Molecular speciation of animal fibres

Paul F Hamlyn, Andrew Bayliffe, Brian J McCarthy and Gordon Nelson of BTTG discuss the new developments in techniques for the objective analysis of animal hair.

Animal hair fibres are based on cross-linked proteins known as keratins. The major commercial fibre in this group is sheep's wool but other fibres of importance are those obtained from animals such as goats (cashmere and mohair), the Bactrian camel and the South American camelids. The latter are known as speciality or luxury fibres due to their softness, lustre and scarcity relative to other fibres.

The development of analytical techniques for the objective analysis of animal fibres has long been desired. Positive identification of animal fibres is important, not only in textiles where regulations govern the labelling of fibre content, but also in forensic science, archaeology and other investigative fields. In the case of the textile industry, garment labelling requirements now demand exact analytical methods, particularly as expensive fibres like cashmere are increasingly found to be adulterated with cheaper fibres such as wool and yak hair.

Traditionally, accurate fibre identification has depended on expert microscopists working with high-powered optical or electron microscopes. Chemically there is little difference between cashmere, wool and yak hair. Therefore microscopists have to rely on physical parameters such as internal morphology, fibre diameter and cuticle scale height. Detailed measurements have to be made on hundreds of individual fibres from each sample received for analysis. However, even skilled personnel working in this area may have great difficulty in positively identifying the components of fibre blends.

The requirement for objectivity has stimulated a variety of research initiatives in recent years. The chemical properties of speciality fibres have been widely examined. Techniques employed include amino acid analysis, the resolution of fibre polypeptides using one- and two-dimensional electrophoresis, and the measurement of the variability of the lipid component of the fibre cell membrane complex. Although some of these techniques are useful for close comparison between specific animal species, problems are found when the effects of different fibre processing regimes, local climate and diet are taken into account. Industry is keen to have access to analytical methods that are rapid, low-cost, accurate, applicable to material at all stages of production, and objective. Methods are required to identify fibres, detect adulteration of supplies and quantify blend components. It is increasingly apparent that a sophisticated and complex high technology solution will be required to satisfy all of these demands.

A breakthrough in speciality fibre analysis was made in the late 1980s when it was demonstrated that DNA (deoxyribonucleic acid), the chemical molecule that carries the hereditary information, was not only present in hair roots but could easily be extracted from animal hair shafts. This was an important development since some fibres, such as wool, are shorn rather than combed. DNA of sufficient quality to be used as a template in DNA hybridisation studies has been successfully isolated from scoured, bleached and dyed fabrics, as well as from raw fibre samples. This has led to the development of DNA fibre profiling techniques at the British Textile Technology Group (BTTG) for the specific identification of goat fibres, wool and yak hair.

Location of DNA in fibres

In the living cells of animals the DNA is contained inside spherical bodies known as nuclei and smaller cellular bodies known as mitochondria. Nuclei contained within individual immature hair follicles can be directly visualised using the technique of *in situ* DNA hybridisation and a high-powered microscope. As the follicle matures, the nuclei become elongated but the hybridisation signal does not extend into the more mature parts of the fibre where keratinisation has taken place (Figure 1). During the process of keratinisation the cells become metabolically inactive. However, remnants of the nuclear material con-

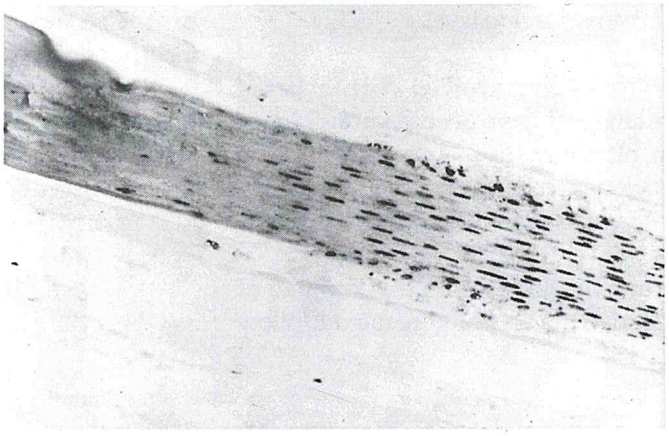


Figure 1
Visualisation of the nuclei inside a hair follicle using the technique of *in situ* hybridisation

taining DNA remain trapped inside the fibre. The recalcitrant and hydrophobic nature of fibres helps to protect the DNA from chemical and biological degradation.

Selection of target DNA sequences

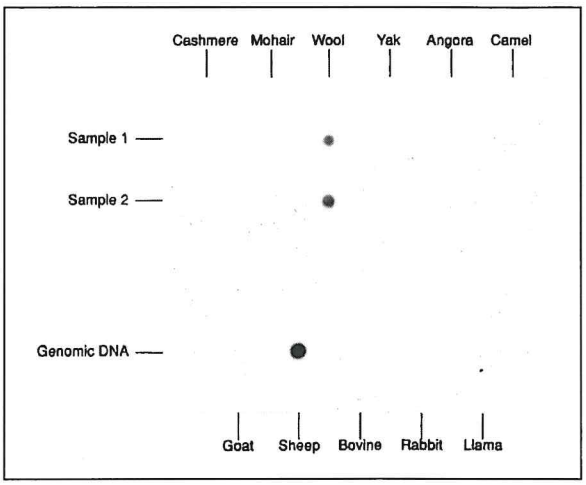
A prerequisite to fibre profiling is the identification of short DNA sequences unique to each species. It is an advantage if these sequences are present as multiple copies since this increases the sensitivity of the test. Once species-specific DNA sequences have been located (e.g. from databases containing published sequence information), complementary DNA sequences known as oligionucleotides can be constructed which, under carefully controlled conditions, specifically hybridise to the target DNA molecule giving a positive signal, confirming the presence of a particular fibre type. Oligonucleotides based on the repetitive DNA region known as Satellite II can be used to distinguish between DNA samples from closely related species such as sheep and goats. Satellite II DNA is present in high concentrations in the nuclei of many higher animals. Evolutionary pressure has produced a series of mutations in this DNA, resulting in natural variations between animal species.

Mitochondrial genes (e.g. cytochrome b) which are also present in high copy numbers have a faster evolution rate than nuclear genes and are proving useful in the design of species-specific primers for the analysis of processed fibres.

Conventional DNA hybridisation analysis

Conventional DNA hybridisation analysis is carried out using a simple dot-blot technique but is only useful for the analysis of raw fibres. DNA extracted from the fibre samples (20 g each) together with control samples of genomic DNA is denatured by boiling to convert the native double-stranded DNA molecules into single strands and then spotted onto a nylon membrane. A single-stranded preparation of the oligonucleotide to be used as the probe is then incubated with the membrane to allow hybridisation to take place between the probe and any complementary strands of DNA present in the sample. After free unhybridised probe has been removed by washing the membrane several times with a detergent solution, the hybrid double-stranded DNA molecules can be visualised by several different methods according to how the probe has been labelled (i.e. by using radioactive, colorimetric or luminescent labels). For example, a sheep-specific oligonucleotide probe can be used to detect the presence of wool but does not give a positive signal with DNA extracted from other types of fibre (Figure 2).

Figure 2
Hybridisation of a sheep-specific oligonucleotide probe to DNA extracted from various animal fibres; a positive signal was only obtained with wool DNA



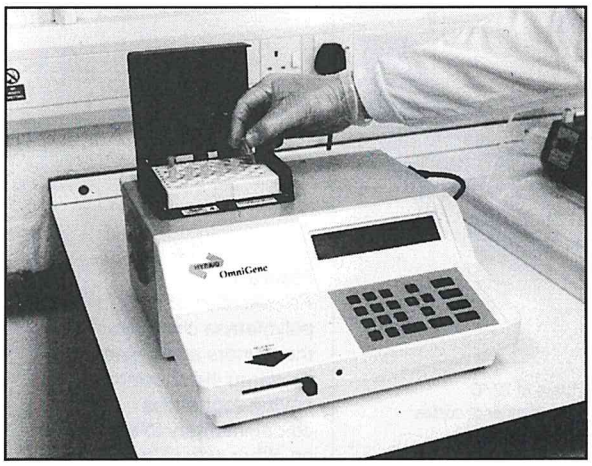


Figure 3
Thermal cycling machine used for carrying out the polymerase chain reaction

DNA amplification technology

With processed materials (scoured, bleached or dyed) and finished garments, the quality and quantity of DNA present within the fibre is much reduced and it is not possible to use conventional methods of DNA analysis. However, the development of *in vitro* DNA amplification technology known as the polymerase chain reaction (PCR) allows for the analysis of minute quantities of DNA. In essence, PCR reproduces what happens when the DNA inside a cell is replicated during cell division. By using an automated temperature cycling block (Figure 3) this enzymatic copying system can be used to produce millions of identical copies of a specified DNA sequence after only 1–2 hours (Figure 4). These copies provide sufficient DNA for further analysis (e.g. sequencing), or can simply confirm the presence of the target sequence in the sample. Species-specific primers have been designed to give different sized amplification products with wool, yak hair and cashmere, and these can be visualised by ultraviolet fluorescence after resolution on an agarose gel containing ethidium bromide (Figure 5). Thus the presence or absence of an adulterant can be quickly determined and this result can later be confirmed by DNA sequencing. The resulting DNA sequence can be compared to published sequences providing courts with evidence of an exact match that leaves no room for doubt. Because

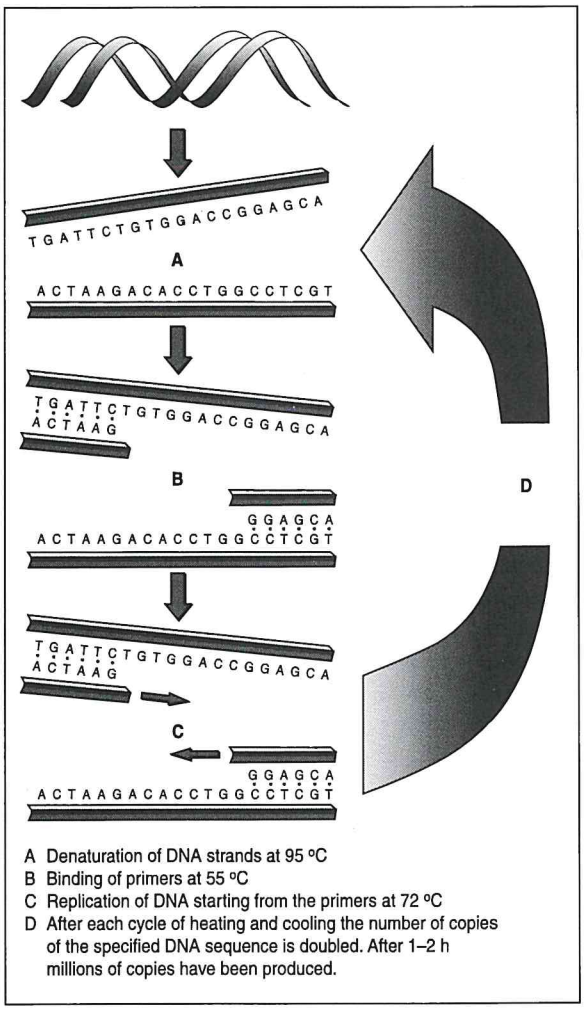


Figure 4
Amplification of DNA using the polymerase chain reaction; the primers are synthetically prepared oligonucleotides having sequences complementary to the DNA on either side of the segment of DNA to be amplified

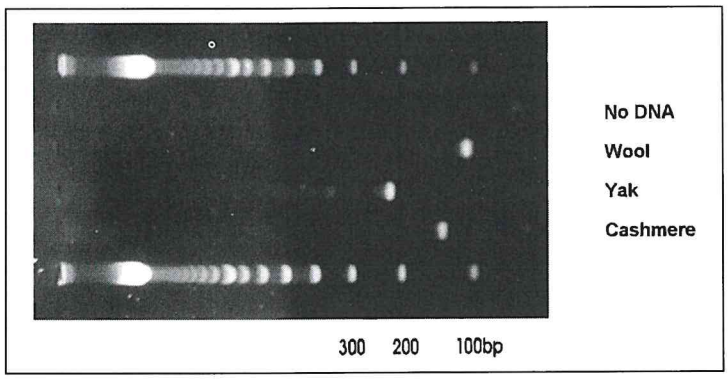


Figure 5
DNA amplification products obtained from wool, yak hair and cashmere using different primer sets

of the prodigious sensitivity of PCR it is essential that negative controls are included with the samples and go through the same extraction process.

Using this approach, DNA has been successfully amplified from 2 g samples of dyed material that did not yield detectable levels of DNA using conventional extraction techniques, and we have shown that PCR is capable of amplifying and therefore allowing rapid detection of minor components in mixed DNA samples. Although this type of analysis is not quantitative it is a simple and rapid test providing useful criteria for the confirmation of fraudulent substitution. In a recent press release, Karl Spilhaus, president of the Cashmere and Camel Hair Manufacturers Institute (Boston, USA), stated that 'mis-statement of fibre content in cashmere and cashmere blend garments constitutes a multi-million dollar fraud on the consuming public and damages legitimate cashmere producers and the image of the fibre. The new technology allows us to state with absolute certainty whether contamination by the other fibres is present at any stage of processing from raw material to finished garment.'

Future developments

A major challenge for the future is to develop techniques for carrying out quantitative fibre analysis. To carry out quantitative analysis it is necessary to identify individual fibre shafts. The development of such technology will enable accurate determination of the composition of unknown fibre blends. Recent work has indicated that *in situ* DNA analysis of single fibre shafts is not practically possible since the DNA is encapsulated inside the waterproof environment of the keratinised cells. Attempts to recover this DNA leads to the dissolution of the fibres. Therefore extraction based techniques have to be employed. A key strategy is to improve the efficiency of recovery of DNA from whole fibres. Improved DNA extraction procedures have now been developed at BTTG such that only 20 mg of a fibre sample are required for analysis.

A second problem relates to the large number of fibres that will have to be analysed to give an accurate result making any test prohibitively costly using manual techniques. However, automated systems are being developed for DNA analysis such as the DNA and RNA Analysis System, DARAS, and the advent of chip-based systems will ultimately lead to low cost procedures that can handle large numbers of samples.

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