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IN THE UNITED STATES DISTRICT COURT

FOR THE DISTRICT OF OREGON

ROBSON BONNICHSEN, et al.,	)	**Civil No. 96-1481-JE**
	)	
Plaintiffs,	)	**FEDERAL DEFENDANTS' MOTION TO MODIFY THE SEPTEMBER 21, 1999 SCHEDULING ORDER TO ALLOW FOR ADDITIONAL TIME TO DO DNA ANALYSIS**
	)	
v.	)	
	)	
UNITED STATES OF AMERICA, et al.,	)	
	)	
Defendants.	)	

INTRODUCTION

By Order dated September 21, 1999, the Court required the federal defendants to respond to the Bonnichsen plaintiffs' study request by March 24, 2000, or be deemed to have denied their study request. Order at 7. In setting this date, the Court recognized that the federal defendants would need to complete a cultural affiliation study before responding to the study request. See Order at 6 ("... some time probably will be needed to complete a cultural affiliation study."). The Court also recognized that DNA analysis may well be part of the affiliation study and, in setting the March 24th deadline, sought to provide the federal defendants "the time needed to carry out this testing, if they so choose." Id.

Last week, the Department of the Interior completed its evaluation of the expert report it commissioned to study the efficacy of conducting DNA testing (see discussion below) and has preliminarily decided to conduct DNA testing as part of its cultural affiliation study, subject to consultation with the Tribes. However, as discussed below, the DNA analysis is estimated to take six months to complete once appropriate experts are hired, and thus the analysis cannot be completed by the March 24th deadline. With this motion, the federal defendants seek to modify the Court's Order to allow an additional six months to respond to the plaintiffs' study request if the Department of the Interior decides, upon completion of its consultation process, to conduct the DNA analysis. If a final decision is made not to conduct DNA analysis, the federal defendants would still be bound by the March 24th deadline.

The decision-making process on whether to conduct DNA analysis began shortly after this Court issued its September 21, 1999 Order, when the federal-defendants began the hiring

process for experts to assess the utility of performing DNA analysis on these ancient human remains. Two experts with extensive expertise in analyzing both modern and ancient DNA– Dr. Noreen Tuross, Senior Research Biochemist, Smithsonian Center for Materials Research and Education, and Dr. Connie J. Kolman, National Institutes of Health– were hired to make this assessment and completed their report in January of this year. Declaration of Dr. Francis McManamon at 2 (Exhibit 1[1]) and January 2000 Report on Potential For DNA Testing of the Human Remains from Columbia Park, Kennewick, Washington, (Attachment A). After considering their report, the Department of the Interior has preliminarily decided to proceed with DNA analysis, pending consultation with the tribes as required by 43 C.F.R. 10.5. The Department of the Interior is in the process of setting up consultation with the tribes and anticipates completing consultation and making a final decision within approximately the next two weeks. Declaration of Dr. Francis McManamon at 2.

If the agency, after consultation with the tribes, makes a final decision to obtain DNA samples, an extension of time to complete DNA analysis is necessary.[2] As the independent laboratories hired to perform the radiocarbon dates discovered, testing of these human remains is much more complicated than testing of more modern remains. Dr. Tuross and Dr. Kolman estimate that completing DNA testing may take at least six months because of the low collagen levels found in the radiocarbon samples of the human remains, the difficulty of performing DNA

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[1] Due to time constraints, a facsimile copy of Dr. McManamon's declaration is being filed at this time. As soon as the original is received, it will be filed with the Court.

[2] As mentioned above, if the federal defendants do not undertake DNA analysis, they would still be bound by the Court's March 24, 2000 deadline. Declaration of Dr. Francis McManamon at 5.

testing on such ancient remains, and the great risk of contamination with contemporary DNA. DNA Report at 3-5, 22-27. Accordingly, the federal defendants request that the court modify its September 21, 1999 Order to grant an extension of time of six months from the time of the court's ruling on this motion to complete the DNA testing and make the final agency determination. The federal defendants are seeking this modification at this time to assist in the consultation and final deliberative process on DNA testing, since the current schedule does not provide sufficient time to initiate and complete the DNA analysis.

ARGUMENT

Since the Court's September 21, 1999 Order requiring the completion of the administrative process by March 24, 2000, the federal defendants have been working simultaneously on the several complicated and time-consuming determinations that must be made in order to meet the Court's deadline: (1) the completion and interpretation of the radiocarbon dating; (2) the determination whether the human remains are Native American; (3) the development of the cultural affiliation study protocols, identification of experts available to perform those studies, and review of the draft studies; and (4) the decision whether to undertake DNA analysis. See Federal Defendants' Tenth Status Report, filed January 3, 2000. The Department of the Interior received the draft cultural affiliation reports on January 15, 2000 and is in the process of commenting on those reports, and will send them out to the five claimant tribes to aid in consultation with the tribes. Declaration of Dr. Francis McManamon at 4.

In order to make a reasoned decision on the utility of performing DNA analysis on these human remains, the Department of the Interior contacted several DNA experts, including Dr.

Tuross and Dr. Kolman, in early October 1999. The first draft of the scope of work detailing the questions to be answered was prepared on or about October 4, 1999 and the final draft was completed on or about October 17, 1999. Declaration of Dr. Francis McManamon at 4-5. In the scope of work, the experts were asked to evaluate a broad range of issues including: (1) the likely extent of preservation of ancient DNA in these remains; (2) how knowing the DNA composition of the Kennewick remains will help in determining cultural affiliation as defined by NAGPRA; and (3) if DNA testing is conducted, how it should be conducted. Declaration of Dr. Francis McManamon at 3. The process of hiring Dr. Tuross and Dr. Kolman was initiated in October 1999; their draft report was submitted to the agency on December 15, 1999; the Department of the Interior provided comments on that draft on December 20, 1999; and the final was received on January 4, 2000. Declaration of Dr. Francis McManamon at 5. Since that time, the Department of the Interior has distributed and evaluated the report internally. After a series of meetings, the first of which was held on January 11, 2000, and internal deliberations concerning DNA testing, the Department of the Interior has preliminarily decided that the agency should proceed with the testing, but is reserving a final decision until after consultation with the tribes, as required by 43 C.F.R. 10.5. Declaration of Dr. Francis McManamon at 5. The

NA results are biological information that will aid in making the cultural affiliation determination.[3] 25 U.S.C. 3005; 43 C.F.R. 10.2(e). Declaration of Dr. Francis McManamon at 3.

DNA testing is not a simple process. As the court is aware from the difficulties in the radiocarbon testing, and as Dr. Tuross and Dr. Kolman state in their report, the age of these human remains, the low collagen level of the samples taken for the radiocarbon date testing, and the risk of contamination with modern DNA greatly complicate the already complex testing process. DNA Report at 3-4. To ensure the accuracy of the testing results, the experts recommend that "two independent laboratories be retained for these painstaking analyses," that a research plan be designed for the analysis to ensure that the data is not contaminated by modern DNA, and that these complex studies will take at least six months. DNA Report at 3-5, 22-27.

Indeed, while the metacarpal submitted to U.C. Davis appears to have sufficient collagen

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[3]NAGPRA and the Department of the Interior's implementing regulations provide general guidance as to the types of evidence the agency should consider in making a cultural affiliation determination. 25 U.S.C. § 3005; 43 C.F.R. 10.2 (e). Both the statute and the regulations provide only general areas of information that should be explored; the particular studies to be done are left to the agency's discretion. Where Congress does not expressly state how an agency shall carry out its statutory responsibilities, it is left to the discretion of the agency and a court should defer to the agency expertise on questions of methodology unless the agency's methodology is arbitrary and capricious. Inland Empire Public Lands Council v. U.S. Forest Service, 88 F.3d 754, 760 (9th Cir. 1996). Further, where an issue "requires a high level of technical expertise, [the court] must defer to the informed discretion of responsible federal agencies." Marsh v. Oregon Natural Resources Council, 490 U.S. 360, 377 (1989). Courts should exercise considerable deference to an "agency's technical expertise and experience," particularly with respect to questions involving " 'engineering and scientific' considerations," FPC v. Florida Power & Light Co., 404 U.S. 453, 463, (1972). Further, "[w]hen specialists express conflicting views, an agency must have discretion to rely on the reasonable opinions of its own qualified experts even if, as an original matter, a court might find contrary views more persuasive." Price Rd. Neighborhood Ass'n., Inc. v. U.S. Dept. of Transportation, 113 F.3d 1505, 1511 (9th Cir. 1997).

to perform DNA analysis if the agency goes forward with DNA testing, the agency will have to collect at least one additional sample from the human remains with sufficient collagen to successfully test. An additional step in this process is to run tests to determine whether any of the human remains have sufficient collagen and, if so, the agency must return to the human remains to take samples for DNA analysis. Once the proper samples are identified and taken, the DNA extraction, amplification, and analysis will take several months. Declaration of Dr. Francis McManamon at 3-4. Careful testing is time-consuming, which in this situation means at least six months. DNA Report at 23- 27. Therefore, the federal defendants request an extension of time of six months from the date of the court's ruling on this motion to complete the DNA analysis and make a final agency determination.

This delay will not overly prejudice the plaintiffs. Both Bonnichsen and Asatru plaintiffs have urged the agency to perform precisely this test. The Bonnichsen plaintiffs have submitted affidavits to the Department of the Interior explaining why they too believe that DNA analysis is important and underscoring some of the difficulties of undertaking such analysis. See Affidavit of Dr. Theodore G. Schurr, Post-Doctoral Scientist, Department of Genetics at the Southwest Foundation for Biomedical Research, January 21, 2000 (Exhibit 2). Indeed, in Dr. Schurr's affidavit, he emphasizes that "analyzing ancient DNA is more complicated than analyzing modern DNA" because it is "usually degraded by normal processes. . . ." and that "extraction and PCR amplification (replication) of these fragments can be difficult." Schurr Affidavit at p.8. Dr. Schurr also emphasizes that "to ensure the reliability of the data obtained, samples from the skeleton should be tested by at least two different laboratories . . . ." Id. Asatru plaintiffs also

requested that DNA analysis be performed. See Asatru Folk Assembly Request For Mitochondrial DNA Analysis, filed September 2, 1999. The Asatru's expert, Dr. Michael D. Brown, Assistant Professor, Emory University, also notes the likelihood of degradation of the DNA, risk of contamination by modern DNA, and the need for careful study by two laboratories. See Asatru Folk Assembly Motion to Conduct Mitochondrial DNA Testing, filed September 2, 1999, at Brown Affidavit at p.8-9.

In addition, the federal defendants would use the extension of time for additional consultation on the issues surrounding cultural affiliation with the tribes. See 43 C.F.R. 10.5. Although the Department of the Interior and the U.S. Army Corps of Engineers held consultation discussions on the cultural affiliation process with the tribes on October 14, 1999 and the Department of the Interior has received the draft reports on cultural affiliation and is in the process of distributing them to the tribes, the additional time to perform DNA analysis would give the agency time to do more detailed consultation with the tribes about the cultural affiliation reports and to again seek whatever information the tribes may have to support their claims. Declaration of Dr. Francis McManamon at 4.

CONCLUSION

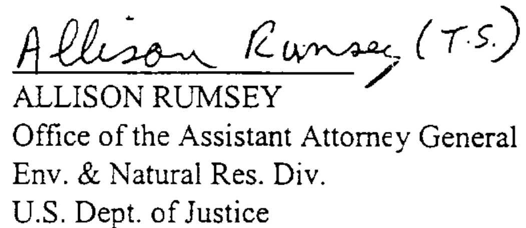
At this time the Department of the Interior has preliminarily decided to attempt DNA analysis and will make its final determination after consultation with the tribes within the next two weeks. The federal defendants respectfully request that the court modify its September 21, 1999 Order to grant the federal defendants an extension of time of six months from the date of the court's ruling on this motion so that the Department of the Interior can complete the DNA

analysis and make a final agency determination. Such an extension would permit the agency to undertake the painstaking DNA analysis in a manner that is most likely to yield meaningful results. Under this proposed modification of the order, the federal defendants would still be bound by the Court's deadline of March 24, 2000, if they decide not to conduct the DNA analysis.

Dated this 1st day of February, 2000.

Respectfully submitted,

LOIS J. SCHIFFER
Assistant Attorney General



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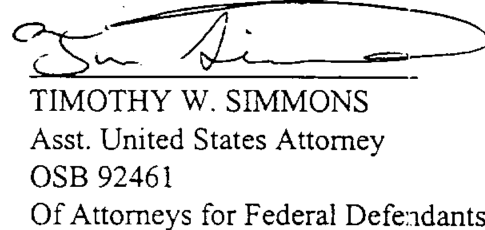

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IN THE UNITED STATES DISTRICT COURT

FOR THE DISTRICT OF OREGON

ROBSON BONNICHSEN, et al., Plaintiffs, v. UNITED STATES OF AMERICA, et al., Defendants.	**DECLARATION OF FRANCIS P. McMANAMON, Ph.D.** Civil No. 96-1481 JE

I, Francis P. McManamon, declare as follows:

1. I am Chief Archeologist of the National Park Service and the Departmental Consulting Archeologist for the U.S. Department of the Interior ("DOI"). My

duties and responsibilities in this capacity are as set forth in my original declaration, which was filed with this Court on May 28, 1998. This declaration is being submitted in support of this filing in the above-styled matter. All information herein is based upon my personal knowledge and upon information furnished to me in my official capacity.

2. Pending consultation with the claimant Indian tribes, the Department of the Interior (DOI) has made a preliminary decision to undertake extraction and, if the extraction is successful, analysis of DNA from the Kennewick remains. We anticipate completing consultation and making a final determination on undertaking DNA analysis in approximately two weeks. This information will be used as part of our determination of whether or not a cultural affiliation, as defined by the Native American Graves Protection and Repatriation Act (NAGPRA), can be determined for these remains. In reaching our preliminary decision in this matter, an important consideration was the information provided in the report, "Potential for DNA Testing of the Human Remains from the Columbia Park, Kennewick, Washington," by two DNA experts, Dr. Noreen Tuross, Senior Biochemist, Smithsonian Center for Materials Research and Education, and Dr. Connie J. Kolman, National Research Council Senior Research Associate, National Institutes of Health. A copy of this report is provided to the court as part of this filing (Attachment A).

3. The report covers three topics: (1) the likely extent of preservation of ancient DNA in these remains; (2) how knowing the DNA composition of the Kennewick remains will help in determining cultural affiliation, as defined by NAGPRA, and (3) if DNA testing is conducted, how it should be conducted.

4. DNA analysis may help in our attempt to determine cultural affiliation. DNA analysis, if a successful extraction and amplification are possible, will provide biological information about the genetic heritage of these remains. NAGPRA identifies biological information as one type of evidence that may establish cultural affiliation. DNA analysis, while relatively new in anthropological, archeological, and genetic investigations is a technique increasingly used for cultural, historical, and biological research.

5. As advised by our experts, we require at least a six month period of time in order to conduct the DNA extraction, amplification, and analysis in a rigorous, systematic manner. Our experts have recommended strongly taking two separate samples and using two independent laboratories for the analysis. Once the proper samples are identified and taken, extraction, amplification, and analysis will require several months. To be done properly and successfully, we must utilize a bone sample with sufficient remaining bone collagen. Our experience with the bone

samples taken for the C14 analysis indicated that the Kennewick remains contain substantial variation in remaining intact bone collagen. Therefore, as an initial step, we propose to sample the skeleton extensively. If we proceed, we will use very small amounts of material for collagen/carbon preservation and select additional new samples for DNA investigation.

6. If the requested time is allowed, in addition to undertaking the DNA sampling, extraction, amplification, and analysis, we would use this time to conduct additional consultation with the claimant Indian tribes. A meeting with the tribes was previously held in November, 1999, at the early stages of our cultural affiliation inquiry. Further consultations would be held to consider the various kinds of cultural affiliation information we have investigated: archeological, linguistic, mortuary, and traditional historical. DOI received draft reports covering these cultural affiliation inquiries on or about January 15, 2000.

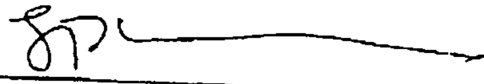
7. DOI's consideration of investigating the ancient DNA of the Kennewick remains has been the subject of extensive internal debate and has strived to consider all of the various perspectives. To meet the Court's deadline, DOI has moved quickly to reach a decision about how to proceed with the investigation of the ancient DNA from the Kennewick remains. In early October, 1999, DOI and the Department of

Justice contacted Drs. Tuross and Kolman. On or about October 4, 1999, a draft scope of work for an expert report on the usefulness of DNA testing was prepared. On or about October 17, the scope of work was finalized. On 15 December 1999, DOI received a first draft DNA report from Drs. Tuross and Kolman. My staff and I reviewed this preliminary draft and returned comments on 20 December to the experts for redrafting. As part of our first comments, information about the low carbon and poor collagen preservation in the bone samples being carbon-dated also was provided for the DNA experts so they could take this information into account in their redraft. A final draft report was received on 4 January 2000, and distributed to DOI officials working on the Kennewick project. We met at the staff level on 11 January, discussed the issues and possible next steps. A meeting was scheduled at the earliest opportunity that all necessary senior DOI officials were available and in the country, and held on 21 January to further discuss the issue and possible alternatives. Following that meeting, and after additional discussions among senior officials, a preliminary decision was made to proceed with DNA testing, pending consultation with the tribes.

8. If DOI does not undertake DNA analysis, the agency is on schedule to meet the court's March 24, 2000 deadline.

I declare under penalty of perjury that the foregoing is true and correct.

Executed the 31 Day of January, 2000.



Francis P. McManamon, Ph.D.

Potential for DNA Testing of the Human Remains from Columbia Park, Kennewick, Washington

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Report to the Department of Justice and Department of Interior
January, 2000

Introduction

At the request of the Department of Justice and Dr. Francis P. McManamon, Departmental Consulting Archaeologist of the National Park Service, Department of the Interior, we supply this discussion of the potential for DNA analysis of the human skeletal remains from Kennewick, Washington that are the objects of the lawsuit now pending (Bonnichsen et al., vs. United States of America, Civil No. 9601481-JE). The purpose of such an analysis would be to determine the genetic affinity of the above individual by isolating DNA from bone, and comparing any data generated with the known range and variation in human mitochondrial[1] DNA.

Summary

The following is a synopsis of the potential results of a mitochondrial DNA analysis of a human skeleton found at Columbia Park in Kennewick, Washington. The possible results are listed in the order that we deem most likely based on our own experience and the data available in the literature. In the text of this document, we have detailed both the reasoning and the published support for these positions. We would emphasize that DNA testing of skeletalized human remains at this time depth is <u>not</u> a routine matter. To our knowledge, DNA data has never been used in whole or in part by the courts or as part of a NAGPRA (Native American Grave Protection Act) request to resolve the identification of an individual skeleton as either as American Indian or as a tribal member.

[1] Mitochondria are cellular components that contain a separate and distinct type of DNA compared with the nucleus. Mitochondrial DNA (mtDNA) is thought to be exclusively maternally inherited and clonal in nature.

Should DNA analysis of the skeleton in question be undertaken, the following are the likely outcomes:

1. No DNA of suitable size or integrity remains in the bone.

This result is likely and is based on three considerations: A) few skeletons of significant age (>1,000 years) have yielded DNA that could be enzymatically amplified and DNA sequenced, B) the skeleton in question was found in a riverbed after eroding out of the bank, and C) the bone organic content is very low relative to modern bone. While it is always possible to invoke the adage, " you won't know until you try," three indicators of DNA preservation are missing in the human skeletal remains in question: recent antiquity, burial in a stable environment, and, most importantly, significant organic content remaining in the bone.

2. A DNA type indicative of an American Indian will be identified.

Mitochondrial DNA of American Indians who self-report only American Indian ancestors, usually fall into one of four groups that are defined by specific changes in the sequence of the DNA relative to a reference sample. In addition to American Indian, these four groupings, called A, B, C and D haplogroups, are all found in Asia. These same genetic groups are absent in individuals of European or African decent who are lacking Asian admixture. A fifth haplogroup, X, has a much lower representation among American Indians, is incompletely characterized, and has a potentially broader distribution worldwide.

3. A DNA type of ambiguous origin will be found.

A mitochondrial DNA type that does not belong to the A, B, C or D groups could be

more difficult to interpret as unambiguously American Indian. The finding of an X type would engender discussion as to the natal origins of this group, as an Asian source population has not yet been identified. There are other possibilities as well. A previously unidentified genetic type might be found, and the assignment of continental origins might not be possible. It is important to note that the finding of a novel mitochondrial type does not necessarily rule out contemporary American Indians as possible direct descendents of this individual. Mitochondrial types can be lost, especially if they are in low frequency in a population.

4. Contaminating DNA from contemporary sources will prevent an ancient DNA analysis.

In spite of all best efforts, the skeleton may have become so contaminated with contemporary DNA that all amplification reactions produce a DNA sequence that does not belong the to skeletalized individual. This scenario is particularly likely when the amount of ancient DNA is low and/or damaged, and when the skeleton has been handled during excavation. While this outcome may be difficult to distinguish from a DNA of ambiguous origin, there are some results that can readily be identified as contemporary human contamination.

None of the outcomes described above will unambiguously allow the remains of the skeleton in question to be assigned to a given tribal authority. Mitochondrial DNA analyses of known American Indian genetic groupings will not resolve questions of <u>cultural</u> affiliation at the tribal or regional level. It is possible that mitochondrial DNA analyses of the skeleton will allow assignment of the skeleton to the <u>biological</u> grouping of American Indian.

It is our considered opinion that the risk of obtaining no DNA from the bone or a result that is complicated by either contamination or ambiguous data is so high that, should DNA analyses be desired, two independent laboratories should be retained for these painstaking analyses.

Ancient DNA Background and a Consideration of Finding No DNA in a Subfossil Bone

Standard molecular genetic analyses of humans and other organisms utilize a technological innovation that was developed in the mid-1980s (Mullis and Faloona 1987). The polymerase chain reaction, or PCR (see Figure 1), enables the specific targeting and amplification of a discrete region of the genome while the remaining bulk of the genomic DNA is excluded from the reaction and effectively stays in the background. Multiple cycles of amplification result in the exponential production of PCR product since product from each cycle serves as template for additional product in each subsequent cycle. In a typical 30-cycle reaction, one billion copies are made of a single initial DNA template.

The exponential amplification of a specific region of DNA from only a few molecules has permitted the investigation of "ancient" DNA samples that are too degraded or damaged for analysis by traditional cloning methods, which require a much higher quantity and quality of DNA. "Ancient" samples are generally those that were not collected for the purpose of immediate DNA or RNA analysis and include archaeological, clinical, and natural history specimens. Since these specimens were not originally collected or preserved for nucleic acid analysis, endogenous DNA is typically damaged to an extent that enzymatic amplification can be quite difficult, if not impossible, to achieve. The types of DNA damage that are primarily encountered include modifications of pyrimidines and sugar residues as well as baseless sites and intermolecular crosslinks (Pääbo 1989). Only limited research has been conducted on the chemistry of ancient (aDNA) damage and possible methods of *in vitro* repair. Various

protocols have been developed to determine the utility of specific ancient specimens for aDNA analysis (Handt *et al.*, 1994, 1996, Richards *et al.*, 1995, Poinar *et al.*, 1996) and these methods have recently been evaluated in a comparative study (Kolman and Tuross, in press).

Although the study of variability in the human genome has long been an area of research, both the ability to retrieve DNA from human skeletal remains and a substantial database with which to compare the results are fairly new scientific developments. The first Ancient DNA meeting was held in Nottingham, England in 1991. Since that time, the development in the field has been slow, and not without controversy. Early, spectacular claims of successful DNA extraction and amplification from extremely old specimens, such as 17-20 million year old (Myr old) *Magnolia* leaf fossils (Golenberg *et al.*, 1990), 25-135 Myr old specimens preserved in amber (DeSalle *et al.*, 1992, Cano *et al.*, 1993), and 80 Myr old dinosaur bones (Woodward *et al.*, 1994), generally have been disproved or cast into serious doubt (Sidow *et al.*, 1991, DeSalle *et al.*, 1993, Hedges and Schweitzer 1995, Austin *et al.*, 1997, Walden and Robertson, 1997, Austin *et al.*, 1998). Later authors, using relatively simple methods, were able to detect contamination in the early studies, such as Hedges and Schweitzer's (1995) phylogenetic analysis of proposed dinosaur DNA that identified it as modern human contamination.

There are relatively few published ancient DNA studies of bone of an age that approaches or exceeds the 9000 years reported (Taylor *et al.*, 1998) for the human skeleton in this case. Horse fossil and subfossil bone were subjected to PCR amplification, and from a total of fifty-two specimens, two bones yielded DNA data: one from Kent's Cavern in Britain (approximately 12,000 BP), and another about 100 years

old (Lister *et al.*, 1998). When nine ancient cattle bones samples were subjected to PCR of a coding region of mitochondrial DNA, no specimen more than 2,000 years old was successfully amplified (Turner *et al.*, 1998). The bones of Pleistocene megafauna have produced DNA that was successfully PCR amplified (Yang *et al.*, 1996; Greenwood *et al.*, 1999; Hanni *et al.*, 1994) from a ground sloth, a cave bear, two mastodons and three mammoths. The later study (Greenwood *et al.*, 1999) reported the retrieval of multi- and single copy nuclear genes, opening up the possibility that permafrost stored fossils may be a good source of a wider range of ancient DNA data.

In the Americas, no ancient DNA study of human skeletal remains comes close to the temporal range ascribed to the skeleton in question. The only large genetic study of ancient American Indians (n=108) was applied to a population that lived approximately 700 years ago (Stone and Stoneking, 1998). Seventy percent of the bone samples produced mtDNA results in this study (Stone and Stoneking, 1999) and mitochondrial haplogroups that are found in contemporary American Indians make up 95% of the ancient genetic types (Stone and Stoneking, 1998). The other genetic types found associated with the human skeletal remains in this study were ascribed to contemporary human DNA contamination or were of ambiguous origin (Stone and Stoneking, 1998), although further analysis of a fifth haplogroup found at Norris Farm associates with Mongolian sequences (Stone and Stoneking, 1999). In general, when high-resolution genetic analyses, such as the sequencing of multiple PCR clones, is used (Handt *et al.*, 1996; Kolman and Tuross, in press), contamination from contemporary human DNA seems to be a persistent albeit not always fatal problem. Specifically, the high-resolution analyses revealed the presence of multiple DNA sequences in several specimens

implying multiple sources of DNA, only one of which could represent ancient, endogenous DNA. The difficulty lies in determining which sequence, if any, is derived from the ancient specimen and is not modern contamination. Where only lower resolution data is available (Parr *et al.*, 1996; Fox, 1996; Merriwether *et al.*, 1997; Kaestle, 1997) it is difficult, and at times impossible, to determine the impact of contemporary human DNA contamination in the form of airborne products, handling or PCR products. In other parts of the world, a robust analysis of a one Neanderthal skeleton has been reported (with accompanying contemporary contamination) (Krings *et al.*, 1997). In this case, the Neanderthal sequence could be discriminated from the contamination because the ancient sequence was completely novel and highly divergent from all previously reported modern human sequences.

Recent information regarding the general organic preservation of the skeleton in question (see pg. 23 of this report) further strengthens the possibility that no DNA remains in a state that is useful for genetic analysis.

Defining a Choice of Genetic Markers With an Emphasis on the New World

Human beings carry two types of DNA, mitochondrial and nuclear, both of which are suitable for genetic analysis. Traditionally, mitochondrial DNA (mtDNA) has been studied much more extensively although the analysis of nuclear markers has become increasingly common in recent years. There are several advantages to the analysis of mtDNA that account for its early popularity in genetic and evolutionary studies. The mitochondrial genome has a higher mutation rate compared to the nuclear genome (although specific loci exist within both genomes that provide exceptions to this statement) such that mutations are generated sufficiently rapidly in the mitochondrial

genome that the process of evolution can be detected and investigated. Furthermore, the region of the mitochondrial genome involved in replication of the genome, the control region, appears to have a mutation rate that is approximately ten times the rate of the mitochondrial genome as a whole. For this reason, many researchers have focused on the control region for evolutionary or population studies. Thousands of mitochondria are present in each cell meaning that thousands of copies of mtDNA are present in contrast to a single copy of each nuclear genome per cell. Due to the high copy number of mitochondrial genomes, mtDNA is relatively easy to isolate in the laboratory although technological advances over the past decade have minimized this technical difference between mitochondrial and nuclear DNA. Finally, mtDNA is characterized by strict maternal inheritance (offspring receive mtDNA only from their mother) and lack of recombination between different regions of the genome in contrast to nuclear DNA, which is biparentally inherited and subject to extensive recombination. The significance of the mitochondrion's simpler mode of transmission from parent to offspring is the ease with which any particular region of the mitochondrial genome can be traced through time and through the maternal lineage. Resent reports suggesting the contribution of parental mitochondria or nuclear copies of mtDNA to mtDNA (e.g., Awadalla *et al.*, 1999; Hagelberg *et al.*, 1999) are unlikely to obscure the utility of markers discussed in this report. It can, therefore, be a straightforward matter to reconstruct evolutionary relationships of populations or individuals through an analysis of mtDNA.

When choosing a particular region of the genome, or locus, to investigate in a genetic study, there are two criteria that must be met. First, the locus must have a level of variability among the individuals or populations being studied such that the individuals or

populations can be differentiated from one another. Second, a comparative database for relevant populations on the same genetic region must be available in order to determine the relationship or identity of the individual or population under study with respect to other populations.

Currently, all genetic analyses are performed using amplification products derived from the polymerase chain reaction as described above. In order to identify regions with the best levels of variability, researchers are constantly testing new regions and assaying their variability in different populations. This means that the comparative database is spread out over many different genetic loci and populations making comparison between specific loci and particular populations difficult. However, due to the long-term focus on the mitochondrial genome for genetic studies, the comparative database for mitochondrial loci is much more extensive than that for nuclear loci. Furthermore, in human populations, DNA sequence determination of the control region is the most frequently generated type of mitochondrial data. Analysis of restriction fragment length polymorphisms (RFLPs), in which the PCR product is cleaved by a restriction enzyme that recognizes a particular DNA sequence, or analysis of regions with large deletions or insertions also is commonly conducted.

Figure 1 illustrates the three types of genetic markers that typically are assayed in New World indigenous populations. After PCR amplification of the region of interest, the PCR product can be analyzed in a number of ways. First, the order of nucleotides, or DNA sequence, of the PCR product, usually the control region, can be determined compared. Second, a marker commonly called the 9bp deletion (located between base pairs [bps] 8272 and 8289 [numbering according to Anderson *et al.*, 1981]) can be

identified. The 9bp deletion represents a region where a stretch of 9bps has been deleted relative to a reference sequence. Based on the size difference between the deleted and non-deleted alleles that is observed by electrophoresis of the PCR product through an agarose gel matrix, it can be determined which allele a particular individual carries. Third, there are certain RFLPs that are highly informative in New World indigenous population that are also assayed based on a size difference between alleles. Any difference between individuals that is detected in the above analyses can be referred to as a marker, a polymorphism, or an allele and the combination of these markers in one individual is referred to as a haplotype.

New World indigenous groups were first assayed for mtDNA RFLPs and the 9bp deletion by Douglas C. Wallace and coworkers in the 1980s. These investigators used a phylogenetic analysis, which is similar to drawing a family tree, to define four clusters of haplotypes, or haplogroups, that were present at varying frequencies in populations distributed throughout the New World (Torroni *et al.*, 1992). Each haplogroup was defined by a single RFLP or deletion and the four clusters were called haplogroups A, B, C, and D. Briefly, a *Hae*III site at bp 663 defined haplogroup A; the 9bp deletion defined haplogroup B; an *Alu*I site at bp 13262 defined haplogroup C; and, loss of an *Alu*I site at bp 5176 defined haplogroup D. These haplogroups were proposed to represent the entire mitochondrial diversity of New World indigenous populations and also to correspond to the founding haplotypes present at the initial colonization of the New World. These haplogroups have now also been defined by specific polymorphisms in the mitochondrial control region (Horai *et al.*, 1993). All diagnostic sites are listed in Table 1.

With the advent of PCR technology in the field of aDNA, the analysis of these markers in ancient individuals or populations would seem obvious though perhaps not as straightforward as originally thought. The RFLPs and deletion defined by Wallace and coworkers represent only a subset of all mitochondrial polymorphisms currently assayed in contemporary New World indigenous populations. Furthermore, contemporary New World populations carry only a fraction of the mitochondrial variation present worldwide. Since modern populations may carry less genetic variation than their ancestors or may be more distantly related to ancient populations than is currently recognized, it is a dangerous strategy to assay prehistoric populations for a restricted set of markers that have been culled from contemporary populations. To assay ancient specimens for only a few diagnostic markers with the justification of damaged aDNA and commensurate increase in time required for aDNA analyses is to invite incorrect haplogroup assignments. In other words, more markers, rather than fewer, should be assayed in ancient specimens relative to modern ones in order to increase the probability of an accurate classification of the ancient specimen.

In order to obtain maximal information and comparability of their data, most researchers assay both control region DNA sequence and RFLP/deletion markers in New World populations, both contemporary and ancient (eg. Ward *et al.*, 1991, Stone and Stoneking 1998, Kolman and Tuross, in press). The assignation of haplogroup using both control region sequence data and RFLP/deletion data provides a quality control check for the accuracy of the data, which is a necessary safeguard in an aDNA study. Furthermore, the existence of databases with only RFLP/deletion data or only control region sequence data means that the comparability of one's data is doubled if both types of loci are

analyzed. Comparability of data is essential if the goal of the study is to determine relatedness or identity of an individual since it is only through comparison with other populations that an identity classification can be made.

Genetic classification of a single archaeological specimen and a consideration of ambiguous genetic results

In the absence of accompanying cultural artifacts, a single, isolated skeleton can often be classified with respect to other human populations using genetic data with the caveat that detailed classifications are more difficult to resolve than more general ones. Classification of an individual as being more closely affiliated with one population than another is based on a measure of distance of some character between the ancient individual and comparable populations. Physical morphological characters, such as cranial measurements or dental characteristics, can be used although these data may be valid only for divergence times of several thousand years as it appears that there is more plasticity in osteological characters than was previously believed, particularly in the New World (Powell 1998). On the other hand, current data suggest that there is very little genetic change measured by mitochondrial DNA over time throughout New World indigenous populations and substantial continuity between ancient and contemporary populations.

As described above, mitochondrial control region DNA sequence data or RFLP data are most commonly used in human evolutionary or population genetic studies. Table 2 provides a summary of data available on contemporary human populations distributed worldwide that have been published in peer-reviewed scientific journals. For the purposes of the question currently being considered, i.e. the genetic classification of

the skeleton found in Washington State, only a representative listing of studies on African and European populations are presented in Table 2. Because of the increased relevance of populations geographically close to the discovery site, all DNA-based studies on Native American populations and ancestral Asian populations are listed. Asian populations are considered ancestral to Native Americans because it is generally accepted by the scientific community that the New World was colonized by ancient Asian population(s) crossing over the Bering land bridge that was exposed during the last Ice Age. Finally, all aDNA studies on human populations also are listed with unreviewed publications included, but listed separately. Only populations with sample sizes greater than 20 were included in the table, with the exception of aDNA studies. Most aDNA studies have smaller sample sizes relative to studies of contemporary populations because many excavated ancient burial-populations are fewer than 20 individuals and may be only a single individual, as in the Kennewick case, and due to the increased difficulty of analyzing ancient specimens. Twenty individuals are generally considered to be the minimal size of a population to be used in a comparative analysis. The type of data generated, RFLP or DNA sequence, in each study is presented. Also, the frequency of New World founding haplogroups A, B, C, and D determined for each population is listed, with all non-A, B, C, D haplotypes pooled together under "Other".

As can be seen in Table 2, the four New World haplogroups are found only in American Indian populations and ancestral Asian populations. Therefore, a distinction between American Indian ancestry and African or European ancestry easily can be made based on the presence or absence of a New World founding haplogroup in the individual under study. This conclusion assumes that contemporary populations accurately reflect

the genetic make-up of their ancestors and that no distinct haplotypes have been lost over time (this point will be discussed more fully below). It is equally clear from Table 2 that contemporary American Indian populations look quite similar to one another from a mitochondrial perspective. All four haplogroups are found in populations distributed throughout the New World with no haplogroup unique to any population, geographic region, or linguistic classification (New World indigenous populations have been divided into three linguistic families, Esk-Aleut, Na-Dene, and Amerind [Greenberg *et al.*, 1986]). Furthermore, New World ancient populations also appear similar to contemporary American Indian populations in that the four haplogroups are found throughout the studied ancient populations and throughout the New World. In general, non-A, B, C, D haplogroups make only a minor contribution to the genetic diversity of ancient New World populations, a result that is mirrored in contemporary populations. Note that 8.8% of haplotypes are listed as "Other" in all ancient New World studies relative to 4.5% "Other" haplotypes in all contemporary New World studies listed in Table 2. However, some of the "Other" haplotypes in aDNA studies are likely to be due to modern DNA contamination thus lowering the number of truly ancient "Other" haplotypes. A comparable continent-wide distribution and high frequency of the four founding haplogroups in ancient and contemporary New World populations suggest that descendant populations accurately represent ancestral populations. This conclusion implies that no haplotypes have become extinct during the human settlement of the New World and that the four haplogroups represent all founding lineages, although a very low frequency founding haplotype in an ancient population could still be missed given the small sampling of ancient populations at present. Asian populations show higher levels

of mitochondrial diversity and more non-A, B, C, D haplotypes. Siberian populations are characterized by a lack of haplogroup B and Southeast Asian populations are characterized by presence of only haplogroup B of the four New World haplogroups. In Asia, all four haplogroups are found only in east central Asian populations. This non-random distribution of the New World haplogroups outside of the New World has been used to support the argument that colonizing populations originated in the greater Mongolia region (Kolman *et al.*, 1996). This interpretation is consistent with the Asian ancestry of American Indians that had been proposed prior to molecular analyses.

Therefore, determination of a haplotype A, B, C, D in a skeletal specimen would strongly suggest American Indian ancestry. However, because of the ubiquity of haplogroups A, B, C, D throughout the New World, a more detailed classification of a single A, B, C, D haplotype to a particular American Indian population or tribal group would be virtually impossible based on a visual inspection of the data. Therefore, mtDNA sequence and RFLP/deletion data such as those presented in Table 2 typically are analyzed using phylogenetic algorithms to determine accurate genetic relationships among the haplotypes.

Phylogenetic analysis of genetic data is a means of determining the most accurate evolutionary relationship of individual haplotypes. The result is generally displayed as a tree, similar to a family tree, with an ancestral root haplotype denoted and branches of related haplotypes referred to as clades. There are basically two types of mathematical models used to derive phylogenetic trees. Cladistic approaches attempt to determine the shortest, most parsimonious, tree needed to accurately represent all of the characters that have been assayed. Phenetic approaches are based on a numerical genetic distance

measured between assayed characters and is reflected in the branch lengths of the tree. Both approaches have advantages and disadvantages depending on the type of data analyzed and its mutation rate, the divergence time of the individuals or populations being studied, and other factors. Due to the difficulty in identifying a superior phylogenetic model for any particular dataset, multiple models and algorithms are typically used so that similarities between approaches are given greater weight relative to relationships that are detected using only a single model. However, all of the models depend on the strength of the signal being greater than any "noise." "Noise" is considered random mutational events or multiple mutations at identical sites that either do not reflect evolutionary history or violate assumptions implicit in the phylogenetic models. In other words, all phylogenetic methods assume that tracking DNA mutations through a given data set will reveal the evolutionary history of the populations being studied and any mutations that violate this assumption will confuse the outcome and compromise the integrity of the phylogenetic analysis. Moreover, any analysis is only as good as the input data. Although a phylogenetic analysis is quite useful for determining the affinity of one population to another, the classification of a single individual or single haplotype as belonging to one particular group, such as a specific Native American tribe, is most likely beyond the power of phylogenetic analysis and, indeed, any analysis. The exception is an individual and comparable population that are so uniquely similar that their relatedness is obvious, in which case no sophisticated analysis would be necessary for proof of the relationship.

Contamination of ancient human specimens with modern DNA

As explained above, PCR enables the specific, exponential amplification of a discrete region of the genome. This ability has permitted the investigation of DNA samples from ancient specimens that typically are much more degraded or damaged than DNA samples from fresh or modern samples. However, the damage to aDNA increases the potential for another characteristic of PCR, that of contamination to intrude into the analysis. Since PCR analysis involves the exponential generation of new, synthetic DNA products from a small number of molecules, contamination with exogenous DNA in one of the initial PCR cycles can result in exclusive amplification of the contaminating DNA. This possibility is increased in aDNA analysis where the contaminant is likely to be undamaged DNA which will be amplified preferentially over the damaged, endogenous DNA. The growing number of aDNA studies published and number of samples and polymorphic sites assayed may give the impression that all technological hurdles associated with aDNA technology have been overcome. However, identification of contamination remains the single most critical issue in aDNA methodology. Standard precautionary measures such as negative extraction and PCR controls, multiple extractions, and "clean" rooms, while necessary, have been proven insufficient to identify complex co-occurrence of endogenous ancient DNA and modern contamination in human skeletal remains (Kolman and Tuross, in press).

The determination of DNA sequence from an ancient human source is uniquely sensitive to contamination simply because every person involved in the study represents a potential source of contaminating DNA. Even ancient pathogenic DNA associated with human skeletons may be analyzed with more straightforward controls on possible

contamination (Kolman *et al.*, 1999). Numerous cases exist in the published literature that indicates researchers have encountered contamination of human remains with modern DNA although many laboratories are reluctant to report examples of contamination. Recent analysis of DNA extracted from the Neanderthal type specimen (Krings *et al.*, 1997) revealed two distinct sets of mitochondrial D-loop sequences, one significantly different from modern humans and proposed to be Neanderthal in origin and one identical to the human reference sequence (Anderson *et al.*, 1981) and presumed to reflect modern human contamination. A second example of contamination is provided by Kaestle (1999) who identified one sample in a collection of western Nevada skeletons as belonging to New World haplogroup B (described below) although the sample also exhibited a second diagnostic site for haplogroup C. Conscientiousness and complete disclosure of results make it possible to assess the types and extent of contamination that may be present in the majority of aDNA studies. Reluctance to report evidence of contamination and/or the use of research strategies that are unlikely to detect contamination, e.g. partial typing of samples, should not be interpreted as absence of contamination or as proof of authenticity of the data.

Richards *et al.* (1995) reported that approximately 50% of nonhuman bones excavated from a site in England exhibited contamination with human DNA sequences. Similar contamination should be assumed for all human bones and measures to identify contaminants should be integrated into the research design of all human aDNA studies. Furthermore, aDNA investigators should be aware of their own genetic haplotype at the markers being studied and constantly screen out any identical aDNA haplotypes as potential contaminants. Again, examples exist in the literature of researchers identifying

themselves as sources of contamination in aDNA studies (Stone and Stoneking 1998; Kolman and Tuross, in press). In short, careful selection of polymorphic markers capable of discriminating between ancient DNA and probable modern DNA contaminants is critical. Research strategies must be designed with a goal of identifying all DNA contaminants in order to differentiate convincingly between contamination and endogenous DNA.

Many laboratories routinely include positive PCR controls to evaluate the effectiveness of the amplification reaction. Although this appears to be an obvious control given the high PCR failure rate of many aDNA samples, use of modern, undamaged DNA as a positive control represents the conscious introduction of a potential DNA contaminant. In the event that identical haplotypes are determined for both the ancient specimen and the control DNA sample, it becomes impossible to prove that the data on the ancient sample do not reflect contamination by the control DNA.

If the inclusion of a single modern DNA sample for use as a positive PCR control is to be avoided in aDNA studies, it must be evident that aDNA studies should not be conducted in laboratories where studies on genetically similar, contemporary populations are ongoing. Studies on contemporary populations typically involve the analysis of hundreds or thousands of modern DNA samples. The standard solution is to physically separate the rooms in which experiments on ancient and modern DNA samples are conducted and incorporate the use of air locks, "sticky" floor mats, dedicated lab ware, etc. However, locating an aDNA laboratory outside of the main laboratory that is still utilized by the same researchers is unlikely to eliminate contamination since DNA can adhere to clothing worn by the researcher. Previous work performed by our group on

natural history specimens of fish provides an example of the pervasiveness of contamination; one year after moving all positive control goldfish DNA to another floor and wing of the building, goldfish contamination was still being detected in ancient fish DNA PCRs performed in the aDNA laboratory. The bottom line is, despite a decade of aDNA research, contamination by modern DNA remains a significant problem because the many sources and modes of contamination are still not known or understood and, therefore, can not be controlled or eliminated.

Suggested research plan for analysis of the skeleton discovered at Kennewick, WA

It must be understood from the outset that bone would have to be destroyed in order to proceed with any DNA analysis. The amount of organic matter remaining in the skeleton is quite low based on the available information supplied by the Department of Interior pursuant to ^{14}C (radiocarbon) dating (pers.comm. F. McManamon). In one case, (Beta Analytic), the amount of organic material produced from the bone was approximately 1.6% the theoretical yield of modern bone, 200 milligrams protein/gram of bone (Herring, 1972). A second laboratory at the University of Arizona (UA AMS Facility) has also reported extremely low yields in carbon from the skeleton in question. Finally, it is not clear, based on information provided from the third radiocarbon laboratories (University of California at Riverside) whether the organic material has any of the major protein, collagen, still remaining in the bone. An amino acid analyses of two bone samples (CENWW.97.L.20b/DOI 2b and CENWW.97.R.24 (Mta)/DOI 1b) were reported to contain a "non-collagen amino acid composition." These preliminary results from three separate laboratories are consistent with extensive degradation of the organic matrix in this human skeleton. Furthermore, these data differ significantly from those

reported in the widely circulated letter (Taylor *et al.*, 1998) on the radiocarbon dating of this skeleton in which a "collagen-like pattern similar to that which is typically obtained from a modern bone" was found.

These most recent reports do not portend well for DNA testing of the skeleton from Kennewick, WA. The accumulating information regarding the organic preservation of the skeleton suggests the bone has very little, if any, of its original protein remaining, and by inference, one would assume very little, if any, DNA remains in a form adequate for genetic analysis. This assessment must involve some speculation because the professional literature is largely silent on the issue. However, a general consideration of organic preservation in the skeleton is a necessary part of planning any proposed genetic analysis.

Bone must be destroyed in order to remove any DNA trapped in the mineral matrix. Upon decalcification, DNA is released into solution, and is purified from this solution for further testing. The amount of bone that is processed for DNA analyses varies, and the amount of starting material generally relates inversely to the amount of total organic matter remaining in the bone—the lower the amount of original organic matter, the greater the amount of bone that has to be used. The low amounts of protein that seem to be preserved in this skeleton would lead many analysts to request large samples of bone (on the order of 15-30 grams). The amount of bone requested is based on the assumption that, if DNA still exists in the mineral matrix, many of the molecules will be damaged beyond use for the required testing, and, thus, a larger sample will give the analyst a greater statistical probability of isolating undamaged DNA templates.

The commonly accepted practice for removing DNA from skeletal remains involves dissolving the bone in a calcium-chelating agent. This gentle decalcifying agent will leave any collagen that does exist in the bone in a form that can be used for radiocarbon dating. Unfortunately, due to the damage caused by halide acids in the form of depurination, the soluble preparations from the previously obtained radiocarbon dates will not be useful for genetic analyses.

Should DNA analysis of this ancient skeleton be attempted, an important criterion in designing a research plan for the molecular analysis is to ensure that the resultant data are not due to contaminating, exogenous DNA. The research plan must be designed to be capable of discriminating between endogenous, ancient DNA and exogenous, contaminating DNA. This is accomplished by assaying markers that differentiate between the endogenous DNA and all potential sources of contaminating DNA. With the stated caveat that it may be difficult to ensure a distinction between endogenous DNA and all sources of contamination, the minimal number of markers that should be assayed for a complete genetic characterization of the skeleton in question are those listed in Table 2. In terms of the specifics of the analysis, a minimum of six PCRs would be required to assay these markers one time. Four independent amplification reactions would be required to assay the three RFLPs and 9bp deletion. Two PCRs are advisable for the control region so that it could be amplified in segments no larger than 150-200 bps, a necessary precaution when dealing with damaged, fragmented ancient DNA. These markers should be assayed at least two times, starting each time from a fresh amplification reaction. The ideal situation would be to generate two DNA extracts from different tissue samples. No positive PCR controls using modern human DNA should

ever be performed. All primer testing and reaction optimization should be performed in an independent, geographically separate laboratory. If there is evidence of contamination, e.g. conflicting results from analysis of two sets of PCRs, the extracted DNA should be cloned into a plasmid vector and multiple clones should be sequenced from each amplification reaction. Ten clones per amplification reaction would be sufficient to identify the contamination and, perhaps, to determine if endogenous DNA could be differentiated from contaminating DNA.

The most important component of the research plan requires that the complete analysis be conducted in two independent laboratories. Neither laboratory should be involved in the analysis of contemporary human populations because the presence of overwhelming amounts of undamaged, potentially contaminating DNA would immediately compromise the results of any analysis. It is difficult to find laboratories that are experienced with the analysis of aDNA, but do not conduct analyses of contemporary populations since, from a scientific perspective, similar questions are addressed with both types of analyses. However, it is essential for the integrity and defensibility of the final results that all possibility of contamination with modern sources of DNA, with the exception of the investigators themselves, be eliminated.

Once data is generated on all of the assayed markers, a haplotype can be constructed that joins all of the polymorphisms. If there is no evidence of contamination, only a single result will have been noted at each marker and only a single haplotype construction will be possible. If contamination has been detected and multiple haplotypes can be constructed, a thorough analysis must be performed in order to determine which haplotype, if any, corresponds to endogenous DNA. Once a single,

endogenous haplotype has been determined, its affiliation with published haplotypes will be determined through a hierarchical analysis. If the haplotype is A, B, C, or D, and the radiocarbon dates indicate a pre-1492 AD date, the skeleton in question is most probably a American Indian ancestor. Phylogenetic analysis of the haplotype with other American Indian haplotypes can be performed but will very likely be unsuccessful in identifying an affiliation of this individual a particular American Indian tribe. If the haplotype is non-A, B, C, D, the skeleton may be non-American Indian or may represent a American Indian haplotype that has become extinct in modern Native American populations. A phylogenetic analysis of the ancient haplotype against contemporary populations distributed worldwide must be performed in order to attempt a general classification of the skeletal haplotype. However, this analysis likely will not be able to distinguish between the two possibilities listed above, i.e. non-American Indian ancestry vs. American Indian ancestry with an extinct haplotype. In the case where contamination has been detected during the analysis and a single, endogenous haplotype cannot be determined, then the analysis is inconclusive and no assignment to a haplogroup can be made. In all circumstances, the final results and conclusions must agree between the two laboratories in which the analyses were performed, and a genial commitment to work toward an accurate and complete genetic analysis of the skeleton is as important as the independence of the two laboratories. If different haplotypes were determined and the differences cannot be reconciled, again, the analysis is contradictory and no conclusions can be made.

Discussion

A complete and convincing genetic analysis of the skeleton would be expensive (many thousands to tens of thousands of dollars), time-consuming (six months or more) and a destructive (30-60 grams of bone) undertaking. Is there potential research value in a genetic typing of any ancient skeleton? In the abstract the answer is "yes", however an isolated skeleton with poor organic preservation found in an erosional environment would not be the first choice in most hypothesis-driven research of any ancient population. A complete delineation of research potential was not the issue we were to address in this document. Rather, the utility of DNA data in assigning a skeleton to potential cultural and/or biological affiliation with contemporary Americans Indians was discussed.

The larger question is what would be done with any genetic typing (or lack thereof) of this skeleton. If haplogroup A, B, C or D is found, and a likely determination of American Indian biological affiliation is made, will this set the standard for all future new finds of human skeletal remains? Will this type of analysis never have to be done again, and will all skeletons that predate the arrival of Europeans to the Americas be assumed to be ancestral to American Indians? If the results are ambiguous or if no DNA remains in the skeleton, how will this be interpreted, and what will be the ramifications? It is our considered opinion that, for all the parties concerned, the genetic analysis of this skeleton may not yield the resolution that is so dearly sought.

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Table 1. Defining polymorphisms for New World mitochondrial mtDNA founding haplogroups

Site[a]	Haplogroup			
	A	B	C	D
Restriction/deletion sites				
*Hae*III:663	+	-	-	-
9 bp region	no deletion	deletion	no deletion	no deletion
*Alu*I:13262	-	-	+	-
*Alu*I:5176	+	+	+	-
Control region polymorphisms				
16189	T/c	C	T	T/c
16217	T	C	T	T
16223	T	C	T	T
16290	T	C	C	C
16298	T	T	C	T
16319	A	G	G	G
16325	T	T	C/t	C
16327	C	C	T	C
16362	C	T	T	C

[a] Restriction/deletion defining sites are provided by Torroni *et al.* (1992) and control region polymorphisms are provided by Horai *et al.* (1993). Presence and absence of a restriction site are indicated by "+" and "–", respectively. For D-loop sequence data, the defining sites are underlined. X/y indicates that X is the predominant nucleotide at that position although y does occur at a low frequency.

Table 2. Summary of Mitochondrial DNA New World Haplogroups in Humans Worldwide

Population	Location	N	RFLP/DNA seq data	New World founding haplogroups A	B	C	D	Other	Reference
Athapaskan	N. America	21	DNA sequence	0.81	0	0.048	0	0.14	1
Haida	N. America	41	DNA sequence	0.85	0	0.073	0.049	0.024	2
Haida	N. America	25	RFLP	0.96	0	0	0.04	0	3
Dogrib	N. America	30	RFLP	1	0	0	0	0	4
Navajo	N. America	48	RFLP	0.58	0.38	0	0	0.042	4
Apache	N. America	25	RFLP	0.64	0.16	0.12	0.08	0	3
Yakima	N. America	42	DNA sequence	0.095	0.62	0.071	0.14	0.071	1
Nuu-Chah-Nulth	N. America	63	DNA sequence	0.44	0.032	0.19	0.19	0.11	5
Bella Coola	N. America	40	DNA sequence	0.62	0.05	0.075	0.25	0	2
Bella Coola	N. America	25	RFLP	0.6	0.08	0.08	0.2	0.04	3
Ojibwa	N. America	43	RFLP	0.51	0.07	0.16	0	0.26	3
Cheyenne/Arapahoe	N. America	26	RFLP	0.31	0.12	0.35	0.15	0.077	6
Siouan	N. America	34	RFLP	0.53	0.18	0.15	0.059	0.088	6
Chickasaw/Choctaw	N. America	27	RFLP	0.67	0.22	0.074	0	0.037	6
Zuni	N. America	22	RFLP	0.18	0.64	0.091	0	0.091	6
Washo	N. America	28	RFLP	0	0.54	0.36	0.11	0	6
Quechan/Cocopa	N. America	23	RFLP	0	0.65	0.3	0	0.043	6
Jemez/Taos/San Idelfonso	N. America	36	RFLP	0	0.86	0.028	0.028	0.083	6
Pima	N. America	30	RFLP	0.067	0.5	0.43	0	0	4
Maya	C. America	27	RFLP	0.52	0.22	0.15	0.074	0.037	4
Mixtec	C. America	29	RFLP	0.83	0.1	0.069	0	0	7
Nahua/Cora	C. America	32	RFLP	0.53	0.34	0.063	0	0.063	6
Chibcha	C. America	109	RFLP&DNA sequence	0.7	0.3	0	0	0	8 and 9
Choco	C. America	75	RFLP&DNA sequence	0.39	0.25	0.35	0.013	0	10
Yanomama	S. America	24	RFLP	0	0.17	0.54	0.29	0	3
Ticuna	S. America	28	RFLP	0.18	0	0.32	0.5	0	4
Mapuche	S. America	38	DNA sequence	0.16	0.39	0.21	0.24	0	11
Mataco	S. America	28	RFLP	0.11	0.36	0	0.54	0	3
Mandenka	Africa	110	DNA sequence	0	0	0	0	1	12
Tuareg	Africa	23	DNA sequence	0	0	0	0	1	13
Fulbe	Africa	60	DNA sequence	0	0	0	0	1	13
Turkana	Africa	37	DNA sequence	0	0	0	0	1	13
Kikuya	Africa	24	DNA sequence	0	0	0	0	1	13
Somali	Africa	27	DNA sequence	0	0	0	0	1	13
Sweden	Europe	37	RFLP	0	0	0	0	1	14
Finland	Europe	49	RFLP	0	0	0	0	1	14
Finland	Europe	29	DNA sequence	0	0	0	0	1	15
Switzerland	Europe	74	DNA sequence	0	0	0	0	1	16
Denmark	Europe	33	DNA sequence	0	0	0	0	1	15
Wales	Europe	92	DNA sequence	0	0	0	0	1	15
Cornwall	Europe	69	DNA sequence	0	0	0	0	1	15
North Germany	Europe	107	DNA sequence	0	0	0	0	1	15
Bavaria	Europe	49	DNA sequence	0	0	0	0	1	15
Tuscany	Europe	48	RFLP	0	0	0	0	1	14
Basques	Europe	61	DNA sequence	0	0	0	0	1	15
Spain	Europe	30	DNA sequence	0	0	0	0	1	15
Portugal	Europe	30	DNA sequence	0	0	0	0	1	15
Turkey	Europe	22	DNA sequence	0	0	0	0	1	15

Population	Location	N	RFLP/DNA seq data	A	B	C	D	Other	Reference
Vietnamese	SE Asia	28	RFLP	0	0.071	0	0	0.93	17
Malayans	SE Asia	32	RFLP	0	0.031	0	0	0.97	17
Malaysians	SE Asia	32	RFLP	0	0.16	0	0	0.84	17
Papua New Guinea, coast	Pacific islands	55	DNA sequence	0	0.42	0	0	0.58	18
Papua New Guinea, highland	Pacific islands	64	DNA sequence	0	0	0	0.031	0.97	18
Vanuatu	Pacific islands	41	DNA sequence	0	0.73	0	0	0.93	19
Mongolians	Central Asia	103	RFLP&DNA sequence	0.048	0.097	0.14	0.2	0.52	20
Tibetans	Central Asia	54	RFLP	0.11	0.056	0.037	0.11	0.68	21
C. Chinese	Central Asia	20	RFLP	0.1	0.25	0.05	0.05	0.55	17
Altai	Siberia	17	DNA sequence	0	0	0.18	0	0.82	1
Sel'kups	Siberia	20	RFLP	0	0	0.35	0	0.65	22
Nganasans	Siberia	49	RFLP	0.02	0	0.39	0.37	0.22	22
Evenks	Siberia	51	RFLP	0.039	0	0.84	0.1	0.02	22
Udegeys	Siberia	45	RFLP	0	0	0.18	0	0.82	22
Nivkhs	Siberia	57	RFLP	0	0	0	0.28	0.72	22
Evens	Siberia	43	RFLP	0	0	0.58	0.07	0.35	22
Yukagirs	Siberia	27	RFLP	0	0	0.59	0.33	0.074	22
Koryak	Siberia	24	RFLP	0.24	0	0.22	0.087	0.46	22
Koryak	Siberia	155	RFLP&DNA sequence	0.052	0	0.36	0.013	0.57	23
Itel'men	Siberia	47	RFLP&DNA sequence	0.064	0	0.15	0	0.79	23
Chukchi	Siberia	38	RFLP	0.38	0	0.17	0.17	0.29	22
Eskimos	Siberia	80	RFLP	0.8	0	0	0.2	0	22
Eskimos	N. America	462	RFLP	0.51	0.006	0.024	0.4	0.054	24
Aleuts	N. America	77	RFLP	0.27	0	0.013	0.65	0.065	24
Inupiaq	N. America	5	DNA sequence	1	0	0	0	0	1
Inuit	N. America	30	RFLP	0.97	0	0	0.033	0	6
Ancient Humans	**Location**	**N**	**RFLP/DNA seq data**	**A**	**B**	**C**	**D**	**Other**	**Reference**
Plains	N. America	5	RFLP&DNA sequence	0	0.4	0.4	0	0.2	25
Ventana Cave	N. America	3	RFLP&DNA sequence	0	0.67	0.33	0	0	26
Oneota	N. America	108	RFLP (some DNA sequence)	0.32	0.12	0.43	0.083	0.056	27
Fremont	N. America	32	RFLP	0	0.75	0.12	0.06	0.06	28
Kaweskar	N. America	19	RFLP	0	0	0.16	0.84	0	29
Aonikenk	N. America	15	RFLP	0	0	0.27	0.73	0	29
Yamana	N. America	11	RFLP	0	0	0.91	0.091	0	29
Selk'nam	N. America	13	RFLP	0	0	0.46	0.46	0.077	29
Maya	N. America	9	RFLP	0	0	0.89	0.11	0	30
Brazilian Amazon	S. America	18	DNA sequence	0.28	0.056	0.056	0.056	0.56	31
Japanese	Asia	10	DNA sequence	0	0	0	0	1	32
Chinese	Asia	23	DNA sequence	0	0.043	0.087	0	0.87	33
Neandertal	Europe	1	DNA sequence	0	0	0	0	1	34
Ice Man	Europe	1	DNA sequence	0	0	0	0	1	35
Un-reviewed publications									
Pyramid Lake, NV	N. America	21	RFLP	0.095	0.28	0.048	0.48	0.095	36

[1]Shields et al. 1993, [2]Ward et al 1993, [3]Torroni et al. 1993a, [4]Torroni et al. 1992, [5]Ward et al. 1991, [6]Lorenz and Smith 1996, [7]Torroni et al. 1994b, [8]Kolman et al. 1995, [9]Batista et al. 1995, [10]Kolman and Bermingham 1997, [11]Ginther et al. 1993, [12]Graven et al. 1995, [13]Watson et al. 1996, [14]Torroni et al. 1996, [15]Richards et al. 1996, [16]Pult et al. 1994, [17]Ballinger et al. 1992, [18]Stoneking et al. 1990, [19]Hagelberg et al. 1999, [20]Kolman et al. 1996, [21]Torroni et al. 1994a, [22]Torroni et al. 1993b, [23]Schurr et al. 1999, [24]Merriwether et al. 1995, [25]Kolman and Tuross 1999, [26]Handt et al. 1996, [27]Stone and Stoneking 1998, [28]Parr et al. 1996, [29]Fox 1996, [30]Merriwether et al. 1997, [31]Ribeiro-dos-Santos et al. 1996, [32]Horai et al. 1991, [33]Oota et al. 1999, [34]Krings et al. 1997, [35]Handt et al. 1994, [36]Kaestle 1997

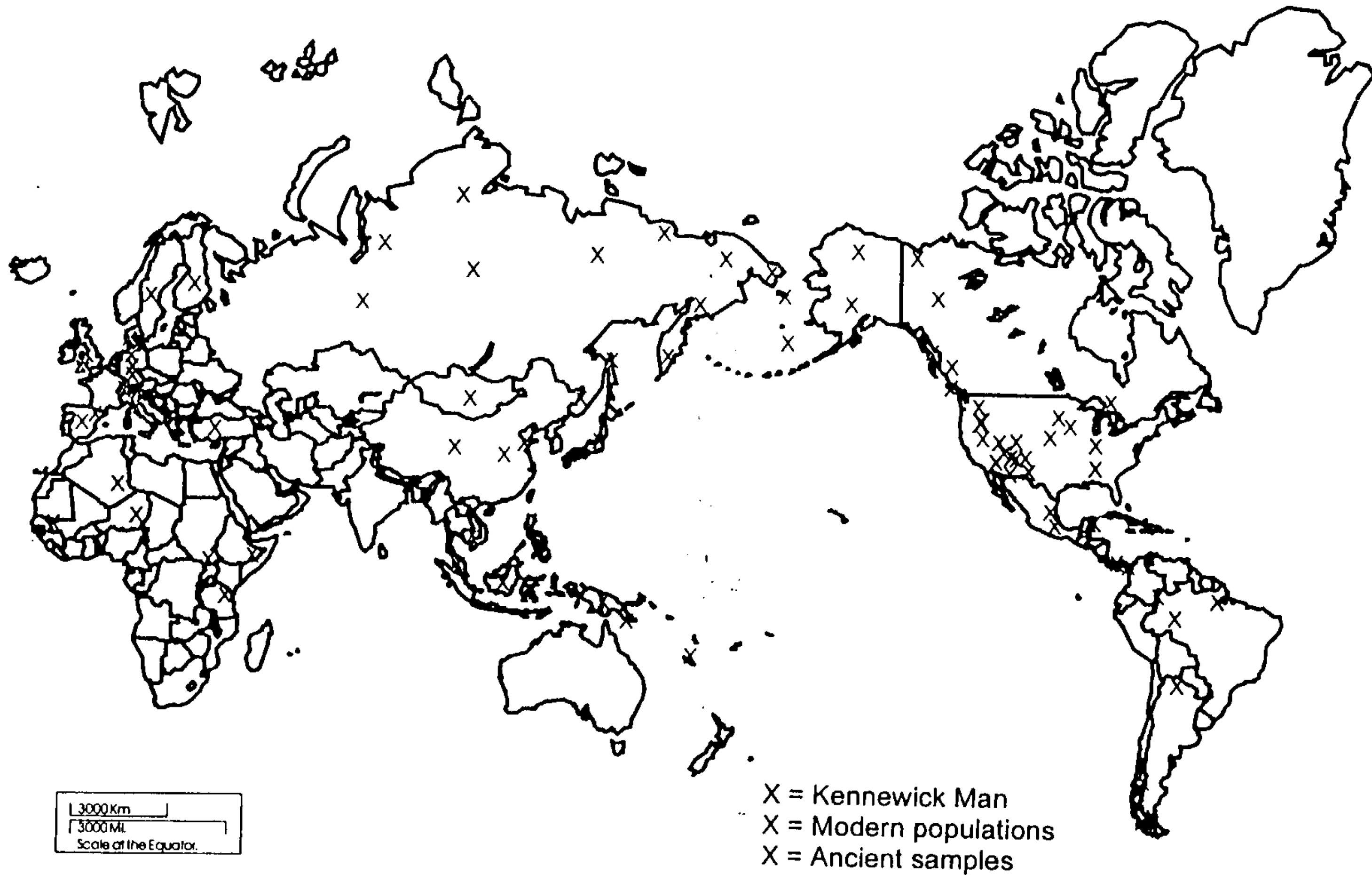

Figure 2. Map of the world marked with populations from Table 2. Contemporary popuilations are marked with a red "X" and ancient populations are marked with a blue "X". The excavation site for Kennewick Man is marked with a large black "X".

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IN THE UNITED STATES DISTRICT COURT

FOR THE DISTRICT OF OREGON

ROBSON BONNICHSEN, et.al., Plaintiffs, v. UNITED STATES OF AMERICA, DEPARTMENT OF THE ARMY, et.al., Defendants.	)	USDC No. CV 96-1481 JE AFFIDAVIT OF THEODORE G. SCHURR

STATE OF TEXAS)
)ss.
County of Bexar)

I, Theodore G. Schurr being first duly sworn, do depose and state as follows:

1. I am a Post-Doctoral Scientist in the Department of Genetics at the Southwest Foundation for Biomedical Research ("SFBR"), San Antonio, Texas. My area of expertise is the study and analysis of mitochondrial DNA ("mtDNA") and Y chromosome variation in modern human populations, in particular,

the indigenous populations of Siberia and the Americas. I make this affidavit in support of the plaintiffs' motion to gain access to the Kennewick Man skeleton for the purpose of undertaking the scientific studies and analyses described in that motion. Specifically, this affidavit will address the following issues: (a) the importance and relevance of performing genetic tests on the skeleton; (b) how such tests should be performed and the results analyzed.

2. My professional qualifications are as follows: I hold an M.A. and Ph.D. in Anthropology which I received from Emory University in 1996 and 1998, respectively, and a Bachelor's degree in Zoology which I received from the University of Georgia in 1983. Between earning my Bachelor's degree and completing the Ph.D., I worked for three years as a Research Technician in the Department of Genetics at the University of Georgia, where I conducted research on genes involved in photosynthesis, and then another five years as a Research Technician in the Department of Genetics and Molecular Medicine at Emory University, where I conducted research into both clinical and anthropological genetics of human populations. After graduating from Emory University, I worked briefly as a Post-Doctoral Fellow in the Center for Molecular Medicine at Emory University. I then took my current Post-Doctoral Scientist position at SFBR. At present, I am participating in a long-term National Institute of Health project called the Strong Heart Family Study which involves the mapping and identification of genes that contribute to cardiovascular disease risk in Native Americans.

3. For the past ten years, the main focus of my work has been investigating the peopling of the Americas from a biogenetic perspective. This work has involved the analysis of mtDNA variation in approximately 1000 native Siberian and approximately 600 Native American individuals from 50 different populations, and the analysis of Y-chromosome variation in the majority of those individuals. While most of my research has taken place in the laboratory, I have also conducted field research with Russian colleagues in northeastern Siberia to gain a better understanding of population histories in that region. In addition to these studies, I have been involved in numerous other molecular genetic analyses of African,

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1 Asian, Aboriginal Australian, and European/Caucasian populations, and these have collectively given me a
2 broad understanding of population genetic variation in human groups. Based on these studies, I have co-
3 authorized nearly 50 scientific articles and papers. These include articles published in scientific journals,
4 review articles, papers presented at scientific conferences, and chapters for books on anthropological issues.

5 4. Genetic research conducted by myself, my colleagues at Emory University, and other scientists
6 over the past decade has provided a number of seminal insights into the peopling of the New World. DNA
7 analyses of modern populations and prehistoric skeletal remains have provided important new information
8 about the timing of human colonization of the Americas, the number of migrations that reached the New
9 World, and the potential source area(s) from which the early New World colonizing population(s)
10 originated. Overall, the data obtained from DNA research imply that the colonization of the Americas was
11 a more complex process than suggested by earlier models, one that has a greater time depth and involves
12 more colonizing groups than previously thought. A general overview of these insights is provided below.
13 More details can be found in Appendixes A and B attached to this affidavit. Appendix A provides technical
14 details concerning the properties of the two genetic systems that have commonly been used for population
15 affiliation studies, the mtDNA and the Y-chromosome. Appendix B describes the genetic characteristics of
16 modern New World native populations. These characteristics provide critical baseline information that are
17 needed for any efforts to determine the population affinities of the Kennewick skeleton.

18 5. For many years, the ruling "paradigm" in scientific thought concerning the peopling of the
19 Americas was the Clovis First Model. According to this model, the New World was first colonized by a
20 small band of Ice Age big-game hunters who gained access to the interior of North America via an ice-free
21 corridor in west-central Canada approximately 11,700 years before present ("YBP"). From the southern
22 end of this ice-free corridor (somewhere in the vicinity of modern Montana), this small band of humans
23 supposedly radiated outward so rapidly that, within less than 1,500 years, their descendants had reached the
24 tip of South America. Modern genetic research has brought these postulates of the Clovis First Model into

question.

A. The Clovis First Model postulates that the New World was colonized by people of Asian origin. DNA data have confirmed this postulate, at least for the most part. The majority of mtDNAs and Y-chromosomes of modern New World native populations contain genetic markers indicating that their ancestors originated in Asia. See Appendix B, Paragraphs 16, 17.

B. The Clovis First Model also postulates that the peopling of the New World is attributable to a single colonizing event. DNA studies do not support that postulate. The most common mtDNA lineages found in modern New World native populations belong to haplogroups A, B, C and D. See Appendix B, Paragraph 2. Two of these haplogroups (A and B) appear to have originated in southeast Siberia or Mongolia, although haplogroup B seems to have a strong East Asian distribution. Appendix B, Paragraph 16A. Haplogroups C and D, on the other hand, may have had multiple source areas in Asia, including southeastern Siberia and the Amur River region. Appendix B, Paragraph 16B. In addition, a mtDNA lineage found in varying frequencies in modern New World populations, haplogroup X, appears to be distantly related to a similar haplogroup found in European populations. Appendix B, Paragraph 13. Although the original source area for haplogroup X has yet to be determined, it does not appear to be east Asia. Such data appear to indicate that the colonizers of the New World did not originate in a single limited region of the Asian landmass. If they did not, then the case for a single colonizing event becomes less plausible.

C. Another postulate of the Clovis First Model is that the original colonizers of the New World consisted of a small band that contained only a few hundred members (or at most a few thousand). One corollary of this postulate is that all modern New World native peoples would share the same degree of biological relationship to one another and to the original colonizing group. Under this view, the genetic and morphological differences between modern native populations would merely be a reflection of the different historical events (e.g., genetic drift, founder effects, natural selection) they experienced after separation in

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1 the New World. However, the DNA data discussed in Paragraph B above does not support these
2 conclusions. If the New World was in fact colonized by multiple groups at different times, then the
3 differences between modern native peoples reflect different genetic inputs as well as their particular historic
4 experiences. As a result, some modern native groups will have a closer, and others a more remote,
5 biological connection to specific early New World populations. For some groups, the connection may be
6 almost nonexistent, or indirect at best.

7 D. Another postulate of the Clovis First Model is that the New World was not colonized until
8 approximately 11,700 YBP. This postulate is inconsistent with dates obtained through statistical analyses
9 of DNA data. Various researchers have used DNA data to estimate the timing of New World colonization
10 by calculating how long ago the genetic lineages found in modern Native Americans split from their
11 progenitors in Asia. The divergence times calculated for the different genetic lineages range on average
12 from 38,139 YBP to 23,097 YBP, depending on the data and methods used. The most probable conclusion
13 is that mtDNA haplogroups A-D arrived in the New World well before 18,000 YBP, with haplogroup X
14 arriving either before or after this time. See Appendix B, Paragraphs 11, 14.

15 6. It is my understanding that answers are being sought to two questions concerning the Kennewick
16 Man skeleton: (a) is it related to present-day U.S. Native Americans; (b) is it affiliated to any of the five
17 tribes that have claimed it? By necessity, any attempt to resolve these questions must rely primarily on
18 biological and genetic analyses of the skeleton. There are no cultural artifacts associated with the skeleton
19 other than the projectile point fragment lodged in its hip. Even if this fragment can be identified as
20 belonging to a particular lithic tradition, there is no objective way to determine whether it was manufactured
21 by Kennewick Man's tribe or by some other, possibly hostile, group of people. Furthermore, utilitarian
22 artifacts such as projectile points may not be the best indicators of group identity because unrelated
23 populations may use similar tools as a result of cultural borrowing or trade. Likewise, arguments based on
24 linguistic criteria will be essentially unhelpful. Since dead men can't speak, there is no way to know what

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language Kennewick Man spoke during his lifetime. Thus, without symbolically interpretable artifacts or evidence of linguistic affiliation, one can only speculate as to whether Kennewick Man's cultural conception of the world, mythology, clan structure and other symbolic elements used to determine his social and cultural identity, were the same as those of any modern Native American tribe.

7. The only things that can definitely be known about Kennewick Man are what his skeleton can tell us. In fact, much can be learned from skeletal and dental studies (i.e., metric measurements and discrete traits observations). These lines of evidence can provide important insights into Kennewick Man's biological affinities to different modern and prehistoric human populations. However, they provide only part of the needed information. Anatomical features such as teeth and cranial features indirectly reflect the underlying genetic relationships between populations and individuals because the genes influencing those traits are not known. In contrast, DNA analyses can measure those relationships directly. Among other things, DNA data can determine whether Kennewick Man is genetically similar to modern Native Americans, or whether he possesses genetic markers not typical of contemporary native populations. In addition, depending upon the specific markers that are found, DNA data may possibly be able to tell us whether Kennewick Man is genetically closer to one tribe (or group of tribes) than to others. Such data, together with skeletal and dental data, can provide an objective and rational basis for assessing this individual's population affinities.

8. If DNA testing of the skeleton is permitted, the testing protocol should be designed to obtain as much information as possible. In this regard, I recommend that, at a minimum, the following tests should be performed:

A. The mtDNA from the skeleton should be subjected to restriction fragment length polymorphism (or "RFLP") analysis. This method determines the extent to which the mtDNAs of different individuals are the same or dissimilar at certain discrete locations (called "recognition sites") in their sequences of nucleotide bases. See Appendix A, Paragraph 3. All of the RFLPs present in a human mtDNA defines its

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1 "haplotype." Haplotypes that share a specific set of RFLPs are said to belong to a "haplogroup" or,
2 alternatively, a "mtDNA lineage", because they are genealogically related. See Appendix A, Paragraph 7.
3 Of these RFLPs, only a small subset of them identify specific haplogroups, and, hence, constitute the
4 diagnostic genetic markers for these mtDNA lineages. To date, the only haplogroups found in modern New
5 World populations that are thought to predate European contact are haplogroups A, B, C, D and X. See
6 Appendix B, Paragraphs 4 and 14. Consequently, Kennewick Man's mtDNA should be screened for the
7 RFLPs that define these haplogroups. If none of them are detected, then the skeleton should be tested for
8 RFLPs which define other known Asian haplogroups.

9 B. DNA testing of the skeleton should also include the direct sequencing of at least the first
10 hypervariable segment ("HVS-I") of the mtDNA control region ("CR"). In contrast to RFLP analysis
11 which scans the genome for isolated sequence changes at selected recognition sites, CR sequencing
12 provides a nucleotide-by-nucleotide decoding of a sizeable piece of the mtDNA. See Appendix A,
13 Paragraph 6. Variation in CR nucleotide sequences often provides information about lineal identity of
14 mtDNAs, and can be used to distinguish otherwise identical RFLP haplotypes from each other. As a result,
15 they increase our ability to reconstruct the genetic histories and relationships of different mtDNA lineages
16 (and of the individuals who share those lineages).

17 C. DNA testing of the skeleton should also include an attempt to define its Y-chromosome
18 haplogroup, or paternal lineage. The Y chromosome is the male counterpart of mtDNA. Whereas mtDNA
19 is inherited from an individual's mother, Y chromosomes are transmitted only through the male members of
20 a family tree (females possess only X chromosomes). To date, two Asian paternal lineages that are thought
21 to predate the era of European contact comprise the vast majority of Y-chromosomes found in modern New
22 World native populations. See Appendix B, Paragraph 17. Tests should be conducted on the Kennewick
23 skeleton for these two haplogroups. If they are not found, tests for other Y-chromosome haplogroups
24 should be performed.
25
26

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9. Analyzing ancient DNA is more complicated than analyzing modern DNA. Ancient DNA is usually degraded (i.e., broken into many small segments) because of normal processes of deterioration in the skeleton, and sometimes because of post-mortem environmental conditions. As a result, extraction and PCR amplification (replication) of these fragments can be difficult. In addition, special care must be taken during the analysis to avoid contamination by DNA from modern sources. Consequently, the testing of the Kennewick skeleton should be conducted by scientists experienced in the unique challenges presented by ancient DNA research. To ensure the reliability of the data obtained, samples from the skeleton should be tested by at least two different laboratories, much as was done with the recently analyzed Neandertal skeleton.

10. Equally critical is the process used for the analysis of the test results. Some of the relevant considerations in this regard include the following:

A. The evaluation and interpretation of the test results should be conducted by scientists who are familiar with both ancient human DNA research and First Americans issues. Not all DNA researchers have the necessary background in these areas. In addition, since individual scientists can differ in their interpretations of data, an effort should be made to obtain as many different viewpoints as possible.

B. The test results should be compared to all relevant published DNA data. Such data should include mtDNA and Y chromosome data for both modern and prehistoric New World native populations, and for relevant groups in Asia and elsewhere in the world. In addition, analyses should be requested from researchers who have databases of unpublished DNA information. For example, I have unpublished DNA data from Siberian and other Asian populations that could be helpful in interpreting any test results from the Kennewick skeleton. Other researchers interested in First Americans issues may also have relevant unpublished information.

C. Since one purpose of this process is to determine if the skeleton can be affiliated to any of the tribes that have claimed it, a special effort should be made to obtain comparative data specific to those

1 tribes. Without such data, any decision upholding their claims would lack an adequate factual foundation.
2 Accordingly, the claiming tribes should be asked if their members will provide blood or bucchal (cheek)
3 cell samples for DNA testing. If they will not, then it may be possible to obtain DNA samples for these
4 tribes from skeletal or other biological materials held in archaeological collections.

5 11. It cannot be predicted in advance what kind of DNA data will be obtained from the Kennewick
6 skeleton if testing is permitted, or what conclusions will be appropriate to draw from those data. There are
7 many possibilities. For example, tribal claims would be enhanced if the skeleton is found to contain one of
8 the genetic lineages (such as mtDNA haplogroups A, B, C, D or X) that are known to predate European
9 contact. All other things being equal, their presence in the skeleton would be consistent with the conclusion
10 that Kennewick Man represents a population that contributed to the ancestry of modern U.S. Native
11 Americans. However, they would not be conclusive proof of ancestry because these haplogroups are not
12 unique to U.S. native populations. On the other hand, it is possible that DNA testing could discover one or
13 more genetic markers that are unique to this skeleton and one of the claiming tribes. If this were the case,
14 then the inference of an ancestral-descendant relationship would be difficult to dispute. This is why all of
15 the abovementioned genetic data should be obtained, as they are needed to delineate between the genetic
16 markers present in Asian/Eurasian DNAs from those appearing in modern New World native populations.
17 Conversely, tribal claims would be weakened if the skeleton were found to contain genetic markers that are
18 not known to be characteristic of modern New World native populations. Once again, however, such data
19 would not be absolutely conclusive.

20 12. In any of these possible scenarios, the final conclusions about the skeleton's population
21 affinities should be made in light of all of the information that can be obtained from it, whether it be
22 genetic, osteological, dental, or biochemical. Should all such information be entirely consistent in pointing
23 to the same conclusion, then our overall interpretation will become more robust. Conversely, if the data
24 obtained from different studies appear to be inconsistent with one another, then each line of evidence must

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1 be carefully reviewed and assessed to determine what it is telling us. In some cases, it may be difficult to
2 reconcile the different data sets and reach an unambiguous conclusion. Such a situation would not
3 necessarily mean that these data are inaccurate or irrelevant, but only that more data are required to make a
4 more certain ascertainment of the skeleton's biological status.

5 13. While DNA data cannot be predicted to conclusively establish Kennewick Man's population
6 affiliations, any decision concerning the skeleton's fate will be deficient if it does not take this line of
7 evidence into account. DNA is the only source of information that directly assesses the underlying genetic
8 relationships (or lack thereof) between and among populations. Only DNA analyses can directly establish
9 the shared genetic characteristics of all human groups and the broad geneological links between populations
10 within various geographic regions, as well as more localized genetic differences between different
11 population subgroups. In situations of this kind, DNA is a line of evidence that cannot be reasonably
12 disregarded.

13

14 14. On a broader level, DNA data from the Kennewick skeleton is important because of the
15 contributions such information could make to our understanding of the processes that resulted in the peopling of
16 the Americas. New statistical analyses of cranial and skeletal data from New World populations have begun to
17 reveal anatomical differences between ancient Paleoamerican or "Paleoindian" human remains and those dating
18 from the Archaic period forward to modern times. However, it is not completely clear what caused these
19 differences. They could be attributable to the occurrence of multiple, temporally distinct migrations from
20 different parts of Asia to the Americas. On the other hand, they could reflect the *in situ* biological differentiation
21 of native populations because of geographic isolation from ancestral populations in Asia, and subsequent contact
22 since that time between widely scattered populations in the Americas. In either case, data from studies of
23 Paleoamerican remains are needed to clarify these questions since such remains represent the earliest known
24 occupants of the New World.

25

26 15. The study of Paleoamerican remains will help scientists more accurately reconstruct the prehistory

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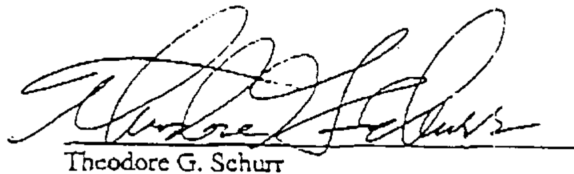
of the Americas. While molecular genetics has enlarged our understanding of the biological links between Asian and Native American peoples, this field has not provided answers to all of the questions concerning the origins and affinities of New World populations. Improvements in our understanding of the timing and processes of the colonization of the New World requires study of the geography and geology of Siberia and the Americas, the languages of modern Native American peoples, the cultural diversity of these populations, and the biological variation present within them. In other words, one must consider the totality of anthropological evidence pertaining to Native American origins to gain the most complete picture of the peopling of the New World, and this includes biological information available through the examination of Paleoamerican skeletons.

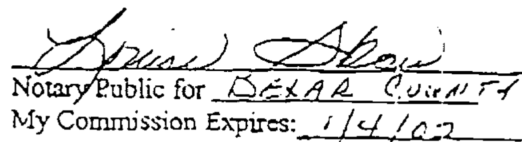
16. I have no personal stake in testing of the Kennewick Man skeleton, nor any prejudices about the ultimate outcome of this study, which I would evaluate fairly and impartially if given the opportunity. Moreover, I have nothing to gain from an erroneous or inaccurate determination of the biological affinities of this skeleton.

DATED this 21st day of January, 2000.

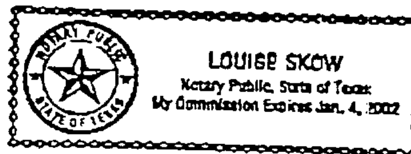


Theodore G. Schurr

SUBSCRIBED and SWORN to before me this 21st day of January, 2000.



Notary Public for BEXAR COUNTY
My Commission Expires: 1/4/02



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CERTIFICATE OF SERVICE BY MAIL

I certify that I made service of the foregoing Motion To Modify the September 21, 1999 Order on the parties herein by faxing and depositing in the United States mail at Portland, Oregon, on February 1st 2000, a copy thereof, enclosed in a postage prepaid envelope, addressed to:

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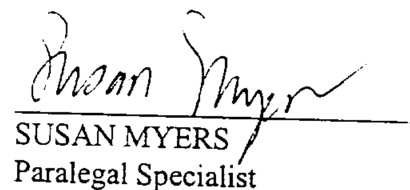

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