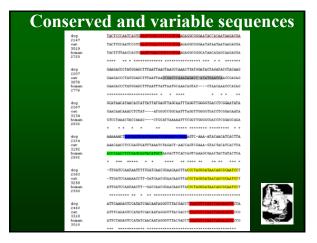
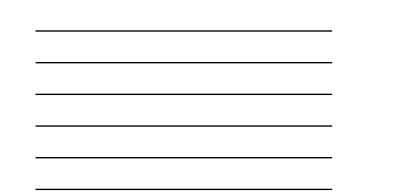
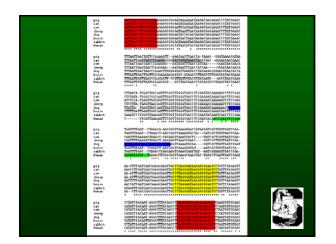


http://www.cstl.nist.gov/biotech/strbase/training/AAFS2008_qPCRworkshop.htm





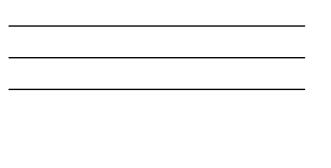


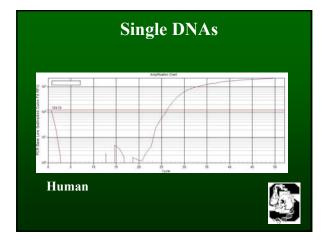


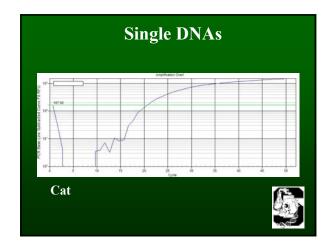
- Optimized
- Cycling
 - BioRad iQ5
 - 94°C: 15s, 54°C 60s
 - 50 cycles



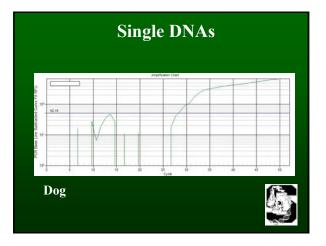
Singl	e DNAs and Th	eir Probe
40g 2147	TATTOCANTARTS	
- 040 90129 browson 2729	TRETEREARCOFFI DAAT DAEED DOOT DE ANALONG ANTALANDAGA TAGANGGA ANTALANDAGA TAGANG ANALONGA TAGANG ANALONGA TAGANG ANTALANDAGA ANTAL	
40-9 22년 20년 20년 20년 20년 20년 27년	ОМАКОЛОГИИ ОКЛОСТИЦИАТИЛАСТИКОССКАКТИИ ТОКИ КАТАКИЛАСТИСИМ ОМАКОЛОГИИ ОЧНАСТИ ТИЛИТИ ОБЛИСТИКАЛИКИМО ИЛИТИКАТАСКИ САЛА ОМАКОЛОГИИ ОЧНАСТИ ТИЛИТИ ОБЛИСТИКАЛИКИМО ИЛИТИКАТИКА ОМАКОЛОГИИ ОКЛОСТИКАТИ И ТИЛИ ОСЛАСКИ ОТЛИСТИКАТИ ОТЛИСТИКА И ПОЛИТИКАТИ ОКЛОСТИКАТИ И ТИЛИТИКАТИ ОТЛИСТИКАТИ ОТЛИСТИКАТИ И ПОЛИТИКАТИ ОТЛИСТИКАТИ И ТИЛИТИКАТИ ОТЛИСТИКАТИ ОТЛИСТИКАТИ И ПОЛИТИКАТИ ОТЛИСТИКАТИ И ТИЛИТИКАТИ ОТЛИСТИКАТИ ОТЛИСТИКАТИ ОТЛИСТИКАТИ И ПОЛИТИКАТИ ОТЛИСТИКАТИ И ТИЛИТИКАТИ ОТЛИСТИКАТИ ОТЛИСТИКАТИ ОТЛИСТИКАТИ ОТЛИСТИКАТИ ОТЛИСТИКАТИ ОТЛИСТИКАТИ И ПОЛИТИКАТИ ОТЛИСТИКАТИ ОТЛИСТИКА И ПОЛИТИКАТИ ОТЛИСТИКАТИ ОТЛИСТИ ОТЛИСТИКАТИ ОТЛИСТИКАТИ ОТЛИСТИКАТИ ОТЛИСТИКАТИ ОТЛИСТИКАТИ ОТЛИСТИКАТИ ОТЛИСТИКАТИ ОТЛИСТИКАТИ ОТИ ОТЛИСТИКАТИ ОТЛИСТИ ОТЛИСТИ ОТЛИСТИ ОТЛИСТИТИ ОТЛИСТИКАТИ ОТЛИСТИКАТИ ОТЛИСТИКАТИ ОТЛИСТИ ОТЛИСТИКАТИ ОТЛИСТИКАТИ ОТЛИСТИКАТИ ОТЛИСТИКИ ОТЛИСТИКИ ОТЛИСТИ ОТЛИСТИ ОТЛИСТИ ОТЛИСТИ ОТЛИСТИТИ ОТЛИСТИИ ОТЛИСТИИ ОТЛИСТИ ОТЛИСТИ ОТЛИСТИ ОТЛИСТИ ОТЛИСТИ ОТЛИ ОТЛИСТИ ОТЛИ ОТЛИСТИ ОТЛИ ОТЛИСТИ ОТЛИ	
40-9 2249 0334 20324 2032	OCATAMENTALEACEDCENTERTERIGHTERGATTERGITTERGENTINGCOTERCICCOGRAFIATA GRACAMENAACEDCECTATREFORCEORCANTERGETTERGENTRACETCOGRAFIATA GTECTAMENTERCEMENTCTORETERBANETTERGETTERGENTRACETCOGRAFIATA	
40-# 2124 	AAAAAACT	
dang 2.545 2.64 2.50 Jonesen 2.800	- TT GA TO AN AN AT T THAT DAA DOMAIDANT A BY TANGA TANAN WORKAN T - TT GA TO ANALOT TT THAT DAA DOMAIDANT A BY TANGA TANAN WORKAN T - TT GA TO ANALOT T- GA TO ANALONI TA BY TANGA TANAN MORE AND TO AN AT T GA TO ANALOT TO AND TANGA T AND TANGA TANAN MORE AND TANK	
4+9 2+43 444 3340 3340 5460	ATTCAMARCAINTGEANTEGEATERS AND AND THE GALL AND	



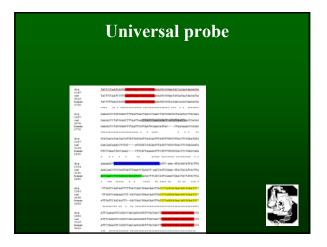




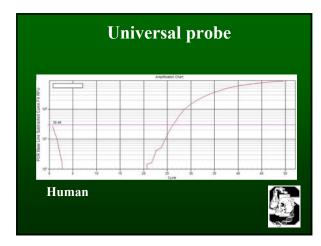


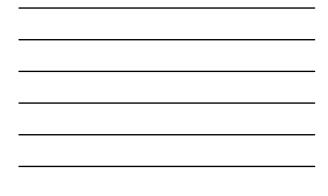


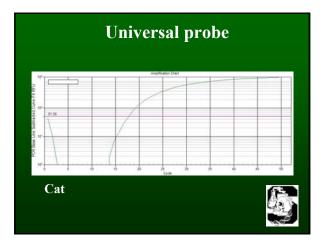


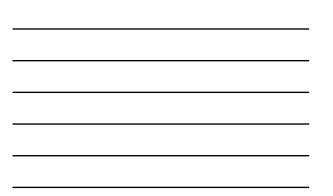


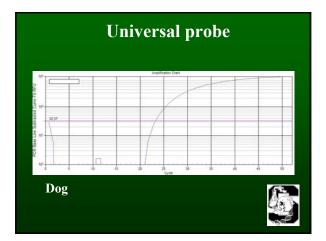




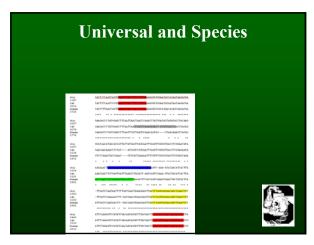


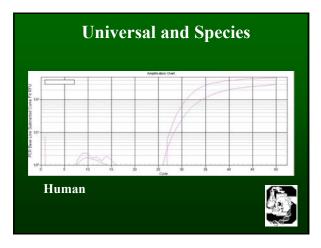




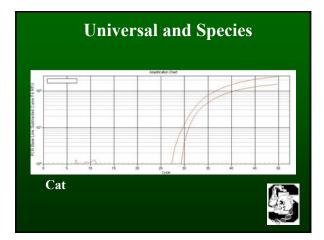




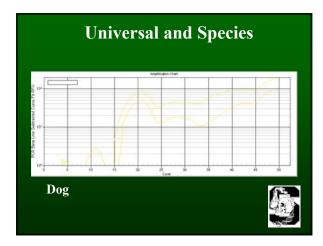


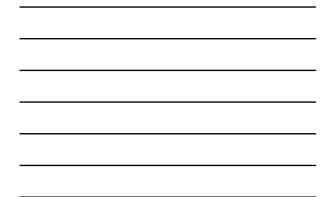


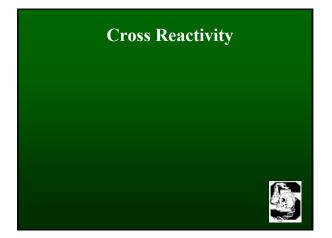


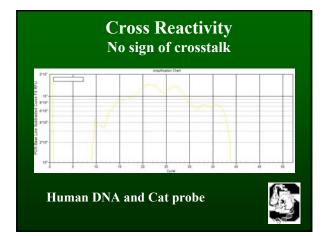


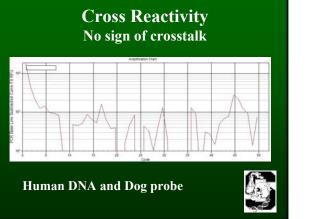


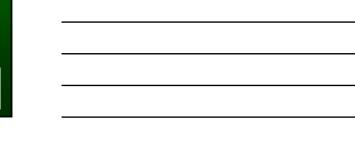


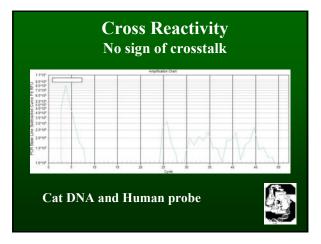




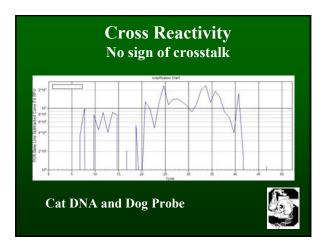




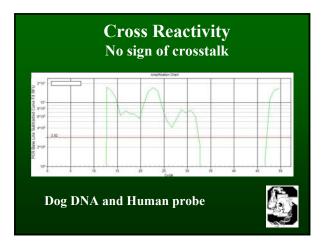


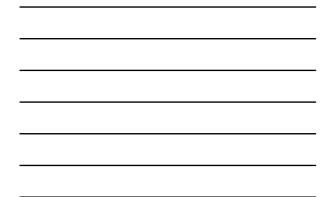


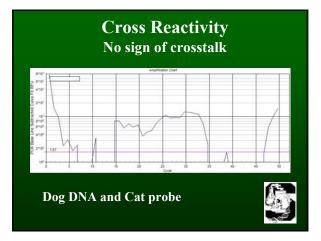


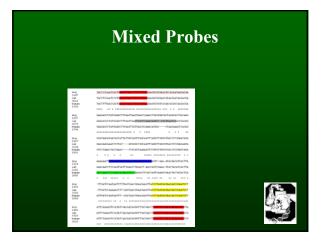




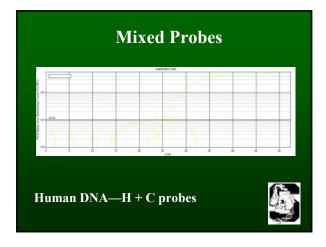




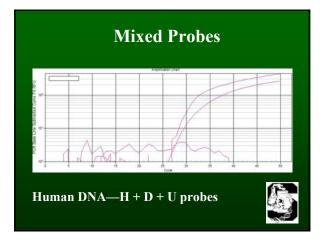




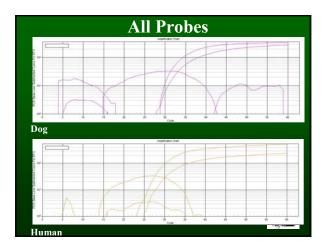




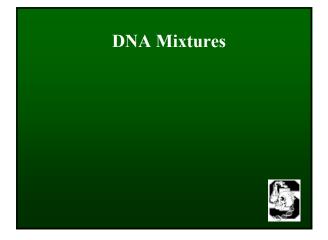


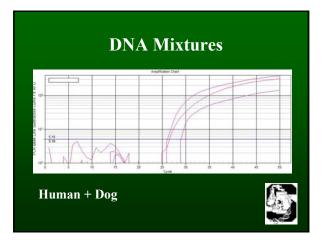




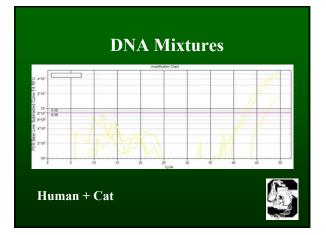




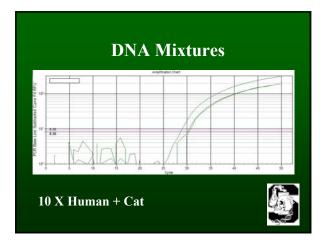


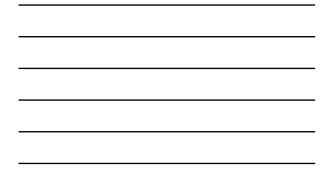


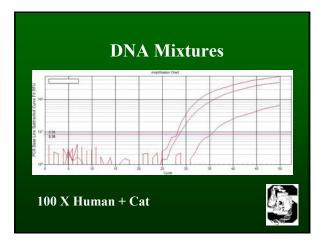


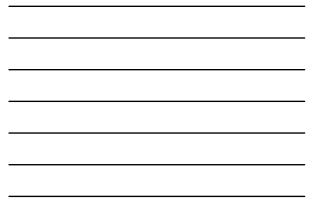


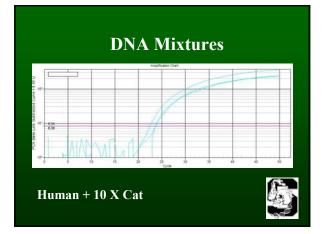




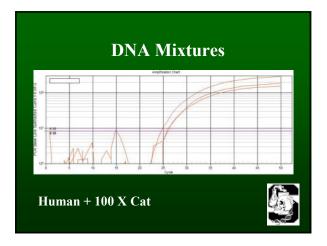


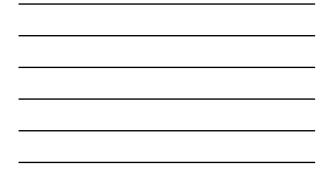




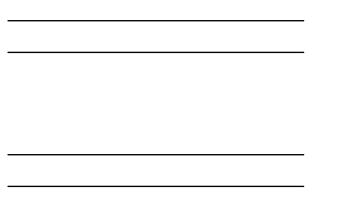




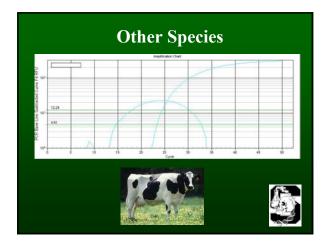




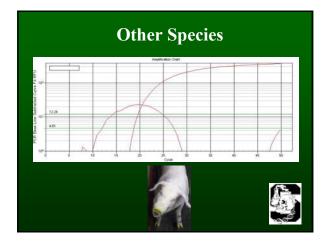
		E.	1	
	11	/		
	11			
VYCA	 1			



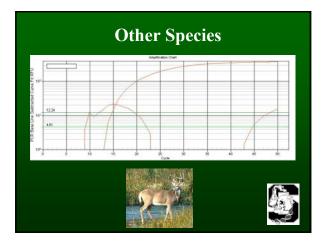




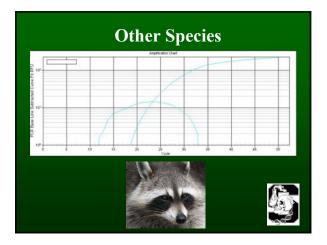




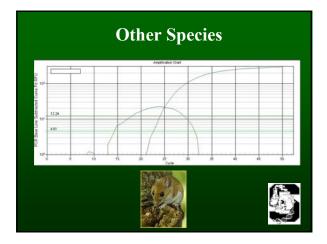




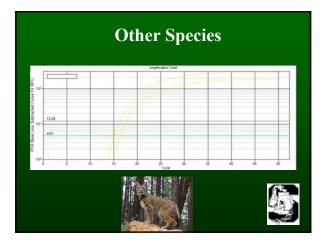




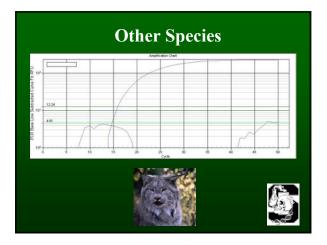




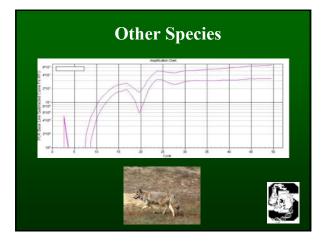


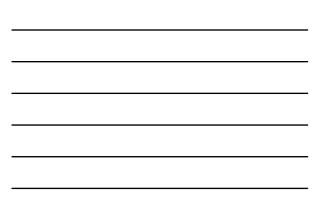


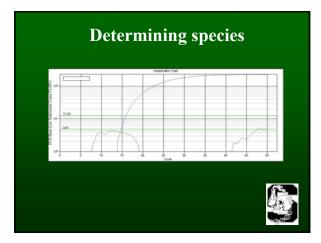




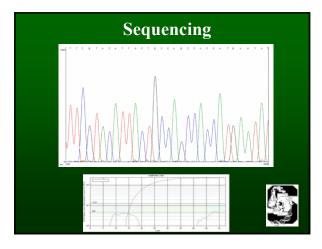










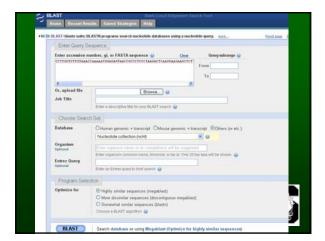




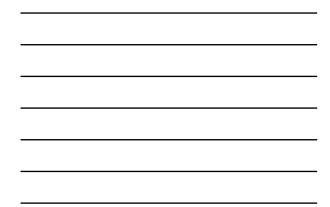
BLAST Searches Basic Local Alignment Search Tool

http://www.ncbi.nlm.nih.gov/blast/Blast.cgi





BLAST Searches						
Accession	Description	Man	Total	Query	- value	
00410435.1	Camie familiaria isolate 1 breed Cocker Spaniel mituchondrico, complete genome	\$90	590	100%	14-165	100
Av056755.1	Canis familiaris isolate 1 breed Sapsaree mitochondrise, complete penome	590	590	100%	14-105	100
81655747.1	Caris familiaria isolate 1 breed Welsh Springer Spariel mitochandrion, complete genor	590	590	100%	1e-165	100
296439.2	Canis familiarie mitochondrion, complete genome	390	590	100%	1+-165	100
A1705522.1	Canis familiaris issiate H48 sytochrome b gene, partial cds; tRNA-Thr and tRNA-Pro gen	130	590	200%	14-165	100
AY705405.1	Canis familiaria issilate HLO ovtochrome b cene, partial ods: ISNA-Thr and IRNA-Pro per	130	590	100%	14-165	100
AV729600.1	Caris familiaris mitochondrian, complete genome	5.54	584	100%	68-164	991
AV656752.1	Canis familiaris isstate 2 breed bish Setter mitochondrion, complete gamime	524	584	100%	64-164	99
87655744.1	Carva familiaria issilate 1 breed English Springer Spaniel mitschondrion, complete genos	584	584	100%	64-264	- 99
AY656747.3	Canis familiaris isslate 1 breed Old English Sheepdrog mitochondrion, complete genome	554	5.84	200%	64-164	- 99
AY7068221	Canis familiaria instate H47 cytochrome b gene, partial ode; IRNA-Thr and IRNA-Pro gei	554	5.84	100%	64-164	0.991
AV706510.1	Canis familiaris isslate KIS cytochrome b gene, partial ofs; tRNA-Thr and tRNA-Pro ger	204	504	100%	64-264	997
AY706409.3	Canis familiaris isolate H14 sytochrome b gene, partial cds; IRNA-Thr and IRNA-Pro ger	504	684	100%	64-164	991
AY706483 1	Canis familiaris isolate H08 cytochrome b gene, partial cds; tRNA-Thr and tRNA-Pro ger	584	584	100%	de-164	991
AV706482.1	Canis familiaris isolate H07 cytochrome b gene, partial ods; IRNA-Thr and IRNA-Pro ger	584	554	100%	6e-164	991
00480496.1	Canis familiaris isolate 1 breed Irish Soft Coated Wheaten Terrier mitochondrion, corro	579	578	100%	3e-162	991
Ave54754.1	Canis familiaria isolate 1 breed Chinese Crested mitochondrion, complete genome	579	579	100%	3e-162	997
Av706518.1	Canis familiaris isolate HA3 cytochrome b gene, partial ods; tRNA-Thr and tRNA-Pro ger	573	579	100%	3e-162	991
A1704503.X	Carvis familiaria isolate H28 cytochrome b gene, partial odi; tRNA-Thr and tRNA-Pro gei	979-	579	200%	3e-162	991
81706495.1	Canis familiaria issiate H20 cytochrome b gene, partial cds; tRNA-Thr and tRNA-Pro ger	529	579	100%	34-162	991
00480498.3	Canis familiaris isolate 1 breed Siberian Musky mitochondrion, complete genome	572	572	100%	Le-160	99
DOMBORRS 3	Canix familiarie isslate 1 breed Hiniature Schnauzer mitschondrion, complete genome	3.7.2	572	1.00%	1e-160	99
0.0480437.3	Canis familieris isslate 1 breed west Highland White Terrier mitochondrion, complete p	3.7.2	\$72	100%	14-160	99
2.26908900	Canis familiaris isolate 1 bread trish Setter mitochondrion, complete genome	522	572	200%	1e-160	.99
A10656751.1	Canis familiaris isolate 1 breed Gordon Setter mitochondrion, complete genome	522	573	300%	1e-160	.99
aye54750.1	Canis familiaris isolate 1 breed Leonberger mitochondrion, complete genome	172	575	100%	1#-100	99
1,987.62674	Canis familiaris isolate 2 breed Saint Bernard mitochondrion, complete genome	522	\$72	100%	1e-160	- 99
AY656748.1	Canis familiaris isolate 1 breed Airedale Terrier mitochondrisn, complete genome	172	872	100%	1e-160	.99
8X65674A.1	Canis familiaris isolate 1 breed Standard Schnauzer mitochondrion, complete genome	572	\$73	100%	14-160	.99
A1655741.1	Caris familiaris isolate 1 breed Irish Setter mitochundrion, complete genome	\$2.2	573	300%	1e-160	.99
81655738.1	Canis familiaris isolate 1 breed Jack Russell Terrier mitochondrion, complete genome.	57.2	573	100%	14-160	- 99
81706511.1	Carris familiaria isolate HD6 cytochrome b gene, partial cds: \$NA-Thr and \$NA-Pro gei	573	62.5	100%	1e-160	1.99



	BLAST Searches
AF064567.1	Canis familiaris breed shepherd 16S ribosomal RNA gene, mitochondrial gene for mitoch
AM711902.1	Canis lupus lupus complete mitochondrial genome
DQ334813.1	Canis lupus voucher UAM 19690 16S ribosomal RNA gene, partial sequence; mitochondr
DQ334812.1	Canis latrans voucher UAM 19791 16S ribosomal RNA gene, partial sequence; mitochon-
DQ480511.1	Canis latrans isolate 2 from Colorado mitochondrion, complete genome
DQ480510.1	Canis latrans isolate 1 from Colorado mitochondrion, complete genome
DQ480509.1	Canis latrans isolate 1 from Nebraska mitochondrion, complete genome
DQ480508.1	Canis lupus isolate 1 from Canada mitochondrion, complete genome
DQ480507.1	Canis lupus isolate 2 from Saudi Arabia mitochondrion, complete genome
DQ480506.1	Canis lupus isolate 1 from Saudi Arabia mitochondrion, complete genome
DQ480505.1	Canis lupus isolate 1 from Spain mitochondrion, complete genome
DQ480504.1	Canis lupus isolate 1 from Sweden mitochondrion, complete genome
DQ480503.1	Canis lupus isolate 1 from Russia mitochondrion, complete genome
DQ480502.1	Canis familiaris isolate 2 breed Jamthund mitochondrion, complete genome
DQ480501.1	Canis familiaris isolate 1 breed Swedish Elkhound mitochondrion, complete genome
DQ480500.1	Canis familiaris isolate 1 breed Shetland Sheepdog mitochondrion, complete genome
DQ480494.1	Canis familiaris isolate 1 breed Poodle mitochondrion, complete genome
DQ480493.1	Canis familiaris isolate 1 breed Black Russian Terrier mitochondrion, complete genome
DQ480492.1	Canis familiaris isolate 1 breed Jamthund mitochondrion, complete genome
DQ480490.1	Canis familiaris isolate 1 breed Flat Coated Retriever mitochondrion, complete genome
DQ480489.1	Canis familiaris isolate 1 breed German Shepherd mitochondrion, complete genome
AY289970.1	Canis aureus isolate Jackal-AP2 16S ribosomal RNA gene, partial sequence; mitochondr
AY289969.1	Canis aureus isolate Jackal-AP1 16S ribosomal RNA gene, partial sequence; mitochondr
AY289968.1	Canis indica isolate GW-Gujarat 16S ribosomal RNA gene, partial sequence; mitochondr
AY289967.1	Canis indica isolate GW-AMM 16S ribosomal RNA gene, partial sequence; mitochondrial
AY289966.1	Canis indica isolate GW-AP3 16S ribosomal RNA gene, partial sequence; mitochondrial
AY289965.1	Canis indica isolate GW-AP2 16S ribosomal RNA gene, partial sequence; mitochondrial
AX2000064-1	Copic indica isolate CW, AB1 165 vibesomal BNA gape, partial sequences mitochendrial

Species ID and Casework

- Blood at scenes
- Endangered species products
- Hairs associated with victims or suspects
- Can combine with nuclear markers
- •



A Highly Sensitive Sex Determination Assay For Low Quality DNA





Brianne Kiley, Carrie Jackson, David Foran Forensic Biology Laboratory Michigan State University



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

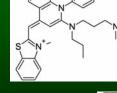
• DYZ1

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



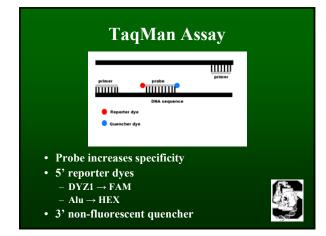
Y-Chromosome Specificity...

• SYBR Green



Some female samples amplified with the DYZ1 primers





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



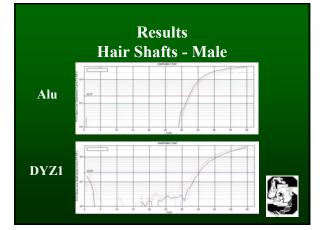
Low Quality DNA Samples

- Hair Shafts
 - Blind study
 - DNA previously extracted (Graffy 2003)
 Roots not included
- Skeletal Remains

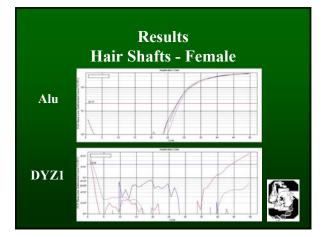
 - Voegtly Cemetery, Pittsburgh, Pennsylvania
 - 150 years old

 - Anthropological sex estimation

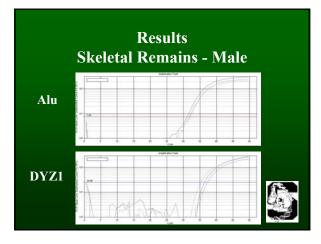




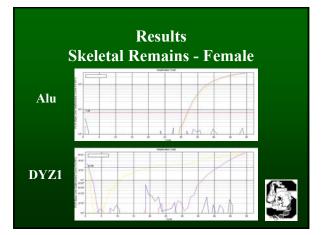












Some Results

- 10 hair shaft samples were tested blind
 - Sex was accurately determined in 8 samples2 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 amplificationLow 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

