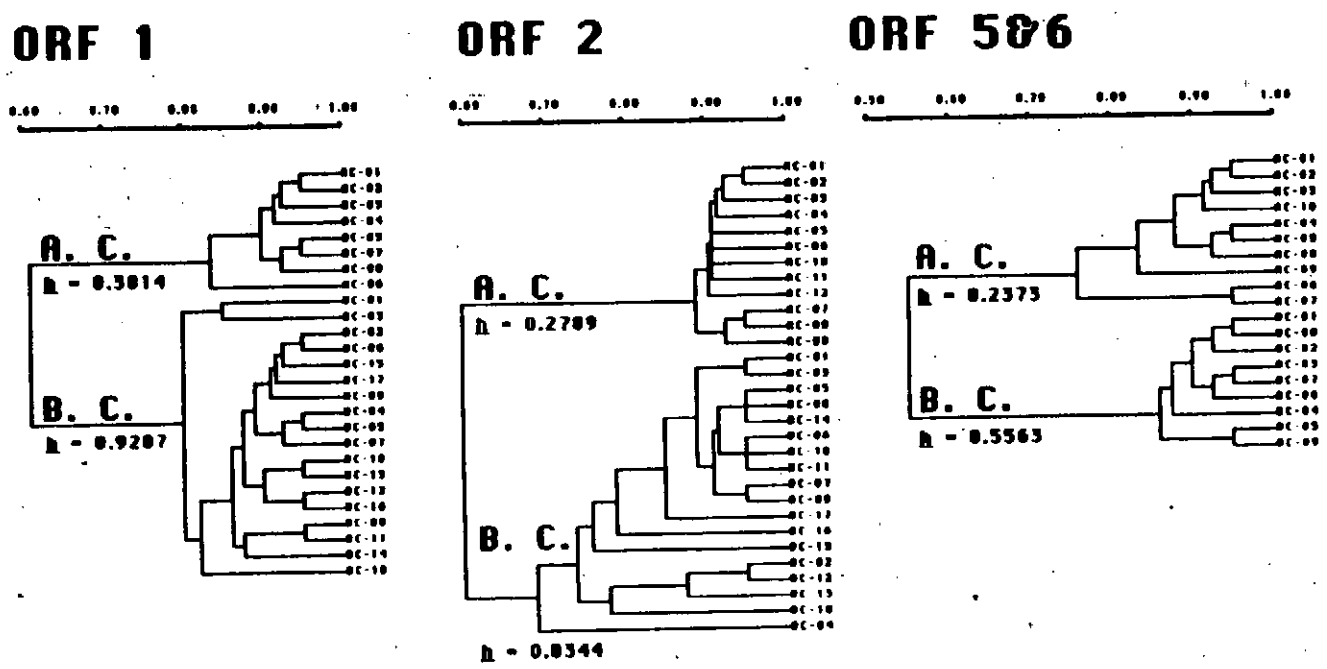

ORF 1
A. C.
h = 0.3814
B. C.
h = 0.9287
ORF 2
A. C.
h = 0.2789
B. C.
h = 0.8344
ORF 5&6
A. C.
h = 0.2373
B. C.
h = 0.5563

TABLE 1. Sample sizes, mitochondrial DNA haplotypes, and nucleon diversity estimates (h) are presented for each population (populations numbered as in Fig. 1). Data are presented as grouped haplotypes as discussed in text and presented in Fig. 4. Fish were identifiec as Arctic or Bering cisco according to mitochondrial DNA haplotype (Fig. 4). For purposes of estimating nucleon diversity, Bering cisco haplotypes were all considered to be a single grouped haplotype and for this reason nucleon diversity was not estimated for population 12. This table includes data from all fish examined including the 264 fish for which a full data set was obtained as well as 224 fish which were scored only for enzymes that defined the grouped haplotypes. All data are from the ORF1, ORF2, and ORF5-6 regions.

		Arctic cisco								Bering cisco	
Localities	N	A1	A2	A3	B1	B2	B3	B4	C1		h
1. Point Barrow	26	7	-	-	-	-	-	-	-	19	0.409
2. Colville River	87	48	-	8	3	-	3	-	1	24	0.616
3. Sagavanirktok	128	86	2	11	9	2	10	2	2	4	0.533
4. Kaktovik	9	7	-	-	-	-	-	-	-	2	0.389
5. Stokes Point	25	17	1	2	-	1	2	1	-	1	0.540
6. Atkinson Point	24	14	-	3	2	-	-	3	-	2	0.385
7. Peel River	45	35	1	1	1	-	3	2	1	1	0.395
8. Arctic Red R.	63	50	-	7	3	-	2	-	1	-	0.363
9. Mountain River	20	13	-	1	4	-	-	1	-	1	0.558
10. Carcajou River	21	15	-	2	1	1	1	-	-	1	0.495
11. Liard River	30	24	-	1	1	1	1	1	-	1	0.365
12. Yukon River	10	-	-	-	-	-	-	-	-	10	
Total	488	316	4	36	24	5	22	10	5	66	

TABLE 2. Haplotypes identified for Arctic (AC01-AC35) and Bering (BC01-BC27) ciscoes. Each of three regions was amplified and then cut with 20 restriction enzymes. The common fragment pattern observed in Arctic cisco for each enzyme was designated A and the common pattern observed in Bering cisco, if different from A, was designated B. Less frequently observed patterns for both species were lettered consecutively as they were identified. Thus, each letter in each column refers to a specific fragment pattern produced by a particular restriction enzyme for the corresponding region of mitochondrial DNA. Phenetic relationships of the 62 haplotypes in this table are shown in Fig. 4.

	ORF1[1]	ORF2[2]	ORF5-6[3]
AC01,	AAAAAAAAAAAAAAAAAAAA	AAAAAAAAAAAAAAAAAAAA	AAAAAAAAAAAAAAAAAAAA
AC02,	AAAAAAAAAAAAAAAAAAAA	AAAAAAAAAAAAAAAAAAAA	AAAAAAACAAAAAAAAAAAA
AC03,	AAAAAAAAAAAAAAAAAAAA	AAAAAAAAAAAAAAAAAAAA	AAAACAAAAAAAAAAAAAAA
AC04,	AAAAAAAAAAAAAAAAAAAA	AAAAAAAAAAAAAAAAAAAA	AEAAAAAAAAAAAAAAAAAA
AC05,	AAAAAAAAAAAAAAAAAAAA	AAAAAAAAAAAAAAAAAAEA	AAAAAAAAAAAAAAAAAAAA
AC06,	AAAAAAAAAAAAAAAAAAAA	AAAAAAAAAAAAAAAAABAA	AAAAAAAAAAAAAAAAAAAA
AC07,	AAAAAAAAAAAAAAAAAAAA	AAAAAAAAAAAAAAAADAAA	AAAAAAAAAAAAAAAAAAAA
AC08,	AAAAAAAAAAAAAAAAAAAA	AAAAAAAAAAAAACAAAAAA	AAAAAAAAAAAAAAAAAAAA
AC09,	AAAAAAAAAAAAAAAAAAAA	AAAAAAAAAACAAAAAAAAA	AAAAAAAAAAAAAAAAAAAA
AC10,	AAAAAAAAAAAAAAAAAAAA	AAAAAAAAAADAAAAAAAAA	AAAAAAAAAAAAAAAAAAAA
AC11,	AAAAAAAAAAAAAAAAAAAA	AAAACAAAAAAAAAAAAAAA	AAAAAAAAAAAAAAAAAAAA
AC12,	AAAAAAAAAAAAAAAAAAAA	AABAAAAAAAAAAAAAAAAA	AAAAAAAAAAAAAAAAAAAA
AC13,	AAAAAAAAAAAAAAAAAAAA	BAAAAAAAAAAAAAAAAAAA	AAAAAAAAAAAAAAAAAAAA
AC14,	AAAAAAAAAAACAAAAAAAA	AAAAAAAAAAAAAAAAAAAA	AAAAAAAAAAAAAAAAAAAA
AC15,	AAAAAAACAAAAAAAAAAAA	AAAAAAAAAAAAAAAAAAAA	AAAAAAAAAAAAAAAAAAAA
AC16,	AAAAAAAAAAAAAAAAAAAA	AAAAAAAAAAAAAAAAAAAA	ADAAAAAAADAAAAAAAAAA

Table 2 continued.

AC17,	AAAAAAAAAAAAAAAAAABA	AAAAAAAAAAAAAAAAAAAA	AAAAAAAAAAAAAAAAAAAA
AC18,	ACAAAAAAAAAAAAAAAABA	AAAAAAAAAAAAAAAAAAAA	AAAAAAAAAAAAAAAAAAAA
AC19,	ACAAAAAAAAAAAAAAAABA	AAAAAAAAAACAAAAAAAAA	AAAAAAAAAAAAAAAAAAAA
AC20,	ACAAAAAAAAAAAAAAAAAA	AAAAAAAAAAAADAAAAAAA	AAAAAAAAAAAAAAAAAAAA
AC21,	AAAAAAAAAAAAAAAAAAAA	AAAAAAAAAACAAAAAAAAA	ACAACAACAAAAAAAAAAAA
AC22,	ABAAAAAAAAAAAAAAAAAA	AAAAAAAAAACAAAAAAAAA	ACAACAACAAAAAAAAAAAA
AC23,	ABAAAAAAAAAAAAAAAAAA	AAAAAAAAAAAAAAAAAAAA	AAAACAACAAAAAAAAAAAA
AC24,	ABAAAAAAAAAAAAAAAAAA	AAAAAAAAAAAAAAAAAAAA	ACAACAACAAAAAAAAAAAA
AC25,	ABAAAAAAAAAAAAAAAAAA	AAAAAAAAAACAAAAAAAAA	AAAAAAACAAAAAAAAAAAA
AC26,	ABAAAAAAAAAAAAAAAAAA	AAAAAAAAAACAAAAAAAAA	AAAACAACAAAAAAAAAAAA
AC27,	ABAAAAAAAAAAAAAAAAAA	AAAAAAAAAACAAAAAAAAA	AAAACAACAAAABAAAAAAA
AC28,	ABAAAAAAAAAAAAAAAAAA	AAAAAAAAAACAAAAAAAEA	AAAACAACAAAAAAAAAAAA
AC29,	ABAAAAAAAAAAAAAAAAAA	AAAAAAAAAAAAAAAAAAAA	AAAAAAAAAAAAAAAAAAAA
AC30,	ABAAAAAAAAAAAAAAAAAA	AAAAAAAAAACAAAAAAAAA	AAAAAAAAAAAAAAAAAAAA
AC31,	ABAAAAAAAAAAAAAAAAAA	AAAAAAAAAACAAAAAAAAA	AAAACAAAAAAAAAAAAAAA
AC32,	ABAAAAAAAAADAAAACAAA	AAAAAAAAAACAAAAAAAAA	AAAAAAAAAAAAAAAAAAAA
AC33,	ABAAAAAAAAAAAAAAAAAA	AAAAAAAAAAAAAAAAAAAA	AAACDAAAAAAAAAADCAAA
AC34,	ABAAAAAAAAAAAAAAAAAA	AAAAAAAAAACAAAAAAAAA	AAACDAAAAAAAAAADCAAA
AC35,	ABAAAAAAAAAAAAAAAAAA	AAAAAAAAAACAAAAAAAAA	AAACCAAAAAAAAAADCAAA
BC01,	ABABAAAAAAAABABAAAAA	BABAAABABAAAAAAAABAB	AAAAABAABBBAAABBAABA
BC02,	ABABABAAABAABABAAAAA	BABAABBABAAAAAAAABAB	AAAAABAABBBAAABBAABA
BC03,	ABBBABAAABAABABAAAAA	BABAABBABAAAAAAAABAB	AAAAABAABBBAAABBAABA
BC04,	ABBBBBAAABAABABAAAAA	BABAABBABAAAAAAAABAB	AAAAABAABBBAAABBAABA
BC05,	ABBBABAAABAABABAAAAA	BABAABBABAAAAAAAABAB	AAAAABAABBBAAACBAABA
BC06,	ABBBABAAABAABABAAAAA	BABAABBABAAAAAAAABAB	AAAAABAACBBAAABBAABA
BC07,	ABBBABAAABAABABAAAAA	BABAABBAHAAAAAAAABAB	AAAAABAABBBAAABBAABA

Table 2 continued.

BC08,	ABBBABAAABBABABAAAAA	BABAABBABAAAAAAAABAB	AAAAABAABBBAAABBAABA
BC09,	ABBBABAAABBACABAAAAA	BABAABBABAAAAAAAABAB	AAAAABAABBBAAABBAABA
BC10,	ABBBABBAABAADABAAAAA	BABAABBABAAAAAAAABAB	AAAAABAABBBAAABBAABA
BC11,	ABBBABAAABAABABAAAAA	BABAABBABAAAAAAAABAB	AAAAABAABBBAAABCAAEA
BC12,	ABBBABAAABAABABBAAAA	BABAABBABAAAAAAAABAC	AAAAABAABBBAAABBAABA
BC13,	ABBBABAAAAAABABAAAAA	BABAAFBABAAAAAAAABAB	AAAAABAABBBAAABBAABA
BC14,	ABBBABAAABADBABAAAAA	BABAAABABAAAAAAAABAB	AAAAABAABBBAAABBAABA
BC15,	ABBBABAABBAABABAAAAA	BABAABBAAAAAAAAAABAB	AAAAABAABBBAAABBAACA
BC16,	ABBBABAABBBABABAAAAA	BABAABBABAAAAAAAABAA	AAAAABAABBBAAABBAABA
BC17,	ABBBABAABBBABABAAAAA	BABAABBAIAAAAAAAABAB	AAAAABAABBBAAABBAABA
BC18,	ABBBABAAABAACABAAAAA	BACAABBABAAAAACACBAB	AAAAABAABBBAAABBAABA
BC19,	ABBBABAAABBABABAAAAA	BABDABBAIAABAAAAABDB	AAAAABAABBBAAABBAABA
BC20,	ABBBABAABBAABABAAAAA	BBBAABBABAAAAAAAABCB	AAABABAABBBAAACBAABA
BC21,	ABABACABABAABABAAAAA	AABAAEBABAAAAAAAABAB	AAAAABAABBBABABBAADA
BC22,	ABBBACAAABAABABAAAAA	AABAABBABAAAAAAAABAB	AAAAABAABCBAAABBAADA
BC23,	ABBBABAAAAAABABBAAAA	BABAABCABAAACAAAABCB	AAAAABAABBBAAABBAADA
BC24,	ABBBABAAABAABABAAAAA	AABAABCABAAACAAAABCB	AAAAABAABBBAAABBAABA
BC25,	ABBBBBAABAAABABAAAAA	BABAABCADABACAAAABCB	AAAAABAABBBAAABBAADA
BC26,	ABBBABAABBAABABAAAAA	DADAABCAAAAACAAAABAB	AAAAABAABBBAAABBAABA
BC27,	ABBBADAABBAABABAAAAA	AABDABACBAABAAAAABAB	AAAAABAABBBAAABBAADA

[1]Restriction enzymes used (in order): RsaI, DdeI, AseI, TaqI, SspI, HinfI, NciI, BstUI, BstNI, BsaJI, HphI, HhaI, MspI, AvaII, Sau3AI, HpaI, Bsp1286I, HincII, HaeIII, AluI.

[2]Restriction enzymes used (in order): RsaI, DdeI, AseI, TaqI, DraI, HinfI, NciI, BstUI, BstNI, BsaJI, HphI, AccI, MspI, HhaI, Sau3AI, Sau96I, Bsp1286I, HincII,

Table 2 continued.

HaeIII, AluI.

[b]Restriction enzymes used (in order): RsaI, DdeI, AseI, TaqI, DraI, HinfI, NciI, SspI, BstNI, BsaJI, HphI, AccI, MspI, HhaI, Sau3AI, Sau96I, Bsp1286I, HincII, HaeIII, BsrI.

TABLE 3. Haplotypes identified for Arctic and Bering ciscoes for the CytB and ORF3-4 gene regions. The number of fragment patterns (phenotypes) ranged from 1-4 (A-D) for each of the 18-19 enzymes that cut these two regions. Only one haplotype, other than the common Arctic cisco haplotype, was found in more than a single individual (haplotype 5).

Haplotype	N	CytB[1]	ORF 3-4[2]
Arctic ciscoes			
1	22	A A A A A A A A A A A A A A A A A A	A A A A A A A A A A A A A A A A A A
2	1	A A A A A A A A A A A A A A A A B A	A A A A A A A A A A A A A A A A A A
3	1	A A A A A A A A A A C A A B A A A A	A A A A A A A A A A A A A A A A A A
4	1	A A A A A A A A A A A A A A A A A A	A A A A A A A A A C A A A A A A A A
5	5	A A A A A A A A A A A A A A A A A A	B A A A A A A A A A A A A A A B A A
6	1	A A A A A A A A A A A A A A A A A A	D A A A A A A A A D A A A A A A A A
7	1	A A A A A A A A A A A A A A A A A A	A A A A A A A A A A A A A A A B A A B
Bering cisco			
8	1	A B B A B B B B A A B A A A B A B A	A B B B B B B A A A A A A A A A A A

[1] Enzymes used were (in order): HaeIII, HincII, NciI, HphI, DdeI, MspI, RsaI, Bsp1286I, HhaI, Sau96, HinfI, BslI, SspI, DpnII, BsaJ, TaqI, MseI, BstN. Five enzymes (not included) did not cut this region: AseI, DraI, BclI, BstU, BsrI.

[2] Enzymes used were (in order): HhaI, NciI, HincII, HphI, DdeI, Sau96, RsaI, DpnII, HaeIII, HinfI, BstU, BstN, MspI, Bsp1286I, BsaJ, BsrI, TaqI, MseI, BslI. Three enzymes (not shown) did not cut this region: AseI, DraI, SspI.

MANUSCRIPT 2

IDENTIFICATION OF ARCTIC AND BERING CISCOES IN THE COLVILLE RIVER FISHERY

John W. Bickham, John C. Patton, Shamone Minzenmayer,
L.L. Moulton, and Benny J. Gallaway

Submitted To American Fisheries Society Symposium on Arctic Fish Biology

IDENTIFICATION OF ARCTIC AND BERING CISCOES IN THE COLVILLE RIVER FISHERY

JOHN W. BICKHAM[2], JOHN C. PATTON[1], SHAMONE MINZENMAYER[2],
L. L. MOULTON[3], AND Benny J. GALLAWAY[1]

[1]LGL ECOLOGICAL GENETICS, INC., 1410 CAVITT ST., BRYAN, TX 77901
[2]DEPARTMENT OF WILDLIFE AND FISHERIES SCIENCES, TEXAS A&M UNIVERSITY, COLLEGE STATION, TX 77843
[3]MJM RESEARCH, 5460 NE TOLO RD., BAINBRIDGE ISLAND, WA 98110

Abstract.--Arctic and Bering ciscoes were obtained from the Colville River Delta during the Fall fishery in 1990. Specimens initially were identified to species based on exomorphological features. Gill raker counts were distinctly bimodal with Bering ciscoes having 21-24 and Arctic ciscoes having 23-30 gill rakers. Mitochondrial DNA haplotypes were different between the two species and all animals identified as Bering ciscoes had haplotypes characteristic of that species. All of the animals identified as Arctic ciscoes had Arctic cisco haplotypes except two that had Bering cisco haplotypes. Those two individuals had gill raker counts of 26 and 28 which are characteristic of Arctic ciscoes. These same fish were assayed electrophoretically for the Gpd-1 locus. Four alleles were identified of which two (B and C) showed strong frequency differences between the two species. One of the two fish with Arctic cisco morphology and gill raker

counts and Bering cisco mitochondrial DNA haplotypes had a Gpd-1 genotype common to Arctic ciscoes and the other had a genotype found at low frequency in both species. It is concluded that Arctic and Bering ciscoes possess diagnostic morphological characters that allow for their accurate separation in the fishery. Moreover, only a low level of apparent introgression of mitochondrial DNA, from Bering cisco into Arctic cisco, was detected. We therefore conclude that these taxa represent distinct species as currently recognized.

The Arctic cisco (Coregonus autumnalis) is an anadromous salmonid fish that spawns in the tributaries of the Mackenzie River in North America (Gallaway et al., 1983). Coastal habitat includes the nearshore, low-saline, neritic waters of the Beaufort Sea from Victoria Island (Canadian Northwest Territories) to Point Barrow (Alaska). The two most important coastal feeding and overwintering areas are the deltas of the Colville River in Alaska and the Mackenzie River in the Northwest Territories. This species and the broad whitefish (Coregonus nasus) are the two most economically important fish along the Alaskan North Slope. Native subsistence fishing and commercial fishing for Arctic ciscoes take place in the Colville River delta (Gallaway et al., 1983).

The most closely related taxon to the Arctic cisco ostensibly is the Bering cisco (Coregonus laurettae) which spawns primarily in the Yukon River system and whose coastal distribution overlaps that of the Arctic cisco from Point Barrow to the Colville River (Craig, 1989). These taxa are superficially very similar and their specific status has been questioned (Dillinger, 1989). Interpretations of the population trends in the Arctic cisco fishery at the Colville River is complicated by the presence of Bering

ciscoes. Because the mechanisms of transport of Arctic and Bering ciscoes from their spawning areas to the Colville River delta is distinctly different, with Arctic ciscoes being transported from the Mackenzie as young of the year by passive transport (Gallaway et al., 1983; Fechhelm and Fissel, 1988) and Bering ciscoes travelling from the Yukon to the Colville as juveniles or adults by active transport, the correct interpretation of the causes of population fluctuations in the fishery requires the ability to accurately distinguish these forms. The purpose of this study is to test the ability of the fishery biologists to distinguish Arctic and Bering ciscoes using morphological, biochemical, and molecular characters taken from field-identified specimens, and to assess the level of genetic introgression and the taxonomic status of these species.

METHODS

Specimens were collected in the field using gill nets during the Fall fishery of 1990. Individuals were identified as Bering ciscoes (N = 20) or Arctic ciscoes (N = 49) based upon the presence of small black dots with faint white halos on the body and (or) white spots on the fins that are visible in live Bering ciscoes and absent in Arctic ciscoes. Fish were frozen whole and shipped to the laboratory where they were dissected to remove tissues (heart, liver, muscle) for allozyme and DNA analyses. The first gill arch was removed from each fish and the gill rakers counted as described by Dillinger (1989). A small piece of the liver of each fish was homogenized for protein electrophoresis. The enzyme glycerol-3-phosphate dehydrogenase (Gpd-1; Enzyme Commission number 1.1.1.8) was analyzed (for all fish except three Arctic ciscoes) as described by Bickham et al. (1989) and Morales et al. (in press). None of the specimens

reported in this study were reported in the previous studies. Mitochondrial DNA was examined for all specimens using the polymerase chain reaction restriction-enzyme assay of the ORF1 gene region as described in Bickham et al. (submitted). The specimens reported in this study also were included in the mitochondrial DNA study of Bickham et al. (submitted).

RESULTS

Gill-raker counts ranged from 23-30 (X = 27) for Arctic ciscoes and 21-24 (X = 21.8) for Bering ciscoes (Fig. 1). There were three Bering and two Arctic ciscoes with gill-raker counts within the range of overlap of the two species (23-24).

Mitochondrial DNA haplotypes were distinctly different for Arctic and Bering ciscoes. Two restriction enzymes (DdeI and HinfI) give diagnostic fragment patterns for the ORFI gene region. Three patterns (A-C) were observed when Arctic cisco DNA was cut with DdeI but only the B pattern was observed in Bering cisco. Two patterns were observed with HinfI (A and B) in Arctic cisco but only the B pattern was observed in Bering cisco. Thus, all Bering cisco had BB haplotypes for these two enzymes which is consistent with the observation of Bickham et al. (submitted) that this is the predominant haplotype in Bering ciscoes taken from spawning runs in the Yukon River. Arctic ciscoes had haplotypes AA (N = 35), BA (N = 6), CA (N = 6), and BB (N = 2). The AA, BA, and CA haplotypes were observed by Bickham et al. (submitted) to be the predominant haplotypes in Arctic ciscoes taken from spawning runs in the Mackenzie River system.

Genotypic and allelic frequencies were computed for Arctic and Bering ciscoes for the GPD-1 locus and analyzed using BIOSYS-1. Allele frequencies for Arctic and Bering ciscoes respectively were: A = .272, 2.75; B = .511, .225; C = .054, .400 and D = .163, .100. Mean heterozygosity (H) was 0.636 for Arctic ciscoes and 0.704 for Bering ciscoes. Chi square tests for Hardy-Weinberg equilibrium (using Levene correction for small sample sizes) was $P < .001$ for Arctic ciscoes and $P = .257$ for Bering ciscoes.

DISCUSSION

The difference in gill-raker counts observed in our samples is consistent with previous workers who have observed lower, but overlapping, counts for Bering ciscoes compared to Arctic ciscoes (Dymond, 1943; McPhail, 1966; Dillinger, 1989). This, combined with the subtle, but consistent, difference in color pattern between the two indicate these two taxa are morphologically distinct, at least in their geographic area of overlap.

Previous genetic studies have shown these taxa to be distinct as well. Bickham et al. (1989) and Morales et al. (in press) found no fixed differences in allele frequencies between these taxa, but they noted strong frequency differences at two loci (Mpi and Gpd-1). Our samples showed allele frequencies for Gpd-1 similar to those found in the previous studies which is another indication that fish were generally assigned correctly to their respective taxa. Lockwood and Bickham (1992) examined genome size variation in Arctic ciscoes taken from Beaufort Sea coastal aggregations and concluded that the pattern of variation observed (lower average genome sizes in the West and higher genome sizes in the East)

was due to the inclusion of higher numbers of Bering ciscoes in the western samples. Thus, Bering ciscoes were thought to have a lower genome size than Arctic ciscoes. Mitochondrial DNA has been shown to be distinctly different between Arctic and Bering ciscoes (Bickham et al., submitted). In our samples, all fish identified as Bering ciscoes had mitochondrial DNA haplotypes (BB) characteristic of that taxon. All fish identified as Arctic ciscoes had mitochondrial DNA haplotypes (AA,BA, CA) characteristic of that taxon except for two specimens that had BB. Thus, these two specimens are potentially animals of hybrid origin or were misidentified.

The two fish that were identified as Arctic ciscoes but had Bering cisco mitochondrial DNA were otherwise typical Arctic ciscoes. Gill-raker counts for these fish were 26 and 27, which is well within the range of Arctic ciscoes and outside the range of Bering ciscoes. Additionally, these fish had Gpd-1 genotypes of BB and AB. The frequencies of BB in Arctic and Bering ciscoes was .4 and 0, respectively. The frequencies of AB genotypes was .045 and .1 respectively. Thus, one of the two fish of questionable identity had an Arctic cisco Gpd-1 genotype and the other had a genotype found in low frequency in both species.

We thus conclude that these fish were not Bering ciscoes misidentified as Arctic ciscoes. Rather, these fish likely were of hybrid origin in possessing a Bering cisco female ancestor. These female ancestors probably were not the mothers of either of these fish; it is likely they were more distant ancestors. Mitochondrial DNA is a particularly sensitive genetic marker for revealing past hybridization events because it is maternally inherited and clonal (Brown, 1983; Avise, 1986; Wilson et al.,

1985). After a few generations of backcrossing to one of the parental species the nuclear genotypes of the descendants of a hybrid cross quickly come to resemble one of the parental species. However, when mitochondrial DNA is introduced by hybridization it is retained in any unbroken female lineage. Because of these features, mitochondrial DNA has been used successfully to track introgressive hybridization in a number of vertebrate species including mule deer and white-tailed deer (Carr et al., 1986; Cronin et al., 1988, 1991; Ballinger et al., 1992; Carr and Hughes, in press).

The frequency of fish with hybrid ancestry is apparently low in Arctic ciscoes. In our sample, only two of 49 Arctic ciscoes had Bering cisco haplotypes, or 4%. Bickham et al. (submitted) found 5.6% of fish from all coastal sites east of the Colville River to have Bering cisco haplotypes and 2% of all fish taken from spawning runs in the Mackenzie River drainage to have Bering cisco haplotypes. Our data from the Colville River fishery is consistent with that of Bickham et al. (submitted) in showing a low frequency of Bering cisco haplotypes within samples ostensibly of Arctic ciscoes. Moreover, the absence of fixed differences in allozymes (Bickham et al., 1989; Morales et al., in press) could be due to a past history of low-level hybridization.

We therefore conclude that hybridization is rare between Arctic and Bering ciscoes and that these taxa are best considered to be different species as presently recognized. Furthermore, it is concluded that consistent morphological differences between these two species are present and that the fisheries biologists at the Colville River can identify these species with virtually 100% accuracy.

ACKNOWLEDGMENTS

We thank the many people who have aided us in obtaining specimens of ciscoes from Canada and Alaska. Many of the specimens used in this study were also examined in previous studies of allozymes and flow cytometry. Additional specimens not used in those studies were provided by L. Moulton, C. George, R. Fechhelm, W. Griffiths, and S. Lockwood. We thank the Canadian Department of Fisheries and Oceans for granting collecting permits. This study was jointly funded by BP Exploration (Alaska) Inc., the Minerals Management Service, and the U.S. Fish and Wildlife Service, Region 8.

LITERATURE CITED

Avise, J. C. 1986. Mitochondrial DNA and the evolutionary genetics of higher animals. Phil. Trans. R. Soc. London, Ser. B 312:325-342.

Ballinger, S. W., L. H. Blankenship, J. W. Bickham, and S. M. Carr. 1992. Allozyme and mitochondrial DNA analysis off a hybrid zone between white-tailed and mule deer (Odocoileus) in West Texas. Biochem. Genet. 30:1-11.

Bickham, J. W., S. M. Carr, B. G. Hanks, D. W. Burton, and B. J. Gallaway. 1989. Genetic analysis of population variation in the Arctic Cisco using electrophoretic, flow cytometric, and mitochondrial DNA restriction analyses. Biol. Pap. Univ. Alaska 24:112-122.

Bickham, J. W., J. C. Patton, B. J. Gallaway, and J. C. Morales. Biogeography, phylogeny, and relative rates of evolution of mitochondrial DNA in Arctic and Bering ciscoes from the western Arctic of North America. Submitted.

Brown, W. M. 1983. Evolution of animal mitochondrial DNA, pp. 62-88. In M. Nei and R. K. Koehn (eds.), Evolution of Genes and Proteins. Sinauer Associates, Sunderland, MA.

Carr, S. M., S. W. Ballinger, J. N. Derr, L. H. Blankenship, and J. W. Bickham. 1986. Mitochondrial DNA analysis of hybridization between sympatric white-tailed and mule deer in west Texas. Proc. Natl. Acad. Sci. U.S.A. 83: 9576-9580.

Carr, S. M., and G. A. Hughes. In Press. The direction of hybridization between species of North American deer (Odocoileus) as inferred from mitochondrial cytochrome B sequences. J. Mammal.

Craig, P. C. 1989. An introduction to anadromous fishes in the Alaskan Arctic. Biol. Pap. Univ. Alaska 24:27-54.

Cronin, M. A., M. E. Nelson, and D. F. Pac. 1991. Spatial heterogeneity of mitochondrial DNA and allozymes among populations of white-tailed deer and mule deer. J. Hered. 82:118-127.

Cronin, M. A., E. R. Vyse, and D. G. Cameron. 1988. Genetic relationships between mule deer and white-tailed deer in Montana. J. Wildl. Manage. 52:320-328.

Dillinger, R. E. 1989. An analysis of the taxonomic status of the Coregonus autumnalis species complex in North America, and an investigation of the life histories of whitefishes and ciscoes (Pisces; Coregoninae) in North America and Eurasia. Ph.D. Diss. Memorial U. of Newfoundland, St. John's.

Dymond, J. R. 1943. The coregonine fishes of northwestern Canada. Trans. Roy Canad. Inst. 24:171-232.

Fechhelm, R. G., and D. B. Fissel. 1988. Wind-aided recruitment of Canadian Arctic cisco (Coregonus autumnalis) into Alaskan waters. Canadian Journal of Fisheries and Aquatic Sciences 45:906-910.

Gallaway, B. J., W. B. Griffiths, P.C. Craig, W. J. Gazey, and J. W. Helmericks. 1983. An assessment of the Colville River delta stock Arctic cisco--migrants from Canada? Biological Papers of the University of Alaska 21:4-23.

Lockwood, S. F., and J. W. Bickham. 1992. Geographic trends in DNA content in Beaufort Sea coastal populations of Arctic cisco (Coregonus autumnalis) identified by flow cytometry. Transactions of the American Fisheries Society 121:13-20.

McPHAIL, J. D. 1966. The Coregonus autumnalis complex in Alaska and northwestern Canada. J. Fish. Res. Board Can. 23:141-148.

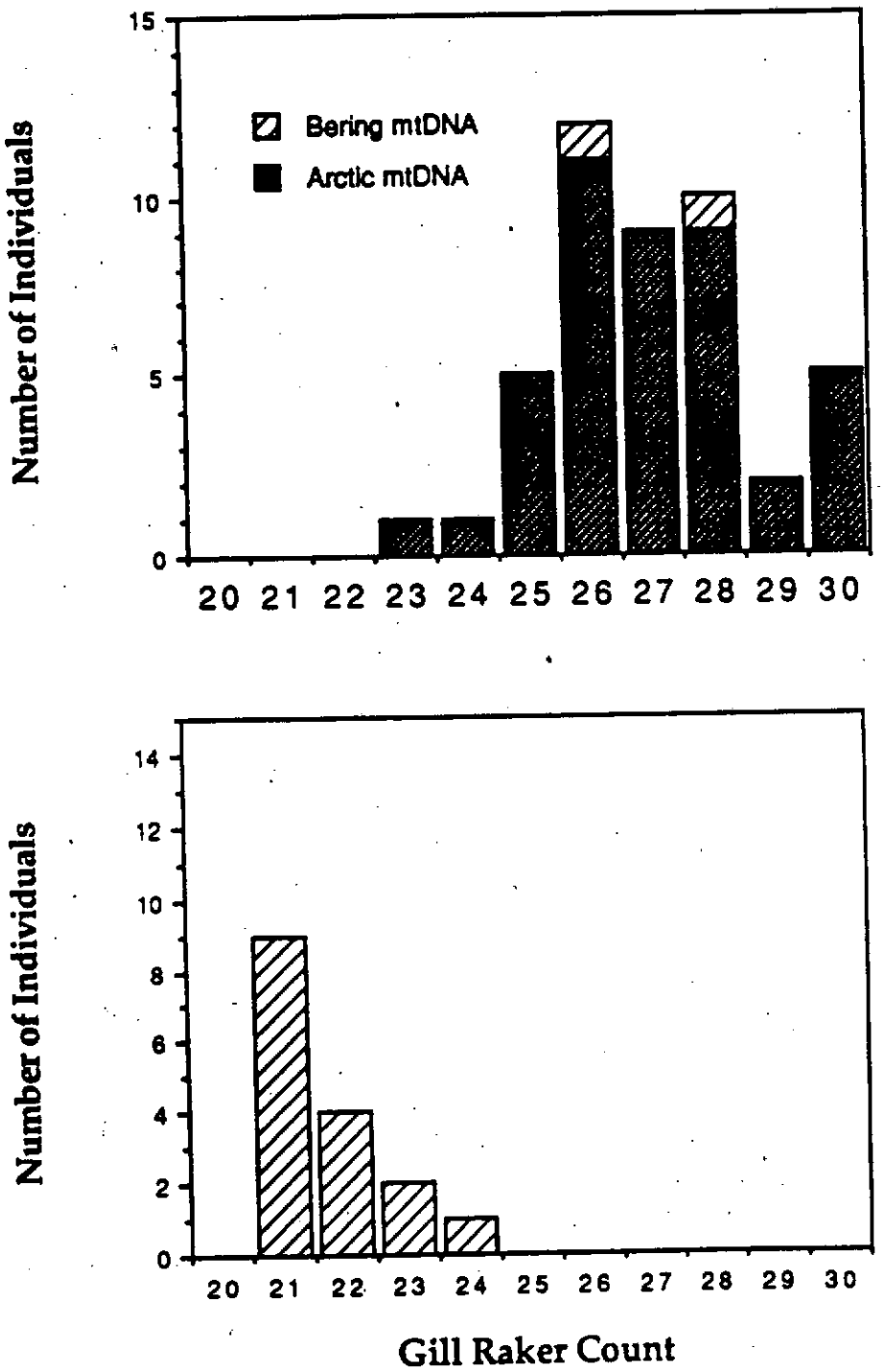
Morales, J. C., J. W. Bickham, J. N. Derr, and B. J. Gallaway. In Press. Genetic analysis of population structure in Arctic cisco (Coregonus autumnalis) from the Beaufort Sea. Copeia.

Wilson, A. C., ET AL. 1985. Mitochondrial DNA and two perspecitives on evolutionary genetics. Biol. J. Linnean Soc. 26:385-400.

FIGURE LEGENDS

FIGURE 1.–Gill-raker counts and mitochondrial DNA haplotypes for 69 fish taken from the Colville River fishery. Individuals were identified in the field as Arctic (top panel) or Bering (bottom panel) ciscoes based upon the presence of dark spots with blue halos in Bering ciscoes that were absent from Arctic ciscoes. Animals identified as Bering ciscoes had lower gill raker counts than most of the fish identified as Arctic ciscoes. Mitochondrial DNA haplotypes previously identified as being characteristic of Bering ciscoes were found in all fish identified as Bering ciscoes. Mitochondrial DNA haplotypes previously identified as being characteristic of Arctic ciscoes were found in all but two Arctic ciscoes. Those two individuals are potentially hybrids or misidentified specimens.

Colville River Ciscoes



MANUSCRIPT 3

GENETIC STOCK ASSESSMENT OF ARCTIC CISCO (COREGONUS AUTUMNALIS) FROM THE BEAUFORT SEA USING NUCLEOTIDE SEQUENCES OF THE MITOCHONDRIAL CONTROL REGION

John W. Bickham, John C. Patton, and Benny J. Gallaway

Submitted To Science

Genetic Stock Assessment of Arctic cisco (Coregonus autumnalis) from the Beaufort Sea Using Nucleotide Sequences of the Mitochondrial Control Region

JOHN W. BICKHAM, JOHN C. PATTON, AND BENNY J. GALLAWAY

ABSTRACT.--The Arctic cisco represents one of the most economically important fish species on the Beaufort Sea coast of Alaska. This population is at risk due to the potential effects of oil-related activities at Prudhoe Bay. For example, the presence of gravel-filled causeways could cause a serious impediment to the passive migration of young of the year Arctic cisco from the Mackenzie River system to the primary Alaskan overwintering habitat in the delta of the Colville River. Mitochondrial DNA haplotypes were identified by nucleotide sequence of the control region and previously published restriction site analysis. MtDNA haplotype frequencies indicate a generally low level of population genetic divergence. There is one haplotype which predominates (46-69%) in all spawning and overwintering sites. Lower levels of spawning-site fidelity (i.e., gene flow) compared to other anadromous fish such as salmon, and the recent (post-Pleistocene) origin of the populations from common ancestral populations are probably responsible for the low level of differentiation. Despite the overall low level of differentiation, statistical analysis of haplotype distributions suggests that stocks in the downstream region, especially the Peel River, contribute more fish to the Alaskan overwintering population than the upstream stocks. Our sampling was limited, and additional analyses are needed for better resolution of the structure of the Mackenzie River stocks and the composition of the wintering populations.

The Arctic cisco (Coregonus autumnalis) is the focus of an exhaustive research and monitoring program designed to evaluate the effects of oil production and related activities on fisheries resources along the Alaskan North Slope. In North America, this species spawns in tributaries of the Mackenzie River system (Fig. 1). Its' coastal distribution is from Victoria Island in the Northwest Territories to Point Barrow in Alaska. Coastal habitat includes the nearshore, low-saline, waters for feeding and deep pools in the deltas of major rivers for overwintering. The primary overwintering habitats are the deltas of the Mackenzie River in the Northwest Territories and the Colville River in Alaska. The Alaskan populations are of special concern because the unique life history of the species makes them particularly vulnerable to impediments (such as the causeways that bisect the nearshore, low-saline waters near Prudhoe Bay) on their migratory route.

Arctic cisco undergo a passive migration, as young of the year, from the Mackenzie River delta into Alaskan waters (Gallaway et al., 1983, 1989). This migration is wind-aided, and it is known to occur primarily in years that have strong, northeasterly winds (Fechhelm and Fissel, 1988). Population studies at both the Colville River and Sagavanirktok River deltas show the year-to-year recruitment of young of the year Arctic cisco to be highly variable and to be correlated with the strength and timing of northeasterly winds (Fechhelm and Griffiths, 1990). Because the primary overwintering habitat for Arctic cisco in Alaska is the delta of the Colville River, it is thought that the survival of the Alaskan population is possibly dependant upon the ability of young of the year fish to complete the migration from the Mackenzie to the Colville River without obstruction. Moreover, this species is important in both a commercial fishery and an Inupiat subsistance fishery in

the Colville River delta (Craig, 1989; Gallaway et al., 1983). All of these reasons, combined with the fact that the species undergoes a transboundary migration, make it the center of a politically, and environmentally, sensitive issue--the effects of gravel filled causeways and oil production on North Slope fish stocks.

Previous studies of genetic variability in Arctic cisco showed this species to have a moderate level of variation in allozyme loci (Bickham et al., 1989; Morales et al., in press), a high level of variation in genome size Lockwood and Bickham (1991, 1992), and a rather low level of variation in mitochondrial DNA (Bickham et al., 1989, submitted a). Genetic assays proved to be useful in distinguishing Arctic ciscoes from the closely related Bering cisco (Coregonus laurettae) and ostensible hybrids (Bickham et al., submitted a, b). Animals with Bering cisco mitochondrial DNA haplotypes were rare in the spawning streams of the Mackenzie River system (2%) and in the coastal sites east of the Colville River (5%; Bickham et al., submitted a).

This study was designed to determine whether or not multiple stocks of Arctic cisco exist within the Mackenzie River system and, if so, which stock or stocks primarily contribute to the makeup of the coastal aggregations of the Colville and Sagavanirktok River deltas in Alaska. An understanding of the stock composition of the Alaskan populations would identify what stock or stocks might be at risk should any environmental mishap occur. Additionally, this knowledge would contribute to a better understanding of fish movements and population structure of Arctic anadromous fishes.

MATERIALS AND METHODS

In this study we investigate genetic stock composition of Arctic cisco populations from four coastal sites from the Beaufort Sea and five spawning-site populations from Mackenzie River tributaries using nucleotide sequence of a 300 bp segment of the control region of the mitochondrial DNA. Oligonucleotide primers were developed to amplify the entire control region. These primers, placed in the $tRNA^{Glu}$ and $tRNA^{Thr}$ genes, amplify a ≈ 1-kb product. A portion of this product near the $tRNA^{Glu}$ primer and inside the control region was sequenced using automated DNA sequence analysis employing dye labelled terminators (Ferl et al., 1991). Nucleotide sequences were obtained from 326 fish which also were examined in an extensive restriction site analysis by Bickham et al. (submitted a). Genetic differentiation of populations and stock definition was accomplished by use of a Chi-square exact test (contingency chi-square analysis using permutation testing based upon an algorithm given by Roff and Bentzen, Mol. Biol. Evol. Vol 6: 539-545, 1989 as interpreted by Alan Templeton 15 May 1990 in Basic and converted to c by John G. Cole) which generates a probability of identity for paired comparisons. We used $\alpha = 0.1$ to indicate statistically significant differences ($P \leq 0.1$) or identities ($P \geq 0.9$) among the paired comparisons. Values of $0.9 < P > 0.1$ were used only to indicate trends.

RESULTS AND DISCUSSION

Sequence analysis of the control region was consistent with the observation of Bickham et al. (submitted a) that Arctic cisco populations are composed primarily of a single, common haplotype (haplotype C, Table 1) and numerous rare or low-frequency haplotypes. Thirteen haplotypes were

identified by restriction analyses (Appendix I). Sequence analysis of the control region identified two nucleotides (positions 47 and 109) both of which were present as either A or T. In addition, a sequence repeat was scored as either present or absent. By combining the restriction-site data with the sequence data, 20 haplotypes were identified (Table 1, Appendices VII and VIII). The distribution of low-frequency haplotypes show evidence of subdivision among the spawning-site populations. A Chi Square Exact Test was conducted which showed the four spawning-site populations to be composed of at least three significantly different populations. The upstream tributaries (Mountain and Carcajou Rivers) were shown to have very high values for probability of identity (P = 0.9) and thus these populations were combined for further analyses. The downstream tributaries (Peel and Arctic Red Rivers) were not significantly identical with each other (P = 0.148) but were significantly different from the combined upstream population (P = 0.065; Table 2).

Chi square analysis showed the probability of identity of the Colville (the western-most coastal site) with other coastal sites to be progressively lower with increased geographic distance (Table 2). The Alaskan coastal samples (Colville and Sagavanirktok) both were significantly different from the upstream stock (P = 0.025 and 0.065; Table 1). Both Alaskan coastal samples were most similar to the the Peel River stock (Table 2). In particular, our sample from the Sagavanirktok River was very similar to the Peel River stock (P = 0.913). From these data we conclude that multiple stocks exist among Arctic cisco populations that spawn within the Mackenzie River system, although the stocks are only weakly differentiated, and that it is primarily the two downstream stocks that contribute to the Alaskan coastal

aggregations. The Peel River appears to be the predominant stock represented in Alaska, although it is likely that other stocks are present as well.

Of the Canadian coastal sites, Stokes Point, which is to the west of the Mackenzie River, is most similar to the Peel River stock. This is consistent with the above observation that Peel River fish predominate in Alaska because they would have to pass Stokes Point on their westward migration. Atkinson Point, which is to the east of the Mackenzie River, is the only coastal site that is most similar to the upstream stock (Table 2). Moreover, it is significantly different from both downstream stocks. It is likely that the population at Atkinson Point also is composed to a high degree of fish from the Liard River, based upon overlap in genome size estimates of these two populations by Lockwood and Bickham (1991, 1992).

The data presented in this study are consistent with previous studies showing the Arctic cisco populations in the Mackenzie River to be weakly differentiated (Bickham et al., 1989). It should be emphasized that weak genetic differentiation does not necessarily mean the stocks are unimportant from a management perspective. In the case of the Arctic cisco, lack of strong genetic differences among populations is more likely the result of historical factors than of population biology. The Mackenzie River system was mostly glaciated during the Wisconsin Period and thus the populations under study are no older than the end of the Pleistocene. It is likely that most of the observed variation in mitochondrial DNA among Arctic cisco populations is the result of different frequencies of haplotypes being present among the founders of the various stocks. The populations are simply not old enough to expect the establishment of many new variants to have taken place. This

study used the relative proportions of low-frequency haplotypes to discriminate stocks. Such low-frequency haplotypes are especially sensitive to stochastic processes such as genetic drift and should show population differentiation more quickly than changes in the proportions of high-frequency haplotypes.

In addition to recency of origin, another factor that might account for low levels of genetic differentiation in the anadromous Arctic cisco is higher than expected levels of gene flow among spawning tributaries. The degree to which such straying occurs in Arctic cisco is unknown. Nevertheless, coregonines are not thought to have the high degree of natal-site fidelity usually attributed to salmon.

The low level of variation in mitochondrial DNA of Arctic ciscoes contrasts markedly with the situation in Bering ciscoes. Bickham et al. (submitted a) showed the latter species to have one of the highest nucleon diversity estimates yet measured for a fish. Those authors attributed this to the fact that the Bering cisco spawns in the Yukon River system which was a glacial refugium. The difference is probably not due to any inherent difference in population biology because the two species are so similar in their natural history.

The conclusion that the downstream stocks, especially the Peel River stock, comprise the majority of the fish that move into Alaskan waters is supported by tag return data and is consistent with the known mechanism of dispersal of young of the year Arctic cisco. Tag returns from ciscoes tagged at the Sagavanirktok River delta show that adults undergo an eastward migration to the Mackenzie River delta (Fechhelm et al., 1989; Gallaway et al.,

1983; Moulton, 1989). More recent tag returns obtained at spawning tributaries include four from the Peel River and one from the Hare Indian River. The Hare Indian River is near the Carcajou and Mountain Rivers and thus could be expected to be part of the upstream stock. No spawning run of Arctic cisco was detected from this river during the field expedition that obtained most of the samples used in this study. Thus, the majority of tag returns are from the Peel River which is consistent with our conclusions. However, it should be cautioned that the tag returns are not the result of an extensive effort to obtain fish from all tributaries and could simply reflect sampling bias in favor of the Peel River.

In order for young of the year Arctic cisco to be transported to Alaskan waters, it is necessary for them to be positioned in the freshwater plume of the Mackenzie in such a way as to be captured by the west-moving, long-shore current (Moulton, 1989). Because the main channel of the Peel River empties into the western end of Mackenzie Bay, as young of the year fish are flushed out of the tributary by spring floods, they would be in the most favorable position to be entrained. In addition, the fish from the northern (downstream) tributaries would most likely arrive in the bay earlier than fish from the upstream tributaries thus making it more likely for them to be transported westward and more likely for them to reach the Colville River delta and successfully overwinter. It thus appears that the similarity between coastal sites to the west of the Mackenzie River and downstream stocks, and between Atkinson Point (east of the Mackenzie River) and the upstream stock is likely due to the timing as to when the young of the year fish reach the Mackenzie River delta.

Concern exists regarding the identity of the stock or stocks of Arctic cisco that could be impacted by the potential impediment to migration resulting from the presence of gravel-filled causeways and other effects (such as spills) of the petroleum industry in the Prudhoe Bay region of Alaska. As a result of the pesent study, attention should focus on the Peel River stock. However, there is presently no estimate of the proportion of the Peel River stock that is transported to Alaska, nor is there significant evidence that the existing causeways or oil-related activities seriously impair the passive migration of young of the year Arctic cisco. Nonetheless, the dynamics of the Alaskan populations of Arctic cisco clearly are indicative of a fragile and dynamic system. Future studies should focus on increasing our ability to differentiate the stocks of Arctic cisco and to assess in a similar way the stock composition of other Arctic anadromous fishes. Our conclusion that the Peel River stock predominates in Alaskan coastal sites should be considered as a hypothesis to be tested in future investigations. We cannot rule out the presence of other stocks in the Alaskan coastal sites, nor do we yet fully understand the importance of the Alaskan Beaufort Sea coast to the survival of any of these stocks.

ACKNOWLEDGMENTS

We thank the many people who have aided us in obtaining specimens of ciscoes from Canada and Alaska. Many of the specimens used in this study were also examined in previous studies of allozymes and flow cytometry. Additional specimens not used in those studies were provided by L. Moulton, C. George, R. Fechhelm, W. Griffiths, and S. Lockwood. We thank the Canadian Department of Fisheries and Oceans for granting collecting permits. This study was jointly funded by BP Exploration (Alaska) Inc., the Minerals Management Service, and the U.S. Fish and Wildlife Service, Region 8.

LITERATURE CITED

Bickham, J. W., S. M. Carr, B. G. Hanks, D. W. Burton, and B. J. Gallaway. 1989. Genetic analysis of population variation in the Arctic Cisco using electrophoretic, flow cytometric, and mitochondrial DNA restriction analyses. Biol. Pap. Univ. Alaska 24:112-122.

Bickham, J. W., J. C. Patton, B. J. Gallaway, and J. C. Morales. Biogeography, phylogeny, and relative rates of evolution of mitochondrial DNA in Arctic and Bering ciscoes from the western Arctic of North America. Submitted a.

Bickham, J. W., J. C. Patton, S. Minzenmayer, L. L. Moulton, and B. J. Gallaway. Identification of Arctic and Bering ciscoes in the Colville River fishery. Submitted b.

Craig, P. C. 1989. An introduction to anadromous fishes in the Alaskan Arctic. Biol. Pap. Univ. Alaska 24:27-54.

Fechhelm, R. G., J. S. Baker, W. B. Griffiths, and D. R. Schmidt. 1989. Localized movement patterns of least cisco (Coregonus sardinella) and Arctic cisco (Coregonus autumnalis) in the vicinity of a solid-filled causeway. Biol. Pap. Univ. Alaska 24:75-106.

Fechhelm, R. G., and D. B. Fissel. 1988. Wind-aided recruitment of Canadian Arctic cisco (Coregonus autumnalis) into Alaskan waters. Canadian Journal of Fisheries and Aquatic Sciences 45:906-910.

Fechhelm, R. G., and W. B. Griffiths. 1990. The effect of wind on the recruitment of Canadian Arctic cisco (Coregonus autumnalis) into the central Alaskan Beaufort Sea. Can. J. Fish. Aquat. Sci. 47:11:2164-2171.

Gallaway, B. J., W. J. Gazey, and L. L. Moulton. 1989. Population trends fro the Arctic cisco (Coregonus autumnalis) in the Colville River of Alaska as reflected by the commercial fishery. Biol. Pap. Univ. Alaska. 24:153-165.

Gallaway, B. J., W. B. Griffiths, P.C. Craig, W. J. Gazey, and J. W. Helmericks. 1983. An assessment of the Colville River delta stock Arctic cisco--migrants from Canada? Biological Papers of the University of Alaska 21:4-23.

Lockwood, S. F., and J. W. Bickham. 1991. Genetic stock assessment of spawning Arctic cisco (Coregonus autumnalis) populations by flow cytometric determination of DNA content. Cytometry 12:260-267.

Lockwood, S. F., and J. W. Bickham. 1992. Geographic trends in DNA content in Beaufort Sea coastal populations of Arctic cisco (Coregonus autumnalis) identified by flow cytometry. Transactions of the American Fisheries Society 121:13-20.

McPHAIL, J. D. 1966. The Coregonus autumnalis complex in Alaska and northwestern Canada. J. Fish. Res. Board Can. 23:141-148.

Morales, J. C., J. W. Bickham, J. N. Derr, and B. J. Gallaway. In Press. Genetic analysis of population structure in Arctic cisco (Coregonus autumnalis) from the Beaufort Sea. Copeia.

Moulton, L. L. 1989. Recruitment of Arctic cisco (Coregonus autumnalis) into the Colville Delta, Alaska, in 1985. Biol. Pap. Univ. Alaska 24:107-111.

Table 1. -- Numbers of individuals per halotype listed by locality and sample sizes per locality (*N*) for Arctic cisco (Coregonus autumnalis). Sample localities are presented in Fig. 1 Haplotypes (A-T) are identified by nucleotide sequence or restriction sites as described in the appendix.

Localities	Halotypes																				
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	*N*
Coastal Sites																					
Colville R.	3		37		2		6						2			2		2			54
Sagavanirktok R.	3		48		10		7			1			5	1		2	1	1	1		80
Stokes Pt.	1		16		1		2		1				3								24
Atkinson Pt.	2		12	1			3		1		1					2					22
Spawning Sites																					
Peel R.	1	1	27		7		1	1		1			2			1	1	1			44
Arctic Red R.			40		9		6							1	1	3				1	61
Upstream Stock	2		19		6	1	3		1		1	2				5	1				41
Sum																					326

Table 2. -- Results of Chi-square exact test for all pairwise comparison of four coastal-site populations (1-4) and three spawning-site stocks (5-7) of Arctic cisco. Numbers reported are the exact prohabilities of identity.

	Col	Sag	Sto	Atk	Peel	ARR	Ups
1. Colville R. (Col)	-	0.743	0.532	0.214	0.197	0.057	0.025
2. Sagavanirktok R. (Sag)		-	0.776	0.095	0.913	0.380	0.065
3. Stokes Pt. (Sto)			-	0.314	0.636	0.018	0.136
4. Atkinson Pt. (Atk)				-	0.060	0.010	0.590
5. Peel R. (Peel)					-	0.148	0.209
6. Arctic Red R. (ARR)						-	0.065
7. Upstream Stock (Ups)							-

APPENDICES

APPENDIX I

Table of Restriction Site Analysis

APPENDIX I. Table of Restriction Site Haplotypes.

Alternate Designation*	Haplotype	ORF 1			ORF 5/6							
		Dde I	Hae III	Hinf I	Dde I	Taq I	Dra I	Ssp I	Sau96 I	Bsp 1286	BsaJ I	Msp I
A	Bering	B	A	B	A	B	A	A	B	A	A	A
B-E	A	A	A	A	A	A	A	A	A	A	A	A
G	C	C	B	A	A	A	A	A	A	A	A	A
H-J	OA	B	A	A	A	A	A	A	A	A	A	A
	OB	A	A	A	A	A	A	C	A	A	A	A
K	1A	B	A	A	A	A	C	A	A	A	A	A
L	1B	B	A	A	A	A	A	C	A	A	A	A
M-N	2A	B	A	A	A	A	C	C	A	A	A	A
O	2C	B	A	A	A	A	C	C	A	A	A	B
F	2D	A	A	A	A	A	D	A	A	A	D	A
P-Q	3A	B	A	A	C	A	C	C	A	A	A	A
R	4A	B	A	A	A	C	C	A	D	C	A	A
S-T	4B	B	A	A	A	C	D	A	D	C	A	A

* Designations used in Chapter 3, Bickham et al.

APPENDIX II

Table for 1992 Restriction Site Analyses for Colville, Sagavanirktok, Peel, Artic Red Rivers and Prudhoe Bay Samples

Restriction Site Analysis for Colville River.

	Sample No.	Helmrick Designation	Gill Raker	ORF I Dde I	Hae III	Hinf I	ORF 5/6 Dde I	Sau 96	Dra I	Ssp I	Taq I	Bsp 1286	Group
GEN	12	ARCS	24	A	A	A							
GEN	14	ARCS	29	B	A	A	A		C	C	A	A	2A
GEN	15	ARCS	28	C	B	A							
GEN	19	ARCS	26	B	A	A	A		C	C	A	A	2A
GEN	20	ARCS	25	A	A	A							
GEN	22	ARCS	30	A	A	A							
GEN	23	ARCS	28	A		A							
GEN	25	ARCS	27	A									
GEN	26	ARCS	26	A	A	A							
GEN	27	ARCS	30	C	B	A							
GEN	31	ARCS	28	A	A	A							
GEN	32	ARCS	28	A	A	A							
GEN	33	ARCS	27	A	A	A							
GEN	34	ARCS	26	A	A	A							
GEN	35	ARCS	30	A	A	A							
GEN	36	ARCS	30	B	A	A	A		C	A	C	C	4A
GEN	38	ARCS	27	A	A	A							
GEN	39	ARCS	28	A	A	A							
GEN	40	ARCS	27	A	A	A							
GEN	41	ARCS	28	A	A	A							
GEN	43	ARCS	24	A	A	A							
GEN	44	ARCS	30	A	A	A							
GEN	45	ARCS	26	A	A	A							
GEN	47	ARCS	26	A	A	A							
GEN	48	ARCS	28	A		A							
GEN	49	ARCS	26	A	A	A							
GEN	50	ARCS	25	C	B	A							
GEN	51	ARCS	26	C	B	A							

G.R.- ARCS, BRCS

Sample No.	Helmrick Designation	Gill Raker	ORF I Dde I	ORF I Hae III	ORF I Hinf I	ORF 5/6 Dde I	ORF 5/6 Sau 96	ORF 5/6 Dra I	ORF 5/6 Ssp I	ORF 5/6 Taq I	ORF 5/6 Bsp 1286	Group
GEN 52	ARCS	27	A	A	A							
GEN 60	ARCS	28	B	A	A	C		C	C	A	A	3A
GEN 63	ARCS	27	A	A	A							
GEN 64	ARCS	27	B	A	A	C		C	C	A	A	3A
GEN 65	ARCS	28	A	A	A							
GEN 67	ARCS	29	C	B	A							
GEN 69	ARCS	26	B	A	B							BRCS
GEN 70	ARCS	26	A	A	A							
GEN 72	ARCS	28	A	A	A							
GEN 73	ARCS	26	A	A	A							
GEN 74	ARCS	26	A	A	A							
GEN 76	ARCS	26	A	A	A							
GEN 78	ARCS	25	C	B	A							
GEN 79	ARCS	26	A		A							
GEN 80	ARCS	27	A		A							
GEN 81	ARCS	26	A	A	A							
GEN 85	ARCS	28	B	A	B							BRCS
GEN 86	ARCS	27	B	A	A							
GEN 87	ARCS	26	A	A	A							
GEN 88	ARCS	27	A	A	A							
GEN 89	ARCS	25	A	A								
GEN 116	BRCS	21	B	A	B							BRCS
GEN 117	BRCS	21	B	A	B							BRCS
GEN 118	BRCS	23	B	A	B							BRCS
GEN 119	BRCS	21	B	A	B							BRCS
GEN 120	BRCS	23	B		B							BRCS
GEN 121	BRCS	22	B	A	B							BRCS
GEN 122	BRCS	21	B	A	B							BRCS
GEN 123	BRCS	21	B	A	B							BRCS
GEN 124	BRCS	21	B	A	B							BRCS

G.R.- ARCS, BRCS

Sample No.	Helmrick Designation	Gill Raker	ORF I			ORF 5/6						Group
			Dde I	Hae III	Hinf I	Dde I	Sau 96	Dra I	Ssp I	Taq I	Bsp 1286	
GEN 125	BRCS	22	B	E	B							BRCS
GEN 126	BRCS	24	B	A	B							BRCS
GEN 127	BRCS	21	B	A	B							BRCS
GEN 129	BRCS	22	B	A	B							BRCS
GEN 131	BRCS	21	B	A	B							BRCS
GEN 132	BRCS	23	B	A	B							BRCS
GEN 133	BRCS	23	B	A	B							BRCS
GEN 134	BRCS	22	B	A	B							BRCS
GEN 135	BRCS	22	B	A	B							BRCS
GEN 136	BRCS	21	B	A	B							BRCS
GEN 137	BRCS	21	B	A	B							BRCS

G.R.- ARCS, BRCS

Restriction Site Analyses for Sagavanirktok River.

Sample No.	ORF I			ORF 5/6							Group
	Dde I	Hae III	Hinf I	Dde I	Sau 96	Dra I	Ssp I	Taq I	Bsp 1286	Bst N	
AK 11700	A	A	A								
AK 11742	B	A	B	A	B	A	A	A	A	B	BRCS
AK 11745	A	A	A								
AK 11746	A	A	A								
AK 11747	A	A	A								
AK 11748	A	A	A								
AK 11749	B	A	A	A	D	C	A	C	C	*	4A
AK 11750	C	B	A								
AK 11751	A	A	A								
AK 11752	A	A	A								
AK 11753	A	A	A								
AK 11754	C	B	A								
AK 11755	B	A	A	A	A	C	C	A	A	*	2A
AK 11756	A	A	A								
AK 11758	C	B	A								
AK 11759	A	A	A								
AK 11761	A	A	A								
AK 11762	A	A	A								
AK 11763	A	A	A								
AK 11765	B	A	A	C	A	C	C	A	A	*	3A
~~AK 11767~~	~~C~~	~~B~~	~~A~~								
AK 11768	B	A	A	C	A	C	C	A	A	*	3A
AK 11769	A	A	A								
AK 11770	A	A	A								
AK 11771	A	A	A								
AK 11772	A	A	A								
AK 11774	B	A	A	C	A	C	C	A	A	*	3A
AK 11775	B	A	A	A	A	C	C	A	A	*	2A
AK 11777	A	A	A								

Sample No.	ORF I			ORF 5/6							
	Dde I	Hae III	Hinf I	Dde I	Sau 96	Dra I	Ssp I	Taq I	Bsp 1286	Bst N	
AK 11778	A	A	A								
AK 11779	B	A	A	A	A	C	C	A	A	*	2A
AK 11780	A	A	A								
AK 11781	A	A	A								
AK 11782	A	A	A								
AK 11783	A	A	A								
AK 11784	A	A	A								
AK 11808	A	A	A								
AK 11810	A	A	A								
AK 11817	A	A	A								
AK 11818	C	B	A								
AK 11819	A	A	A								
AK 11821	A	A	A								
AK 11822	A	A	A								
AK 11823	B	A	A	A	A	C	C	A	A	*	2A
AK 11825	B	A	A	A	A	C	C	A	A	*	2A
AK 11827	C	B	A								
AK 11828	A	A	A								
AK 11832	A	A	A								
AK 11833	A	A	A								
AK 11834	A	A	A								

Restriction Site Analysis for Peel and Arctic Red Rivers, NWT.

Location	Sample No.	ORF I			ORF 5/6						Group
		Dde I	Hae III	Hinf I	Dde I	Sau 96	Dra I	Ssp I	Taq I	Bsp 1286	
Peel River, NWT	MS 1	B	A	A	A	A	C	C	A	A	2A
	MS 2	A	A	A							
	MS 3	A	A	A							
	MS 4	A	A	A							
	MS 6	B	A	A	C	A	C	C	A	A	3A
	MS 7	A	A	A							
	MS 8	C	B	A							
	MS 9	A	A	A							
	MS 10	B	A	A	A	A	C	C	A	A	2A
	MS 11	A	A	A							
	MS 12	A	A	A							
	MS 13	A	A	A							
	MS 14	A	A	A							
	MS 15	A	A	A							
	MS 16	A	A	A							
Arctic Red River, NWT	SR 232	B	A	A	C	A	C	C	A	A	3A
	SR 233	A	A	A							
	SR 234	A	A	A							
	SR 235	A	A	A							
	SR 236	A	A	A							
	SR 237	A	A	A							
	SR 238	A	A	A							
	SR 239	A	A	A							
	SR 240	A	A	A							
	SR 241	A	A	A							
	SR 242	A	A	A							
	SR 243	A	A	A							
	SR 244	A	A	A							
	SR 245	A	A	A							

Location	Sample No.	ORF I Dde I	ORF I Hae III	ORF I Hinf I	ORF 5/6 Dde I	ORF 5/6 Sau 96	ORF 5/6 Dra I	ORF 5/6 Ssp I	ORF 5/6 Taq I	ORF 5/6 Bsp 1286	
	SR 246	A	A	A							
	SR 247	A	A	A							
	SR 248	B	A	A	C	A	C	C	A	A	3A
	SR 249	A	A	A							
	SR 250	A	A	A							
	SR 251	A	A	A							
Arctic Red	SR 253	C	B	A							
River, NWT	SR 256	A	A	A							
	SR 257	A	A	A							
	SR 258	C	B	A							
	SR 259	A	A	A							
	SR 260	A	A	A							
	SR 262	A	A	A							
	SR 264	A	A	A							
	SR 267	B	A	A	C	A	C	C	A		3A
	SR 268	A	A	A							
	SR 269	A	A	A							
	SR 271	A	A	A							
	SR 275	A	A	A							

Restriction Site Analysis for Prudhoe Bay.

Sample No.	ORF I		ORF 5/6						
	Dde I	Hae III	Hinf I	Dde I	Sau 96	Dra I	Ssp I	Taq I	Bsp 1286	Group
GEN 259	A		A							
GEN 260	B		A	A		C	C	A	A	
GEN 261	A		A							
GEN 262	C		A							
GEN 263	B		A	A		C	C	A	A	
GEN 264	C		A							
GEN 265	A		A							
GEN 266	A		A							
GEN 267	A		A							
GEN 269	B		A	C		C	C	A	A	
GEN 270	B		A	C	A	C	C	A	A	3A
GEN 271	A		A							
GEN 272	A		A							
GEN 273	A		A							
GEN 274	A		A							
GEN 275	A		A							
GEN 277	B		A	A	A	C	C	A	A	2A
GEN 278	A		A							
GEN 279	B		B	A	B	A	A	B	A	BRCS
GEN 280	A		A							
GEN 281	B		A	A	A	C	A	A	A	1A
GEN 282	A		A							
GEN 283	A		A							
GEN 284	A		A							
GEN 285	A		A							
GEN 286	B		A	C	A	C	C	A	A	3A
GEN 287	A		A							
GEN 288	A		A							
GEN 289	B		A	A	A	C	C	A	A	2A

	ORF I		ORF 5/6						
	Dde I	Hae III	Hinf I	Dde I	Sau 96	Dra I	Ssp I	Taq I	Bsp 1286	
GEN 290	B		A	A	A	C	C	A	A	2A
GEN 291	A		A							
GEN 292	A		A							
GEN 293	A		A							
GEN 294	A		A							
GEN 295	B		A	C	A	C	C	A	A	3A
GEN 296	C									
GEN 297	A		A							
GEN 298	A		A							
GEN 299	A		A							
GEN 300	A		A							
GEN 301	A		A							
GEN 302	A		A							
GEN 303	B		A	C	A	C	C	A	A	3A
GEN 304	A									

APPENDIX III

Chromatograms of Different Sequence Haplotypes

HAPLOTYPE A (Standard)

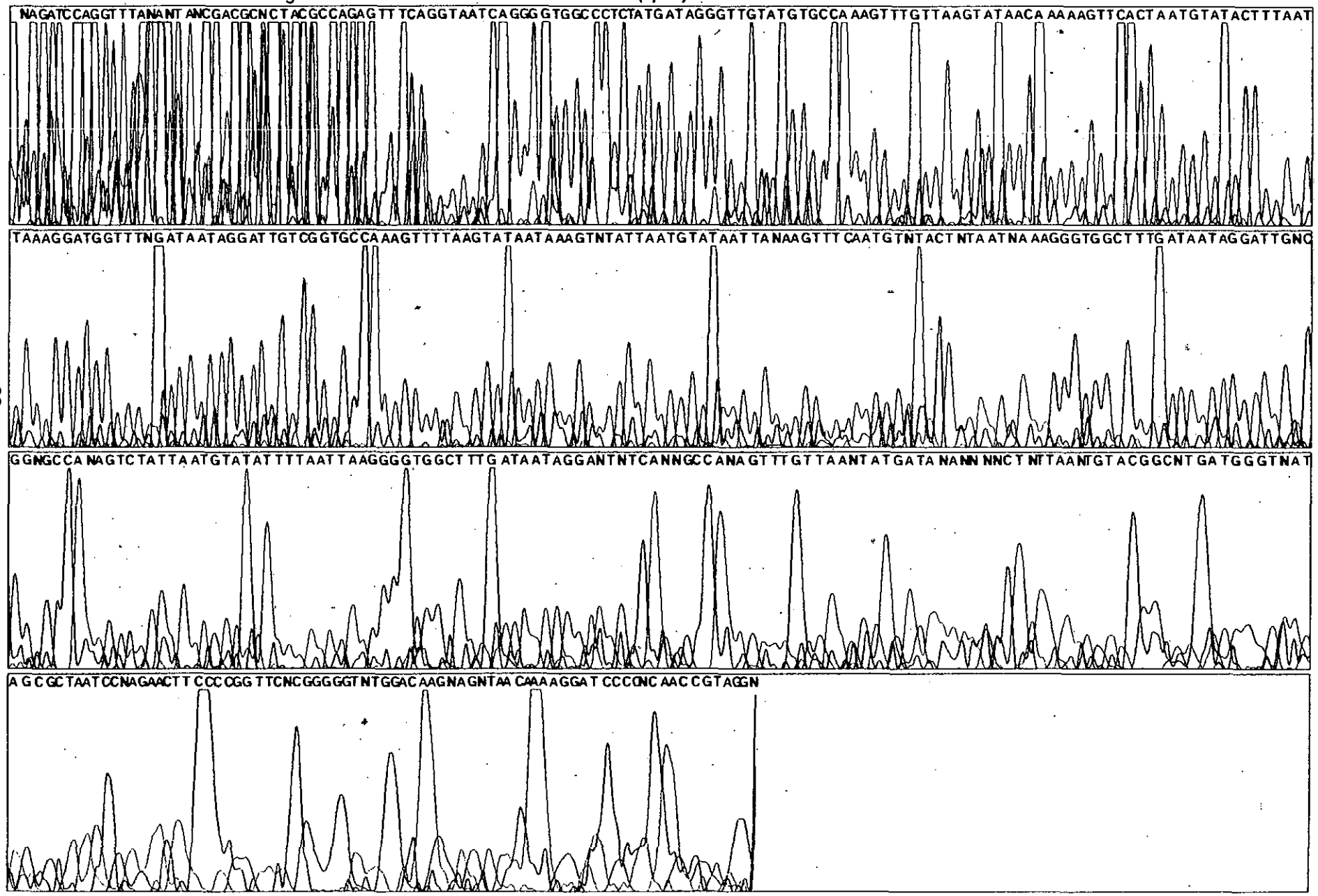
Applied Biosystems

Model 373A
Version 1.0.2

ARCS 7/9/92 24
DyeTerminator{AnyPrimer}
Lane 24
Signal: G:268 A:121 T:98 C:30

Points 888 to 5546 Base 1: 888
330 MATRIX FILE
AK 11781-M13R
P-19 (4/3X)

Thu, Jul 9, 1992 10:20 PM
X: 0 to 5364 Y: 0 to 1200



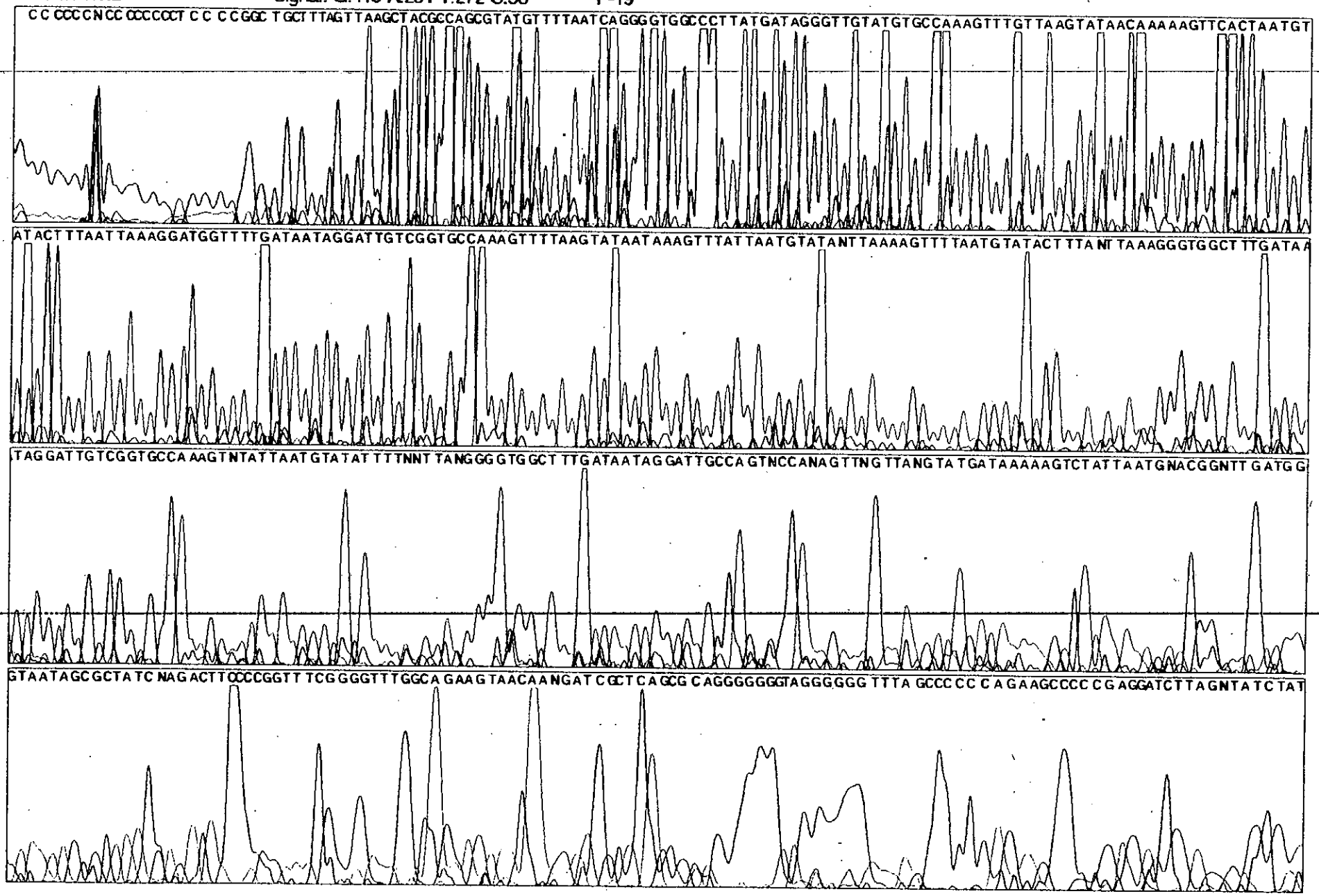
HAPLOTYPE A

Applied Biosystems
Model 373A
Version 1.0.2

3 11 92 Arcs 22.Res
DyeTerminator{AnyPrimer}
Lane 22
Signal: G:415 A:231 T:272 C:38

Points 576 to 5536 Base 1: 576
330 MATRIX FILE
AK 12472-279S
P-19

Wed, Mar 11, 1992 9:32 PM
X: 0 to 6597 Y: 0 to 1200



HAPLOTYPE A

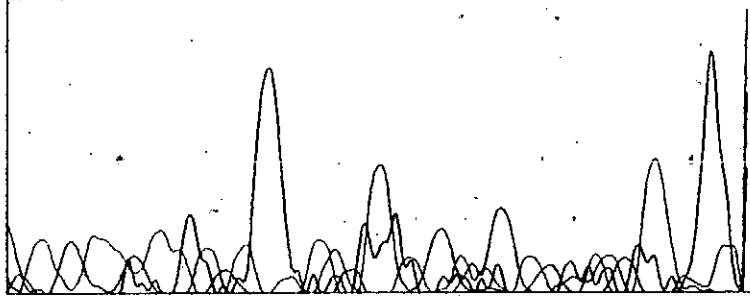
Applied Biosystems
Model 373A
Version 1.0.2

3 11 92 Arcs 22.Res
DyeTerminator[AnyPrimer]
Lane 22
Signal: G:415 A:231 T:272 C:38

Points 576 to 5536 Base 1: 576
330 MATRIX FILE
AK 12472-279S
P-19

Wed, Mar 11, 1992 9:32 PM
X: 0 to 6597 Y: 0 to 1200

NAAGGAAAGAAAC TGACCNNT CACCGGATTTAAGCC NGCANNCNT



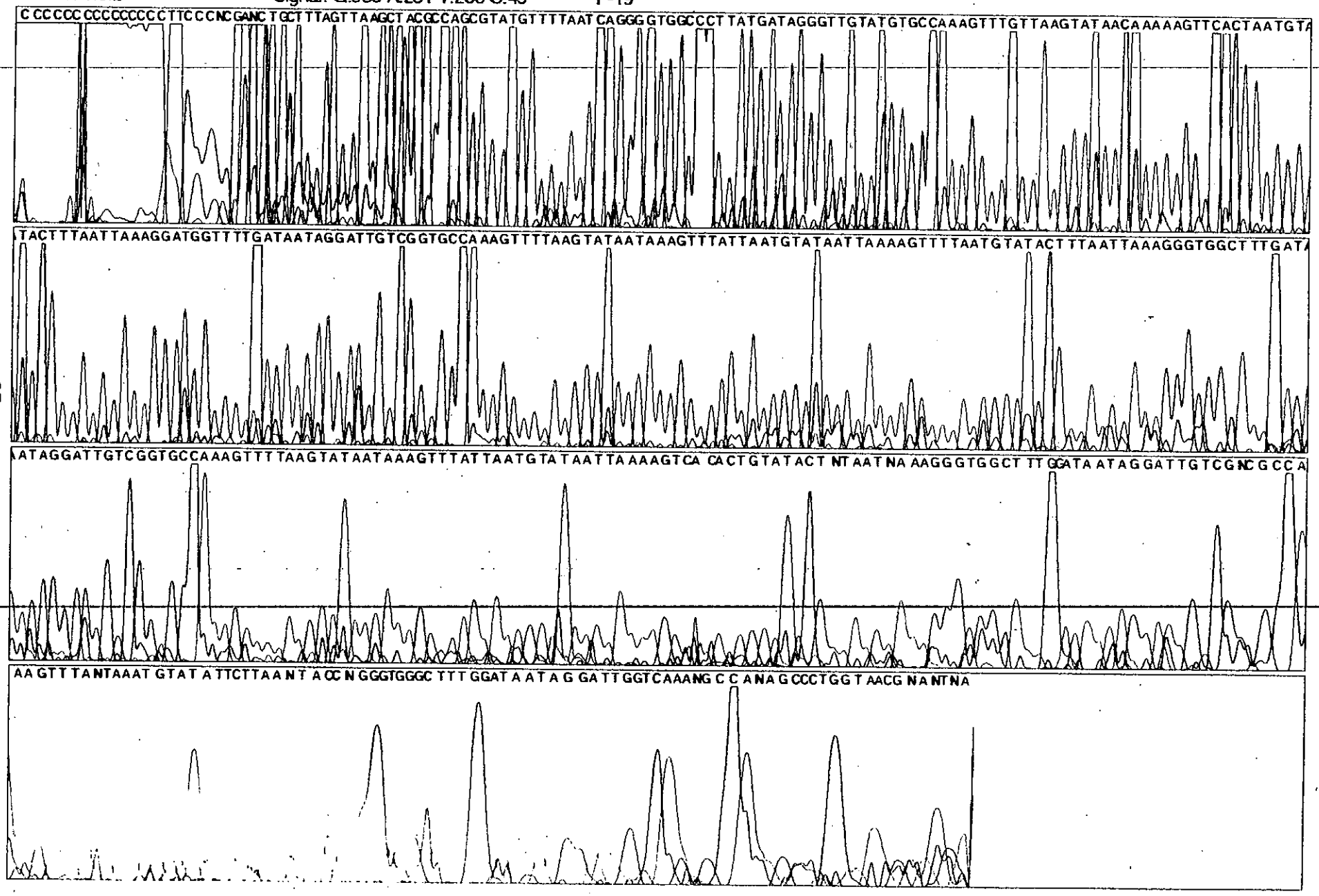
HAPLOTYPE A-III

Applied Biosystems
Model 373A
Version 1.0.2

3 12 92 Arcs 20.
DyeTerminator{AnyPrimer}
Lane 20
Signal: G:380 A:231 T:266 C:49

Points 632 to 5376 Base 1: 632
330 MATRIX FILE
AK 12414-279S
P-19

Wed, Mar 25, 1992 3:33 PM Page 1 of 1
X: 0 to 5620 Y: 0 to 1200



95

HAPLOTYPE C

Applied Biosystems

Model 373A

Version 1.0.2

3 12 92 Arcs 09.

DyeTerminator[AnyPrimer]

Lane 9

Signal: G:337 A:216 T:236 C:55

Points 658 to 5376 Base 1: 658

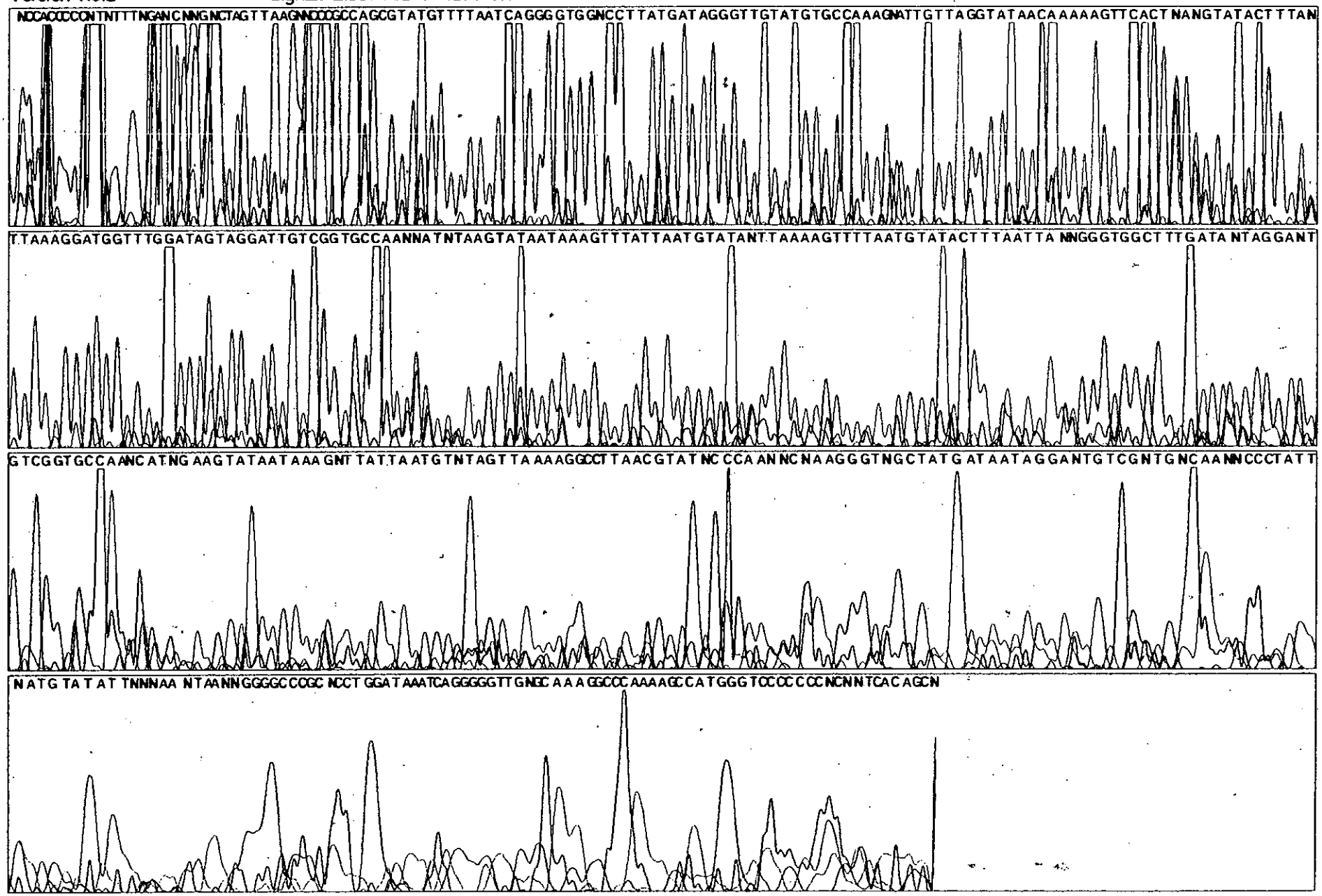
330 MATRIX FILE

AK 12404-279S

P-19

Wed, Mar 25, 1992 3:33 PM Page 1 of 1

X: 0 to 5567 Y: 0 to 1200

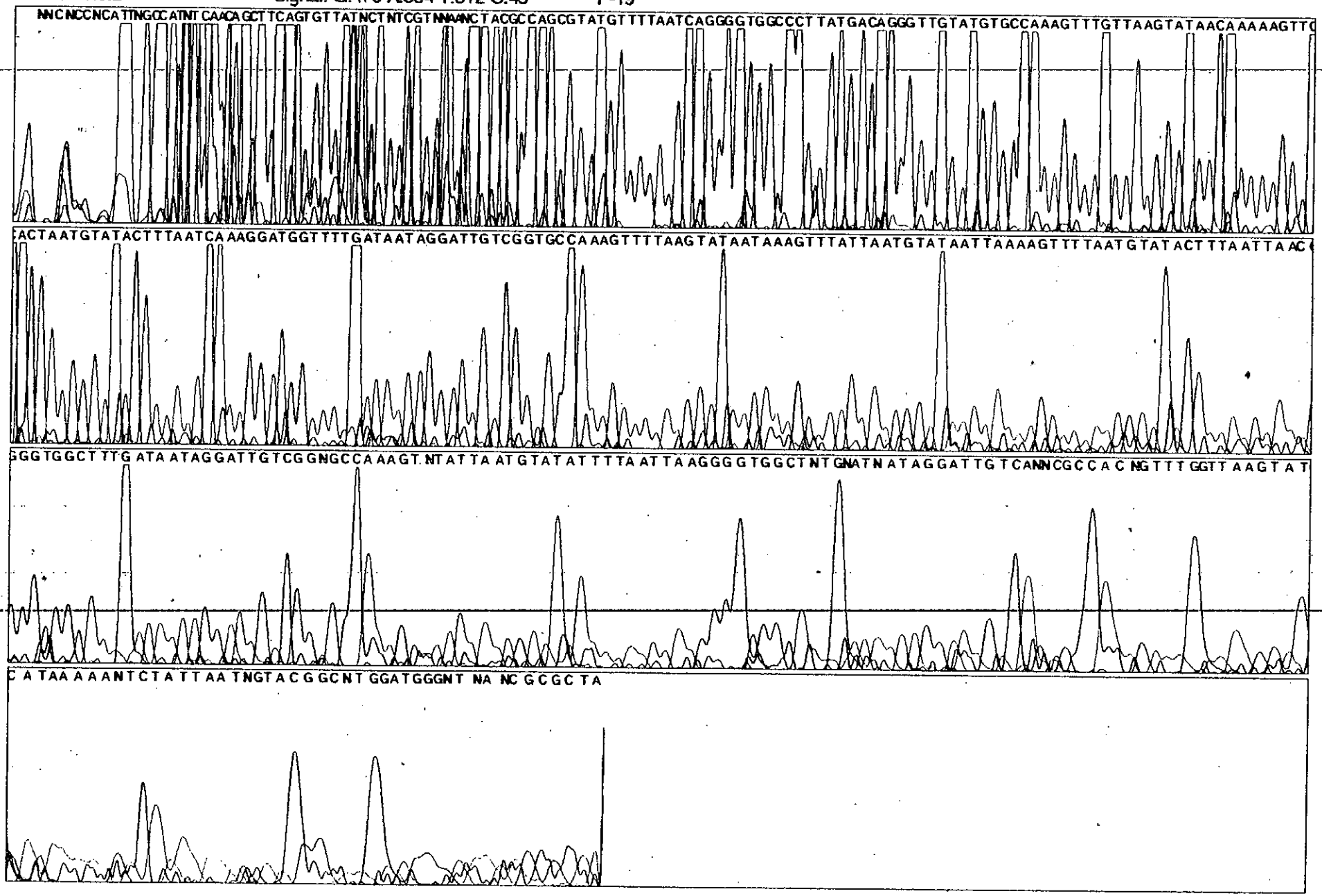


Applied Biosystems
Model 373A
Version 1.0.2

HAPLOTYPE OA
4/26/92 ARCS 13
DyeTerminator{AnyPrimer}
Lane 13
Signal: G:470 A:384 T:312 C:45

Points 713 to 5536 Base 1: 713
330 MATRIX FILE
AK 12197-M13R
P-19

Sun, Apr 26, 1992 2:47 PM
X: 0 to 5193 Y: 0 to 1200



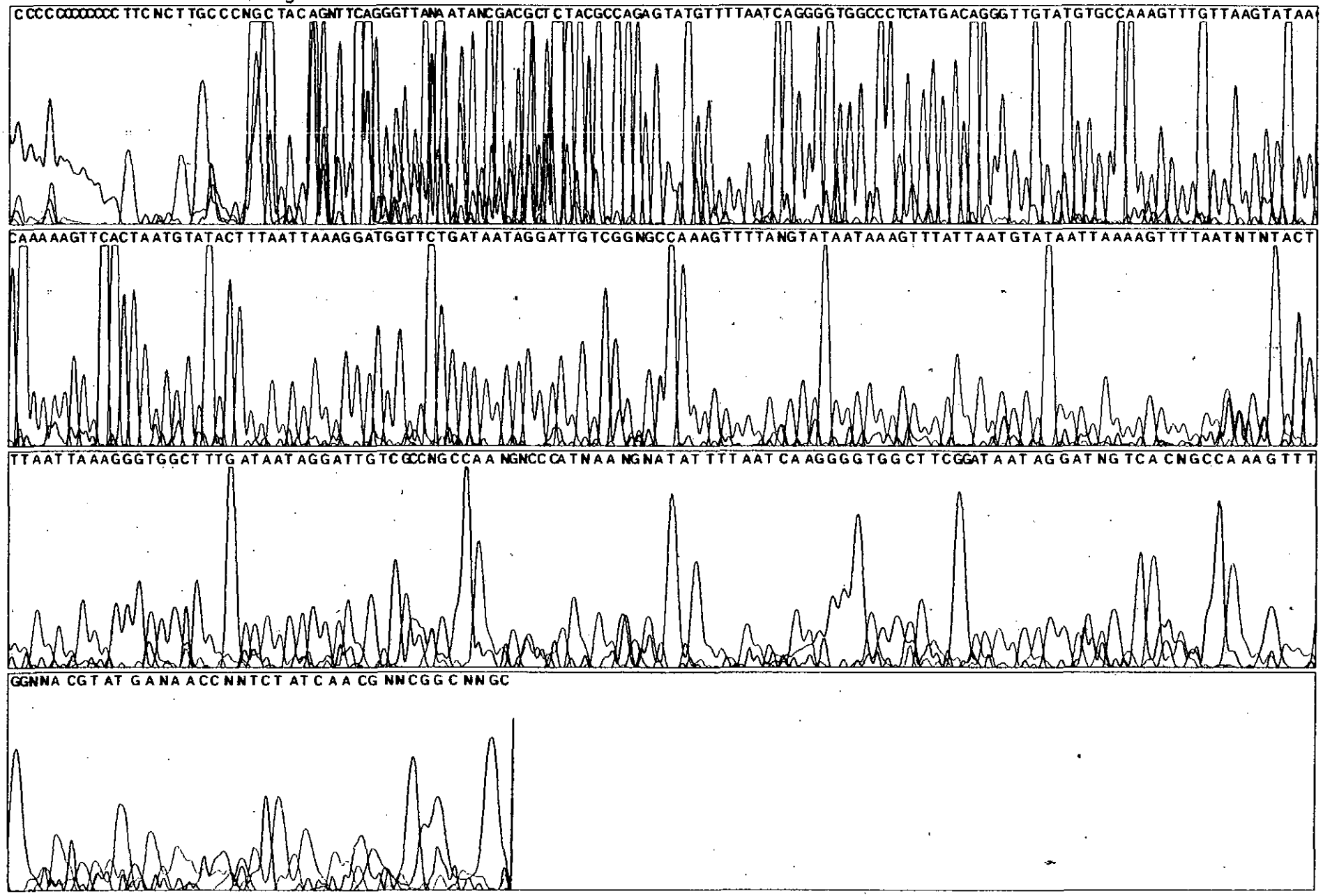
HAPLOTYPE OA

Applied Biosystems
Model 373A
Version 1.0.2

4/28/92 ARCS 02
DyeTerminator{AnyPrimer}
Lane 2
Signal: G:295 A:199 T:156 C:37

Points 668 to 5536 Base 1: 668
330 MATRIX FILE
AK 12253-M13R
P-19

Tue, Apr 28, 1992 7:55 PM
X: 0 to 5086 Y: 0 to 1200

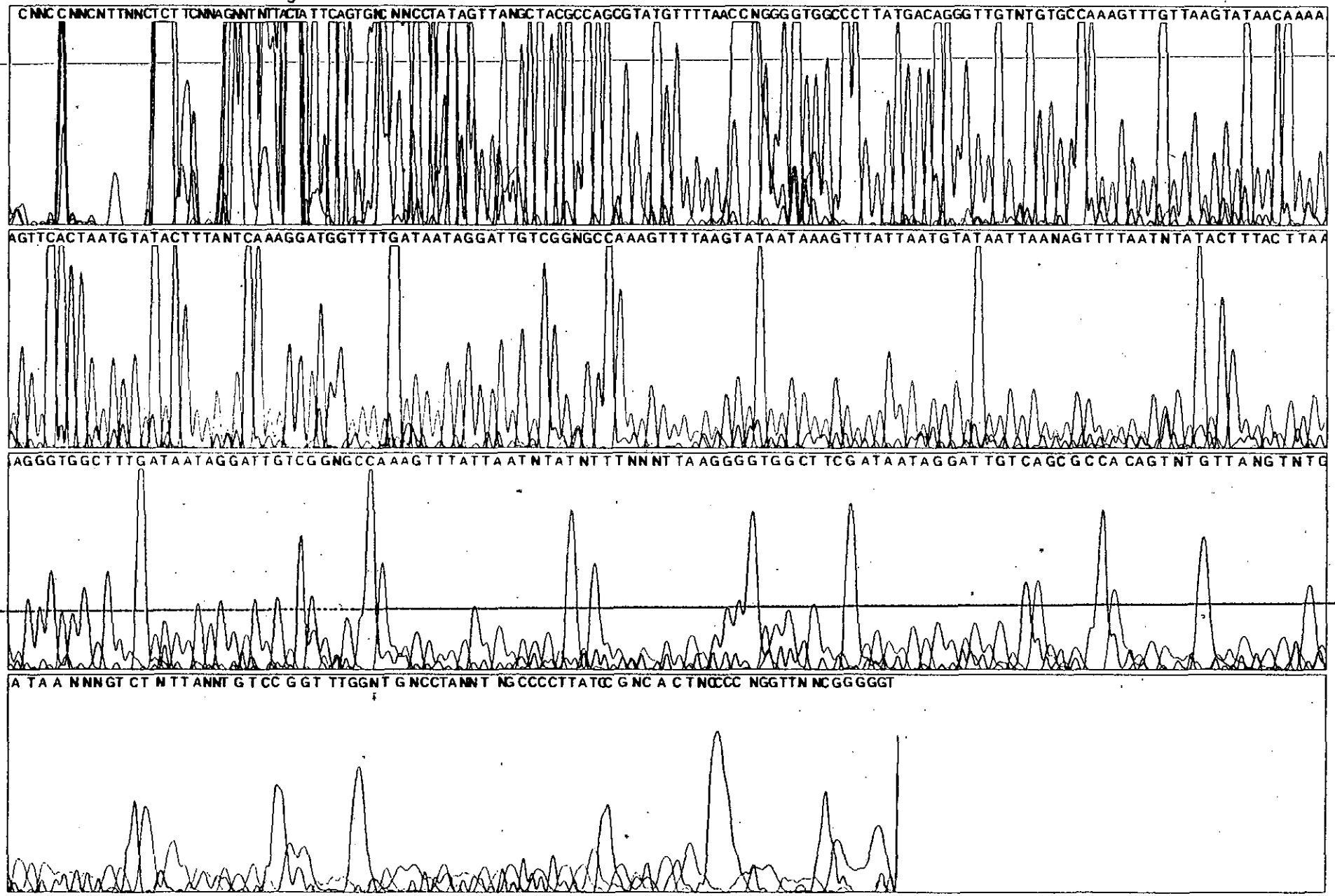


Applied Biosystems
Model 373A
Version 1.0.2

HAPLOTYPE 1A
4 30 92 Arcs 06.Res
DyeTerminator{AnyPrimer}
Lane 6
Signal: G:442 A:390 T:294 C:255

Points 654 to 5536 Base 1: 654
330 MATRIX FILE
AK 12278-M13R
P-19

Thu, Apr 30, 1992 9:21 PM
X: 0 to 5517 Y: 0 to 1200



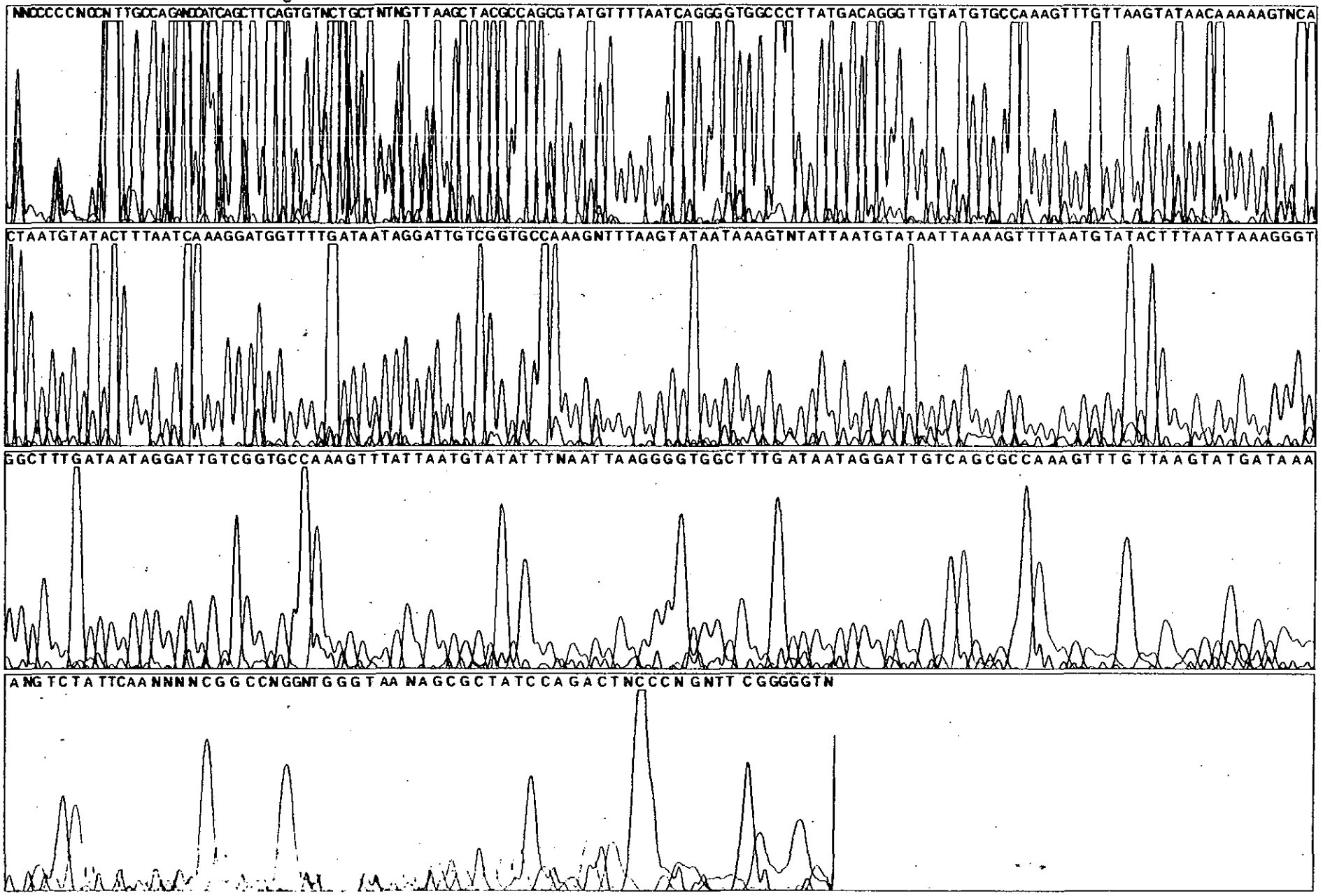
HAPLOTYPE 1B

Applied Biosystems
Model 373A
Version 1.0.2

4 30 92 Arcs 15.Res
DyeTerminator{AnyPrimer}
Lane 15
Signal: G:566 A:316 T:301 C:95

Points 659 to 5536 Base 1: 659
330 MATRIX FILE
AK 12326-M13R
P-19

Thu, Apr 30, 1992 9:21 PM
X: 0 to 5455 Y: 0 to 1200

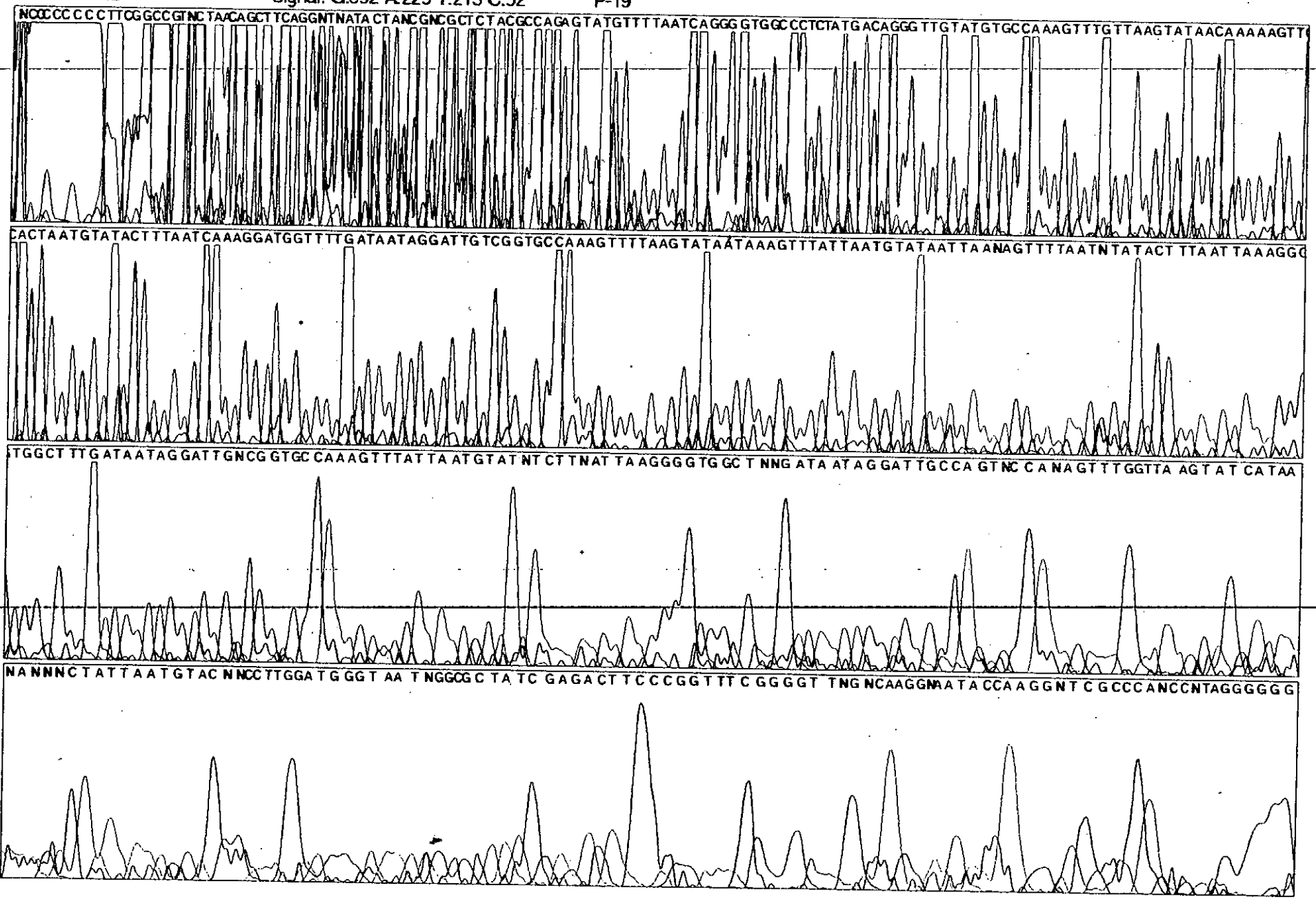


Applied Biosystems
Model 373A
Version 1.0.2

HAPLOTYPE 2A
4/14/92 ARCS 12
DyeTerminator{AnyPrimer}
Lane 12
Signal: G:392 A:225 T:213 C:52

Points 705 to 6784 Base 1: 705
330 MATRIX FILE
AK 11823-M13R
P-19

Tue, Apr 14, 1992 9:56 PM
X: 0 to 7051 Y: 0 to 1200



HAPLOTYPE 2A

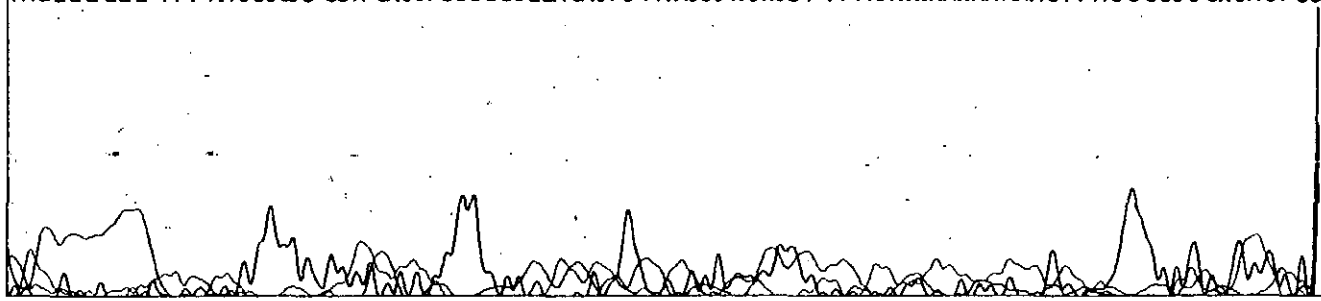
Applied Biosystems
Model 373A
Version 1.0.2

4/14/92 ARCS 12
DyeTerminator{AnyPrimer}
Lane 12
Signal: G:392 A:225 T:213 C:52

Points 705 to 6784 Base 1: 705
330 MATRIX FILE
AK 11823-M13R
P-19

Tue, Apr 14, 1992 9:56 PM
X: 0 to 7051 Y: 0 to 1200

TAGGGGGGG TTT AACCCCCC CCA G NAA CCCCCCGGA G NA C TTNACCC NCNCCT TTTAA NNAANAA AAACTT ACCCCCC CNCACT CC

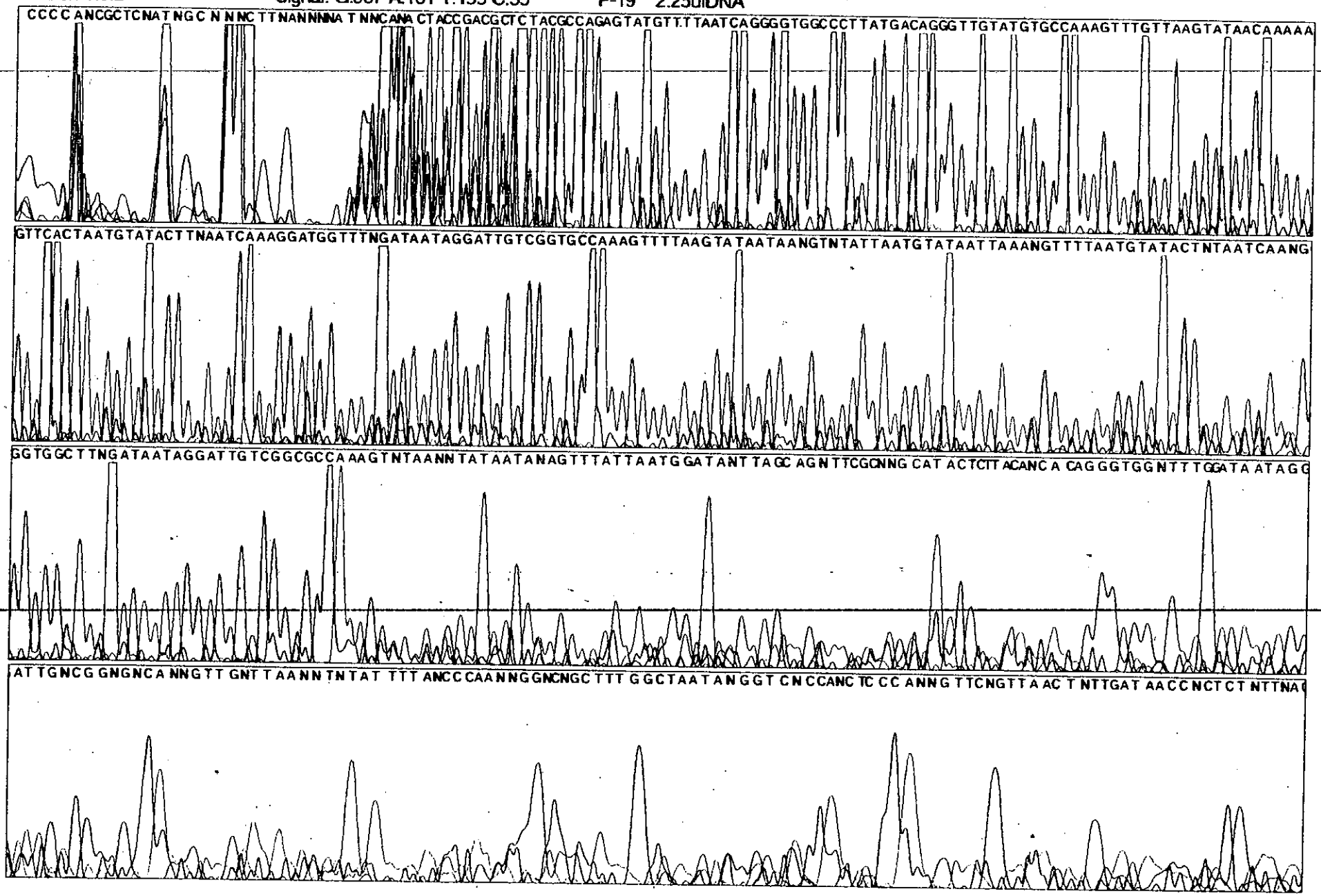


Applied Biosystems
Model 373A
Version 1.0.2

HAPLOTYPE 2A-III
3 25 92 Arcs 06.Res
DyeTerminator{AnyPrimer}
Lane 6
Signal: G:307 A:181 T:155 C:35

Points 496 to 5504 Base 1: 496
330 MATRIX FILE
AK 12238-279SM13R
P-19 2.25ulDNA

Wed, Mar 25, 1992 5:21 PM
X: 0 to 6084 Y: 0 to 1200



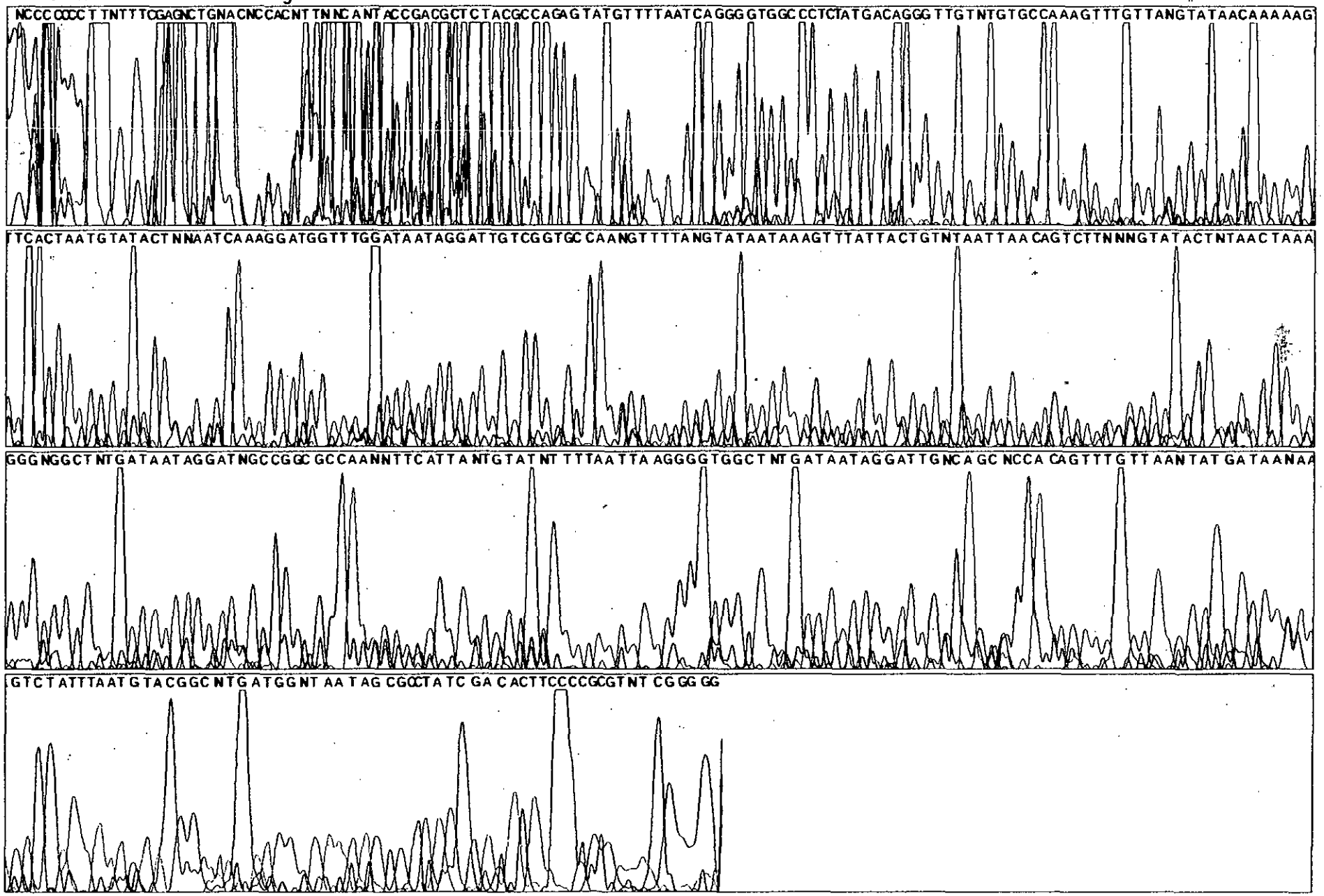
HAPLOTYPE 3A

Applied Biosystems
Model 373A
Version 1.0.2

3 26a 92 Arcs 19.Res
DyeTerminator{AnyPrimer}
Lane 19
Signal: G:251 A:117 T:115 C:76

Points 683 to 5536 Base 1: 683
330 MATRIX FILE
AK 12341-279SM13R
P-19 2.25ul

Thu, Mar 26, 1992 5:54 PM
X: 0 to 5327 Y: 0 to 1200



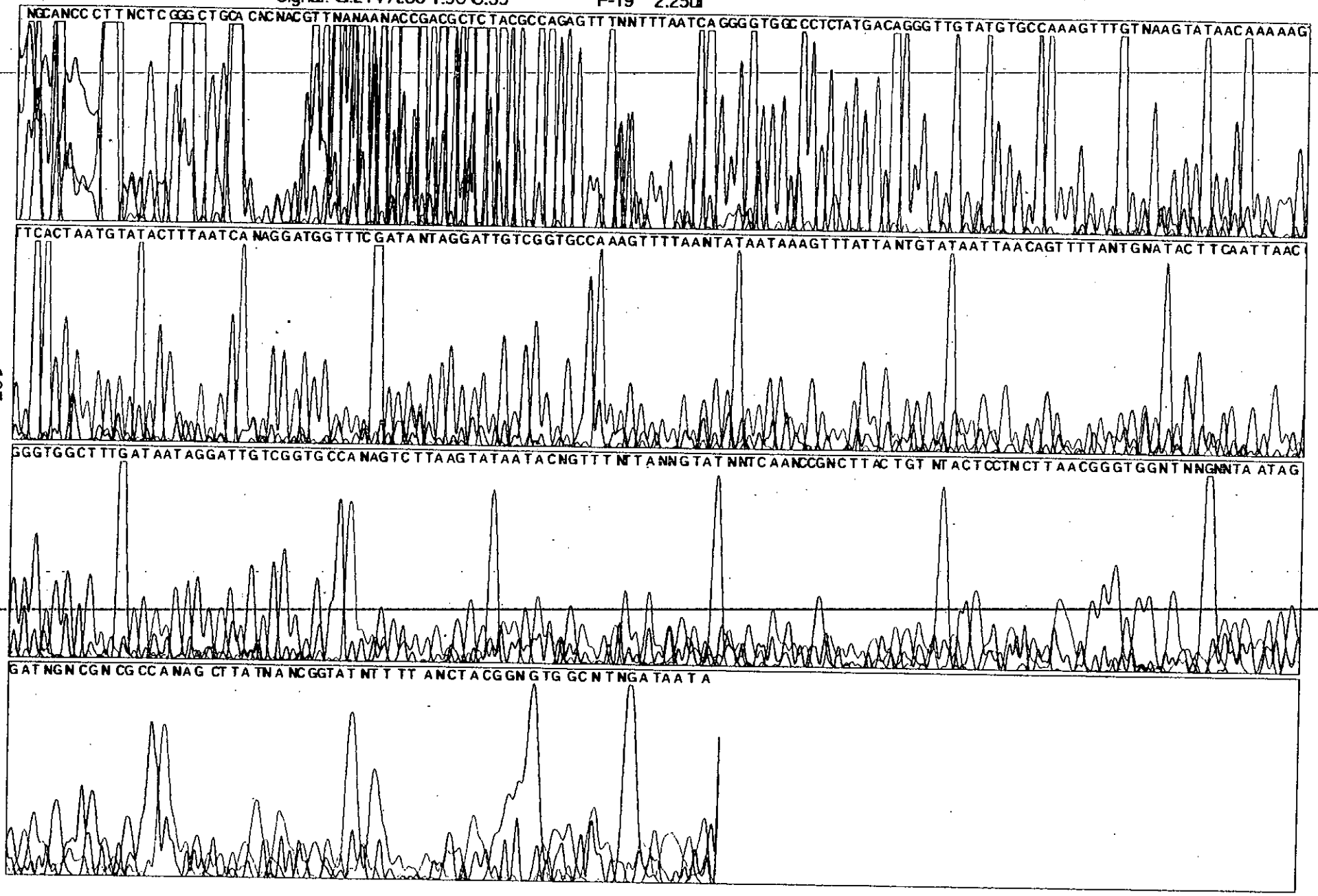
HAPLOTYPE 3A-III

Applied Biosystems
Model 373A
Version 1.0.2

3 26a 92 Arcs 15.Res
DyeTerminator{AnyPrimer}
Lane 15
Signal: G:214 A:86 T:90 C:35

Points 679 to 5536 Base 1: 679
330 MATRIX FILE
AK 12335-279SM13R
P-19 2.25ul

Thu, Mar 26, 1992 5:54 PM
X: 0 to 5331 Y: 0 to 1200



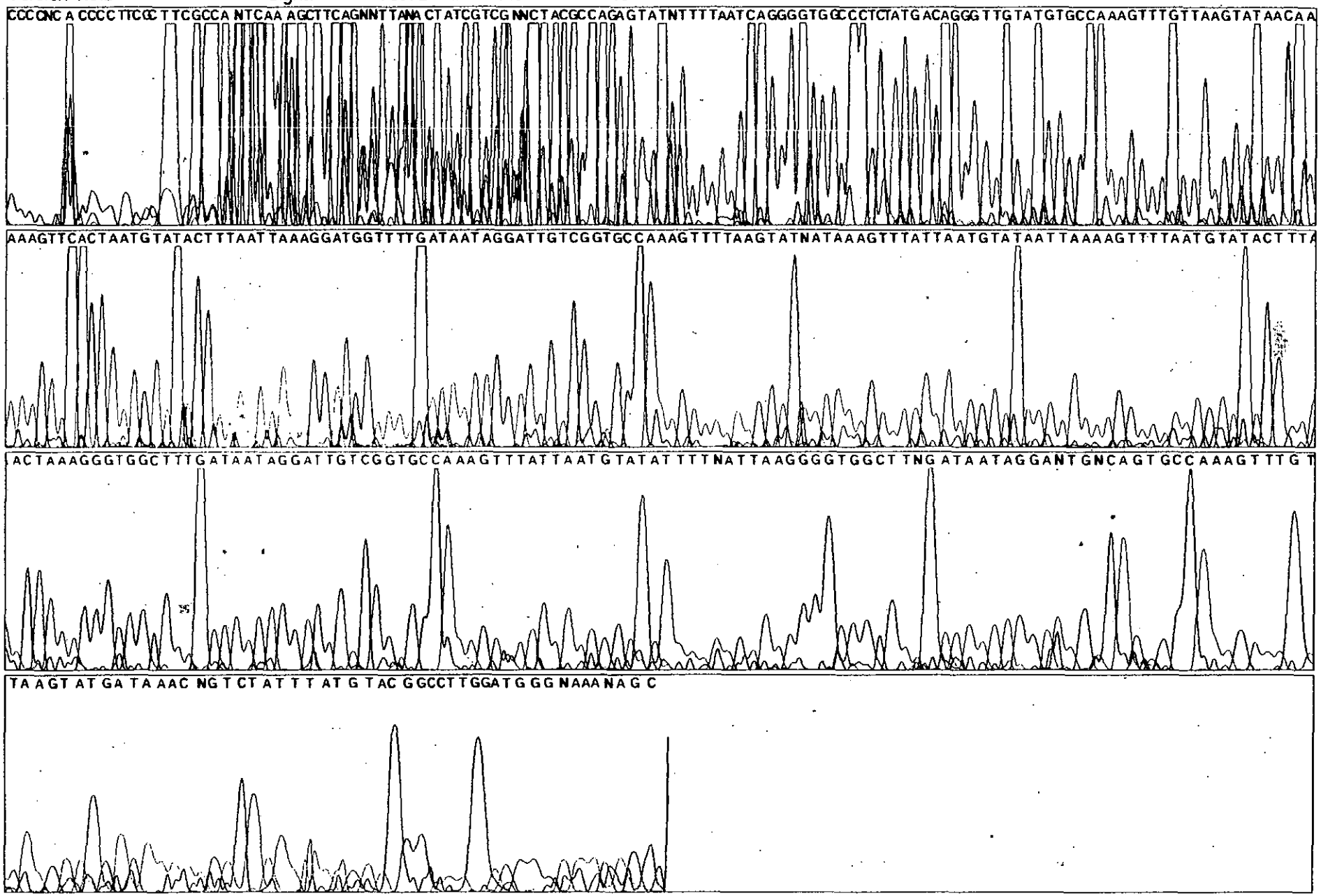
HAPLOTYPE 4A

Applied Biosystems
Model 373A
Version 1.0.2

4/26/92 ARCS 10
DyeTerminator{AnyPrimer}
Lane 10
Signal: G:461 A:372 T:266 C:49

Points 703 to 5536 Base 1: 703
330 MATRIX FILE
AK 12183-M13R
P-19

Sun, Apr 26, 1992 2:47 PM
X: 0 to 5265 Y: 0 to 1200

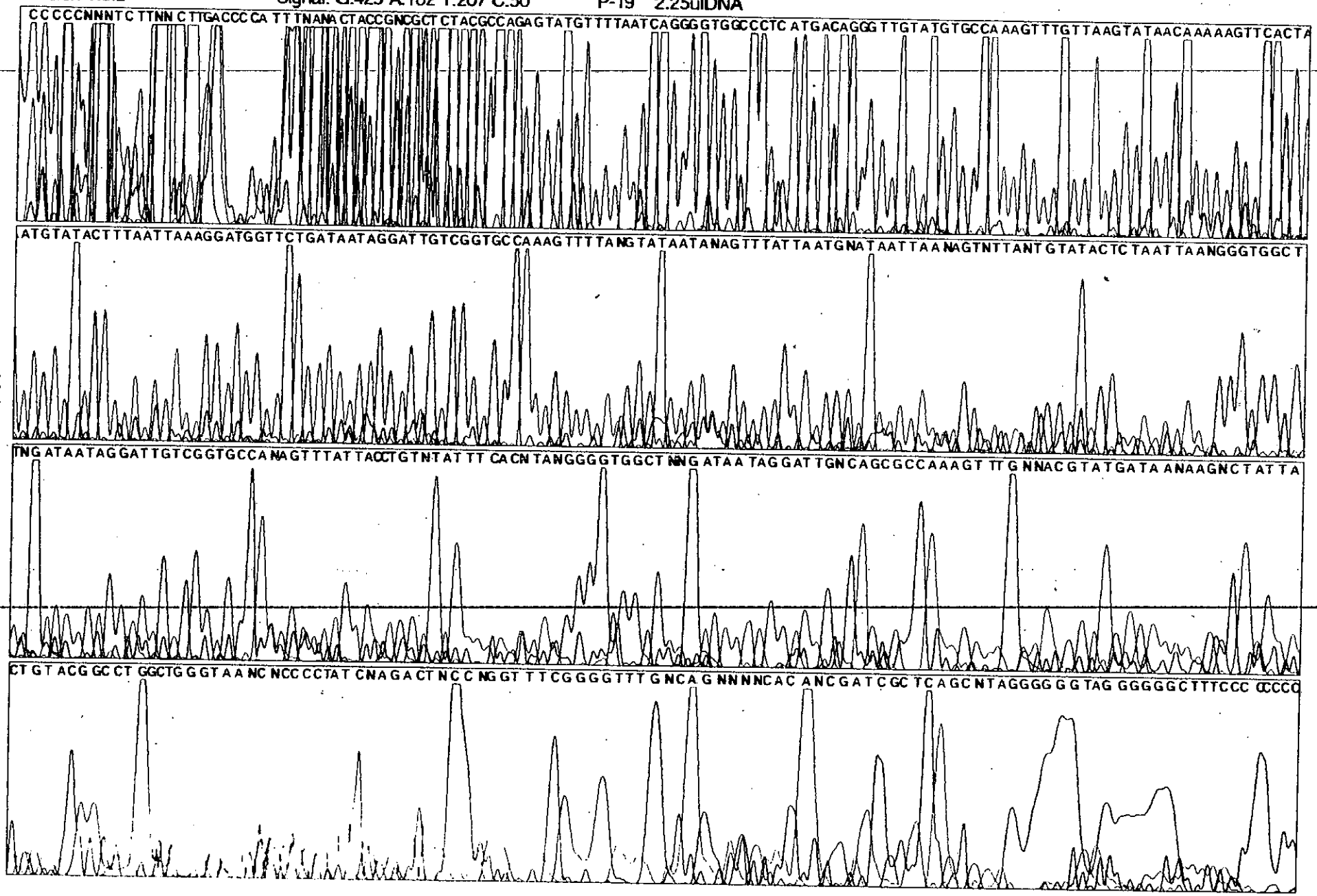


Applied Biosystems
Model 373A
Version 1.0:2

HAPLOTYPE 4B
3 25 92 Arcs 13.Res
DyeTerminator[AnyPrimer]
Lane 13
Signal: G:425 A:182 T:207 C:50

Points 519 to 5504 Base 1: 519
330 MATRIX FILE
AK 12245-279SM13R
P-19 2.25ulDNA

Wed, Mar 25, 1992 5:21 PM
X: 0 to 6011 Y: 0 to 1200

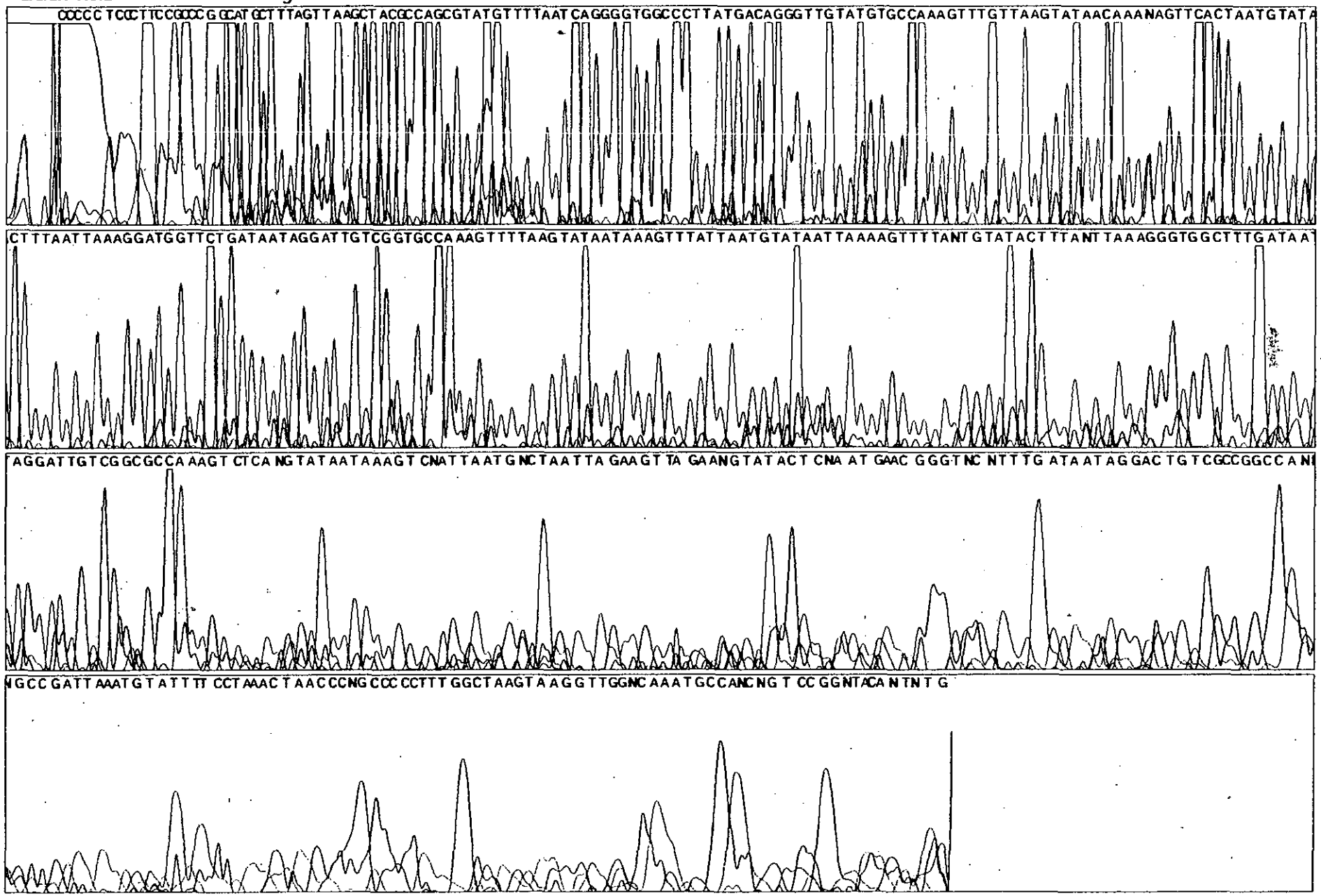


Applied Biosystems
Model 373A
Version 1.0.2

HAPLOTYPE 4B-III
3 12 92 Arcs 14.
DyeTerminator{AnyPrimer}
Lane 14
Signal: G:356 A:229 T:228 C:43

Points 647 to 5376 Base 1: 647
330 MATRIX FILE
AK 12409-279S
P-19

Wed, Mar 25, 1992 3:33 PM Page 1 of 1
X: 0 to 5588 Y: 0 to 1200



APPENDIX IV

Sequence Alignments of Arctic Cisco Haplotypes

HAPLOTYPE COMPARISON 7/13/92 3:43 PM

	10	20	30	40	50	60	70
STANDARD	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
A	GTTAAGCTAC	GCCAGCGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
A III	GTTAAGCTAC	GCCAGCGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
C	GTTAAGCTAC	GCCAGCGTAT	GTTTTAATCA	GGGGTGGNCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
OA	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGACAGG	GTTGTATGTG	CCAAAGTTTG
OA	GTTAAGCTAC	GCCAGCGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGACAGG	GTTGTATGTG	CCAAAGTTTG
1A	GTTAAGCTAC	GCCAGCGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGACAGG	GTTGTATGTG	CCAAAGTTTG
1B	GTTAAGCTAC	GCCAGCGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGACAGG	GTTGTATGTG	CCAAAGTTTG
2A	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGACAGG	GTTGTATGTG	CCAAAGTTTG
2A III	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGACAGG	GTTGTATGTG	CCAAAGTTTG
3A	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGACAGG	GTTGTATGTG	CCAAAGTTTG
3A III	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGACAGG	GTTGTATGTG	CCAAAGTTTG
4A	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGACAGG	GTTGTATGTG	CCAAAGTTTG
4B	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGACAGG	GTTGTATGTG	CCAAAGTTTG
4B III	GTTAAGCTAC	GCCAGCGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGACAGG	GTTGTATGTG	CCAAAGTTTG

HAPLOTYPE COMPARISON 7/13/92 3:45 PM

	80	90	100	110	120	130	140
STANDARD	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
A	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
A III	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
C	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
OA	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	CTGATAATAG	GATTGTCGGT
OA	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATCA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
1A	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATCA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
1B	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATCA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
2A	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATCA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
2A III	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATCA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
3A	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATCA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
3A III	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATCA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
4A	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
4B	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	CTGATAATAG	GATTGTCGGT
4B III	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	CTGATAATAG	GATTGTCGGT

HAPLOTYPE COMPARISON 7/13/92 3:47 PM

	150	160	170	180	190	200	210
STANDARD	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
A	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
A III	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
C	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
OA	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
OA	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
1A	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
1B	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
2A	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
2A III	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
3A	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAACTAAAG
3A III	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
4A	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAACTAAAG
4B	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
4B III	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG

HAPLOTYPE COMPARISON 7/13/92 3:49 PM

```
                 220        230        240        250        260        270        280
STANDARD         GGTGGCTTTG ATAATAGGAT TGTCGGTGCC AAAGTT---- ---------- ---TATTAAT GTATA-----
                 ---------- ---------- ---------- ---------- ---------- ---------- ----------
A                GGTGGCTTTG ATAATAGGAT TGTCGGTGCC AAAGTT---- ---------- ---TATTAAT GTATA-----
                 ---------- ---------- ---------- ---------- ---------- ---------- ----------
A III            GGTGGCTTTG ATAATAGGAT TGTCGGTGCC AAAGTTTTAA GTATAATAAA GTTTATTAAT GTATAATTAA
                 ---------- ---------- ---------- ------IIII IIIIIIIIII III------- -----IIIII
C                GGTGGCTTTG ATAATAGGAT TGTCGGTGCC AAAGTTTTAA GTATAATAAA GTTTATTAAT GTATAATTAA
                 ---------- ---------- ---------- ------IIII IIIIIIIIII III------- -----IIIII
OA               GGTGGCTTTG ATAATAGGAT TGTCGGAGCC AAAGTT---- ---------- ---TATTAAT GTATA-----
                 ---------- ---------- ------*--- ---------- ---------- ---------- ----------
OA               GGTGGCTTTG ATAATAGGAT TGTCGGTGCC AAAGTT---- ---------- ---TATTAAT GTATA-----
                 ---------- ---------- ---------- ---------- ---------- ---------- ----------
1A               GGTGGCTTTG ATAATAGGAT TGTCGGTGCC AAAGTT---- ---------- ---TATTAAT GTATA-----
                 ---------- ---------- ---------- ---------- ---------- ---------- ----------
1B               GGTGGCTTTG ATAATAGGAT TGTCGGTGCC AAAGTT---- ---------- ---TATTAAT GTATA-----
                 ---------- ---------- ---------- ---------- ---------- ---------- ----------
2A               GGTGGCTTTG ATAATAGGAT TGTCGGTGCC AAAGTT---- ---------- ---TATTAAT GTATA-----
                 ---------- ---------- ---------- ---------- ---------- ---------- ----------
2A III           GGTGGCTTTG ATAATAGGAT TGTCGGTGCC AAAGTTTTAA GTATAATAAA GTTTATTAAT GTATAATTAA
                 ---------- ---------- ---------- ------IIII IIIIIIIIII III------- -----IIIII
3A               GGTGGCTTTG ATAATAGGAT TGTCGGTGCC AAAGTT---- ---------- ---TATTAAT GTATA-----
                 ---------- ---------- ---------- ---------- ---------- ---------- ----------
3A III           GGTGGCTTTG ATAATAGGAT TGTCGGTGCC AAAGTTTTAA GTATAATAAA GTTTATTAAT GTATAATTAA
                 ---------- ---------- ---------- ------IIII IIIIIIIIII III------- -----IIIII
4A               GGTGGCTTTG ATAATAGGAT TGTCGGTGCC AAAGTT---- ---------- ---TATTAAT GTATA-----
                 ---------- ---------- ---------- ---------- ---------- ---------- ----------
4B               GGTGGCTTTG ATAATAGGAT TGTCGGTGCC AAAGTT---- ---------- ---TATTAAT GTATA-----
                 ---------- ---------- ---------- ---------- ---------- ---------- ----------
4B III           GGTGGCTTTG ATAATAGGAT TGTCGGTGCC AAAGTTTTAA GTATAATAAA GTTTATTAAT GTATAATTAA
                 ---------- ---------- ---------- ------IIII IIIIIIIIII III------- -----IIIII
```

HAPLOTYPE COMPARISON 7/13/92 3:51 PM

```
                290       300       310       320       330       340       350
STANDARD        ---------- ---------- -----TTTTA ATTAAGGGGT GGCTTTGATA ATAGGATTGT CAGCGCCAAA
                ---------- ---------- ---------- ---------- ---------- ---------- ----------
A               ---------- ---------- -----TTTTA ATTAAGGGGT GGCTTTGATA ATAGGATTGT CAGCGCCAAA
                ---------- ---------- ---------- ---------- ---------- ---------- ----------
A III           AAGTTTTACT GTATACTTTA ATTAA
                IIIIIIIIII IIIIIIIIII IIIII
C               AAGTTTTAAT GTATACTTTA ATTAA
                IIIIIIIIII IIIIIIIIII IIIII
OA              ---------- ---------- -----TTTTA ATTAAGGGGT GGCTTTGATA ATAGGATTGT CAGCGCCAAA
                ---------- ---------- ---------- ---------- ---------- ---------- ----------
OA              ---------- ---------- -----TTTTA ATTAAGGGGT GGCTTTGATA ATAGGATTGT CAGCGCCAAA
                ---------- ---------- ---------- ---------- ---------- ---------- ----------
1A              ---------- ---------- -----TTTTA ATTAAGGGGT GGCTTTGATA ATAGGATTGT CAGCGCCAAA
                ---------- ---------- ---------- ---------- ---------- ---------- ----------
1B              ---------- ---------- -----TTTTA ATTAAGGGGT GGCTTTGATA ATAGGATTGT CAGCGCCAAA
                ---------- ---------- ---------- ---------- ---------- ---------- ----------
2A              ---------- ---------- -----TTTTA ATTAAGGGGT GGCTTTGATA ATAGGATTGT CAGTGCCAAA
                ---------- ---------- ---------- ---------- ---------- ---------- ---*------
2A III          AAGTTTTACT GCATACTTTA ATTAA
                IIIIIIIIII IIIIIIIIII IIIII
3A              ---------- ---------- -----TTTTA ATTAAGGGGT GGCTTTGATA ATAGGATTGT CAGCGCCAAA
                ---------- ---------- ---------- ---------- ---------- ---------- ----------
3A III          AAGTTTTACT GTATACTTTA ATTAA
                IIIIIIIIII IIIIIIIIII IIIII
4A              ---------- ---------- -----TTTTA ATTAAGGGGT GGCTTTGATA ATAGGATTGT CAGCGCCAAA
                ---------- ---------- ---------- ---------- ---------- ---------- ----------
4B              ---------- ---------- -----TTTTA ATTAAGGGGT GGCTTTGATA ATAGGATTGT CAGCGCCAAA
                ---------- ---------- ---------- ---------- ---------- ---------- ----------
4B III          AAGTTTTACT GTATACTTTA ATTAA
                IIIIIIIIII IIIIIIIIII IIIII
```

APPENDIX V

Sequence Alignments of A Haplotypes

ALIGNED "A" VARIANTS 7/14/92 11:40 AM

	10	20	30	40	50	60	70
1 STANDARD 7/9-24	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
23	----------	----------	----------	----------	----------	----------	----------
2 3 26a 92 Arcs 10	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
24	----------	----------	----------	----------	----------	----------	----------
3 3 26a 92 Arcs 11	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
25	----------	----------	----------	----------	----------	----------	----------
4 3 26a 92 Arcs 12	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
26	----------	----------	----------	----------	----------	----------	----------
5 3 26a 92 Arcs 13	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
27	----------	----------	----------	----------	----------	----------	----------
6 3 26a 92 Arcs 14	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
28	----------	----------	----------	----------	----------	----------	----------
7 3 26a 92 Arcs 16	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
29	----------	----------	----------	----------	----------	----------	----------
8 3 26a 92 Arcs 17	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
30	----------	----------	----------	----------	----------	----------	----------
9 3 26a 92 Arcs 20	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
31	----------	----------	----------	----------	----------	----------	----------
10 3 26a 92 Arcs 22	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
32	----------	----------	----------	----------	----------	----------	----------
11 3 27 92 Arcs 03.	GTTAAGCTAC	GTCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
33	----------	-*--------	----------	----------	----------	----------	----------
12 3 27 92 Arcs 07.	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
34	----------	----------	----------	----------	----------	----------	----------
13 3 27 92 Arcs 08.	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
35	----------	----------	----------	----------	----------	----------	----------
14 3 27 92 Arcs 12.	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
36	----------	----------	----------	----------	----------	----------	----------
15 3 27 92 Arcs 13.	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
37	----------	----------	----------	----------	----------	----------	----------
16 3 27 92 Arcs 14.	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
38	----------	----------	----------	----------	----------	----------	----------
17 3 27 92 Arcs 15.	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
39	----------	----------	----------	----------	----------	----------	----------
18 3 27 92 Arcs 17.	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
40	----------	----------	----------	----------	----------	----------	----------
19 3 27 92 Arcs 18.	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
41	----------	----------	----------	----------	----------	----------	----------
20 3 27 92 Arcs 20.	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
42	----------	----------	----------	----------	----------	----------	----------
21 3 27 92 Arcs 21.	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG

ALIGNED "A" VARIANTS 7/14/92 11:41 AM

43 22 3 27 92 Arcs 23. 44	---------- ---------- ---------- ---------- ---------- ---------- ---------- GTTAAGCTAC GCCAGAGTAT GTTTTAATCA GGGGTGGCCC TTATGATAGG GTTGTATGTG CCAAAGTTTG ---------- ---------- ---------- ---------- ---------- ---------- ----------

ALIGNED "A" VARIANTS 7/14/92 11:44 AM

	80	90	100	110	120	130	140
1 STANDARD 7/9-24	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
23	----------	----------	----------	----------	----------	----------	----------
2 3 26a 92 Arcs 10	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
24	----------	----------	----------	----------	----------	----------	----------
3 3 26a 92 Arcs 11	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
25	----------	----------	----------	----------	----------	----------	----------
4 3 26a 92 Arcs 12	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
26	----------	----------	----------	----------	----------	----------	----------
5 3 26a 92 Arcs 13	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
27	----------	----------	----------	----------	----------	----------	----------
6 3 26a 92 Arcs 14	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
28	----------	----------	----------	----------	----------	----------	----------
7 3 26a 92 Arcs 16	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
29	----------	----------	----------	----------	----------	----------	----------
8 3 26a 92 Arcs 17	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
30	----------	----------	----------	----------	----------	----------	----------
9 3 26a 92 Arcs 20	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
31	----------	----------	----------	----------	----------	----------	----------
10 3 26a 92 Arcs 22	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
32	----------	----------	----------	----------	----------	----------	----------
11 3 27 92 Arcs 03.	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
33	----------	----------	----------	----------	----------	----------	----------
12 3 27 92 Arcs 07.	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
34	----------	----------	----------	----------	----------	----------	----------
13 3 27 92 Arcs 08.	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
35	----------	----------	----------	----------	----------	----------	----------
14 3 27 92 Arcs 12.	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
36	----------	----------	----------	----------	----------	----------	----------
15 3 27 92 Arcs 13.	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
37	----------	----------	----------	----------	----------	----------	----------
16 3 27 92 Arcs 14.	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
38	----------	----------	----------	----------	----------	----------	----------
17 3 27 92 Arcs 15.	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	GAGGATGGTT	TTGATAATAG	GATTGTCGGT
39	----------	----------	----------	----------	*---------	----------	----------
18 3 27 92 Arcs 17.	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
40	----------	----------	----------	----------	----------	----------	----------
19 3 27 92 Arcs 18.	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
41	----------	----------	----------	----------	----------	----------	----------
20 3 27 92 Arcs 20.	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
42	----------	----------	----------	----------	----------	----------	----------
21 3 27 92 Arcs 21.	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT

ALIGNED "A" VARIANTS 7/14/92 11:46 AM

43 22 3 27 92 Arcs 23. 44	---------- ---------- ---------- ---------- ---------- ---------- ---------- TTAAGTATAA CAAAAAGTTC ACTAATGTAT ACTTTAATTA AAGGATGGTT TTGATAATAG GATTGTCGGT ---------- ---------- ---------- ---------- ---------- ---------- ----------

ALIGNED "A" VARIANTS 7/14/92 11:48 AM

	150	160	170	180	190	200	210
1 STANDARD 7/9-24	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
23 2 3 26a 92 Arcs 10	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
24 3 3 26a 92 Arcs 11	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
25 4 3 26a 92 Arcs 12	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
26 5 3 26a 92 Arcs 13	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
27 6 3 26a 92 Arcs 14	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
28 7 3 26a 92 Arcs 16	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
29 8 3 26a 92 Arcs 17	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
30 9 3 26a 92 Arcs 20	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
31 10 3 26a 92 Arcs 22	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
32 11 3 27 92 Arcs 03.	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
33 12 3 27 92 Arcs 07.	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
34 13 3 27 92 Arcs 08.	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAAT-AAAG
35 14 3 27 92 Arcs 12.	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	-----D---- TTAATTAAAG
36 15 3 27 92 Arcs 13.	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
37 16 3 27 92 Arcs 14.	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
38 17 3 27 92 Arcs 15.	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
39 18 3 27 92 Arcs 17.	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
40 19 3 27 92 Arcs 18.	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
41 20 3 27 92 Arcs 20.	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
42 21 3 27 92 Arcs 21.	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG

ALIGNED "A" VARIANTS 7/14/92 11:50 AM

43 22 3 27 92 Arcs 23. 44	---------- ---------- ---------- ---------- ---------- ---------- ---------- GCCAAAGTTT TAAGTATAAT AAAGTTTATT AATGTATAAT TAAAAGTTTT AATGTATACT TTAATTAAAG ---------- ---------- ---------- ---------- ---------- ---------- ----------

ALIGNED "A" VARIANTS 7/14/92 11:53 AM

	220	230	240	250	260	270	280
1 STANDARD 7/9-24	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTATT	AATGTATATT	TTAATTAAGG	GGTGGCTTTG
23	----------	----------	----------	----------	----------	----------	----------
2 3 26a 92 Arcs 10	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTATT	AATGTATATT	TTAATTAAGG	GGTGGCTTTG
24	----------	----------	----------	----------	----------	----------	----------
3 3 26a 92 Arcs 11	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTATT	AATGTATATT	TTAATTAAGG	GGTGGCTTTG
25	----------	----------	----------	----------	----------	----------	----------
4 3 26a 92 Arcs 12	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTATT	AATGTATATT	TTAATTAAGG	GGTGGCTTTG
26	----------	----------	----------	----------	----------	----------	----------
5 3 26a 92 Arcs 13	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTATT	AATGTATATT	TTAATTAAGG	GGTGGCTTTG
27	----------	----------	----------	----------	----------	----------	----------
6 3 26a 92 Arcs 14	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTATT	AATGTATATT	TTAATTAAGG	GGTGGCTTTG
28	----------	----------	----------	----------	----------	----------	----------
7 3 26a 92 Arcs 16	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTATT	AATGTATATT	TTAATTAAGG	GGTGGCTTTG
29	----------	----------	----------	----------	----------	----------	----------
8 3 26a 92 Arcs 17	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTATT	AATGTATATT	TTAATTAAGG	GGTGGCTTTG
30	----------	----------	----------	----------	----------	----------	----------
9 3 26a 92 Arcs 20	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTATT	AATGTATATT	TTAATTAAGG	GGTGGCTTTG
31	----------	----------	----------	----------	----------	----------	----------
10 3 26a 92 Arcs 22	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTATT	AATGTATATT	TTAATTAAGG	GGTGGCTTTG
32	----------	----------	----------	----------	----------	----------	----------
11 3 27 92 Arcs 03.	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTATT	AATGTATATT	TTAATTAAGG	GGTGGCTTTG
33	----------	----------	----------	----------	----------	----------	----------
12 3 27 92 Arcs 07.	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTATT	AATGTATATT	TTAATTAAGG	GGTGGCTTTG
34	----------	----------	----------	----------	----------	----------	----------
13 3 27 92 Arcs 08.	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTATT	AATGTATATT	TTAATTAAGG	GGTGGCTTTG
35	----------	----------	----------	----------	----------	----------	----------
14 3 27 92 Arcs 12.	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTATT	AATGTATATT	TTAATTAAGG	GGTGGCTTTG
36	----------	----------	----------	----------	----------	----------	----------
15 3 27 92 Arcs 13.	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTATT	AATGTATATT	TTAATTAAGG	GGTGGCTTTG
37	----------	----------	----------	----------	----------	----------	----------
16 3 27 92 Arcs 14.	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTATT	AATGTATATT	TTAATTAAGG	GGTGGCTTTG
38	----------	----------	----------	----------	----------	----------	----------
17 3 27 92 Arcs 15.	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTATT	AATGTATATT	TTAATTAAGG	GGTGGCTTTG
39	----------	----------	----------	----------	----------	----------	----------
18 3 27 92 Arcs 17.	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTATT	AATGTATATT	TTAATTAAGG	GGTGGCTTTG
40	----------	----------	----------	----------	----------	----------	----------
19 3 27 92 Arcs 18.	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTATT	AATGTATATT	TTAATTAAGG	GGTGGCTTTG
41	----------	----------	----------	----------	----------	----------	----------
20 3 27 92 Arcs 20	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTATT	AATGTATATT	TTAATTAAGG	GGTGGCTTTG
42	----------	----------	----------	----------	----------	----------	----------
21 3 27 92 Arcs 21	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTATT	AATGTATATT	TTAATTAAGG	GGTGGCTTTG

ALIGNED "A" VARIANTS 7/14/92 11:55 AM

43 22 3 27 92 Arcs 23. 44	---------- ---------- ---------- ---------- ---------- ---------- ---------- GGTGGCTTTG ATAATAGGAT TGTCGGTGCC AAAGTTTATT AATGTATATT TTAATTAAGG GGTGGCTTTG ---------- ---------- ---------- ---------- ---------- ---------- ----------

ALIGNED "A" VARIANTS 7/14/92 11:57 AM

	290 300 310 320 330 340 350
1 STANDARD 7/9-24	ATAATAGGAT TGTCAGCGCC AAAG
23	---------- ---------- ----
2 3 26a 92 Arcs 10	ATAATAGGAT TGTCAGTGCC AAAG
24	---------- ------*--- ----
3 3 26a 92 Arcs 11	ATAATAGGAT TGTCAGCGCC AAAG
25	---------- ---------- ----
4 3 26a 92 Arcs 12	ATAATAGGAT TGTCAGCGCC AAAG
26	---------- ---------- ----
5 3 26a 92 Arcs 13	ATAATAGGAT TGTCAGCGCC AAAG
27	---------- ---------- ----
6 3 26a 92 Arcs 14	ATAATAGGAT TGTCAGCGCC AAAG
28	---------- ---------- ----
7 3 26a 92 Arcs 16	ATAATAGGAT TGTCAGCGCC AAAG
29	---------- ---------- ----
8 3 26a 92 Arcs 17	ATAATAGGAT TGTCAGCGCC AAAG
30	---------- ---------- ----
9 3 26a 92 Arcs 20	ATAATAGGAT TGTCAGCGCC AAAG
31	---------- ---------- ----
10 3 26a 92 Arcs 22	ATAATAGGAT TGTCAGCGCC AAAG
32	---------- ---------- ----
11 3 27 92 Arcs 03.	ATAATAGGAT TGTCAGCGCC AAAG
33	---------- ---------- ----
12 3 27 92 Arcs 07.	ATAATAGGAT TGTCAGCGCC AAAG
34	---------- ---------- ----
13 3 27 92 Arcs 08.	ATAATAGGAT TGTCAGTGCC AAAG
35	---------- ------*--- ----
14 3 27 92 Arcs 12.	ATAATAGGAT TGTCAGCGCC AAAG
36	---------- ---------- ----
15 3 27 92 Arcs 13.	ATAATAGGAT TGTCAGCGCC AAAG
37	---------- ---------- ----
16 3 27 92 Arcs 14.	ATAATAGGAT TGTCAGAGCC AAAG
38	---------- ------*--- ----
17 3 27 92 Arcs 15.	ATAATAGGAT TGTCAGCGCC AAAG
39	---------- ---------- ----
18 3 27 92 Arcs 17.	ATAATAGGAT TGTCAGCGCC AAAG
40	---------- ---------- ----
19 3 27 92 Arcs 18.	ATAATAGGAT TGTCAGCGCC AAAG
41	---------- ---------- ----
20 3 27 92 Arcs 20.	ATAATAGGAT TGTCAGCGCC AAAG
42	---------- ---------- ----
21 3 27 92 Arcs 21.	ATAATAGGAT TGTCAGCGCC AAAG

ALIGNED "A" VARIANTS' 7/14/92 11:59 AM

43 22 3 27 92 Arcs 23. 44	---------- ---------- ---- ATAATAGGAT TGTCAGCGCC AAAG ---------- ---------- ----

APPENDIX VI

Sequence Alignments of C Haplotypes

ALIGNED "C" VARIANTS 7/15/92 1:56 PM

		10	20	30	40	50	60	70
1	4/28/92 ARCS 11	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
19		----------	----------	----------	----------	----------	----------	----------
2	4/13/92 ARCS 01	GTTAAGCTAC	GTCAGAGTAT	GTTTTANTCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
20		----------	-*--------	------*---	----------	----------	----------	----------
3	4/14/92 ARCS 18	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTGTGATAGG	GTTGTAGGTG	CCAAAGTTTG
21		----------	----------	----------	----------	--*-------	------*---	----------
4	4/21/92 ARCS 15	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
22		----------	----------	----------	----------	----------	----------	----------
5	4/21/92 ARCS 18	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
23		----------	----------	----------	----------	----------	----------	----------
6	4/23/92 ARCS 20	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
24		----------	----------	----------	----------	----------	----------	----------
7	4/26/92 ARCS 08	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
25		----------	----------	----------	----------	----------	----------	----------
8	4/26/92 ARCS 14	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
26		----------	----------	----------	----------	----------	----------	----------
9	4/26/92 ARCS 19	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
27		----------	----------	----------	----------	----------	----------	----------
10	4/30/92 ARCS 08	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
28		----------	----------	----------	----------	----------	----------	----------
11	4/30/92 ARCS 10	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
29		----------	----------	----------	----------	----------	----------	----------
12	4/30/92 ARCS 11	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
30		----------	----------	----------	----------	----------	----------	----------
13	4/30/92 ARCS 20	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
31		----------	----------	----------	----------	----------	----------	----------
14	4/7/92 ARCS 10	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
32		----------	----------	----------	----------	----------	----------	----------
15	4/7/92 ARCS 11	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
33		----------	----------	----------	----------	----------	----------	----------
16	4/7/92 ARCS 17	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
34		----------	----------	----------	----------	----------	----------	----------
17	5/5/92 ARCS 21	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
35		----------	----------	----------	----------	----------	----------	----------
18	5/5/92 ARCS 22	GTTAAGCTAC	GCCAGAGTAT	GTTTTAATCA	GGGGTGGCCC	TTATGATAGG	GTTGTATGTG	CCAAAGTTTG
36		----------	----------	----------	----------	----------	----------	----------

ALIGNED "C" VARIANTS 7/15/92 1:58 PM

		80	90	100	110	120	130	140
1	4/28/92 ARCS 11	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
19		----------	----------	----------	----------	----------	----------	----------
2	4/13/92 ARCS 01	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
20		----------	----------	----------	----------	----------	----------	----------
3	4/14/92 ARCS 18	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
21		----------	----------	----------	----------	----------	----------	----------
4	4/21/92 ARCS 15	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
22		----------	----------	----------	----------	----------	----------	----------
5	4/21/92 ARCS 18	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
23		----------	----------	----------	----------	----------	----------	----------
6	4/23/92 ARCS 20	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
24		----------	----------	----------	----------	----------	----------	----------
7	4/26/92 ARCS 08	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
25		----------	----------	----------	----------	----------	----------	----------
8	4/26/92 ARCS 14	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
26		----------	----------	----------	----------	----------	----------	----------
9	4/26/92 ARCS 19	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
27		----------	----------	----------	----------	----------	----------	----------
10	4/30/92 ARCS 08	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
28		----------	----------	----------	----------	----------	----------	----------
11	4/30/92 ARCS 10	TTAAGTATAA	CAAAAAGTTC	ACTAATGCAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
29		----------	----------	-------*--	----------	----------	----------	----------
12	4/30/92 ARCS 11	TTAAGT--AA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
30		------DD--	----------	----------	----------	----------	----------	----------
13	4/30/92 ARCS 20	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
31		----------	----------	----------	----------	----------	----------	----------
14	4/7/92 ARCS 10	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
32		----------	----------	----------	----------	----------	----------	----------
15	4/7/92 ARCS 11	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
33		----------	----------	----------	----------	----------	----------	----------
16	4/7/92 ARCS 17	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
34		----------	----------	----------	----------	----------	----------	----------
17	5/5/92 ARCS 21	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
35		----------	----------	----------	----------	----------	----------	----------
18	5/5/92 ARCS 22	TTAAGTATAA	CAAAAAGTTC	ACTAATGTAT	ACTTTAATTA	AAGGATGGTT	TTGATAATAG	GATTGTCGGT
36		----------	----------	----------	----------	----------	----------	----------

ALIGNED "C" VARIANTS 7/15/92 2:00 PM

		150	160	170	180	190	200	210
1	4/28/92 ARCS 11	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
19		----------	----------	----------	----------	----------	----------	----------
2	4/13/92 ARCS 01	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
20		----------	----------	----------	----------	----------	----------	----------
3	4/14/92 ARCS 18	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAGT	TAAAAGTTTT	GACGTATACT	TTAATTAAAG
21		----------	----------	----------	---------*-	----------	*-*-------	----------
4	4/21/92 ARCS 15	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
22		----------	----------	----------	----------	----------	----------	----------
5	4/21/92 ARCS 18	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
23		----------	----------	----------	----------	----------	----------	----------
6	4/23/92 ARCS 20	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
24		----------	----------	----------	----------	----------	----------	----------
7	4/26/92 ARCS 08	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
25		----------	----------	----------	----------	----------	----------	----------
8	4/26/92 ARCS 14	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
26		----------	----------	----------	----------	----------	----------	----------
9	4/26/92 ARCS 19	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
27		----------	----------	----------	----------	----------	----------	----------
10	4/30/92 ARCS 08	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
28		----------	----------	----------	----------	----------	----------	----------
11	4/30/92 ARCS 10	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
29		----------	----------	----------	----------	----------	----------	----------
12	4/30/92 ARCS 11	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
30		----------	----------	----------	----------	----------	----------	----------
13	4/30/92 ARCS 20	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
31		----------	----------	----------	----------	----------	----------	----------
14	4/7/92 ARCS 10	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
32		----------	----------	----------	----------	----------	----------	----------
15	4/7/92 ARCS 11	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
33		----------	----------	----------	----------	----------	----------	----------
16	4/7/92 ARCS 17	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
34		----------	----------	----------	----------	----------	----------	----------
17	5/5/92 ARCS 21	GCCAAAGTTT	TAATTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
35		----------	---*------	----------	----------	----------	----------	----------
18	5/5/92 ARCS 22	GCCAAAGTTT	TAAGTATAAT	AAAGTTTATT	AATGTATAAT	TAAAAGTTTT	AATGTATACT	TTAATTAAAG
36		----------	----------	----------	----------	----------	----------	----------

ALIGNED "C" VARIANTS 7/15/92 2:03 PM

		220	230	240	250	260	270	280
1	4/28/92 ARCS 11	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTTAA	GTATAATAAA	GTTTATTAAT	GTATAATTAA
19		----------	----------	----------	----------	----------	----------	----------
2	4/13/92 ARCS 01	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTTAA	GTATAATAAA	GTTTATTAAT	GTATAATTAA
20		----------	----------	----------	----------	----------	----------	----------
3	4/14/92 ARCS 18	GATGGTCTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTTAA	GTATAATAAA	GTTTATTAAT	GTATAGTTAA
21		-*---**	----------	----------	----------	----------	----------	------*---
4	4/21/92 ARCS 15	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTTAA	GTATAATAAA	GTTTATTAAT	GTATAATTAA
22		----------	----------	----------	----------	----------	----------	----------
5	4/21/92 ARCS 18	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTTAA	GTATAATAAA	GTTTATTAAT	GTATAATTAA
23		----------	----------	----------	----------	----------	----------	----------
6	4/23/92 ARCS 20	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTTAA	GTATAATAAA	GTTTATTAAT	GTATAATTAA
24		----------	----------	----------	----------	----------	----------	----------
7	4/26/92 ARCS 08	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTTAA	GTATAATAAA	GTTTATTAAT	GTATAATTAA
25		----------	----------	----------	----------	----------	----------	----------
8	4/26/92 ARCS 14	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTTAA	GTATAATAAA	GTTTATTAAT	GTATAATTAA
26		----------	----------	----------	----------	----------	----------	----------
9	4/26/92 ARCS 19	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTTAA	GTATAATAAA	GTTTATTAAT	GTATAATTAA
27		----------	----------	----------	----------	----------	----------	----------
10	4/30/92 ARCS 08	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTTAA	GTATAATAAA	GTTTATTAAT	GTATAATTAA
28		----------	----------	----------	----------	----------	----------	----------
11	4/30/92 ARCS 10	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTTAA	GTATAATAAA	GTTTATTAAT	GTATAATTAA
29		----------	----------	----------	----------	----------	----------	----------
12	4/30/92 ARCS 11	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTTAA	GTATAATAAA	GTTTATTAAT	GTATAATTAA
30		----------	----------	----------	----------	----------	----------	----------
13	4/30/92 ARCS 20	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTTAA	GTATAATAAA	GTTTATTAAT	GTATAATTAA
31		----------	----------	----------	----------	----------	----------	----------
14	4/7/92 ARCS 10	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTTAA	GTATAATAAA	GTTTATTAAT	GTATAATTAA
32		----------	----------	----------	----------	----------	----------	----------
15	4/7/92 ARCS 11	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTTAA	GTATAATAAA	GTTTATTAAT	GTATAATTAA
33		----------	----------	----------	----------	----------	----------	----------
16	4/7/92 ARCS 17	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTTAA	GTATAATAAA	GTTTATTAAT	GTATAATTAA
34		----------	----------	----------	----------	----------	----------	----------
17	5/5/92 ARCS 21	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTTAA	GTATAATAAA	GTTTATTAAT	GTATAATTAA
35		----------	----------	----------	----------	----------	----------	----------
18	5/5/92 ARCS 22	GGTGGCTTTG	ATAATAGGAT	TGTCGGTGCC	AAAGTTTTAA	GTATAATAAA	GTTTATTAAT	GTATAATTAA
36		----------	----------	----------	----------	----------	----------	----------

ALIGNED "C" VARIANTS 7/15/92 2:05 PM

		290 300 310 320 330 340 350
1 4/28/92 ARCS 11		AAGTTTTAAT GTATACTTTA ATTAA
19		---------- ---------- -----
2 4/13/92 ARCS 01		AAGTTTTAAT GTATACTTTA ATTAA
20		---------- ---------- -----
3 4/14/92 ARCS 18		AAGTTTTGAC GTATACTTTA ATTAA
21		-------*-* ---------- -----
4 4/21/92 ARCS 15		AAGTTTTAAT GTATACTTTA ATTAA
22		---------- ---------- -----
5 4/21/92 ARCS 18		AAGTTTTAAT GTATACTTTA ATTAA
23		---------- ---------- -----
6 4/23/92 ARCS 20		AAGTTTTAAT GTATACTTTA ATTAA
24		---------- ---------- -----
7 4/26/92 ARCS 08		AAGTTTTAAT GTATACTTTA ATTAA
25		---------- ---------- -----
8 4/26/92 ARCS 14		AAGTTTTAAT GTATACTTTA ATTAA
26		---------- ---------- -----
9 4/26/92 ARCS 19		AAGTTTTAAT GTATACTTTA ATTAA
27		---------- ---------- -----
10 4/30/92 ARCS 08		AAGTTTTAAT GTATACTTTA ATTAA
28		---------- ---------- -----
11 4/30/92 ARCS 10		AAGTTTTAAT GTATACTTTA ATTAA
29		---------- ---------- -----
12 4/30/92 ARCS 11		AAGTTTTAAT GTATACTTTA ATTAA
30		---------- ---------- -----
13 4/30/92 ARCS 20		AAGTTTTAAT GTATACTTTA ATTAA
31		---------- ---------- -----
14 4/7/92 ARCS 10		AAGTTTTAAT GTATACTTTA ATTAA
32		---------- ---------- -----
15 4/7/92 ARCS 11		AAGTTTTAAT GTATACTTTA ATTAA
33		---------- ---------- -----
16 4/7/92 ARCS 17		AAGTTTTAAT GTATACTTTA ATTAA
34		---------- ---------- -----
17 5/5/92 ARCS 21		AAGTTTTAAT GTATACTTTA ATTAA
35		---------- ---------- -----
18 5/5/92 ARCS 22		AAGTTTTAAT GTATACTTTA ATTAA
36		---------- ---------- -----

APPENDIX IV

Sequence Alignments of Arctic Cisco Haplotypes

Appendix VII. Table of Composite Haplotypes.

Alternate Designation*	Haplotype	Restriction	NUC 47	NUC 109	Sequence Repeat
A	BRCS	BRCS	T	T	N
C	A1	A	T	T	N
B	A2	A	C	T	N
D	A3	A	T	C	N
F	A4	2D	T	T	N
E	A-III	A	T	T	III
G	C	C	T	T	III
H	OA1	OA	T	C	N
I	OA2	OA	C	T	N
J	OA3	OA	C	C	N
K	1A	1A	C	C	N
L	1B	1B	C	C	N
M	2A	2A	C	C	N
N	2A-III	2A	C	C	III
O	2C	2C	C	C	N
P	3A	3A	C	C	N
Q	3A-III	3A	C	C	III
R	4A	4A	C	T	N
T	4B	4B	C	T	N
S	4B-III	4B	C	T	III

* Designations used in Chapter 3, Bickham et al.

APPENDIX VIII

Tables of Scorings of Individuals for Composite Haplotypes

Cisco Restriction and Sequence Comparison

Common Name	Sample Number	Locality	Restriction Analysis	Sequence Analysis	Sequence Repeat
Bering Cisco	6341	Yukon River, AK			
Bering Cisco	6342	Yukon River, AK			
Bering Cisco	6343	Yukon River, AK			
Bering Cisco	6344	Yukon River, AK			
Bering Cisco	6345	Yukon River, AK			
Bering Cisco	6346	Yukon River, AK			
Bering Cisco	6347	Yukon River, AK			
Bering Cisco	6348	Yukon River, AK			
Bering Cisco	6349	Yukon River, AK			
Bering Cisco	6350	Yukon River, AK			
Cisco	12486	Point Barrow, AK	A	T-T	
Cisco	12487	Point Barrow, AK	A	T-T	C
Cisco	12488	Point Barrow, AK	A	T-T	C
Cisco	12489	Point Barrow, AK			
Cisco	12490	Point Barrow, AK			
Cisco	12491	Point Barrow, AK			
Cisco	12492	Point Barrow, AK			
Cisco	12493	Point Barrow, AK			
Cisco	12494	Point Barrow, AK			
Cisco	12495	Point Barrow, AK			
Cisco	12497	Point Barrow, AK			
Cisco	6352	Point Barrow, AK	A	T-T	
Cisco	6353	Point Barrow, AK	A	T-T	
Arctic Cisco	12432	Kogru River, AK	A	T-T	
Arctic Cisco	12433	Kogru River, AK			
Arctic Cisco	12434	Kogru River, AK	A	T-T	
Arctic Cisco	12435	Kogru River, AK	A	T-T	
Arctic Cisco	12436	Kogru River, AK		T-T	
Arctic Cisco	12437	Kogru River, AK	C	T-T	C
Arctic Cisco	12438	Kogru River, AK	4A	C-T	
Arctic Cisco	12439	Kogru River, AK	A	T-T	
Arctic Cisco	12463	Colville River, AK	A	T-T	
Arctic Cisco	12464	Colville River, AK	A	T-T	
Arctic Cisco	12465	Colville River, AK	A	T-T	
Arctic Cisco	12466	Colville River, AK	A	T-T	
Arctic Cisco	12467	Colville River, AK	A	T-T	
Arctic Cisco	12468	Colville River, AK	A	T-T	
Arctic Cisco	12469	Colville River, AK	A	T-T	
Arctic Cisco	12470	Colville River, AK	A	T-T	
Arctic Cisco	12471	Colville River, AK	A	T-T	
Arctic Cisco	12472	Colville River, AK	A	T-T	
Arctic Cisco	GEN 12	Colville River, AK	A	T-T	

Common Name	Sample Number	Locality	Restriction Analysis	Sequence Analysis	Sequence Repeat
Arctic Cisco	GEN 14	Colville River, AK	2A	C-C	
Arctic Cisco	15	Colville River, AK	C	T-T	C
Arctic Cisco	19	Colville River, AK	2A	C-C	
Arctic Cisco	20	Colville River, AK	A	T-T	
Arctic Cisco	22	Colville River, AK	A	T-T	
Arctic Cisco	23	Colville River, AK	A		
Arctic Cisco	25	Colville River, AK	A	T-T	
Arctic Cisco	26	Colville River, AK	A	T-T	
Arctic Cisco	27	Colville River, AK	C	T-T	C
Arctic Cisco	31	Colville River, AK	A	T-T	
Arctic Cisco	32	Colville River, AK	A	T-T	
Arctic Cisco	33	Colville River, AK	A	T-T	C
Arctic Cisco	34	Colville River, AK	A	T-T	
Arctic Cisco	35	Colville River, AK	A	T-T	
Arctic Cisco	36	Colville River, AK	4A	C-T	
Arctic Cisco	38	Colville River, AK	A	T-T	
Arctic Cisco	39	Colville River, AK	A	T-T	
Arctic Cisco	40	Colville River, AK	A		
Arctic Cisco	41	Colville River, AK	A		
Arctic Cisco	43	Colville River, AK	A	T-T	
Arctic Cisco	44	Colville River, AK	A		
Arctic Cisco	45	Colville River, AK	A		
Arctic Cisco	47	Colville River, AK	A	T-T	
Arctic Cisco	48	Colville River, AK	A		
Arctic Cisco	49	Colville River, AK	A	T-T	
Arctic Cisco	50	Colville River, AK	C	T-T	C
Arctic Cisco	51	Colville River, AK	C		
Arctic Cisco	52	Colville River, AK	A	T-T	
Arctic Cisco	60	Colville River, AK	3A	C-C	
Arctic Cisco	63	Colville River, AK	A	T-T	
Arctic Cisco	64	Colville River, AK	3A	C-C	
Arctic Cisco	65	Colville River, AK	A	T-T	C
Arctic Cisco	67	Colville River, AK	C	T-T	C
Cisco	69	Colville River, AK	BRCS	T-T	
Arctic Cisco	70	Colville River, AK	A	T-T	
Arctic Cisco	72	Colville River, AK	A	T-T	
Arctic Cisco	73	Colville River, AK	A	T-T	
Arctic Cisco	74	Colville River, AK	A	T-T	
Arctic Cisco	76	Colville River, AK	A	T-T	
Arctic Cisco	78	Colville River, AK	C	T-T	C
Arctic Cisco	79	Colville River, AK	A	T-T	
Arctic Cisco	80	Colville River, AK	A	T-T	
Arctic Cisco	81	Colville River, AK	A	T-T	
Cisco	85	Colville River, AK	BRCS	T-T	
Arctic Cisco	86	Colville River, AK	B	C-C	
Arctic Cisco	87	Colville River, AK	A		
Arctic Cisco	88	Colville River, AK	A	T-T	

Common Name	Sample Number	Locality	Restriction Analysis	Sequence Analysis	Sequence Repeat
Arctic Cisco	GEN 89	Colville River, AK	A	T-T	
Cisco	116	Colville River, AK	BRCS		
Cisco	117	Colville River, AK	BRCS		
Cisco	118	Colville River, AK	BRCS		
Cisco	119	Colville River, AK	BRCS		C
Cisco	120	Colville River, AK	BRCS		
Cisco	121	Colville River, AK	BRCS		
Cisco	122	Colville River, AK	BRCS		
Cisco	123	Colville River, AK	BRCS		
Cisco	124	Colville River, AK	BRCS		
Cisco	125	Colville River, AK	BRCS		
Cisco	126	Colville River, AK	BRCS		
Cisco	127	Colville River, AK	BRCS		
Cisco	129	Colville River, AK	BRCS		
Cisco	131	Colville River, AK	BRCS		
Cisco	132	Colville River, AK	BRCS		
Cisco	133	Colville River, AK	BRCS		
Cisco	134	Colville River, AK	BRCS		
Cisco	135	Colville River, AK	BRCS		
Cisco	136	Colville River, AK	BRCS		
Cisco	137	Colville River, AK	BRCS		
Arctic Cisco	12396	Sag. River, AK	A	T-T	
Arctic Cisco	12397	Sag. River, AK	A	T-T	
Arctic Cisco	12398	Sag. River, AK	A	T-T	
Arctic Cisco	12399	Sag. River, AK	A	T-T	
Arctic Cisco	12400	Sag. River, AK	A	T-T	
Arctic Cisco	12401	Sag. River, AK	A	T-T	
Arctic Cisco	12402	Sag. River, AK	3A	C-C	
Arctic Cisco	12403	Sag. River, AK	3A	T-T	
Arctic Cisco	12404	Sag. River, AK	C	T-T	C
Arctic Cisco	12405	Sag. River, AK	A	T-T	
Arctic Cisco	12406	Sag. River, AK	A	T-T	
Arctic Cisco	12407	Sag. River, AK	2A	C-C	
Arctic Cisco	12408	Sag. River, AK	A	T-T	C
Arctic Cisco	12409	Sag. River, AK	4B	C-T	C
Arctic Cisco	12410	Sag. River, AK	A	T-T	
Arctic Cisco	12411	Sag. River, AK	A	T-T	
Arctic Cisco	12412	Sag. River, AK	C	T-T	C
Arctic Cisco	12413	Sag. River, AK	A	T-T	C
Arctic Cisco	12414	Sag. River, AK	A	T-T	C
Arctic Cisco	12415	Sag. River, AK	A	T-T	
Arctic Cisco	12416	Sag. River, AK	A		
Arctic Cisco	12417	Sag. River, AK	A	T-T	
Arctic Cisco	12418	Sag. River, AK	A	T-T	
Arctic Cisco	12419	Sag. River, AK	A	T-T	
Arctic Cisco	12420	Sag. River, AK	OA	C-C	

Common Name	Sample Number	Locality	Restriction Analysis	Sequence Analysis	Sequence Repeat
Arctic Cisco	12421	Sag. River, AK	A	T-T	
Arctic Cisco	12422	Sag. River, AK			
Arctic Cisco	12423	Sag. River, AK	A	T-T	C
Arctic Cisco	12424	Sag. River, AK	A	T-T	
Arctic Cisco	12425	Sag. River, AK	A	T-T	
Arctic Cisco	12426	Sag. River, AK			
Arctic Cisco	12428	Sag. River, AK	A	T-T	
Arctic Cisco	12429	Sag. River, AK	A	T-T	
Arctic Cisco	12430	Sag. River, AK	A	T-T	
Arctic Cisco	11700	Sag. River, AK	A	T-T	
Cisco	11742	Sag. River, AK	BRCS	T-T	
Arctic Cisco	11745	Sag. River, AK	A	T-T	
Arctic Cisco	11746	Sag. River, AK	A	T-T	
Arctic Cisco	11747	Sag. River, AK	A	T-T	
Arctic Cisco	11748	Sag. River, AK	A	T-T	
Arctic Cisco	11749	Sag. River, AK	4A	C-T	
Arctic Cisco	11750	Sag. River, AK	C	T-T	C
Arctic Cisco	11751	Sag. River, AK	A	T-T	
Arctic Cisco	11752	Sag. River, AK	A	T-T	C
Arctic Cisco	11753	Sag. River, AK	A	T-T	
Arctic Cisco	11754	Sag. River, AK	C	T-T	C
Arctic Cisco	11755	Sag. River, AK	2A	C-C	
Arctic Cisco	11756	Sag. River, AK	A	T-T	C
Arctic Cisco	11758	Sag. River, AK	C	T-T	C
Arctic Cisco	11759	Sag. River, AK	A	T-T	
Arctic Cisco	11761	Sag. River, AK	A	T-T	
Arctic Cisco	11762	Sag. River, AK	A	T-T	
Arctic Cisco	11763	Sag. River, AK	A	T-T	
Arctic Cisco	11765	Sag. River, AK	3A	C-C	
Arctic Cisco	11767	Sag. River, AK	C	T-T	C
Arctic Cisco	11768	Sag. River, AK	3A	C-C	
Arctic Cisco	11769	Sag. River, AK	A	T-T	
Arctic Cisco	11770	Sag. River, AK	A	T-T	
Arctic Cisco	11771	Sag. River, AK	A	T-T	
Arctic Cisco	11772	Sag. River, AK	A	T-T	
Arctic Cisco	11774	Sag. River, AK	3A	C-C	C
Arctic Cisco	11775	Sag. River, AK	2A	C-C	C
Arctic Cisco	11777	Sag. River, AK	A	T-T	C
Arctic Cisco	11778	Sag. River, AK	A	T-T	C
Arctic Cisco	11779	Sag. River, AK	2A	C-C	
Arctic Cisco	11780	Sag. River, AK	A	T-T	
Arctic Cisco	11781	Sag. River, AK	A	T-T	
Arctic Cisco	11782	Sag. River, AK	A	T-T	C
Arctic Cisco	11783	Sag. River, AK	A	T-T	
Arctic Cisco	11784	Sag. River, AK	A	T-T	
Arctic Cisco	11808	Sag. River, AK	A	T-T	
Arctic Cisco	11810	Sag. River, AK	A	T-T	

Common Name	Sample Number	Locality	Restriction Analysis	Sequence Analysis	Sequence Repeat
Arctic Cisco	11817	Sag. River, AK	A	T-T	C
Arctic Cisco	11818	Sag. River, AK	C		
Arctic Cisco	11819	Sag. River, AK	A		
Arctic Cisco	11821	Sag. River, AK	A	T-T	
Arctic Cisco	11822	Sag. River, AK	A	T-T	C
Arctic Cisco	11823	Sag. River, AK	2A	C-C	
Arctic Cisco	11825	Sag. River, AK	2A	C-C	
Arctic Cisco	11827	Sag. River, AK	C	T-T	C
Arctic Cisco	11828	Sag. River, AK	A	T-T	
Arctic Cisco	11832	Sag. River, AK	A	T-T	
Arctic Cisco	11833	Sag. River, AK	A	T-T	
Arctic Cisco	11834	Sag. River, AK	A		
Arctic Cisco	12474	Kaktovik Lagoon, AK	A	T-T	
Arctic Cisco	12475	Kaktovik Lagoon, AK	A	T-T	
Arctic Cisco	12476	Kaktovik Lagoon, AK	A	T-T	
Arctic Cisco	12477	Kaktovik Lagoon, AK	A	T-T	
Arctic Cisco	12478	Kaktovik Lagoon, AK	A	T-T	
Arctic Cisco	12479	Kaktovik Lagoon, AK	A	T-T	
Arctic Cisco	12481	Kaktovik Lagoon, AK	A	T-T	
Arctic Cisco	12483	Kaktovik Lagoon, AK			
Arctic Cisco	12484	Kaktovik Lagoon, AK			
Arctic Cisco	12247	Stokes Point, YT	A	T-T	
Arctic Cisco	12248	Stokes Point, YT	A	T-T	
Arctic Cisco	12249	Stokes Point, YT			
Arctic Cisco	12250	Stokes Point, YT	A	T-T	
Arctic Cisco	12251	Stokes Point, YT	A	T-T	
Arctic Cisco	12252	Stokes Point, YT	A	T-T	
Arctic Cisco	12253	Stokes Point, YT	OA	C-T	
Arctic Cisco	12254	Stokes Point, YT	A	T-T	
Arctic Cisco	12284	Stokes Point, YT	A	T-T	
Arctic Cisco	12285	Stokes Point, YT	A	T-T	
Arctic Cisco	12286	Stokes Point, YT	A	T-T	
Arctic Cisco	12287	Stokes Point, YT	C	T-T	C
Arctic Cisco	12288	Stokes Point, YT	A	T-T	
Arctic Cisco	12289	Stokes Point, YT	A		
Arctic Cisco	12290	Stokes Point, YT	A	T-T	C
Arctic Cisco	12291	Stokes Point, YT	A	T-T	
Arctic Cisco	12292	Stokes Point, YT	A	T-T	
Arctic Cisco	12293	Stokes Point, YT	C	T-T	C
Arctic Cisco	12294	Stokes Point, YT	A	T-T	
Arctic Cisco	12295	Stokes Point, YT	2A	C-C	
Arctic Cisco	12296	Stokes Point, YT	A	T-T	
Arctic Cisco	12297	Stokes Point, YT	2A	MIX	
Arctic Cisco	12298	Stokes Point, YT	2A	C-C	
Arctic Cisco	12299	Stokes Point, YT	A	T-T	

Common Name	Sample Number	Locality	Restriction Analysis	Sequence Analysis	Sequence Repeat
Arctic Cisco	12300	Stokes Point, YT	A	T-T	
Arctic Cisco	12255	Atkinson Point, NWT	A	T-T	
Arctic Cisco	12256	Atkinson Point, NWT	A	T-C	
Arctic Cisco	12257	Atkinson Point, NWT	C	T-T	
Arctic Cisco	12258	Atkinson Point, NWT	A	T-T	
Arctic Cisco	12259	Atkinson Point, NWT			
Arctic Cisco	12260	Atkinson Point, NWT	3A	C-C	
Arctic Cisco	12262	Atkinson Point, NWT			
Arctic Cisco	12263	Atkinson Point, NWT	A	T-T	
Arctic Cisco	12264	Atkinson Point, NWT	B	C-T	
Arctic Cisco	12265	Atkinson Point, NWT	A	T-T	
Arctic Cisco	12266	Atkinson Point, NWT	OA	T-T	
Arctic Cisco	12267	Atkinson Point, NWT	C	T-T	C
Arctic Cisco	12268	Atkinson Point, NWT	A	T-T	
Arctic Cisco	12269	Atkinson Point, NWT	A	T-T	
Arctic Cisco	12270	Atkinson Point, NWT	A	T-T	
Arctic Cisco	12271	Atkinson Point, NWT	3A	C-C	
Arctic Cisco	12272	Atkinson Point, NWT	A	T-T	
Arctic Cisco	12273	Atkinson Point, NWT	C	T-T	C
Arctic Cisco	12274	Atkinson Point, NWT	A	T-T	C
Arctic Cisco	12275	Atkinson Point, NWT	A	T-T	
Arctic Cisco	12276	Atkinson Point, NWT	A	T-T	
Arctic Cisco	12277	Atkinson Point, NWT	A	T-T	
Arctic Cisco	12278	Atkinson Point, NWT	1A	C-C	
Arctic Cisco	12279	Atkinson Point, NWT	OA		
Arctic Cisco	12130	Peel River, NWT	A	T-T	
Arctic Cisco	12137	Peel River, NWT			
Arctic Cisco	12138	Peel River, NWT	A	T-T	C
Arctic Cisco	12141	Peel River, NWT	A	T-T	
Arctic Cisco	12142	Peel River, NWT	A	T-T	
Arctic Cisco	12143	Peel River, NWT	A	T-T	
Arctic Cisco	12151	Peel River, NWT	A	T-T	
Arctic Cisco	12154	Peel River, NWT	A	T-T	
Arctic Cisco	12155	Peel River, NWT	A	T-T	C
Arctic Cisco	12161	Peel River, NWT	A	T-T	
Arctic Cisco	12162	Peel River, NWT	A	T-T	C
Arctic Cisco	12163	Peel River, NWT	A	T-T	
Arctic Cisco	12175	Peel River, NWT	A	T-T	
Arctic Cisco	12178	Peel River, NWT	A	T-T	
Arctic Cisco	12183	Peel River, NWT	4A	C-T	
Arctic Cisco	12184	Peel River, NWT	A	T-T	
Arctic Cisco	12185	Peel River, NWT	A	T-T	
Arctic Cisco	12186	Peel River, NWT	A	T-T	
Arctic Cisco	12187	Peel River, NWT	A	T-T	
Arctic Cisco	12188	Peel River, NWT	A	T-T	

Common Name	Sample Number	Locality	Restriction Analysis	Sequence Analysis	Sequence Repeat
Arctic Cisco	12189	Peel River, NWT	A	T-T	
Arctic Cisco	12190	Peel River, NWT	2A	C-C	
Arctic Cisco	12191	Peel River, NWT	A	T-T	
Arctic Cisco	12192	Peel River, NWT	A	T-T	
Arctic Cisco	12193	Peel River, NWT	B	C-C	
Arctic Cisco	12194	Peel River, NWT	A	T-T	
Arctic Cisco	12195	Peel River, NWT	A	T-T	C
Arctic Cisco	12196	Peel River, NWT	OA	T-C	
Arctic Cisco	12197	Peel River, NWT	OA	C-C	
Arctic Cisco	12198	Peel River, NWT	A	T-T	
Arctic Cisco	MS 1	Peel River, NWT	2A	C-C	
Arctic Cisco	2	Peel River, NWT	A	T-T	
Arctic Cisco	3	Peel River, NWT	A	T-T	C
Arctic Cisco	4	Peel River, NWT	A	T-T	C
Arctic Cisco	6	Peel River, NWT	3A	C-C	
Arctic Cisco	7	Peel River, NWT	A	T-T	
Arctic Cisco	8	Peel River, NWT	C	T-T	C
Arctic Cisco	9	Peel River, NWT	A	C-T	
Arctic Cisco	10	Peel River, NWT	3A	C-C	C
Arctic Cisco	11	Peel River, NWT	A	T-T	
Arctic Cisco	12	Peel River, NWT	A	T-T	
Arctic Cisco	13	Peel River, NWT	A	T-T	
Arctic Cisco	14	Peel River, NWT	A	T-T	
Arctic Cisco	15	Peel River, NWT	A	T-T	C
Arctic Cisco	16	Peel River, NWT	A	T-T	
Arctic Cisco	12211	Arctic Red River, NWT	A	T-T	C
Arctic Cisco	12212	Arctic Red River, NWT	A	T-T	
Arctic Cisco	12213	Arctic Red River, NWT	A	T-T	
Arctic Cisco	12214	Arctic Red River, NWT	A	T-T	
Arctic Cisco	12215	Arctic Red River, NWT	A	T-T	
Arctic Cisco	12216	Arctic Red River, NWT	A	T-T	
Arctic Cisco	12217	Arctic Red River, NWT	C	T-T	C
Arctic Cisco	12218	Arctic Red River, NWT	A	T-T	C
Arctic Cisco	12219	Arctic Red River, NWT	A	T-T	
Arctic Cisco	12220	Arctic Red River, NWT	C	T-T	C
Arctic Cisco	12221	Arctic Red River, NWT	A	T-T	
Arctic Cisco	12222	Arctic Red River, NWT	A	T-T	C
Arctic Cisco	12223	Arctic Red River, NWT	A	T-T	
Arctic Cisco	12224	Arctic Red River, NWT	A	C-C	
Arctic Cisco	12225	Arctic Red River, NWT	2C	T-T	C
Arctic Cisco	12226	Arctic Red River, NWT	A	T-T	
Arctic Cisco	12227	Arctic Red River, NWT	A	T-T	
Arctic Cisco	12228	Arctic Red River, NWT	A	T-T	
Arctic Cisco	12229	Arctic Red River, NWT	C	T-T	C
Arctic Cisco	12230	Arctic Red River, NWT	C	T-T	C
Arctic Cisco	12231	Arctic Red River, NWT	A	T-T	

Common Name	Sample Number	Locality	Restriction Analysis	Sequence Analysis	Sequence Repeat
Arctic Cisco	12233	Arctic Red River, NWT	A	T-T	
Arctic Cisco	12238	Arctic Red River, NWT	2A	C-C	C
Arctic Cisco	12239	Arctic Red River, NWT	A	T-T	
Arctic Cisco	12240	Arctic Red River, NWT	A	T-T	
Arctic Cisco	12241	Arctic Red River, NWT	A	T-T	
Arctic Cisco	12242	Arctic Red River, NWT	A	T-T	
Arctic Cisco	12243	Arctic Red River, NWT	A	T-T	
Arctic Cisco	12244	Arctic Red River, NWT	A	T-T	
Arctic Cisco	12245	Arctic Red River, NWT	4B	C-T	
Arctic Cisco	SR 232	Arctic Red River, NWT	3A	C-C	
Arctic Cisco	233	Arctic Red River, NWT	A	T-T	
Arctic Cisco	234	Arctic Red River, NWT	A	T-T	
Arctic Cisco	235	Arctic Red River, NWT	A	T-T	C
Arctic Cisco	236	Arctic Red River, NWT	A	T-T	
Arctic Cisco	237	Arctic Red River, NWT	A	T-T	
Arctic Cisco	238	Arctic Red River, NWT	A	T-T	
Arctic Cisco	239	Arctic Red River, NWT	A	T-T	
Arctic Cisco	240	Arctic Red River, NWT	A	T-T	
Arctic Cisco	241	Arctic Red River, NWT	A	T-T	
Arctic Cisco	242	Arctic Red River, NWT	A	T-T	
Arctic Cisco	243	Arctic Red River, NWT	A	T-T	
Arctic Cisco	244	Arctic Red River, NWT	A	T-T	C
Arctic Cisco	245	Arctic Red River, NWT	A	T-T	
Arctic Cisco	246	Arctic Red River, NWT	A	T-T	
Arctic Cisco	247	Arctic Red River, NWT	A	T-T	
Arctic Cisco	248	Arctic Red River, NWT	3A	C-C	
Arctic Cisco	249	Arctic Red River, NWT	A	T-T	
Arctic Cisco	250	Arctic Red River, NWT	A		
Arctic Cisco	251	Arctic Red River, NWT	A	C-T	
Arctic Cisco	253	Arctic Red River, NWT	C	T-T	C
Arctic Cisco	256	Arctic Red River, NWT	A	T-T	
Arctic Cisco	257	Arctic Red River, NWT	A	T-T	
Arctic Cisco	258	Arctic Red River, NWT	C	T-T	C
Arctic Cisco	259	Arctic Red River, NWT	A	T-T	C
Arctic Cisco	260	Arctic Red River, NWT	A	T-T	C
Arctic Cisco	262	Arctic Red River, NWT	A	T-T	
Arctic Cisco	264	Arctic Red River, NWT	A	T-T	
Arctic Cisco	267	Arctic Red River, NWT	3A	C-C	
Arctic Cisco	268	Arctic Red River, NWT	A	T-T	
Arctic Cisco	269	Arctic Red River, NWT	A	T-T	C
Arctic Cisco	271	Arctic Red River, NWT	A	T-T	
Arctic Cisco	275	Arctic Red River, NWT	A	T-T	
Arctic Cisco	12303	Mountain River, NWT	3A	C-C	
Arctic Cisco	12304	Mountain River, NWT	A	T-T	
Arctic Cisco	12305	Mountain River, NWT	A	T-T	
Arctic Cisco	12306	Mountain River, NWT	3A	C-C	

Common Name	Sample Number	Locality	Restriction Analysis	Sequence Analysis	Sequence Repeat
Arctic Cisco	12307	Mountain River, NWT	A	T-T	
Arctic Cisco	12308	Mountain River, NWT	A	T-T	C
Arctic Cisco	12309	Mountain River, NWT			C
Arctic Cisco	12310	Mountain River, NWT	A	T-T	
Arctic Cisco	12311	Mountain River, NWT	C	T-T	C
Arctic Cisco	12312	Mountain River, NWT	A	C-C	
Arctic Cisco	12313	Mountain River, NWT	1B	T-T	C
Arctic Cisco	12314	Mountain River, NWT	A	T-T	
Arctic Cisco	12315	Mountain River, NWT	A	T-T	
Arctic Cisco	12316	Mountain River, NWT	3A	C-C	
Arctic Cisco	12317	Mountain River, NWT	A	T-T	
Arctic Cisco	12318	Mountain River, NWT	A	T-T	C
Arctic Cisco	12319	Mountain River, NWT	A	T-T	
Arctic Cisco	12320	Mountain River, NWT	3A	C-C	
Arctic Cisco	12321	Mountain River, NWT	A	T-T	
Arctic Cisco	12322	Mountain River, NWT	OA	C-T	
Arctic Cisco	12323	Carcajou River, NWT	A	T-T	
Arctic Cisco	12324	Carcajou River, NWT	C	T-T	C
Arctic Cisco	12325	Carcajou River, NWT	A	T-T	
Arctic Cisco	12326	Carcajou River, NWT	1B	C-C	
Arctic Cisco	12327	Carcajou River, NWT	A	T-T	C
Arctic Cisco	12328	Carcajou River, NWT	A	T-T	C
Arctic Cisco	12329	Carcajou River, NWT	A	T-T	
Arctic Cisco	12330	Carcajou River, NWT	A	T-T	
Arctic Cisco	12331	Carcajou River, NWT	A	T-T	
Arctic Cisco	12332	Carcajou River, NWT			
Arctic Cisco	12333	Carcajou River, NWT	A	T-T	
Arctic Cisco	12334	Carcajou River, NWT	A	T-T	
Arctic Cisco	12335	Carcajou River, NWT	3A	C-C	C
Arctic Cisco	12336	Carcajou River, NWT	A	T-T	
Arctic Cisco	12337	Carcajou River, NWT	C	T-T	C
Arctic Cisco	12338	Carcajou River, NWT	2D	T-T	
Arctic Cisco	12339	Carcajou River, NWT	A	T-T	C
Arctic Cisco	12340	Carcajou River, NWT	A	T-T	
Arctic Cisco	12341	Carcajou River, NWT	3A	C-C	
Arctic Cisco	12342	Carcajou River, NWT	A	T-T	
Arctic Cisco	12343	Carcajou River, NWT	1A	T-T	
Arctic Cisco	12358	Liard River, NWT	A	T-T	
Arctic Cisco	12359	Liard River, NWT	A	T-T	
Arctic Cisco	12360	Liard River, NWT	2A	C-C	
Arctic Cisco	12361	Liard River, NWT	A	T-T	
Arctic Cisco	12362	Liard River, NWT	A	T-T	
Arctic Cisco	12363	Liard River, NWT	A	T-T	
Arctic Cisco	12364	Liard River, NWT	3A	C-C	
Arctic Cisco	12365	Liard River, NWT	A	T-T	

Common Name	Sample Number	Locality	Restriction Analysis	Sequence Analysis	Sequence Repeat
Arctic Cisco	12366	Liard River, NWT	A	T-T	
Arctic Cisco	12367	Liard River, NWT	C	T-T	C
Arctic Cisco	12368	Liard River, NWT	2A	C-C	
Arctic Cisco	12369	Liard River, NWT	A	T-T	
Arctic Cisco	12370	Liard River, NWT	A	T-T	
Arctic Cisco	12372	Liard River, NWT	A	T-T	
Arctic Cisco	12373	Liard River, NWT	A	T-T	
Arctic Cisco	12374	Liard River, NWT	A	C-C	
Arctic Cisco	12375	Liard River, NWT	A	T-T	
Arctic Cisco	12376	Liard River, NWT	A	T-T	
Arctic Cisco	12377	Liard River, NWT	A	T-T	
Arctic Cisco	12378	Liard River, NWT	A	T-T	
Arctic Cisco	12379	Liard River, NWT	OA	C-C	
Arctic Cisco	12380	Liard River, NWT			
Arctic Cisco	12381	Liard River, NWT	A	T-T	C
Arctic Cisco	12382	Liard River, NWT	A	T-T	
Arctic Cisco	12383	Liard River, NWT	A	T-T	
Arctic Cisco	12384	Liard River, NWT	A	T-T	
Arctic Cisco	12385	Liard River, NWT	A	T-T	C
Arctic Cisco	12386	Liard River, NWT	A	T-T	
Arctic Cisco	12387	Liard River, NWT	A	T-T	
Arctic Cisco	12388	Liard River, NWT	A	T-T	

APPENDIX IX

Table of Composite Haplotype Distributions by Locality

APPENDIX IX. Table of composite haplotype distributions by locality.

Location	Bering	A1	A2	A3	A4	A-III	C	OA1	OA2	OA3	1A	1B	2A	2A-III	2C	3A	3A-III	4A	4B	4B-III
Point Barrow	8	3	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Colville River	3	37	-	-	-	2	6	-	-	-	-	-	2	-	-	2	-	2	-	-
Sagavanirktok River	3	48	-	-	-	10	7	-	-	1	-	-	5	1	-	2	1	1	-	1
Kaktovik	2	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stokes Point	1	16	-	-	-	1	2	-	1	-	-	-	3	-	-	-	-	-	-	-
Atkinson Point	2	12	-	1	-	-	3	-	1	-	1	-	-	-	-	2	-	-	-	-
Peel River	1	27	1	-	-	7	1	1	-	1	-	-	2	-	-	1	1	1	-	-
Artic Red River	0	40	-	-	-	9	6	-	-	-	-	-	-	1	1	3	-	-	1	-
Mountain River	1	9	-	-	-	3	1	-	1	-	-	1	-	-	-	4	-	-	-	-
Carcajou River	1	10	-	-	1	3	2	-	-	-	1	1	-	-	-	1	1	-	-	-
Liard River	1	23	-	-	-	2	1	-	-	1	-	-	2	-	-	1	-	-	-	-
	A	C	B	D	F	E	G	H	I	J	K	L	M	N	O	P	Q	R	T	S

(Designations used in Chapter 3, Bickham et al.)

APPENDIX X

Nuclear DNA Investigations

GENETIC STUDIES OF THE ARCTIC CISCO (*COREGONUS AUTUMNALIS*) IN ALASKA—PILOT STUDIES OF NUCLEAR DNA

INTRODUCTION

As described in the main body of our report, our genetic studies of the Arctic cisco (*Coregonus autumnalis*) have focused on mitochondrial DNA. In this year's investigation, a pilot study of nuclear DNA was also conducted. The main goal of the study was to evaluate the utility of different approaches that might be used to assay population-level variation in nuclear DNA. The methodologies evaluated included 1) Randomly Amplified Polymorphic DNA (RAPD), 2) analysis of variation within specific genes, and 3) analysis of loci containing microsatellites or VNTR (Variable Number Tandom Repeats) elements. The specific objectives were to:

1) Develop and test up to 6 primers for RAPD analyses,

2) Develop and test up to 5 PCR-directed primers for specific genes, and

3) Develop and test up to 3 microsatellite primers.

If suitable primers were developed, then they were to be used to assay a total of 25 to 30 specimens consisting of Arctic cisco from spawning rivers, and king (*Oncorhynchus tshawytscha*) or red salmon (*Oncorhynchus nerka*) which were used as outgroups.

RESULTS

Fourteen primers were developed for each of the RAPD and PCR-directed approaches, and two microsatellite primers were developed and tested (Table 1). A summary of the test results is provided below by primer type.

RAPD

Three of the RAPD primers were tested for variation under a number of conditions and with several replications (Table 1). The

Table 1. Nuclear primers developed and evaluated as part of the 1992 Arctic cisco genetic studies.

PRIMER TYPE	GENE	PRIMER NUMBER	SEQUENCE 5' TO 3'	PRIMER PAIR TESTED	SAMPLE SPECIES/NO.
RAPD		939	AGCGATCGAG	939-939	Sockeye Salmon-20
		1000	GCTCACTGAT	1000-1000	Arctic Cisco-2
		1001	GGACACTGAT	1001-1001	Bering Cisco-2
		1002	GCTCACTGAG		
		1003	GCTCACTGAA		
		1004	GCTCACTGAC		
		1005	GGACACTGAG		
		1006	GGACACTGAA		
		1007	GGACACTGAC		
		1008	AGCGATCGAA		
		1009	AGCGATCGAC		
		1100	ATGCTCACTGA		
		1101	GTGCTCACTGA		
		1102	TTGCTCACTGA		
PCR DIRECTED					
	Growth Hormone	180	(C/T)CGGGTCAGTCGGGT(G/A)CAACA	180 - 181	Arctic Cisco - 2
		181	CTTGTGCATGTCCTTCCTGAAGC		King Salmon - 2
	Zinc Finger	331	CAAATCATGCAAGGATAGAC	331 - 335	Arctic Cisco - 2
		335	AGACCTGATTCCAGACAGTACCA	335 - 337	King Salmon - 2
		336	ATAATCACATGGAGAGCCACAAGCT	336 - 337	
		337	GCACTTCTTTGGTATCTGAGAAAGT		
	Major-Histo-Immune Complex	200	GATGGAGATGAGG(A/T)GTTTTA(C/T)GTGGA	200 - 201	Arctic Cisco - 2
		201	CCAC(G/A)G(A/T)CAACCGCAGGGCACA	200 - 202	King Salmon - 2
		202	CCCAGTGCTCCACT(C/T)TGCAGTCATA	201 - 203	
		203	GACTGCA(G/A)AGTGGAGCACTGGGG	201 - 201	
	ASP-ATase	100	GTTGA(G/A)ATGGGCC(C/T)CCAGATCC	100 - 101	Arctic Cisco - 1
		101	GTTCTCACCCAGGGC(C/T)AGTTC	102 -103	King Salmon - 1
		102	GCTGACCGCATCAT(C/T)ACGATGAG		
		103	AG(G/A)GAGATTCGGCCATCCTTTGT	100-100	Arctic Cisco - 2
MICROSATELLITES		mic1	ACAAGGGAAATTCTGAGAGA	mic1 - mic1	Arctic Cisco - 2
		mic2	TGTTCCCAAATTCTCTCTCT	mic1 - 100	King Salmon - 2
				mic1 - 101	
				mic1 - 102	
				mic1 - 103	
				mic2 - mic2	
				mic2 - 100	
				mic2 - 101	
				mic2 - 102	
				mic2 - 103	

technique unquestionably allowed the visualization of DNA variation in salmonoids. However, the technique was found to be extremely sensitive to minor variations in tissue preservation and the type of tissue from which the genomic DNA had been extracted. As a result, the likelihood of observed variation being an experimental artifact was virtually as great as the variant having a genetic basis. We confirmed that other researchers were experiencing the same problem with the RAPD approach in laboratories throughout the research community. Therefore, after numerous protocol modifications designed to minimize the variation due to experimental effect, we , like a number of other laboratories, have abandoned this approach.

PCR-Directed Primers

Directed PCR analysis of specific genes was conducted with the anticipation that some of the directed nuclear products from specific genes could be produced from published sequence data and these would include microsatellite loci which could be independently analyzed. Two problems were immediately encountered. First, for many of the genes of interest published sequences for the human or mouse were readily available, but sequences reported for other organisms could not be obtained for our use in this study. Second, although numerous genes have been sequenced and found to possess one or more microsatellite loci, for none of the known microsatellite bearing genes could we find sufficient published sequence data to allow development of a PCR directed set of primers for fish. The available published sequence data enabled us to develop directed PCR protocols for four nuclear genes or gene families; growth hormone (GH), the zinc finger X gene (ZFX), the class II alpha subunit of the major histocompatibility complex (MHC II), and aspartate amino transferase (AAT).

Growth Hormone

Two primers were developed for GH and tested extensively under a number of different experimental conditions. A DNA fragment was produced and it was of the appropriate size based on published sequence data from salmon. However, three additional bands were also produced

which could not be eliminated from the reactions without additional research beyond the scope of this project.

Zinc Finger X

For the ZFX gene, four primers were developed and tested. One of the primers did not yield a reaction product. Using the remaining three primers in a semi-nested PCR protocol, it was possible to obtain a single-band product of the ZFX gene. The sequence of the amplified region confirmed the identity of the gene. However, the portion of the gene which we were able to characterize was in an extremely conserved region and was thus of no value in intraspecific population screening.

Major Histocompatibility Complex

Four primers were generated in an effort to obtain data for MHC II genes. Only one primer pair yielded a product that could be analyzed, but even here multiple bands were produced. Through an extensive protocol, a single band was ultimately produced and tested. While this product shows promise, extensive additional research would be necessary to enable its use in population studies.

Aspartate Amino Transaminase

A final effort was made to obtain DNA data for the commonly analyzed protein locus, aspartate amino transaminase (AAT). Four primers were developed for this gene. All primers proved ineffective in producing the desired results. Each of these primer pairs consistently, under numerous experimental conditions, produced multiple bands. Even when individual primers were tested under conditions of high stringency, multiple bands were produced.

Microsatellite Primers

Two microsatellite DNA primers were designed which possessed perfect microsatellite repeats on their 3' ends. These primers were used individually in reactions to produce PCR-generated fragments which, due to primer design, would recognize only microsatellite containing regions

of DNA. The generated fragments that were less than 500 base pairs could then be excised and sequenced yielding the information necessary to design a primer for a specific microsatellite locus.

The results of amplifications using the initial primers were encouraging, but far too many bands were produced to allow simple band extraction and sequencing. We attempted then to reduce the number of bands produced by pairing one of the microsatellite directed primers with one of the nuclear gene directed primers. While this approach did reduce the number of bands, the resulting patterns were still not sufficiently simple to allow single-band extraction from the gel matrix and subsequent sequencing of that band. The only alternative therefore appears to be cloning of the generated fragments and then sequencing them.

CONCLUSIONS

Based upon the results of our pilot study of nuclear DNA variation in salmonids, we conclude:

1) RAPD approaches are not useful in the study of natural populations due to unacceptably high levels of variation attributable to sources other than genetic diversity.

2) The body of basic research data and information dealing with the molecular genetics of fish is not presently sufficient to enable development of primer sets for nuclear genes. Without this information, studies of population variation based upon analysis of nuclear DNA is neither efficient nor cost effective.

Researchers wishing to contribute in this area should focus on the development of genetic libraries for salmonids, isolation of regions of interest, and sequence analysis of those regions. Once this basic research has been conducted, population analysis based upon variation in nuclear DNA will become straightforward.

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering the wisest use of our land and water resources, protecting our fish and wildlife, preserving the environmental and cultural values of our national parks and historical places, and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to assure that their development is in the best interest of all our people. The Department also has a major responsibility for American Indian reservation communities and for people who live in Island Territories under U.S. Administration.

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering the wisest use of our land and water resources, protecting our fish and wildlife, preserving the environmental and cultural values of our national parks and historical places, and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to assure that their development is in the best interest of all our people. The Department also has a major responsibility for American Indian reservation communities and for people who live in Island Territories under U.S. Administration.