



University of
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Identification of Animal Species Using the Cytochrome *b* Gene on the Mitochondrial Genome

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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

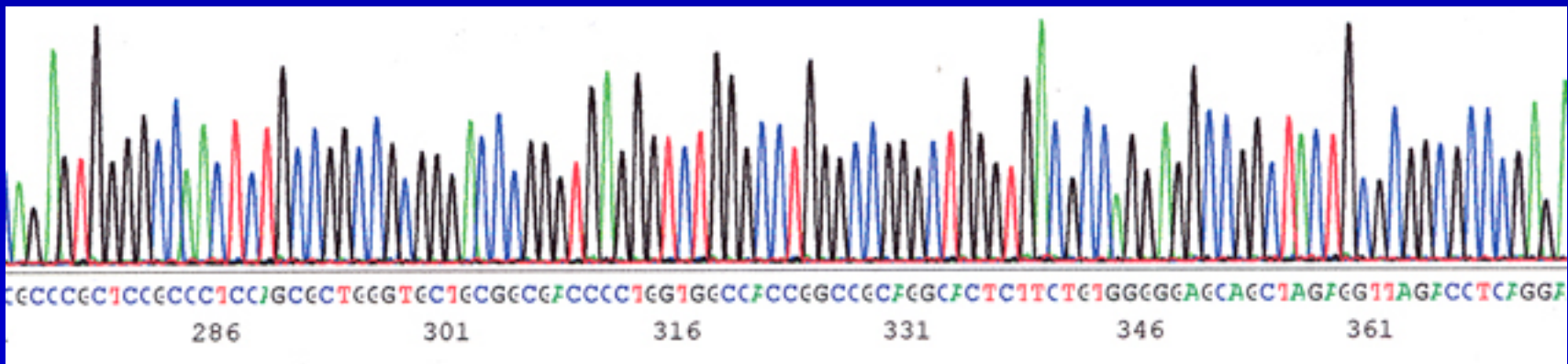
- Gross
 - Microscopy
 - Osteology

- 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



Problems With Sequencing

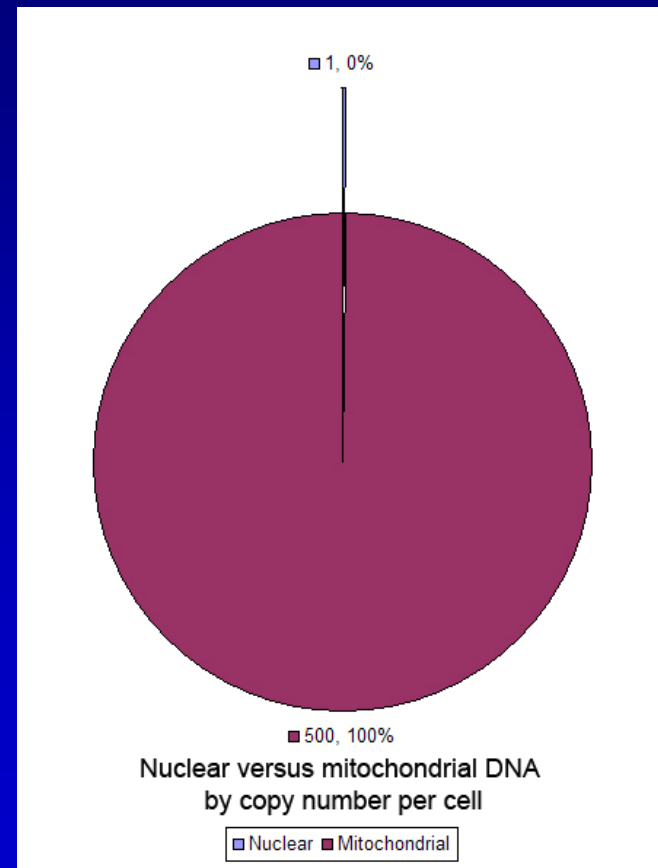
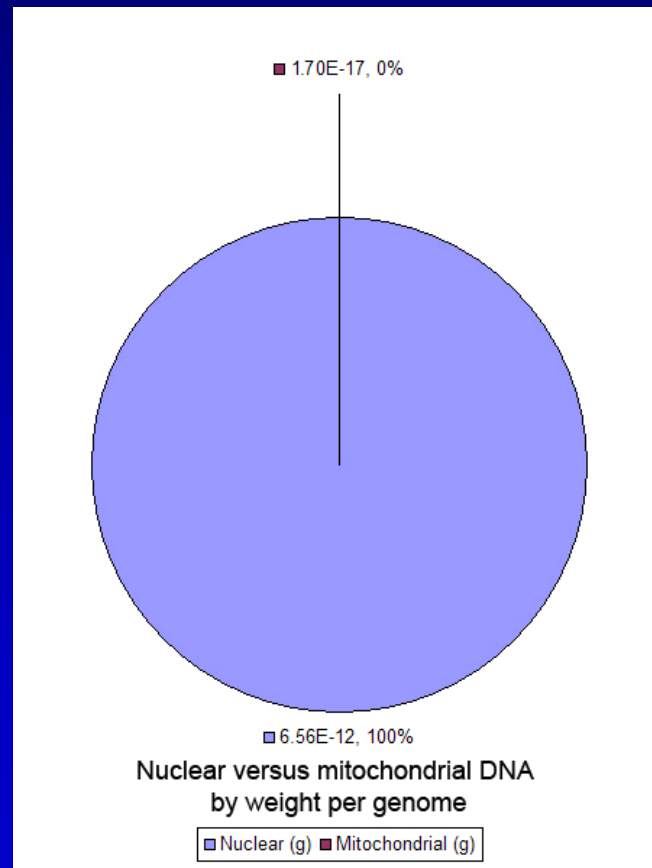
- Costly and labour intensive
- Mixtures cannot be separated reliably
- Degraded samples may not yield enough sequence data for identification

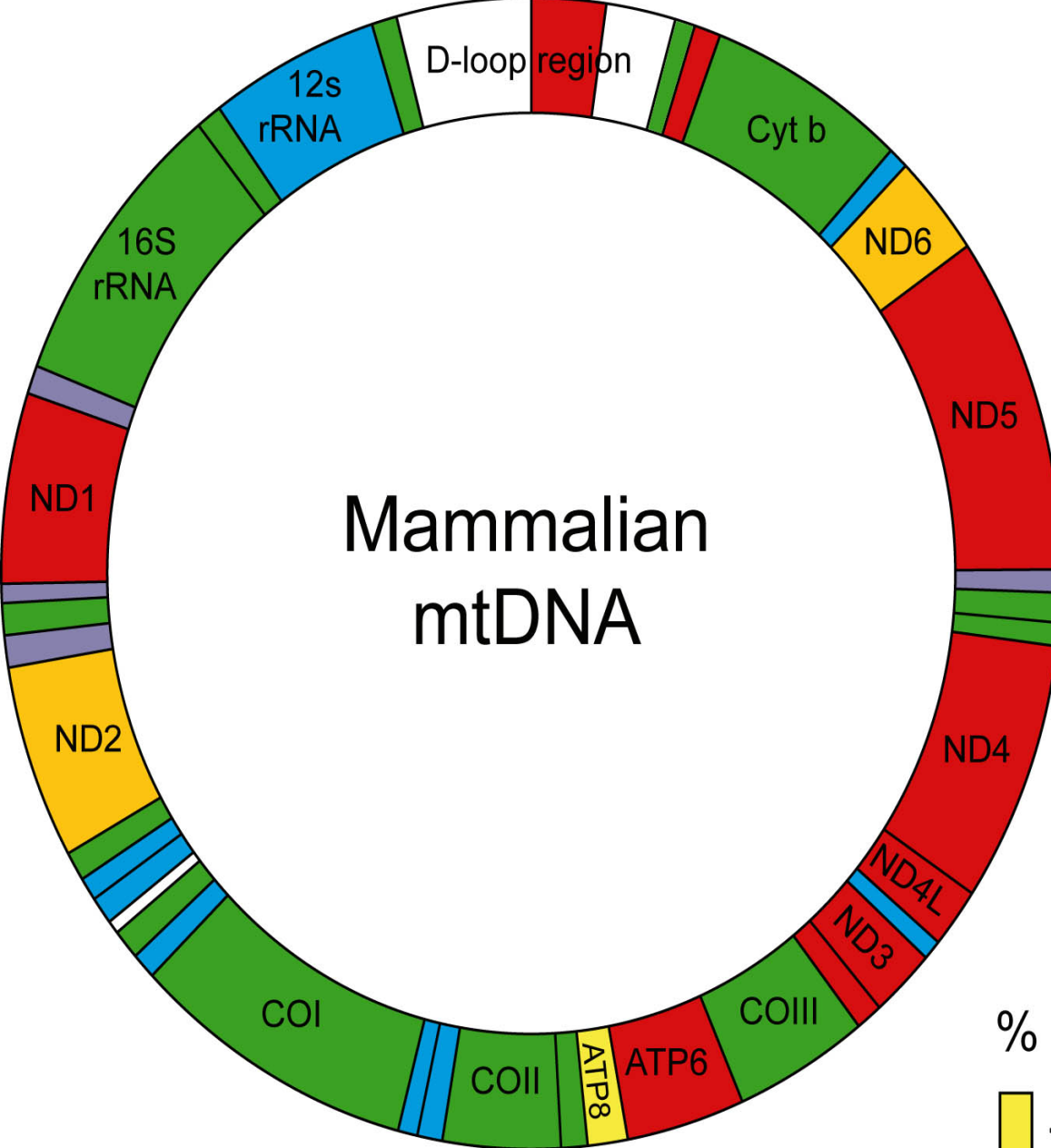
Requirements For Identification

- 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





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

% Similarity

< 65

70 - 75

80 - 85

65 - 70

75 - 80

> 85

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

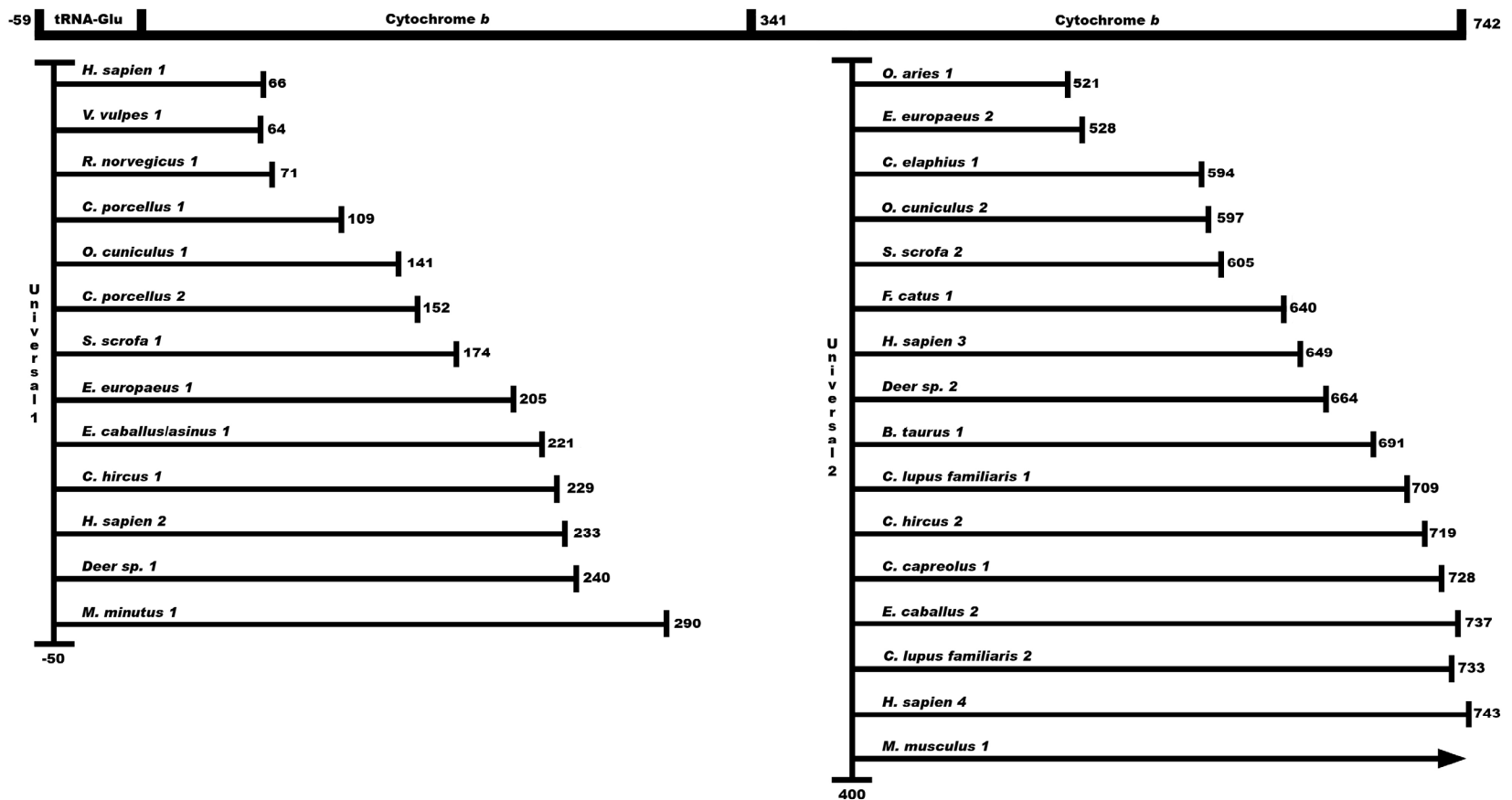
Base Pair Differences At The Cytochrome *b* Gene

	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	

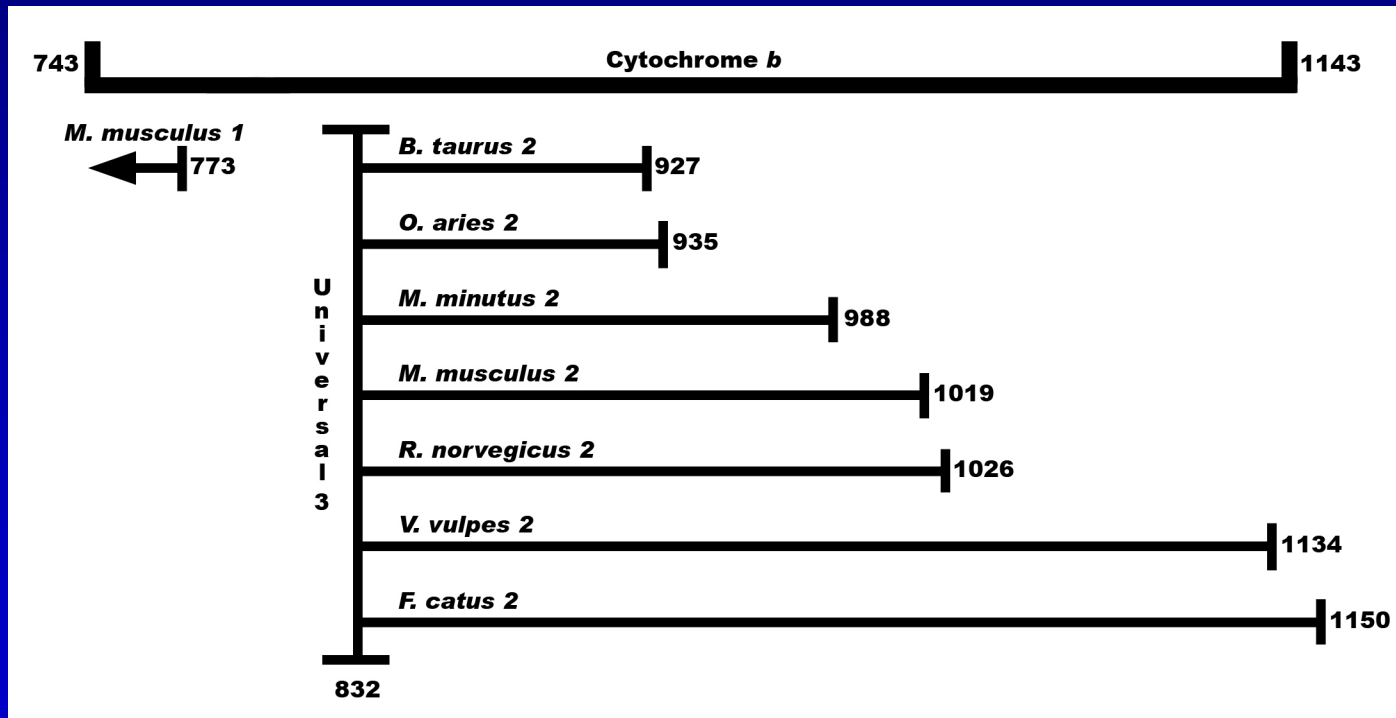
Primer Design

<i>B. taurus</i>	TCACAGTAATAGCCACAGCATTATAGGATACGTCCTACCATGAGGACAAATATCATTCT	479
<i>H. grypus</i>	TCACCATCATAGCCACGGCATTATGCGCTACGTCCTACCATGAGGACAAATATCATTCT	479
<i>S. scrofa</i>	TTACCGTTATAGCAACAGCCTTTCATAGGCTACGTCCTGCGCTGAGGACAAATATCATTCT	479
<i>F. catus</i>	TTACAGTCATAGCCACAGCCTTTTATGGGATACGTCCTACCATGAGGCCAAATATCCTTCT	478
<i>H. sapien</i>	TTGCAACTATAGCAACAGCCTTTCATAGGCTATGTCCTCCCGTGGGCCAAATATCATCTCT	479
Universal 2		
<i>B. taurus</i>	GAGGAGCAACAGTCATCACCAACCTCTTATCAGCAATCCCATACATCGGCACAAATTTAG	539
<i>H. grypus</i>	GAGGGGCAACAGTCATTACCAATCTACTATCAGCAATCCCCTATATCGGAACCGACCTTG	539
<i>S. scrofa</i>	GAGGAGCTACGGTCATCACAAATCTACTATCAGCTATCCCTTATATCGGAACAGACCTCG	539
<i>F. catus</i>	GAGGAGCAACCGTAATCACTAACCTCCTGTCAGCAATTCCATACATCGGGACTGAACCTAG	538
<i>H. sapien</i>	GAGGGCCACAGTAATTACAAACTTACTATCCGCCATCCCATACATTGGGACAGACCTAG	539
<i>B. taurus</i>	TCGAATGAATCTGAGGCGGATTCTCAGTAGACAAAGCAACCCCTTACCCGATTCTTCGCTT	599
<i>H. grypus</i>	TACAATGAATCTGAGGAGGATTTTTCAGTAGACAAAGCAACCCCTTAAACAGGATTCTTCGCTT	599
<i>S. scrofa</i>	TAGAATGAATCTGAGGGGGCTTTTCCGTCGACAAAGCAACCCCTCACACGATTCTTCGCTT	599
<i>F. catus</i>	TAGAATGAATCTGAGGGGGGTTCTCAGTAGACAAAGCCACCCCTAACACGATTCTTTGGCT	598
<i>H. sapien</i>	TTCAATGAATCTGAGGAGGCTACTCAGTAGACAGTCCCACCCCTCACACGATTCTTTACCT	599
H. grypus 1 - 187bp		
<i>B. taurus</i>	TCCATTTTATCCTTCCATTTATCATCATAGCAATTGCCATAGTCCACCTACTATTCCCTCC	659
<i>H. grypus</i>	TCCACTTCATCCTACCAATTCCTAGTATTAGCACTAGCAGCAGTCCACCTACTATTCCCTAC	659
<i>S. scrofa</i>	TCCACTTTATCCTGCCATTTCATCATTACCGCCCTCGCAGCCCTACATCTCCTATTCCCTCC	659
<i>F. catus</i>	TCCACTTCATTCTTCCATTTCATTATCTCAGCCTTAGCAGGAGTACACCTCTTATTCCCTTC	658
<i>H. sapien</i>	TTCACTTCATCTTACCCCTTCATTATTCAGCCCTAGCAGCACTCCACCTCCTATTCTTCC	659
H. sapien 3 - 246bp		
<i>B. taurus</i>	ACGAAACAGGCTCCAAACAAACCCCAACAGGAATTTCTCAGACGTAGACAAAATCCCATTCC	719
<i>H. grypus</i>	ACGAAACAGGATCAAAACAAACCCCTCCGGAATCATACCCGACTCAGACAAAATCCCATTCC	719
<i>S. scrofa</i>	ACGAAACCGGATCCAAACAAACCCCTACCGGAATCTCATCAGACATAGACAAAATTCCTATTCC	719
<i>F. catus</i>	ATGAAACAGGATCTAAACAAACCCCTCAGGAATTACATCCGATTTCAGACAAAATCCCATTCC	718
<i>H. sapien</i>	ACGAAACGGGATCAAAACAAACCCCTAGGAATCACCTCCCATTCGGATAAAATCACCTTCC	719
<i>B. taurus</i>	ACCCCTACTATACCATTAAGGACATCTTAGGGGCCCTCTTACTAATTCTAGCTCTAATAAC	779
<i>H. grypus</i>	ACCCGTACTATACAATTAAGACATCCTAGGAGCCCTGCTTCTCATTCTAGTCCTGACAC	779
<i>S. scrofa</i>	ACCCATACTACACTATTAAAGACATTCTAGGAGCCCTATTTATAATACTAATCCTACTAA	779
<i>F. catus</i>	ACCCATACTATACAATCAAGACATCCTAGGTCTTCTAGTACTAGTTTAAACACTCATAC	778
<i>H. sapien</i>	ACCCTTACTACACAATCAAGACGCCCTCGGCTTACTTCTCTTCTCTCTCTCTCTTAATGA	779

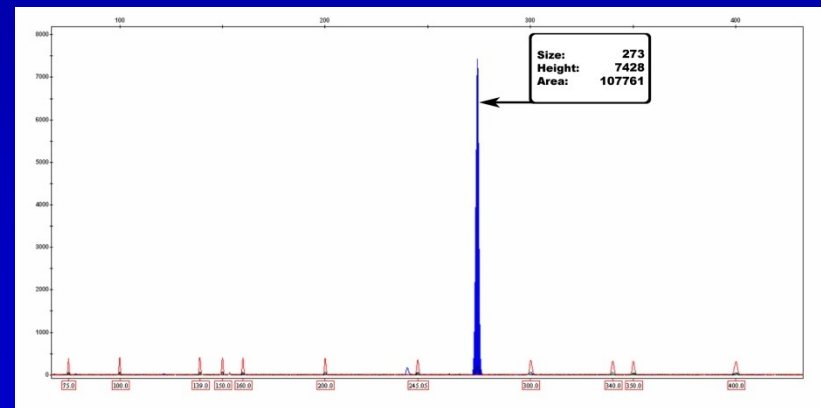
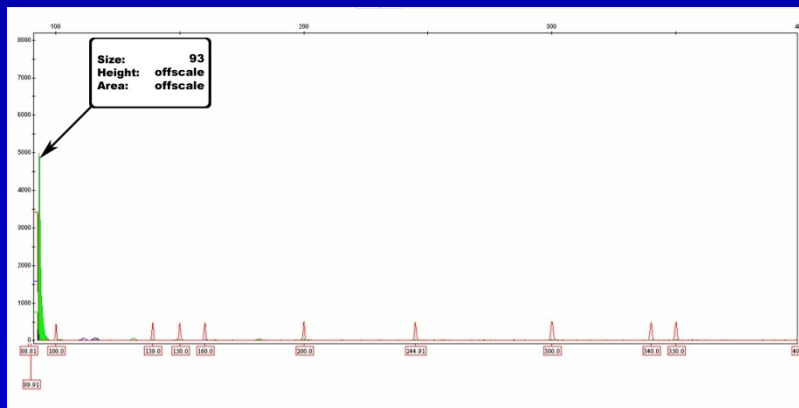
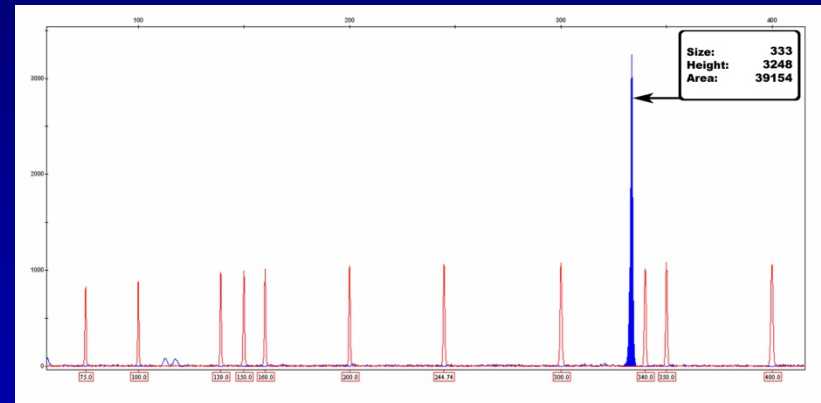
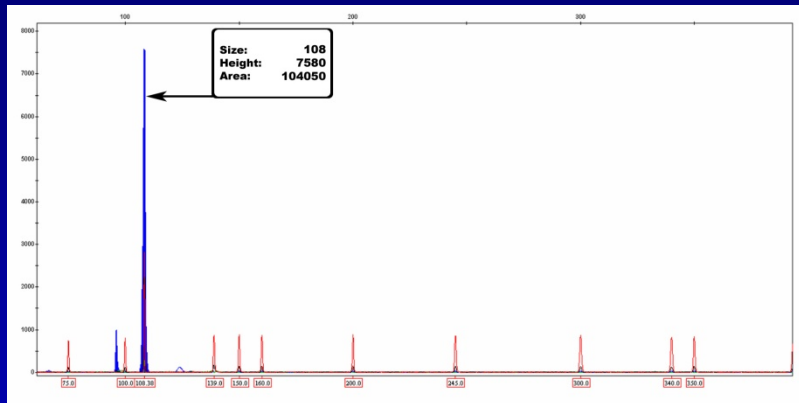
Species-Specific and Universal Primers



Species-Specific and Universal Primers

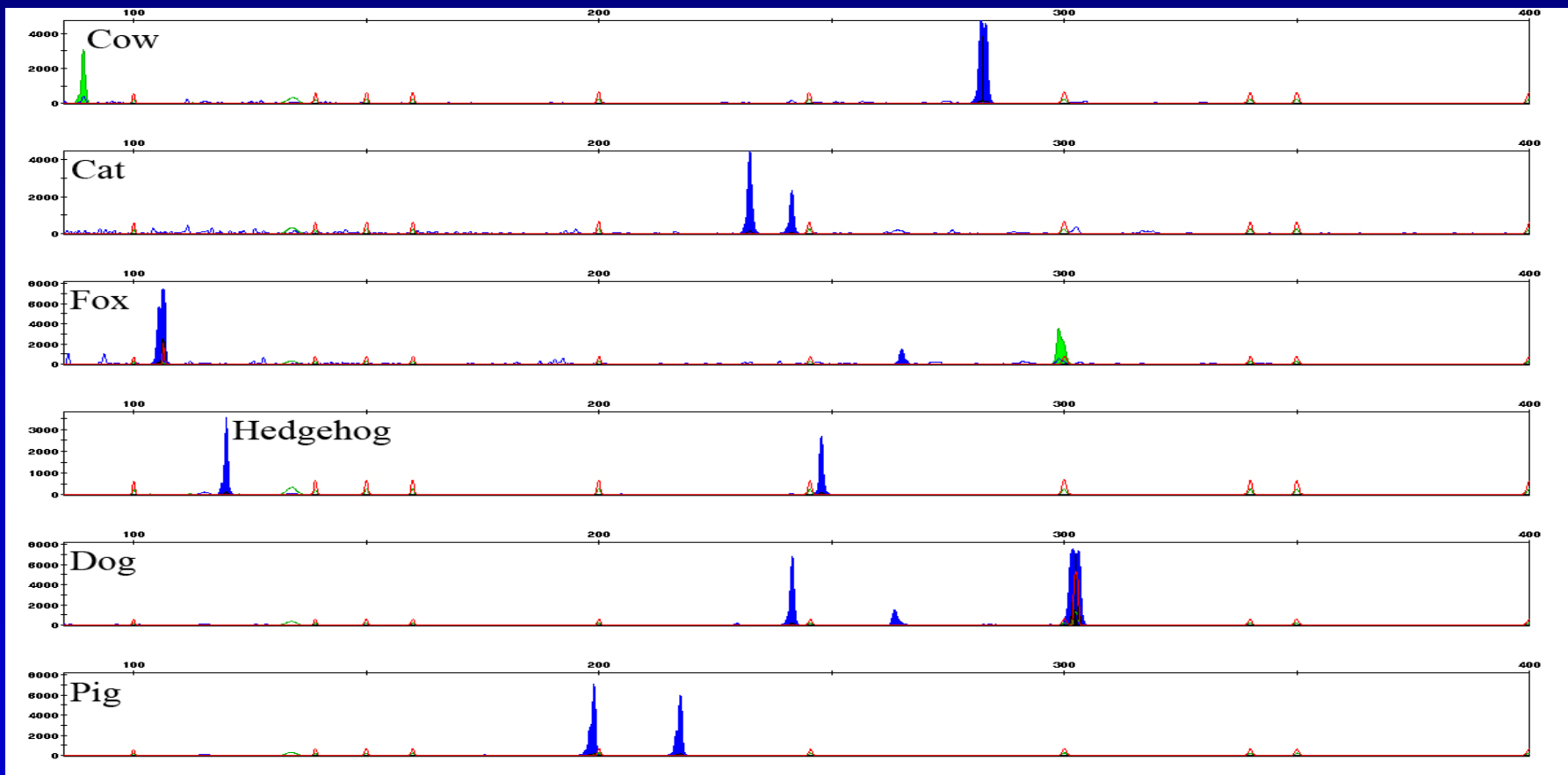


Single Animal Reactions

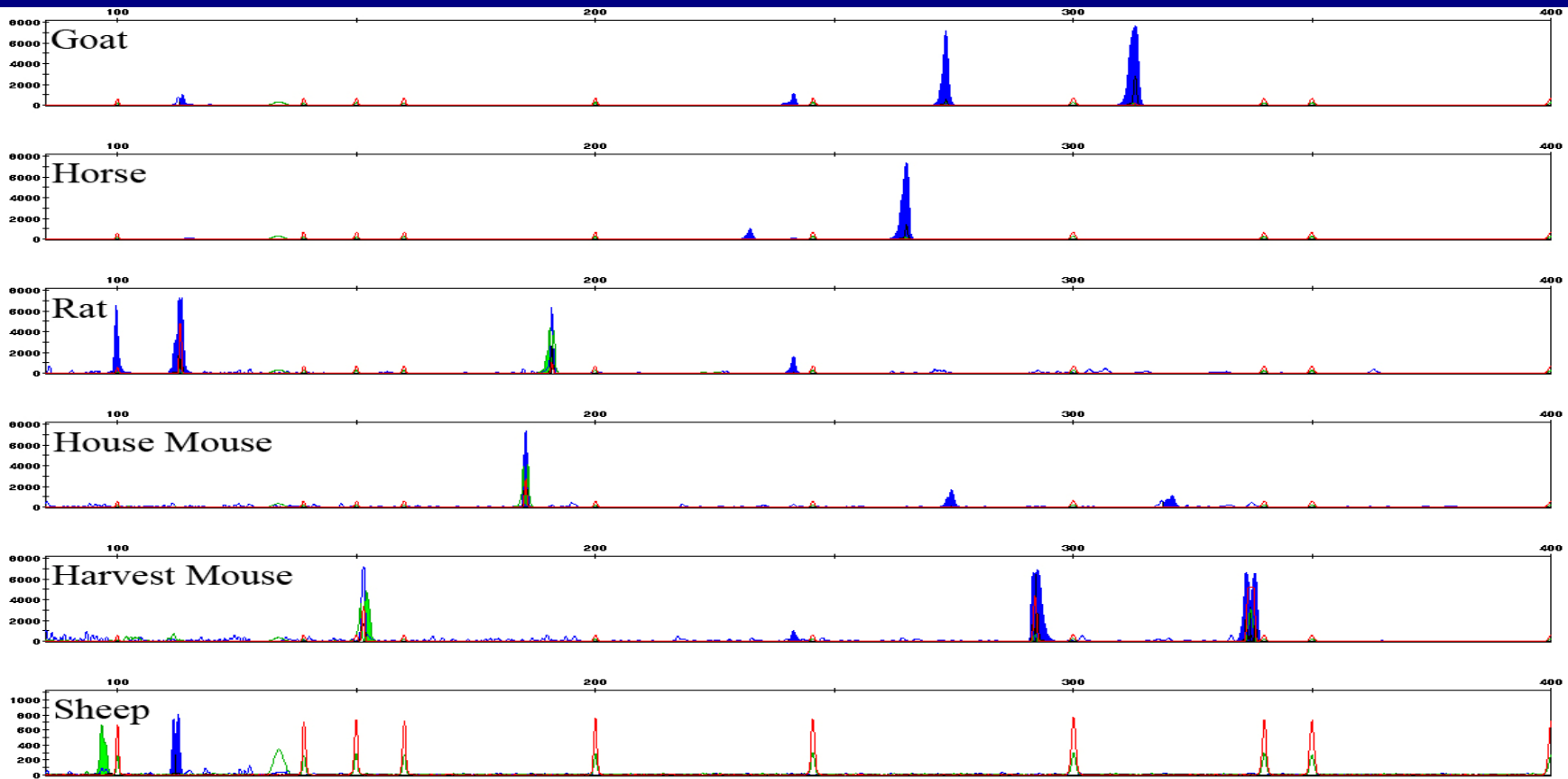


From top left: Human, Horse, Cow and Goat

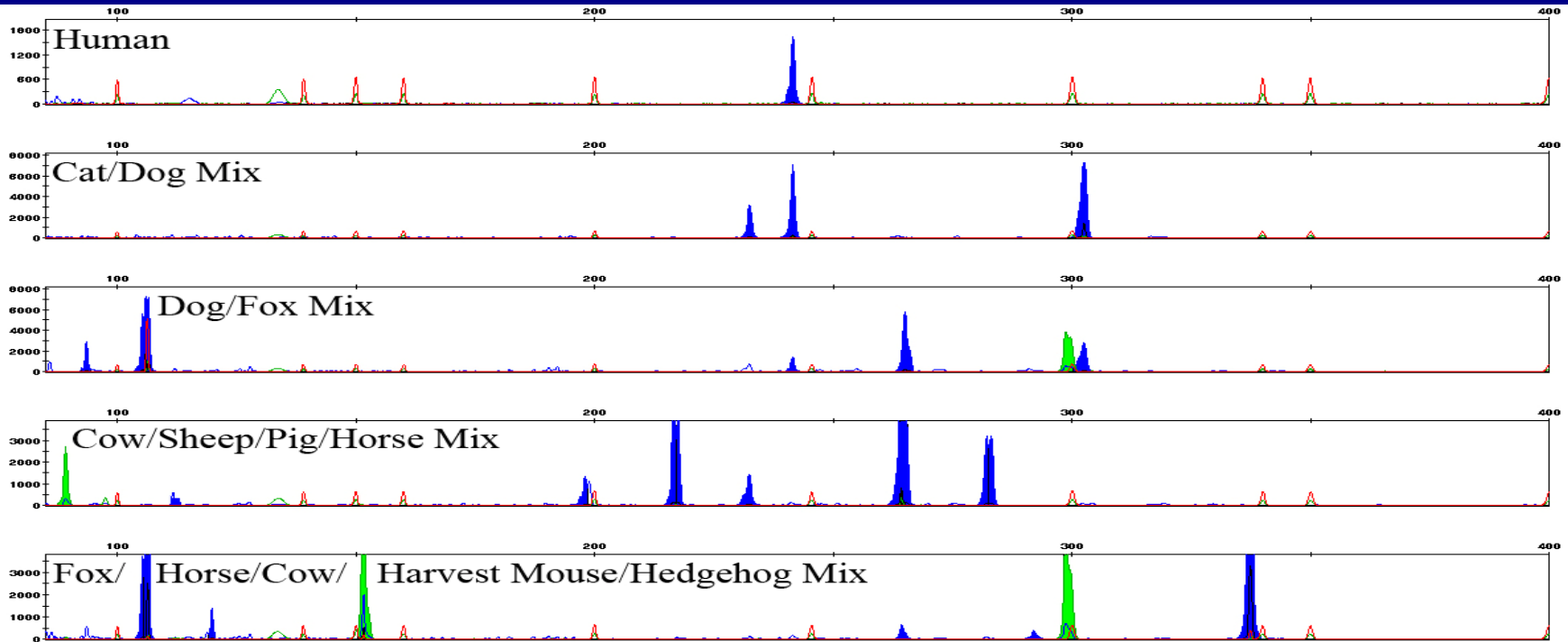
Multiplex Species Test



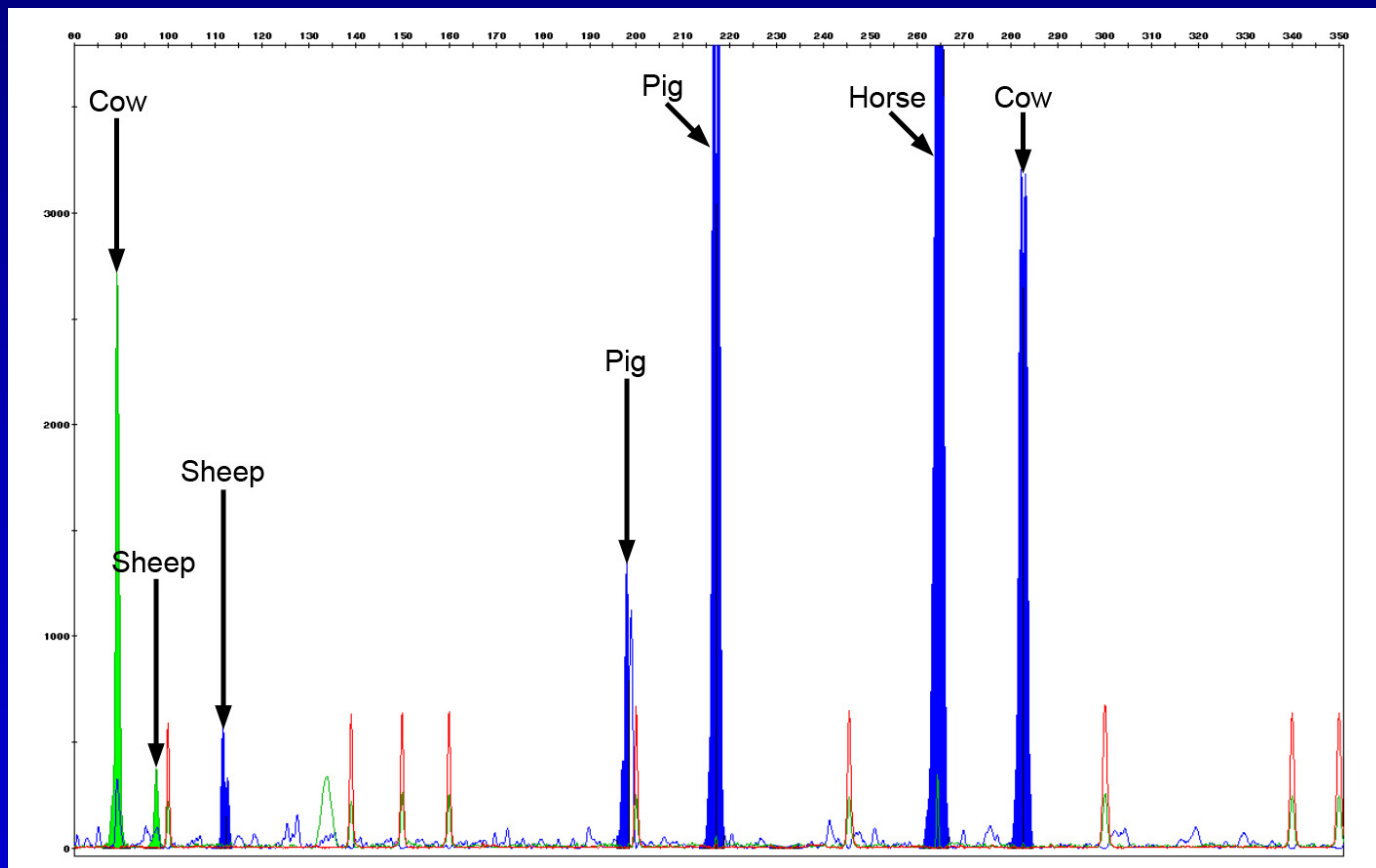
Multiplex Species Test



Multiplex Species Test



Cow/Sheep/Pig/Horse Mixture



Future Goals

- Quantification of all samples
 - Balance out the species multiplex
- Validation

THANK YOU