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Intermolecular attraction between DNA and fibers: A robust template for *in-situ* processing and PCR

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ABSTRACT

It is thought that the operation of strong attractive intermolecular forces that can exist between DNA and chains of natural fibers like cotton and wool have made microsatellites amenable to in-situ processing and PCR multiplexing. In this study, the binding properties of six types of fabrics of different monomeric composition with DNA were investigated. Blood dried on cotton, rayon, nylon, wool, acrylic and polyester were tested. Multiplex PCR using the AmpFlSTR® Identifiler kit on a 1mm to 2mm diameter bloodstain disc successfully amplified the DNA in-situ from cotton, rayon, nylon and wool. There was partial amplification from acrylic and null amplification from polyester. The efficiency of the in-situ technique for multiplex genotyping of microsatellites provides the capacity for (I) Genotyping large sample numbers such as in DNA databasing work, (II) A platform for employment of robotics, and (III) The versatility and robustness of solid-media processing.

KEYWORDS

Intermolecular bonding;
Microsatellite;
AmpF*l*STR Identifiler;
in-situ DNA profiling;
Bloodstain typing on fabrics.

INTRODUCTION

The binding capacity of fibrous materials such as nitrocellulose and nylon for DNA has been extensively utilized in techniques like Southern Blotting after the procedure was developed by Edward M. Southern at Edinburgh University in the 1970s[1]. Direct solid phase purification and profiling of blood and buccal DNA on FTA cards has utilized the binding of DNA to a cellulose-based matrix[2,3,4].

Fibers are long, thin, threadlike bits of polymeric material characterised by great tensile strength in the direction of the fiber[5]. Natural cellulose fibers, e.g. cotton or man-made cellulose fibers, e.g. rayon chemically consists of linked glucose units with free hydroxyl units which can provide sites for secondary and tertiary hydrogen bond linkages. Similarly, natural protein fibers like wool or synthetic polyamide fibers such as nylon are long chains of polyamide with amide(-CONH-) linkages with the potential for secondary and tertiary linkages held by hydrogen bonding(N—H—O bond) between chains. By comparison, the 'introvert'-like manufactured fibers of polyester and acrylic are long chain synthetic polymers composed respectively of units of ester of a substituted aromatic carboxylic acid and acrylonitrile which are less polar with no capacity for hydrogen bonding[6].

Biological fluids e.g. blood, semen or saliva are im-

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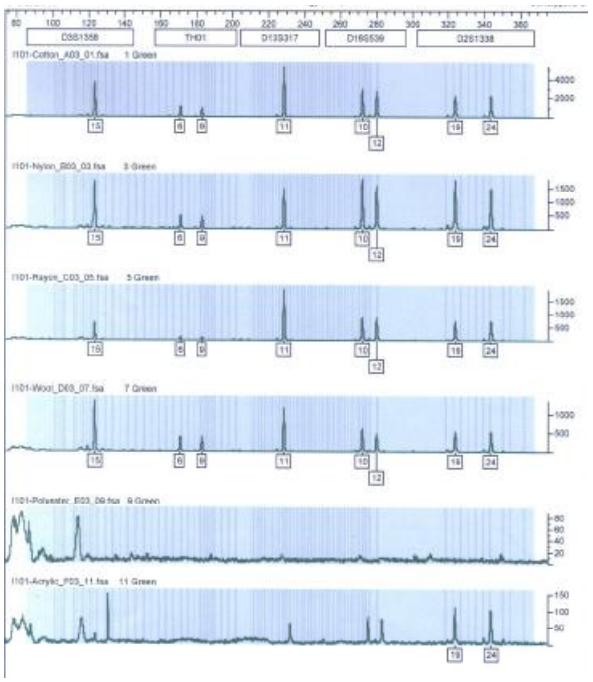

Figure 1

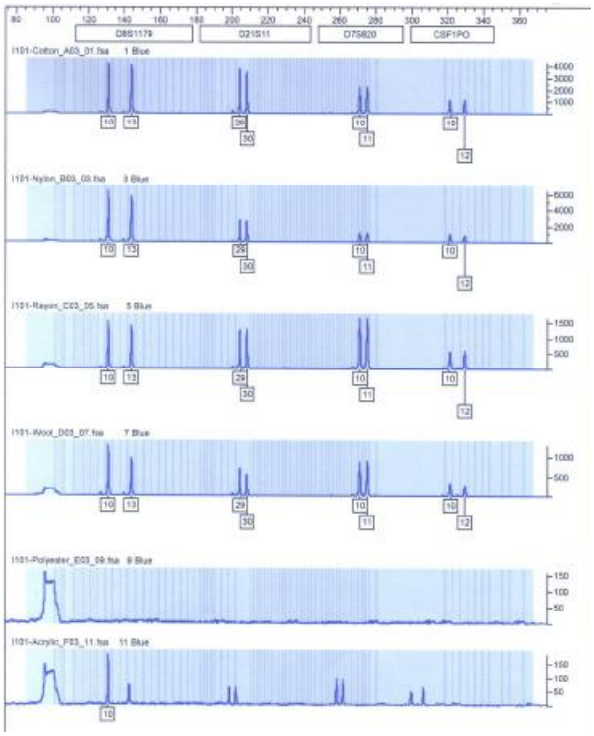

Figure 2

portant and reliable sources of DNA and when stained on a binding fiber present a robust format for processing and PCR (Polymerase Chain Reaction)[7]. The ability to perform multiplex genotyping by *in-situ* amplification from a small stained area(approximately 1-4mm^2 area) without prior extraction of DNA from the fabric exploits the attractive intermolecular forces between DNA and chains of fiber. This technique of DNA genotyping was investigated with various types of fabrics(fibers).

EXPERIMENTAL

A preserved blood sample(with normal blood counts) was loaded and dried on six types of standard white fabrics namely cotton, rayon, nylon, wool, acrylic and polyester. A 1.2-mm diameter bloodstain disc was punched from the cotton, rayon, nylon and wool fabrics, and a 2-mm diameter disc was taken from acrylic and polyester fabrics due to the relatively bigger spread area. The stains were purified *in-situ* with one wash in 10mM sodium hydroxide and rinsed twice with TE(Tris-EDTA) buffer and subsequently amplified directly using the AmpF*l*STR® Identifiler™ kit(a 16-loci STR [short tandem repeats] amplification kit) following manufacturer's instructions. The DNA typing was carried out on the Applied Biosystems 3100 Genetic Analyzer and genotypes were analyzed by GeneScan (version 3.7) and Genotyper 3.7 software[7].

RESULTS

The conclusions from the experiments were derived from the concordance of at least three experiments and were found to be consistent when the experiments were repeated with blood specimens from other sources.

The DNA from cotton, nylon, rayon and wool fabrics were successfully amplified generating full DNA profiles at all 16 loci of the AmpF*l*STR Identifiler kit(see Figures 1,2,3 & 4). A partial profile was obtained from acrylic and no DNA profile was derived from polyester. It is interesting to note that for acrylic, amplification for the larger alleles was generally more successful.

DISCUSSION

The gross characteristics of fibers are reflected on the molecular level-the molecules, too, are long, thin and threadlike and lined up stretched out in the direction of the fiber. The key requirements of a fiber are a linear molecular shape that permits side-by-side align-

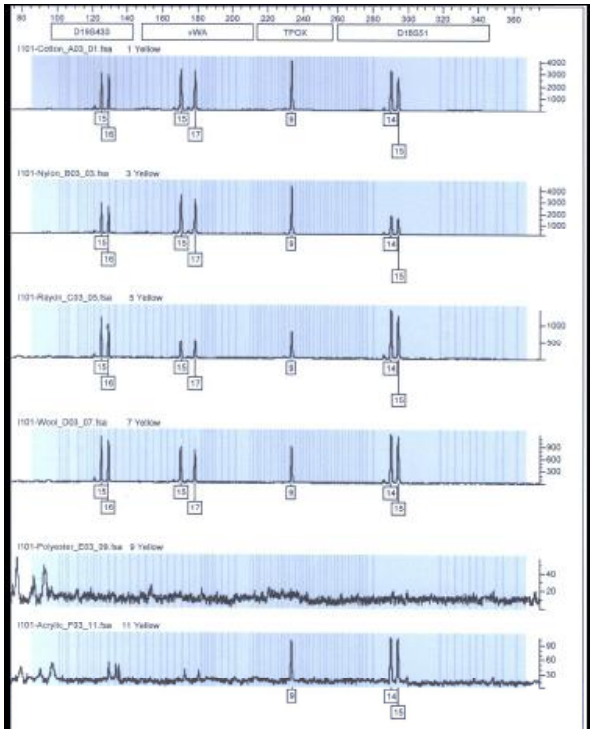

Figure 3

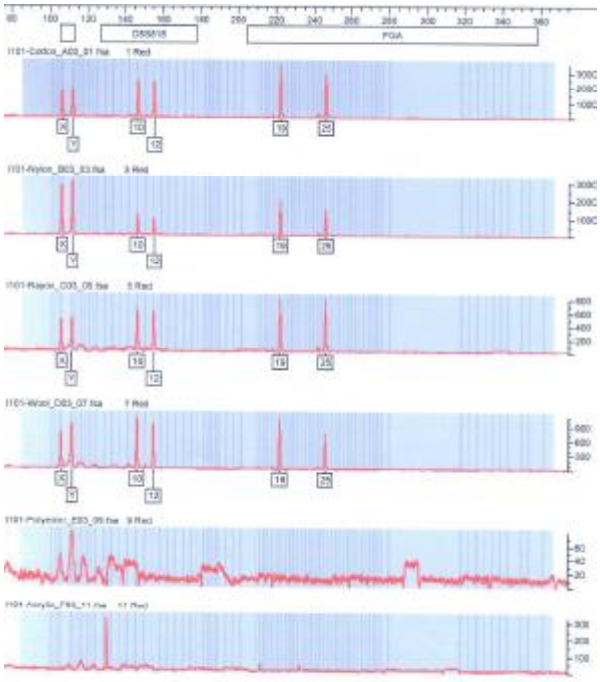

Figure 4

ment, and strong intermolecular forces to maintain this alignment. The presence of functional groups on fibers permit molecular chains (whether intrinsic or extrinsic like nucleic acid chains) to be held together by the operation of strong intermolecular forces-hydrogen bonding, dipole-dipole attractions and van der Waals forces[5]. The O-H groups of cotton and rayon and the N-H groups of nylon and wool are capable of strong hydrogen bonding with nucleic acid chains resulting in powerful intermolecular attractions. Polyester and acrylic contain polar carbonyl (C=O) and cyano (C≡N) groups which may permit weak dipole-dipole attraction with nucleic acid chains.

Hydrogen bonding between the molecular chains of nucleic acids and fibers like cotton, nylon, rayon and wool permit side-by-side alignment with strong intermolecular attractive forces to maintain this alignment[5], hence presenting an efficient format for *in-situ* amplification of the DNA on the solid template. The apparent weaker dipole-dipole attractions between acrylic with nucleic acid chains may not facilitate efficient direct solid-template amplification. The null amplification from polyesters could be due to ineffective intermolecular forces between polyester and nucleic acids. The efficiency of the *in-situ* technique for the six different fabrics in decreasing order are cotton=nylon>rayon>wool> acrylic>polyester.

The successful genotyping of DNA directly from binding fabrics like cotton and nylon without an extraction process is time-and-cost efficient and provides the capacity for genotyping work on a large-scale such as in population studies. As it involves solid-media processing this technique becomes amenable to robotics employment with the added advantages of robustness and versatility in tracking and storage.

Sources of DNA from other biological fluids such as semen and saliva are indicated in future studies.

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