**Commentary on** Linch CA, Smith SL, Prahlow JA. Evaluation of the human hair root for DNA typing subsequent to microscopic comparison. J Forensic Sci. 1998; 43(2):305–14.

Sir:

It was with some dismay that we read the above-cited article by Linch et al., who reported that, in their experience, the technique of Fluorescence In Situ Hybridization (FISH) was unsuitable for gender determination of hair.

Linch et al., reported that they failed to attain hybridization of commercially (VYSIS) available X- and Y-chromosome-specific alpha-satellite FISH probes to both archived and fresh hair samples. We had previously reported that FISH, using these probes, could correctly identify the gender of hair (1). In addition, we have reported using FISH successfully to identify the gender of cells in a number of different sample types as it could be applicable in forensic analysis (2–7). As a result of this discrepancy, we reviewed their methodology. The technique used was essentially that reported in our article (1) with one major exception. The cells were heat fixed to the slides. In our original report, cells from the hair bulb were attached using liquid nitrogen (2). The step of heating cells represents a critical error in their FISH methodology. In our experience with FISH, those of other colleagues, reports and recommendations in the literature (8,9) and "trouble shooting" recommendations by commercial companies (VYSIS, Venatana-Oncor), heating, baking, or flaming a slide prior to the hybridization step severely inhibits the efficient hybridization of DNA probes to the cells. Hence, probes do not hybridize well, if at all, and may result in inconclusive results and/or cause false hybridization signals. This appears to be the case with the observation made by Linch et al., wherein they report seeing either no signals (i.e., no hybridization), some hybridization or false hybridization. A simple change in the way they made slides would have solved their lack of FISH hybridization.

Linch et al. attempted to justify their negative results by stating that "FISH probes have inherent problems even when used with fresh viable cells. Loss of target DNA, poor penetration of probe, and incomplete or non-specific hybridization are problems associated with apoptotic, necrotic, and keratinizing cells. FISH requires examination of a large number of cells, the use of control cells on the same microscope slide as the evidence slide (due to critical temperature requirement) and sophisticated statistical analysis" (10). Those statements may have had validity some years ago, however they are no longer of critical concern with newer techniques and commercial probes. Techniques have been so well standardized that FISH is now used routinely for prenatal, postnatal and leukemia diagnosis (11–15). In fact, in microdeletion syndrome cases such as DiGeorge or William syndromes, FISH is the only truly confirmatory test. A number of the currently available probes have been FDA approved for clinical testing.

It is our recommendation that Linch et al. or any other investigator planning to use FISH, first thoroughly familiarize themselves with the technique and its potential pitfalls, before reporting conflicting information in the literature.

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## Authors' Response

Sir:

Early reviewers and colleagues suggested we separate the paper into three articles: (1) Fluorescence in situ hybridization (FISH) gender typing of telogen hair club material, (2) Transmission electron microscopy (TEM) of telogen hair club material and anagen hair bulb material, and, (3) Polymerase Chain Reaction (PCR) nuclear DNA typing of all hair root stages. We protested however because we hoped the reader would appreciate the relationship between hair root morphology and expected DNA typing results if the three parts were taken as a whole. One of the main goals of the paper was to urge the reader to microscopically evaluate hair roots prior to attempting biotechnical methods.

A complete read of the paper will show that we had FISH X-Y probe failure with telogen hair club material (trichilemmal keratin) and not with anagen hair bulb cells. Telogen hair clubs have no intact nuclei and anagen hair bulbs do, as revealed by the TEM part of the study. In 1997 FISH X-Y probes required interphase nuclei or metaphase chromosomes for success. We did not attempt FISH gender typing of the anagen hair bulb material because the practicing forensic community prefers the STR, amelogenin typing of such material for obvious reasons. FISH gender typing of trichilemmal keratin would be similar to FISH gender typing of fingernails absent soft tissue. There is a 1993 report of successful FISH gender typing in which the slides containing "sheath cells from the shaft of the hair roots" were heated to 80 degrees C for 20 minutes prior to the dehydration steps (1). It was refreshing to see investigators actually identify the material they were testing but, again, these types of hairs (anagen) are a waste of time for FISH X-Y forensic analysis since more informative methods exist for such cell rich materials (STR, amelogenin).

The commentators' use of the term "hair bulb" indicates their focus on anagen phase hairs which we did not use. Investigators not experienced with hair root microscopy do not know if they are testing clubs or bulbs, each of which may, or may not, also have follicular tissue present. In Prahlow et al., (2), Dr. Pettenati, Dr. Rao, and Dr. Prahlow reported successful FISH typing of "pulled" and "combed" hairs from autopsy patients without benefit of microscopic examination of the hair roots prior to typing. It is extremely difficult to comb the hair of an autopsy patient without obtaining some hairs that contain either sheath cells or bulb cells (not telogen clubs).

Forensic scientists do not have the luxury of testing clinical diagnostic material. Our brief touch of the micro slide to the hot plate to evaporate the acetic acid, as complained about, was a minor tissue insult compared to that suffered by hairs left at crime scenes. Forensic validation guidelines require that degradative environmental and matrix studies be performed on specimens prior to implementation of such biotechnologies for crime lab use (3–5). In other words, subject the telogen club (trichilemmal keratin) material to extreme temperatures, humidity, direct sunlight, dyes, soils, and foreign blood/semen/saliva contaminants; wash with an appropriate method (5), and then, attempt FISH gender typing if one expects to find interphase nuclei in keratin material. We did contact Vysis technical support about our results, March 1997, and they recommended purchase of their FISH apoptosis detection kit. (The telogen club is the final product of an apoptosis process that shrinks the hair root stem from the active (anagen) growth stage to the resting (telogen) stage). At that time the Vysis technical staff was not concerned about our brief specimen heat fixation method.

The focus of the FISH portion of the study was the telogen hair club since its exploitation for gender typing would be an addition to comparison microscopy and mitochondrial DNA D-loop sequence analysis, the only currently useful techniques for forensic comparison of such. Biomedical and forensic investigators should take the time to learn proper hair histiogenic micro structure and language. "Shed", "combed", "pulled", and "plucked" hair specimen categories only add to the confusing data that have been published using FISH, nuclear DNA PCR, and mitochondrial DNA PCR sequence methods. One must know the nature of the material actually being tested and account for the potential environmental insults the material may have had prior to arriving at the sterile laboratory.

We have no doubt that FISH is a useful methodology for clinical specimens. We have no doubt that FISH X-Y probes work on anagen hairs. FISH X-Y probes will not work on telogen hair clubs (absent attached follicular cells) no matter what methodology is used.

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**Commentary on** Willey P, Scott DD. Who's buried in Custer's grave? J Forensic Sci 1999;44(3):656–65.

Sir:

The excellent article, referenced above, was absolutely fascinating!

As a forensic dentist and a clinical dentist, I have the following comments. The suggestion that skull (Burial 8B) was a tobacco user and specifically a pipe smoker, due to "pipestem abrasion" on the left mandibular premolar teeth may not be perfectly accurate for the following reasons:

1. All of the left posterior teeth depict a degree of occlusal abrasion, but I believe that this abrasion was the result of bruxism. (I am sure that soldiers over 125 years ago had plenty of problems over which to clench and grind their teeth.)
2. I am not sure what pipestems were made of in the 1870's, but I cannot think of many materials suitable for pipestems harder than enamel, thus, I would expect the stem to yield before the enamel structure of the teeth.
3. If the individual were a pipe smoker, and clenched the stem in a chronic fashion, more than likely the stem would have caused a vertical downward movement of the involved tooth or teeth, much like an orthodontic appliance.

The bottom line: I would not think that one of the elements in eliminating Custer should be the fact that he was disdainful of smoking, simply because I don't believe there is ample evidence that the abrasion came from a pipestem in the first place! Eliminate him on other factors if you will, but not on that particular one.

Again, I thank the authors for a meticulous and interesting account of the events surrounding the death of Gen. Custer. The photographs, sketches and maps were very illustrative and engrossing.

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**Authors' Response**

Sir:

We appreciate Dr. Norman Sperber's comments and insights concerning our assessment of Burial 8B. We concur with many of his statements, particularly those concerning the exceptional service that the *Journal of Forensic Sciences's* editor and