DEPARTMENT OF THE ARMY
CORPS OF ENGINEERS, NORTHWESTERN DIVISION
PO BOX 2870
PORTLAND OR 97208-2870

REPLY TO
ATTENTION OF

Office of Counsel

AUG 23 2011

Dr. Thomas W. Stafford, Jr.
Stafford Research, Inc.
200 Acadia Avenue
Lafayette, Colorado 80026

Dear Dr. Stafford:

We have reviewed the "Preliminary Report of aDNA Analyses on the Kennewick Human Skeleton" by Dr. Cristina Valdiosera of the Center for GeoGenetics at the Geolgogiske Museum in Copenhagen, Denmark, and have discussed the need for additional Kennewick remains to complete the DNA analysis. Additionally, we have reviewed your electronic message, dated May 5, 2011, in which you provide us with images of two previously sampled fragments that would be good candidates for this further analysis. Based on these reviews, as well as the advice of my staff, Burke personnel, and the government's conservators, I am approving your request for the destructive analysis of one of the fragments identified in your May 5 message. The "potential gain in scientific...information" will outweigh the loss of the object, particularly given that these two fragments have already been sampled from the remains. 36 C.F.R. § 79.10(d)(5).

Any reports written on the results of this research must be provided to the Corps' Mandatory Center of Expertise for the Curation and Management of Archaeological Collections (MCX-CMAC) in the St. Louis District within one (1) year of the date of this letter for inclusion with the Kennewick collection's associated records. I also ask that you coordinate the retrieval of the sample with Mr. Chris Pulliam, the assistant director of MCX-CMAC, at 314-331-8481.

I look forward to reading the report from this analysis. Any questions should be directed to either to Mr. Pulliam or Ms. Jennifer Richman, Office of Counsel, at 503-808-3763.

Sincerely,

John R. McMahon, P.E.
Brigadier General, US Army
Division Commander

Enclosure

DEPARTMENT OF THE ARMY
ST. LOUIS DISTRICT CORPS OF ENGINEERS
1222 SPRUCE STREET
ST. LOUIS, MISSOURI 63103-2833

REPLY TO
ATTENTION OF:

CEMVS-EC-Z

16 August 2011

MEMORANDUM FOR Commander, U.S. Army Corps of Engineers, Northwestern Division, ATTN: CECC-NWD (Richman), P.O. Box 2870, Portland, OR 97208-2870

SUBJECT: Request by Thomas W. Stafford, Jr., for One Previously Sampled Fragment to Complete DNA Analysis

1. My staff, the government's contracted conservators (Dr. Nancy Odegaard and Dr. Vicki Cassman), and staff from the Burke Museum (Laura Phillips, Collections Manager, and Megon Noble, Assistant Collections Manager) have reviewed and discussed Dr. Cristina Valdiosera's (Center for GeoGenetics, Geologiske Museum, Copenhagen, Denmark) "Preliminary Report of aDNA Analyses of the Kennewick Human Skeleton" (May 1, 2011), which is an integral part of Dr. Thomas Stafford's ongoing research on sampled fragments of the Kennewick remains. While not specifically outlined in the October 2002 Plaintiffs' Study Plan, this research is contemplated in Dr. Stafford's April 2005 research plan (as submitted in Alan Schneider's April 4, 2005, letter to Sydney Cook). Dr. Stafford's

> geochemical studies on archived bones will provide: 1) more precise and accurate data about the distribution and preservation of collagen, and therefore, DNA, in all bones....Equally important, the amount of new intact bone that will be needed for future DNA and other tests will be dramatically reduced by having first performed quantitative tests on bone already removed from the skeleton (Schneider Letter to Cook, April 4, 2005, page 3).

Comments from all reviewers indicate that this research has the potential to yield meaningful data. It is also important to note that this specific approach will minimize destruction to the main collection by using a previously sampled remnant. As a result, I find the proposal consistent with the Corps' obligations under the curation regulations (36 CFR Part 79), and I recommend that you inform Dr. Stafford that he will be permitted to use one of the two identified samples for Dr. Valdiosera's analysis. Please ask Dr. Stafford to make arrangements with us for the transfer of the sample to him.

2. In addition, I respectfully request that Dr. Stafford adhere to the following conditions, which I feel are consistent with requests for the study of archaeological collections (including human remains) that the St. Louis District recently has reviewed.

- Any material that remains after testing is the property of the government and will be returned in the original containers once testing is complete.

- We will provide transfer forms that will be signed by Dr. Stafford and a Burke Museum representative. Copies of the fully executed forms will be provided to Dr. Stafford.
- All testing will be completed, and remaining material returned to the government, within six months of receipt of the material by Dr. Stafford or his agent.
- Copies of all raw data and photographs will be provided to the government for inclusion with the Kennewick associated records.
- All publications resulting from the study will acknowledge the U.S. Army Corps of Engineers, and the Corps will be provided with one copy of all publications generated from this research.

3. If you have any questions about this issue, please do not hesitate to contact Mr. Chris Pulliam at (314) 331-8481.

FOR THE COMMANDER:

MICHAEL K. TRIMBLE, Ph.D.
Director, Mandatory Center of Expertise
for the Curation and Management
of Archaeological Collections

Preliminary Report of aDNA Analyses on the Kennewick Human Skeleton

Cristina Valdiosera, Ph.D.
Center for GeoGenetics, Geologiske Museum
Copenhagen, Denmark

May 1, 2011

I am Dr. Cristina Valdiosera working with Drs. Eske Willserlev and Thomas Stafford at the Center for GeoGenetics in Copenhagen. My current research project is on the peopling of the Americas, using ancient DNA from a number of Native American samples. Among these, one of the most interesting if not the most scientifically important, is the Kennewick Man skeleton. I have been working full time with this sample as a top priority because it is of great interest to get this specimen to yield ancient DNA (aDNA).

In order to do this I have been using the most novel methods and technologies available to the field of ancient DNA. Despite these techniques however, there are chemical inhibitors that are preventing complete recovery of aDNA. These "inhibitors" are compounds that enter the bone during burial, form over time during diagenesis, or both and which prevent or hinder aDNA recovery. I have now resolved these inhibition issues by diluting the DNA extract a series of times and am now using Next Generation Sequencing Technologies through a technique termed "shotgun sequencing", which has yielded my initial results.

From these data I can definitely say that I have ancient human DNA and that further experiments are justified. The only issue now is the small size of my existing material, which has been used for the present experiments. I started with 500 mg that Dr. Stafford originally sent. I propose using a technique called "Custom designed Microarrays for DNA Capture" (Hodges et al,2009) (one of the latest and most informative techniques for ancient material, see Burbano et al, 2010) with a sample in the near future, now that I have solved the inhibition problems. The protocol involves using at least 3 to 5 DNA libraries (in the case of Kennewick I can anticipate that more will be needed due to its degraded state), each of which uses 16 µl of DNA extract. This DNA capture technique has already given us good results in other degraded material (Burbano et al, 2010, and on some unpublished data from our center in Copenhagen) so

I am confident that this will be the best approach for the recovery of DNA from Kennewick Man. Although it is expensive to build several libraries, and use several microarrays, this cost is not an issue due to the scientific importance of the Kennewick skeleton. We are willing to do everything it takes to make this sample work; however, the amount of sample I have remaining will be too small to complete the Capture method. Now that I have been able to see that it is possible to obtain DNA from the Kennewick bone, I will need to obtain more material to interpret the origins of this individual.

The array based sequence capture method I mentioned above is the best approach we can use at this time. It requires additional DNA, which I will need to acquire. Once I have this aDNA I will be able to trace thousands of ancestry-informative markers and hundreds of potential phenotypic traits, as well as the complete mitochondrial genome. This would provide the most complete information recoverable today from such a geologically ancient and degraded specimen. We have been able to do this already with other individuals, and it will be our top priority to do this on the Kennewick Man.

References

1.- Emily Hodges, Michelle Rooks, Zhenyu Xuan, Arindam Bhattacharjee, D Benjamin Gordon, Leonardo Brizuela, W Richard McCombie, Gregory J Hannon. (2009) *Nature Protocols.*

2.- Hernán A. Burbano, Emily Hodges, Richard E. Green, Adrian W. Briggs, Johannes Krause, Matthias Meyer, Jeffrey M. Good, Tomislav Maricic, Philip L. F. Johnson, Zhenyu Xuan, Michelle Rooks, Arindam Bhattacharjee, Leonardo Brizuela, Frank W. Albert, Marco de la Rasilla, Javier Fortea, Antonio Rosas, Michael Lachmann, Gregory J. Hannon, Svante Pääbo. 2010. *Science*

Please do not hesitate to contact me if you have any questions.

Best regards.

Cristina Valdiosera, Ph.D.

Research Scientist

Center for GeoGenetics, Geologiske Museum

Copenhagen, Denmark

Cristina Valdiosera <cvaldioser@yahoo.es>

Richman, Jennifer R NWD

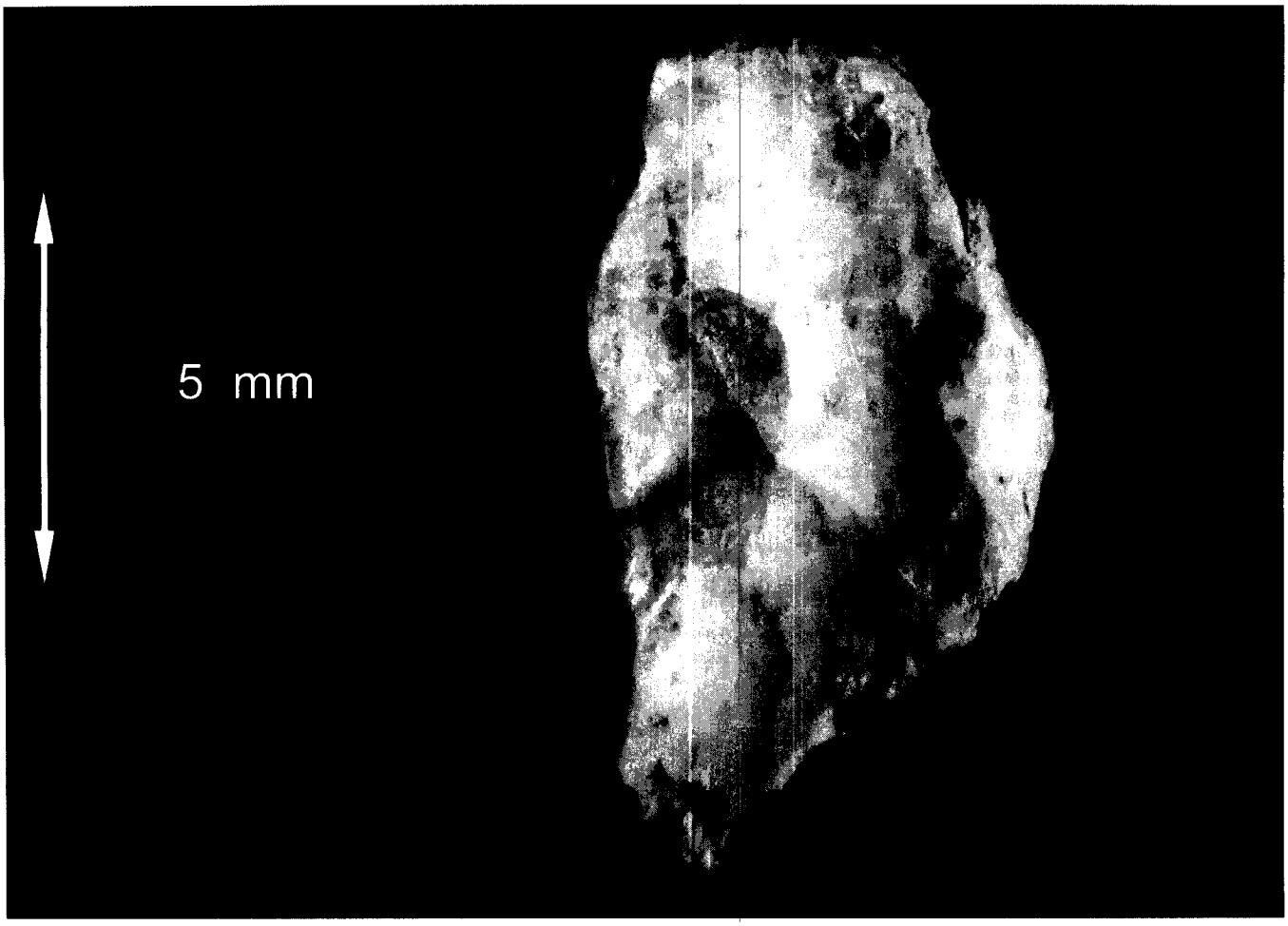
From: Thomas Stafford, Jr. [TWSTAFFORD@stafford-research.com]
Sent: Thursday, May 05, 2011 1:37 PM
To: Pulliam, Christopher B MVS
Subject: Kennewick aDNA
Attachments: SR-7094 KENNEWICK (K#1588EC.jpg; SR-7094 KENNEWICK (K#1588EE.jpg

Chris,

These are photos of the two fragments in question and which I'd propose using for the next aDNA tests.

Thanks for the ideas today.

Tom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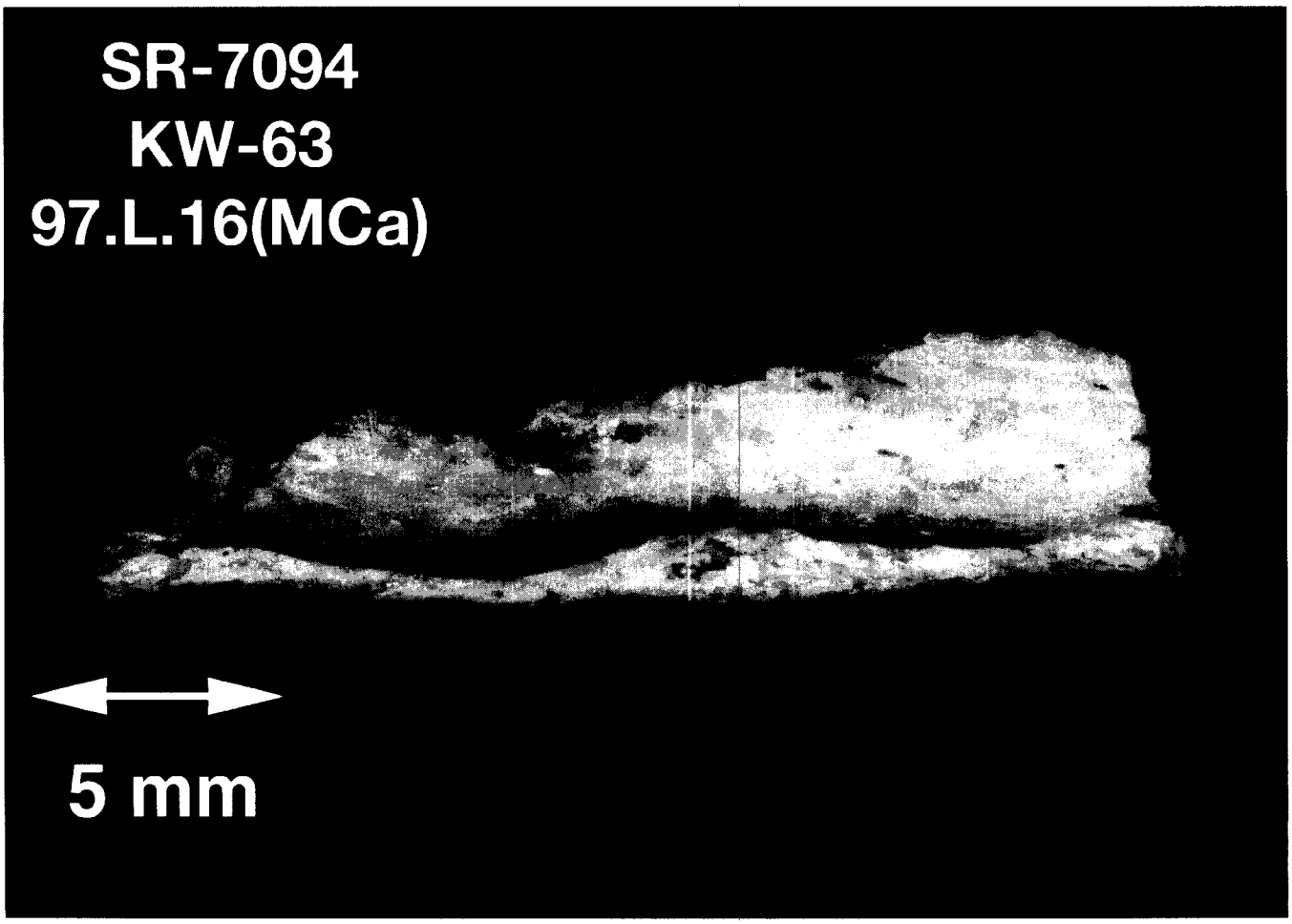
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