Wildlife Genetics Proficiency Testing Program –Test # 022013

Consensus Report 05/03/2013

Test Start Date -02/20/2013
Test Due Date -04/26/2013

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 mule deer. The suspect claims the meat in his freezer is coming from one mule deer. 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	Mule Deer *(Odocoileus hemionus)*	White-tailed Deer (*Odocoileus virginianus)*	White-tailed Deer (*Odocoileus virginianus)*
Gender Origin	Male	Female	Female
Accession No.	QA2L56-QA2M24	QA2P73-QA3A41	QA2P73-QA3A41
Provider	Wyoming Game and Fish	Idaho Fish and Game	Idaho Fish and Game
Original ID	STK02, HA81/82 Platte River Wilderness Area	GMU32/39, Hwy55 Gardena and Banks	GMU32/39, Hwy55 Gardena and Banks

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	*Odocoileus hemionus*	*Odocoileus virginianus*	*Odocoileus virginianus*
I3K48M-2	*Odocoileus hemionus*	*Odocoileus virginianus*	*Odocoileus virginianus*
M3B22N	*Odocoileus hemionus* Mule Deer	*Odocoileus virginianus* WTD	*Odocoileus virginianus* WTD
J4L18F	Mule Deer	White-tailed Deer	White-tailed Deer
B5H06W	*Odocoileus hemionus* Mule Deer	*Odocoileus virginianus* White-tailed Deer	*Odocoileus virginianus* White-tailed Deer
B7H20L	*Odocoileus sp.*	*Odocoileus sp.*	*Odocoileus sp.*
D6S24F	*Odocoileus hemionus*	*Odocoileus virginianus*	*Odocoileus virginianus*
S2F23G	*Odocoileus sp.*	*Odocoileus sp.*	*Odocoileus sp.*
R2J94A-1	*Odocoileus sp.* White-tail vs Mule deer not determined due to lack of geographic information	*Odocoileus sp.* White-tail vs Mule deer not determined due to lack of geographic information	*Odocoileus sp.* White-tail vs Mule deer not determined due to lack of geographic information
R2J94A-2	*Odocoileus sp.* Note: Unable to differenciate between White-tailed deer and Mule deer due to lack of geographical information	*Odocoileus sp.* Note: Unable to differenciate between White-tailed deer and Mule deer due to lack of geographical information	*Odocoileus sp.* Note: Unable to differenciate between White-tailed deer and Mule deer due to lack of geographical information
R2J94A-3	*Odocoileus sp.* Cannot differentiate between White-tailed + Mule deer due to lack of geographic information	*Odocoileus sp.* Cannot differentiate between White-tailed + Mule deer due to lack of geographic information	*Odocoileus sp.* Cannot differentiate between White-tailed + Mule deer due to lack of geographic information
P2W87T-1	*Odocoileus hemionus* Mule Deer	*Odocoileus virginianus* White-tailed Deer	*Odocoileus virginianus* White-tailed Deer
P2W87T-2	*Odocoileus hemionus hemionus* Mule Deer	*Odocoileus virginianus* White-tailed Deer	*Odocoileus virginianus* White-tailed Deer
K2R46H	*Odocoileus hemionus* Mule Deer	*Odocoileus virginianus* White-tailed Deer	*Odocoileus virginianus* White-tailed Deer
D3H13G-1	*Odocoileus hemionus* Mule Deer	*Odocoileus virginianus* WTD	*Odocoileus virginianus* WTD
D3H13G-2	*Odocoileus hemionus* Mule Deer	*Odocoileus virginianus* WTD	*Odocoileus virginianus* WTD
D3H13G-3	*Odocoileus hemionus* Mule Deer	*Odocoileus virginianus* WTD	*Odocoileus virginianus* WTD
C3F65S	*Odocoileus hemionus*	*Odocoileus virginianus*	*Odocoileus virginianus*
M1S68R	*Odocoileus hemionus*	*Odocoileus virginianus*	*Odocoileus virginianus*

	Mule Deer	White-tailed Deer	White-tailed Deer
B4W11V-1	*Odocoileus hemionus*	*Odocoileus virginianus*	*Odocoileus virginianus*
B4W11V-2	*Odocoileus hemionus*	*Odocoileus virginianus*	*Odocoileus virginianus*
B4W11V-3	*Odocoileus hemionus*	*Odocoileus virginianus*	*Odocoileus virginianus*
J2R15F-1	*Odocoileus hemionus* Mule Deer	*Odocoileus virginianus* White-tailed Deer	*Odocoileus virginianus* White-tailed Deer
J2R15F-2	*Odocoileus hemionus* Mule Deer	*Odocoileus virginianus* White-tailed Deer	*Odocoileus virginianus* White-tailed Deer
K1W95S-1 K1W95S-3 K1W95S-4	*Odocoileus sp.* (most likely *O. hemionus* Mule Deer)	*Odocoileus sp.* (most likely *O. virginianus* White-tailed Deer)* *Based on WDFW's Washington baseline	*Odocoileus sp.* (most likely *O. virginianus* White-tailed Deer)* *Based on WDFW's Washington baseline
K1W95S-2 K1W95S-3 K1W95S-4	*Odocoileus spp.* Deer	*Odocoileus spp.* Deer	*Odocoileus spp.* Deer
R4R65C-1 R4R65C-2	*Odocoileus sp.*	*Odocoileus sp.*	*Odocoileus sp.*
C3F54C	Inconclusive* failure to amplify some of the markers used to sort Mule deer from White-tail using structure analysis *We do not guarantee our customers the ability to discern White-tail deer from Mule deer, this is clearly stated on our Fee schedule form	White-tailed deer	White-tailed deer
B4C27D	*Odocoileus virginianus* or *hemionus*	*Odocoileus virginianus*	*Odocoileus virginianus*
R9H57A	*Odocoileus hemionus*	*Odocoileus sp.* Inconsistent results in NCBI-Blast: come as *O.virginianus* and *O. hemionus*	*Odocoileus sp.* Inconsistent results in NCBI-Blast: come as *O.virginianus* and *O. hemionus*
J3V67H	*Odocoileus hemionus*	*Odocoileus virginianus*	*Odocoileus virginianus*

2 Methods Used

Lab	Methods/ Genetic Marker(s)
I3K48M-1	DNA Sequence Analysis/ D-loop Hemopexin-5
I3K48M-2	DNA Sequence Analysis/ mtDNA d-loop, Bexon 4 (serum albumin)
M3B22N	DNA Sequence Analysis/ Cyt b sequence STR Analysis/ Cervid STR panel
J4L18F	DNA Sequence Analysis/ Cyt-b seq analysis STR Analysis/ Cervid STR panel
B5H06W	DNA Sequence Analysis/ Portion of the Cyt B/Central Region genes STR Analysis/8 STR loci – Deer STR Panel
B7H20L	DNA Sequence Analysis
D6S24F	DNA Sequence Analysis/ Cytochrome b STR Analysis/ Cervid panel
S2F23G	DNA Sequence Analysis/ mtDNA cytochrome b, 5' end
R2J94A-1	DNA Sequence Analysis/ Fragment of mitochondrial 16S ribosomal RNA gene
R2J94A-2	DNA Sequence Analysis/ Fragment of mitochondrial 16S ribosomal RNA gene
R2J94A-3	DNA Sequence Analysis/ segment of mitochondrial 16S ribosomal RNA gene
P2W87T-1	DNA Sequence Analysis/ Analysis of cytochrome b region
P2W87T-2	DNA Sequence Analysis/ Analysis of cytochrome B region of mtDNA
K2R46H	Immunodiffusion/ Ouchterlony – (Cervid, Bovine & Ursid) Isozyme Analysis/ PGI, SOD & EAP Isoelectric Focusing
D3H13G-1	Isoelectric Focusing/ Phosphoglucose Isomerase and Albumin Counter Immunoelectrophoresis
D3H13G-2	Isoelectric Focusing/ Phosphoglucose Isomerase and Albumin Counter Immunoelectrophoresis
D3H13G-3	Isoelectric Focusing/ Phosphoglucose Isomerase and Albumin Counter Immunoelectrophoresis
C3F65S	Immunodiffusion/ Counter immunoelectrophoresis Isoelectric Focusing/ Phosphoglucose Isomerase Other/ Albumin Western blot
M1S68R	Immunodiffusion/ Ouchterlony - Deer anti-serum Isoelectric Focusing/ PGI (IEF3-9); EAP (IEF5-8)
B4W11V-1	DNA Sequence Analysis/ Cytochrome b STR Analysis/ FCB193
B4W11V-2	DNA Sequence Analysis/ Cytochrome b STR Analysis/ FCB193
B4W11V-3	DNA Sequence Analysis/ Cytochrome b STR Analysis/ FCB193
J2R15F-1	Immunodiffusion/ Ouchterlony (Anti-Cervid) Isozyme Analysis/ PGI & EAP with Phast System
J2R15F-2	Immunodiffusion/ Ouchterlony using Cervid antiserum Isoelectric Focusing/ PGI & EAP with Phast System

K1W95S-1 K1W95S-3 K1W95S-4	DNA Sequence Analysis/ 12s rRNA
K1W95S-2 K1W95S-3 K1W95S-4	DNA Sequence Analysis/ 12s rRNA mtDNA sequencing
R4R65C-1 R4R65C-2	DNA Sequence Analysis/ Cytochrome B
C3F54C	DNA Sequence Analysis/ Cytochrome b Structure Analysis A data set using the genotypes of 50 Mule deer & 50 White-tail deer was used to run a structure analysis.
B4C27D	DNA Sequence Analysis/ COI and Cyt B – Big Dye Terminator v.3.1 STR Analysis/ Custom 19 marker panel
R9H57A	DNA Sequence Analysis/ Cyt b - Sanger Sequencing
J3V67H	DNA Sequence Analysis/ cytochrome – B, D-loop

II) Compilation of Gender Origin Results

1 Gender Origin

Lab	Item 1	Item 2	Item 3
I3K48M-1	Male	Female	Female
I3K48M-2	Male	Female	Female
M3B22N	Male	Female	Female
J4L18F	Male	Female	Female
B5H06W	Male	Female	Female
B7H20L	Male	Female	Female
D6S24F	Male	Female	Female
S2F23G	Male	Female	Female
R2J94A-1	Test not performed because it is not validated for casework	Test not performed because it is not validated for casework	Test not performed because it is not validated for casework
R2J94A-2	This test was not performed because it is not validated for case work	This test was not performed because it is not validated for case work	This test was not performed because it is not validated for case work
R2J94A-3	not performed because no validated sex-typing test for deer in lab	not performed because no validated sex-typing test for deer in lab	not performed because no validated sex-typing test for deer in lab
P2W87T-1	Male	Female	Female
P2W87T-2	Male	Female	Female
K2R46H	Male	Female	Female
D3H13G-1	Male	Female	Female
D3H13G-2	Male	Female	Female
D3H13G-3	Male	Female	Female
C3F65S	Male	Female	Female
M1S68R	Male	Female	Female
B4W11V-1	Male	Female	Female
B4W11V-2	Male	Female	Female
B4W11V-3	Male	Female	Female
J2R15F-1	Male	Female	Female
J2R15F-2	Male	Female	Female
K1W95S-1 K1W95S-3 K1W95S-4	Male	Female	Female
K1W95S-2 K1W95S-3 K1W95S-4	Male	Female	Female
R4R65C-1 R4R65C-2	Male	Female	Female
C3F54C	Male	Female	Female
B4C27D	Male	Female	Female

R9H57A	Male	Female	Female
J3V67H	Male	Female	Female

2 Methods Used

Lab	Methods/ Genetic Marker(s)
I3K48M-1	PCR ZF/XY amplimers
I3K48M-2	PCR ZF/XY amplimers
M3B22N	PCR amplification of portions of the SRY locus, visualization by capillary electrophoresis & laser detection of male band
J4L18F	PCR of SRY region
B5H06W	Fragment analysis/ C. Electrophoresis testing for the presence or absence of a portion of the SRY gene linked to the male sex chromosome of mammals.
B7H20L	PCR amplification of sex-linked DNA markers
D6S24F	PCR amplification of a portion of the SRY gene
S2F23G	PCR amplification of diagnostic mammalian sex chromosome markers SRY (HMG region); ZFX (Last exon, 5' end).
R2J94A-1	-
R2J94A-2	-
R2J94A-3	N/A
P2W87T-1	Amplified nuclear DNA (Zfx/Zfy genes on X & Y chromosomes) and ran product on 1.5% Agarose gel.
P2W87T-2	PCR amplification of nuclear DNA using 2 sets of primers – one specific to region on Y chromosome + the other specific to region on X chromosome (3C & 3D, Zfx & Zfy). The amplified product was run on a gel to determine the gender (2 bands=male, 1 band=female).
K2R46H	PCR amplification/analysis of the ZFX/ ZFY control region and SRY region through capillary electrophoresis fragment analysis
D3H13G-1	Amplification of the zinc finger protein of the X- chromosome and the testes determining factor of the Y- chromosome (if present) using PCR
D3H13G-2	Amplification of the zinc finger protein of the X- chromosome and the testes determining factor of the Y- chromosome (if present) using PCR
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
C3F65S	Amplification of the Zfx region on the X-chromosome and the SRY region of the Y-chromosome
M1S68R	PCR amplification of SRY gene visualized using capillary electrophoresis
B4W11V-1	Amplification of SRY and CELB9 using dye-labeled primers
B4W11V-2	Amplification of SRY and CELB9 using dye-labeled primers
B4W11V-3	Amplification of SRY and CELB9 using dye-labeled primers
J2R15F-1	ZFX/ SRY PCR gender typing
J2R15F-2	PCR-based typing using ZFx & Sry genetic markers
K1W95S-1 K1W95S-3 K1W95S-4	SRY - sex- determining region Y chromosome ZF - zinc finger (X chromosome control)

K1W95S-2 K1W95S-3 K1W95S-4	SRY - sex- determining region Y chromosome ZF - zinc finger (X chromosome control)
R4R65C-1 R4R65C-2	USFW method 'PCR Gender Typing of Mammals' NFWFL DNA-016 ver. 01-09-2009 Detection of ZFX/ZFY and SRY genes
C3F54C	PCR amplification of ZFX on the X chromosome & SRY on the Y chromosome
B4C27D	PCR fragment analysis markers Sry & Zfx
R9H57A	PCR amplification of amelogenin and agarose gel electrophoresis diagnosis using two set of primers: K41/K42 - Yamauchi et al.(2000) SE47/SE48 – Ennis and Gallagher (1994)
J3V67H	PCR and gel electrophoresis using primer set CerSRY and primer set CerzFXY

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	Item 2 & Item 3
M3B22N	-	2	Item 2 and Item 3
J4L18F	-	2	Items 2 & 3
B5H06W	-	2	Item 2 & Item 3
B7H20L	-	-	-
D6S24F	-	2	PT-2 & PT-3
S2F23G	-	-	-
R2J94A-1	-	Two (2)	Item 2 and Item 3
R2J94A-2	-	Two (2)	Item 2 and Item 3
R2J94A-3	none	2	Item 2 and Item 3
P2W87T-1	N/A	2	Sample 2 & Sample 3 (Based on 8 microsatellite loci, Sample 2 & Sample 3 cannot be excluded as originating from the same animal).
P2W87T-2	-	-	-
K2R46H	none	2	Item 2 and Item 3
D3H13G-1	-	2	Items #2 and 3
D3H13G-2	-	2	Items #2 and 3
D3H13G-3	-	2	Items #2 and 3
C3F65S	*Odocoileus hemionus*	2	Items 2 and 3
M1S68R	-	2	Item 2 and Item 3
B4W11V-1	-	2	Items 2 & 3
B4W11V-2	-	2	Items 2 & 3
B4W11V-3	-	2	Items 2 & 3
J2R15F-1	*Odocoileus virginianus* White-tailed Deer	-	-
J2R15F-2	*Odocoileus virginianus* White-tailed Deer	-	-
K1W95S-1 K1W95S-3 K1W95S-4	-	2	Items #2 and #3 have the same genetic profile
K1W95S-2 K1W95S-3 K1W95S-4	-	2	Items 2 and 3 were both identified as female deer (*Odocoileus spp.*) and had identical STR genotypes.

R4R65C-1 R4R65C-2	Item 1, 2 and 3	-	-
C3F54C	-	2	Sample #2 & Sample #3
B4C27D	-	2	Item 2 and Item 3
R9H57A	-	2	Item 2 and 3
J3V67H	-	-	-

3 Methods Used

Lab	Methods/ Genetic Marker(s)
I3K48M-1	STR Analysis/ Cervid Loci (microsat) BM4513, BM1225, RT24, D, N
I3K48M-2	STR Analysis/ RT9, BM1225, RT24, D, N
M3B22N	STR Analysis/ Cervid 1, BM1225, T159S, RT24, BM4208, T7, BM4107, RT7, SRY
J4L18F	STR Analysis/ CBR1, BM1225, BM4207, RT24, BM4208, T7, T159S, RT7
B5H06W	STR Analysis/ 8 STR loci; Cervid 1, BM1225, BM4107, RT24, BM4208, T7, T159S, RT7
B7H20L	-
D6S24F	STR Analysis/ Cervid 1, BM1225, BM4107, RT24, BM4208, T7, T159S, RT7+SRY
S2F23G	-
R2J94A-1	STR Analysis/ Deer Multiplex 1-BM4107, T7, OvirA, Rt30, Rt7 Deer Multiplex 2-Rt5, BM1225, OheN, BM4208, OheQ
R2J94A-2	STR Analysis/ Multiplex 1-BM4107, T7, OvirA, Rt30, Rt7 Multiplex 2-Rt5, BM1225, OheN, BM4208, OheQ
R2J94A-3	STR Analysis/ BM4107, T7, OvirA, Rt30, Rt7, Rt5, BM1225, OheN, BM4208, OheQ
P2W87T-1	STR Analysis / MAP2C, BM1225, RT9, RT24, IGF, BM4208, FCB193, RT30
P2W87T-2	-
K2R46H	STR Analysis/ CDFG's Suite of 8 nuclear loci: P, M, Q, D, N, K, R, O
D3H13G-1	STR Analysis/ RT1, RT5, INRA040, RT13, RT24, RT30, CERV1, ETH152, BM1225, BM4107, T7 and BM4208
D3H13G-2	STR Analysis/ RT1, RT5, INRA040, RT13, RT24, RT30, CERV1, ETH152, BM1225, BM4107, T7 and BM4208
D3H13G-3	STR Analysis/ RT1, RT5, INRA040, RT13, RT24, RT30, CERV1, ETH152, BM1225, BM4107, T7 and BM4208
C3F65S	STR Analysis/ RT1, RT5, RT13, RT24, RT30, INRA040, Cerv1, Eth152, BM4107, BM4208, T7
M1S68R	STR Analysis/ Protocol DNA020C/ Cervid1, BM1225, BM4107, RT24, BM4208, T7, T159S, RT7
B4W11V-1	STR Analysis/ ADCYC, AGLA226, BL42, BM203, BM4107, BM4208, BM6438, BM6506, CELB9, CELJP15, CERVID1, CERVID2, ETH152, FCB193, GNZ204, RM006, SRCRSP1, TGLA94
B4W11V-2	STR Analysis/ ADCYC, AGLA226, BL42, BM203, BM4107, BM4208, BM6438, BM6506, CELB9, CELJP15, CERVID1, CERVID2, ETH152, FCB193, GNZ204, RM006, SRCRSP1, TGLA94
B4W11V-3	STR Analysis/ ADCYC, AGLA226, BL42, BM203, BM4107, BM4208, BM6438, BM6506, CELB9, CELJP15, CERVID1, CERVID2, ETH152, FCB193, GNZ204, RM006, SRCRSP1, TGLA94
J2R15F-1	-
J2R15F-2	-

K1W95S-1 K1W95S-3 K1W95S-4	STR Analysis/WDFW deer STR microsatellite panel/ BM1225, BM4107, C89, Cervid1, CRSP-1, RT24, RT5, RT7, T159, T7, Texan-4
K1W95S-2 K1W95S-3 K1W95S-4	STR Analysis/WDFW deer STR microsatellite panel/ BM1225, BM4107, C89, Cervid1, CRSP-1, RT24, RT5, RT7, T159, T7, Texan-4
R4R65C-1 R4R65C-2	-
C3F54C	STR Analysis/ RT1, INRA040, BM4107, BM1225, Cerv1, T7, RT5, BM4208, RT24, BM203, ETH152, RT9
B4C27D	STR Analysis/ Cervid, RT7, L, BM6506, N, RT5, INRA, Q, S, OAR, O, BM6438, BL25, P, K, RT13, D, BL42 Gender Markers – Sry & Zfx
R9H57A	STR Analysis/ OarFCB304, BMC1009, BM1706, RT1, RT13, T156, BM188, BM757, RT7, T26 References: (Talbot et al.1996), (Bishop et al.1994), (Wilson et al.1997), (Jones et al.2002), (Barendse et al.1994), (Bouchard and Crowford 1993)
J3V67H	-

Response Summary Total Participants: 34

Confirmation	Item 1	Item 2	Item 3
Species Origin	33 (97%)	34 (100%)	34 (100%)
Gender Origin	31 (91%)	31 (91%)	31 (91%)
Individual Identification		26 (76%)	

Inconclusive	Item 1	Item 2	Item 3
Species Origin	1 (3%)	0 (0%)	0 (0%)
Gender Origin	0 (0%)	0 (0%)	0 (0%)
Individual Identification		0 (0%)	

N/A	Item 1	Item 2	Item 3
Species Origin	0 (0%)	0 (0%)	0 (0%)
Gender Origin	3 (9%)	3 (9%)	3 (9%)
Individual Identification		8 (24%)	

END OF REPORT