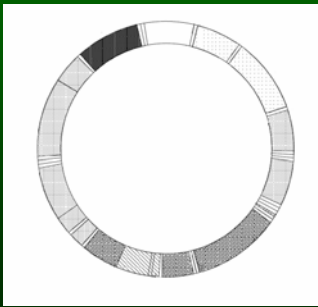


Real Time PCR and Species Identification

David Foran
Kim Anderson, Ashley McClellan
Scott Grammer
Forensic Biology Laboratory
Michigan State University



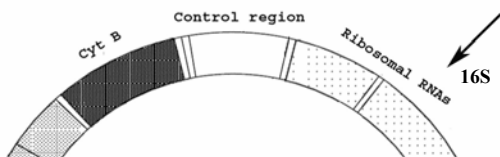
Mammalian mitochondrial DNA

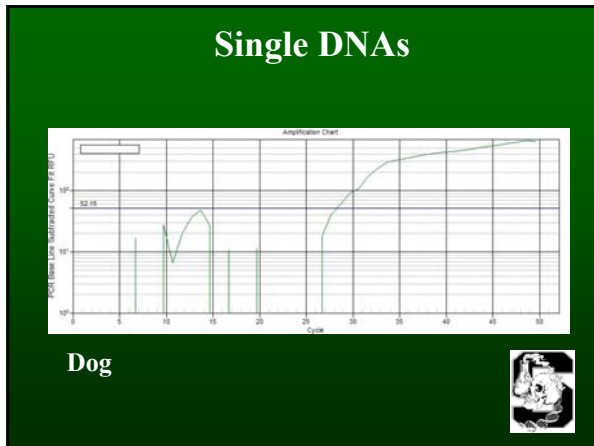


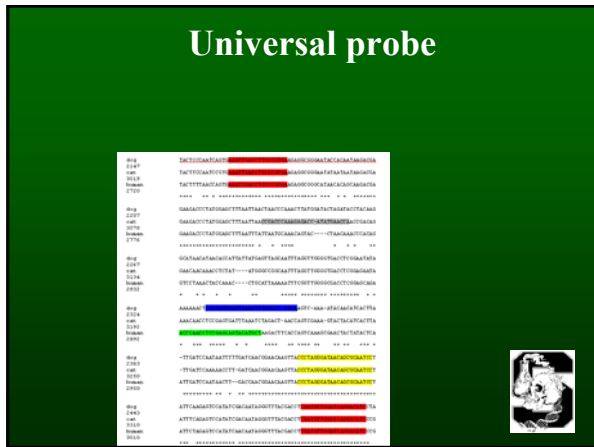
- 16569 bp in humans
- 15 genes
- 22 tRNAs

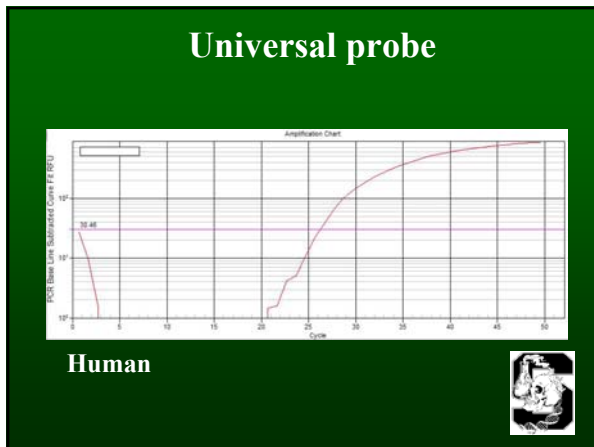


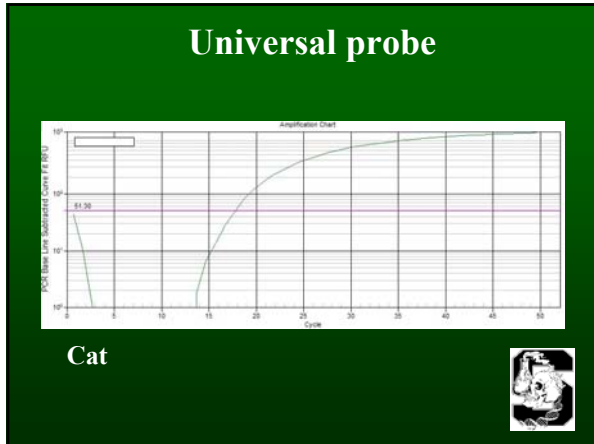
Mammalian mitochondrial DNA common conserved regions

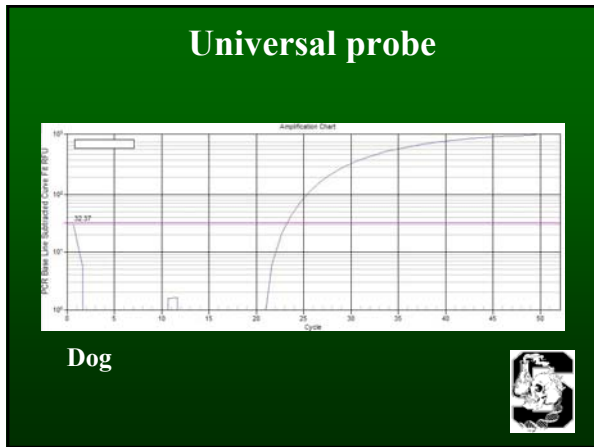


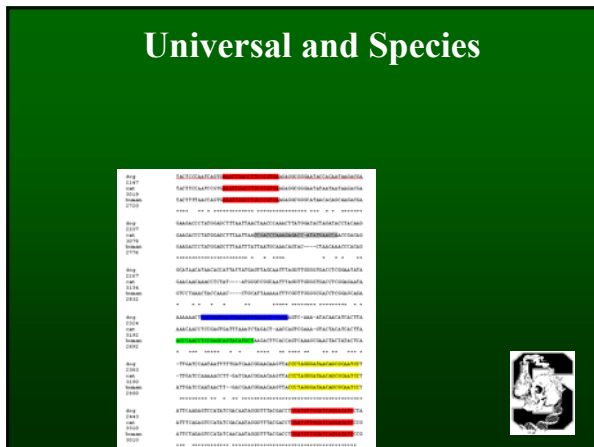




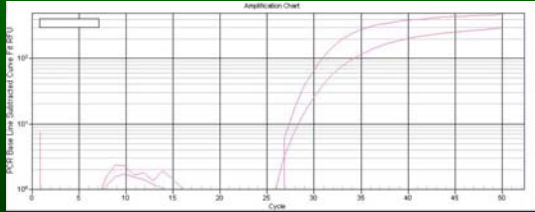








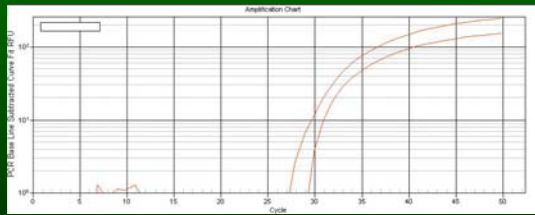
Universal and Species



Human



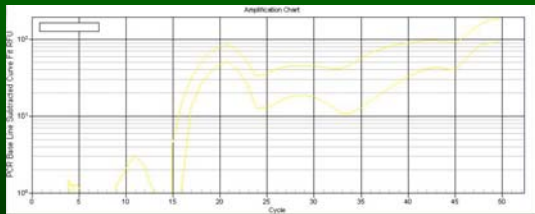
Universal and Species



Cat



Universal and Species



Dog



Cross Reactivity



Cross Reactivity No sign of crosstalk



Human DNA and Cat probe

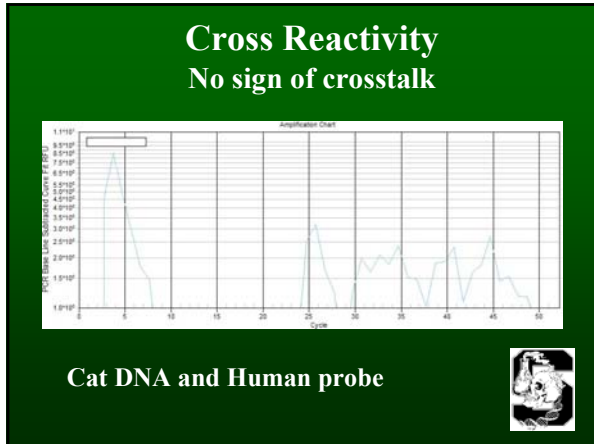


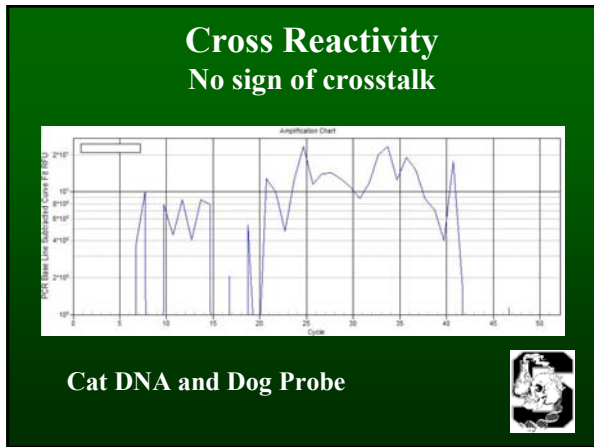
Cross Reactivity No sign of crosstalk

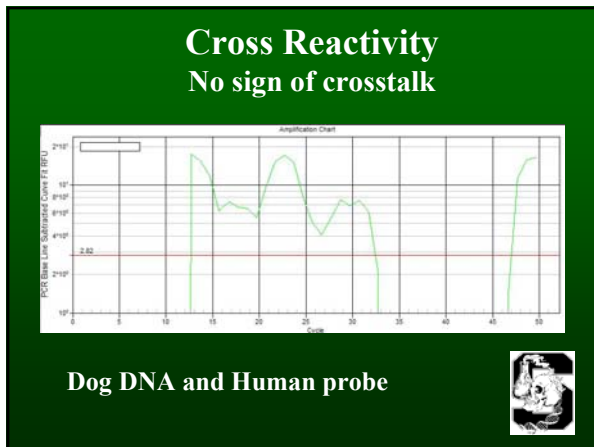


Human DNA and Dog probe










Cross Reactivity No sign of crosstalk

Amplification Chart

Log₁₀ (Fluorescence Intensity) (RFU)


Cycle

Dog DNA and Cat probe



Mixed Probes

Sequence alignment showing DNA sequences with highlighted regions in red, green, and blue, indicating cross-reactivity between probes.




Mixed Probes

Amplification Chart

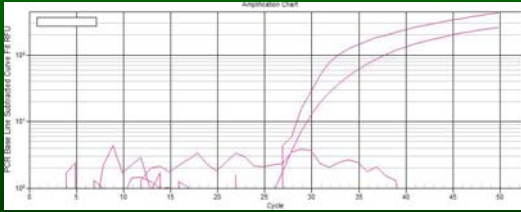
Log₁₀ (Fluorescence Intensity) (RFU)

Cycle

Human DNA—H + C probes



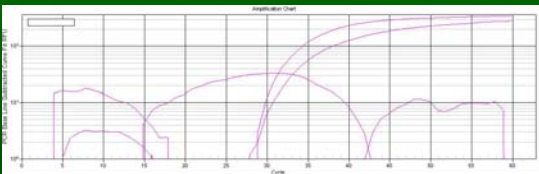
Mixed Probes



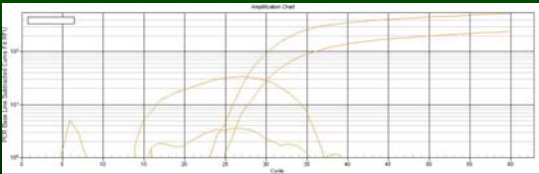
Human DNA—H + D + U probes



All Probes



Dog

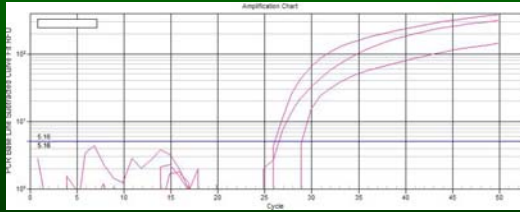


Human

DNA Mixtures

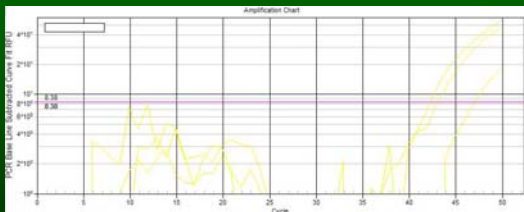


DNA Mixtures



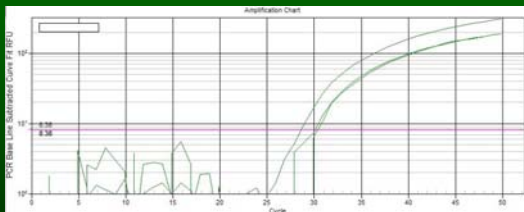
Human + Dog

DNA Mixtures



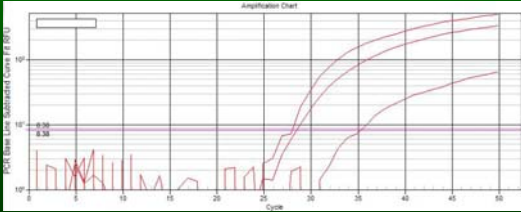
Human + Cat

DNA Mixtures



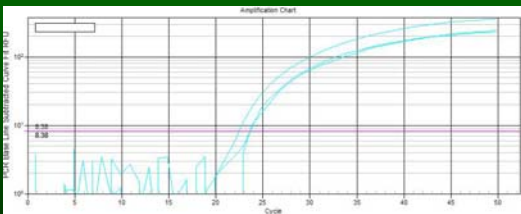
10 X Human + Cat

DNA Mixtures



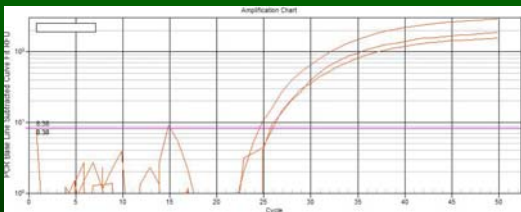
100 X Human + Cat

DNA Mixtures



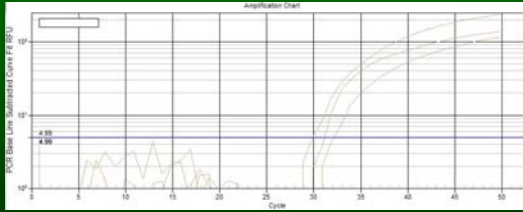
Human + 10 X Cat

DNA Mixtures



Human + 100 X Cat

DNA Mixtures



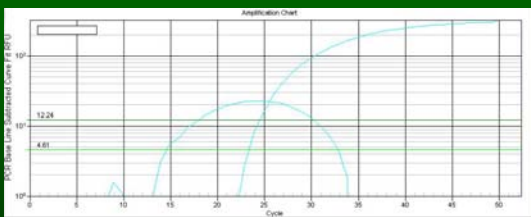
Cat + 100 X Dog

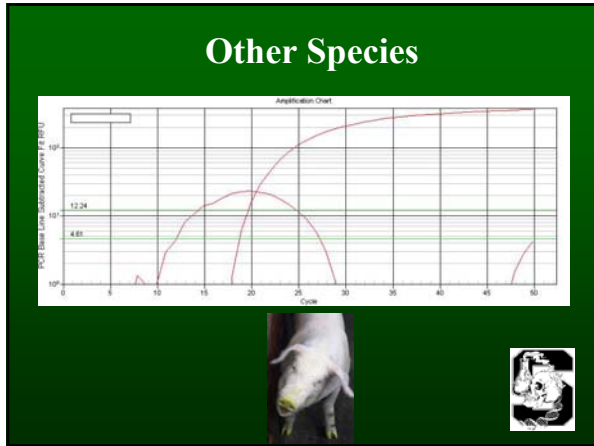


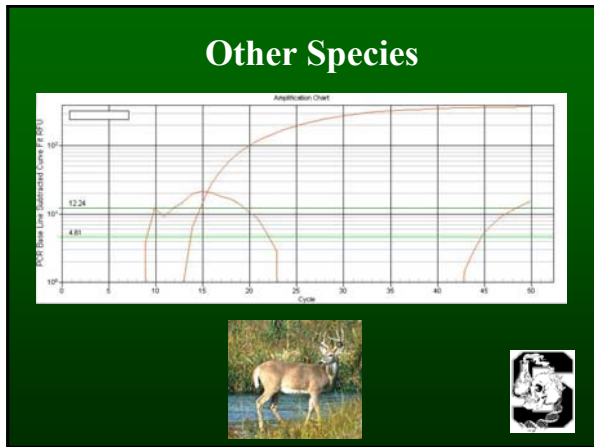
Non-target Species

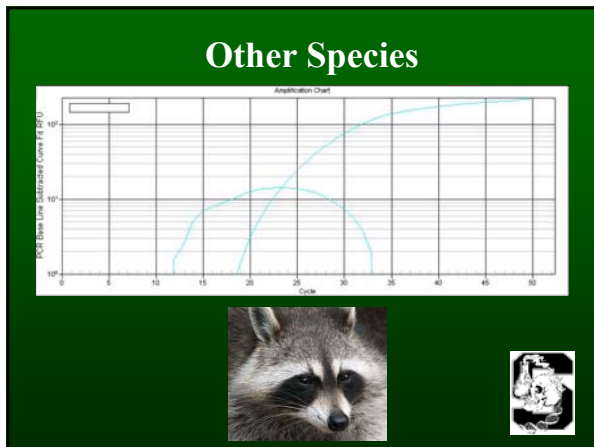


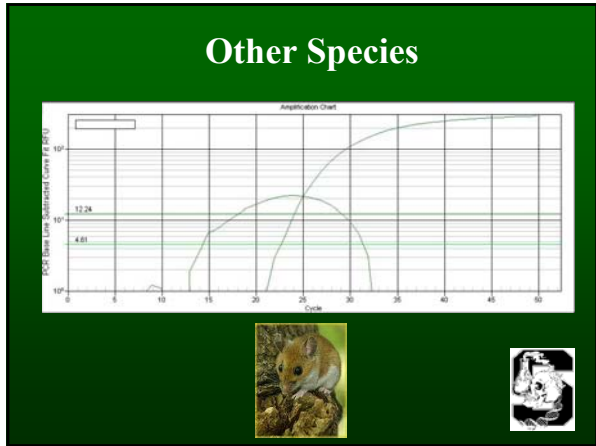
Other Species

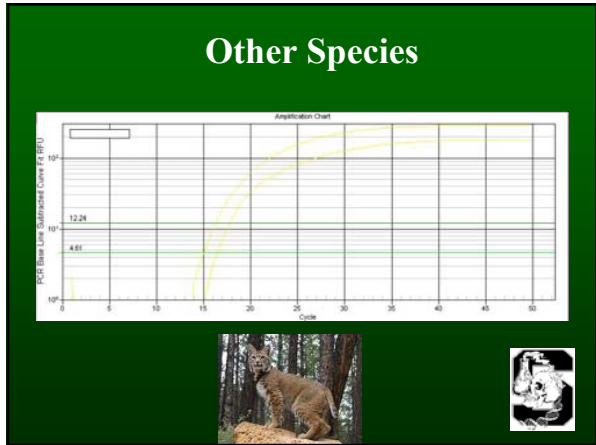


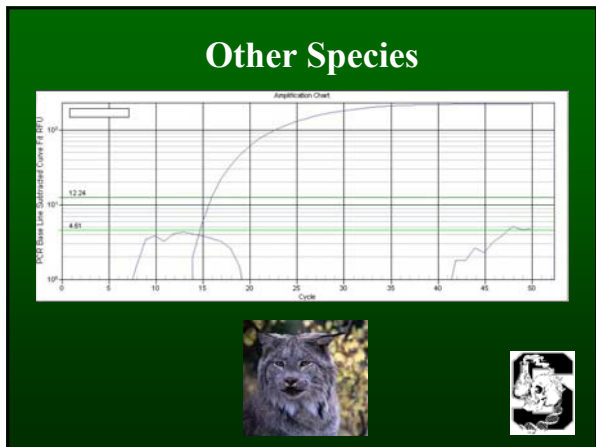












Previous Sexing Techniques



- Amelogenin
- SRY (Sex-determining Region Y)
- X or autosome



A More Sensitive Assay

- TaqMan Technology
- Y-chromosome repeat sequence (DYZ1)
- Autosomal repeat sequence (Alu)



Autosomal Repetitive Sequence (Nicklas and Buel, 2005)

- Alu
 - 280 bp
 - Most prevalent repeat
 - 10% of human genome
- Female/Positive Control



Y-Chromosome Repetitive Sequences

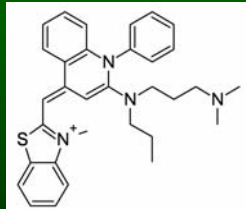
- **DYZ1**
 - Long arm of the Y-chromosome
 - 3.4 kb long
 - 2000 – 4000 copies



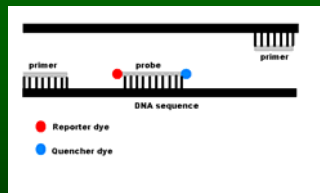
Y-Chromosome Specificity...

- **SYBR Green**

Some female samples amplified with the DYZ1 primers



TaqMan Assay



- Probe increases specificity
- 5' reporter dyes
 - DYZ1 → FAM
 - Alu → HEX
- 3' non-fluorescent quencher



Protocol

- 15µL reactions in triplicate
- Primer Concentrations
 - 500 nM Alu-F, DYZ1-F & R
 - 900 nM Alu-R
- Probe Concentrations
 - 250 nM Alu, DYZ1
- 2x iQ5 Supermix (Bio-Rad)
- 0.5µL BSA at 20 µg/µL
- 1µL DNA



Protocol

- Real-time Parameters
 - 94°C – 10 min
 - 50 Cycles
 - 95°C – 15 sec
 - 60°C – 1 min
- Assay performed on a iQTM5 Multicolor Real-Time Detection System and iCycler (Bio-Rad)
- Data analyzed using Bio-Rad iQ5 Standard Edition software



Control Assay

- Positive Female Control
 - High molecular weight female DNA (Promega)
 - Triplicates of 100pg and 7pg
- Positive Male Control
 - Single source high molecular weight male DNA
 - Triplicates of 100pg and 7pg



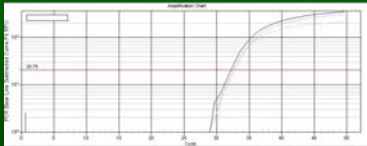
Low Quality DNA Samples

- Hair Shafts
 - Blind study
 - DNA previously extracted (Graffy 2003)
 - Roots not included
- Skeletal Remains
 - Voegtly Cemetery, Pittsburgh, Pennsylvania
 - 150 years old
 - Anthropological sex estimation

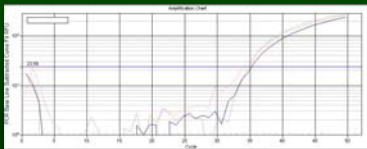


Results Hair Shafts - Male

Alu



DYZ1

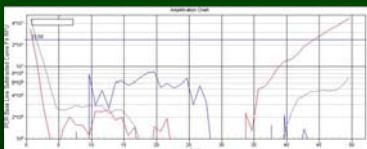


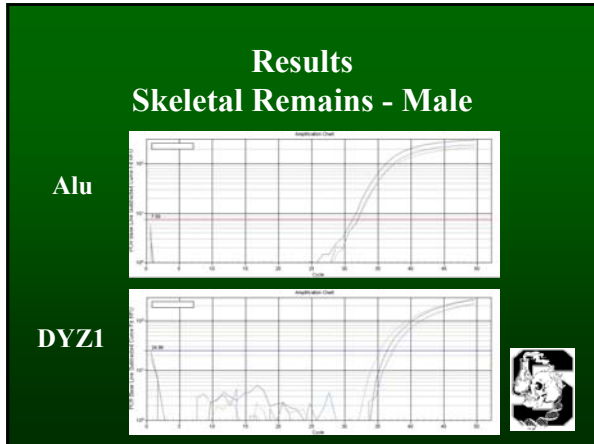
Results Hair Shafts - Female

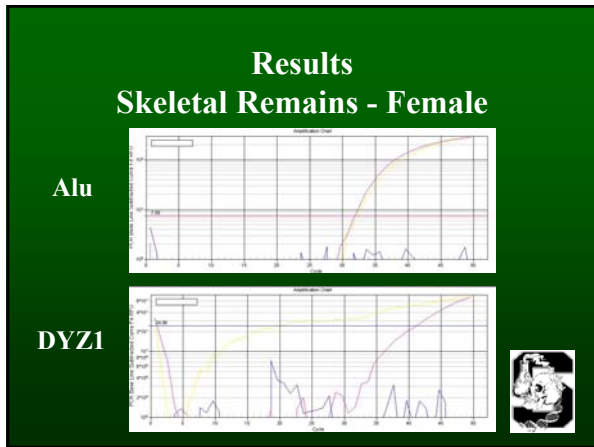
Alu



DYZ1








Some Results

- 10 hair shaft samples were tested blind
 - Sex was accurately determined in 8 samples
 - 2 samples did not amplify
- 6 skeletal remains samples were tested
 - Sex was determined from 5 samples
 - 1 sample did not amplify



Sensitivity

- Higher amounts of DNA (greater than 5pg)
 - Great amplification
- Low amounts of DNA
 - Observed stochastic effects with high molecular weight DNA for DYZ1
 - Better amplification of the DYZ1 locus with degraded DNA



Findings to Date

- Successful amplification of low quality DNA
- Sex determination possible for both hair shafts and skeletal remains
- Still to do
 - Sensitivity assays of degraded DNA
 - Other forensic samples



Acknowledgements

- Dr. Janice Nicklas of the Vermont Forensic Laboratory
- Elizabeth Graffy
- Lisa Misner
- Michigan State University Forensic Biology Laboratory
- Michigan State University Graduate School