



University of
Strathclyde
Glasgow

A Single Step Multiplex PCR to Identify Mammalian Species in the United Kingdom

Shanan S. Tobe M.Sc.; Adrian M.T. Linacre D.Phil.

Centre for Forensic Science
University of Strathclyde
Glasgow, United Kingdom

*This is an ongoing PhD research project.

Need for Species Identification

- Non-human biological evidence encountered:
 - Abuse (both against people and animals)
 - Poaching
 - Components in food
 - Linking suspects to crimes
 - As components in TCM
 - Other crimes

Current Methods

- Gross
 - Microscopy
 - Osteology

- Trace
 - Antigen-antibody
 - DNA analysis
 - Sequencing

Current Methods

Sequencing

Generally part or all of a mitochondrial gene is amplified and sequenced for comparison to known sequences on Genbank

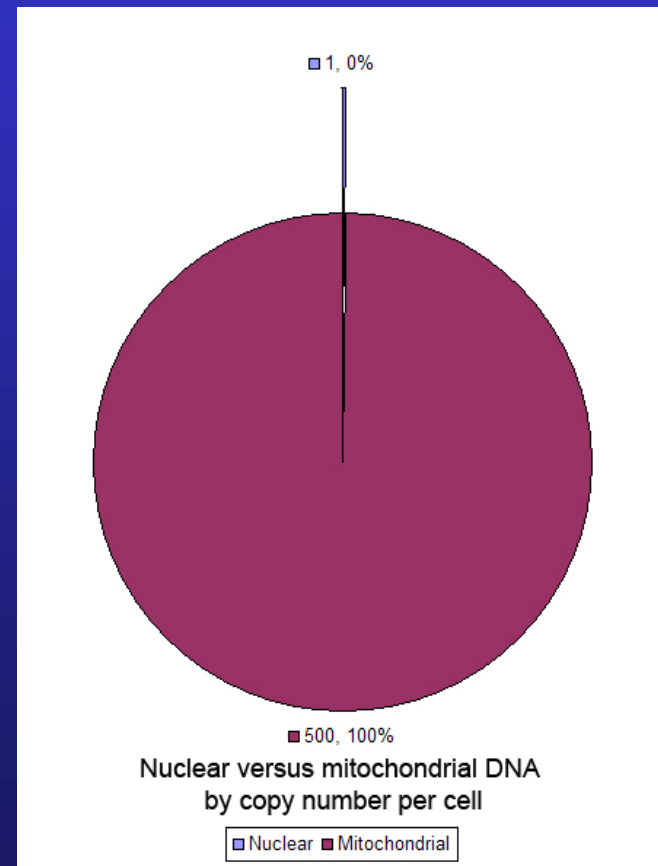
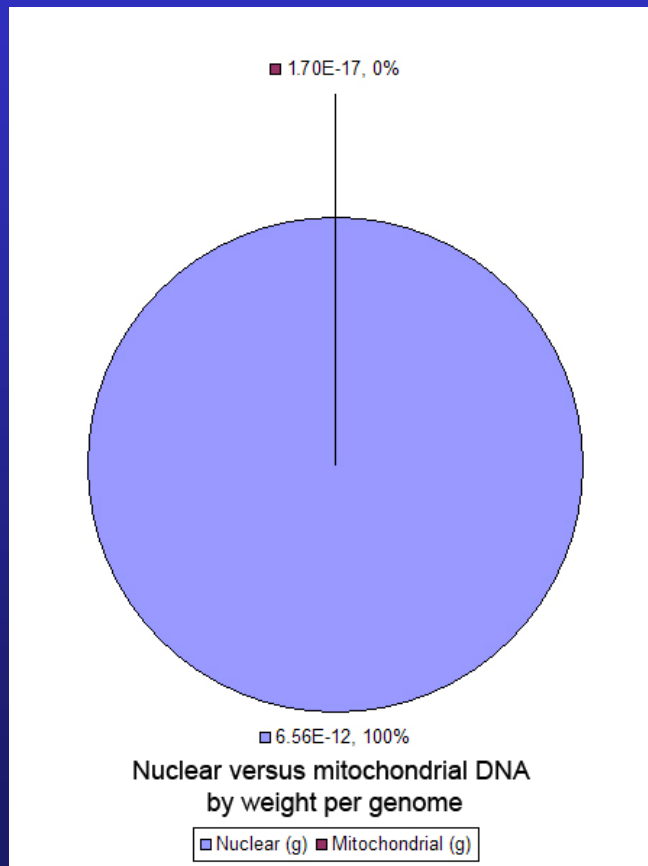
Problems With Sequencing

- Costly and labour intensive
- Mixtures cannot be separated reliably
- Degraded samples may not yield enough sequence data for identification

Requirements For Identification

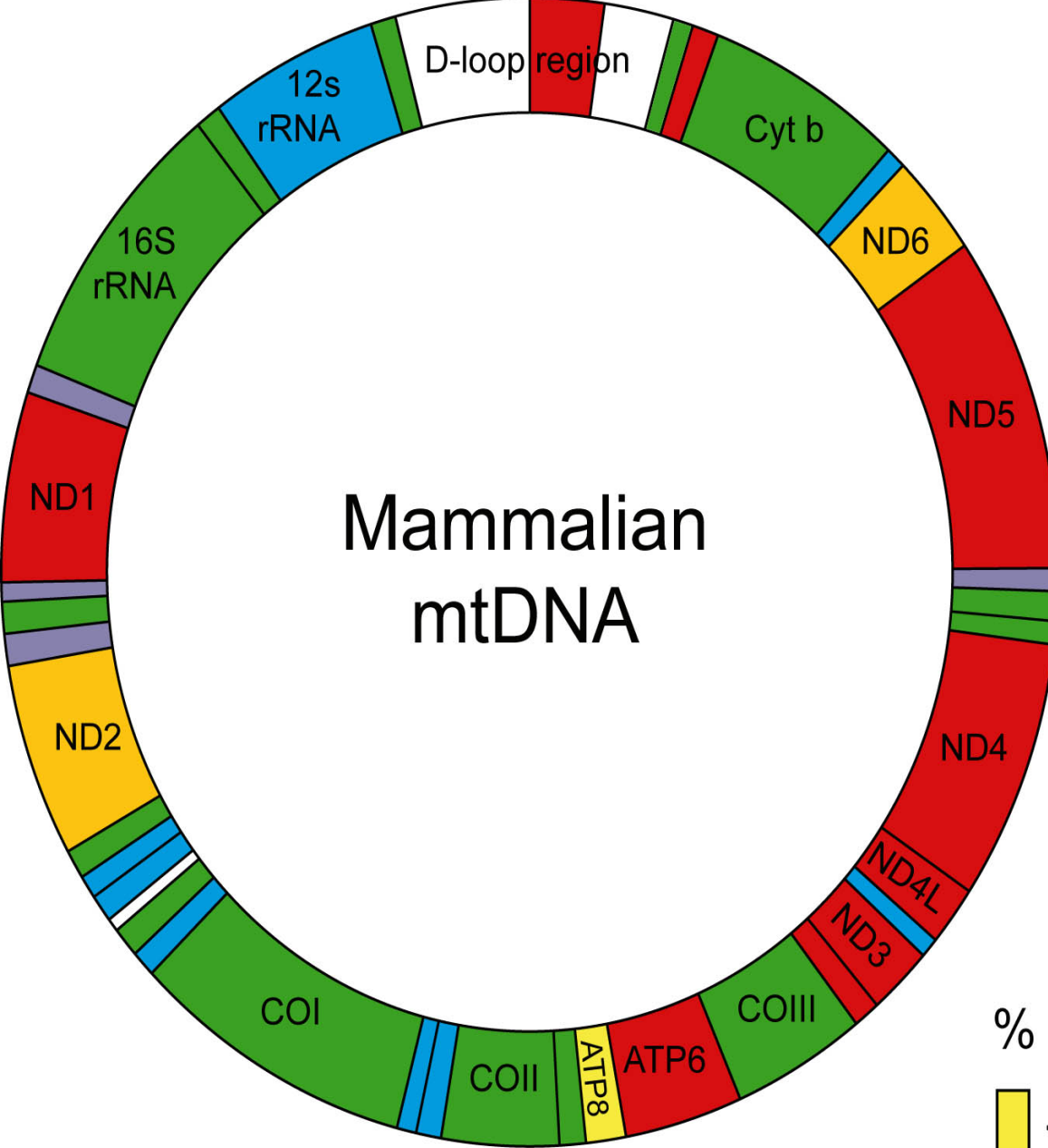
- Any test must work
 - on poor or powdered remains
 - on samples that have been subjected to environmental insult
 - on mixtures
- The test must also be cost and labour effective

Nuclear vs. Mitochondrial DNA



Other Advantages of mtDNA

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



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

Locus Qualities Required For Species Identification

- Locus must show inter species variation
- Locus must also show little intra species variation

Choosing a Locus

- Several genes show inter species variation but little intra species variation
- Cytochrome b is one such gene
 - commonly used 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 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

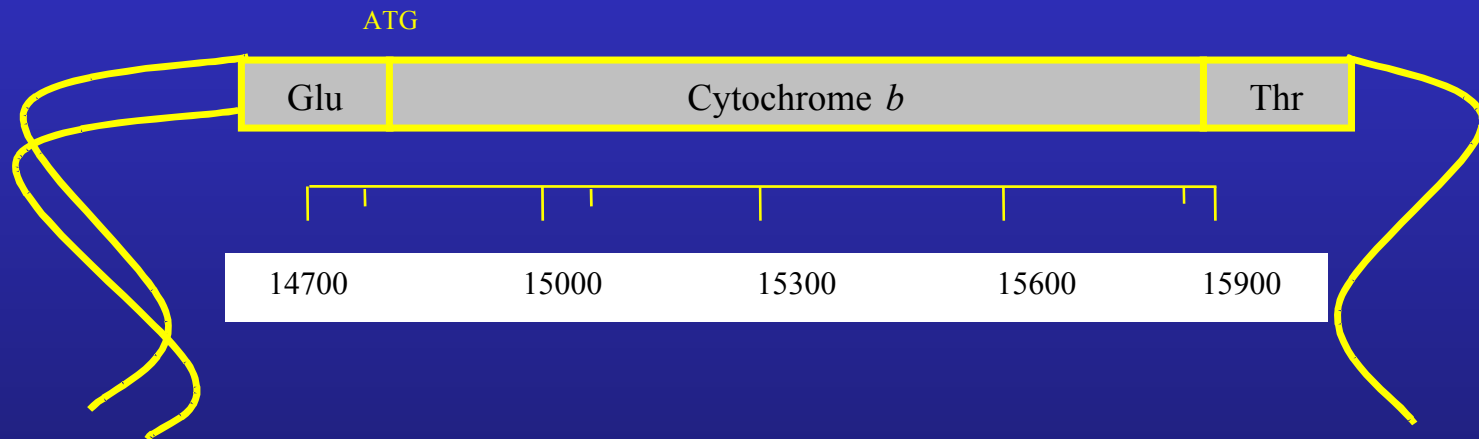
Taxonomy of Mammals

| | 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 |

Base Pair Differences At The Cytochrome *b* Gene

| | 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*

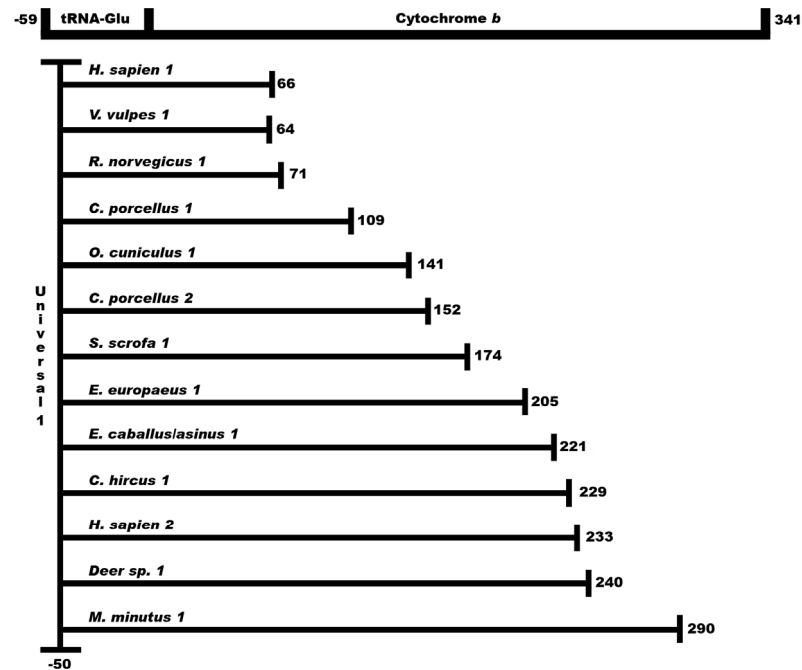


As the DNA sequence for many mammals is well conserved there are numerous possible sites to design either universal primers or species specific primers

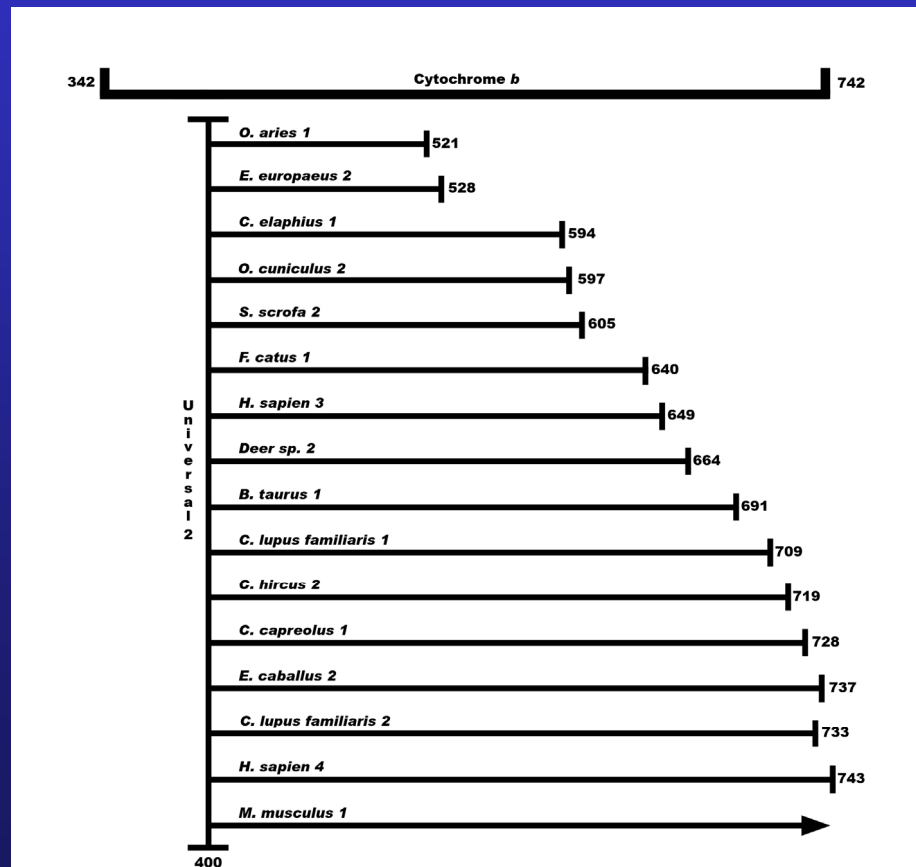
Primer Design

- Universal primers were developed from previously published studies
- Potential species specific priming sites identified
- Primers put through:
 - B.L.A.S.T.
 - Oligonucleotide calculator

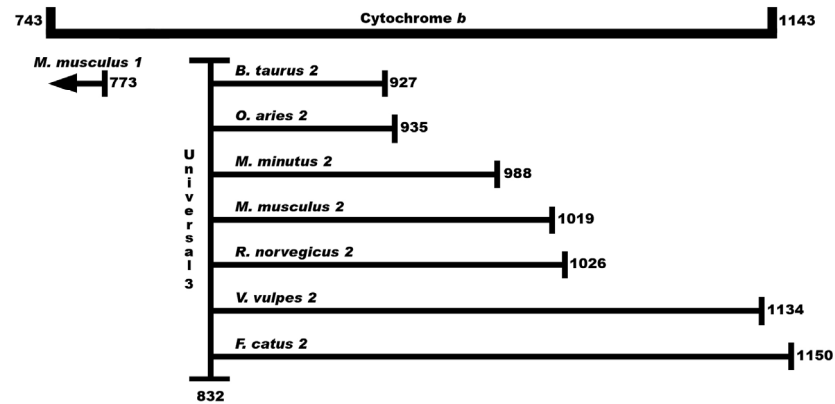
Universal Site 1



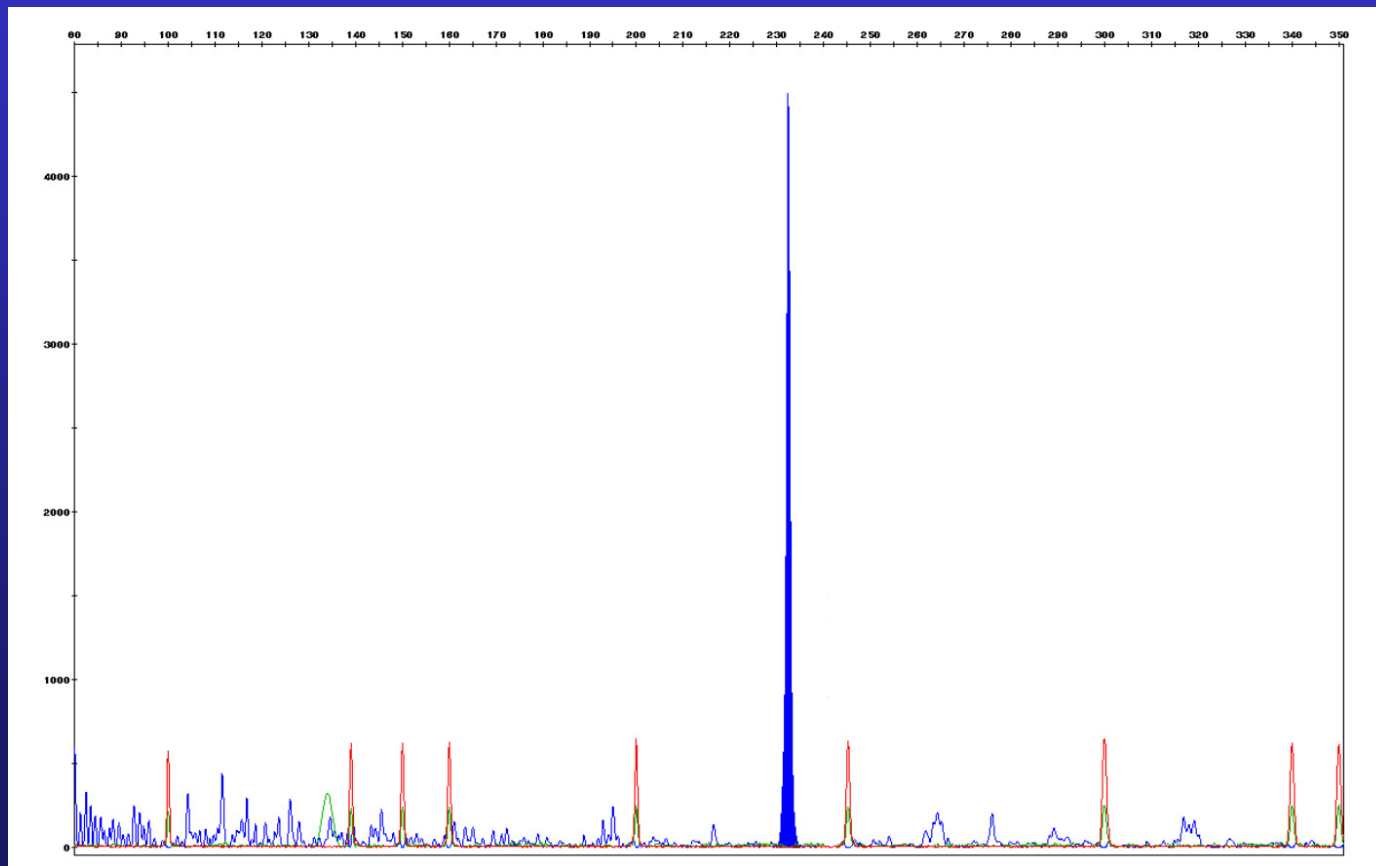
Universal Site 2



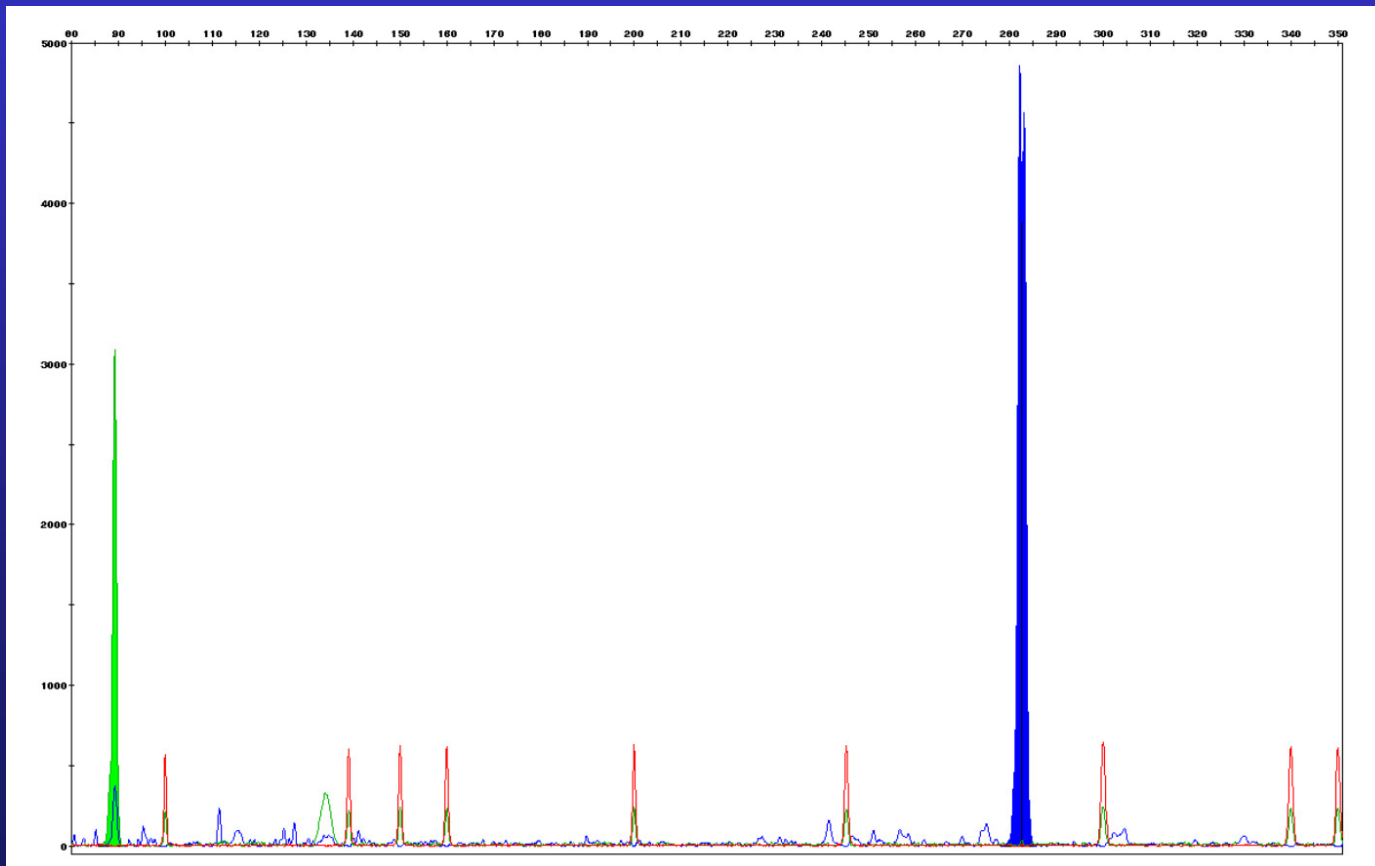
Universal Site 3



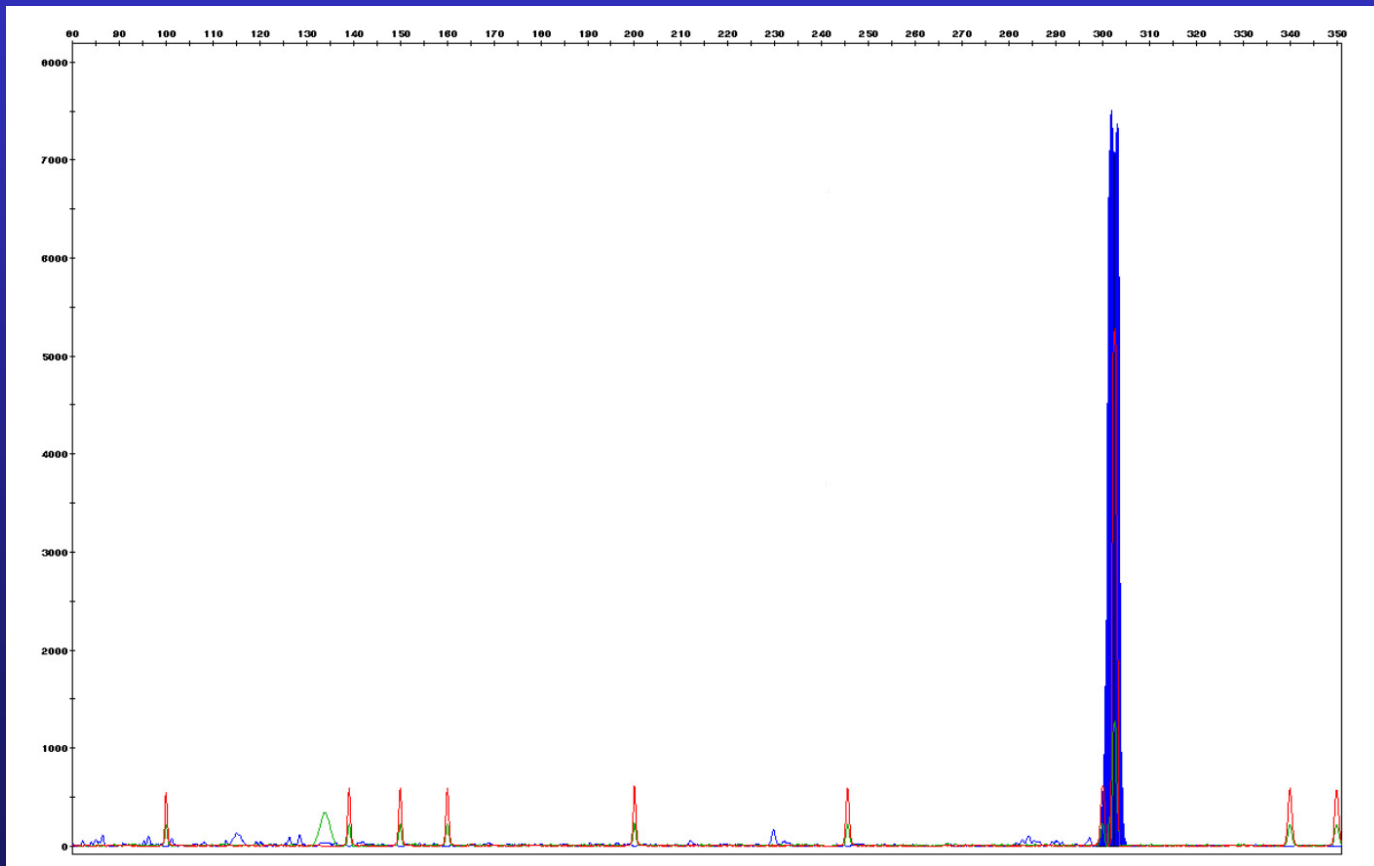
Cat Peak at 232 bp



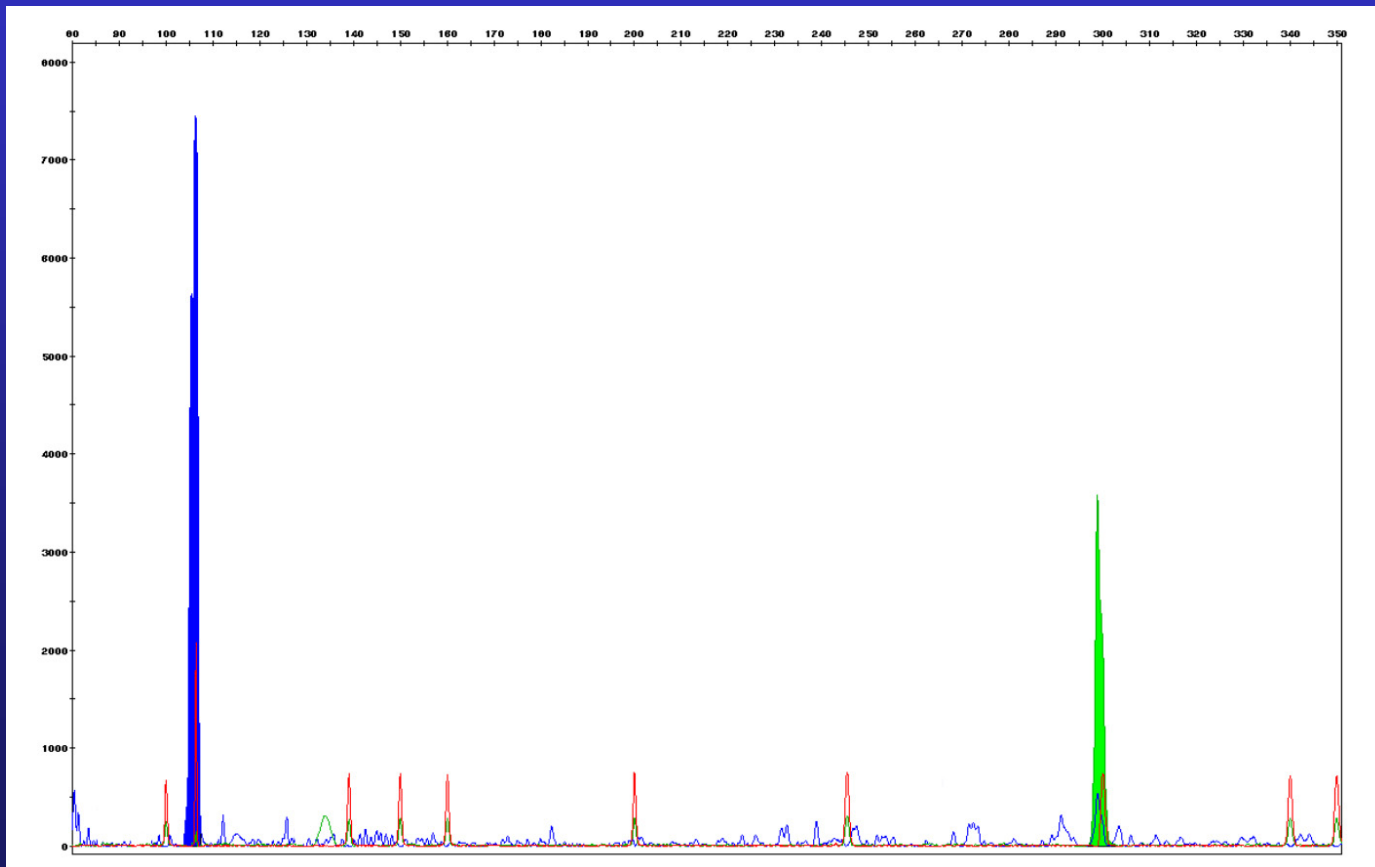
Cow Peaks at 89 and 282 bp



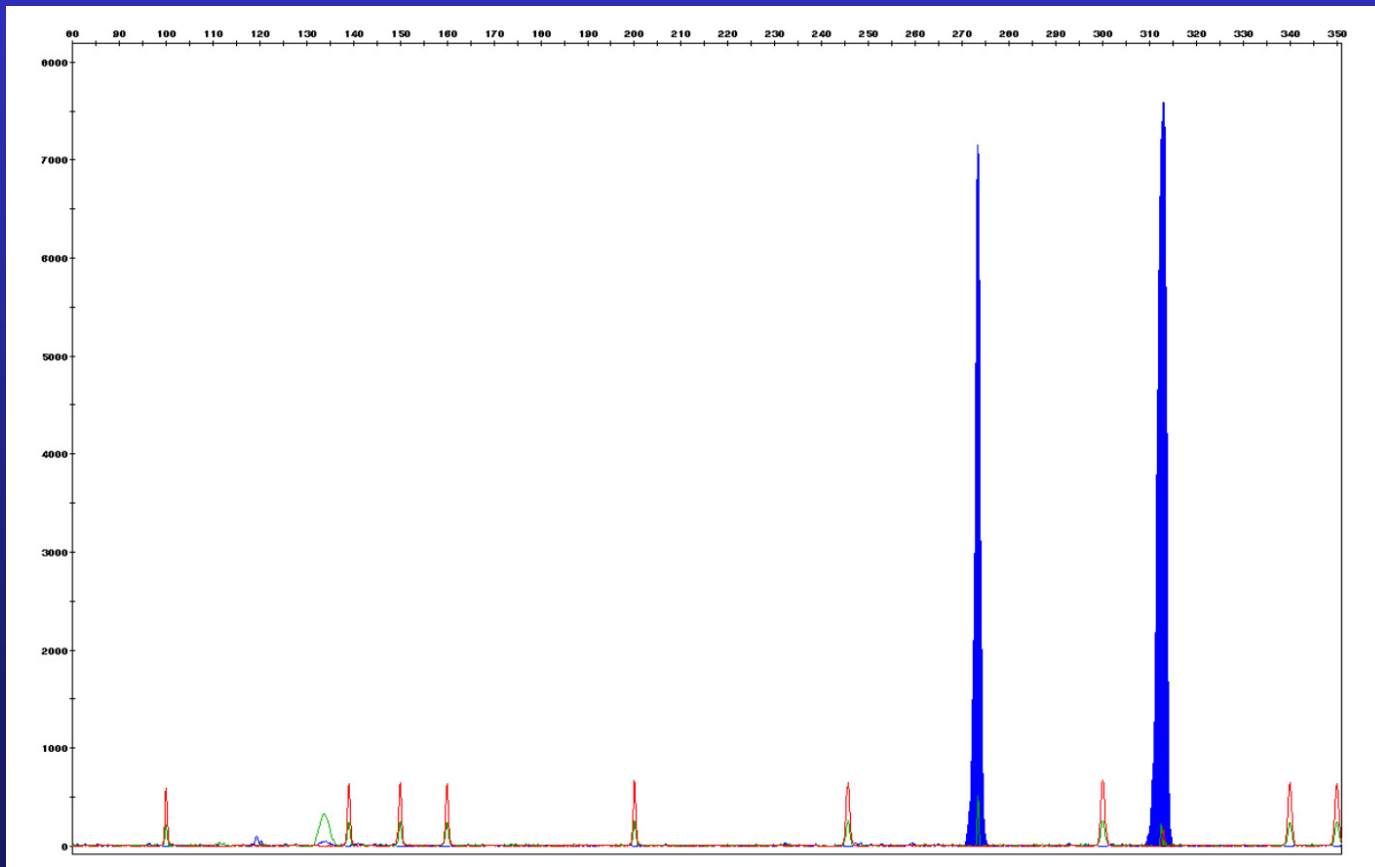
Dog Peak at 303 bp



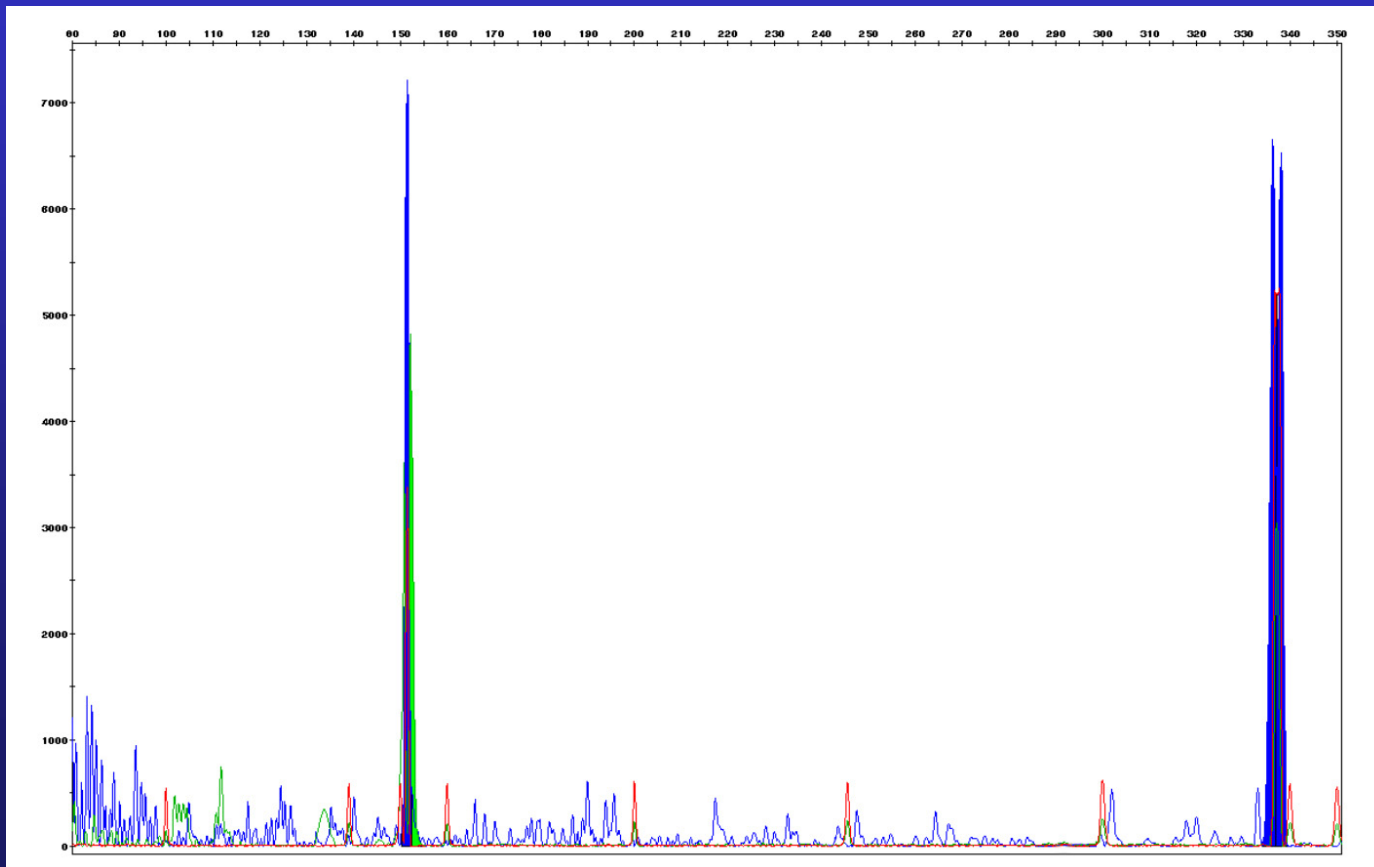
Fox Peaks at 106 and 298 bp



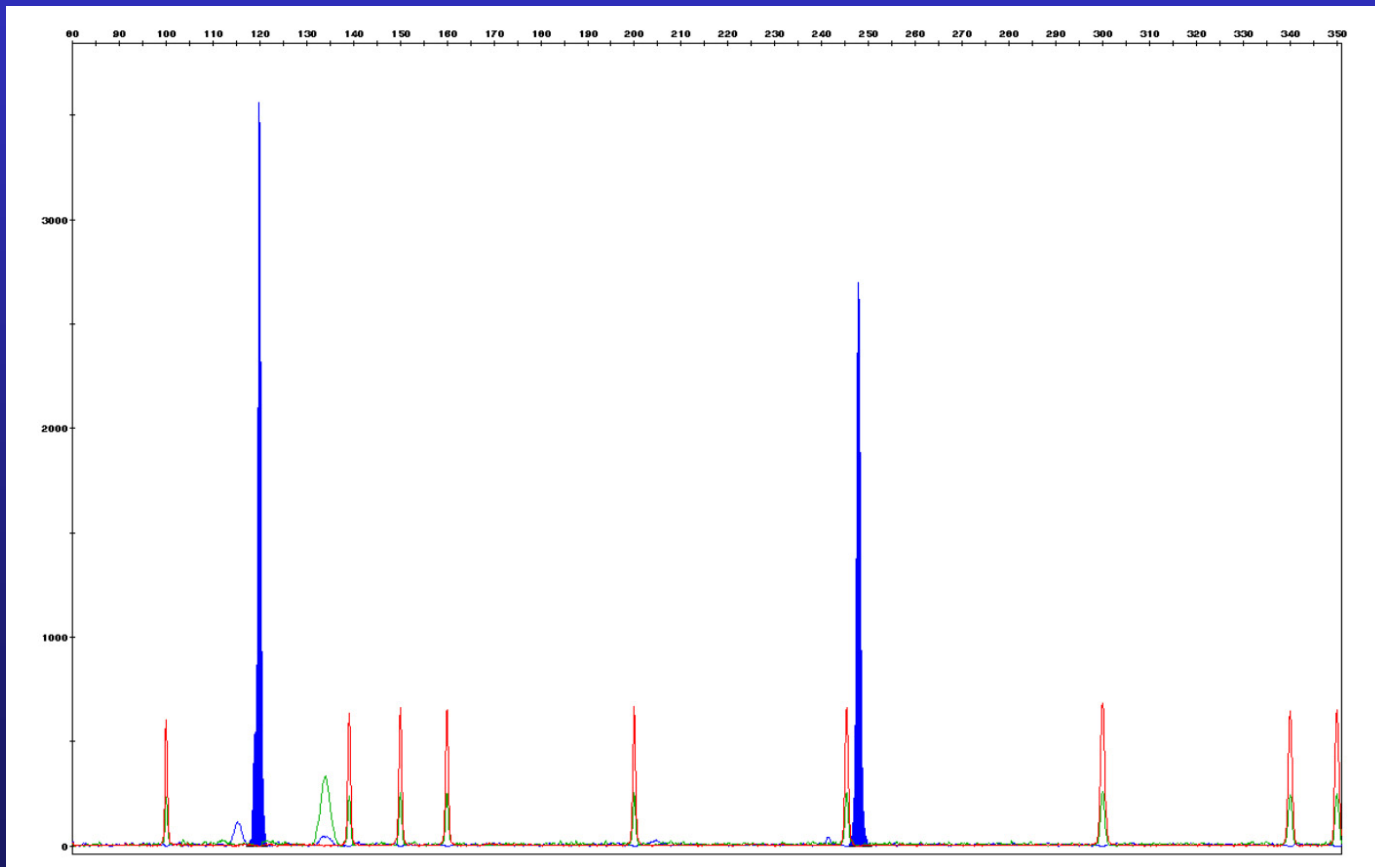
Goat Peaks at 273 and 313 bp



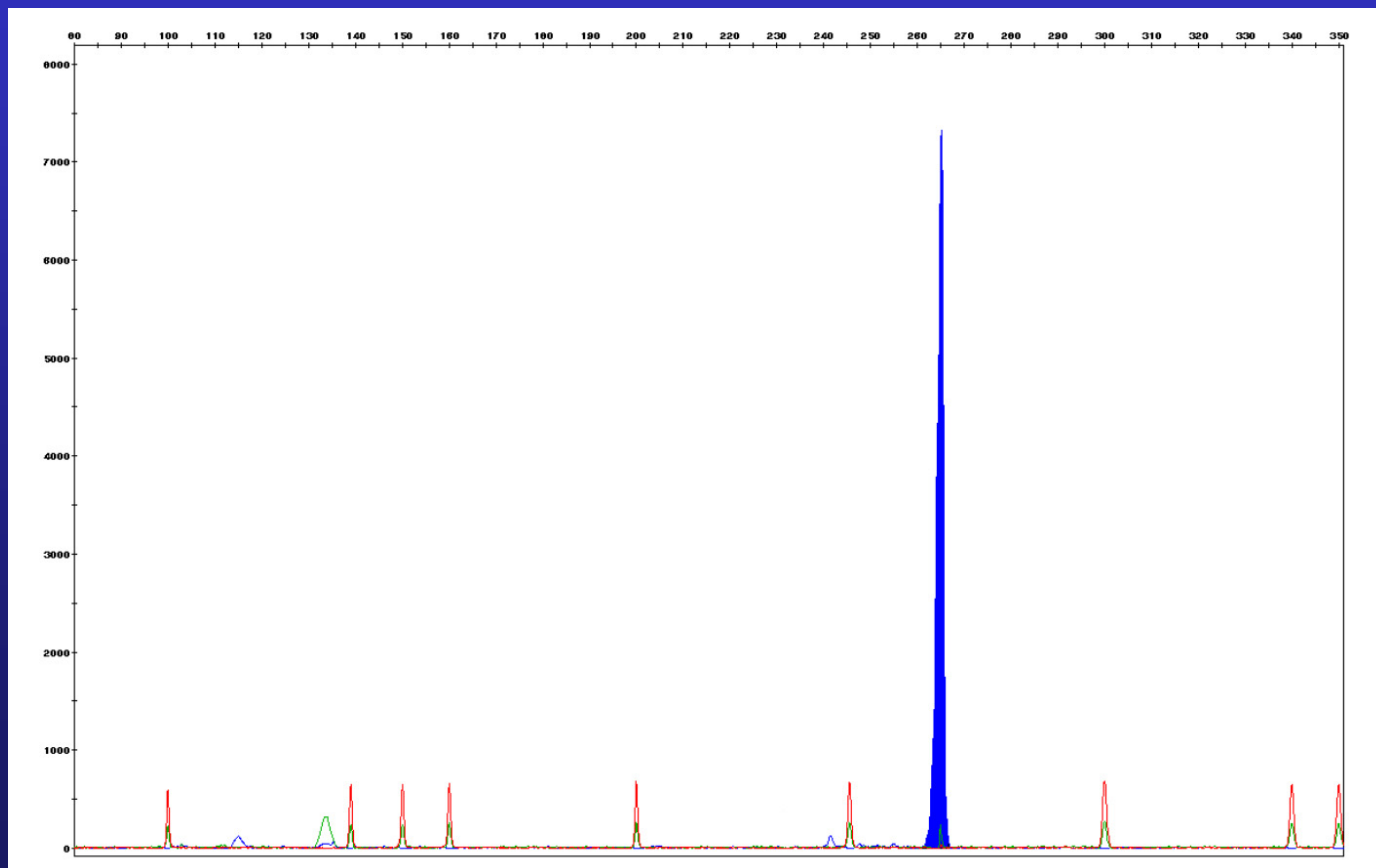
Harvest Mouse Peaks at 152 and 338 bp



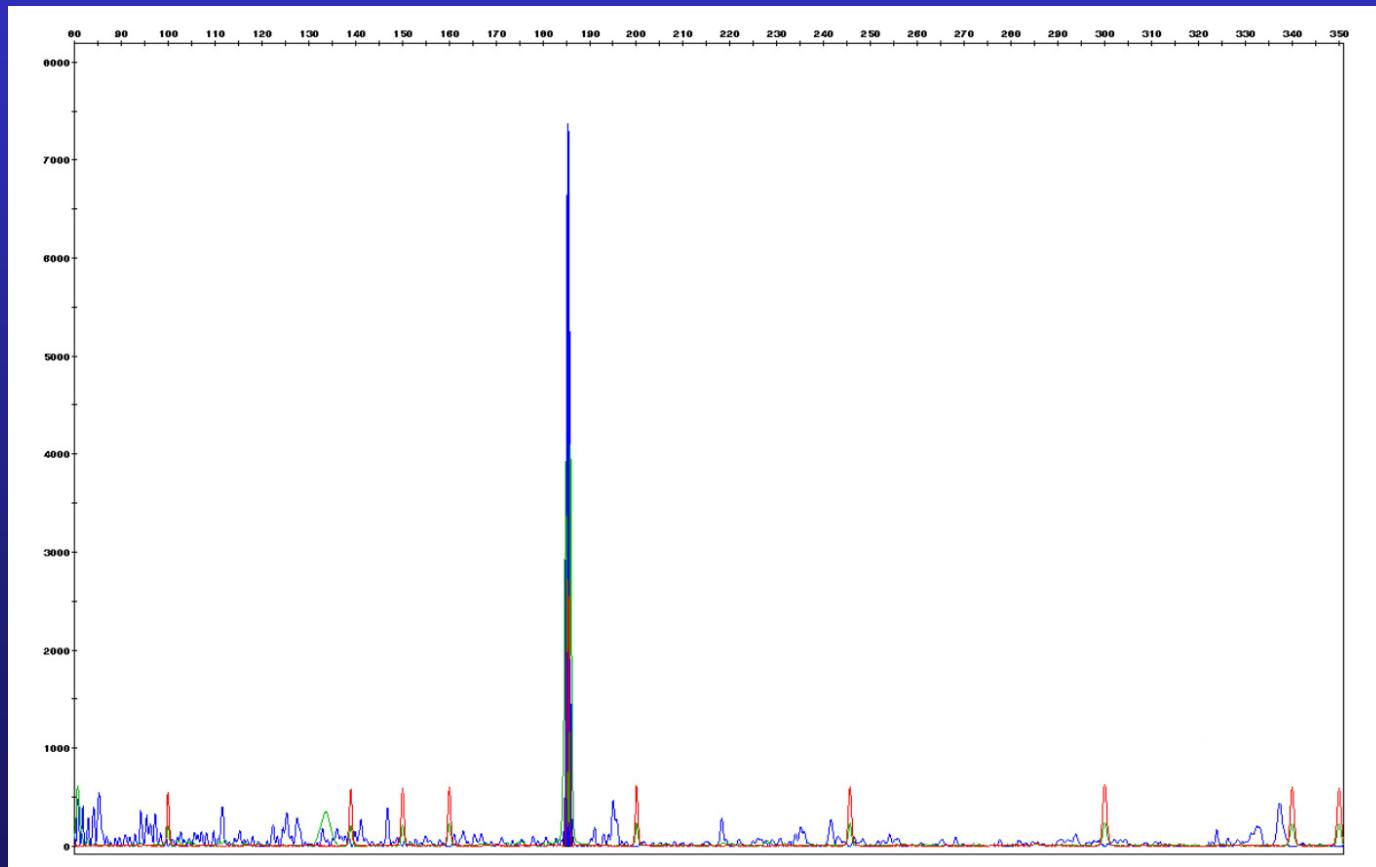
Hedgehog Peaks at 120 and 247 bp



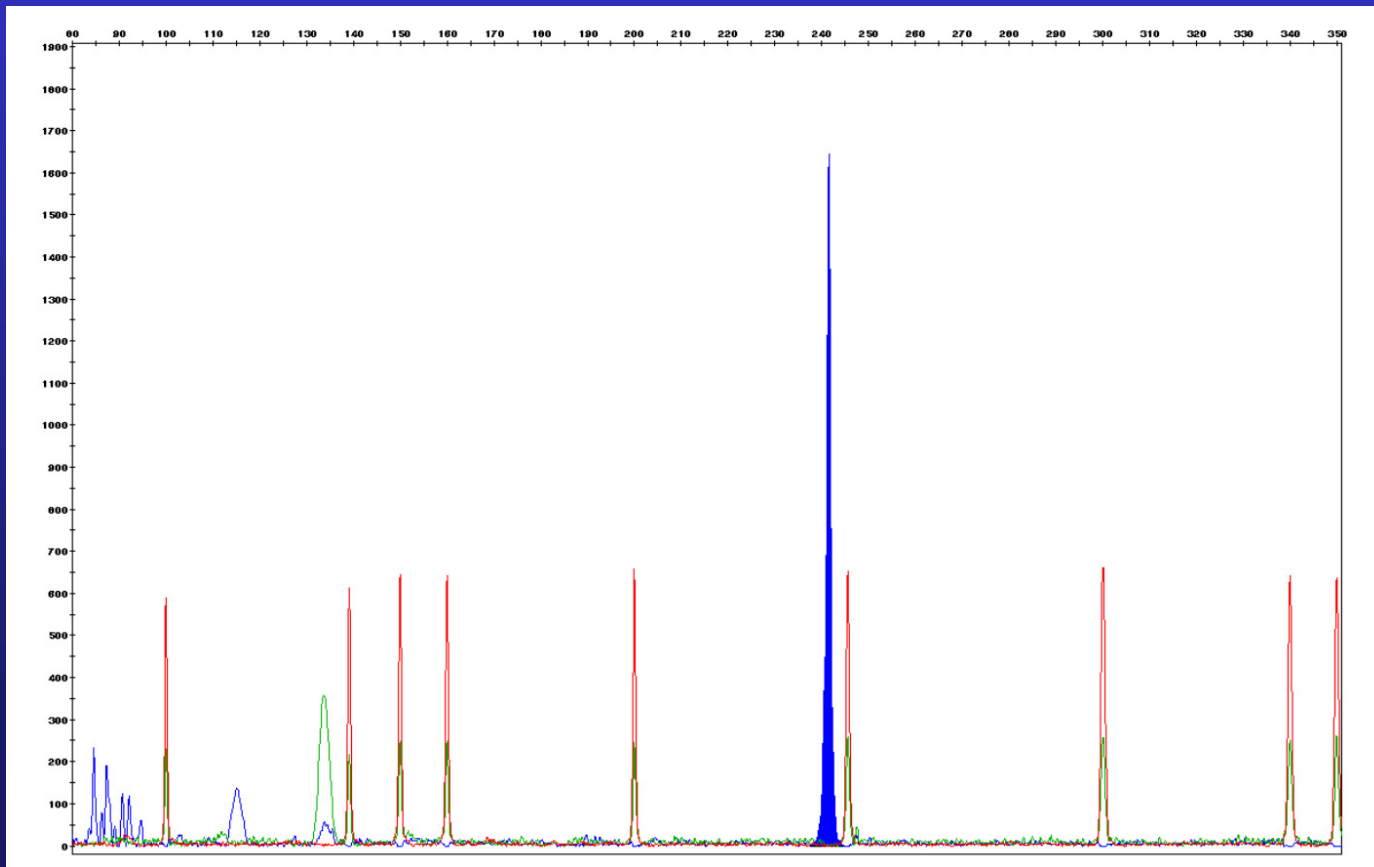
Horse Peak at 265 bp



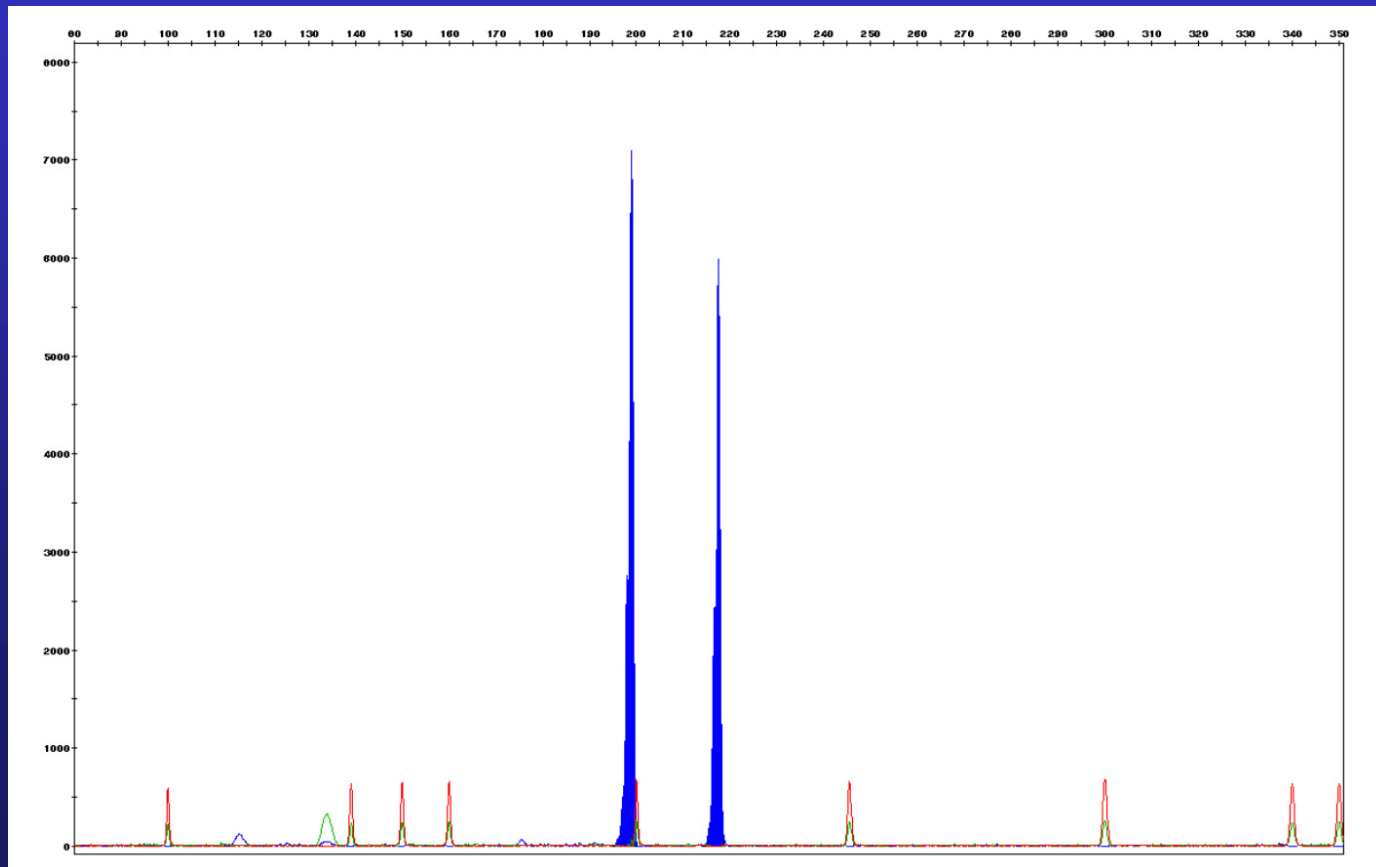
House Mouse Peak at 185 bp



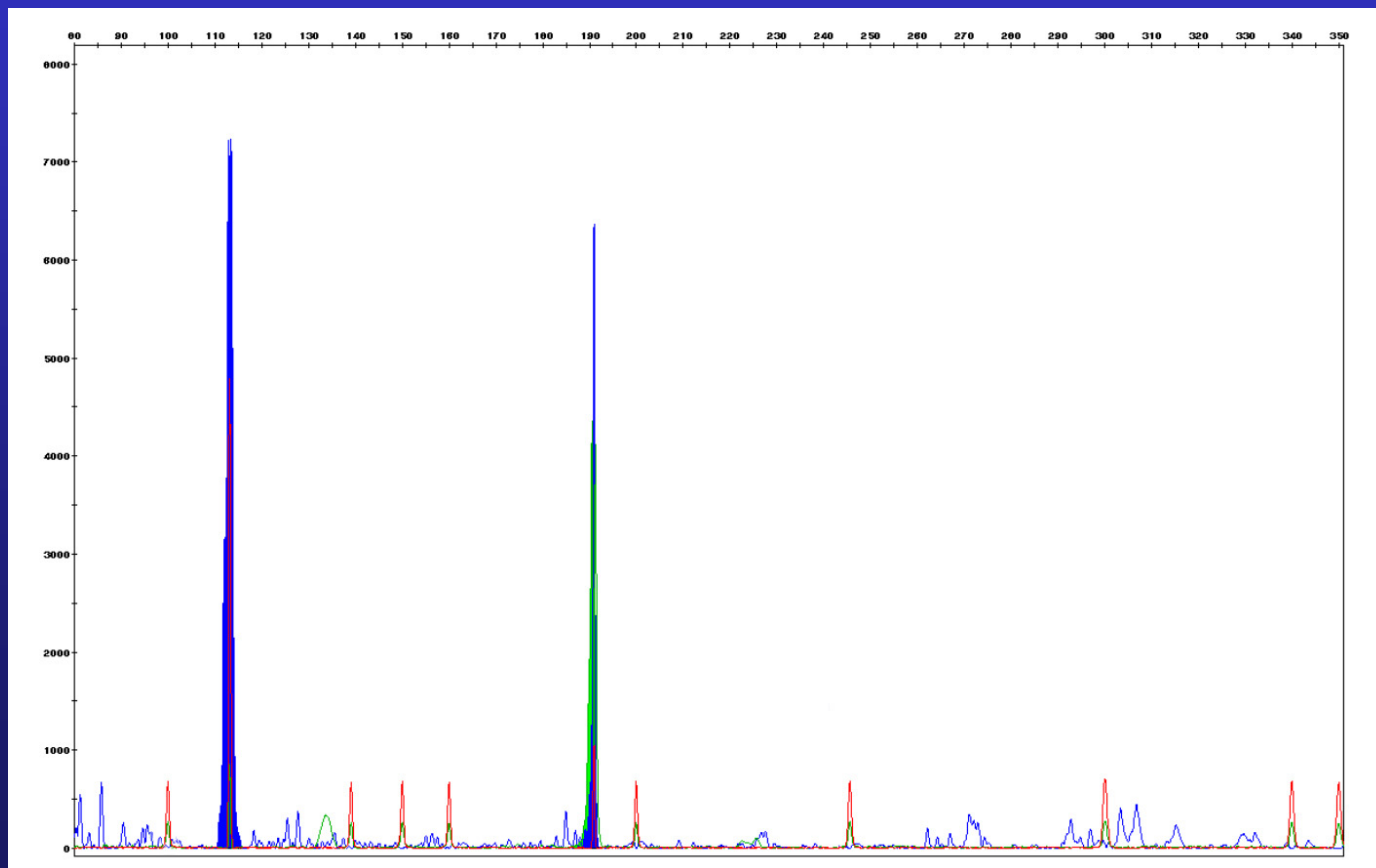
Human Peak at 241 bp



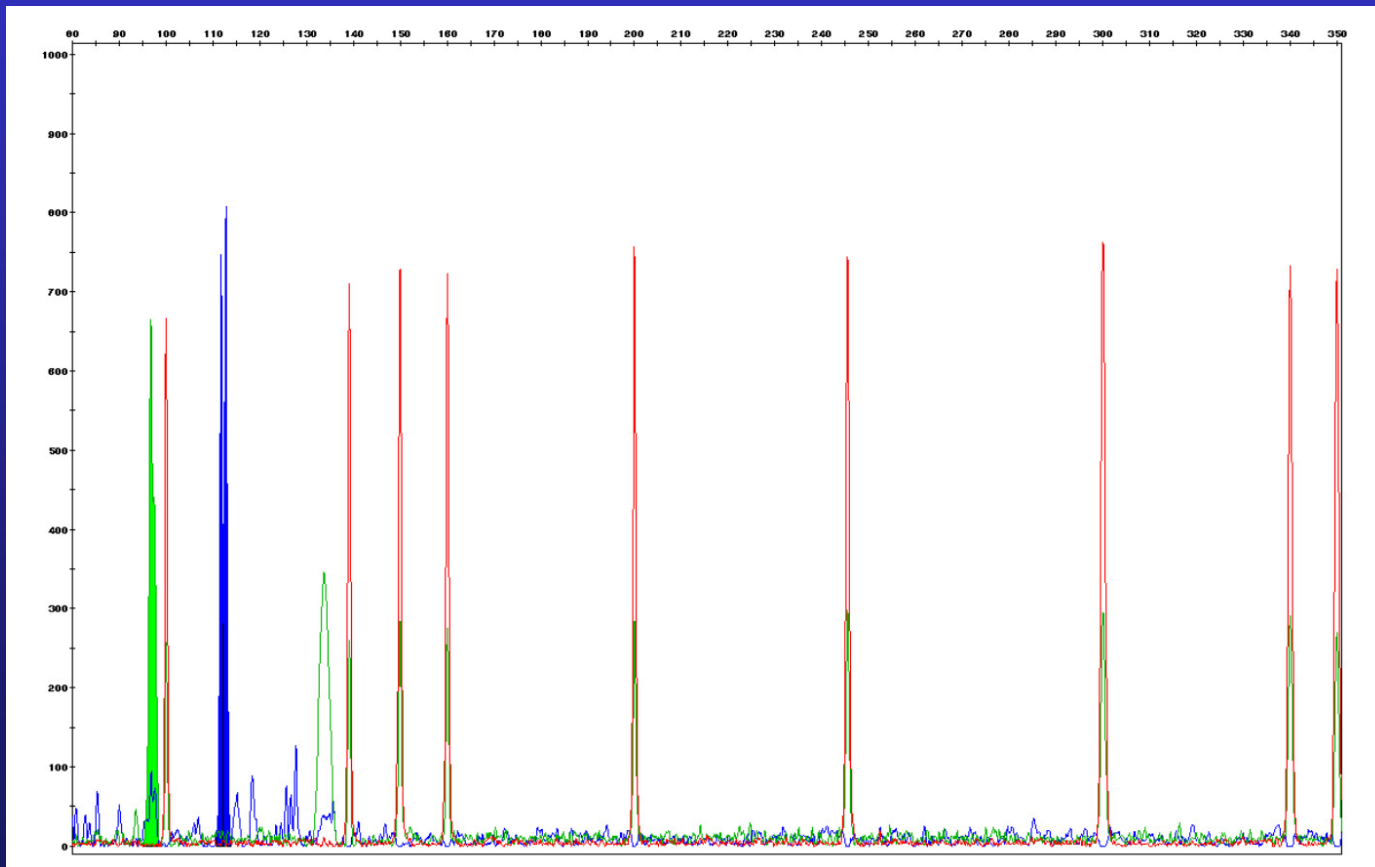
Pig Peaks at 199 and 217 bp



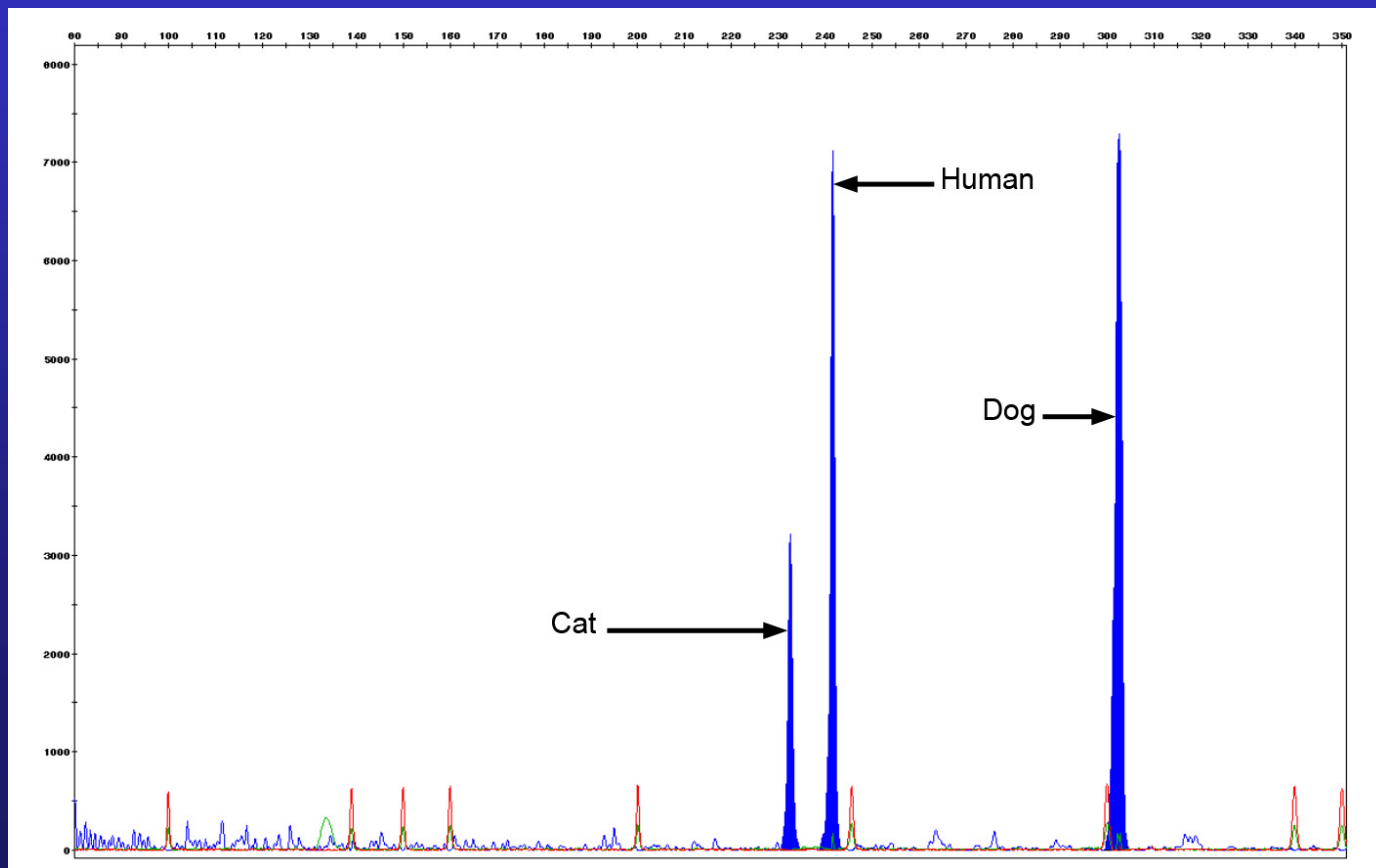
Rat Peaks at 114 and 191 bp



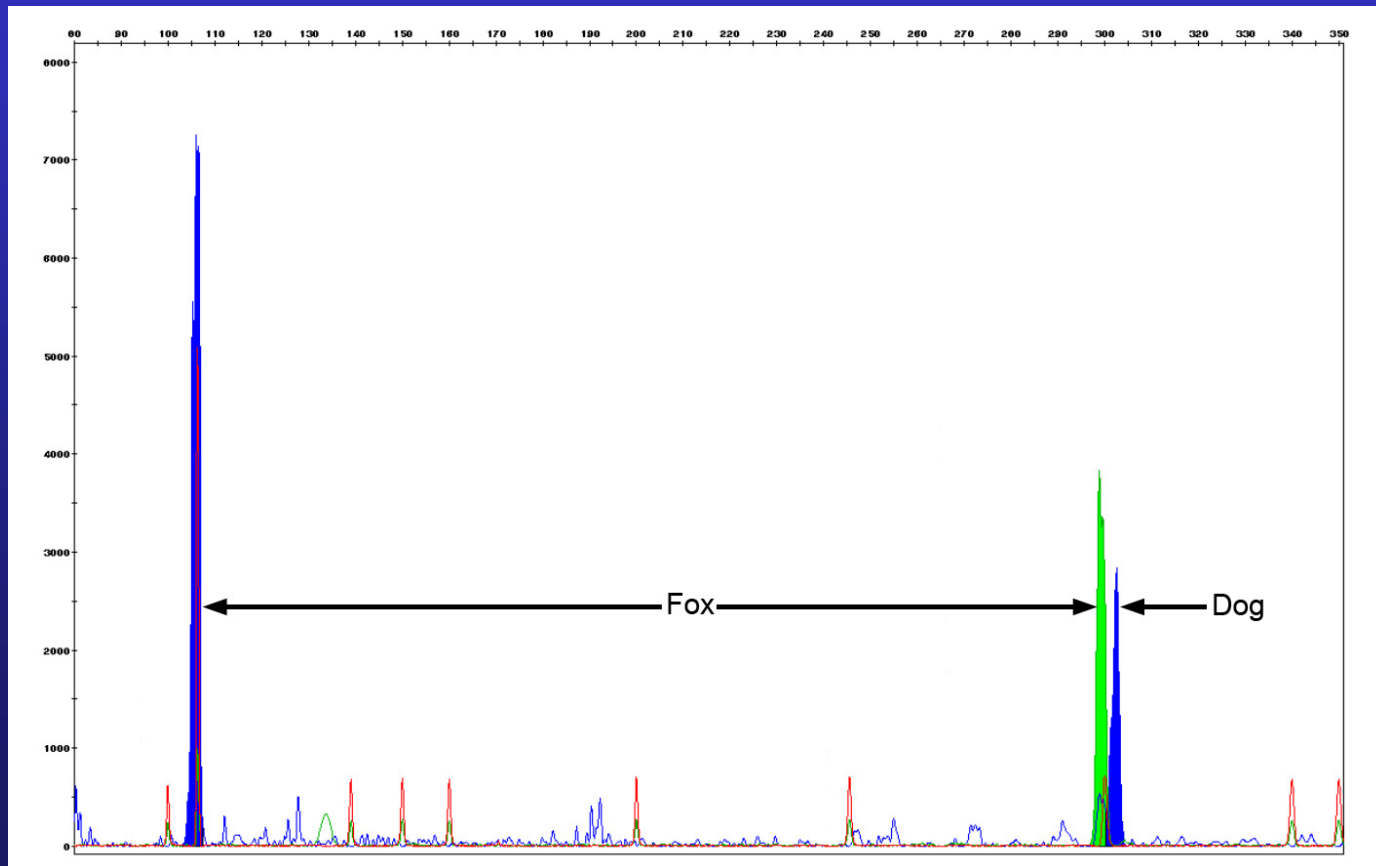
Sheep Peaks at 96 and 112 bp



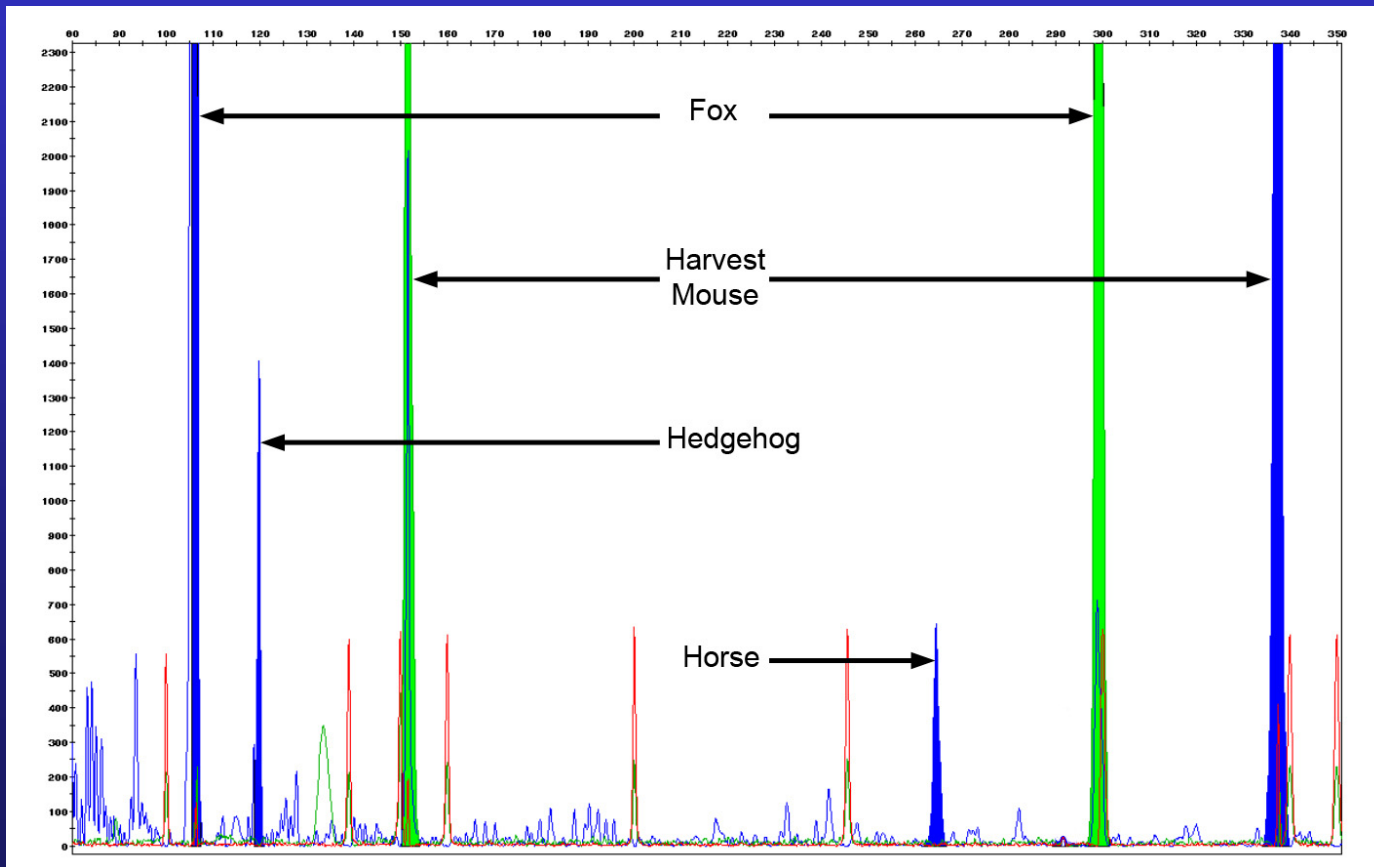
Cat/Dog/Human Mixture



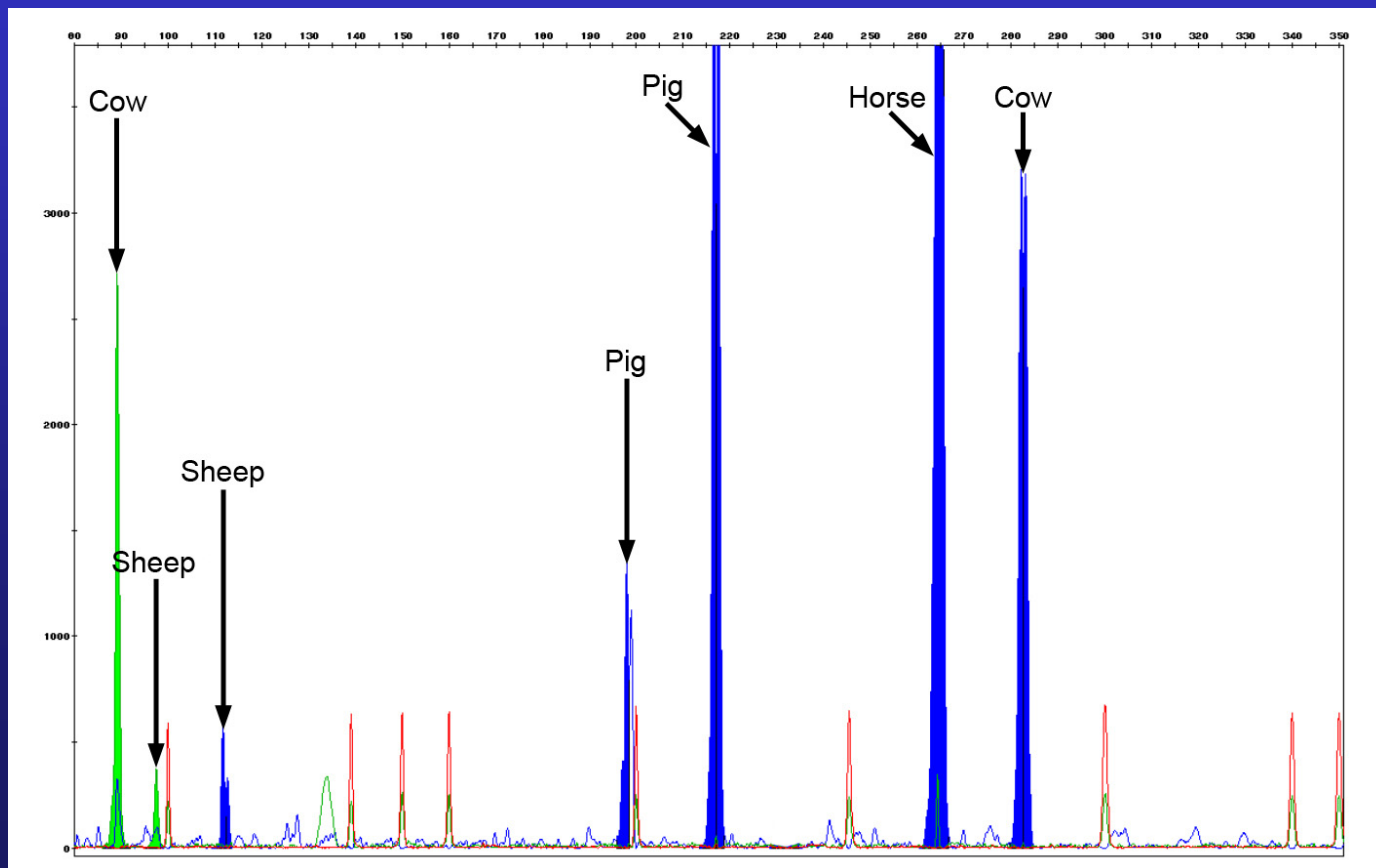
Dog/Fox Mixture



Fox/Harvest Mouse/ Hedgehog/Horse Mixture



Cow/Sheep/Pig/Horse Mixture



Future Directions

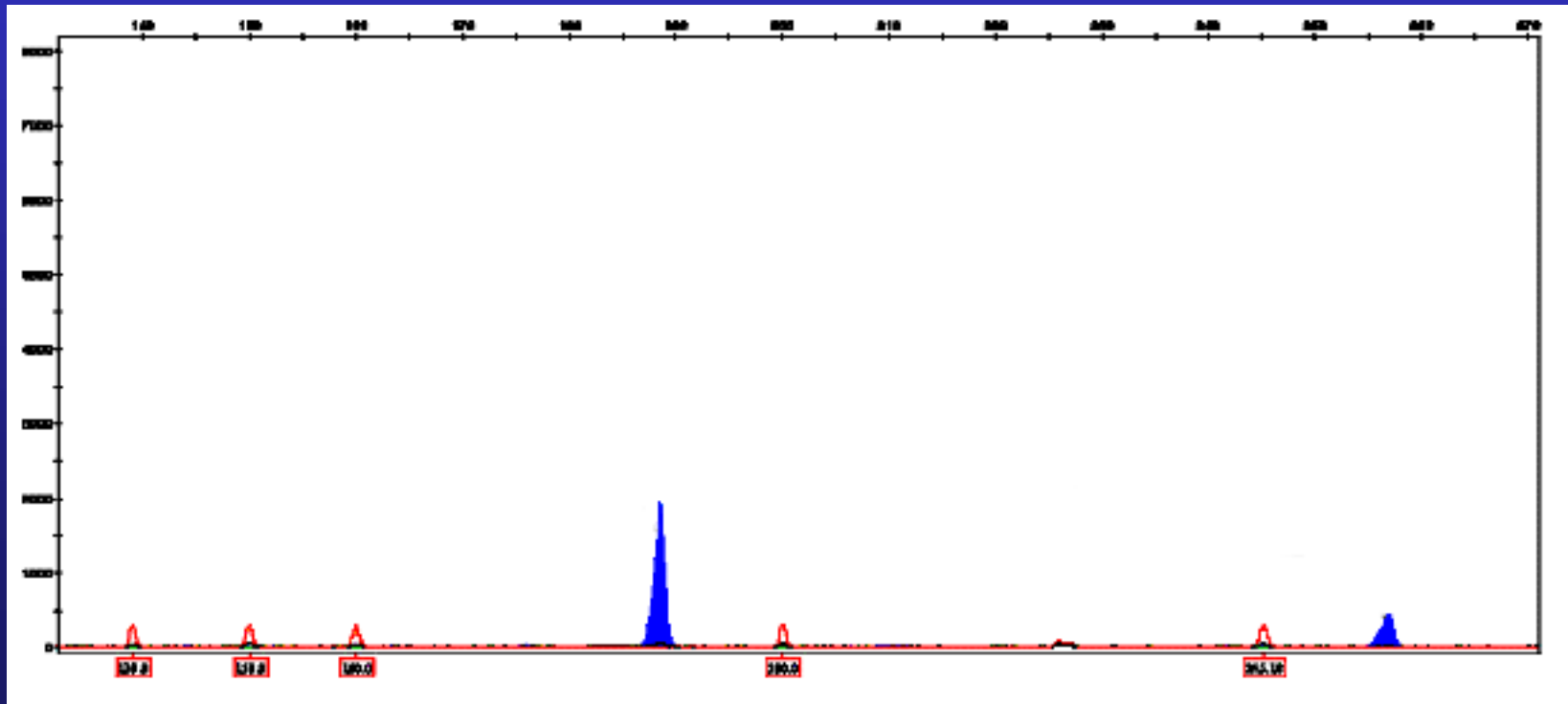
- Primers developed:
 - Red deer
 - Guinea Pig
 - Rabbit and Hare
- Additional animals can be added
- Accurate quantification
- Expansion to include other genes

Case 1: Poaching

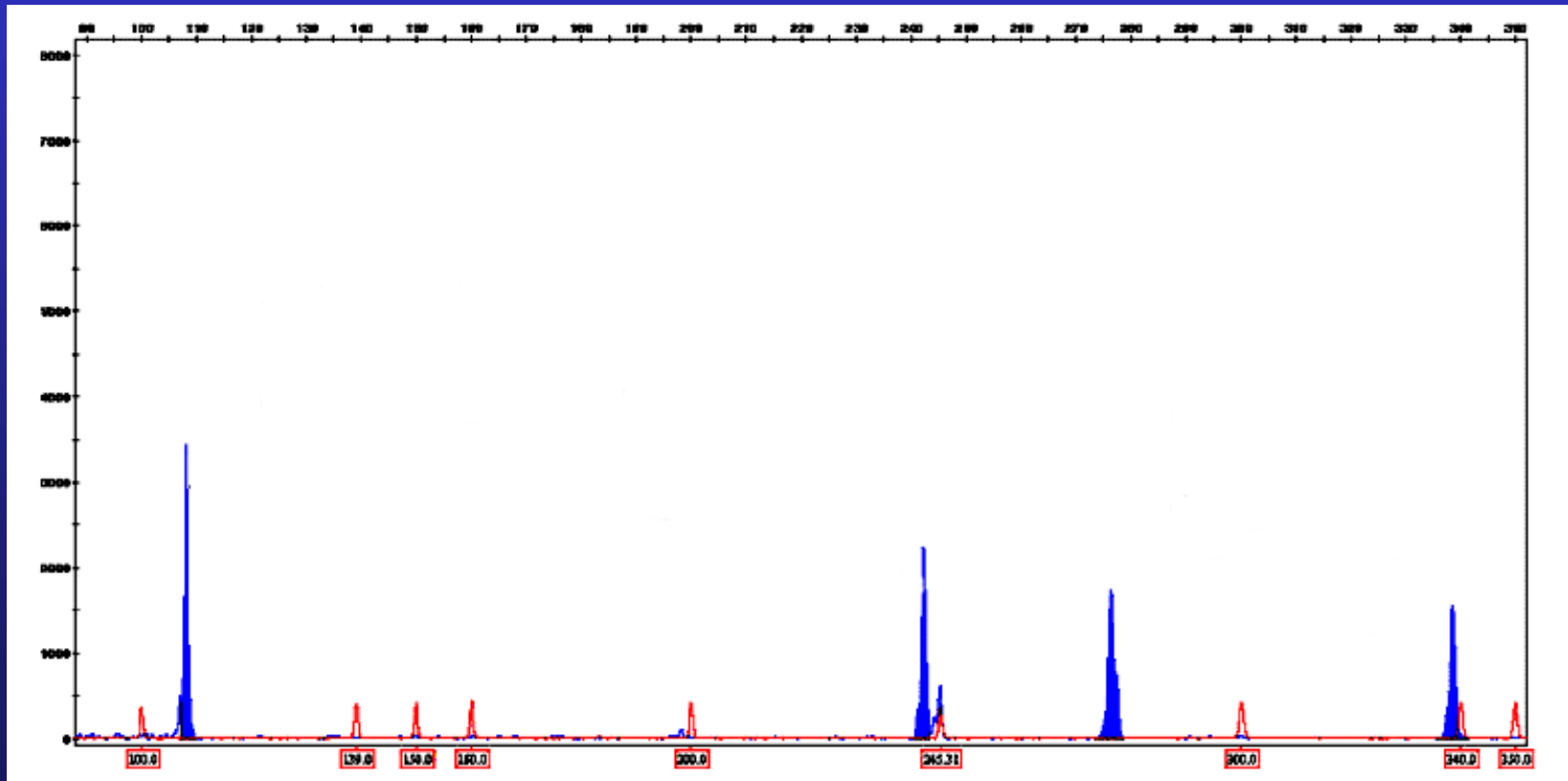
- Man found in army fatigues with blood spatter
- Suspected of poaching red deer
- Police were interested if the blood was that of red deer



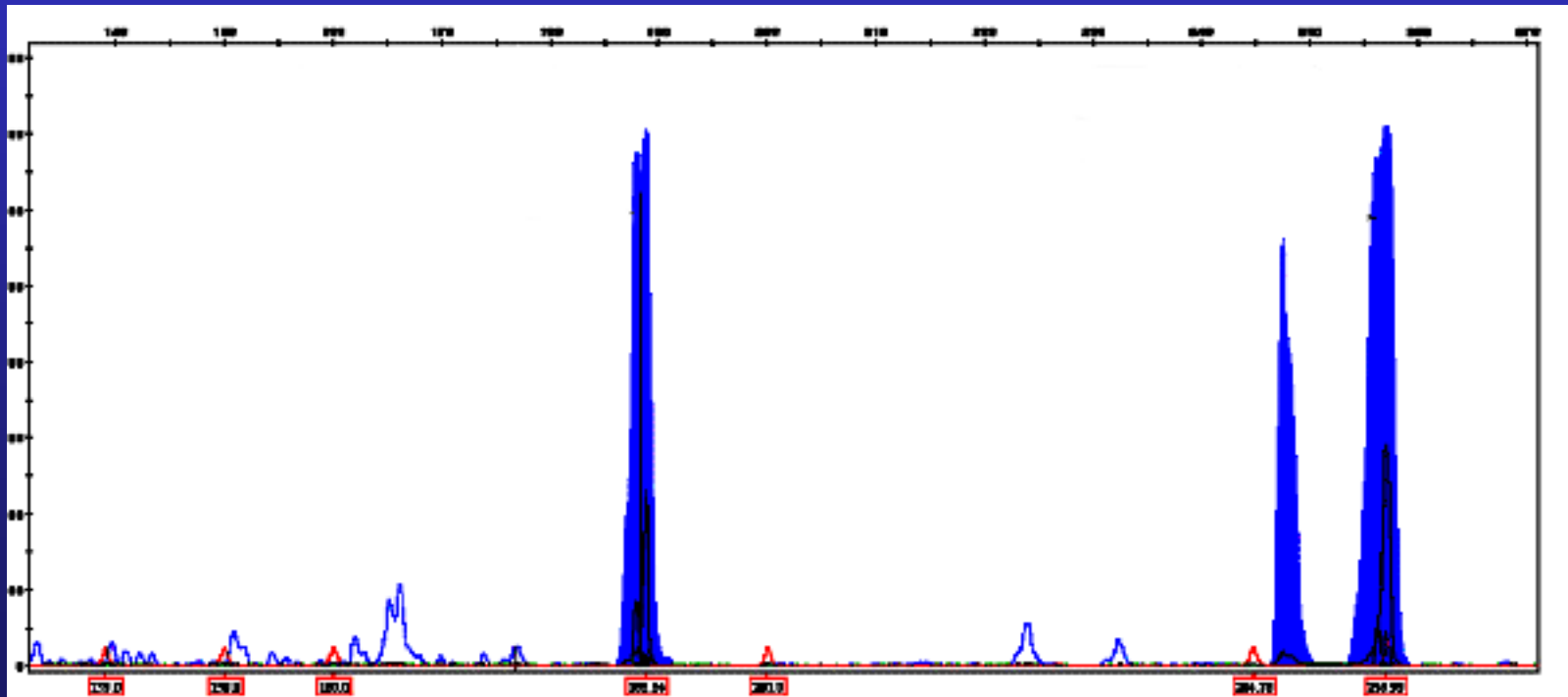
Case 1: Poaching Red Deer Primers Control



Case 1: Poaching Human Primers

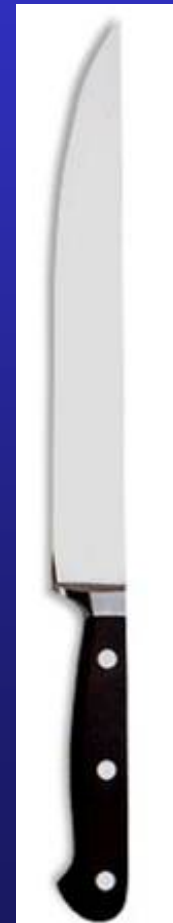


Case 1: Poaching Red Deer Primers



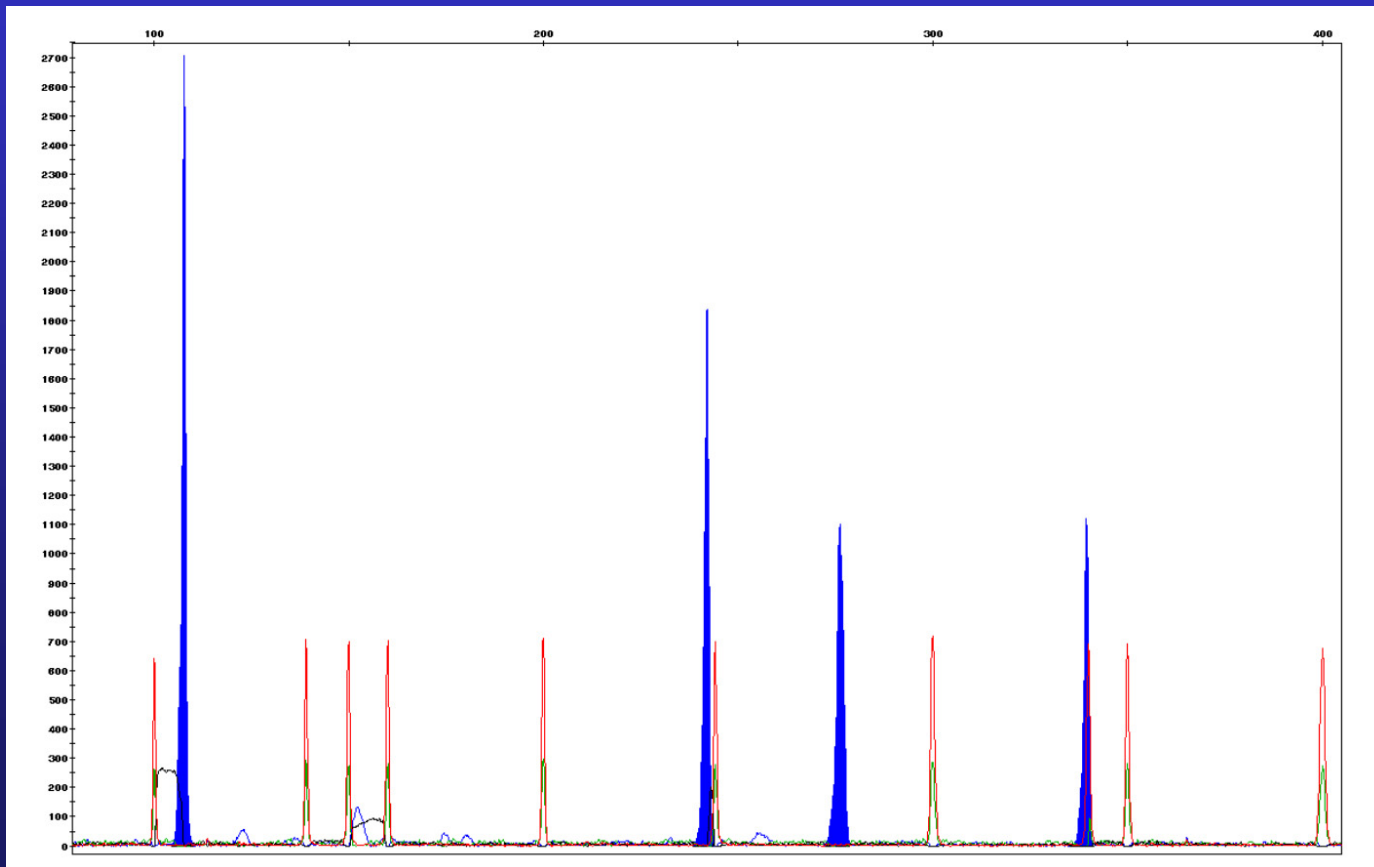
Case 2: Murder

- Knife tested positive for blood
- Interested if blood was human
- Partial human STR profile obtained
- Confirmation with species specific primers



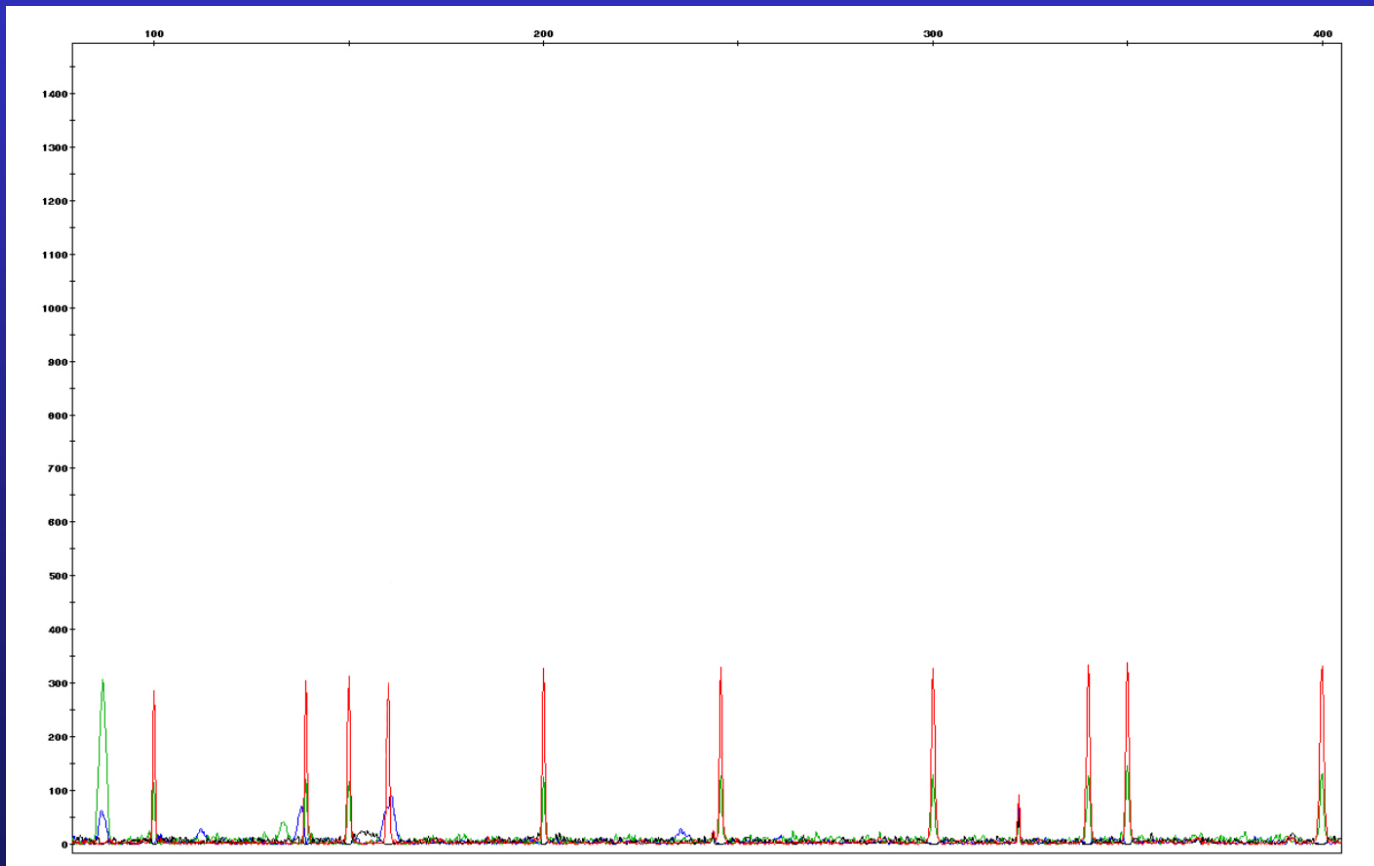
Case 2: Murder

Sample with Human Primers



Case 2: Murder

Sample with Mammalian Primers





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THANK YOU

QUESTIONS?