DNA in Your Jeans? Effect of Abrasion and Bleaching on DNA Tagged Denim

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Introduction

The United States produces 20 million bales of cotton fiber per year.[1] The most commonly-produced US variety is *Gossypium hirsutum*, which is popularly known as Upland cotton. This type of cotton is ideal for denim because of its strength and durability.

Having the ability to label your fibers' origin could add value to American brands that produce jeans. Denim jeans have become both a fashion item and a commodity product, with US sales reaching US$13.7 billion dollars in 2016.[1] Americans enjoy wearing cotton and look for the cotton label when making purchases. US consumers would like to know that the clothes they wear were made using American Upland cotton. Manufactures of apparel products, such as denim jeans, may gain sales if they could definitively identify this cotton's origin, but there is a lack of commercial test methods to determine where the fiber was grown. Most American cotton is shipped overseas and combined with other cotton. In these types of blends, American cotton fiber can lose its identity.

This project investigated if fibers tagged with a unique DNA molecular tag applied prior to stone and bleach washing could be identified after jeans were treated with the tagged fibers. A denim fabric was chosen because it is a unique product, distinguished by its washed styles and distressed look. Denim jeans are typically exposed to a stone and bleach wash, which is the harshest type of treatment made to any apparel product. If we could identify the markers after this type of wash, it is believed that any cotton product could be identified at any stage in the supply chain.

The DNA molecule is used to store most of the information required to support life. In human beings, the DNA differs enough among individuals that it is unique and may be used as a molecular tag. Although cotton contains its own DNA, it may be compromised by typical industrial processing such that a textile made from it lacks enough information for high resolution DNA identification of the fiber.

Applied DNA Sciences recently developed a technology to produce small DNA fragments or identifiers on an industrial scale. Rather than producing such DNA in a living creature, purified enzymes are used to manufacture DNA fragments, each one containing enough information to be used as a DNA-based "molecular bar code," much like an ordinary ink bar code on a label. There are billions of different DNA tag bar code combinations possible for any small DNA fragment produced enzymatically.

DNA tags can be applied during the cotton ginning phase, allowing the fiber to be tracked and authenticated throughout the entire supply chain, from the raw fiber stage all the way to the retail shelf. An aqueous solution of the DNA tag and water wicks into the hydrophilic cotton fiber, where the tag binds firmly to the cotton using a non-covalent bond and resides there throughout the manufacturing processes. DNA tagging protects the cotton supply chain by providing assurance of quality and provenance, and helps brands guarantee label claims with certainty. By using different DNA tags, different products can be tracked. For instance, one tag can be used for a particular farm growing Pima cotton and another, different tag for Upland cotton grown at a different farm. This will help ensure fiber transparency throughout the supply chain.

DNA Tag Production

The two DNA tags used for this study were prepared at Applied DNA Sciences on an industrial scale, via very large-scale enzymatic production. The DNA was produced at the 1-liter scale via polymerase chain reaction (PCR) technology. Following PCR production, the DNA tags were concentrated, purified by preparative anion exchange chromatography, and desalted by ethanol precipitation. The

resulting DNA tags were diluted into a proprietary activation solution to enhance its adsorption to the cotton fabric.

DNA Analysis by PCR-Capillary Electrophoresis

The DNA tags were analyzed before and after stone washing and bleaching treatments using standard forensic methods of PCR[2,3] followed by capillary electrophoresis (CE).[4,5] For analysis, a 1–cm^2 denim square was cut from each of the samples and then soaked in 0.5 mL of extraction buffer at 95 °C for 30 minutes. After heat extraction, the water solution phase was recovered and subjected to ChargeSwitch (Thermo Corp) magnetic-bead DNA processing, which was used to both purify and concentrate the DNA eluted from the denim.[6]

The DNA recovered from the magnetic beads was then subjected to analytical PCR. Briefly, the DNA concentrate was subjected to PCR under conditions optimized to be specific for the DNA tags used, and not for others. Following PCR, 2 µL of the PCR-amplified product was subjected to high resolution CE on an ABI-3500 capillary electrophoresis device (Applied Biosystems).

The DNA used to tag the denim migrated as sharp, specifically-amplified, and discrete DNA bands in the resulting CE profile. Positive control experiments (with genuine DNA stock material) and negative controls (where PCR was performed on a water control) were performed in parallel to confirm both sensitivity and specificity.

PCR

PCR is a fundamental tool used routinely throughout the world in forensic science and molecular biology. This method allows a small sample of DNA to be amplified up to 10^9 times its initial quantity. The procedure is composed of three distinct temperature-dependent stages in which the template molecule (in this case the unique DNA tags used for denim stone washing) is first denatured. Then, primers are annealed to each end of the resulting single strands.[7] Primers are designed to uniquely identify a specific region of a DNA fragment to ensure that any other DNA in the sample will not amplify (in this case, it would avoid the endogenous cotton DNA that would be mixed into the sample).[8] Once the PCR primers bind to the appropriate sites on the DNA tag, the polymerase enzymes extend the new strand by incorporating building blocks (nucleotides) onto the primers.[7] The building blocks are laid down so as to complement the template strand. In the final stage of the cycle, the enzyme ligates the nucleotide building blocks that are incorporated into the new strand. The thermal cycle is repeated about 35 times, thus exponentially increasing the quantity of template DNA in the sample. Once PCR has completed, the DNA is analyzed by CE, where DNA fragments are separated by size and detected via fluorescent signals to visualize the DNA electronically.[8]

CE

CE is used to determine the fragment size of a DNA strand that has been amplified via PCR. To identify the unique tag used for stone washing, the PCR amplified product is electrokinetically injected into the CE column (a long, thin glass capillary coated with a polymer).[9] As the DNA gets pulled through the capillary column under the influence of an electric field, fragments are separated based on length because they move through the polymer with a speed that is inversely proportional to the length.

DNA Tagging of Cotton Fiber

In the laboratories at the Fashion Institute of Technology, a State University of New York, a denim fabric was treated with two unique DNA tags, stone and bleach washed, and then analyzed for those tag signatures. The denim fabric contained 98% cotton and 2% spandex. The warp was indigo dyed and the filling was a white yarn. The fabric weighed 7.0 oz/yd^2 and was constructed with a 3 × 1 right-handed twill weave. Four samples measuring 15 × 15 in. were prepared for this experiment. The first sample was to ensure the DNA tags were absorbed by the fiber (Fig. 1). The second sample was DNA-tag treated and washed to a medium shade of indigo, typically seen in the fall season (Fig. 2). The third sample was DNA-tag treated and washed to a lighter indigo shade that would be seen in the spring and summer fashion seasons (Fig. 3). Sample four was the control sample with no DNA material (Fig. 4). A hand-held spray bottle was used to apply the DNA solution (concentration of both fragments was 0.5−1.0 ng /µL) to the denim fabric to the point where the activated DNA-water solution would increase the weight of the denim fabric, and then the samples were dried for 20 minutes using a Sussex garment oven set at 210 °F.

A 30-lb capacity soft mount continental washer/extractor model EHO30 frontloading machine (Girbau Inc.) was used for the denim wash using the following formulas.

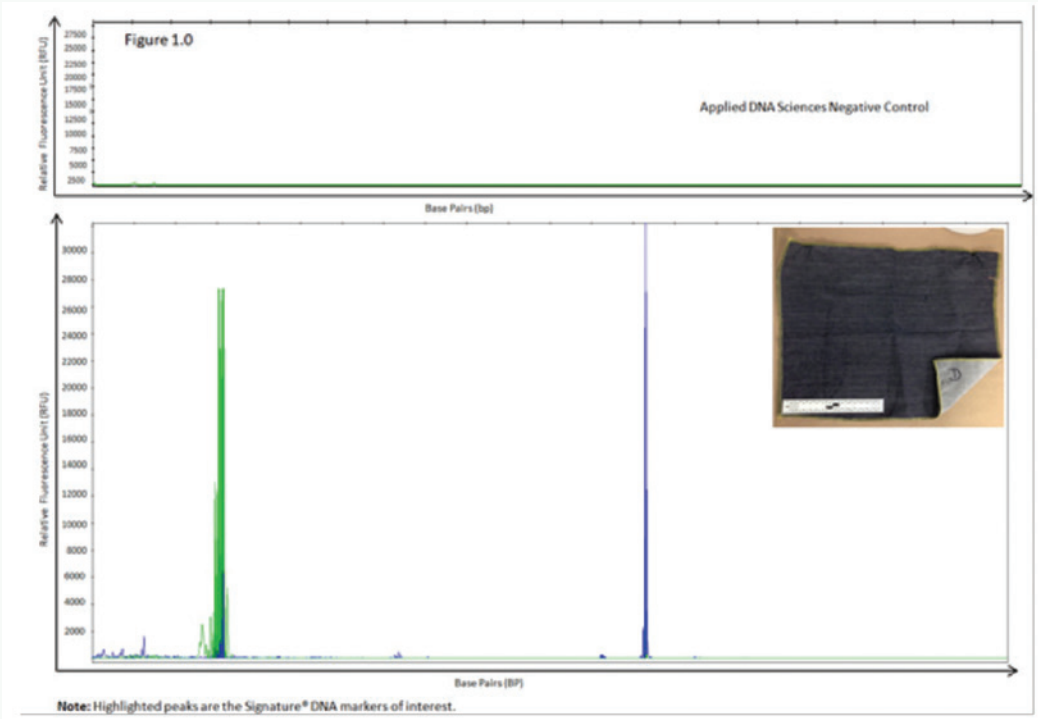

Fig. 1. CE of the denim sample that absorbed the DNA solution, but was not stone and bleach washed. Both fragments gave a strong DNA signal.

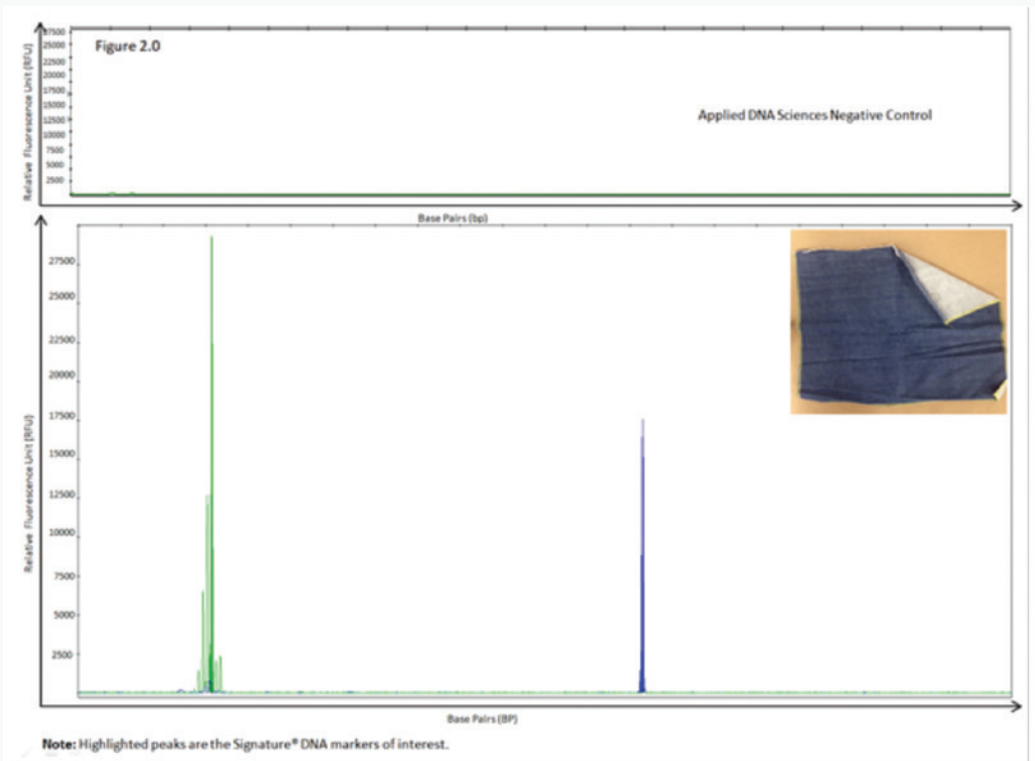

Fig. 2. CE of the Medium Wash denim sample confirmed the presence of DNA. The picture shows the color of the denim faded, yet the DNA signal remained strong as shown by the height of the peaks on the y-axis. Sodium hypochlorite can destroy DNA through oxidative damage. Cleaving of DNA inhibits PCR, which contributes to a lower yield of DNA when analyzed via CE.

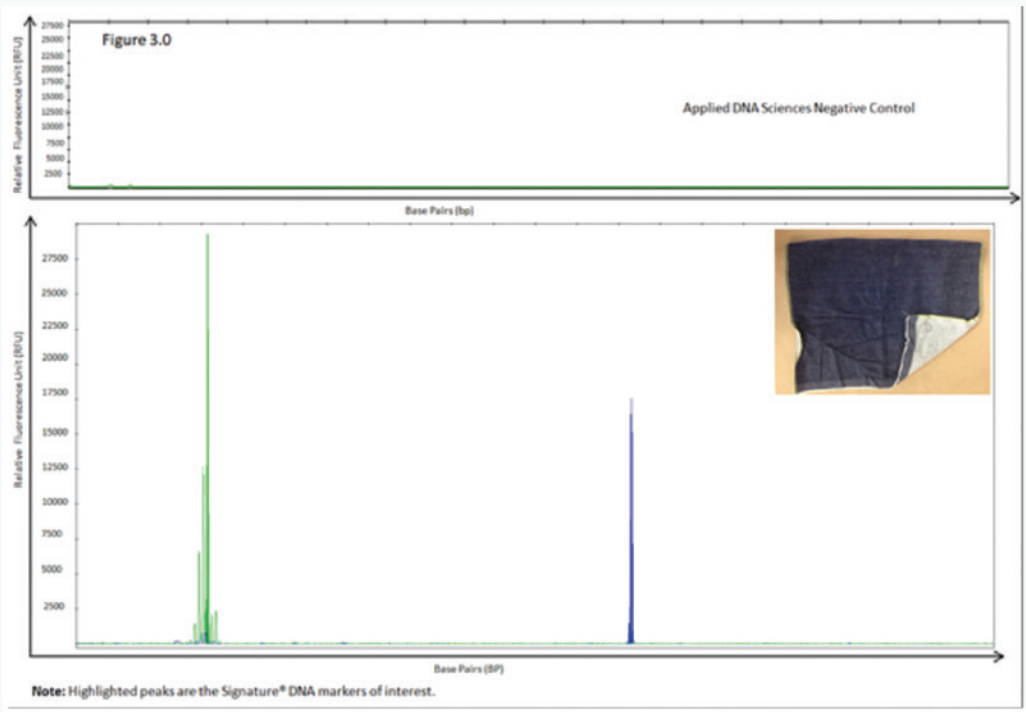

Fig. 3. CE of the Light Wash denim sample confirmed the presence of DNA. The color of the denim faded as indicated in the image on the top right corner of the CE scan; however, the DNA signal remained strong, indicating that the DNA survived the stone washing process.

Medium Wash

Fill machine with 30 liters of water and soak for 15 minutes to wet out the denim panel. Drain, refill with 1:1 ratio of pearl-lite for abrasion and one liter of chlorine bleach for shade change and soak for 15 minutes. Drain and rinse using hot water with a silicone softener for 10 minutes. Tumble dry at medium heat for 20 minutes.

Light Wash

Fill machine with 30 liters of water and soak for 15 minutes to wet out the denim panel. Drain, refill with 1:1 ratio of pearl-lite for abrasion and two liters of chlorine bleach for shade change and soak for 15 minutes. Drain and rinse using hot water with a silicone softener for 10 minutes. Tumble dry medium heat for 20 minutes.

Results

The samples were analyzed in the laboratories of Applied DNA Sciences for the DNA tags. The CE graph produced two peaks, one green (small DNA tag) and one blue (large DNA tag); both represent different DNA fragments (Figs. 1–4). The x-axis represents the size of the fragment in base pairs. FIT was given a combination of two tags (one large and one small) as opposed to just one, which allowed for a greater number of unique markers to be created within the Applied DNA Sciences database. Each fragment required a different set of primers for PCR amplification and each primer had a different fluorescent tag, which can be visualized by the different colors in the figures. The untreated sample did not contain these tags. The three samples that received the DNA yielded strong signals indicated by the height of the blue and green highlighted peaks on the y-axis (labeled RFU, relative fluorescence units). During the electrokinetic injection, where DNA is pulled through the CE capillary, the DNA is separated based on length. The fluorescent tag on the primer is also detected, which emits a signal that can be visualized as a peak on the graph in the figures. The strength of the fluorescent signal correlates to the amount of DNA that is being pulled through the capillary. Although CE can be viewed as "semi-quantitative," it cannot determine the exact amount of DNA in a given sample; CE is used more qualitatively to determine DNA "presence." However, a strong signal on the y-axis of a CE graph still indicates a plentiful DNA in the starting sample. When reviewing CE data, a strong signal is a peak that is >10,000 relative fluorescence units (RFUs). The instrument's highest limit of detection is 30,000 RFUs.

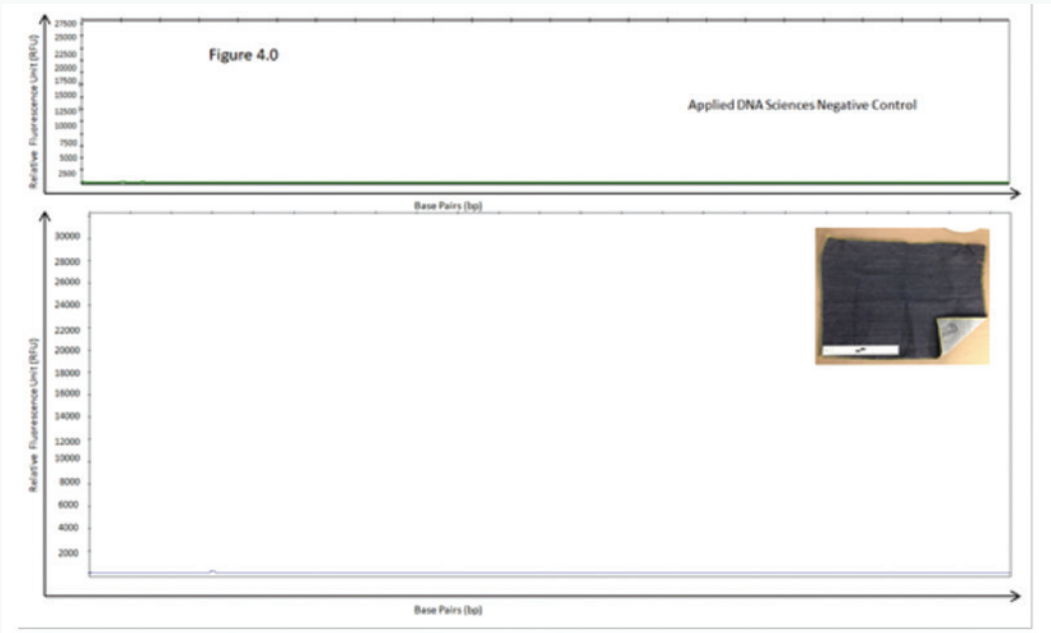

Fig. 4. CE of the control sample, to which no DNA was added. It shows that there was no signature DNA marker in this sample.

Conclusions

This experiment provided a laboratory proof of concept that after a typical distress wash following DNA tag application, a substantial fraction of the original DNA molecular tag was recovered and identified. These results are a first step in pursuit of an industrial scale model of DNA fabric tagging, where the tag is introduced at the ginning mill, the first process step after harvest.

The data suggests that a substantial fraction of the DNA tag remained intact and suitable for high-quality, forensic-scale PCR-CE analysis, even after a highly destructive finishing protocol such as stone washing. Based on that observed stability, along with future work on preparing denim fabric from DNA tagged cotton, DNA tags of this kind may soon be ready for testing at a full manufacturing facility to verify the authenticity of the finished denim garment.

Based upon the laboratory success revealed here, and rapidly growing sophistication in the use of DNA as a molecular tag, it is now possible to assign several different (unique) DNA molecular tags to any cotton product.

Future projects will tag cotton intended for denim use and then tag other premium fibers such as cashmere. Some manufactures may want to use this tool to identify their products and prevent counterfeit product from entering the market and diluting their brand image. Other projects could tag products such as recycled polyesters and tracking it through the supply chain. This process, extended to incorporating DNA tagged fibers into fabrics, would add much needed transparency to the textile industry by validating products from the fiber to the finished product.

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