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Forensic Testing Using Species Specific Primers

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Wildlife Crimes

- Many species of animals and plants are on the verge of extinction
- Many mammalian species are endangered due to the trade in their body parts
- It is the job of the Forensic laboratory to investigate wildlife crimes
- This presentation will highlight the use of mitochondrial DNA in Species Identification

CITES

- Convention on the International Trade in Endangered Species
- Established in 1973 by 21countries
- Currently 166 countries are members
- Membership is voluntary but members are bound by the regulations and can be expelled if they do not adhere to resolutions

CITES

- The species protected by CITES are on 3 Appendices
- Appendix I
  - Most at risk – no trade at all allowed
- Appendix II
  - Need to control trade to protect the species
- Appendix III
  - A species protected in at least one country

Species on CITES

- 28,000 plant species
- 5,000 animal species
- High profile are Rhino, Panda, Whale but also protected are orchids, pitcher plants, hard woods, and numerous invertebrate species
- Trade in these species is illegal and therefore can be subject to a forensic investigation

Scope of Trade

- Food products
  - Bushmeat, whale & dolphin
- Musical instruments or wooden furniture
  - Hard woods such as mahogany
- Tourist curios
  - Coral reef, skins & hides
- Traditional Chinese Medicines (TCM)
  - Tiger bone, bear bile

In Addition to Cites

- In addition to CITES protected species the forensic lab is often asked to analyse non-human samples.
- Often these samples are left unanalysed to lack of standardised testing and costs.

Role of Forensic Laboratory

- Species Identification – what species is this and is it protected
- Population – African or Asian Elephant
- Lineage – common ancestor
- Individualisation – linking hairs or bone to one or more animal

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Species Identification

- Method of identification depends upon whether gross physical features are present or whether trace or powdered remains are seized
- Gross
  - microscopy
- Trace
  - DNA

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DNA Loci in Species Testing

- Any test must show sufficient inter species variation so that closely related species can be separated

- Any test must show little intra species variation so that all members of the same species will be included by the same test

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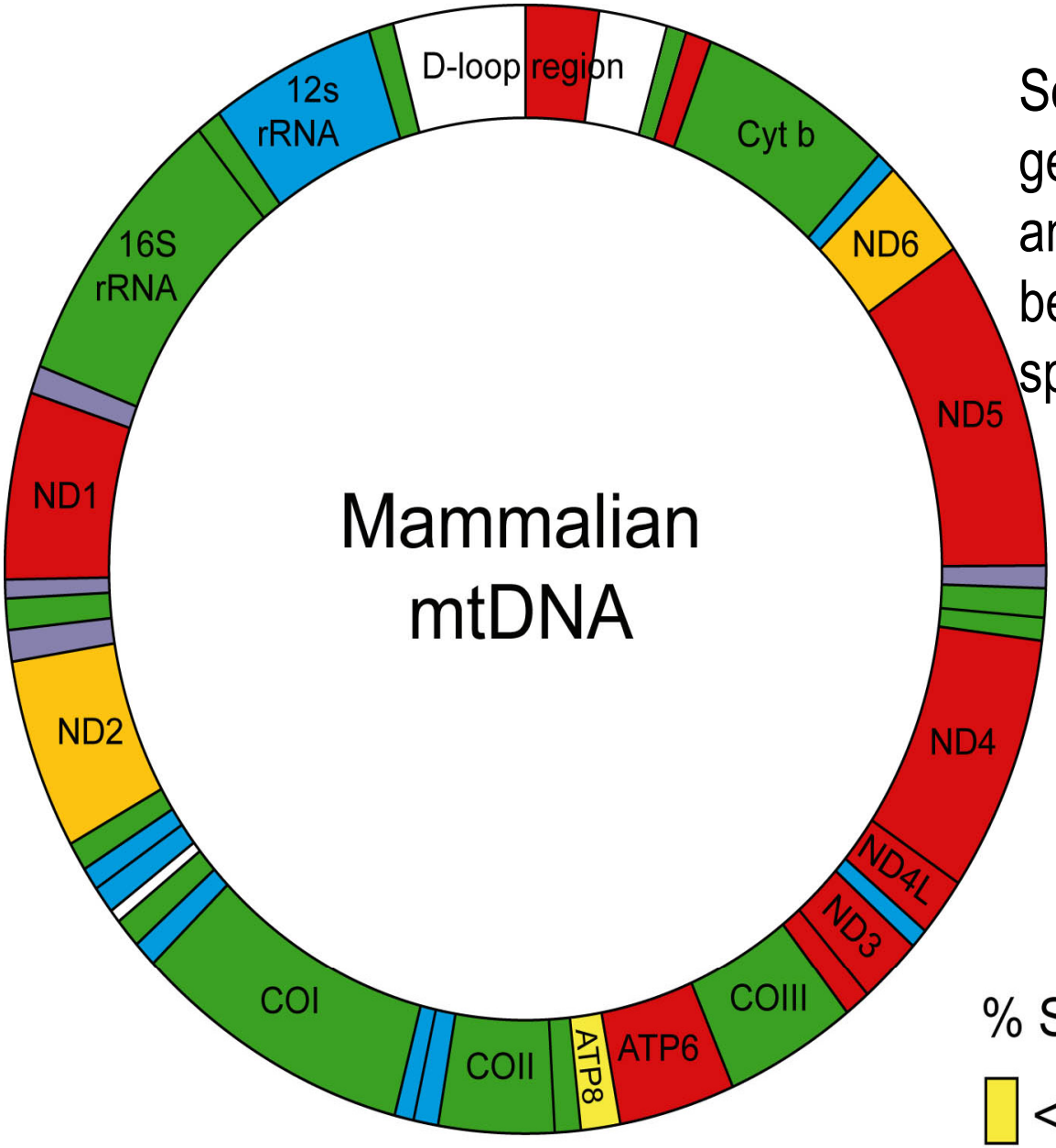
DNA Loci in Species Testing

- Any test must work on poor or powdered remains
- Any test must work on samples that have been subjected to environmental insult
- Few DNA loci meet these requirements, except mitochondrial DNA



Some parts of the mitochondrial genome are well conserved and show little variation between different mammalian species

37 genes are encoded by the mtDNA. The mammalian mtDNA is between 15 – 17 Kb in size

% Similarity

< 65

65 - 70

70 - 75

75 - 80

80 - 85

> 85

mtDNA Structure

- The order of genes on the mitochondrial DNA is very similar for most species
- Most of the mtDNA in encoding
  - 13 genes for proteins
  - 24 RNA molecules encoded
- Order and structure of the vertebrate mtDNA used in taxonomic studies

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Cytochrome b

- There are a number of genes that show inter species variation but little intra species variation
- Cytochrome b is one such gene and the most commonly used gene for taxonomy
- It is situated near to the D-loop and encodes a 380 amino acid protein and is ~1,140 bp in size

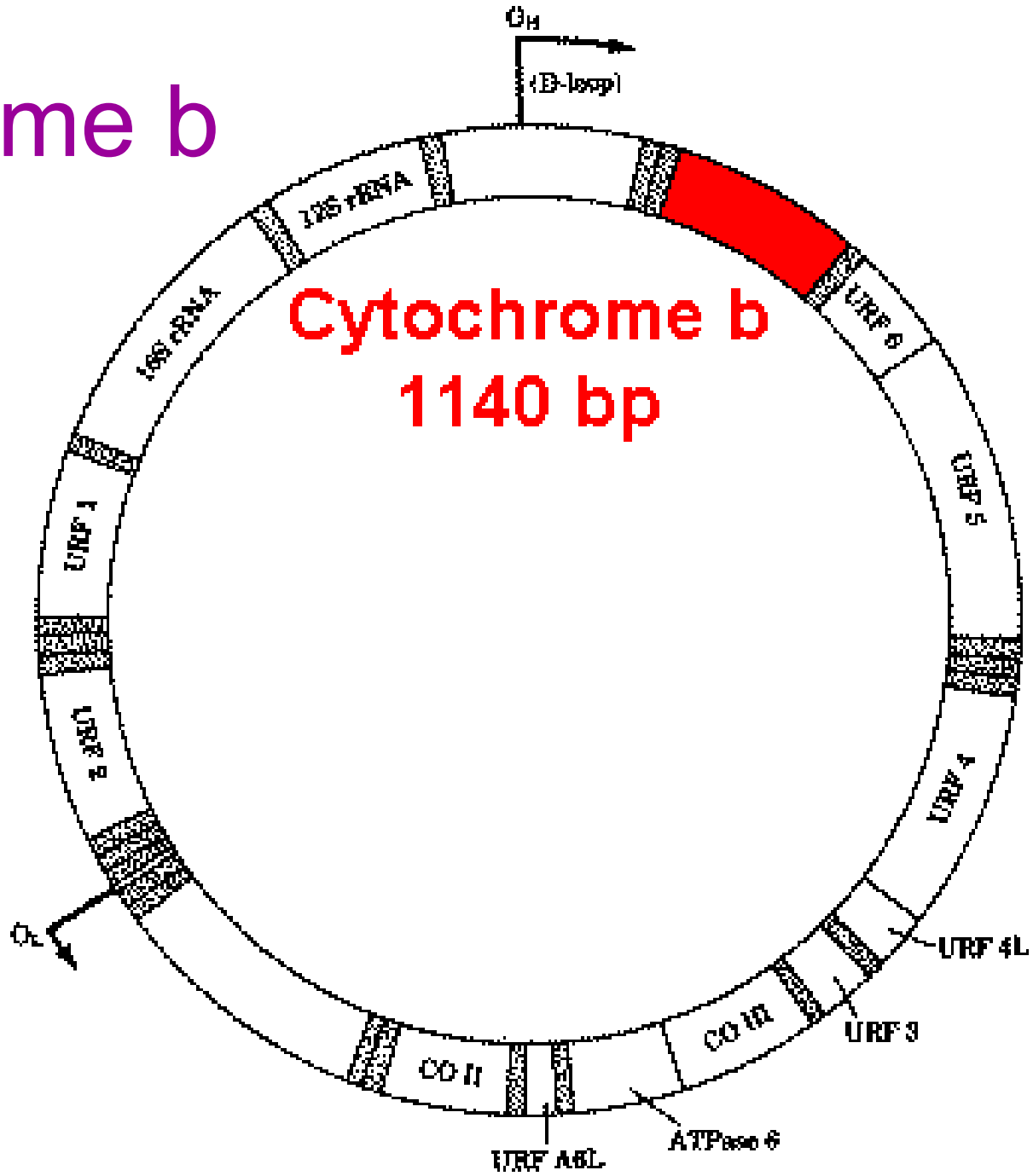
Cytochrome b

The gene is 1140 bp

It encodes a 380 amino acid protein

It is commonly used in taxonomy & evolutionary studies

Now used as a species marker in forensic science



Using the Cytochrome b Gene

- The DNA sequence for many animal and plant species is known for the cytochrome b gene
- DNA Databases exist
  - EMBL DNA Database (www.ebi.ac.uk)
  - GenBank® (www.ncbi.nih.gov)
  - Currently there are over 32 million sequence records on these databases

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Taxonomy of Mammals

	Wolf	Dog	Fox	Cat	Human
Phylum	Chordata				
Class	Mammalia				
Order	Carnivora				Primate
Family	Canidae			Felidae	Hominid
Genus	Canis		Vulpes	Felis	Homo
Species	lupus	familiaris	vulpes	catus	sapiens

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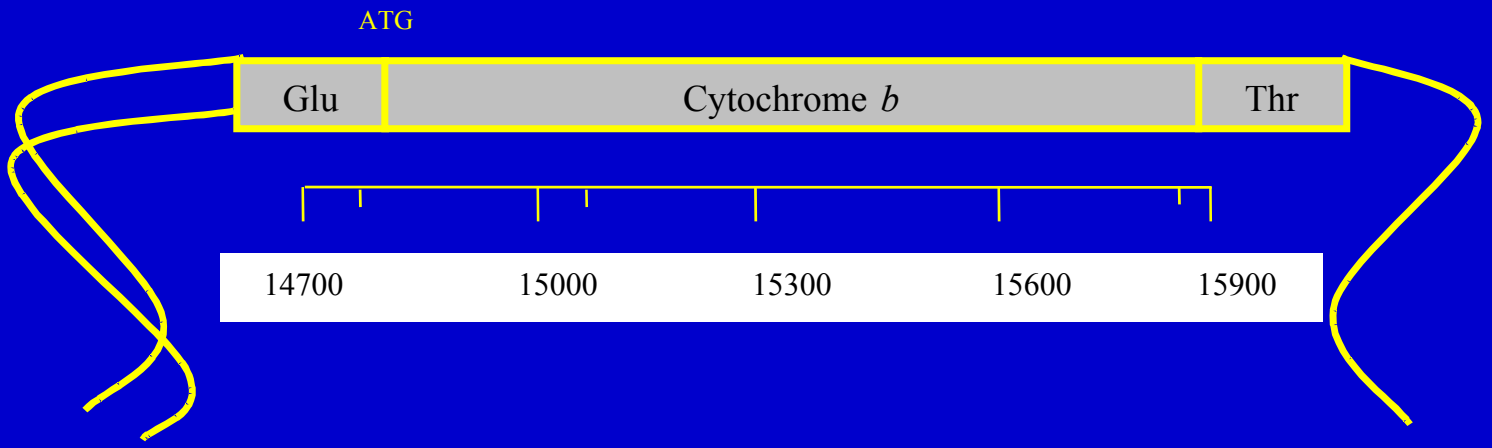
Difference at the cytochrome B Gene (bp)

	Wolf	Dog	Fox	Cat	Human
Wolf		99.6%	84.0%	79.0%	74.5%
Dog	4		83.7%	78.9%	74.3%
Fox	182	186		78.6%	73.8%
Cat	239	240	244		76.6%
Human	291	293	299	267	

Cytochrome B Gene



As the DNA sequence for many mammals is well conserved there are numerous possible sites to design either universal primers or species specific primers

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Database Searches

- New sequences of DNA can be compared to the sequences on the database to determine homology or degree of conservation
- Basic Local Alignment Search Tool (BLAST) is the bioinformatic tool for the comparison of nucleic acid of amino acid sequences
- Can be used for primer design or sequence comparison.

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Species Testing

- Currently part or all of the gene is amplified and sequenced
- It is known that only some regions are polymorphic
- Primers can be designed to species specific sequences
- Species specific primers used in conjunction with one universal primer will produce a PCR product of known size for each species

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Current Methods for Species Identification

- PCR of the cytochrome b gene will make double stranded DNA molecule of a particular size and a particular sequence
  - The length and sequence will be dependant upon the species being studied
- The sequence can be determined by standard DNA sequence methods
- The sequence can then be compared to the EMBL or NCBI databases

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Map showing Universal & Species Specific Priming Sites

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Currently

- Primers have been designed for 20 species of mammals.
- More than one specific primer for each species for most of the mammals.
- Are currently being multiplexed together.
- Outcome: A single step test for species identification.

Case Example 1

- Alleged that a dog was involved in a sexual attack.
- Victim claimed she was held down while dog was allowed to lick her.
- Swabs were taken 3 days after the alleged incident.
- Asked to test for the presence of dog.

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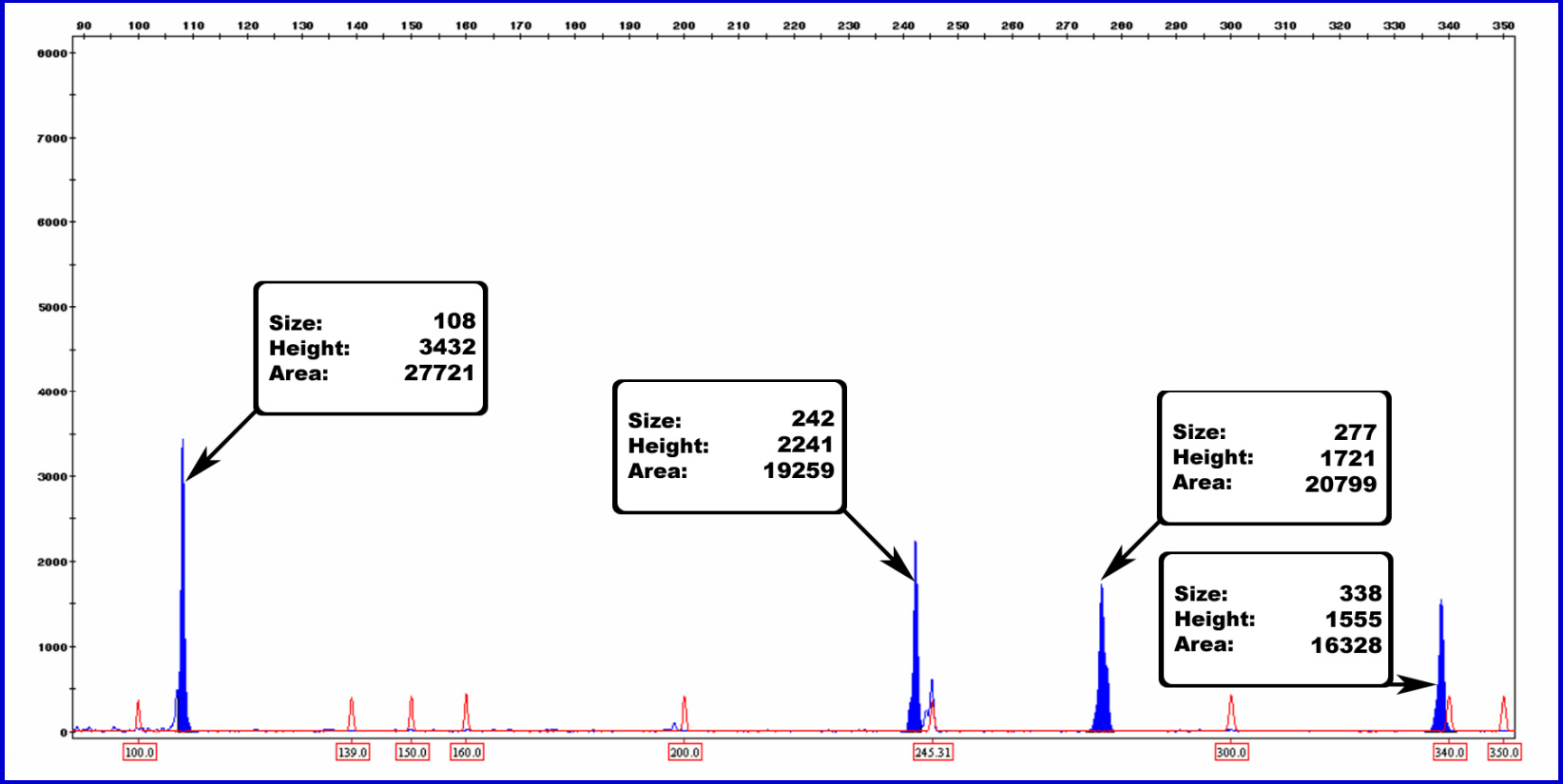
Human Specific Fragments From Dog/Human Control Mixture



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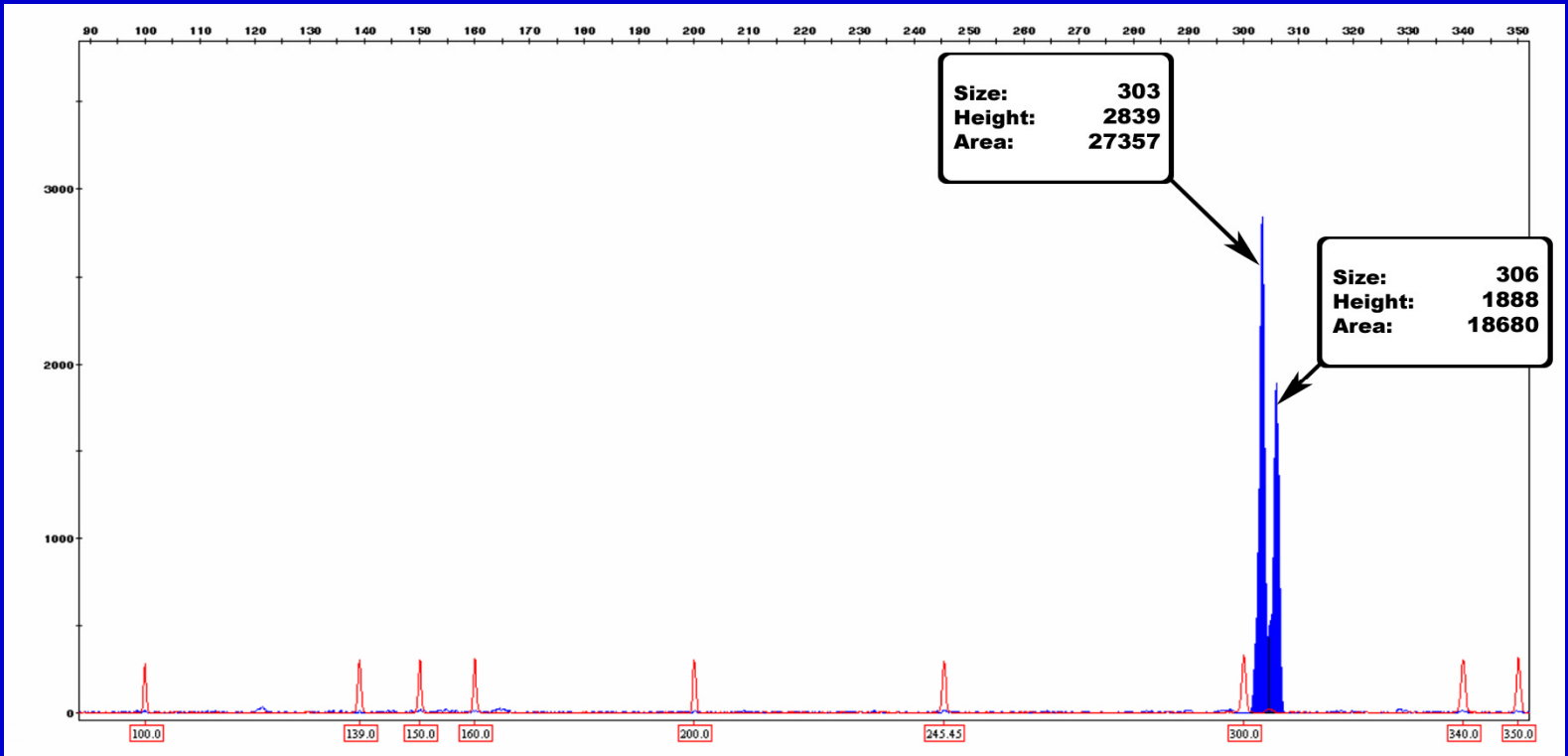

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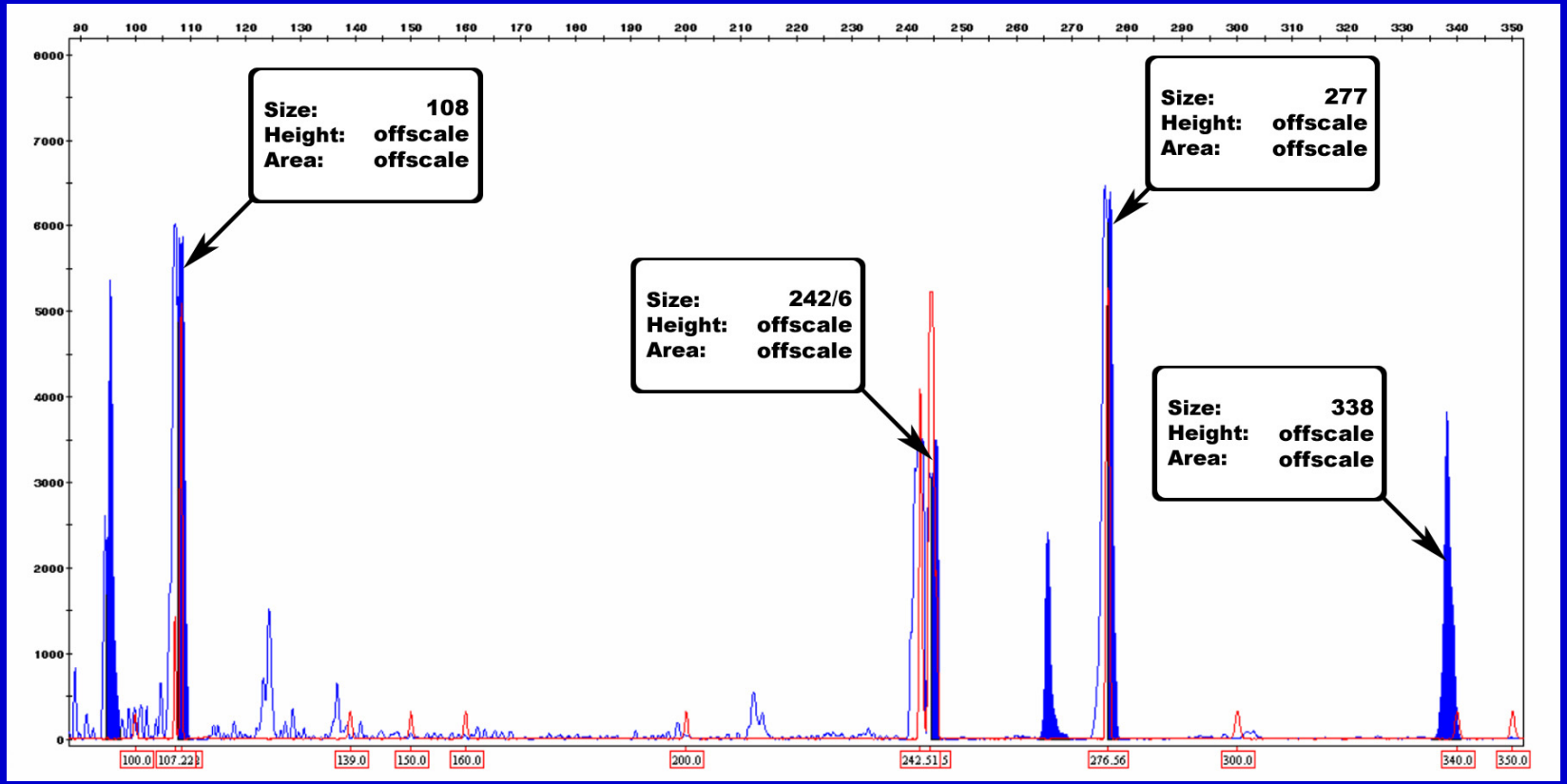
Swab Results Using Human Specific Primers



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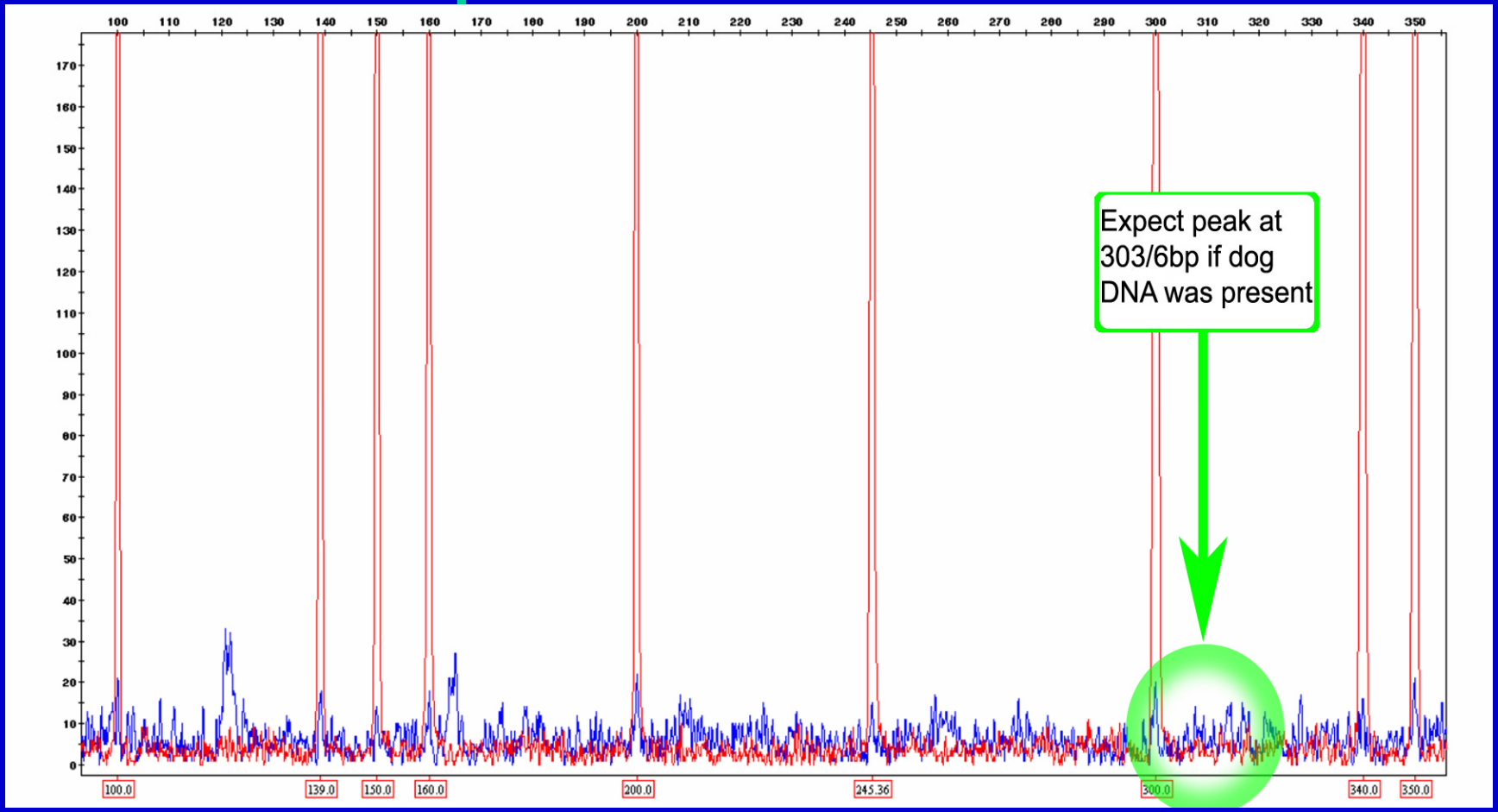
Swab Results Using Dog Specific Primers



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Case 1: Conclusions

- No trace of dog DNA was found.
  - Dog was not involved in attack.
  - DNA was lost due to the extended period of time between the incident and the examination in which the swab was taken.

Case Example 2

- Man found in army fatigues with blood spatter.
- Suspected of poaching red deer.
- Police were interested if the blood was that of red deer.

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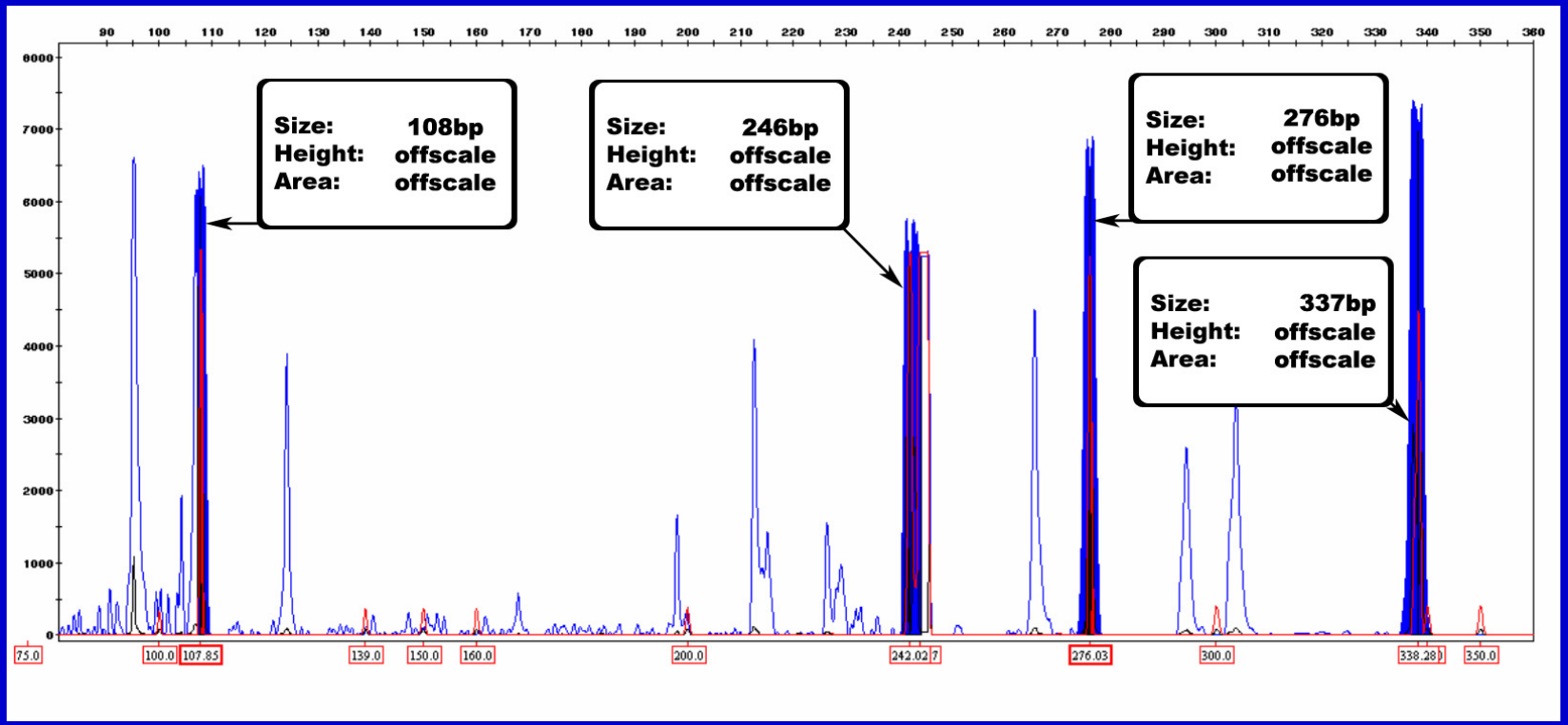
Human Control



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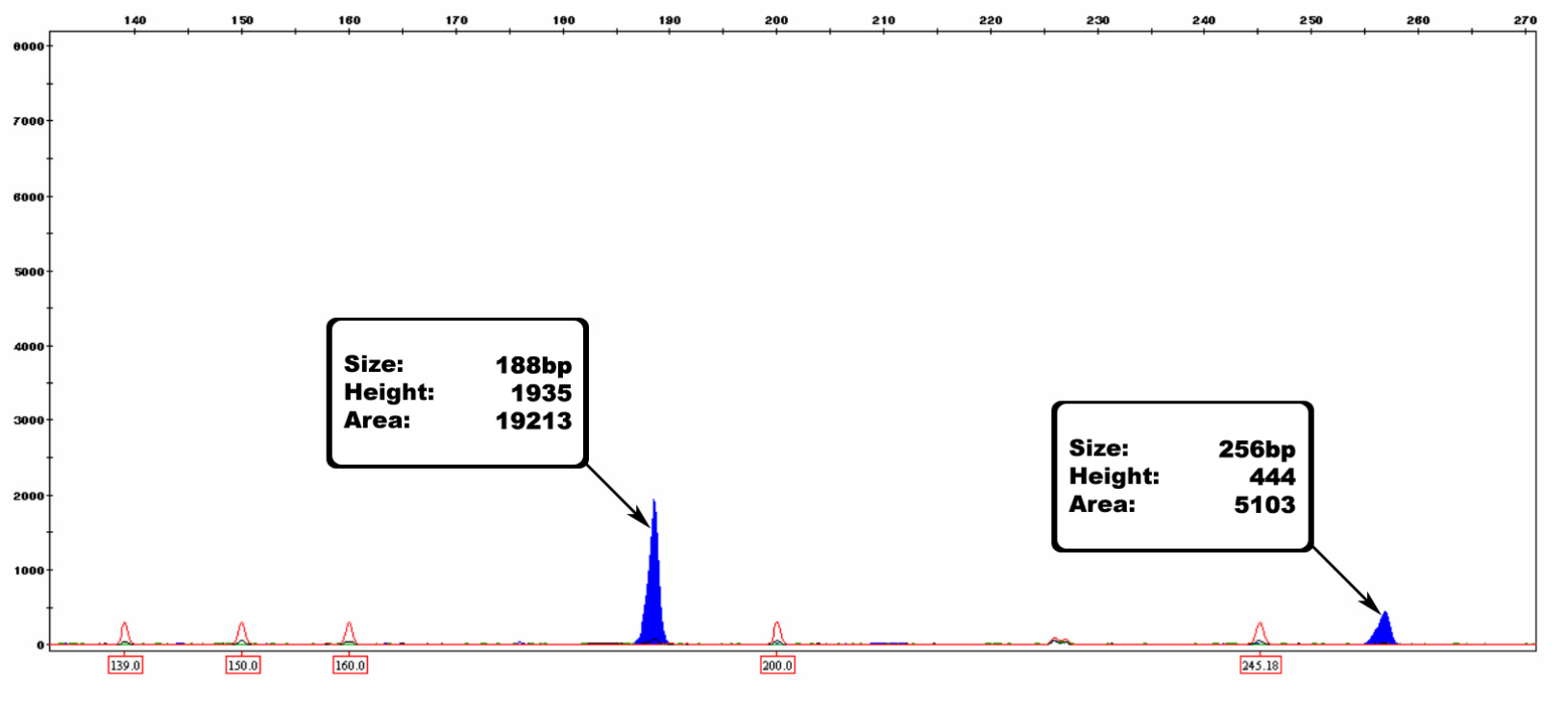
Red Deer Control



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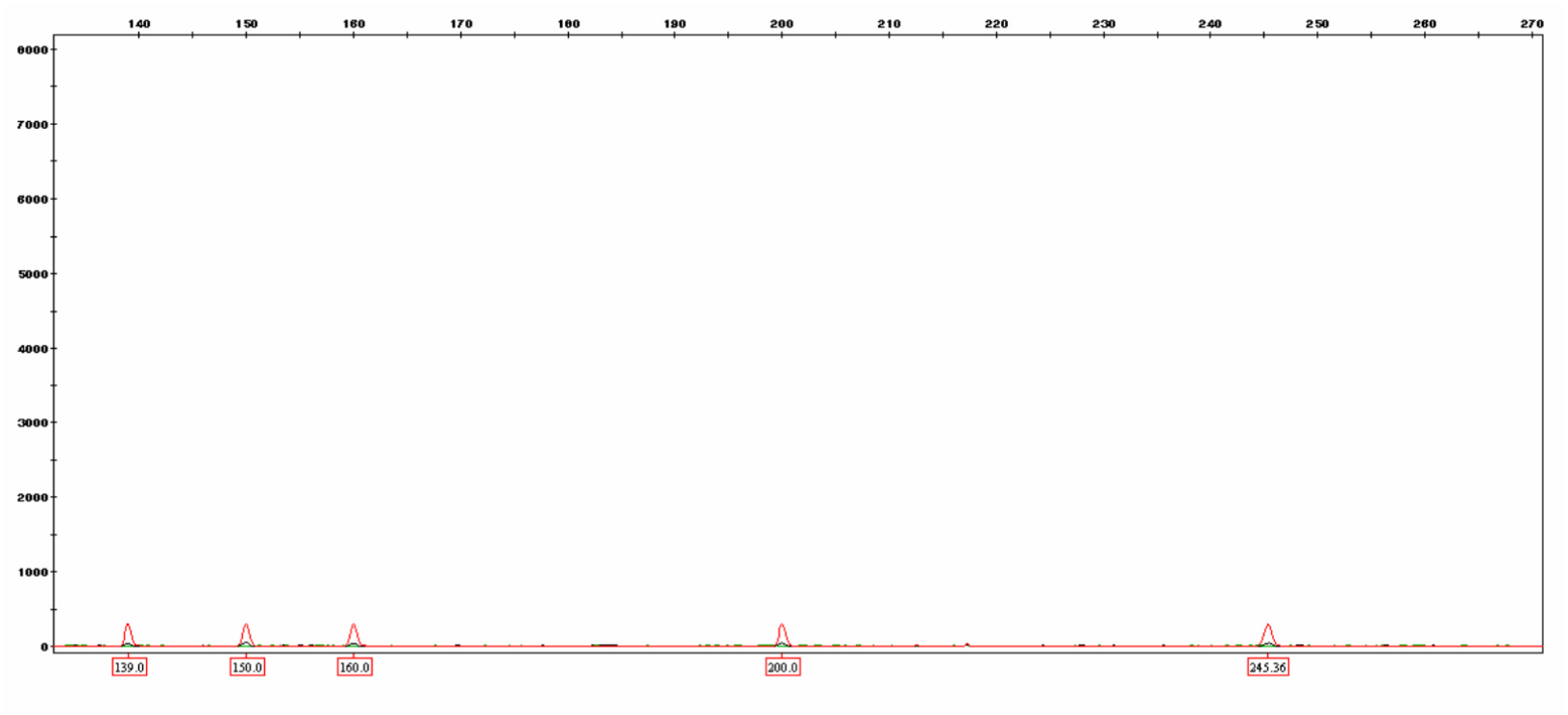
Control Human With Red Deer Primers



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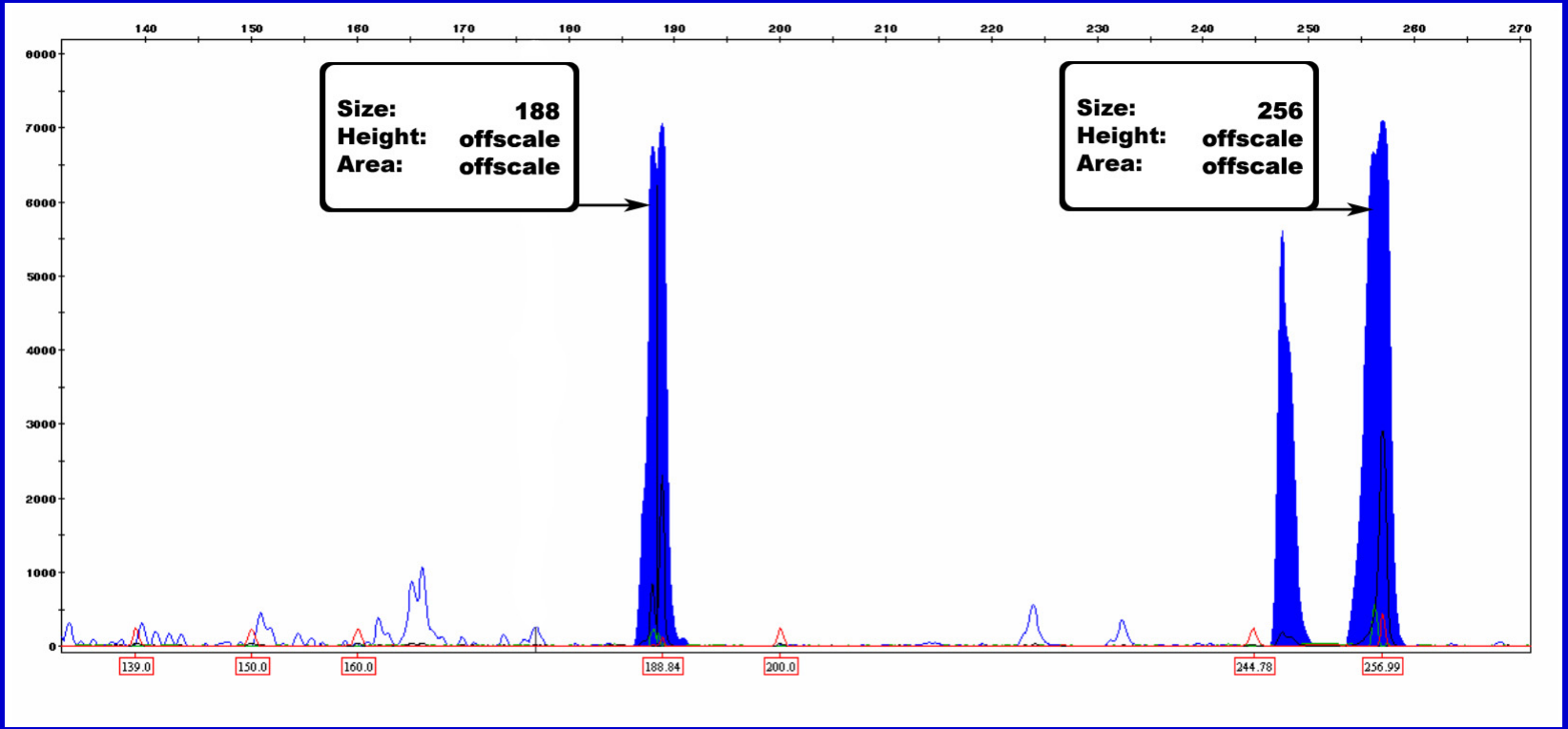
Unknown Sample With Red Deer Primers



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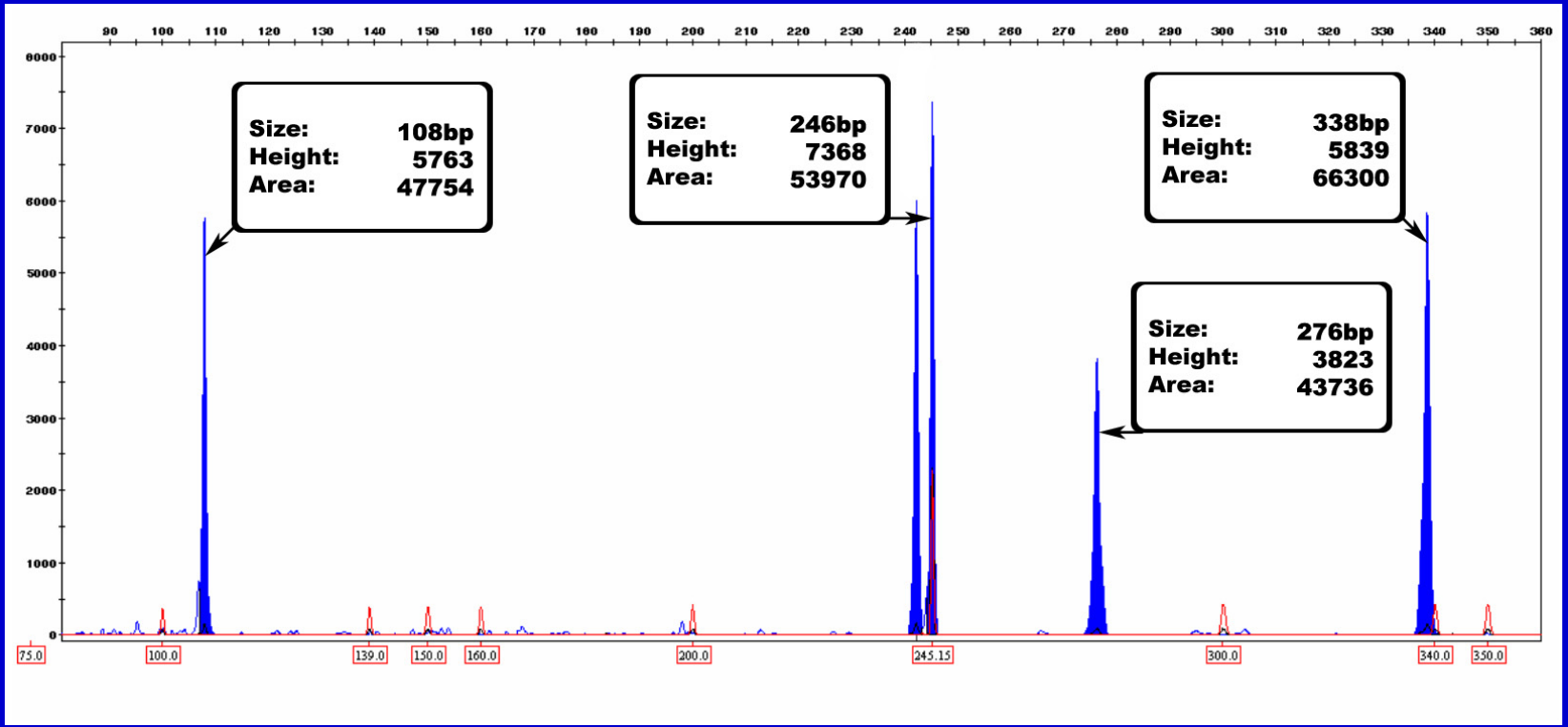
Unknown Sample With Human Primers



Case 2: Conclusions

- Expect a human result due to epithelial cells on trousers.
- Red deer result only obtained if blood was that of deer.
- Obtained a mixture of human and red deer DNA.
- Blood on the trousers was from a red deer.

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Aims for the Future

- SNP typing will be possible for a range of mammalian species
- An unknown sample can be rapidly and reliably identified to genus, species or even subspecies level
- Closely related species will require a test designed to identified polymorphisms