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TECHNICAL NOTE

Mignon Dunbar,[1,†] *M.S. and Terence M. Murphy,*[1] *Ph.D.*

DNA Analysis of Natural Fiber Rope*

ABSTRACT: When rope is found at a crime scene, the type of fiber is currently identified through its microscopic characteristics. However, these characteristics may not always unambiguously distinguish some types of rope from others. If rope samples contain cells from the plants of origin, then DNA analysis may prove to be a better way to identify the type of rope obtained from a crime scene. The objective of this project was to develop techniques of DNA analysis that can be used to differentiate between ropes made from *Cannabis sativa* L. (hemp), *Agave sisalana* Perrine (sisal), *Musa textilis* Née (abaca, "Manila hemp"), *Linum usitatissimum* L. (flax), and *Corchorus olitorus* L. (jute). The procedures included extracting the DNA from the rope, performing polymerase chain reaction (PCR) using the extracted DNA as a template, and analyzing the DNA products. A primer pair for PCR, chosen from within a chloroplast gene for the large subunit of ribulose bisphosphate carboxylase/oxygenase, was designed to be specific for plant DNA and complementary to the genes from all five plants. The resulting PCR fragments were approximately 771 base pairs long. The PCR fragments, distinguished through base sequence analysis or restriction enzyme analysis, could be used to identify the five different rope types. The procedure provides a useful addition to visual methods of comparing rope samples.

KEYWORDS: forensic science, DNA typing, rope, polymerase chain reaction, base sequence, restriction analysis, ribulose bisphosphate carboxylase/oxygenase

Rope, as a constraint or a weapon, plays a role in a significant proportion of violent crimes (1). Scientific analysis of a rope sample can provide clues to link a suspect to a crime. For example, if sisal rope is found at a crime scene, and a suspect has sisal rope in his possession, a possible link is present between the suspect and the crime. Natural fibers that have traditionally been used for rope manufacture include *Gossypium* spp. (cotton), *Cannabis sativa* L. (hemp), *Agave sisalana* Perrine (sisal), and *Musa textilis* Née (Manila hemp, also known as abaca) (2). Other natural fibers, such as *Linum usitatissimum* L. (flax) and *Corchorus olitorus* L. (jute), have also been used to make rope. Although ropes of synthetic fibers, such as polypropylene, polyethylene, polyester and polyamide, have become more prevalent, natural fiber rope is still imported from countries outside the U.S. (2,3).

While microscopy is currently used to match cut ends of a rope (4) and to analyze the cellular composition of ropes in order to help in identifying rope types, DNA analysis may be another method to identify the types of rope. Natural fiber rope primarily consists of fiber cells, which at maturity are typically dead and no longer have a nucleus or other cytoplasmic organelles containing DNA. However, the crude fiber extraction process, which involves crushing stems or leaves to isolate fiber bundles, may allow parenchymal, collenchymal, and epidermal cells that have nuclei or cytoplasmic organelles to remain attached to the fibers. These cells may contain DNA, allowing analysis of the rope components.

DNA analysis requires that DNA be extracted, a particular gene be copied by polymerase chain reaction (PCR), and the amplified DNA fragments be identified, either through restriction analysis or base sequence. The main objectives of this project were to see if DNA could be obtained from rope and if so, to develop techniques to differentiate between ropes of hemp, sisal, abaca, flax, and jute. In this research, the gene that was chosen for analysis was a plastid gene for a protein known as ribulose bisphosphate carboxylase/oxygenase large subunit (rbcL). The rbcL gene was chosen because it is plant specific, present in multiple copies per cell, and present in all plant species (5,6). The plant specificity of the rbcL gene was important because contaminating DNA from animals or fungi could be eliminated. The strong conservation of the rbcL gene among plant species allowed the gene to be amplified by PCR with one set of primers, while base pair variations allowed the different ropes or plants to be identified.

[1]Department of Plant Biology, University of California, One Shields Avenue, Davis, CA 95616.

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[†]Present address: Scientific Investigation Division, Los Angeles Police Department, 1800 Paseo Rancho Castilla, Los Angeles, CA 90032.

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Materials and Methods

Materials

Dried plant tissue samples of the following species were obtained from the University of California, Davis Center for Plant Diversity: *Cannabis sativa* L. (hemp); *Linum usitatissimum* L. (flax); *Corchorus olitorus* L. (jute). Fresh *Agave sisalana* Perrine (sisal) was obtained from the University of California, Berkeley Botanical Garden. Fresh *Musa cavendishii* Lamb. ex Paxt. (*Musa acuminata* Colla.) (banana) came from the University of California, Davis Plant Conservatory (fresh plant material of *Musa textilis* Née (abaca) from which rope is actually made could not be obtained).

The sequences of two rbcL genes from possible rope sources, hemp and *Boehmeria nivea* (L.) Gaud (ramie), were found in GenBank (http://www.ncbi.nlm.nih.gov/Genbank/index.html). Comparison of the two plant sequences (GenBank accessions AJ390068 and AJ235801; 5, 6) revealed identical regions that were used for the design of two forward and two reverse primers. One primer pair, which resulted in a 771 bp amplicon, worked well with all five subject species of plants [forward: 5′-(1) TGTTTACTTCCATTGTGGGTAATG-3′; reverse: 5′-CTGGTAGAGAGACCCAATCTTGA (749)-3′; numbers in parentheses indicate the position of the adjacent base in the rbcL gene].

DNA Extraction

Samples of rope made from jute, sisal, flax, abaca, and hemp were obtained from hardware stores. The numbers of independent samples were: jute, 4 (from two separate stores); sisal, 2; abaca, hemp, and flax, 1 each. Multiple extractions were made from each sample, although not every extract provided good DNA template. Rope samples (0.02–0.09 g) were ground in liquid nitrogen with a mortar and pestle, combined with 350 μL of buffer containing 2% w/v cetyl-trimethyl-ammonium bromide, 1.4 M NaCl, 100 mM Tris HCl pH 8.0, and 20 mM EDTA and 350 μL of 10 mM Tris HCl pH 8.0, 1 mM EDTA buffer, and then incubated at 65°C for 10 min (7). The mixture was allowed to cool; then 4 μL of 5.6 mg/ml pancreatic RNAse were added; and the mixture was incubated at 37°C for at least 20 min. The samples were cooled, extracted with 350 μL of chloroform, and precipitated with an equal volume of isopropanol. The precipitate was centrifuged, washed with 500 μL of 70% ethanol, air dried, and dissolved in 20–30 μL of H_2O. DNA was quantified using spot densitometry (8) and standards of purified human DNA, the concentrations of which were determined spectrophotometrically (8).

PCR

The 50 μL PCR mixture contained 29.8 μL of water, 10 μL of 5× Green Go Taq Buffer (Promega Corporation, Madison, WI), 4 μL dNTPs (2.5 mM of each dNTP), 0.625 μL *Taq* (Go Taq DNA Polymerase, 5 u/μL; Promega), 1.5 μL of each primer (forward and reverse, 20 μM), 2.5 μL template DNA (concentration varied with extraction and type of rope). PCR conditions were 96°C for 1 min; 35 cycles of 94°C for 45 sec, 55°C for 45 sec, and 72°C for 2.5 min; 72°C for 5 min; 4°C hold. The unusually long extension time may have been necessary because of the presence of inhibitors in the extracts (9). On occasion, no PCR product was obtained. The presence of inhibitory factors was tested by assembling a PCR mixture with 0.5 μL of a known effective template and 0.5 μL of the template in question. If no PCR product was seen, an inference was made that inhibitors were present in the template in question. Depending on the sample of rope, the inhibition was relieved by diluting the template, adding bovine serum albumin (1 μL at 1 mg/mL), and increasing the concentration of magnesium.

Sequencing

After PCR products were obtained from confirmed plant samples of hemp, sisal, flax, jute, banana, and abaca rope, the amplicons were sent for sequencing to the DNA Sequencing Facility at the University of California, Davis. The resulting sequences were analyzed using NEBcutter version 2 and Vector NTI (10,11) to find restriction sites (see Table 2 for full restriction analysis).

Restriction Digest

A 50 μL PCR mixture was apportioned to provide 8 μL for each restriction enzyme: *Acc*I (New England Biolabs, Inc., Beverly, MA), *Bam*HI (Promega), *Pst*I (Promega), *Dra*I (Promega), and *Sac*II (Promega). 10 μL were left as an "uncut" standard. 8 μL of PCR product were combined with 1 μL of the restriction enzyme and 1 μL of appropriate 10× restriction buffer, and then incubated at 37°C for at least 1.5 h. The digest mix was then placed at 65°C for 15 min to inactivate the restriction enzymes. The 10 μL mixtures were then run on a 2% agarose gel in 0.04 M Tris-acetate, 1 mM EDTA (7) together with a 1 Kb DNA ladder from Gibco BRL (Invitrogen Corporation, Gaithersburg, MD).

Histological Examination of Rope

Rope samples were macerated by placing them in a solution of 1:4:5 hydrogen peroxide (30%): water: glacial acetic acid. The samples were incubated at 60°C for 2 days or until the samples were translucent. The samples were rinsed and vigorously shaken with water for several minutes. Four drops of 1% Safranin O solution were added; the samples were again shaken and allowed to sit for several hours. A small amount of each sample was then examined under an Olympus Vanox-AHBT (Olympus America, Melville, NY) compound light microscope linked to a Pixera 600ES digital camera (Pixera Corporation, San Jose, CA).

Results

Histology

Microscopic examination of the rope after maceration and Safranin O staining revealed that parenchyma cells were present in all samples (Fig. 1). Seeing parenchyma cells is not uncommon in rope samples, as reported by Catling and Grayson (2). During the rope manufacturing process, when the fibers are being extracted, the methods are crude enough that attached parenchyma cells easily mix with the extracted fibers. The parenchyma cells in plants possess both nuclei and plastids and therefore may contain both nuclear and plastid DNA.

DNA Extraction

Three different methods of extracting DNA from samples of sisal rope were tested. The first technique involved using a mortar and pestle cooled with liquid nitrogen to grind the rope into a powder and small fiber pieces. The second technique involved using a plastic drill bit and liquid nitrogen to grind the rope in a plastic microfuge tube. The third technique involved shaking rope pieces together with ball bearings with a vortex mixer at room temperature in the presence of buffer. The different extraction methods resulted in different amounts of DNA. In a single test, using samples from the same rope, we obtained: mortar and pestle, 14.7 μg DNA/g of rope; plastic drill bit, 8.7 μg/g; ball bearings and vortex, 1.7 μg/g. The liquid-nitrogen/mortar-pestle technique was selected for grinding the rope samples in all experiments described below.

Sequencing

PCR-derived rbcL amplicons of hemp, sisal, flax, jute, and banana plant samples and abaca rope were sent to the DNA Sequencing Facility at the University of California, Davis. The sequence of hemp rbcL that was obtained from the DNA Sequencing Facility matched the GenBank sequence (12, accession AJ390068) with a 97% identity. Out of the 771 bp, there were about 18 bp differences between the GenBank sequence and the sequencing done in this work, none of which affected the restriction enzymes that were selected. With the help of two computer programs, Vector NTI and NEBcutter2 (10,11), all the sequences were analyzed for predicted restriction sites. Table 1 shows the sizes of fragments predicted to be formed from cleavage by the set of restriction enzymes chosen to distinguish the types of ropes.

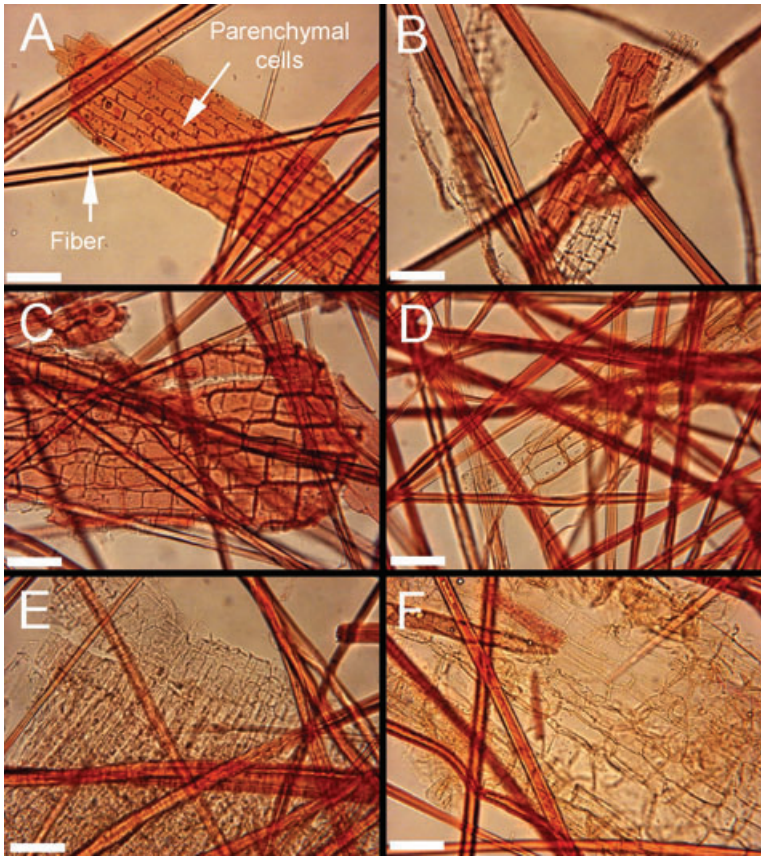

FIG. 1—*Micrographs of ropes made from: A, abaca leaves; B, flax stems; C, hemp stems; D, jute stems; E, hemp stems (sample sold as jute); F, sisal leaves. All types of ropes in this case, whether made from stems or leaves, had both fibers and parenchyma cells present. (Scale bars = 50μm.)*

TABLE 1—*Restriction analysis of rope types.*

	Hemp	Sisal	Flax	Jute	Abaca
*Acc*I GT′MKAC M = A/C K = G/T	518 + 60 + 193	230 + 541	578 + 193	230 + 288 + 253	230 + 541
*Bam*HI G′GATCC	115 + 656	–	–	115 + 656	–
*Pst*I CTGCA′G	386 + 385	386 + 385	–	–	–
*Dra*I TTT′AAA	–	–	35 + 736	332 + 439	–
*Sac*II CCGC′GG	–	–	385 + 386	734 + 37	–

This table shows the DNA from different types of rope along the top, and the different restriction enzymes and the sequences at which they cut along the left side.

The numbers in a box indicate the sizes of fragments produced by each restriction enzyme.

A dash indicates that the enzyme is not predicted to cut that DNA amplicon, resulting in an uncut fragment of 771 bp.

The table shows that the DNA from each different type of rope is cut by a different combination of restriction enzymes and yields a characteristic set of restriction fragments.

Restriction Analysis of Rope Samples

DNAs extracted from commercially obtained samples of rope were digested with the collection of restriction enzymes listed in Table 1. The results are shown in Fig. 2. Patterns predicted from the sequence analysis of the DNA were obtained for all species. It is notable, however, that the identification by DNA did not always match the packaging label. A sample of rope labeled "hemp" was shown to be made of flax (Fig. 2*D*). One sample of "jute" rope was shown to be made of hemp (Fig. 2*E*). A second sample of jute rope, obtained from a different hardware store, clearly contained jute but showed restriction bands indicating a second component (Fig. 2*F*). The second component was found in three independent subsamples, representing four extractions of the jute rope sample.

The cutting pattern of the second DNA in the sample from jute rope was similar to that of abaca. However, the second type of plant may be *Hibiscus* L., as *Hibiscus* is commonly added to jute to make it manageable (2). The identification of this jute rope was done with the aid of Table 1. *Pst*I did not cut, excluding sisal and hemp. Although *Sac*II cut, it did not give bands of 385 bp and 386 bp, thereby eliminating flax. The presence of abaca could not be disproved. As the sample contained a mixture of plants, a definitive identification was difficult. Additional sequence analysis would need to be performed to confirm the identity of the second plant DNA in the sample.

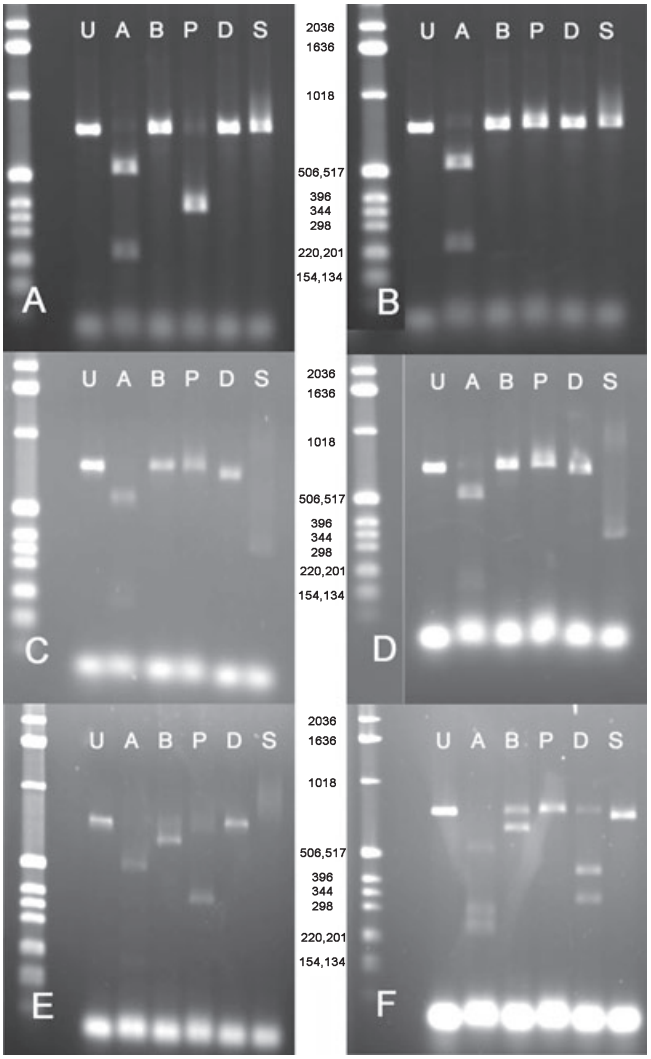

FIG. 2—*The patterns of DNA fragments from restriction digests of rbcL amplicons produced using template DNA from different rope samples. A, sisal; B, abaca; C, flax; D, flax (sample sold as ''hemp''); E, hemp (sample sold as ''jute''); F, jute. Numbers in the center column give the sizes of DNA standard fragments in base-pairs. Column labels indicate the restriction enzyme used: U, Uncut standard; A,* Acc*I; B,* Bam*HI; P,* Pst*I; D,* Dra*I; S,* Sac*II.*

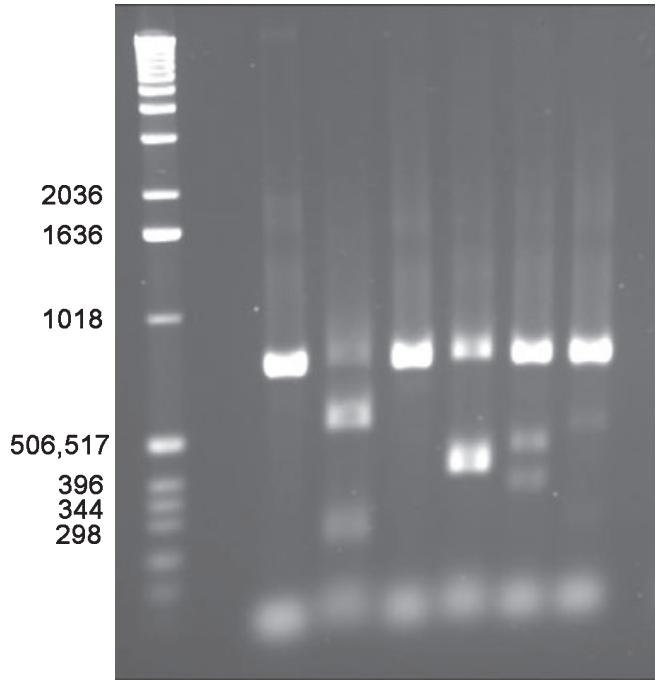

FIG. 3—*The pattern of DNA fragments from restriction digests of the rbcL amplicon produced using template DNA from grass-stained sisal rope. Compare the pattern to that in Fig. 2A.*

Testing Contaminated Rope

The rbcL gene was used in this experiment so that mammalian DNA and fungal DNA could be eliminated as contaminants. But what happens if a piece of rope is found covered with a plant contaminant, for example grass? Is it possible to make an identification of a rope species in the presence of the contamination? Sisal rope was rubbed with lawn grass (species undetermined), DNA was extracted, and an amplicon synthesized and cut with the standard collection of restriction enzymes (Fig. 3). The contaminated rope gave results different from uncontaminated rope. It would be difficult to determine which bands belong to the contaminant and the actual rope if one did not know the identity of the actual rope.

Discussion

The main objectives of this project were to see if DNA could be obtained from rope and used to differentiate between ropes made from flax, sisal, abaca, hemp, and jute. While there has been a considerable amount of work with plants in forensic science (13), no work using DNA analysis to identify the plant material comprising rope has been previously reported.

The experiments in this research demonstrated that parenchyma cells accompany the rope fibers during the crude fiber extraction process, and that DNA, presumably from the parenchyma cells, is present and can be obtained from rope samples. The identity of the DNA that was extracted from the rope was shown to be species specific and was matched to DNA from plants identified by experts, such as dried plants obtained from a herbarium and fresh samples from greenhouses. In most cases, the results of the restriction analysis from rope samples matched the results predicted from the sequences of well-identified plant species. For some rope samples, the results were not as expected, but discrepancies could be shown to result from misidentification of the rope by the manufacturer or by the inclusion of fibers of more than one species.

The results of the restriction digest of the grass stain-covered sisal rope indicated that if this were an unknown piece of rope, it would be difficult to determine its identity. It was difficult to determine which bands belonged to the rope and which to the contaminant (Fig. 3). Through analysis of restriction bands, three out of five types of rope could be eliminated, resulting in the unknown being either sisal rope with contamination or abaca rope with contamination. A detailed analysis of the base sequence of the mixed DNA amplicons was performed, but was not helpful. It is possible that other restriction enzymes could differentiate between these two types of rope (Table 2). Cloning the PCR amplicons and analyzing individual clones to separate DNA components might be necessary. As a general rule to minimize contamination, samples should be taken from the interior strands of a rope. However, it will still be difficult to distinguish between a ''mixed'' rope, for example one containing jute and *Hibiscus*, and a rope where contamination is present. Extensive contamination by the same unique, complex mixture of contaminants might be used to support, although not to

TABLE 2—*Restriction enzymes that differentiate pairs of rbcL amplicons (13).*

	Rope Type that is not Cut				
	Hemp	Abaca	Jute	Flax	Sisal
Rope Type that is Cut:					
Sisal	*Acu*I, *Bsm*FI, *Ban*II, *Bsr*BI, *Bcl*I, *Bsr*I, *Bmr*I, *Tau*I, *Eco*57MI, *Fnu*4HI, *Psh*AI, *Sac*I, *Sfa*NI	*Acu*I, *Bcl*I, *Bfu*AI, *Bmr*I, *Fnu*4HI, *Sfa*NI, *Sfc*I, *Spe*I, *Tau*I	*Acu*I, *Afl*III, *Apo*I, *Ban*II, *Bcl*I, *Bfu*AI, *Bmr*I, *Bpm*I, *Bsi*HKAI, *Bsp*1286I, *Bsp*MI, *Bsr*BI, *Pci*I, *Bsr*I, *Bst*API, *Eco*57MI, *Psh*AI, *Psi*II, *Sac*I, *Sfa*NI, *Sfc*I, *Sna*BI, *Spe*I, *Sph*I	*Afl*II, *Alw*I, *Apo*I, *Bcl*I, *Bfu*AI, *Bsi*HKAI, *Bsm*FI, *Bsp*MI, *Bsr*BI, *Bst*XI, *Bst*YI, *Bts*I, *Eco*RI, *Fnu*4HI, *Pci*I, *Psh*AI, *Sac*I, *Sfa*NI, *Sfc*I, *Spe*I, *Tau*I, *Tsp*RI	
Flax	*Acc*65I, *Aju*I, *Ban*I, *Ban*II, *Bcc*I, *Bmr*I, *Bpm*I, *Bsi*EI, *Bsp*CNI, *Bsr*I, *Bsu*36I, *Btg*ZI, *Dra*I, *Eco*57MI, *Fau*I, *Hpa*II, *Hpy*99I, *Kpn*I, *Msp*A1I, *Msp*I	*Acc*65I, *Aju*I, *Ale*I, *Ban*I, *Bmr*I, *Bsi*EI, *Bsp*CNI, *Bst*Z17I, *Btg*I, *Dde*I, *Hpy*99I, *Msp*A1I, *Nla*IV, *Taq*I	*Acc*65I, *Aju*I, *Ban*I, *Ban*II, *Bcc*I, *Bmr*I, *Bsp*1286I, *Bsp*CNI, *Bsr*I, *Bst*API, *Bst*Z17I, *Btg*ZI, *Eco*57MI, *Hpy*99I, *Kpn*I, *Mae*III, *Msp*I, *Psi*I, *Sna*BI, *Sph*I, *Tsp*GWI		*Acc*651, *Aju*I, *Ale*I, *Ban*I, *Bcc*I, *Bsi*EI, *Bst*Z171, *Btg*I, *Btg*ZI, *Hpa*II, *Kpn*I, *Mae*III, *Mme*I, *Asp*A1I, *Msp*I, *Nla*IV, *Taq*I, *Tsp*GWI, *Xcm*I
Jute	*Bsi*EI, *Bsm*FI, *Bss*KI, *Bst*NI, *Fau*I, *Fnu*4HI, *Hin*dIII, *Hph*I, *Msp*A1I, *Psp*GI, *Scr*FI, *Sty*D4I, *Tau*I, *Xba*I	*Ale*I, *Bsi*EI, *Bss*KI, *Bst*NI, *Bst*XI, *Btg*I, *Dde*I, *Fnu*4HI, *Hin*dIII, *Msp*A1I, *Nla*IV, *Psp*GI, *Scr*FI, *Sty*D4I, *Taq*I, *Tau*I, *Xba*I		*Alw*I, *Bsm*FI, *Bss*KI, *Bst*NI, *Bst*XI, *Bst*YI, *Bts*I, *Fnu*4HI, *Hin*dIII, *Psp*GI, *Scr*FI, *Sty*D4I, *Tsp*RI, *Xba*I	*Ale*I, *Bst*NI, *Btg*I, *Hin*dIII, *Hph*I, *Mme*I, *Msp*A1I, *Nla*IV, *Psp*GI, *Scr*FI, *Sty*D41, *Taq*I, *Xba*I, *Xcm*I
Abaca	*Ban*II, *Bcc*I, *Bpm*I, *Bsm*FI, *Bsr*BI, *Bsr*FI, *Bsr*I, *Btg*ZI, *Eco*57MI, *Fau*I, *Hpa*II, *Hph*I, *Msp*I, *Psh*AI, *Sac*I, *Tsp*45I,		*Afl*III, *Apo*I, *Ban*II, *Bcc*I, *Bpm*I, *Bsi*HKAI, *Bsp*1286I, *Bsr*BI, *Bsr*FI, *Bsr*I, *Bst*API, *Btg*ZI, *Eco*57MI, *Eco*RI, *Hpa*II, *Mae*III, *Msp*I, *Pci*I, *Nde*I, *Psh*AI, *Psi*I, *Sac*I, *Sna*BI, *Sph*I, *Tsp*45I, *Tsp*GWI	*Alw*I, *Apo*I, *Bsi*HKAI, *Bsm*FI, *Bsr*BI, *Bsr*FI, *Bst*YI, *Bts*I, *Eco*RI, *Nde*I, *Pci*I, *Psh*AI, *Sac*I, *Tsp*45I, *Tsp*RI	*Bcc*I, *Bsr*FI, *Hpa*II, *Hph*I, *Mae*III, *Mme*I, *Msp*I, *Nde*I, *Tsp*451, *Tsp*GWI, *Xcm*I
Hemp		*Ale*I, *Alo*I, *Alw*NI, *Ase*I, *Ava*II, *Bbs*I, *Bfu*AI, *Bsg*I, *Bsp*DI, *Bsp*MI, *Bss*HII, *Bst*XI, *Bst*Z17I, *Btg*I, *Cla*I, *Dde*I, *Eco*O109I, *Hpy*188I, *Nla*IV, *Sfc*I, *Spe*I, *Taq*I, *Xmn*I	*Afl*III, *Alo*I, *Alw*NI, *Apo*I, *Ase*I, *Ava*II, *Bbs*I, *Bfu*AI, *Bsg*I, *Bsi*HKAI, *Bsp*1286I, *Bsp*DI, *Bsp*MI, *Bss*HII, *Bst*API, *Bst*Z17I, *Cla*I, *Eco*O109I, *Eco*RI, *Nde*I, *Hpy*188I, *Mae*III, *Pci*I, *Psi*I, *Sfc*I, *Sna*BI, *Spe*I, *Sph*I, *Tsp*GWI, *Xmn*I	*Afl*III, *Alo*I, *Apo*I, *Ase*I, *Ava*II, *Bbs*I, *Bfu*AI, *Bsg*I, *Bsi*HKAI, *Bsp*DI, *Bsp*MI, *Bss*HII, *Bst*XI, *Bst*YI, *Bts*I, *Cla*I, *Xmn*I, *Eco*O109I, *Eco*RI, *Hpy*188I, *Nde*I, *Pas*I, *Pci*I, *Sfc*I, *Spe*I, *Tsp*RI	*Ale*I, *Bsg*I, *Alo*I, *Bsp*DI, *Alw*NI, *Bss*HII, *Ase*I, *Bst*Z171, *Ava*II, *Btg*I, *Bbs*I, *Cla*I, *Dde*I, *Mme*I, *Hpy*1881, *Mae*III, *Eco*O109I, *Nde*I, *Nla*IV, *Taq*I, *Tsp*GWI, *Xcm*I, *Xmn*I

This table shows restriction enzymes that can aid in distinguishing between the listed rope sources to support a conclusion based on the recommended procedure (Table 1, Fig. 2).

prove, a finding that two samples represent segments of the same rope.

Analyzing the DNA of natural fiber rope components can be valuable for several reasons. First, DNA analysis may require less experience than microscopy. In microscopy, rope is identified through crystals, pits, the color, lumen, cell wall, and cross-markings. According to Wiggins (14), a considerable amount of experience and skill is needed to identify rope fibers through microscopy. Second, the current microscopic examination method may not be capable of unambiguously characterizing all natural fibers. DNA analysis can strengthen identification. Third, DNA analysis may have other applications, such as in archaeology—determining the source, local or imported, of cordage found at an excavation. Finally, with advancements in technology, DNA analysis could eventually provide a background for identifying individual samples of rope, in addition to the rope's botanical origin.

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Additional information and reprint requests:
Terence M. Murphy, Ph.D.
Department of Plant Biology
University of California
Davis, CA 95616
E-mail: tmmurphy@ucdavis.edu