

Wildlife Genetics Proficiency Testing Program –Test # 022112

Consensus Report

Test Start Date -02/21/2012

Test Due Date -04/27/2012

This document reports the results of the Wildlife Genetics Proficiency Testing Program. The National Fish and Wildlife Forensic Laboratory was the duty Lab and was responsible for sample preparation, sample verification, distribution, and gathering and reporting the results.

The results are self explanatory and are divided into three sections:

1. Results of Test for Species Origin
2. Results of Determination of Gender Origin
3. Results of Individual Identification

Each section contains the following:

1. The species source that you identified for Items 1, 2 and 3.
2. The methods used to make these identifications.

Scenario

A Wildlife Agent is investigating a poaching incident involving North American elk. The suspect claims the meat in his freezer is coming from one North American elk. All three tissue samples were recovered from the suspect's freezer.

The Agent requests that the species and gender origins of all submitted evidence be determined. He is also interested in knowing whether the three submitted evidence items are from the same individual animal. It is not known where the poaching incident occurred.

Items Submitted

Item 1: Tissue from suspect's freezer.

Item 2: Tissue from suspect's freezer.

Item 3: Tissue from suspect's freezer.

Wildlife Genetics Proficiency Testing Program Answers:

	Item 1	Item 2	Item 3
Species Origin	American Black Bear (<i>Ursus americanus</i>)	Elk (<i>Cervus elaphus</i>)	Elk (<i>Cervus elaphus</i>)
Gender Origin	Male	Male	Male
Accession No.	QA3G21-QA3G50	QA1D26-QA1D70	QA1D26-QA1D70
Provider	USDA/APHIS-WS	Wyoming Game and Fish	Wyoming Game and Fish
Original ID	ID#24063 Sixes Unit, OR	Q11, E668 Gold Stnd#4, HA23	Q11, E668 Gold Stnd#4, HA23

Items 2 and 3 are from the same individual

The results of pre-distribution testing confirmed the expected results.

I) Compilation of Species Origin Results

1 Species Source

Lab	Item 1	Item 2	Item 3
I3K48M-1	<i>Ursus americanus</i>	<i>Cervus elaphus</i>	<i>Cervus elaphus</i>
I3K48M-2	<i>Ursus americanus</i>	<i>Cervus elaphus</i>	<i>Cervus elaphus</i>
I3K48M-3	<i>Ursus americanus</i>	<i>Cervus elaphus</i>	<i>Cervus elaphus</i>
M3B22N	<i>Ursus americanus</i>	<i>Cervus elaphus</i>	<i>Cervus elaphus</i>
J4L18F	American Black Bear	North American Elk	North American Elk
B5H06W	American Black Bear (<i>Ursus americanus</i>)	N. American Elk (<i>Cervus elaphus</i>)	N. American Elk (<i>Cervus elaphus</i>)
B7H20L	N. American Black Bear	N. American Elk	N. American Elk
D6S24F	<i>Ursus americanus</i>	<i>Cervus elaphus</i>	<i>Cervus elaphus</i>
S2F23G	North American Black Bear (<i>U. americanus</i>)	North American Elk (<i>Cervus elaphus</i>)	North American Elk (<i>Cervus elaphus</i>)
R2J94A-1	Black Bear (<i>Ursus americanus</i>)	Elk (<i>Cervus elaphus</i>)	Elk (<i>Cervus elaphus</i>)
R2J94A-2	Black Bear (<i>Ursus americanus</i>)	Elk (<i>Cervus elaphus</i>)	Elk (<i>Cervus elaphus</i>)
R2J94A-3	Black Bear	Elk	Elk
B1V83W	North American Black Bear	North American Elk	North American Elk
P2W87T-1 P2W87T-2	<i>Ursus americanus</i> (American Black Bear)	<i>Cervus elaphus</i> (Elk)	<i>Cervus elaphus</i> (Elk)
K2R46H	Black Bear (<i>Ursus americanus</i>)	Elk (<i>Cervus elaphus</i>)	Elk (<i>Cervus elaphus</i>)
D3H13G-1 D3H13G-2 D3H13G-3	Black Bear	Elk	Elk
M1S68R	Black Bear (<i>Ursus americanus</i>)	Elk (<i>Cervus elaphus</i>)	Elk (<i>Cervus elaphus</i>)
B4W11V-1 B4W11V-2	<i>Ursus americanus</i>	<i>Cervus elaphus</i>	<i>Cervus elaphus</i>
B4W11V-1 B4W11V-3	<i>Ursus americanus</i>	<i>Cervus elaphus</i>	<i>Cervus elaphus</i>
J2R15F	<i>Ursus americanus</i>	<i>Cervus elaphus</i>	<i>Cervus elaphus</i>
K1W95S-1 K1W95S-3 K1W95S-4 K1W95S-5	American Black Bear (<i>Ursus americanus</i>)	Elk (<i>Cervus elaphus</i>)	Elk (<i>Cervus elaphus</i>)
K1W95S-2 K1W95S-3 K1W95S-4 K1W95S-5	Black Bear (<i>Ursus americanus</i>)	Elk (<i>Cervus elaphus</i>)	Elk (<i>Cervus elaphus</i>)
R4R65C-1 R4R65C-2 R4R65C-3 R4R65C-4	<i>Ursus americanus</i> (Black Bear)	Inconclusive	Inconclusive
C3F54C-1	<i>Ursus americanus</i>	<i>Cervus elaphus</i>	<i>Cervus elaphus</i>
C3F54C-2	North American Black Bear (<i>Ursus americanus</i>)	North American Elk (<i>Cervus canadensis</i>)	North American Elk (<i>Cervus canadensis</i>)
B4C27D	<i>Ursus americanus</i>	<i>Cervus elaphus</i>	<i>Cervus elaphus</i>
R9H57A	<i>Ursus americanus</i>	<i>Cervus elaphus</i>	<i>Cervus elaphus</i>

2 Methods Used

Lab	Methods/ Genetic Marker(s)
I3K48M-1	DNA Sequence Analysis/ d-loop mtDNA
I3K48M-2	DNA Sequence Analysis/ mtDNA d-loop
I3K48M-3	DNA Sequence Analysis/ mtDNA partial d-loop
M3B22N	DNA Sequence Analysis/ Seq analysis of portion of Cyt b
J4L18F	DNA Sequence Analysis/ Portion of cyt-b gene
B5H06W	DNA Sequence Analysis/ portion of the Cyt B gene
B7H20L	DNA Sequence Analysis/ Portion of cytochrome b gene
D6S24F	DNA Sequence Analysis/ Seq analysis of a portion of cytochrome b
S2F23G	DNA Sequence Analysis/ TP-DNA503
R2J94A-1	DNA Sequence Analysis STR Analysis
R2J94A-2	DNA Sequence Analysis/ PCR, CE STR Analysis/ PCR, Capillary electrophoresis
R2J94A-3	DNA Sequence Analysis STR Analysis
B1V83W	Immunodiffusion/ Deer and Bear Antisera Isoelectric Focusing/ PGI Staining
P2W87T-1 P2W87T-2	DNA Sequence Analysis/ mtDNA Cytochrome B
K2R46H	Immunodiffusion/ Ouchterlony – Cervid & Ursid α – serum Isozyme Analysis/ PGI, SOD Isoelectric Focusing STR Analysis
D3H13G-1 D3H13G-2 D3H13G-3	Isoelectric Focusing/ Phosphoglucose Isomerase and Albumin Counter Immunoelectrophoresis
M1S68R	Immunodiffusion/ Ouchterlony - Deer anti-sera and Bear anti-sera Isoelectric Focusing/ PGI (IEF3-9); EAP(IEF5-8)
B4W11V-1 B4W11V-2	DNA Sequence Analysis/ Cytochrome b sequencing
B4W11V-1 B4W11V-3	DNA Sequence Analysis/ Sequencing of Cytochrome b
J2R15F	Immunodiffusion/ Ouchterlony (Anti-Cervid; Anti-Bear) Isozyme Analysis/ *Please note our PhastSystem is broken and out for repair, unable to complete normal PGI & EAP analysis STR Analysis/ CDFG Elk Panels
K1W95S-1 K1W95S-3 K1W95S-4 K1W95S-5	DNA Sequence Analysis/ 12s rRNA
K1W95S-2 K1W95S-3 K1W95S-4 K1W95S-5	DNA Sequence Analysis/ 12s rRNA mtDNA sequencing
R4R65C-1 R4R65C-2 R4R65C-3 R4R65C-4	DNA Sequence Analysis/ Cytochrome b
C3F54C-1	DNA Sequence Analysis/ Cytochrome b
C3F54C-2	DNA Sequence Analysis/ Cytochrome b

B4C27D	DNA Sequence Analysis/ Cyt B + COI PCR followed by Sequencing STR Analysis/ Multiplex PCR with STR analysis
R9H57A	DNA Sequence Analysis/ Cyt B and COI

II) Compilation of Gender Origin Results

1 Gender Origin

Lab	Item 1	Item 2	Item 3
I3K48M-1	Male	Male	Male
I3K48M-2	Male	Male	Male
I3K48M-3	Male	Male	Male
M3B22N	Male	Male	Male
J4L18F	Male	Male	Male
B5H06W	Male	Male	Male
B7H20L	Male	Male	Male
D6S24F	Male	Male	Male
S2F23G	Male	Male	Male
R2J94A-1	Male	Not done	Not done
R2J94A-2	Male	Not tested	Not tested
R2J94A-3	Male	Not done, no validated test for elk sex typing in our lab	Not done, no validated test for elk sex typing in our lab
B1V83W	Male	Male	Male
P2W87T-1	Male	Male	Male
P2W87T-2			
K2R46H	Male	Male	Male
D3H13G-1	Male	Male	Male
D3H13G-2			
D3H13G-3			
M1S68R	Male	Male	Male
B4W11V-1	Male	Male	Male
B4W11V-2			
B4W11V-1	Male	Male	Male
B4W11V-3			
J2R15F	Male	Male	Male
K1W95S-1	Male	Male	Male
K1W95S-3			
K1W95S-4			
K1W95S-5			
K1W95S-2	Male	Male	Male
K1W95S-3			
K1W95S-4			
K1W95S-5			
R4R65C-1	Male	Male	Male
R4R65C-2			
R4R65C-3			
R4R65C-4			
C3F54C-1	Male	Male	Male
C3F54C-2	Male	Male	Male
B4C27D	Male	Male	Male
R9H57A	NA we don't have the markers for this sp.	Male	Male

2 Methods Used

Lab	Methods/ Genetic Marker(s)
I3K48M-1	PCR ZF/X/Y amplimers
I3K48M-2	PCR ZF/XY amplimers
I3K48M-3	PCR ZF/XY
M3B22N	PCR amplification of portions of ZFX/Y & SRY genes followed by gel electrophoresis
J4L18F	X and Y linked markers
B5H06W	PCR amplification and gel electrophoresis of a portion of the ZFX/Y and SRY genes linked to the X & Y chromosomes of mammals. Also, as part of the individual identification analysis, Items 2 and 3 were analyzed for the presence or absence of a portion of the SRY gene.
B7H20L	PCR amplification of X. and Y. linked genetic markers (ZFX/Y & SRY) Capillary electrophoresis & visualize results
D6S24F	PCR amplification of portions of ZFX/Y + SRY genes followed by gel electrophoresis
S2F23G	PCR amplification of a segment of the last exon of ZFX/Y as control and the HMG segment of match specific SRY gene as male identifier
R2J94A-1	PCR and CE of amelogenin
R2J94A-2	Amelogenin – PCR and CE
R2J94A-3	STR – Bovine amelogenin
B1V83W	PCR amplification of portions of the ZFX/Y and SRY genes linked to the X and Y chromosomes
P2W87T-1 P2W87T-2	We identified the gender of samples labeled Item 1, Item 2 and Item 3 through amplification with two primers sets: the first specific to the ZFX/ ZFY genes located on the X- and Y-chromosomes respectively (Fain and Lemay 1995) and the second pair targets the <i>sry</i> gene located on the Y chromosome of mammals (Aasen and Medrano 1990, Wasser et al.1997). Post amplification electrophoresis was performed on a 1.5% agarose gel, stained with ethidium bromide and visualized with UV light. The ZFX/ZFY primers are included to ensure amplification success of females lacking the <i>sry</i> gene. The ZFX/ZFY and the <i>sry</i> fragments were co-amplified. The mammalian female would exhibit one band (ZFX/ZFY) during post-amplification electrophoresis, while a mammalian male would exhibit two bands (ZFX/ZFY and <i>sry</i> gene).
K2R46H	PCR amplification/analysis of ZFX/ ZFY, SRY and Amelogen through capillary electrophoresis
D3H13G-1 D3H13G-2 D3H13G-3	Amplification of the zinc finger protein of the X- chromosome and the testes determining factor of the Y- chromosome (if present) using PCR
M1S68R	PCR amplification and analysis of ZFX and ZFY using PAGE
B4W11V-1 B4W11V-2	Bear - Amelogenin, capillary electrophoresis using dye-labeled primers Elk - SRY, capillary electrophoresis using dye-labeled primers
B4W11V-1 B4W11V-3	Amelogenin: Capillary electrophoresis using dye-labeled primers SRY: Capillary electrophoresis using dye-labeled primers
J2R15F	ZFX/ SRY PCR gender typing
K1W95S-1 K1W95S-3 K1W95S-4 K1W95S-5	SRY - sex- determining region Y chromosome ZF - zinc finger (X chromosome control)
K1W95S-2 K1W95S-3 K1W95S-4 K1W95S-5	SRY - sex- determining region Y chromosome ZF - zinc finger (X chromosome control)

R4R65C-1 R4R65C-2 R4R65C-3 R4R65C-4	USFW method 'Polymerase Chain Reaction (PCR) Gender Typing of Mammals' NFWFL DNA-016 ver. 01-09-2009 Detection of ZFX/ZFY and SRY genes
C3F54C-1	PCR amplification of ZFX on the X-chromosome and SRY on the Y-chromosome
C3F54C-2	PCR on the ZFX on the X-chromosome & SRY on the Y-chromosome
B4C27D	PCR fluorescent fragment analysis ZFX/ZFY genes + SRY gene
R9H57A	Amplification of the amelogenin gene and visualisation on a 2% Agarose gel. Primer sets K41/K42 (Yamauchi, Hori et al 2000) SE47/SE48 (Pfeiffer & Brening, 2005)

III) Compilation of Individual Identification Results

Lab	Individual typing is not performed on the following species identified in this proficiency test	1) What could be the minimum number of animals represented in these 3 samples?	2) Which samples have the same genetic profile?
I3K48M-1	-	2	Item 2 & Item 3
I3K48M-2	-	2	Items 2 + 3
I3K48M-3	-	2	Items 2 & 3
M3B22N	<i>Ursus americanus</i> & <i>Cervus elaphus</i> ; not routinely tested by this analyst	2 – different species	NA
J4L18F	1, 2, 3	2	-
B5H06W	Am. Black Bear	2	Item 2 and Item 3
B7H20L	Black Bear, Elk	-	-
D6S24F	<i>Ursus americanus</i> + <i>Cervus elaphus</i> are not routinely tested by this analyst	2 different species	NA
S2F23G	√	-	-
R2J94A-1	Black Bear	2	Item 2 and Item 3
R2J94A-2	Black Bear	2	Item 2 and Item 3
R2J94A-3	Black Bear	2	Item #2 and Item #3
B1V83W	Bear, Elk	2	NA
P2W87T-1 P2W87T-2	Item 1 <i>Ursus americanus</i>	2	Items 2 and 3
K2R46H	Item 1 (Black Bear) <i>Ursus americanus</i>	2	Item 2 and Item 3
D3H13G-1 D3H13G-2 D3H13G-3	-	2	Items #2 and #3
M1S68R	-	2	Item 2 and Item 3
B4W11V-1 B4W11V-2	-	2	Items 2 and 3
B4W11V-1 B4W11V-3	-	2	Items 2 & 3
J2R15F	-	2	Item #2 and Item #3
K1W95S-1 K1W95S-3 K1W95S-4 K1W95S-5	Item 1: This item was the only evidence sample identified as Black Bear	2	Items #2 and #3 have the same genetic profile
K1W95S-2 K1W95S-3 K1W95S-4 K1W95S-5	Item 1 – identified as Black Bear (<i>Ursus americanus</i>)	2	Item 2 and Item 3 were both identified as Elk (<i>Cervus elaphus</i>) and had identical STR genotypes
R4R65C-1 R4R65C-2 R4R65C-3 R4R65C-4	Black Bear + Item 2/ Item 3	-	-
C3F54C-1	<i>Ursus americanus</i>	2	Items 2 and 3
C3F54C-2	<i>Ursus americanus</i>	2	Sample #2 and Sample #3
B4C27D	-	2	Item 2 + Item 3
R9H57A	<i>Ursus americanus</i>	2	Item 2 and 3

3 Methods Used

Lab	Methods/ Genetic Marker(s)
I3K48M-1	STR Analysis/ CERVID Loci BM4513, BM1225, RT24, D, N
I3K48M-2	STR Analysis/ RT7, BM1225, RT24, D, N
I3K48M-3	STR Analysis/ RT7, RT24, BM1225, OVD, OVN
M3B22N	NA
J4L18F	-
B5H06W	STR Analysis/ BL42, BMC1009, BM203, BM4208, BM4107, BM888, BM5004
B7H20L	-
D6S24F	NA
S2F23G	-
R2J94A-1	STR Analysis/ Elk MPX1: BM4028, BM4107, BM203, BM4513, BM888, BL42, OvirH, INRA107 Elk MPX2: BM6506, Rt13, CSSM041, Rt1, BM3507, BM1225, BM848
R2J94A-2	STR Analysis/ BM4208, BM4107, BM203, BM4513, BM888, BL42, OvirH, INRA107, BMC1009, BM6506, Rt13, CSSM041, Rt1, BM3507, BM1225, BM848
R2J94A-3	STR Analysis/ BM4028, BM4107, BM203, BM4513, BM888, BL42, OvirH, INRA107, BMC1009, BM6506, Rt13, CSSM041, Rt41, BM3507, BM1225, BM848
B1V83W	NA
P2W87T-1 P2W87T-2	STR Analysis @ 10 markers/ BM415, BM5004, AF102246, BM4208, BM1009, BM4513, BM1225, IGF, BM848, AF102257
K2R46H	STR Analysis/ Elk STR panel: BM203, BL42, BMC1009, BM5004, BM4208, BM4107, BM888, Amelo
D3H13G-1 D3H13G-2 D3H13G-3	STR Analysis/ VH110, BM888, BM4513, BM1225, RM006, INRA040, BM4208, BMC1009, RT1 and BOVRBP
M1S68R	STR Analysis/ Protocol DNA020B/ BL42, BMC1009, BM203, BM4208, BM4107, BM888, BM5004
B4W11V-1 B4W11V-2	STR Analysis/ Elk: BL42, BM203, BM4107, BM6506, BM888, BMC1009, CELB9, CELJP23, FCB193, FCB5, CELJP15, GNZ204, IRBP, OARCP26, T108b, T26, TGLA94 Bear: G10B, G10C, G10H, G10J, G10L, G10O, G10P, G10X, G1A, G1D, UarMu50, UarMU59
B4W11V-1 B4W11V-3	STR Analysis/ Elk: BL42, BM203, BM4107, BM6506, BM888, BMC1009, CELB9, CELJP23, FCB193, FCB5, CELJP15, GNZ204, IRBP, OARCP26, T108b, T26, TGLA94 Bear: G10B, G10C, G10H, G10J, G10L, G10O, G10P, G10X, G1A, G1D, UarMu50, UarMU59
J2R15F	STR Analysis/ CDFG Elk STR panels (16 loci)
K1W95S-1 K1W95S-3 K1W95S-4 K1W95S-5	STR Analysis/WDFW Elk STR microsatellite panel/ BM1225, BM4107, BM4208, BM4513, BM5004, BM888, BMC1009, ETH152, RT7
K1W95S-2 K1W95S-3 K1W95S-4 K1W95S-5	STR Analysis/WDFW Elk STR microsatellite panel/ BM1225, BM4107, BM4208, BM4513, BM5004, BM888, BMC1009, ETH152, RT7
R4R65C-1 R4R65C-2 R4R65C-3 R4R65C-4	-

C3F54C-1	STR Analysis/ BM4208, BM1225, VH110, ETH152, BMC1009, BM4513, RT13, BL42, BM203, BM4107, RT1
C3F54C-2	STR Analysis/ BM4208, BM1225, VH110, ETH152, BMC1009, BM4513, RT13, BL42, BM4107, BM203, RT1
B4C27D	STR Analysis/ 18 STR Marker Panel: Cervid 1, BM6506, BM6438, BL25, INRA001, OARFCB193, RT5, RT7, RT13, BL42, D, K, L, N, O, P, Q, S Sequencing/ Cyt B, COI could not exclude
R9H57A	STR Analysis/ BM1706, RT13, HUI1177, RT1, OarFCB5, BMC1009, RT7, T26, BM757, T156, BM188, OarFCB304