Six samples of various materials were received for DNA analysis. The samples consisted of the following;
1. Filter paper containing a dried blood spot (Betty)
2. A samples of a comb (Barney)
3. Dress section labelled "lining stain"
4. Dress section labelled "Swathe mid-section"
5. Dress section labelled "Swathe right sleeve"
6. Dress section labelled "Swathe left sleeve"

DNA extraction

The dress sections and dried blood stain were cut into small pieces using a fresh sterile scalpel blade for each sample. Material from the comb was scrapped off using a sterile scalpel blade into a clean DNA extraction tube. The DNA was then extracted from the samples using a Guanidinium based extraction buffer. After addition of the buffer the dress samples dissolved completely. The DNA was then purified by phenol:chloroform:iso-amyl-alcohol extraction. The phenol:chloroform:iso-amyl-alcohol was then back extracted with buffer to ensure complete recovery of any DNA fragments. The DNA was then ethanol precipitated and resuspended in 20µl of DNA buffer.

PCR Amplification

The following regions were chosen to design PCR primers.
- 18S Ribosomal DNA
- 12S Ribosomal DNA
- 5.8S Ribosomal DNA
- 5S Ribosomal DNA
- Human mitochondrial D-loop

Examples of PCR primers sequences are outlined below.
18 F1: CTGGTTGATYCTGCCAGT
18 R1: TCTCCGGRRTCGARCCCT
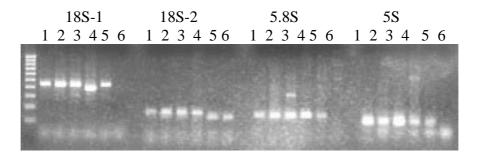
18 F2: TTTGYACACACCGCCCGTCG
18 R2: CYGCAGGTTCACCTACRG

5.8 F: ACTCTWARCGGTGGATCAC
5.8 R: RAGCGACVCTCAGRCAGGCG

5 F: GTCTACGRCCAYACCACSCTG
5 R: GCCWACRRCACCTGGTATTCCC

The above regions are amongst the most conserved region regions of DNA between all species of animals and insects and are ideal regions for the typing of DNA of unknown origin.

To ensure that the PCR primers performed correctly they were first tested on a wide range of animals (see figure 1).

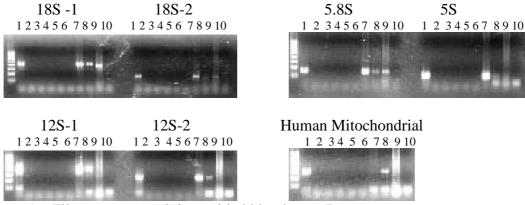Figure 1. PCR amplification using the above PCR primers.

1. Human DNA
2. Mouse DNA
3. Rabbit DNA
4. Spider DNA
5. Rat DNA
6. No DNA control

As can be seen from the above result all primers sets were positive for the amplification of DNA from this diverse sample set.

The DNA extracted from the samples was then subjected to PCR for the above regions and also 12S and human mitochondrial DNA (see figure 2).

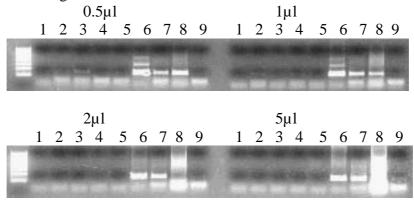Figure 2. PCR amplification from the extracted DNA sample.

18S -1   18S-2
1 2 3 4 5 6 7 8 9 10   1 2 3 4 5 6 7 8 9 10

5.8S   5S
1 2 3 4 5 6 7 8 9 10   1 2 3 4 5 6 7 8 9 10

12S-1   12S-2
1 2 3 4 5 6 7 8 9 10   1 2 3 4 5 6 7 8 9 10

Human Mitochondrial
1 2 3 4 5 6 7 8 9 10

1. Filter paper containing a dried blood spot (Betty)
2. A samples of a comb (Barney)
3. Dress section labelled "Swathe Left Sleeve"
4. Dress section labelled "lining stain"
5. Dress section labelled "Mid section"
6. Dress section labelled "Swathe Right sleeve"
7. Mouse DNA
8. Human DNA (2 genome equivalents)
9. Spider DNA
10. Negative control.

From the above results it can be seen that although all the controls were positive no samples except for the dried blood stain were positive in any test for DNA. Increasing the number of cycles to improve the sensitivity merely resulted in contamination of the negative control (data not shown).

In an attempt to resolve the problem various amounts of starting purified DNA were PCR amplified to determine if the lack of positive signals was due to very low DNA concentrations using the 5.8S PCR reaction (see figure 3).
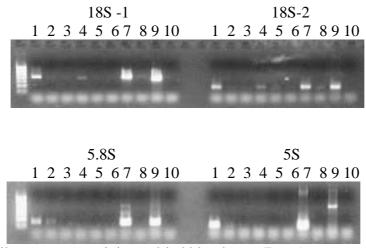
Figure 3. Increasing the volume of DNA added to the reaction.

0.5µl
1 2 3 4 5 6 7 8 9

1µl
1 2 3 4 5 6 7 8 9

2µl
1 2 3 4 5 6 7 8 9

5µl
1 2 3 4 5 6 7 8 9

1. A samples of a comb (Barney)
2. Dress section labelled "Swathe Left Sleeve"
3. Dress section labelled "lining stain"
4. Dress section labelled "Mid section"
5. Dress section labelled "Swathe Right sleeve"
6. Mouse DNA
7. Human DNA (2 genome equivalents)
8. Spider DNA
9. Negative control.

As can be seen the best amplification occurred with the lowest volume of DNA suggesting that an inhibitory substance was present in the dress. Thus the volume of the PCR reaction was increased and the volume of DNA decreased and the samples all re-tested see figure 4.
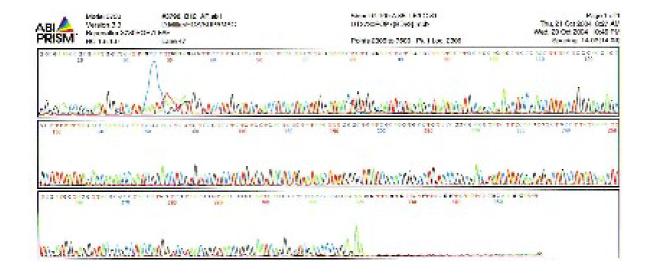
Figure 4. Re-amplification

18S -1   18S-2
1 2 3 4 5 6 7 8 9 10   1 2 3 4 5 6 7 8 9 10

5.8S   5S
1 2 3 4 5 6 7 8 9 10   1 2 3 4 5 6 7 8 9 10

1. Filter paper containing a dried blood spot (Betty)
2. A samples of a comb (Barney)
3. Dress section labelled "Swathe Left Sleeve"
4. Dress section labelled "lining stain"
5. Dress section labelled "Mid section"
6. Dress section labelled "Swathe Right sleeve"
7. Mouse DNA
8. Human DNA (2 genome equivalents)
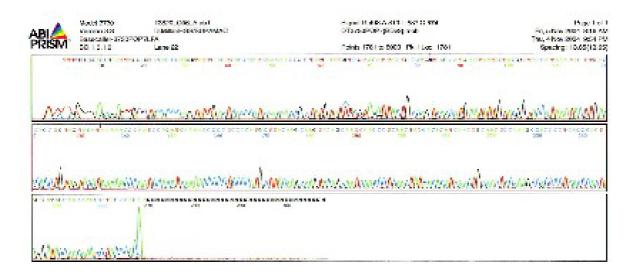9. Spider DNA
10. Negative control.

As can now be seen the samples are now giving positive PCR signals. In addition a human mitochondrial DNA sequence was amplified from swathe right sleeve (data not shown)
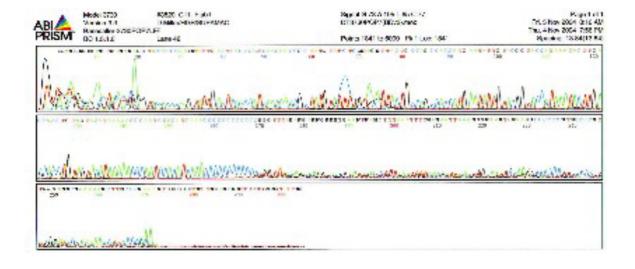
All positive samples amplified during the course of the study were sequenced to determine the species of organism. In total the following samples were sequenced.

- Lining stain
- Swathe mid-section"
- Dress section labelled "Swathe right sleeve"
- Dress section labelled "Swathe left sleeve"

Examples of sequence profiles are shown below.

NCBI Blast sequence alignments.

Uncultured Alpha Proteobacterium (swathe left sleeve).
GAATTCACTAGTGATTTTTGTACACACCGCCCGTCGCACCATGGGAGTTGG
GTTTACCCGAAGGCAGTGCGCTAACCGCAAGGGGGCAGCTGACCACGGTA
GGCTCAGCGACTGGGGTGAAGTCGTAACAAGGTAGCCGTAGGTGAACCTG
CAGAATCGAATTC

Human ribosomal DNA/ Mouse gene for 18S/ Bos taurus external transcribed spacer, partial sequence; 18S (mid-section).
GAATTCGATTCTGCAGGTTCACCTACGGAAACCTTGTTACGACTTTTACCT
CCTCTAGATAGTCAAGTTCGACCGTCTTCTCAGCGCTCCGCCAGGGCCGTG
GGCCGACCCCGGCGGGGCCGATCCGAGGGCCTCACTAAACCATCCAATCG
GTAGTAGCGACGGGCGGTGTGTGCAAAAATCACTAGTGAATTC

Betty DNA sequence
Homo sapiens isolate Ice587 mitochondrial control region
GACTCACCCATCAACAACCGCTATGTATTTCGTACATTACTGCCAGTCACC
ATGAATATTGTACGGTACCATAAATACTGGACCACCTGTAGTACATAAAA
ACCCAATCCACATCAAAACCCCCTCCCCATGCTTACAAGCAAGTACAGCA
ATCAACCCTCAACTATCACACATCAACTGCAACTCCAAAGCCACCCCTCA
CCCACTAGGATACCAACAAACCTACCCACA

Unknown Human mitochondrial sequence (right sleeve)
Homo sapiens isolate YYM22 D-loop, partial sequence; mitochondrial
TITLE: Genetic Structure of Hmong-Mien Speaking Populations in East Asia as Revealed by mtDNA Lineages
CCACCCAAGTATTGACTCACCCACTCAACAACCGCTATGTATTTCGTACAT
TACTGCCAGCCACCATGAATATTGTACGGTACCATAAATACTTGACCACCT
GTAGTACATAAAAACCCAATCCACATCAAACCCCCCCCCCCC

Dress Lining Stain. Coelotes terrestris 18S rRNA gene
TTCCTTGGATCGTACCTCACTACTTGGATAACTGTGGCAATTCTAGAGCTA
ATACATGCAGCAGAGCTCCGACCTTTACTGGGACGAGCGCTTTTATTAGA
CCAAAACCAATCGGACCTCGTGTCCGTCCTCTGTGGTGACTCTGTATAACT
TTGGGCTGATCGCACGGGCCTGTCCCGGCGACGTTCTTTCAAGTGTCTGCC
TTATCAACTTTCGATGGTAGGTTACGCGCCTACCATGGTCGTAACGGGTAA
CGGGGAATCAGGGCTCGACCCCGGAGAA

END OF REPORT