

C.nippon1	CTAATCTACAAATCCTCACAGGCCCTATTCCTAGCAATACACTATACATCCGACACAATA	180
C.nippon2	CTAATCCTACAAATCCTCACAGGCCCTATTCCTAGCAATACACTATACATCCGACACAATA	180
C.nippon3	CTAATCCTACAAATCCTCACAGGCCCTATTCCTAGCAATACACTATACATCCGACACAATA	180
C.elaphus2	CTAATCTACAAATTTCTCACAGGCCCTATTCCTAGCAATACACTATACATCCGATACAATA	180
C.elaphus1	CTAATCCTACAAATCCTCACAGGCCCTATTCCTAGCGATACACTATACATCTGTATACAATA	180
C.elaphus3	CTAATCCTACAAATCCTCACAGGCCCTATTCCTAGCGATACACTATACATCTGTATACAATA	180
D.dama1	TTAATCCTACAAATCCTCACAGGCCCTATTCCTAGCAATACACTACACATCTGTATACAATA	180
D.dama2	TTAATCCTACAAATCCTCACAGGCCCTATTCCTAGCAATACACTACACATCTGTATACAATA	180
C.capreolus1	CTAATCTACAAATCCTCACAGGCCCTATTCCTAGCAATACACTACACATCCGACACAATA	180
C.capreolus2	CTAATCTACAAATCCTCACAGGCCCTATTCCTAGCAATACACTACACATCCGACACAATA	180
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C.nippon1	ACAGCATTTCTCTCTGTCAACCATATCTGTGCGAGATGTCAATTATGGTTGAATTATTCGA	240
C.nippon2	ACAGCATTTCTCTCTGTCAACCATATCTGTGCGAGATGTCAATTATGGTTGAATTATTCGA	240
C.nippon3	ACAGCATTTCTCTCTGTCAACCATATCTGTGCGAGATGTCAATTATGGTTGAATTATTCGA	240
C.elaphus2	ACAGCATTTCTCTCTGTCAACCATATCTGTGCGAGATGTCAATTATGGTTGAATTATTCGA	240
C.elaphus1	ACAGCATTTCTCTCTGTCAACCATATCTGTGCGAGATGTCAATTATGGCTGAATTATTCGA	240
C.elaphus3	ACAGCATTTCTCTCTGTCAACCATATCTGTGCGAGATGTCAATTATGGCTGAATTATTCGA	240
D.dama1	ACAGCATTTCTCTCTGTCAACCATATCTGCCGAGACGTCAATTACGGTTGAATCATTCGA	240
D.dama2	ACAGCATTTCTCTCTGTCAACCATATCTGCCGAGACGTCAATTACGGTTGAATCATTCGA	240
C.capreolus1	ACAGCATTTCTCTCTGTCAACCATATCTGCCGAGACGTAACTATGGCTGAATTATTCGA	240
C.capreolus2	ACAGCATTTCTCTCTGTCAACCATATCTGCCGAGACGTAACTATGGCTGAATTATTCGA	240
	***** * * * * *	
C.nippon1	TACATACACGCAAAACGGGGCATCAATATTTTTCATCTGCGCTATTTCATACACGTAGGACGA	300
C.nippon2	TACATACACGCAAAACGGGGCATCAATATTTTTCATCTGCGCTATTTCATACACGTAGGACGA	300
C.nippon3	TACATACACGCAAAACGGGGCATCAATATTTTTCATCTGCGCTATTTCATACACGTAGGACGA	300
C.elaphus2	TACATACACGCAAAACGGGGCATCAATATTTTTCATCTGCGCTATTTCATACATGTAGGACGA	300
C.elaphus1	TATATACACGCAAAACGGGGCATCAATATTTTTCATCTGCTATTTCATACATGTAGGACGA	300
C.elaphus3	TACATACACGCAAAACGGGGCATCAATATTTTTCATCTGCTGTTTATACATGTAGGACGA	300
D.dama1	TACATGACACGCAAAACGGAGCATCAATATTTTTCATCTGCGCTATTTCATCCATGTAGGACGA	300
D.dama2	TACATGACACGCAAAACGGAGCATCAATATTTTTCATCTGCGCTATTTCATGTAGGACGA	300
C.capreolus1	TATATACATGCAAAACGGAGCATCAATATTTTTCATCTGCTATTTCCTACATGTAGGACGA	300
C.capreolus2	TATATACATGCAAAACGGAGCATCAATATTTTTCATCTGCTATTTCCTACATGTAGGACGA	300
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C.nippon1	GGTCTGTACTACGGATCATATACTTTTCTAGAGACATGAAACATCGGAGTAATTTCTCCTA	360
C.nippon2	GGTCTGTACTACGGATCATATACTTTTCTAGAGACATGAAACATCGGAGTAATTTCTCCTA	360
C.nippon3	GGTCTGTACTACGGATCATATACTTTTCTAGAGACATGAAACATCGGAGTAATTTCTCCTA	360
C.elaphus2	GGCCTATACTACGGGTCATATACTTTTCTAGAGACATGAAACATTTGGAGTAATCTCTCCTA	360
C.elaphus1	GGCCTGTACTACGGATCATATACTTTTCTAGAGACGTGAAACATCGGAGTATTTCTCTCTA	360
C.elaphus3	GGCCTGTACTACGGATCATATACTTTTCTAGAGACGTGAAATATCGGAGTAGTTCTCTCTA	360
D.dama1	GGTCTATACTACGGATCATACACTTTTCTAGAGACATGAAACATCGGAGTAATTTCTCCTA	360
D.dama2	GGCCTATACTATGGATCTTACACTTTTCTAGAGACATGAAACATTTGGAGTAATTTCTCCTA	360
C.capreolus1	GGCCTATATTTATGGATCTTACACTTTTCTAGAGACATGAAACATTTGGAGTAATTTCTCCTA	360
C.capreolus2	GGCCTATACTATGGATCTTACACTTTTCTAGAAACATGAAACATTTGGAGTAATTTCTCCTA	360
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C.nippon1	TTTACAGTCATAGCCACAGCATTCGTAGGATATGTCCTACCATGAGGACAAATATCATTC	420
C.nippon2	TTTACAGTCATAGCCACAGCATTCGTAGGATATGTCCTACCATGAGGACAAATATCATTC	420
C.nippon3	TTTACAGTCATAGCCACAGCATTCGTAGGATACGTCTACCATGAGGACAAATATCATTC	420
C.elaphus2	TTTACAGTTATAGCCACAGCATTCGTAGGATATGTCCTACCATGAGGACAAATATCATTC	420
C.elaphus1	TTTACAGTTATAGCCACAGCATTCGTAGGATATGTCCTACCATGAGGACAAATATCATTC	420
C.elaphus3	TTTACAGTTATAGCCACAGCATTCGTAGGATATGTCCTACCATGAGGACAAATATCATTC	420
D.dama1	TTTACAGTTATAGCTACAGCATTCGTAGGATACGTCTACCATGAGGACAAATATCATTC	420
D.dama2	TTTACAGTTATAGCTACAGCATTCGTAGGATACGTCTACCATGAGGACAAATATCATTC	420
C.capreolus1	TTTACAGTAATAGCCACGGCATTTGTAGGATACGTTTACCATGAGGACAAATATCATTC	420
C.capreolus2	TTTACAGTAATAGCCACGGCATTTGTAGGATACGTTTACCATGAGGACAAATATCATTC	420
	** * * * * * * * * * *	
C.nippon1	TGAGGAGCAAAGTCAATTACCAACCTTCTCTCAGCAATTCCATACATTGGCACAAACCTA	480
C.nippon2	TGAGGAGCAAAGTCAATTACCAACCTTCTCTCAGCAATTCCATACATTGGCACAAACCTA	480
C.nippon3	TGAGGAGCAAAGTCAATTACCAACCTTCTCTCAGCAATTCCATATATTGGCACAAACCTA	480
C.elaphus2	TGAGGAGCAAAGTCAATTACCAACCTTCTCTCAGCAATTCCATACATTGGCACAAACCTA	480
C.elaphus1	TGAGGAGCAAAGTCAATCACCAACCTTCTCTCAGCAATTCCATATATTGGGACAAACCTA	480
C.elaphus3	TGAGGAGCAAAGTCAATTACCAACCTTCTCTCAGCAATTCCATATATTGGTACAAACCTA	480
D.dama1	TGAGGAGCAAAGTATTATACCAACCTTCTCTCAGCAATCCCATACATTGGTACAAACCTA	480
D.dama2	TGAGGAGCAAAGTCAATTACTAATCTCTCTCAGCAATCCCATACATTGGTACAAACCTA	480
C.capreolus1	TGAGGAGCAAAGTATTATACCAATCTCTCTCAGCAATTCCATATATCGGTACAAACCTA	480
C.capreolus2	TGAGGAGCAAAGTATTATACCAATCTCTCTCAGCAATTCCATATATCGGTACAAACCTA	480
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C.nippon2	GTCGAATGGATCTGAGGAGGCTTTTTCAGTAGATAAAGCAACCTTAACCCGATTTTTCGCT	540
C.nippon3	GTCGAATGGATCTGAGGAGGCTTTTTCAGTAGATAAAGCAACCTTAACCCGATTTTTCGCT	540
C.elaphus2	GTCGAATGGATCTGAGGAGGCTTTTTCAGTAGATAAAGCAACCTTAACCCGATTTTTCGCT	540
C.elaphus1	GTCGAATGGATCTGAGGAGGCTTTTTCAGTAGATAAAGCAACCTTAACCCGATTTTTCGCT	540
C.elaphus3	GTCGAATGGATCTGAGGAGGCTTTTTCAGTAGATAAAGCAACCTTAACCCGATTTTTCGCT	540
D.dama1	GTTGAATGAATCTGAGGAGGCTTTTTCAGTAGATAAAGCAACCTTAACCTCGATTTCTCGCT	540
D.dama2	GTTGAATGAATCTGAGGAGGCTTTTTCAGTAGATAAAGCAACCTTAACCTCGATTTCTCGCT	540
C.capreolus1	GTTGAATGAATCTGAGGAGGCTTTTTCAGTAGATAAAGCAACCTTAACCCGATTTTTCGCT	540
C.capreolus2	GTTGAATGAATTTGAGGAGGCTTTTTCAGTAGATAAAGCAACCTTAACCTCGATTTTTCGCT	540
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C.nippon1	TTCCACTTTATTCTTCCATTTATCATCACAGCACTCGCTATAGTACACTTACTCTTCCTT	600
C.nippon2	TTCCACTTTATTCTTCCATTTATCATCACAGCACTCGCTATAGTACACTTACTCTTCCTT	600
C.nippon3	TTCCACTTTATTCTTCCATTTATCATCACAGCACTCGCTATAGTACACTTACTCTTCCTT	600
C.elaphus2	TTCCACTTTATTCTTCCATTTATCATCGCAGCACTCGCTATAGTACACTTACTCTTCCTT	600
C.elaphus1	TTCCACTTTATTCTTCCATTTATCATCGCAGCACTCGCTATAGTACACTTACTCTTCCTT	600
C.elaphus3	TTCCACTTTATTCTTCCATTTATCATCGCAGCACTCGCTATAGTACACTTACTCTTCCTT	600
D.dama1	TTCCACTTTATTCTTCCATTTATCATTTGCGGCACTTGTATAGTACATTTACTCTTTCTT	600
D.dama2	TTCCACTTTATTCTTCCATTTATCATTTGCAAGCACTTGTATAGTACATTTACTCTTCCTC	600
C.capreolus1	TTCCACTTTATTCTTCCATTTATCATTTGCAAGCACTTGTATAGTCCATTTACTTTTCCTC	600
C.capreolus2	TTCCACTTTATCTCTCCATTTATCATTTGCAAGCACTTGTATAGTCCATTTACTTTTCCTC	600
	***** ** ***** * ***** ***** ** ***** ** *	
C.nippon1	CACGAGACAGGATCCAAACCAACCAAGGAATCCCATCGGACGCAGACAAAATCCCCTTC	660
C.nippon2	CACGAGACAGGATCCAAACCAACCAAGGAATCCCATCGGACGCAGACAAAATCCCCTTC	660
C.nippon3	CACGAGACAGGATCCAAACCAACCAAGGAATCCCATCGGACGCAGACAAAATCCCCTTC	660
C.elaphus2	CACGAGACAGGATCCAAATAAACCAACCAAGGAATCCCATCGAGCGCAGACAAAATCCCCTTC	660
C.elaphus1	CACGAAACAGGATCTAATAACCAACCAAGGAATCCCATCGAGCGCAGACAAAATCCCCTTC	660
C.elaphus3	CACGAAACAGGATCTAATAACCAACCAAGGAATCCCATCGAGCGCAGACAAAATCCCCTTC	660
D.dama1	CACGAGACAGGATCCAAATAAACCAACCAAGGAATCCCATCGAGTGTAGATAAAATCCCCTTC	660
D.dama2	CACGAGACAGGATCCAAATAAACCAACCAAGGAATCCCATCGAGTGCAGATAAAATCCCCTTC	660
C.capreolus1	CACGAAACAGGATCAAAACCAACCGCAGGAATCCCATCAACCGTGGACAAAATTCCTATT	660
C.capreolus2	CACGAAACAGGATCAAAACCAACCGGATAGGAATCCCATCAACCGCGGACAAAATTCCTATT	660
	***** ***** ** ***** * ***** ***** * * ** ***** ** *	
C.nippon1	CATCCTTACTATACCATTAAGATATCTTAGGCATCTTACTTCTAGTACTCTTCTGATA	720
C.nippon2	CATCCTTACTATACCATTAAGATATCTTAGGCATCTTACTTCTAGTACTCTTCTGATA	720
C.nippon3	CATCCTTACTATACCATTAAGATATCTTAGGCATCTTACTTCTAGTACTCTTCTGATA	720
C.elaphus2	CATCCTTACTATACCATTAAGATATCTTAGGCATCTTACTTCTAGTACTCTTCTGATA	720
C.elaphus1	CATCCTTATTATACCATTAAGATATCTTAGGCATCTTACTTCTAGTACTCTTCTGATA	720
C.elaphus3	CATCCTTATTATACCATTAAGATATCTTAGGCATCTTACTTCTAGTACTCTTCTGATA	720
D.dama1	CATCCTTACTATACCATTAAGATATCTTAGGCATCTTACTTCTAGTACTCTTCTGATA	720
D.dama2	CACCCCTACTATACCATTAAGATATCTTAGGGGCACTACTTATAATTTCTTCTTCTGATA	720
C.capreolus1	CACCCCTACTATACCATTAAGATATCTTAGGGGTTCTACTCTTAAATCTTCTTCTGATA	720
C.capreolus2	CACCCCTACTATACCATTAAGATATCTTAGGAGTTCTACTTCTTCTTCTTCTGATA	720
	** * * * * ***** ***** ***** * * * * * * * * * * * * *	
C.nippon1	TTACTAGTATTATTCGCACCAAGACCTGCTTGGAGATCCAGACAACTACACCCAGCAAA	780
C.nippon2	TTACTAGTATTATTCGCACCAAGACCTGCTTGGAGATCCAGACAACTACACCCAGCAAA	780
C.nippon3	TTACTAGTATTATTCGCACCAAGACCTGCTTGGAGATCCAGACAACTACACCCAGCAAA	780
C.elaphus2	TTACTAGTATTATTCGCACCAAGACCTGCTTGGAGATCCAGACAACTATACCCAGCAAA	780
C.elaphus1	TTACTAGTATTATTCGCACCAAGACCTACTTGGAGATCCAGATAACTACACCCAGCAAA	780
C.elaphus3	TTACTAGTATTATTCGCACCAAGACCTACTTGGAGATCCAGATAACTACACCCAGCAAA	780
D.dama1	ACACTAGTACTATTTCGCACCAAGCTTGGAGATCCAGACAACTACACTCCAGCAAA	780
D.dama2	ATACTAGTACTATTTCGCACCAAGCTTGGAGATCCAGACAACTACACCCAGCAAA	780
C.capreolus1	TTACTAGTCTATTTCGCACCAAGACCTGCTTGGAGATCCAGATAACTACACCCAGCAAA	780
C.capreolus2	TTACTAGTCTATTTCGCACCAAGACCTGCTTGGAGATCCAGATAACTACACCCAGCAAA	780
	***** ***** ***** ***** * * ***** * * * * * * * * *	
C.nippon2	CCGCTCAACACACCCCTCACATCAAACTGAATGATATTTCTATTTCGATACGCAATC	840
C.nippon3	CCGCTCAACACACCCCTCACATCAAACTGAATGATATTTCTATTTCGATACGCAATC	840
C.elaphus2	CCACTCAACACACCCCTCACATTAACCTGAATGATATTTCTATTTCGATACGCAATC	840
C.elaphus1	CCACTCAACACACCCCTCATATTAACCTGAATGATATTTCTATTTCGATACGCAATC	840
C.elaphus3	CCACTCAACACACCCCTCATATTAACCTGAATGATATTTCTATTTCGATACGCAATC	840
D.dama1	CCACTCAACACACCTCTCATATTAACCTGAATGATATTTCTATTTCGATACGCAATC	840
D.dama2	CCACTCAACACTCCTCTCATATTAAGCCAGAAATGATATTTCTATTTCGATACGCAATC	840
C.capreolus1	CCACTTAACACACCCCTCACATTAACCCAGAAATGGTACTTCTATTTCGATACGCAATC	840
C.capreolus2	CCACTTAACACACCCCTCACATTAACCCAGAAATGATATTTCTATTTCGATACGCAATC	840
	** * * ***** ** *	
C.nippon1	CTACGATCAATTCCCAACAAACTAGGAGGAGTCTTAGCCCTAGTCTCATCTATCCTAATC	900
C.nippon2	CTACGATCAATTCCCAACAAACTAGGAGGAGTCTTAGCCCTAGTCTCATCTATCCTAATC	900
C.nippon3	CTACGATCAATTCCCAACAAACTAGGAGGAGTCTTAGCCCTAGTCTCATCTATCCTAATC	900
C.elaphus2	CTACGATCGATTCCCAACAAACTAGGAGGAGTCTTAGCCCTAGTCTCATCTATCCTAATC	900
C.elaphus1	CTACGATCAATTCCCAACAAACTAGGAGGAGTCTTAGCCCTAGTCTCATCTATCCTAATC	900
C.elaphus3	CTACGATCAATTCCCAACAAACTAGGAGGAGTCTTAGCCCTAGTCTCATCTATCCTAATC	900
D.dama1	CTACGATCAATTCCCAATAAATTAGGAGGGGTGTTAGCCCTAGTCTCATCTATCCTAATC	900
D.dama2	CTACGATCAATCCCTAACAAACTAGGAGGAGTCTTAGCACTAGTCTCTCTATTCTCATC	900
C.capreolus1	CTACGATCTATTCCTAACAAACTAGGAGGAGTCTTAGCCCTAATCTCATCAATCCTAATC	900
C.capreolus2	CTACGATCTATTCCTAACAAACTAGGAGGAGTCTTAGCCCTAATCTCATCAATCCTAATC	900
	***** ** *	
C.nippon1	CTGATCCTCATGCCTCTTCTTACACGTCCAAACAAACGAGCATGATATTCGACCATTC	960
C.nippon2	CTGATCCTCATGCCTCTTCTTACACGTCCAAACAAACGAGCATGATATTCGACCATTC	960
C.nippon3	CTGATCCTCATGCCTCTTCTTACACGTCCAAACAAACGAGCATGATATTCGACCATTC	960
C.elaphus2	TTGATTCTCATGCCTCTTCTTACACGTCCAAACAAACGAGCATGATATTCGACCATTC	960
C.elaphus1	TTAATTCTCATGCCTCTTCTTACACATCCAAACAAACGAGCATGATATTCGACCATTC	960
C.elaphus3	TTAATTCTCATGCCTCTTCTTACACATCCAAACAAACGAGCATGATATTCGACCATTC	960
D.dama1	CTAATTCTTATACCTTCTCTCCACATCCAAACAAACGAGCATGATATTCGACCATTC	960
D.dama2	CTTATCTTTATACCTCTCTCCACATCCAAACAAACGAGCATGATATTCGACCATTC	960
C.capreolus1	TTGATCCTTATACCCCTCTCTCATATCTAAACAAACGAGTATAATGTTCCGCGCATTC	960
C.capreolus2	TTGATCCTTATACCCCTCTCTCATATCTAAACAAACGAGTATAATGTTCCGCGCATTC	960
	* *	

C.nippon1	AGCCAAATGCCTGTTCTGAATCTTAGTAGCAGACCTACTAACTCAGATGAATTGGAGGA	1020
C.nippon2	AGCCAAATGCCTGTTCTGAATCTTAGTAGCAGACCTACTAACTCAGATGAATTGGAGGA	1020
C.nippon3	AGCCAAATGCCTGTTCTGAATCTTAGTAGCAGACCTACTAACTCAGATGAATTGGAGGA	1020
C.elaphus2	AGCCAAATGCCTATTCTGAATTTTAGTAGCAGACCTACTAACTCAGATGAATTGGAGGA	1020
C.elaphus1	AGTCAATGCCTATTCTGAATCTTAGTAGCAGATCTACTAACTTACATGAATCGGAGGA	1020
C.elaphus3	AGCCAAATGCCTATTCTGAATCTTAGTAGCAGACCTATTAACTCAGATGAATCGGAGGA	1020
D.dama1	AGTCAATGCCTATTCTGAGTCCTAGTAGCAGACCTACTAACTTACATGAATCGGAGGA	1020
D.dama2	AGCCAAATGCCTATTTTGAATCCTTGTAGCAGACCTACTAACTAACATGAATCGGAGGG	1020
C.capreolus1	AGTCAATGCCTATTCTGAATCCTAGTAGCTGACCTATTAACTAACATGAATTGGAGGC	1020
C.capreolus2	AGTCAATGCCTATTCTGAATCCTAGTAGCTGACCTATTAACTAACATGAATTGGAGGC	1020
** * * * * *		
C.nippon1	CAACCAAGTTGAATACCCCTTTATTATTATTGGACAACTAGCATCTGTCTTATCTTCTTC	1080
C.nippon2	CAACCAAGTTGAATACCCCTTTATTATTATTGGACAACTAGCATCTGTCTTATCTTCTTC	1080
C.nippon3	CAACCAAGTTGAATACCCCTTTATTATTATTGGACAACTAGCATCTGTCTTATCTTCTTC	1080
C.elaphus2	CAGCCAGTTGAATACCCCTTTATTATTATTGGACAACTAGCATCTGTCTTATCTTCTTC	1080
C.elaphus1	CAACCAAGTCGAATACCCCTTTATCATTATTGGACAACTAGCATCTGTCTTATCTTCTTC	1080
C.elaphus3	CAACCAAGTTGAATATCCCTTTATCATTATTGGACAACTAGCATCTGTCTTATCTTCTTC	1080
D.dama1	CAACCAAGTCGAATATCCTTTTATTACCATTTGGACAACTAGCATCTATCTTATCTTCTTC	1080
D.dama2	CAACCAAGTTGAACACCCATTTATTATCATTGGACAACTAGCATCTATCTTATCTTCTTC	1080
C.capreolus1	CAACCAAGTCGAATACCCCTTTATCGCTATTGGCCAAATTGCATCTATTATGTACTTCTTC	1080
C.capreolus2	CAACCAAGTCGAATACCCCTTTATCGCTATTGGCCAAATTGCATCTATTATGTACTTCTTC	1080
** * * * * *		
C.nippon1	ATTATCCTAGTCCTTATACCGATCACCAGCAATCGAAAAAACCTCCTAAAAATGAAGA	1140
C.nippon2	ATTATCCTAGTCCTTATACCGATCACCAGCAATCGAAAAAACCTCCTAAAAATGAAGA	1140
C.nippon3	ATTATCCTAGTCCTTATACCGATCACCAGCAATCGAAAAAACCTCCTAAAAATGAAGA	1140
C.elaphus2	ATTATCCTAGTCCTTATACCAATTACCGACCAATCGAAAAAACCTCCTAAAAATGAAGA	1140
C.elaphus1	ATTATCCTAGTCCTCATACCAATCACCAGCAATCGAAAAAACCTCCTAAAAATGAAGA	1140
C.elaphus3	ATTATCCTAGTCCTCATACCAATCACCAGCAATCGAAAAAACCTCCTAAAAATGAAGA	1140
D.dama1	ATTATCCTAGTCCTTATACCGATCACCAGCAATCGAAAAAACCTCCTAAAAATGAAGA	1140
D.dama2	ATTATCCTAGTACTAATACCGATCACCAGCAATCGAAAAAACCTCCTAAAAATGAAGA	1140
C.capreolus1	ATTATCCTAGTACTCATACCAATTACTAGCACAATCGAAAAAACCTCCTAAAAATGAAGA	1140
C.capreolus2	ATTATCCTAGTACTCATACCAATTACTAGCACAATCGAAAAAACCTCCTAAAAATGAAGA	1140

A = adenine, T = thymine, C = cytosine, G = guanine, * = same base for all species
Yellow is forward primers, green is reverse primers, blue is the SNP.

Due to the close phylogenetic relationship of the deer species in the UK, and the low sequence variation, it was not possible to design species-specific primers such as had been developed for earlier experiments. Instead, areas of high homology were found that bordered on a single base that was different for at least one of the deer species. After identifying a large number of these highly conserved regions, a series of primers to amplify the single nucleotide polymorphisms (SNPs) was developed to determine the species present (see Table A10.2).

These primers would be unlabeled and would bind to the target DNA one base pair away from the base of interest (the SNP). Labelled ddNTPs would then attach themselves during the PCR process. As the ddNTPs are labelled, no further extension of the primer will proceed after the first base has been attached. This is because the dye label is attached to the dNTPs at the 3' end allowing for no further extension. Each base has a different colour: ddGTP is yellow; ddATP is green; ddTTP is red and; ddCTP is blue. As long as the primers differ in length by several bases, a series of colour coded bases will result. Based on this resulting series of bases, the species of deer can be determined.

Table A10.2: Species-specific deer primers and their location on the cytochrome *b* gene.

Primer	Location *	5'-----Sequence-----3'
Deer039	39	CCGAAAAWCYCACCCAYTRATAAAAAT
Deer066	66	GTAAAYAACGCATTYATTGAYCTCCC
Deer096	96	GCCCCATCRAATATYTCATCMTGATGAAA
Deer127	127	TAGGCCTGTRAGGATTTGTA
Deer165	165	TTCCTAGCRATACTA
Deer174	174	GTGACAGAGGARAATGCTGTTATTGT
Deer397	397	GTTGCTCCTCAGAATGATATTTGTCCTCATGGTA
Deer523	523	TGAGGRGGCTTTTCAGTAGAYAAAGCAACC
Deer555	555	CGATTYTTTCGCYTTCCACTTTATYCT
Deer823	823	GATCGTAGGATTGCGTATGCRAATA

* from the start of cytochrome *b* which was given the designation of 1

A = adenine, T = thymine, C = cytosine, G = guanine, R=A/G, Y=C/T, M=A/C, K=G/T, S=G/C, W=A/T, H=A/T/C, B=G/T/C, D=G/A/T, V=G/A/C, N=A/T/C/G

The primers themselves should not bind to human or any other animal DNA. There is a chance that primers might produce a result with closely related species. If this occurred, not all primers would bind to the non-deer species and an incomplete series of bases would be obtained. The fragment sizes are very small so that even with highly degraded DNA a full result should be obtained. An incomplete series of SNPs (anything less than the full 10) would exclude any deer species as being present in a sample. Even if all ten primers did bind and extend, the resulting series of bases would not correspond to any of the deer species (Table A10.3).

Table A10.3: The resulting SNPs from the primer sets designed for the four deer species and human.

Species/Position	39	66	96	127	165	174	397	523	555	823
Roe	T	A	C	A	C	G	A	C	C	A
Fallow	C	A	T	G	C	A	G	T	A	G
Sika	T	C	T	G	T	G	G	C	T	G
Red	T	A	T	G	T	A	G	C	C	G
Human	T	C	T	G	C	C	C	A	G	T

A = adenine, T = thymine, C = cytosine, G = guanine

The primers and test were designed and tested electronically using the NCBI-BLAST sequence comparison tool. At the request of the investigating officer no further work was carried out and no practical testing was performed. The test however, is completely designed. All that needs to be done to employ this identification technique is order the pre-designed primers, test some deer reference samples, and test outlier samples (other unrelated and closely related animals) to validate the test.

Identification of red deer (*Cervius elaphus*) and human from a mixed biological sample using the cytochrome *b* gene on the mitochondrial genome

Introduction:

A test was required to identify deer species from a mixture. New molecular techniques involving use of the variation in the mtDNA cytochrome *b* gene has been used for species identification. However, these techniques encounter problems when dealing with a mixture of more than one species resulting in an uninterpretable profile.

Case History

In February 2004, a man was caught in camouflage fatigues covered in blood. The police suspected him of poaching red deer and were interested if the blood was that of a deer. Due to the expectation of a mixture of DNA from the accused and whatever provided the bloodstains, normal procedures to determine the species present were not possible. Sequencing of the mitochondrial cytochrome *b* gene would result in uninterpretable results. A novel test was developed to rapidly distinguish between human mtDNA and that of different deer species.

Materials and Methods:

Samples and Standards

The trousers were examined for possible bloodstains with phenolphthalein (KM). Several areas tested positive for the presence of what could be blood. A stain that tested positive for KM was excised for use in DNA extraction.

Control samples of human DNA were obtained from lab staff control DNA. Red deer (*Cervus elaphus*) hair was obtained from Edinburgh Zoo and tissue was obtained from venison meat sausages.

DNA extraction and amplification

DNA was isolated using Qiagen Micro kits (Qiagen, Crawley UK) for all samples. A sample measuring roughly 3 mm² of the questioned bloodstain was used. All samples were processed according to the manufacturer's guidelines for the type of sample.

Universal forward primers were used in conjunction with species-specific reverse primers (Table A10.4). Species-specific primers will only produce a product with the species for which they were designed. Primers were designed such that fragment sizes varied enough to be distinguished on a genetic analysis machine.

Table A10.4: Universal and deer specific primers, their location on the cytochrome b gene, the fragment length, name, and which universal primer the reverse primers correspond to.

Animal	Location *	5'-----Sequence-----3'	Fragment Length (bp)
Universal-50 [†]	-50	FAM6-GACCAATGATATGAAAAACCATCGTTGT	N/A
Universal+400 [†]	400	FAM6-TGAGGACAAATATCATTYTGAGGRGC	N/A
<i>H. sapiens</i>	36	GGGAGGTCGATGAATGAGTGGTTAATTAAT	108
<i>H. sapiens</i>	208	TTCAGCCATAATTTACGTCTCGAGT	277
<i>C. elaphus</i>	567	AGTAAGTGACTATAGCGAGTGCTGCG	188
<i>H. sapiens</i> [‡]	624	ATCGGAATGGGAGGTGATTCCTAGG	242
Deer sp.	639	ATGAAAGGGGATTTTGTCTGCGTCT	256
<i>H. sapiens</i>	720	CTGGTGAGAATAGTGTTAATGTC	338

[†] From Pääbo *et al.* [1]

[‡] From Panvisavas [2]

* in relation to the start of the cytochrome *b* gene which was given the designation of 1

bp = base pairs, A = adenine, T = thymine, C = cytosine, G = guanine, R=A/G, Y=C/T, M=A/C, K=G/T, S=G/C, W=A/T, H=A/T/C, B=G/T/C, D=G/A/T, V=G/A/C, N=A/T/C/G

Two sets of PCRs were prepared: one multiplex specific to human; the other specific to deer. PCRs contained buffer, dNTPs, 1 unit Taq (Platinum Taq, Invitrogen, Paisley UK), universal and species-specific primers (at a final concentration of 0.5 µM each), 1 µL of template DNA and water to 20 µL. The PCR cycle proceeded for 32 cycles at 95 °C for 45 seconds, 60 °C for 45 seconds and 72 °C for 1 minute followed by a final extension step of 20 minutes at 72 °C.

Samples were then analysed on an ABI 310 Genetic Analyser.

Results and Discussion:

Results were obtained and peak sizes in base pairs (bp) were compared. For the human specific multiplex the human control had peaks at 108, 277, 242 and 338 bp. Both the red deer hair and tissue control samples did not react with the human primers. For the red deer specific multiplex the hair and tissue samples had peaks at 188 and 256 bp. The human control did not react with the red deer primers.

The unknown bloodstain sample reacted with the human specific primers giving peaks at 108, 277, 242 and 338 bp indicating the presence of human mtDNA. This is expected from shed epithelial cells on the inside of the trousers from the wearer. The unknown bloodstain also reacted with the deer specific primers with peaks at 188 and 256 bp indicating the presence of deer.

Identification of Asiatic Black Bear (*Ursus thibetanus*), Tiger (*Panthera tigris* sp.), Leopard (*Panthera pardus*) and Musk Deer (*Moschus* sp.) in products of Traditional East Asian Medicine (TEAM)

The Centre for Forensic Science at the University of Strathclyde was approached and asked if it would be possible to identify *Ursus* sp. from highly degraded samples such as those found in Traditional East Asian Medicine (TEAM). It was later approached by the BBC and asked if it would be possible to identify tiger (*Panthera tigris*), leopard (*P. pardus*) and musk deer (*Moschus* sp.) from TEAM.

All of the species thought to be in TEAM are listed on Appendix I or II of CITES [3]. Appendix I of CITES is described by the CITES UK website [4] as:

Appendix I includes species that may be threatened with extinction and which are, or may be, affected by international trade. International trade in wild specimens of these species is subject to strict regulation and is normally only permitted in exceptional circumstances. Trade in artificially propagated or captive-bred specimens is allowed, subject to license. Over 800 species are included in Appendix I at present, including tigers, great apes, certain parrots and certain species of orchids and cacti.

Appendix II is described as:

Appendix II includes species not considered to be under the same threat as those in Appendix I, but which may become so if trade is not regulated. International trade in these species is monitored through a licensing system to ensure that trade can be sustained without detriment to wild populations. Trade in wild, captive bred and artificially propagated specimens is allowed, subject to permit. Approximately 29,000 different species are included in Appendix II, including polar bears, Asiatic cobras, orchids, cacti and carnivorous plants [4].

Even with the strict regulations brought about by the CITES laws, the use of endangered species products in TEAM is still an ever present and ongoing problem throughout the world. Of the Traditional Medicine shops surveyed more than half, in most cases, had some form of bear derived product [5]. The countries surveyed and the percent of products containing bear in them from Country (%) are: Australia (49); Canada and the US (75); Indonesia (62); Japan (76); Malaysia (78); Singapore (73); and Taiwan (30) [5]. In addition to its use in TEAM, bear bile is now being used in

many other non-medicinal commercial products. The WSPA [5] claims bear bile can now be found in wines, eye drops, teas and tonics.

Tigers are poached for their body parts. Recently an article by Graham-Rowe [6] suggests that China is farming tigers for their body parts to be sold in international markets. In a rebuttal, Gubbi [7], claim that poaching is not the cause of the decline in tiger populations, but rather loss of habitat and encroachment of humans. However, even if habitat loss is a main cause of tiger decline, poaching is still a significant problem and will still have major ramifications on the populations and conservation efforts.

Musk deer are killed to remove the musk pod found in mature males [8]. The high value of the pods makes the illegal trade very profitable. Musk deer pods are used for medicinal purposes in relation to pulmonary disease [8]. TEAM claiming to contain tiger, leopard and musk derived products can be bought in shops around Edinburgh and Glasgow, UK.

The WSPA in conjunction with Wildlife DNA Services have developed an immunoassay to detect bear protein in products containing bear [9]. The test is still under development but will function much like a home pregnancy test. Samples will be placed into a mix tube to release the proteins, placed in a comb and then inserted into the detection device [9]. Samples will move by capillary action past three columns fixed within the device. One column will contain anti-bear antibodies, another bear antigen and the last column contains free antibody-microsphere complexes. It will be an antigen-antibody reaction using polyclonal antibodies to detect the albumins of seven bear species [9]. Results, as they are expected, appear to be confusing with two lines indicating a negative result, three lines indicating a low to moderate positive result and one line indicating a strong positive [9].

The test claims to be suitable for field use and has a total reaction time of five minutes with the first three minutes used for the preparation of the sample and device [9]. There are however several problems with antigen-antibody reactions. One

problem with this technique is that an antibody must first be produced and isolated [10]. Some antiserum, such as horse (H type), work only in a narrow range of concentrations and, under unstable conditions, can give rise to multiple precipitates [10]. Other commercial antisera often suffer from low titre and cross-reactivity [10]. Saferstein [10] states that very strict controls are needed and that the same batch of antiserum must be used for all samples and only in this way 'can the forensic serologist possibly maintain a degree of certainty and reproducibility required for a reliable species determination'.

This test will be of great use in testing samples on the spot, but may only be effective as a presumptive test. Cross reactivity as described by Saferstein [10] could preclude this test as being definitive. More testing and validation studies will be needed before this test can be reliably used to determine the presence of bear in products.

Two tests have been developed to identify tigers: one by Wan and Fang [11]; the other by Wetton *et al.* [12]. Wan and Fang [11] designed their test based on the cytochrome *b* gene and were able to successfully amplify from meat, faeces, dried skin and single hairs. Using cytochrome *b* sequences obtained from GenBank, they were able to identify a single base that was identical in all tiger species but different from all other species being compared [11]. This test was shown to work well with samples from meat and from a single hair [11]. The fragment produced is 408 bp and may be too large to detect highly degraded tiger which is expected in TEAM.

The other test by Wetton *et al.* [12] used primers specific to the tiger cytochrome *b* gene. They achieved successful amplification from blood, hair and bone, and from a range of TEAM samples spiked with 0.5 % tiger bone [12]. Primers were tested for cross reactivity from a large number of closely related species of cat as well as from animals such as dog, goat, pig, badger, human, swan, snake and others and were found to be unreactive [12]. This test is a good example of one which would be useful in forensic science, and indeed was developed to test claims of tiger bone in TEAM. For the current test the Centre for Forensic Science will create a multiplex to detect bear, tiger, leopard and musk deer components in commercial products

simultaneously, so new primer sets were developed to work in conjunction with each other.

No test has been located to identify musk deer from samples.

A quick, easy, cost effective and definitive test to identify bear, tiger, leopard and musk deer components in commercial products is therefore needed to help stem the trade in those species. This test would be able to aid customs officials in identifying products containing derivatives from endangered species so that those items could be held back from the markets of the world and charges could be brought against those responsible.

Materials and Methods

Sequence data for 28 *Ursus* species, 21 *Panthera* species and 7 *Moschus* species can be seen in Table A10.5. Sequence alignment can be found in tables A10.6, A10.7 and A10.8 respectively. Sequences were aligned using ClustalW available from the European Molecular Biology Laboratory European Bioinformatics Institute (EMBL-EBI) (<http://www.ebi.ac.uk/Information/>) and possible priming sites were identified.

Table A10.5: *Ursus* species, *Panthera* species and *Moschus* species used for alignment, their letter designation and accession number.

Species	Designa- tion	Accession Number	Species	Accession Number
<i>Ursus</i> species			<i>Panthera</i> species	
<i>U. thibetanus</i>	A	DQ349065	<i>P. pardus</i>	AB211407
<i>U. thibetanus</i>	B	UTU23558	<i>P. pardus</i>	AB211405
<i>U. thibetanus</i>	C	AY522429	<i>P. pardus</i>	AB211404
<i>U. thibetanus</i>	D	AY522430	<i>P. pardus</i>	AB211403
<i>U. americanas</i>	A	U23556	<i>P. pardus</i>	AB211401
<i>U. americanas</i>	B	AF303109	<i>P. pardus</i>	AB211406
<i>U. americanas</i>	C	AF268271	<i>P. pardus</i>	AB211402
<i>U. americanas</i>	D	AF268268	<i>P. pardus</i>	AY773080
<i>U. americanas</i>	E	AF268264	<i>P. tigris tigris</i>	AF053019
<i>U. americanas</i>	F	AF268261	<i>P. tigris tigris</i>	AF053024
<i>U. americanas</i>	G	AF268258	<i>P. tigris tigris</i>	AF053018
<i>U. americanas</i>	H	NC_003426	<i>P. tigris altaica</i>	AF053029
<i>U. americanas</i>	I	X82307	<i>P. tigris altaica</i>	AF053026
<i>U. arctos</i>	A	X82308	<i>P. tigris altaica</i>	AF053038
<i>U. arctos</i>	B	AF303110	<i>P. tigris corbetti</i>	AF053051
<i>U. arctos</i>	C	AB020908	<i>P. tigris corbetti</i>	AF053049
<i>U. arctos</i>	D	AB020906	<i>P. tigris corbetti</i>	AF053050
<i>U. arctos</i>	E	AB020905	<i>P. tigris sumatrae</i>	AF053040
<i>U. arctos</i>	F	U18897	<i>P. tigris sumatrae</i>	AF053047
<i>U. arctos</i>	G	U18870	<i>P. tigris sumatrae</i>	AF053043
<i>U. arctos</i>	H	NC_003427	<i>P. leo</i>	AF053052
<i>U. arctos</i>	I	AB020909		
<i>U. arctos</i>	J	AB020907	<i>Moschus</i> species	
<i>U. maritimus</i>	A	X82309	<i>M. fuscus</i>	AY184427
<i>U. maritimus</i>	B	AJ428577	<i>M. chrysogaster</i>	AY184426
<i>U. maritimus</i>	C	U18898	<i>M. leucogaster</i>	AY921577
<i>U. maritimus</i>	D	NC_003428	<i>M. berezovskii</i>	AY184425
<i>U. maritimus</i>	E	AF303111	<i>M. moschiferus</i>	AY327518
			<i>M. moschiferus</i>	AY121988
			<i>M. moschiferus</i>	AY184428

Table A10.6: Cytochrome *b* alignment for the 28 bear sequences with *Ursus* sp. primers (dark grey) and *U. thibetanus* specific primer (light grey) shown. Only those sections of the gene relating to the primers are shown

U.MaritimusA	CTTCCAAACCATCAAACATCTCAGCATGATGAACTTTGGATCCCTCCTTGGAGTGTGT	120
U.MaritimusB	CTTCCAAACCATCAAACATCTCAGCATGATGAACTTTGGATCCCTCCTTGGAGTGTGT	120
U.MaritimusC	CTTCCAAACCATCAAACATCTCAGCATGATGAACTTTGGATCCCTCCTTGGAGTGTGT	120
U.MaritimusD	CTTCCAAACCATCAAACATCTCAGCATGATGAACTTTGGATCCCTCCTTGGAGTGTGT	120
U.MaritimusE	CTTCCAAACCATCAAACATCTCAGCATGATGAACTTTGGATCCCTCCTTGGAGTGTGT	120
U.ArctosG	CTTCCAAACCATCAAACATCTCAGCATGATGAACTTTGGATCCCTCCTTGGAGTGTGT	120
U.ArctosD	CTTCCAAACCATCAAACATCTCAGCATGATGAACTTTGGATCCCTCCTTGGAGTATGT	120
U.ArctosE	CTTCCAAACCATCAAACATCTCAGCATGATGAACTTTGGATCCCTCCTTGGAGTATGT	120
U.ArctosA	CTTCCAAACCATCAAACATCTCAGCATGATGAACTTTGGATCCCTCCTTGGAGTATGT	120
U.ArctosF	CTTCCAAACCATCAAACATCTCAGCATGATGAACTTTGGATCCCTCCTTGGAGTATGT	120
U.ArctosJ	CTTCCAAACCATCAAACATCTCAGCATGATGAACTTTGGATCCCTCCTTGGAGTATGT	120
U.ArctosC	CTTCCAAACCATCAAACATCTCAGCATGATGAACTTTGGATCCCTCCTCGGAGTATGT	120
U.ArctosI	CTTCCAAACCATCAAACATCTCAGCATGATGAACTTTGGATCCCTCCTCGGAGTATGT	120
U.ArctosB	CTTCCAAACCATCAAACATCTCAGCATGATGAACTTTGGATCCCTCCTCGGAGTATGT	120
U.ArctosH	CTTCCAAACCATCAAACATCTCAGCATGATGAACTTTGGATCCCTCCTCGGAGTATGT	120
U.ThibetanusA	CTCCAGCACCATCAAATATCTCAGCATGATGAACTTTGGATCCCTCCTCGGAGTATGC	120
U.ThibetanusD	CTCCAGCACCATCAAATATCTCAGCATGATGAACTTTGGATCCCTCCTCGGAGTATGC	120
U.ThibetanusC	CTCCAGCACCATCAAATATCTCAGCATGATGAACTTTGGATCCCTCCTCGGAGTATGC	120
U.ThibetanusB	CTCCAGCACCATCAAATATCTCAGCATGATGAACTTTGGATCCCTCCTCGGAGTATGC	120
U.AmericanusF	CTCCAGCACCATCAAATATCTCAGCATGATGAACTTCGGGTCCCTCCTCGGAGTATGT	120
U.AmericanusI	CTCCAGCACCATCAAATATCTCAGCATGATGAACTTCGGGTCCCTCCTCGGAGTATGT	120
U.AmericanusG	CTCCAGCACCATCAAATATCTCAGCATGATGAACTTCGGGTCCCTCCTCGGAGTATGT	120
U.AmericanusA	CTCCAGCACCATCAAATATCTCAGCATGATGAACTTCGGGTCCCTCCTCGGAGTATGT	120
U.AmericanusB	CTCCAAACCATCAAATATCTCAGCATGATGAACTTCGGATCTCTCCTCGGAGTATGT	120
U.AmericanusH	CTCCAAACCATCAAATATCTCAGCATGATGAACTTCGGATCTCTCCTCGGAGTATGT	120
U.AmericanusE	CTCCAAACCATCAAATATCTCAGCATGATGAACTTCGGATCTCTCCTCGGAGTATGT	120
U.AmericanusC	CTCCAAACCATCAAATATCTCAGCATGATGAACTTCGGATCCCTCCTCGGAGTATGT	120
U.AmericanusD	CTCCAAACCATCAAATATCTCAGCATGATGAACTTCGGATCCCTCCTCGGAGTATGT	120
	** ** *	
U.MaritimusA	TTAATTCTACAGATTCTAACAGGCCTGTTTCTAGCCATACACTATACATCAGACACAACC	180
U.MaritimusB	TTAATTCTACAGATTCTAACAGGCCTGTTTCTAGCCATACACTATACATCAGACACAACC	180
U.MaritimusC	TTAATTCTACAGATTCTAACAGGCCTGTTTCTAGCCATACACTATACATCAGACACAACC	180
U.MaritimusD	TTAATTCTACAGATTCTAACAGGCCTGTTTCTAGCCATACACTATACATCAGACACAACC	180
U.MaritimusE	TTAATTCTACAGATTCTAACAGGCCTGTTTCTAGCCATACACTATACATCAGACACAACC	180
U.ArctosG	TTAATTCTACAGATTCTAACAGGCCTGTTTCTAGCCATACACTATACATCAGACACAACC	180
U.ArctosD	TTAATTCTACAGATTCTAACAGGCCTGTTTCTAGCCATACACTATACACACAGACACAACC	180
U.ArctosE	TTAATTCTACAGATTCTAACAGGCCTGTTTCTAGCCATACACTATACACACAGACACAACC	180
U.ArctosA	TTAATTCTACAGATTCTAACAGGCCTGTTTCTAGCCATACACTATACACACAGACACAACC	180
U.ArctosF	TTAATTCTACAGATTCTAACAGGCCTGTTTCTAGCCATACACTATACACACAGACACAACC	180
U.ArctosJ	TTAATTCTACAGATTCTAACAGGCCTGTTTCTAGCCATACACTATACACACAGACACAACC	180
U.ArctosC	TTAGTTCTACAGATTCTAACAGGCCTGTTTCTAGCCATACACTATACATCAGACACAACC	180
U.ArctosI	TTAGTTCTACAGATTCTAACAGGCCTGTTTCTAGCCATACACTATACATCAGACACAACC	180
U.ArctosB	TTAATCCTACAGATTCTAACAGGCCTGTTTCTAGCCATACACTATACATCAGACACAACC	180
U.ArctosH	TTAATCCTACAGATTCTAACAGGCCTGTTTCTAGCCATACACTATACATCAGACACAACC	180
U.ThibetanusA	CTAATCCTACAGATTCTGACAGGCCTATTTCTAGCTATACACTACACATCAGACGCGACT	180
U.ThibetanusD	CTAATCCTACAGATTCTGACAGGCCTATTTCTAGCTATACACTACACATCAGACGCGACT	180
U.ThibetanusC	CTAATCCTACAGATTCTGACAGGCCTATTTCTAGCTATACACTACACATCAGACGCGACT	180
U.ThibetanusB	CTAATCCTACAGATTCTGACAGGCCTATTTCTAGCTATACACTACACATCAGACGCGACT	180
U.AmericanusF	TTAGTACTACAAATTCTAACGGGCCTATTCCTAGCTATACACTATACATCAGACACAAC	180
U.AmericanusI	TTAGTACTACAAATTCTAACGGGCCTATTCCTAGCTATACACTATACATCAGACACAAC	180
U.AmericanusG	TTAGTACTACAAATTCTAACGGGCCTATTCCTAGCTATACACTATACATCAGACACAAC	180
U.AmericanusA	TTAGTACTACAAATTCTAACGGGCCTATTCCTAGCTATACACTATACATCAGACACAAC	180
U.AmericanusB	TTAGTACTACAAATTCTAACGGGCCTATTTCTAGCCATACACTACACATCAGATACAAC	180
U.AmericanusH	TTAGTACTACAAATTCTAACGGGCCTATTTCTAGCCATACACTACACATCAGATACAAC	180
U.AmericanusE	TTAGTACTACAAATTCTAACGGGCCTATTTCTAGCCATACACTACACATCAGATACAAC	180
U.AmericanusC	TTAGTACTACAAATTCTAACGGGCCTATTTCTAGCCATACACTACACATCAGATACAAC	180
U.AmericanusD	TTAGTACTACAAATTCTAACGGGCCTATTTCTAGCCATACACTACACATCAGATACAAC	180
	** * *	

U. MaritimusA	ACAGCTTTTTCATCAGTCA	CCCCA	TTTGCCGAGACGTTCA	CTACCGGGT	GAGTTATCCGA	240
U. MaritimusB	ACAGCTTTTTCATCAGTCA	CCCCA	TTTGCCGAGACGTTCA	CTACCGGGT	GAGTTATCCGA	240
U. MaritimusC	ACAGCTTTTTCATCAGTCA	CCCCA	TTTGCCGAGACGTTCA	CTACCGGGT	GAGTTATCCGA	240
U. MaritimusD	ACAGCTTTTTCATCAGTCA	CCCCA	TTTGCCGAGACGTTCA	CTACCGGGT	GAGTTATCCGA	240
U. MaritimusE	ACAGCTTTTTCATCAGTCA	CCCCA	TTTGCCGAGACGTTCA	CTACCGGGT	GAGTTATCCGA	240
U. ArctosG	ACAGCTTTTTCATCAGTCA	CCCCA	TTTGCCGAGACGTTCA	CTACCGGGT	GAGTTATCCGA	240
U. ArctosD	ACAGCTTTTTCATCGGTCA	CCCCA	TTTGCCGAGACGTTCA	CTACCGGGT	GAGTTATCCGA	240
U. ArctosE	ACAGCCTTTTTCATCGGTCA	CCCCA	TTTGCCGAGACGTTCA	CTACCGGGT	GAGTTATCCGA	240
U. ArctosA	ACAGCTTTTTCATCGGTCA	CCCCA	TTTGCCGAGACGTTCA	CTACCGGGT	GAGTTATCCGA	240
U. ArctosF	ACAGCTTTTTCATCGGTCA	CCCCA	TTTGCCGAGACGTTCA	CTACCGGGT	GAGTTATCCGA	240
U. ArctosJ	ACAGCTTTTTCATCGGTCA	CCCCA	TTTGCCGAGACGTTCA	CTACCGAT	GAGTTATCCGA	240
U. ArctosC	ACAGCTTTTTCATCGGTCA	CCCCA	TTTGCCGAGACGTTCA	CTACCGAT	GAGTTATCCGA	240
U. ArctosI	ACAGCTTTTTCATCGGTCA	CCCCA	TTTGCCGAGACGTTCA	CTACCGAT	GAGTTATCCGA	240
U. ArctosB	ACAGCTTTTTCATCAGTCA	CCCCA	TTTGCCGAGACGTTCA	CTACCGAT	GAGTTATCCGA	240
U. ArctosH	ACAGCTTTTTCATCAGTCA	CCCCA	TTTGCCGAGACGTTCA	CTACCGAT	GAGTTATCCGA	240
U. ThibetanusA	ACAGCCTTTTTCATCAGTC	CGCCCAT	TTTGCCGAGACGTCCAT	TACCGATGA	ATTATCCGA	240
U. ThibetanusD	ACAGCCTTTTTCATCAGTC	CGCCCAT	TTTGCCGAGACGTCCAT	TACCGATGA	ATTATCCGA	240
U. ThibetanusC	ACAGCCTTTTTCATCAGTC	CGCCCAT	TTTGCCGAGACGTCCAT	TACCGATGA	ATTATCCGA	240
U. ThibetanusB	ACAGCCTTTTTCATCAGTC	CGCCCAT	TTTGCCGAGACGTCCAT	TACCGATGA	ATTATCCGA	240
U. AmericanusF	ACAGCCTTTTTCATCAATCA	CCCCAT	TTTGCCGAGATGTTCA	CTACCGATGA	ATTATCCGA	240
U. AmericanusI	ACAGCCTTTTTCATCAATCA	CCCCAT	TTTGCCGAGATGTTCA	CTACCGATGA	ATTATCCGA	240
U. AmericanusG	ACAGCCTTTTTCATCAATCA	CCCCAT	TTTGCCGAGATGTTCA	CTACCGATGA	ATTATCCGA	240
U. AmericanusA	ACAGCCTTTTTCATCAATCA	CCCCAT	TTTGCCGAGATGTTCA	CTACCGATGA	ATTATCCGA	240
U. AmericanusB	ACAGCCTTTTTCATCAATCA	CCCCAT	TTTGCCGAGATGTTCA	CTACCGATGA	ATTATCCGA	240
U. AmericanusH	ACAGCCTTTTTCATCAATCA	CCCCAT	TTTGCCGAGATGTTCA	CTACCGATGA	ATTATCCGA	240
U. AmericanusE	ACAGCCTTTTTCATCAATCA	CCCCAT	TTTGCCGAGATGTTCA	CTACCGATGA	ATTATCCGA	240
U. AmericanusC	ACAGCCTTTTTCATCAATCA	CCCCAT	TTTGCCGAGATGTTCA	CTACCGATGA	ATTATCCGA	240
U. AmericanusD	ACAGCCTTTTTCATCAATCA	CCCCAT	TTTGCCGAGATGTTCA	CTACCGATGA	ATTATCCGA	240
*****						240

[illegible]

U. MaritimusA	CTACGATCCATCCCTAATAAACTAGGAGGAGTACTAGCACTAATTTTCTCCATTCTAATC	900
U. MaritimusB	CTACGATCCATCCCTAATAAACTAGGAGGAGTACTAGCACTAATTTTCTCCATTCTAATC	900
U. MaritimusC	CTATGATCCATCCCTAATAAACTAGGAGGAGTACTAGCACTAATTTTCTCCATTCTAATC	900
U. MaritimusD	CTACGATCCATCCCTAATAAACTAGGAGGAGTACTAGCACTAATTTTCTCCATTCTAATC	900
U. MaritimusE	CTACGATCCATCCCTAATAAACTAGGAGGAGTACTAGCACTAATTTTCTCCATTCTAATC	900
U. ArctosG	CTACGATCCATCCCTAATAAACTAGGAGGAGTACTAGCACTAATTTTCTCCATTCTAATC	900
U. ArctosD	CTACGATCCATCCCTAATAAACTAGGAGGAGTACTAGCACTAATTTTCTCCATTCTAATC	900
U. ArctosE	CTACGATCCATCCCTAATAAACTAGGAGGAGTACTAGCACTAATTTTCTCCATTCTAATC	900
U. ArctosA	CTACGATCCATCCCTAATAAACTAGGAGGAGTACTAGCACTAATTTTCTCCATTCTAATC	900
U. ArctosF	CTACGATCCATCCCTAATAAACTAGGAGGAGTACTAGCACTAATTTTCTCCATTCTAATC	900
U. ArctosJ	CTACGATCCATCCCTAATAAACTAGGAGGAGTACTAGCACTAATTTTCTCCATTCTAATC	900
U. ArctosC	CTGCGATCCATCCCTAATAAACTAGGAGGAGTACTAGCACTAATTTTCTCCATTCTAATC	900
U. ArctosI	CTGCGATCCATCCCTAATAAACTAGGAGGAGTACTAGCACTAATTTTCTCCATTCTAATC	900
U. ArctosB	CTACGATCCATCCCTAATAAACTAGGAGGAGTACTAGCACTAATTTTCTCCATTCTAATC	900
U. ArctosH	CTACGATCCATCCCTAATAAACTAGGAGGAGTACTAGCACTAATTTTCTCCATTCTAATC	900
U. ThibetanusA	CTACGATCCATCCCCAACAACTAGGAGGAGTACTAGCGCTAATCTTCTCTATCCTAATC	900
U. ThibetanusD	CTACGATCCATCCCCAACAACTAGGAGGAGTACTAGCGCTAATCTTCTCTATCCTAATC	900
U. ThibetanusC	CTACGATCCATCCCCAACAACTAGGAGGAGTACTAGCGCTAATCTTCTCTATCCTAATC	900
U. ThibetanusB	TTACGATCCATCCCCAACAACTAGGAGGAGTACTAGCGCTAATCTTCTCTATCCTAATC	900
U. AmericanusF	CTACGGTCCATCCCCAACAACTAGGAGGGGTACTAGCACTAATTTTCTCTATCCTAATC	900
U. AmericanusI	CTACGGTCCATCCCCAACAACTAGGAGGGGTACTAGCACTAATTTTCTCTATCCTAATC	900
U. AmericanusG	CTACGGTCCATCCCCAACAACTAGGAGGGGTACTAGCACTAATTTTCTCTATCCTAATC	900
U. AmericanusA	CTACGGTCCATCCCCAACAACTAGGAGGGGTACTAGCACTAATTTTCTCTATCCTAATC	900
U. AmericanusB	CTACGGTCCATCCCCAACAACTAGGAGAGTGCTAGCACTAATTTTCTCTATCCTAATC	900
U. AmericanusH	CTACGGTCCATCCCCAACAACTAGGAGAGTGCTAGCACTAATTTTCTCTATCCTAATC	900
U. AmericanusE	CTACGGTCCATCCCCAACAACTAGGAGAGTGCTAGCACTAATTTTCTCTATCCTAATC	900
U. AmericanusC	CTACGGTCCATCCCCAACAACTAGGAGAGTGCTAGCACTAATTTTCTCTATCCTAATC	900
U. AmericanusD	CTACGGTCCATCCCCAACAACTAGGAGAGTGCTAGCACTAATTTTCTCTATCCTAATC	900
	* * * * *	

A = adenine, T = thymine, C = cytosine, G = guanine, N=A/T/C/G, * = homologous bases.

Table A10.7: Sequence alignment of the cytochrome *b* gene for lion, tiger, leopard and human. *Panthera* priming regions are shown in yellow. Tiger specific priming region is shown in green and leopard specific is shown in blue. The consensus sequence is that of the leopard.

Leopard	1	ATGACCAACATTTCGAAAATCACACCCCTCATCAAAATTATTAATCACTCATTTCATT
Lion	1TG.....
Tiger	1T.....
Human	1	...CCA..A..C...ATTA.....A..A...T..A.....C.....C
consensus	1	ATGACCAACATTTCGAAAATCACACCCCTCATCAAAATTATTAATCACTCATTTCATT
Leopard	58	GATCTCCCGCTCCATCCAACATCTCAACATGATGGAACCTTTGGCTCCCTATTAGG
Lion	58T...A.....C....T.....G.....A.....T.....
Tiger	58	..C..A.....C.....T..T..G.....A.....T..C....
Human	58	..C...A..C.....CG.....A.....C.....A..CC..T..
consensus	61	GATCTCCCGCTCCATCCAACATCTCAACATGATGGAACCTTTGGCTCCCTATTAGG
Leopard	114	GGTATGTTTAAATCCTACAAATTCTCACCGGCCTCTTTCTAGCCATACATTATACAT
Lion	114	A.....C.....C...C
Tiger	114	..G..C.....T.....C.....T.....C..C....
Human	114	C..CC..CC.G.....C.....CAC...A..A..A..C.....C..CT..C
consensus	121	GGTATGTTTAAATCCTACAAATTCTCACCGGCCTCTTTCTAGCCATACATTATACAT
Leopard	170	CAGACACAACAACCGCTTTCTCATCAGTTACCCATATCTGCCGCGATGTAAATTAT
Lion	170T.....C.....C...T.....C...
Tiger	170T.....A.....C..T.....C.....C..C
Human	170G..CT.....C..T.....A..CG....C...ACT..A..C.....
consensus	181	CAGACACAACAACCGCTTTCTCATCAGTTACCCATATCTGCCGCGATGTAAATTAT
Leopard	226	GGCTGAATTATCCGGTATCTACACGCCAATGGAGCCTCCATATTCTTTATCTGCCT
Lion	226C.....C.....
Tiger	226G.....A.....T.....C.....T..
Human	226C.....C..C..T.....C.....A.....
consensus	241	GGCTGAATTATCCGGTATCTACACGCCAATGGAGCCTCCATATTCTTTATCTGCCT
Leopard	282	ATACATACATGTAGGACGAGGGATATACTATGGCTCCTACACTTTCTCAGAGACAT
Lion	282G.....A.....T.....A.....
Tiger	282G..C.....A.....C.....C.....A.....
Human	282	C..T..C....CA..C..G....CC....T..C..A..A..TTCTC..A.....A..C..
consensus	301	ATACATACATGTAGGACGAGGGATATACTATGGCTCCTACACTTTCTCAGAGACAT
Leopard	338	GGAACATTGGAGTCGTATTATTGTTTCACAGTTATGGCCACAGCCTTCATAGGATAT
Lion	338	.A.....A..T.....G.....A..T.....
Tiger	338	.A..T..C...GA..T..GC.....T..G..C.....T.....
Human	338	.A.....C..CA..TA..CC..CC..C..TG..AC...A..A.....C...
consensus	361	GGAACATTGGAGTCGTATTATTGTTTCACAGTTATGGCCACAGCCTTCATAGGATAT
Leopard	394	GTCTTACCG
Lion	394
Tiger	394A
Human	394	...C..C...
consensus	421	GTCTTACCG

A = adenine, T = thymine, C = cytosine, G = guanine, N=A/T/C/G, '.' = homologous bases.

Table A10.8: Alignment of the 12S rRNA gene for musk deer. Species-specific primers are shown in yellow.

fuscusAY184427	CATAGGTTTGGTCCCAGCCTTCCTATTGATTCTTAATAAACTTACACATGCAAGCATCCA	60
chrysogasterAY184426	CATAGGTTTGGTCCCAGCCTTCCTATTGATTCTTAATAAACTTACACATGCAAGCATCCA	60
leucogasterAY921577	CATAGGTTTGGTCCCAGCCTTCCTATTGATTCTTAATAAACTTACACATGCAAGCATCCA	60
berezovskiiAY184425	CATAGGTTTGGTCCCAGCCTTCCTATTGATTCTTAATAAACTTACACATGCAAGCATCCA	60
moschiferusAY327518	CACAGGTTTGGTCCCAGCCTTCCTATTGATTCTTAATAAACTTACACATGCAAGCATCCA	60
moschiferusAY121988	CACAGGTTTGGTCCCAGCCTTCCTATTGATTCTTAATAAACTTACACATGCAAGCATCCA	60
moschiferusAY184428	CACAGGTTTGGTCCCAGCCTTCCTATTGATTCTTAATAAACTTACACATGCAAGCATCCA	60
** *****		
fuscusAY184427	CACCCCGGTGAAAATGCCCTCCGAGTCTAACGGAACCAAGAGGAGCAGGTATCAAGCACA	120
chrysogasterAY184426	CACCCCGGTGAAAATGCCCTCCGAGTCTAACGGAACCAAGAGGAGCAGGTATCAAGCACA	120
leucogasterAY921577	CATCCCGGTGAAAATGCCCTCCGAGTCTAACGGAACCAAGAGGAGCAGGTATCAAGCACA	120
berezovskiiAY184425	CACCCCGGTGAAAATGCCCTCCGAGTCTAACGGAACCAAGAGGAGCAGGTATCAAGCACA	119
moschiferusAY327518	CGCCCGGTGAAAATGCCCTCCGAGTCTAACGGAACCAAGAGGAGCAGGTATCAAGCACA	119
moschiferusAY121988	CGCCCGGTGAAAATGCCCTCCGAGTCTAACGGAACCAAGAGGAGCAGGTATCAAGCACA	119
moschiferusAY184428	CGC CACCCGTGAAAATGCCCTCCGAGTCTAACGGAACCAAGAGGAGCAGGTATCAAGCACA	119
* *****		
fuscusAY184427	CACCTTGTAGCTCACAACACCTTGCTTAACCACACCCCAACGGGAAACAGCAGTGATAGA	180
chrysogasterAY184426	CACCTTGTAGCTCACAACACCTTGCTTAACCACACCCCAACGGGAAACAGCAGTGATAGA	180
leucogasterAY921577	CACCTTGTAGCTCACAACACCTTGCTTAACCACACCCCAACGGGAAACAGCAGTGATAGA	180
berezovskiiAY184425	CACCTTGTAGCTCACAACACCTTGCTTAACCACACCCCAACGGGAAACAGCAGTGATAGA	179
moschiferusAY327518	CACCTTGTAGCTCATAACACCTTGCTTAACCACACCCCAACGGGAAACAGCAGTGATAGA	179
moschiferusAY121988	CACCTTGTAGCTCATAACACCTTGCTTAACCACACCCCAACGGGAAACAGCAGTGATAGA	179
moschiferusAY184428	CACCTTGTAGCTCATAACACCTTGCTTAACCACACCCCAACGGGAAACAGCAGTGATAGA	179
*** *****		
fuscusAY184427	AATTAAGCCATAAACGAAAGTTTGACTAAGTTATATTAACTAGGGTTGGTAAATCTCGTG	240
chrysogasterAY184426	AATTAAGCCATAAACGAAAGTTTGACTAAGTTATATTAACTAGGGTTGGTAAATCTCGTG	240
leucogasterAY921577	AATTAAGCCATAAACGAAAGTTTGACTAAGTTATATTAACTAGGGTTGGTAAATCTCGTG	240
berezovskiiAY184425	AATTAAGCCATAAACGAAAGTTTGACTAAGTTATATTAACTAGGGTTGGTAAATCTCGTG	239
moschiferusAY327518	AATTAAGCCATAAACGAAAGTTTGACTAAGTTATATTAACTAGGGTTGGTAAATCTCGTG	239
moschiferusAY121988	AATTAAGCCATAAACGAAAGTTTGACTAAGTTATATTAACTAGGGTTGGTAAATCTCGTG	239
moschiferusAY184428	AATTAAGCCATAAACGAAAGTTTGACTAAGTTATATTAACTAGGGTTGGTAAATCTCGTG	239

fuscusAY184427	CCAGCCACCGCGGTGATACGATTGACCCAAATTAATAGGAATACGGCGTAAAGCGTGTTA	300
chrysogasterAY184426	CCAGCCACCGCGGTGATACGATTGACCCAAATTAATAGGAATACGGCGTAAAGCGTGTTA	300
leucogasterAY921577	CCAGCCACCGCGGTGATACGATTGACCCAAATTAATAGGAATACGGCGTAAAGCGTGTTA	300
berezovskiiAY184425	CCAGCCACCGCGGTGATACGATTGACCCAAATTAATAGGAATACGGCGTAAAGCGTGTTA	299
moschiferusAY327518	CCAGCCACCGCGGTGATACGATTGACCCAAATTAATAGGAATACGGCGTAAAGCGTGTTA	299
moschiferusAY121988	CCAGCCACCGCGGTGATACGATTGACCCAAATTAATAGGAATACGGCGTAAAGCGTGTTA	299
moschiferusAY184428	C CAGCCACCGCGGTGATACGATTGACCCAAATTAATAGGAATACGGCGTAAAGCGTGTTA	299

fuscusAY184427	AAGCCTTCAATCAAATAGAGTTAAATTTTAATTAAGCTGTAAAAAGCCACAATTATAACA	360
chrysogasterAY184426	AAGCCTTCAATCAAATAGAGTTAAATTTTAATTAAGCTGTAAAAAGCCACAATTATAACA	360
leucogasterAY921577	TAGCCTTCAATCAAATAGAGTTAAATTTTAATTAAGCTGTAAAAAGCCACAATTATAACA	360
berezovskiiAY184425	AAGCCTTCAATCAAATAGAGTTAAATTTTAATTAAGCTGTAAAAAGCCACAATTATAACA	359
moschiferusAY327518	AAGCCTTCAATCAAATAGAGTTAAATTTTAATTAAGCTGTAAAAAGCCACAATTATAACA	359
moschiferusAY121988	AAGCCTTCAATCAAATAGAGTTAAATTTTAATTAAGCTGTAAAAAGCCACAATTATAACA	359
moschiferusAY184428	AAGCCTTCAATCAAATAGAGTTAAATTTTAATTAAGCTGTAAAAAGCCACAATTATAACA	359
**** * *		
fuscusAY184427	AAAATAAACGACGAAAGTAACCTCTACAAACCGCTGACACACTATAGCTAAGACCCAACTG	420
chrysogasterAY184426	AAAATAAACGACGAAAGTAACCTCTACAAACCGCTGACACACTATAGCTAAGACCCAACTG	420
leucogasterAY921577	AAAATAAACGACGAAAGTAACCTCTACAAACCGCTGACACACTATAGCTAAGACCCAACTG	420
berezovskiiAY184425	AAAATAAATGACGAAAGTAACCTCTACAAACCGCTGACACACTATAGCTAAGACCCAACTG	419
moschiferusAY327518	AAAATAAATGACGAAAGTAACCTCTACAAACCGCTGATACACTATAGCTAAGACCCAACTG	419
moschiferusAY121988	AAAATAAATGACGAAAGTAACCTCTACAAACCGCTGATACACTATAGCTAAGACCCAACTG	419
moschiferusAY184428	AAAATAAATGACGAAAGTAACCTCTACAAACCGCTGATACACTATAGCTAAGACCCAACTG	419

A = adenine, T = thymine, C = cytosine, G = guanine, N=A/T/C/G, * = homologous bases.

Primers were ordered from Sigma Genosys®. The final list of designed primers can be seen in Table A10.9 as well as the name they were given, their sequence and T_m as supplied by Sigma Genosys®. Primers arrived lyophilized and were mixed with

sterile water to a concentration of 100 μ M. A dilution of each primer was made to 10 μ M in volumes of 100 μ L. Primer concentrations of 10 μ M were stored at 4 °C until use and the 100 μ M primers were stored frozen.

Table A10.9: Universal and species specific primers, their labels and T_m.

Animal	Label	5'-----Sequence-----3'	T _m (°C)
<i>Ursus</i> sp.	Ursus 1 st For	TET-CACCATCAAAYATCTCAGCATGATG	66.1
<i>U. thibetanus</i>	U.thib Rev	GATGAAAAGGCTGTAGTCGCG	65.8
<i>Ursus</i> sp.	Ursus 1 st Rev	CATGTTTCTGRGARTAGGTATGAGCCATA	67.2
<i>Ursus</i> sp.	Ursus 2 nd For	FAM6-GCACYCCRCCCCACAT	59.5
<i>Ursus</i> sp.	Ursus 2 nd Rev	CCTCGTTGYTTGGAYGTGTGTAG	65.3
<i>Panthera</i> sp.	Panthera1F	FAM6 – ATGACCAACATTCGAAAATCACACC	68.4
<i>Panthera</i> sp.	Panthera1R	GCTAGRAAGAGGCCRGTGAG	58.3
<i>Panthera</i> sp.	Panthera2F	TET – CCTCCATATTCTTTATCTGCCTATACAT	63.1
<i>Panthera</i> sp.	Panthera2R	GGTAAGACATATCCTATGAAGGCTGT	63.2
<i>P. tigris</i> sp.	Tiger R	CAGTGAGGATTTGTAAGATTAAGCAC	62.8
<i>P. pardus</i>	Leopard R	GTGAACAATAATACGACTCCAATGTTC	64.2
<i>Moschus</i> sp.	Musk F	FAM6 – CCCGGTGAAAATGCCCTCCGAGTC	77.1
<i>Moschus</i> sp.	Musk R	TATGACCGCGGTGGCTG	67.2

FAM6, 6-carboxyfluorescein; and TET, 6-carboxy-4,7,2',7'-tetrachlorofluorescein are dye labels of blue and green, respectively.

bp = base pairs, A = adenine, T = thymine, C = cytosine, G = guanine, R=A/G, Y=C/T, N=A/T/C/G

Samples consisted of hair from *U. martimus*, *U. americanus*, *U. thibetanus*, *P. tigris* sp. and *Moschus* sp. and were donated from zoos (Edinburgh Zoo, Belfast Zoo, Dudley Zoo, the Isle of Wight Zoo and a sample provided by Kathryn Tobe). Seven samples of TCM were also obtained from HMRC in the form of pills (containing bear) and from the BBC (containing tiger and musk) in the form of plasters.

Hair samples were extracted using the QIAamp® DNA Micro Kit using the protocol for hair samples. Extraction from the plasters and from the pills was accomplished by following the protocol for extraction of DNA from tissue. Due to the low expected yield of DNA from the pill samples the DNA isolate was further purified and

concentrated using MicroCon® spin columns (Millipore Co, USA), which remove inhibitory substances and concentrate the sample.

Primers were initially tested in small multiplexes for each set of species (bear, tiger and musk deer). PCRs contained buffer, dNTPs, 1 unit Taq, forward and reverse primers (at a final concentration of 0.5 µM each), 1 µL of template DNA and water to 20 µL. The PCR cycle proceeded for 32 cycles at 95 °C for 45 seconds, 60 °C for 45 seconds and 72 °C for 1 minute followed by a final extension step of 20 minutes at 72 °C.

Samples were then analysed on an ABI 310 Genetic Analyser.

Results, Discussion, Conclusion and Future Work

Results from initial testing of the bear specific primers can be found in Figure A10.1; for tiger in Figure A10.2 and for musk deer in A10.3. No leopard samples were obtained so the testing of the leopard primers has not been completed.

The results of the two pill samples reacted with the multiplex were negative, even when spiked with positive control DNA. One of the plasters showed a positive result for tiger.

Primers produced products of the expected colour and size in bp. Cross species testing and validation has started but not been completed. The species-specific primer for *U. thibetanus* did not react with *U. maritimus* or *U. arctos* and the leopard primers did not react with the tiger species. More tiger samples have been obtained and will be tested. Other *Panthera* and big cat species have also been obtained for testing.

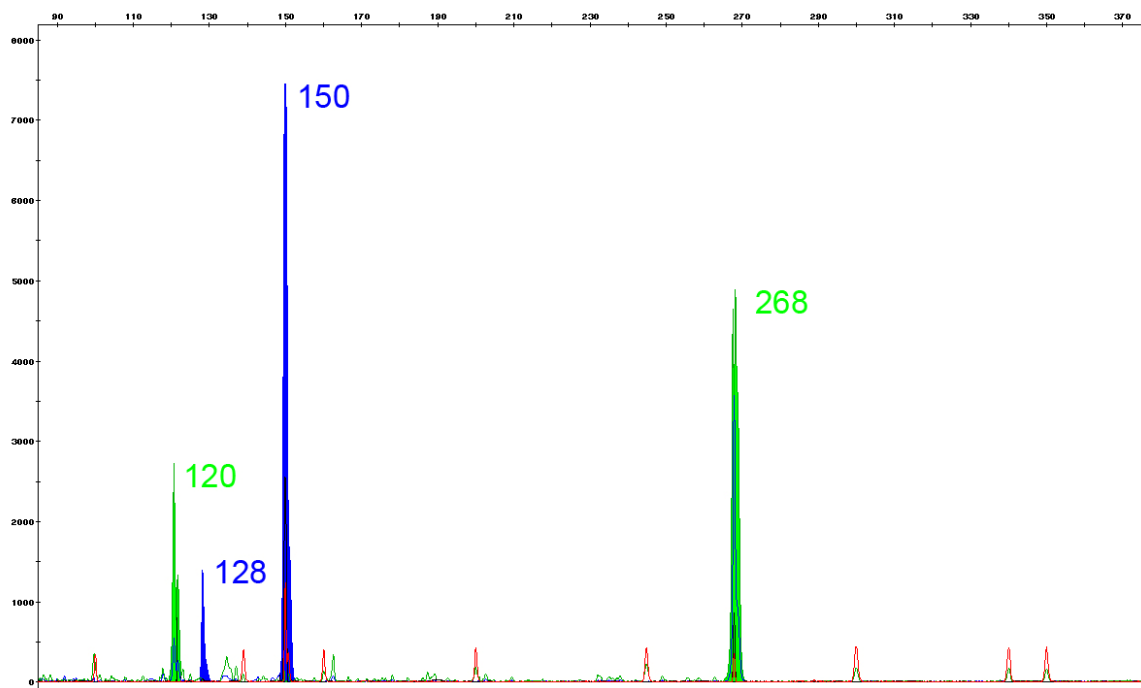


Figure A10.1: Ursus 1st F and R, Ursus 2nd F and R and UthibRev multiplexed together and reacted with *U. thibetanus* DNA with a 2 second injection. The *U. thibetanus* specific peak can be seen at 120 bp and was expected at 127 bp. Peaks are all on-scale and there is only one extra peak at 128 bp.

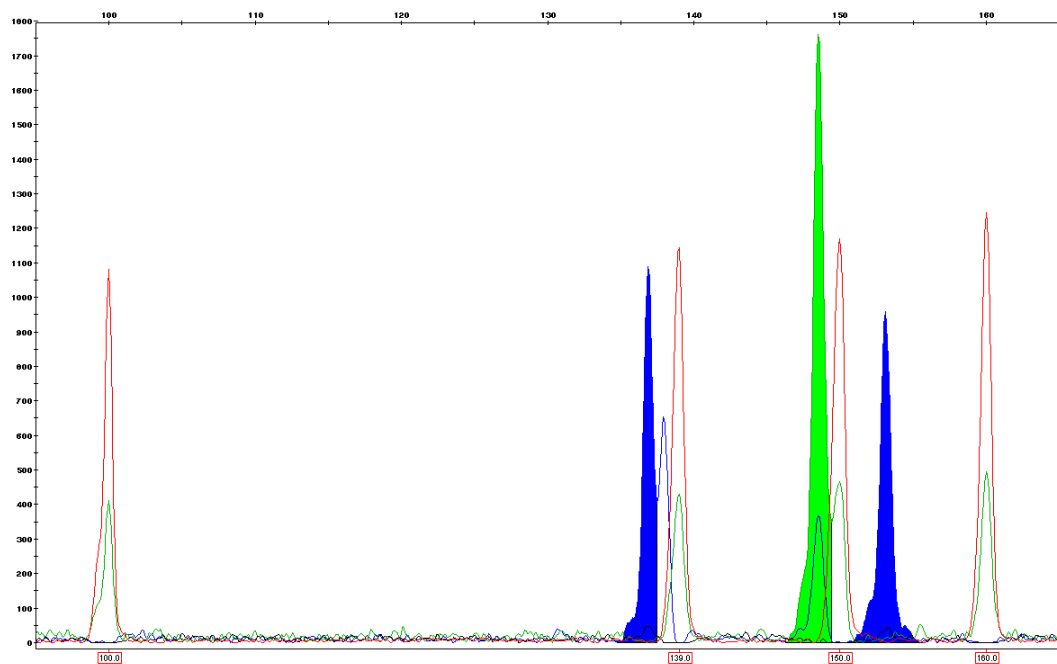


Figure A10.2: Panthera test showing tiger specific peak in blue at 136 bp, and Panthera specific peaks in green and blue at 147 and 152 bp, respectively.

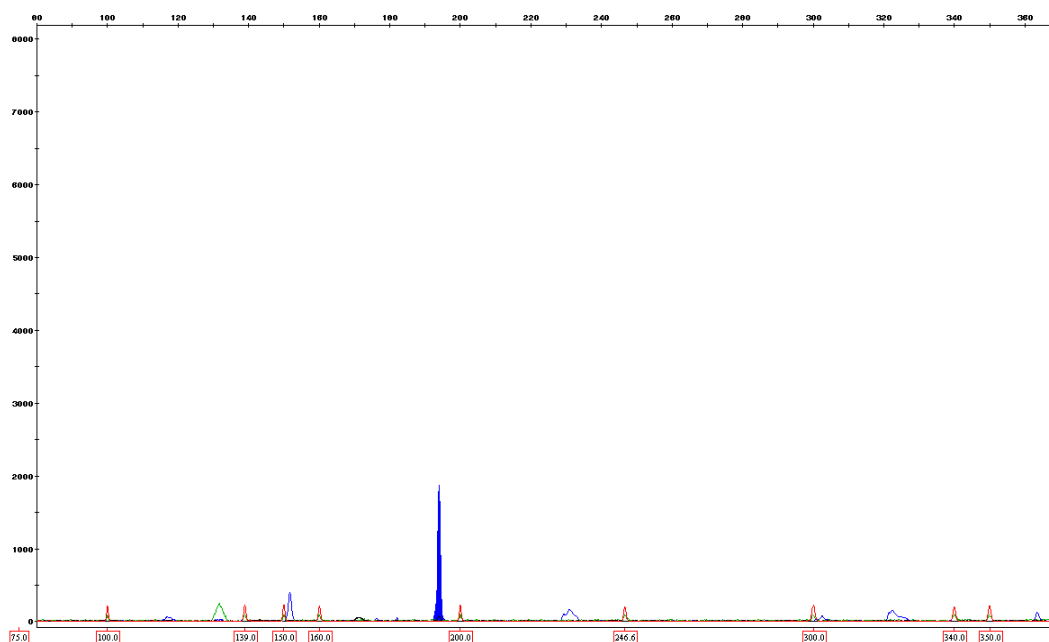


Figure A10.3: Musk specific peak at approximately 190 bp.

Further testing needs to be undertaken to determine the limits of sensitivity for the primers. Primer concentrations and PCR cycle numbers can be altered to ensure the maximum sensitivity so that samples with even minute amounts of DNA can be identified. Following this the primers will be multiplexed together to create a simple test to identify mammalian components in TEAM.

In addition many more voucher samples will be obtained to ensure the test will react with all of the species for which the test was designed. The primers will also need to be tested with a multitude of other animals to determine if any other species will produce a positive result.

Once the test is optimized for maximum sensitivity and efficiency it will be a very useful tool in the analysis of commercial products and TEAM for animal derived material. This test will help stem the illegal trade in endangered species, which ultimately will help with conservation. It will also help customs by providing molecular evidence to prosecute offenders.

A molecular test to differentiate between *Sciurus vulgaris* (red squirrel) and *S. carolinensis* (grey squirrel) using the cytochrome *b* gene on the mitochondrial genome

The Centre for Forensic Science (CFS) was recently contacted and asked if we could differentiate between red (*Sciurus vulgaris*) and grey (*S. carolinensis*) squirrels. The sex of the animal would also need to be determined.

A literature review was undertaken and only one article was found for the identification of squirrels which was based on microsatellite loci [13]. This article did not describe the differentiation between the sexes and will not differentiate between the two species [13].

A new set of primers was therefore designed. Cytochrome *b* sequences (partial) were obtained from GenBank. Sequences were aligned using the alignment tool ClustalW, available from the European Molecular Biology Laboratory European Bioinformatics Institute (EMBL-EBI) (<http://www.ebi.ac.uk/Information/>). Table A10.10 shows the alignment file and the location of possible species-specific primer for the two species of squirrel. The shaded bases are potential primers and demonstrate where enough sequence variation exists which can be used for species-specific primers for either species.

Table A10.10: Partial mitochondrial cytochrome *b* sequences for red and grey squirrel. Possible species-specific primers are highlighted in grey.

Grey	-----ATGACAAATATCCGCAAAACCCACCCACTACTAAAAATGTTAATCACTCTTTT	54
Red	AACATAATGACAAATATCCGCAAAACCCACCCTCTAATCAAAATCGTTAATCACTCTTTT	120

Grey	ATCGACCTTCCCGCTCCTTCAAACATTTAGCCTGATGAACTTTGGCTCCCTCCTTGA	114
Red	ATTGACCTCCCAGCTCCCTCAAACATCTCAGCCTGATGAACTTTGGCTCACTCCTTGA	180
	** *****	
Grey	CTTTCCTTCTAATCCAAATCTTAACGGCCTATTTCTAGCCATACACTACACCTCCGAC	174
Red	CTTGTCTCCTAATTCAAATCTTAACGGCCTATTTCTAGCTATACACTATACATCAGAC	240

Grey	ACAATAACAGCCTTCTCCTCTGTAAACATATTTGCCGAGACGTAAACTACGGCTGACTA	234
Red	ACAATAACAGCTTTTCTTCCGTTACTCACATCTGCCGAGACGTAAATATGGCTGATTA	300

Grey	ATTCGATATATACATGCTAATGGTGCACTTTATTCTTTATTTGCTTGTTCCTTCATGTA	294
Red	ATCCGATACATGATGCCAACGGCGCTTCCCTATTCTTCATTGTCCTGTTCTTCATGTA	360
	** *****	
Grey	GGTCGAGGACTTTACTACGGCTCTTACACTTCTTGAAACCTGAAATATTGGAGTAATT	354
Red	GGCCGTGGCCTTTATTATGGTTCCTACACTTCTTGAAACCTGAAATATTGGAGTTATT	420
	** ** *	

Grey	CTCCTATTGCGAGTAATAGCAACTGCCTTTATAGGTTATGTCCTTACCATGAGGACAAATA	414
Red	CTTCTCTTTGCGAGTAATAGCCACTGCAATTCATAGGCTATGTACTTCCATGAGGACAGATA	480
	** ** *	
Grey	TCATTCTGAGGCGCAACAGTTATTACTAACCACCTATCAGCTATTCCTTATATCGGCACT	474
Red	TCATTTTGAGGCGCCACAGTTATCACAAACCTCTGTCTGCTATTCCTTATATTGGAACC	540
	***** *	
Grey	ACCTTAGTAGAGTGAATCTGAGGTGGGTCTCAGTTGACAAAGCAACTCTAACA-----	528
Red	ACCCTAGTTGAATGAATCTGAGGCGGATTCTCAGTAGATAAAGCAACTCTAACACGATTTC	600
	*** ** *	

Although these primers will not be able to determine the sex of the squirrel it should be possible to include a primer that will. The sex determining region of the Y chromosome, the SRY gene, is exclusively present on the Y chromosome [14]. It is highly conserved in most mammals and is ideal for sex determination [14]. A single primer set could be designed to amplify part of the SRY gene that would bind to both species of squirrel. This would allow simultaneous detection of sex and species if the sample was either a red or grey squirrel.

No other action was taken after the identification of possible priming sites. The CFS is waiting to find out if the individual interested in this test is able to obtain funding before further testing is conducted.

A molecular test to differentiate between European beaver (*Castor fiber*) and American beaver (*Castor canadensis*) using the cytochrome *b* gene on the mitochondrial genome

The Centre For Forensic Science was asked by Edinburgh Zoo to determine if a beaver, under their keeping, was either a European beaver (*Castor fiber*) or an American beaver (*Castor canadensis*). Mammalian species identification can be confirmed by microscopy of hairs, but with closely related species this may not be possible. There are regions of DNA that show variation even with closely related species allowing identification of the species to be possible. A DNA test was used on samples labelled as being taken from a beaver to determine if it was *C. fiber* or *C. canadensis*.

Sequence data for the cytochrome *b* gene was obtained from GenBank and aligned using ClustalW (Table A10.11). A *Castor* sp. forward primer, a *C. fiber* and *C. canadensis* reverse primers were identified.

Table A10.11: Sequence alignment of part of the cytochrome *b* gene for Canadian beaver (C) and European beaver (E). Primers are highlighted.

C-AF155879	ACAGCATTTTCTTCAGTCGCACACATCTGCCGAGACGTAAACTACGGCTGAATCATCCGG	144
E-DQ088706	ACAGCATTTTCTTCAGTCGCACACATCTGCCGAGACGTAAACTACGGCTGAATCATCCGG	240
E-AJ389529	ACAGCATTTTCTTCAGTCGCACACATCTGCCGAGACGTAAACTACGGCTGAATCATCCGG	240
E-DQ088704	ACAGCATTTTCTTCAGTCGCACACATCTGCCGAGACGTAAACTACGGCTGAATCATCCGG	240
E-DQ088705	ACAGCATTTTCTTCAGTCGCACACATCTGCCGAGACGTAAACTACGGCTGAATCATCCGG	240
E-DQ088708	ACAGCATTTTCTTCAGTCGCACACATCTGCCGAGACGTAAACTACGGCTGAATCATCCGG	240
E-DQ088707	ACAGCATTTTCTTCAGTCGCACACATCTGCCGAGACGTAAACTACGGCTGAATCATCCGG	240
C-AF293348	ACAGCATTTCTCTTCAGTCGCACATATTTGCCGAGACGTCAACTACGGCTGAATTATCCGA	144
C-AF155878	ACAGCATTTCTCTTCAGTCGCACATATTTGCCGAGACGTCAACTACGGCTGAATTATCCGA	144
C-AY793641	ACAGCATTTCTCTTCAGTCGCACATATTTGCCGAGACGTCAACTACGGCTGAATTATCCGA	144
C-AY793642	ACAGCATTTCTCTTCAGTCGCACATATTTGCCGAGACGTCAACTACGGCTGAATTATCCGA	144
C-AY793644	ACAGCATTTCTCTTCAGTCGCACATATTTGCCGAGACGTCAACTACGGCTGAATTATCCGA	144
C-AY793643	ACAGCATTTCTCTTCAGTCGCACATATTTGCCGAGACGTCAACTACGGCTGAATTATCCGA	144

C-AF155879	TACCTTCACGCCAACGGAGCATCCATATTTCTTCATCTGCCTGTTTCCTACACGCAGGACGA	204
E-DQ088706	TACCTTCACGCCAACGGAGCATCCATATTTCTTCATCTGCCTGTTTCCTACACGCAGGACGA	300
E-AJ389529	TACCTTCACGCCAACGGAGCATCCATATTTCTTCATCTGCCTGTTTCCTACACGCAGGACGA	300
E-DQ088704	TACCTTCACGCCAACGGAGCATCCATATTTCTTCATCTGCCTGTTTCCTACACGCAGGACGA	300
E-DQ088705	TACCTTCACGCCAACGGAGCATCCATATTTCTTCATCTGCCTGTTTCCTACACGCAGGACGA	300
E-DQ088708	TACCTTCACGCCAACGGAGCATCCATATTTCTTCATCTGCCTGTTTCCTACACGCAGGACGA	300
E-DQ088707	TACCTTCACGCCAACGGAGCATCCATATTTCTTCATCTGCCTGTTTCCTACACGCAGGACGA	300
C-AF293348	TATCTTCATGCCAACGGAGCCTCCATATTTCTTTATCTGCCTATTCATACAGTACGAGGACGA	204
C-AF155878	TATCTTCATGCCAACGGAGCCTCCATATTTCTTTATCTGCCTATTCATACAGTACGAGGACGA	204
C-AY793641	TATCTTCATGCCAACGGAGCCTCCATATTTCTTTATCTGCCTATTCATACAGTACGAGGACGA	204
C-AY793642	TATCTTCATGCCAACGGAGCCTCCATATTTCTTTATCTGCCTATTCATACAGTACGAGGACGA	204
C-AY793644	TATCTTCATGCCAACGGAGCCTCCATATTTCTTTATCTGCCTATTCATACAGTACGAGGACGA	204
C-AY793643	TATCTTCATGCCAACGGAGCCTCCATATTTCTTTATCTGCCTATTCATACAGTACGAGGACGA	204
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C-AF155879      GGAATCTACTACGGATCCTACACCTTTACAGAAACATGAAATATTGGTATCATCTTACTA 264
E-DQ088706      GGAATCTACTACGGATCCTACACCTTTACAGAAACATGAAATATTGGTATCATCTTACTA 360
E-AJ389529      GGAATCTACTACGGATCCTACACCTTTACAGAAACATGAAATATTGGTATCATCTTACTA 360
E-DQ088704      GGAATCTACTACGGATCCTACACCTTTACAGAAACATGAAATATTGGTATCATCTTACTA 360
E-DQ088705      GGAATCTACTACGGATCCTACACCTTTACAGAAACATGAAATATTGGTATCATCTTACTA 360
E-DQ088708      GGAATCTACTACGGATCCTACACCTTTACAGAAACATGAAATATTGGTATCATCTTACTA 360
E-DQ088707      GGAATCTACTACGGATCCTACACCTTTACAGAAACATGAAATATTGGTATCATTTTACTA 360
C-AF293348      GGGGTCTACTATGGATCATATACCTTCACAGAGACATGGAACATTGGCATCATTTCTACTA 264
C-AF155878      GGGGTCTACTATGGATCATATACCTTCACAGAGACATGGAACATTGGCATCATTTCTACTA 264
C-AY793641      GGGGTCTACTATGGATCATATACCTTCACAGAGACATGGAACATTGGCATCATTTCTACTA 264
C-AY793642      GGGATCTACTATGGATCATATACCTTCACAGAGACATGGAACATTGGCATCATTTCTACTA 264
C-AY793644      GGGATCTACTATGGATCATATACCTTCACAGAGACATGGAACATTGGCATCATTTCTACTA 264
C-AY793643      GGGATCTACTATGGATCATATACCTTCACAGAGACATGGAACATTGGCATCATTTCTACTA 264
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C-AF155879      CTTACAGTTATAGCCACTGCTTTTCATAGGATATGTACTCCCA----- 306
E-DQ088706      CTTACAGTTATAGCCACTGCTTTTCATAGGATATGTACTCCCATGAGGACAAATATCCTTC 420
E-AJ389529      CTTACAGTTATAGCCACTGCTTTTCATAGGATATGTACTCCCATGAGGACAAATATCCTTC 420
E-DQ088704      CTTACAGTTATAGCCACTGCTTTTCATAGGATATGTACTCCCATGAGGACAAATATCCTTC 420
E-DQ088705      CTCACAGTTATAGCCACTGCTTTTCATAGGATATGTACTCCCATGAGGACAAATATCCTTC 420
E-DQ088708      CTTATAGTTATAGCCACTGCTTTTCATAGGATATGTACTCCCATGAGGACAAATATCCTTC 420
E-DQ088707      CTTATAGTTATAGCCACTGCTTTTCATAGGATATGTACTCCCATGAGGACAAATATCCTTC 420
C-AF293348      TTCACAGTCATAGCAACCGCCTTTATAGGATACGTCCTCCCG----- 306
C-AF155878      TTCACAGTCATAGCAACCGCCTTTATAGGATACGTCCTCCCG----- 306
C-AY793641      TTCACAGTCATAGCAACCGCCTTTATAGGATACGTCCTCCCGTGAAGGCAAAATATCCTTT 324
C-AY793642      TTCACAGTCATAGCAACCGCCTTTATAGGATACGTCCTCCCGTGAAGGCAAAATATCCTTT 324
C-AY793644      TTCACAGTCATAGCAACCGCCTTTATAGGATACGTCCTCCCGTGAAGGCAAAATATCCTTT 324
C-AY793643      TTCACAGTCATAGCAACCGCCTTTATAGGATACGTCCTCCCGTGAAGGCAAAATATCCTTT 324
                * * * * *

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Primers were ordered from Sigma Genosys and can be found in Table A10.12 with their sequence.

Table A10.12: Primers ordered for beaver identification

Name	Sequence 5'-----3'	T _m
BeavF	TET – ACA GCA TTY TCT TCA GTC GCA CA	67.84
CndBv	GAC GTA TCC TAT AAA GGC GGT TG	64.23
EurBv	ATT TCA TGT TTC TGT AAA GGT GTA GG	62.06

PCRs contained buffer, dNTPs, 1 unit Taq, forward and reverse primers (at a final concentration of 0.5 µM each), 1 µL of template DNA and water to 20 µL. The PCR cycle proceeded for 35 cycles at 95 °C for 45 seconds, 60 °C for 45 seconds and 72 °C for 1 minute followed by a final extension step of 20 minutes at 72 °C.

PCR product was analysed on an ABI 310 genetic analyser (Figure A10.4). Results indicated that the unknown beaver was in fact a European beaver (*Castor fiber*) based on the expected peak at approximately 160 base pairs.

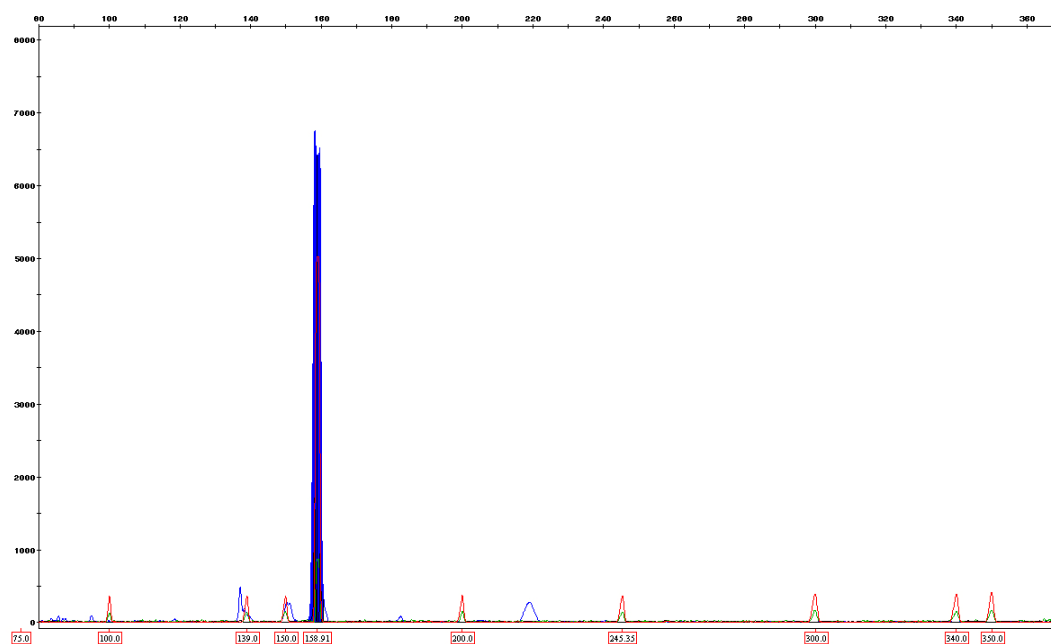


Figure A10.4: Results of test with unknown beaver sample showing a reaction with European beaver primer.

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