3-2019

Construction of a Reference Allelic Ladder for an Odocoileus STR Multiplex

Jolene Strand
University of New Haven

Comments

Erin Meredith also served on this student's advisory committee.

THE UNIVERSITY OF NEW HAVEN

CONSTRUCTION OF A REFERENCE ALLELIC LADDER FOR AN *ODOCOILEUS* STR MULTIPLEX

A THESIS

Submitted in partial fulfillment

of the requirements for the degree of

MASTER OF SCIENCES IN FORENSIC SCIENCE

BY

Jolene Strand

University of New Haven
West Haven, Connecticut
March, 2019

ACKNOWLEDGEMENTS

I would like to thank Dr. David San Pietro, Dr. R. Christopher O'Brien, Dr. Mary Burnham-Curtis, Brian Hamlin and Erin Meredith for their input and support.

Special thanks to the USFWS National Fish & Wildlife Forensics Laboratory and California Department of Fish and Wildlife, Wildlife Forensics Laboratory for providing DNA samples and the Society for Wildlife Forensic Science for helping provide funding.

ABSTRACT

Odocoileus is a genus of Cervidae (deer) consisting of white-tailed deer (*Odocoileus virginianus*, Zimmerman, 1780), mule deer (*Odocoileus hemionus,* Rafinesque, 1817) and black-tailed deer (*Odocoileus hemionus hemionus,* Rafinesque, 1817). Hunting *Odocoileus* is only legal with proper permits according to laws that dictate when, where, how, and what can be hunted. Anything outside the legal limits is considered to be poaching. A useful tool for investigating poaching cases is Short Tandem Repeat (STR) DNA analysis. This is commonly used in human forensic casework to link DNA evidence found at crime scenes to either victims or suspects. In poaching cases, it can be used to link DNA from a deer carcass to DNA from a deer product in a poacher's possession.

Currently, each wildlife crime lab uses their own STR multiplex for this, unlike in human DNA forensic analysis where there is a standardized commercially available STR multiplex for use by the labs. Without a standardized STR multiplex, this means that it is not possible to have a database to search DNA profiles from other labs' cases. This means that potential links between cases might remain unknown. To address this, there is a collaborative effort going on to develop a STR multiplex to be used by wildlife crime labs (Odoplex). In addition to the STR multiplex, a reference allelic ladder is needed to make sure that alleles are being called consistently between labs. The objective of this thesis research was to develop a reference allelic ladder for this *Odocoileus* STR multiplex, "Odoplex".

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES

CHAPTER I

Introduction

Hunting is not only a hobby, but a tool used to control the population size and demographics such as sex and age of *Odocoileus* (Zimmerman 1780, Rafinesque 1817). To avoid drastically altering the population or demographics in a negative manner, a certain amount of hunting permits for each of the various deer demographics are issued. In addition, there are only specific times of the year that hunting is permitted (Long et al. 2008). Even with these management regulations in place to keep the white-tailed deer (*Odocoileus virginianus*, Zimmerman, 1780) population at its best possible fitness, the laws do not stop hunters from killing whichever deer they choose, even if it negatively impacts the fitness of the deer population. If poachers can be linked back to the deer they have been suspected of killing illegally, they can be charged and tried in a court of law. An effective way to do this is to match animal parts in the possession of suspect poachers to parts found at the crime scene through DNA analysis.

STR analysis has been a well-established technique in human forensics to determine if an unknown sample came from a known source (Lygo et al. 1994). In addition to linking samples from crime scenes, the development of national standards for human DNA analysis led to the creation of the Combined DNA Index System (CODIS,), allowing crime labs from different parts of the country to share DNA information with each other (Budowle et al. 1998). This allows investigators to be aware of multiple crimes committed by someone even when they cross jurisdictions. In cases where the suspect has not been identified, combining evidence from the multiple crimes might provide enough information to come up with an identification that would not happen if each crime were investigated individually. In the case of wildlife forensics, STR

analysis has already been applied as a tool to aid in solving poaching cases (Szabolcsi et al. 2014) . While this is useful, at the moment there is no database standardization, or CODIS-like database, that can be used. Since poachers can cross jurisdictions, and different jurisdictions may have wildlife forensic capabilities, a shared DNA database in place could prevent the chance for potential leads to be overlooked.

The development of a standard *Odocoileus* STR panel for use by all wildlife forensic labs provides the foundation for the creation of a common database. While there has been research done in the past for the development of a microsatellite multiplex (Anderson et al. 2002), there is no standard panel used by all the labs. Currently, a universal standard panel called Odoplex is being developed with the idea that it will be used by all of the wildlife crime labs. However the development of a standard panel is just one element in creating a useful shared DNA database. There can be different designations of alleles between labs, despite the fact both labs are analyzing the same sample (De Valk et al. 2009). Without some sort of a way to ensure that alleles are being consistently called between labs, a standard STR panel would still be ineffective as two samples from the same source may not possibly provide the same DNA profiles if analyzed at two separate labs using different allele binning criteria. In the case of human forensics, allelic ladders have been the solution to deal with this (Moretti et al. 2001). Allelic ladders contain the most commonly occurring alleles at each locus in the panel (Fujii et al. 2004) and therefore can be used as a reference to match alleles from a sample to so they are properly uniformly labelled. An allelic ladder would eliminate concern about the alleles not being interpreted the same way between different wildlife crime labs. With this in mind, the objective of this study was to develop a method and begin constructing an allelic ladder that can be used

with the Odoplex STR panel to aid in providing a consistent designation and interpretation of alleles.

Literature Review

Odocoileus is a genus within the Cervidae family. There are two species of deer within this genus: White tailed deer (*Odocoileus virginianus)* and Mule deer (*Odocoileus hemionus)*. Each species consists of multiple subspecies. For white tailed deer, there are over 38 subspecies across North, Central and South America. Of these 38 subspecies, 17 are found in North America (Smith 1991) (Fig 1 and Table 1).

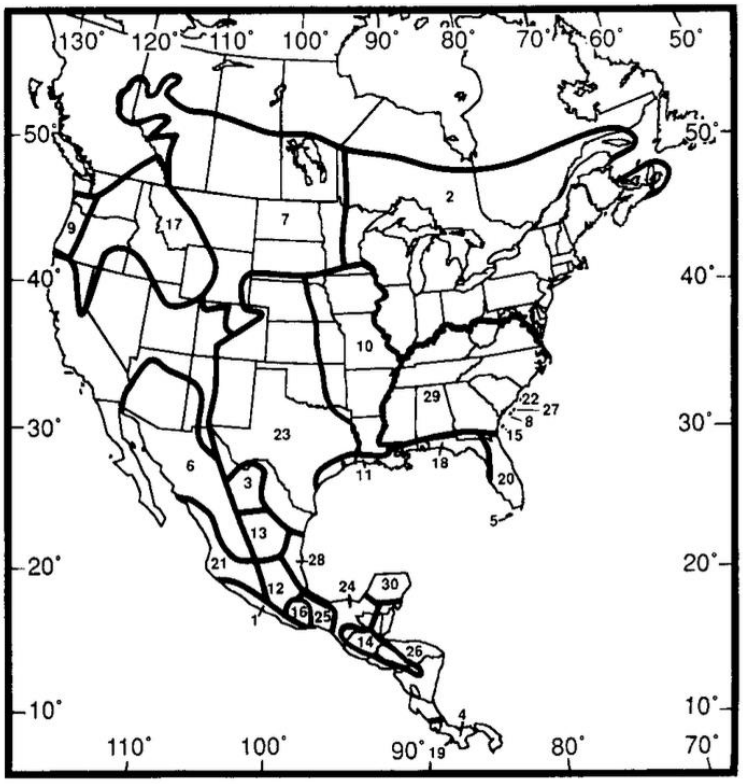


Figure 1. Map of white-tailed deer subspecies. (Smith 1991)

Common Name	Scientific Name	Location
Northern white-tailed deer	*O. v.. borealis* (Miller)	2
Carmen Mountains white-tailed deer	*O. v. carminis* (Goldman and Kellogg)	3
Key deer	*O. v.. clavium* (Barbour and Allen)	5
Couse white-tailed deer	*O. v.. couesi* (Coues and Yarrow)	6

Dakota white-tailed deer	*O. v.. dacotensis* (Goldman and Kellogg)	7
Hilton Head Island white-tailed deer	*O. v.. hiltonesis* (Goldman and Kellogg)	8
Columbian white-tailed deer	*O. v.. leucurus* (Douglas)	9
Kansas white-tailed deer	*O. v.. macrourus* (Rafinesque)	10
Avery Island white-tailed deer	*O. v.. mcilhennyi* (Miller)	11
Blackbeard Island white-tailed deer	*O. v.. nigribarbis* (Goldman and Kellogg)	15
Northwestern white-tailed deer	*O. v.. ochrourus* (Bailey)	17
Florida coastal white-tailed deer	*O. v.. osceola* (Bangs)	18
Florida white-tailed deer	*O. v.. seminolus* (Goldman and Kellogg)	20
Bull Island white-tailed deer	*O. v.. taurinsulae* (Goldman and Kellogg)	22
Texas white-tailed deer	*O. v.. texanus* (Mearns)	23
Hunting Island white-tailed deer	*O. v.. venatorius* (Goldman and Kellogg)	27
Virginia white-tailed deer	*O. v. virginianus* (Zimmerman)	29

Table 1. Table of common and scientific names for white-tailed deer subspecies. Location number refers to location in Figure 1 map where subspecies can be found. (Smith 1991)

Mule deer subspecies are typically grouped into two groups: the mule deer group which has seven subspecies, and the black-tailed deer group which has 2 subspecies (Anderson and Wallmo 1984) (Fig 2 and Table 2).

Figure 2. Map of mule deer subspecies. (Anderson and Wallmo 1984)

Common Name	Scientific Name	Location
Mule Deer Group		
Rocky mountain mule deer	*O. h. hemionus* (Rafinesque)	1
California mule deer	*O. h. californicus* (Caton)	7
Cedros Island mule deer	*O. h. cerrosensis* (Merriam)	5
Desert mule deer	*O. h. eremicus* (Mearns)	2
Southern mule deer	*O. h. fuliginatus* (Cowan)	6
Peninsula mule deer	*O. h. peninsulae* (Lydekker)	4
Tiburon Island mule deer	*O. h. sheldoni* (Goldman)	3
Black-tailed Deer Group		
Columbian black-tailed deer	*O. h. columbianus* (Richardson)	8
Sitka black-tailed deer	*O. h. sitkensis* (Merriam)	9

Table 2. Table of common and scientific names for mule deer subspecies. Location number refers to location in Figure 2 map where subspecies can be found. (Anderson and Wallmo 1984)

White-tailed deer and mule deer are medium sized Cervids. In the case of white-tailed deer, on average their shoulder height is around 91cm. The weight of adult females is on average 100 pounds and the weight of adult males is on average 150 pounds (Sauer 1984). However, the size of different subspecies will vary. The smaller subspecies of white-tailed deer are typically smaller than subspecies found on the mainland. The males of one of the smallest subspecies, Key deer on average weigh only 70 pounds and females on average weigh 64 pounds. On the other hand, white-tailed deer males in the Northeast and Great Lakes region on average weigh 220 pounds and females on average weigh 145 pounds (Geist 1998).

Mule deer are similar to white-tailed deer in the fact that their size will vary depending on subspecies. On average, a male's shoulder height is around 1m and they weigh between 154 to 330 pounds (Geist 1981). Black-tailed deer are the smaller subspecies. The males typically weigh 90-130 pounds and the females typically weigh 70-90 pounds (Dassmann 1954). In comparison, male mule deer on the Roosevelt National Forest weigh on average 148 pounds and females weigh on average 118 pounds (Anderson 1974).

Both white-tailed deer and mule deer have coats that will vary in color based on the season. In the summer, their coats with be more reddish-brown in color. In the winter their coats will darken, and vary in color in the gray to dark brown range (Anderson and Wallmo 1983, Smith 1991). In the case of white-tailed deer, their markings consist of a white band on their nose, and white patches around their orbital region and on their throat. Each side of their chin has a black labial spot; all their underparts are white including the end of their tail (Guthrie 1971). In addition, their ears will be approximately 1/2 the length of their head and their antlers have one main beam with vertical tines (Smith 1991). In the case of mule deer, they have a rump patch that may be either white or yellow. Some mule deer will have one white throat patch, but it is also possible for them to have two. They will have a V-shaped dark mark that starts at their eyes and extends upwards. One very noticeable difference between white-tailed deer and mule deer is that the tail of a mule deer will end with black hair instead of being all white (Geist 1981). In addition, their ears will be approximately 2/3 the length of their head that their antlers will be dichotomously forked instead of being one main beam (Smith 1991).

White-tailed deer and mule deer can live in a variety of habitat types ranging anywhere from coniferous forests, to open plains and deserts, to alpine habitats (Hamlin and Mackie 1989). Within all these different types of habitats, there is a tendency to prefer edge habitats, especially in areas where forage and cover habitats are not well interspersed. Edge habitats are boundary regions between forage and cover habitats (Fulbright and Ortega-S 2006). This kind of habitat is preferable because the forage provides them with food that can provide nutrition year-round while they can use the coverage to hide, especially during hunting season (Fulbright and Ortega-S 2006). In addition, just as they are general in the types of habitats they occupy, they also consume a wide variety of plants for their diet. They consume everything from the stalks,

flowers, fruits and seeds of grasses and forbs along with the buds, fruits, seeds, stems, leaves and bark of trees and shrubs (Rogers et al. 1981).

The habitat that white-tailed deer and mule deer occupy will have an impact on whether they migrate or not. Typically, deer living in more mountainous regions migrate either up or down in elevation depending on weather and seasonal changes that may impact their access to forage. For Mule deer, these migration distances can be anywhere from less than one mile to around 100 miles (Mackie et al. 2003). For white-tailed deer, these migration distances can be anywhere from 4 to 55 miles (McShea and Schwede n.d.). Deer that live at lower elevations will be less likely to migrate (Hanley 1984). In addition, a population of deer might not all migrate or stay in the same location. A population of deer can consist of both migratory and nonmigratory individuals (Hygnstrom et al. 2008).

Breeding season for both will range in the September to March time frame. They have similar gestation periods with white-tailed deer being 187-213 days (Smith 1991) and mule deer being 183-218 days (Anderson and Wallmo 1984). Both white-tailed deer and mule deer typically breed with their own subspecies, but it should be noted that subspecies interbreeding does happen where they coexist. In addition, the diploid number of chromosomes is 70 for both species (68 autosomes and 2 sex chromosomes) (Hsu and Benirschke 1967) which makes hybridization between white-tailed deer and mule deer is possible. However, it is not common (Anderson and Wallmo 1984).

Some subspecies of both white-tailed deer and mule deer are federally listed endangered species. For white-tailed deer, there are two species: The Key deer is listed as Endangered throughout its range and the Columbian white-tailed deer is listed as Threatened in portions of the Columbia River Basin. For mule deer, it is one subspecies: the Cedros Island mule deer is

listed as Endangered throughout its range. These listings are important to note because since these subspecies are more vulnerable to extinction compared to other subspecies, any poaching activity negatively impact their populations to a much greater degree than other subspecies. For all other subspecies of white-tailed deer and mule deer, hunting is permitted as long as it is within hunting season and the proper permits are obtained and followed. Hunting season will vary between states, but will typically fall within the September to December timeframe. If a deer is killed outside of a state's designated hunting season, or if the proper hunting license is not obtained or followed, then that is poaching.

Short Tandem Repeat (STR) analysis is based on nuclear DNA characteristics that are inherited biparentally. The technique has long been validated and used in forensic casework (Lygo et al. 1994). It can be used for analysis of a variety of samples such as bloodstains, semen stains and saliva stains regardless if they are old or degraded samples to obtain DNA profiles (Lygo et al. 1994) because the DNA itself is fairly robust. These profiles can then be used to link samples to victims or suspects by examining shared alleles. This is important because in forensic casework, evidence can come in any sort of condition, so having a form of analysis that can hold up to challenging and less than pristine samples is essential. However, STR analysis is only one part of the process. On many occasions, there may be minimal or no leads in a case. While STR analysis can provide evidentiary DNA profiles, if there is no suspect from which to obtain a DNA profile to compare to, it cannot provide useful information, unless there is a database that the unknown sample is searched against. The FBI created the Combined DNA Index System (CODIS) to deal with this issue for human crimes and implemented it nationally in 1998. It is a hierarchical database of DNA identification records. Initially the DNA sources for the records came from convicted felons, victims, and missing persons, with three indices being the

Convicted Offender Index, Victims Index and Forensic Index (Budowle et al. 1998). This has since changed to the following categories: Arrestee, Convicted Offender, Detainee, Forensic Mixture, Forensic Partial, Forensic Unknown, Juvenile, Legal, and Multi-allelic Offender (FBI 2017). A database as extensive as this is a useful tool in forensic investigations because unknown sample profiles from a crime scene can be matched back to a sample record in the database, providing an investigative lead that would not be possible otherwise. Especially in cases where an offender may be from out of town, a tool such as this is an effective way to provide an investigative lead in the form of a potential suspect. The availability of such databases is also useful for excluding individuals under suspicion and exonerating unjustly accused suspects in crimes (Norris 2017).

STR analysis is not just a useful tool for helping solve human crimes. It can be applied to wildlife forensic investigations as well. Poaching is a big problem in wildlife management and STR analysis is a very useful forensic tool for to assist law enforcement in dealing with this problem. In one case (Lorenzini 2005), a poacher snared and stabbed a wild boar sow with a knife. He hid the carcass with the intention of waiting until nightfall to return and retrieve it. The carcass was discovered before the poacher could return. Officers waited for his return, but he claimed that he discovered the boar after it was already dead. Upon further investigation, a knife was discovered in the poacher's home and trace amounts of blood-like smears were found along the edge of the blade. This indeed turned out to be blood and was genotyped back to the boar carcass using a panel of 12 highly polymorphic microsatellites (Lorenzini 2005). This case demonstrates one way that STR analysis can be applied to wildlife investigations. While it is not entirely the same as in human forensics, because rather than linking DNA from a crime scene to the donor suspect, analysts link DNA from a poached carcass to an object in the possession of a

suspect, thereby helping to link the suspect to the victim and/or the crime scene. In addition, in poaching cases, there might not always be an easily identifiable suspect. If the poacher had not returned to retrieve the carcass, this case would have been more difficult to solve. STR analysis might not have necessarily helped in this case without a known suspect because the suspect's house had to be searched to find the incriminating knife. However, a database where all wildlife crime labs are inputting DNA profiles could be helpful in other cases. For example, a headless carcass of a deer could be discovered in one jurisdiction, with someone found to be in possession of a deer head in a different jurisdiction. Unless there is some sort of DNA database that can compare samples from these two samples/jurisdictions, neither lab would know that they were actually working on the same case. Just like in human forensics, however, a uniform STR panel used by all wildlife crime labs is necessary for the database to actually work.

Wildlife forensic science is more complicated and broader than human forensics in the sense that each of the various species of interest needs a unique STR panel and associate database. There are some species that can be grouped together due to being closely related (e.g. while-tailed deer, mule deer, and black-tailed deer), but there is still a significant amount of STR panels required as compared to human forensics. For example, there has already been some development for different species of interest. One of these is a STR panel that has been developed for Eurasian Badger (*Meles meles)* (Dawnay et al. 2008). Eurasian badgers are a protected species in the United Kingdom under legislative acts such as the Protection of Badgers Act (1992) and the Wildlife and Countryside Act (1981). However, they are still killed through provoking dogs into attacking badgers, poisoning and illegal snaring. A STR multiplex that could link specific samples from blood swabs or hairs back to something in a suspect's possession would greatly help investigators in their investigations. A ten loci panel was

developed to help provide this DNA profile information. Eight of the ten loci were dinucleotides and the other two were complex repeats. The panel was tested for cross-species amplification, which was only observed in European otter (*Lutra lutra).* In addition, an allele frequency database was constructed with a total of 1083 individuals from over twenty different badger populations. There was also an allelic ladder developed to go along with it based on this allele frequency information.

Another example is a STR multiplex called SkydancerPlex. It has been developed for Hen harrier (*Circus cyaneus)* (Van Hoppe et al. 2016). Like the Eurasian Badger, hen harrier are protected under the Wildlife and Countryside Act (1981), but are still hunted. SkydancerPlex is a STR multiplex consisting of eight loci. It is different than the Eurasian Badger STR multiplex in the fact it consists of tetranucleotides instead of dinucleotides. The advantage of using tetranucleotides is that there will not be as much stutter and therefore tetranucleotides are easier to interpret. This multiplex was tested with a variety of case type samples including tissue, buccal swabs, egg shells and feather. Allele frequencies were calculated from sixty-three individuals and an allelic ladder was constructed containing fifty-five out of the fifty-eight alleles. Cross-species amplification was tested and it was found that amplification of STR markers was frequency observed in closely related birds which is to be expected.

Besides Eurasian badgers and hen harriers, there have also been STR multiplexes developed for deer species. In Hungary, red deer *(Cervus elaphus hippelaphus)* are threatened by poaching due to being a highly prized big game trophy. Having a STR panel as an investigative tool would greatly help these investigations. Like with SkydancerPlex, a tetranucleotide multiplex was developed (DeerPlex I-II). DeerPlex I-II is a ten STR loci panel (Szabolcsi et al. 2014). However, instead of being a single multiplex like both the Eurasian badger and hen

harrier panels, it was optimized into two five loci multiplexes instead of a single ten loci multiplex. Cross-species amplification was tested and it was found that while some amplification as observed with mouflon and bovine DNA, the entire panel did not amplify. This panel has already demonstrated its potential in that it has been used to aid an investigation into a serial poacher. However, while this is important as it shows how useful a STR panel can be in aiding deer poaching investigations, it is still not designed specifically for *Odocoileus*. In addition, there was no mention about the intention of DeerPlex I-II becoming a standardized panel. It's not that *Odocoileus* needs a multiplex, it's that a standardized one needs to be developed along with associated databases to answer investigative and conservation questions.

One way to ensure all the wildlife crime labs use the same panels is by encouraging a collaborative effort among them. Currently, this is happening between the USFWS National Fish & Wildlife Forensics Laboratory, Wyoming Game & Fish Wildlife Forensics & Fish Health Laboratory, California Department of Fish & Wildlife Forensic Laboratory, Tennessee Wildlife Resource Agency Unit and DNA Solutions, Inc., with the focus of the panel being deer in the Genus *Odocoileus*: white-tailed deer (*Odocoileus virginianus*, Zimmerman, 178), mule deer *(Odocoileus hemionus*, Rafinesque, 1817) and black-tailed deer (*Odocoileus hemionus hemionus*, Rafinesque, 1817). For this type of collaborative effort, *Odocoileus* is a good option to start out on because they have high levels of heterozygosity, even among white-tailed deer in the southeastern USA who faced near extirpation during the late 19th and early 20th centuries. In Mississippi, the statewide population was estimated to be less than 500 deer in 1933. Harvest regulation and restocking efforts helped the population to over 260,000 in 1969 (Blackard 1971), however whenever a species faces that much of a loss in population it may result in bottleneck or founder effects impacting the genetic diversity of said population (Knapp and Connors 1999).

Because there was such a loss in the population, that also means there is also a loss in the diversity of alleles in the population. This means that when the population rebounds, there are less alleles in the population. While in restoration efforts, there were deer translocated from outside populations which could potentially help with genetic diversity, these translocations frequently involved only a small number of individuals. By only bringing in a small number of individuals, this can result in what is known as a founder effect. It's a similar concept to a bottleneck where there is still a limited diversity in the allele pool to rebuild the population with, the only difference is that the deer have been brought in from a different location. In the case of the southeast United States, efforts to restore the *Odocoileus* population were successful from a wildlife management standpoint. However, drastic losses in populations can cause high levels of homozygosity resulting in a loss in genetic diversity (DeYoung et al. 2003). This could cause a potential problem for wildlife forensic labs as increased levels of homozygosity would result in the STR profiles not being as discriminating in individualizing samples. However, DeYoung et al found that despite the drastic loss in population *Odocoileus* faced in the southeastern United States, only six of sixteen populations in Mississippi they looked at indicated that a genetic bottleneck had occurred. And even despite the fact that there were some populations that indicated they had faced a genetic bottleneck, the populations still had high levels of heterozygosity and allelic diversity (DeYoung et al. 2003). This indicates that there is enough genetic diversity within *Odocoileus* for a STR panel to be an effective investigative tool.

In addition to high levels of genetic diversity, another reason why *Odocoileus* is a good starting point in developing standardized STR panels for wildlife forensic labs is that previous research has been done in finding STR markers for *Odocoileus* that could be incorporated into the panel. Seven of these STR markers were reported by DeWoody et al. They were designed

using white-tailed deer making them an ideal possibility for an *Odocoileus* panel (J. A. DeWoody, R. L. Honeycutt 1995). However, it should be noted that these markers are dinucleotides and therefore are more prone to stutter than a tetranucleotide marker would be. Because tetranucleotides and much more preferable for wildlife forensic casework than dinucleotides, the California Department of Fish and Game isolated 21 tetranucleotide loci. Out of the 21, eight loci were selected to be used in four-duplex reactions (Jones et al. 2000), This panel was specifically designed for the intention for it to be used in casework and in fact the majority of the loci incorporated into Odoplex come from this study. This is not the only panel that has been developed for *Odocoileus*. A 21 loci panel was developed (Anderson et al. 2002) using Cervid 1 from DeWoody et al and seven of the loci from Jones et al along with 12 STR markers that had been initially designed for cattle (Moore et al. 1992, Vaiman et al. 1992, Brezinsky et al. 1993, Buchanan and Crawford 1993, Bishop et al. 1994)and one that had been designed for sheep (Moore et al. 1992).

What has been done before shows it is possible to develop a STR panel for *Odocoileus*. What has yet to happen however is that there has not been a panel developed as a single multiplex reaction that's intended to be used by all the wildlife forensic labs. This collaborative effort in creating this *Odocoileus* multiplex will allow for this to happen.

One of the goals behind this STR panel is that it will allow for a database to be developed that will provide for more effective communication between wildlife crime labs. For this collaborative database to become a reality however, the STR panel is only one part of this process. The International Society of Forensic Genetics (ISFG) recommends that an allelic ladder be developed to go along with a STR panel for the use of non-human DNA in forensic genetic investigations (Linacre et al. 2011). The reasoning behind this recommendation is that

due to varying instrumentation and conditions between labs, there can be non-concordance between base pair sizes for the same allele (De Valk et al. 2009). This could be a big problem for collaborative databases because without a form of normalization, two samples might be from the same source, but not appear to be because of inconsistencies in how alleles are characterized and labelled. By using the same STR panel, with a corresponding allelic ladder, alleles will be consistently called the same way no matter which lab is doing the analysis. While the sizes of the fragments may vary between labs, alleles labeled in comparison to the allelic designations in the ladder will not.

The first part of constructing an allelic ladder is determining what the most common alleles are for each locus in the panel. While there have been allele frequencies calculated for *Odocoileus* loci there has not been a United States wide analysis of allele frequencies for *Odocoileus.* The majority of the loci in Odoplex come from Jones et al. Allele frequency calculations were done in the initial study, however they were only done with the eight loci that were in the finalized STR panel and they were only done with California *Odocoileus* populations (Jones et al. 2000). In addition, the naming of the alleles is different from what is being used in Odoplex as the alleles are now based off repeat count instead of an arbitrary number.

There are three different methods for allelic ladder development that can be found in the literature. The first involves using a plasmid vector (Fujii et al. 2004, Wang et al. 2014). This method works well and has the advantage of not being as susceptible to degradation. However, the *Odocoileus* STR panel and its respective allelic ladder are just the start of a much bigger picture. Eventually the idea is to develop standardized panels for all species that are common in wildlife forensic laboratories. Keeping this in mind, the methodology of the allelic ladder construction needs to incorporate as many techniques commonly used in wildlife crime labs to

streamline the process for efficiency and economy. Plasmid vectors are not a part of the analytical techniques commonly used in wildlife forensics and therefore are not the best option.

The second method is the use of a gel to isolate individual alleles amplified with polymerase chain reaction (PCR), which are then purified and combined into a single ladder. There are many different variations on how this is done. In one study, when making an allelic ladder for HUMCD4, a 9% polyacrylamide gel was used to separated alleles. The desired bands were eluted from the gel, purified using Wizards PCR Preps DNA Purification System (Promega), diluted and amplified a second time. Equal aliquots of the amplified products were then combined to build the allelic ladder (Glock et al. 1995). Since it was not specified how the dilution step was performed, only that it was performed, makes this paper not very informative when it comes to reproducing what they did. In addition, this was only for a single locus, not a panel of loci like in the case of Odoplex. Overall while this paper is helpful in showing that it is possible to construct an allelic ladder using gel electrophoresis to isolate alleles, without detailed specifics on the dilution and combination steps it is only a starting point.

Another paper detailing allelic ladder construction methodology is an allelic ladder created to accompany DogFiler (Wictum et al. 2013), DogFiler is an STR panel designed to help aid investigations where domestic dogs may be involved and their DNA could provide valuable information. This STR panel consists of 15 loci and one sex marker. This paper lays out the methodology they developed for allelic ladder construction. For the construction, homozygotes where chosen as much as possible to minimize the use of gel electrophoresis. When it was not possible to use a homozygote for an allele they wanted to incorporate into the allelic ladder gel electrophoresis was used. However, unlike with Glock et al., instead of using a polyacrylamide gel, an agarose gel was used instead. Once the alleles had been isolated, a 5 uL aliquot of the

resulting product was diluted into 1500uL of water to create a working stock for each allele. A 5uL aliquot of this working stock for each allele in a locus was then combined together. Serial dilutions of this combined working stock were then done (1:10-1:10^6) and then amplified and run using capillary electrophoresis to determine the optimal dilution factor. This was very detailed methodology and especially with the fact that this allelic ladder was constructed for a panel of loci, just like Odoplex is, made it very promising in a methodology to be adapted for Odoplex.

DogFiler was not the only panel that had a detailed methodology on how its corresponding allelic ladder was constructed, however. SkydancerPlex, the panel created for hen harrier also had a corresponding allelic ladder(Van Hoppe et al. 2016). The methodology is similar in the sense that it uses gel electrophoresis to isolate alleles and then follows that with amplification and dilution steps. However, the amplification and dilution steps are not exactly the same. The percent of the agarose gel used was dependent on how far apart the two heterozygote alleles were. For alleles with more than 20bp of separation, a 2.5% gel was used. For alleles with less than 20bp of separation, a 3-4% gel was used. Bands were excised from the gel and purified using either a GenCatch™ Advanced Extraction kit of the EZNA® Gel Extraction kit. The extracted alleles were then amplified and run on capillary electrophoresis. Based on those results a working stock of approximately 500RFU/uL (Relative Fluorescent Units) was prepared. After this, 1:10, 1:1000 and 1:100,000 dilutions were prepared from each working stock. An aliquot of 1uL was used in a second singleplex amplification. Capillary electrophoresis was performed again and the best dilution amplicon was chosen to be included in the ladder. Finally, all of the chosen amplicons were combined together into one single allelic ladder solution. This is another method that showed potential as it was designed specifically for a panel of STR loci.

One of the disadvantages with using a gel electrophoresis based methodology to create an allelic ladder is that agarose has the potential to inhibit PCR amplifications if the extracted DNA is not purified properly. This means that with every allele that is isolated on the gel, there is the additional purification step, making it an even more time consuming process. Because the making of the Odoplex allelic ladder will not be a one-time thing, minimizing the number of steps required to do so would be ideal. One way to do this is to see if it would be possible to minimize the amount of work needed to purify the sample after excising the alleles from the agarose gel (Burgos et al. 2015). This was tried when constructing allelic ladders for two human tetranucleotide mini STRs, D14S1434 and D10S1248. Instead of excising the band using a scalpel, the band was punctured with a 10uL pipette tip and the allele was excised that way. For the following PCR amplification, 32 cycles were done instead of the established 26 of the parameters they were using. There was still a purification step as the amplicons were diluted to 500uL and purified using microcon-100 system (Millipore). The purification step is not completely eliminated, but it uses an alternative that may be simpler than a full blown gel purification kit. Another variation this methodology has is that up until this point, the primers for PCR amplification are not fluorescently tagged. After the microcon-100 purification, aliquots of purified alleles are diluted to 1:1000 and 1:10,000. Aliquots of this diluted product are then finally amplified. They found that they were able to still successfully amplify DNA despite not using a purification kit specifically designed for samples excised from an agarose gel.

Using a gel to isolate alleles has potential, especially since there is already published methodology using this technique on STR panels, however it still has the disadvantage that slab gel electrophoresis is not a commonly used technique in wildlife crime labs. The ideal

methodology would be one that would only require techniques commonly used in wildlife crime labs.

The third method only involves two steps; PCR amplifications and dilution, both which are techniques commonly used in wildlife forensic labs. Alleles to be incorporated in the allelic ladder are first amplified in singleplex reactions, (one source, one locus) which is similar to the start of the second method; however, instead of isolating alleles using gel electrophoresis, all of the PCR amplicons from the singleplex reactions are combined together and diluted. A portion of this diluted product is then amplified in a second PCR reaction, resulting in a completed allelic ladder (LaHood et al. 2002). While no purification step is performed, the dilution allows for a clean looking allelic ladder. This methodology streamlines the process as much as possible, making it the most preferred method to use in the Odoplex allelic ladder construction. However, the paper using this technique only combined alleles for a single locus together for the second amplification. As Odoplex is a panel of 15 loci and two sex markers, part of this research was to see if this technique could be expanded to combining alleles from multiple loci together for the second amplification to streamline the process even further.

One last element of Odoplex that makes constructing its allelic ladder more difficult than the previously mentioned ladders is that while the majority of the loci are tetranucleotides, it does contain one dinucleotide, FCB193. Dinucleotides have a tendency to have prominent stutter and FCB193 is no exception. Stutter happens during PCR amplification. When copying the template strand, strand slippage happens which results in a mispairing of the two DNA strands. The copied strand will end up a different length and therefore a different repeat unit amount than what it was supposed to be. Typically this results in the DNA strand being one repeat unit shorter than the original DNA template strand (Hauge and Litt 1993). Any PCR amplification that is

done will result in stutter and since any methodology to construct an allelic ladder will require PCR amplification, stutter in the final allelic ladder in unavoidable. However, the stutter does not necessarily have to be seen as a problem, and instead can be used in the actual construction of the allelic ladder. When constructing an allelic ladder for *Aspergillus fumigatus,* stutter was actually used to fill in for alleles that they did not have (De Valk et al. 2009). This potentially could be used with FCB193 where alleles are chosen in such a way that space is intentionally left between them for the stutter to fill in.

CHAPTER II

Materials/Methods

DNA samples were provided by the USFWS National Fish & Wildlife Forensics Laboratory and the California Department of Fish and Wildlife, Wildlife Forensic Laboratory. These DNA samples were from white-tailed deer (*Odocoileus virginianus*, Zimmerman, 1780), mule deer (*Odocoileus hemionus,* Rafinesque, 1817) and black-tailed deer (*Odocoileus hemionus hemionus,* Rafinesque, 1817).

Collection of Odoplex DNA profile data was done at the USFWS National Fish & Wildlife Forensic Laboratory in Ashland, Oregon. DNA samples were already extracted and were contained in 96-well plates. Due to this setup, they were first quantified using CytoFluor (Life Technologies, Foster City, CA). Because quantification results greater than around 15ng/uL were not reliable, any samples found to be at or above this concentration were diluted and quantified again using Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA) and the dsDNA HS Assay Kit. After samples were quantified, normalized 2ng/uL working dilution plates were created using QIAgility (Qiagen, Inc. Hilden, Germany). The new working dilution plate contained 100uL. Samples that were already below the 2ng/uL were not diluted and instead 100uL of the sample were transferred over into the working dilution plate.

After quantification and normalization, samples were amplified with the Odoplex STR panel to determine which alleles were present using the QIAGEN Multiplex PCR Kit (Qiagen, Inc. Hilden, Germany). This panel consists of 15 loci and two sex markers. The loci and their corresponding Fluorescent Dye are listed in Table 3.

Fluorescent Dye	Loci	Fluorescent Dye	Loci
6-FAM	OheT256 V	**VIC**	OheC142 F
	OheC273 M		OheT217 K
	OheT7 P		OheT27r N
	OheC229a L	**PET**	OheC10 B
	ZFX		Cervid SRY
NED	FCB193		OheT159 O
	OHEC89 D		OheT32 Q
	CheC186 J		OheC165 H
	CheC50 C		

Table 3. Fluorescent Dyes used in Odoplex and their corresponding loci.

Amplifications were performed with 10μL reactions consisting of 2μL of DNA template, 5μL master mix, 1μL primer mix, and 2μL dH_2O. The PCR amplification was done on a Bio-Rad C1000 thermocycler with the protocol consisting of a 15 minutes activation at 95°C followed by 28 cycles of 30s at 95°C, 90s at 56°C and 60s at 72°C with a final extension for 10 min at 72°C.

Odoplex DNA profiles from 1882 *Odocoileus* samples were obtained on a ABI 3500 Genetic Analyzer capillary electrophoresis unit (CE) to calculate nation-wide allele frequency information. GeneScan 600 LIZ dye Size Standard v2.0 was used as the internal size standard. The majority of the samples were white-tailed deer, with some mule deer and black-tailed deer. The data was analyzed using GeneMarker® Genotyping software (Life Technologies, Foster City, CA) to determine what alleles each profile contained. The analytical threshold was initially set at 50 RFUs, however after initially running the data for analysis, numerous peaks were being

called as alleles when they were clearly not. The analytical threshold was increased to 300RFUs. In instances where the profile have overall lower RFU's, any peaks that were clearly alleles and below that threshold were manually edited back in as alleles. Each DNA profile was looked at to determine that only true alleles were being called and bins for new alleles were added for each locus.

Due to the fact that some samples may have been on multiple plates, or the original sample may have been extracted multiple times and received multiple accession numbers, there were duplicate profiles within the 1882 samples. To accurately determine the allele frequencies duplicates needed to be removed. This was done using GenAlex 6.5 (Genetic Analysis in Excel) (Peakall and Smouse 2006, 2012). GenAlex is population genetics software used by the USFWS National Fish & Wildlife Forensics laboratory in Ashland, Oregon. One of its functions is to compare DNA profiles to each other and provide a list of samples with matching sample profiles. This was done with the deer DNA profiles and based on this information, duplicates were removed from the data leaving 1529 unique DNA profiles.

After repeats were removed, allele frequencies were calculated. This was done using Excel. For each locus, the total number of occurrences of a particular allele was counted. This was divided by 2N, with N representing the number of samples for a locus.

Based on allele frequency calculations, alleles/samples were chosen to be included in the final allelic ladder. Primarily whole repeats were chosen, that being a unit of four bases in all cases except for the single dinucleotide in the panel, FCB193, with more frequent microvariant alleles included when possible. Individual samples were chosen to be included in the allelic ladder that would cover all the determined alleles. The majority of samples chosen contained alleles in heterozygous form. Samples were picked in such a way that the combination of all the

heterozygotes would cover all the alleles chosen to be in the ladder. Homozygotes were chosen to fill in where necessary. In addition, samples with similar RFU values within a locus were selected when possible. This was done to help make sure that the alleles in the final allelic ladder would have relatively balanced peak heights without major adjustments.

For the construction of the allelic ladder, the first step was to see if what was done in LaHood et al. could be applied to Odoplex. Because the methodology had been for a single locus in LaHood et al., a single locus was chosen to start with. Locus OheT256 V was chosen to be this locus because based on allele frequency data it was a more straightforward locus with only seven alleles and no microvariants. The chosen samples containing the alleles to construct the allelic ladder were amplified in singleplex reactions with the OheT256 V primer pair. This was done initially on the Bio-Rad C1000 in Ashland, Oregon but then later repeated back at the University of New Haven on an Applied Biosystems GeneAmp PCR System 9700 Thermal Cycler. These samples were again initially run on a ABI 3500 Genetic Analyzer with POP-7 polymer in Ashland, but later repeated on an ABI 3130xl Genetic Analyzer with a POP-4 polymer at the University of New Haven to confirm amplification success. After this, 1ul of each amplified sample was combined (seven alleles in total for the locus) and diluted to 10mL using sterile water. A 2uL aliquot of this product was taken and amplified using the same thermal cycling conditions in a second singleplex reaction for a completed OheT256 V allelic ladder. A diagram of this process can be found in Appendix I.

This process was then repeated to amplify samples from four loci, one from each of the different fluorescent dyes. OheT256 V was used for 6-FAM as it had already been established to work. OheC143 F was selected for VIC because like with OheT256 V it was a simple locus without many alleles or microvariants. OheC186 J was chosen for NED and OheC10 B for PET

for the same reasons. Samples representing specific alleles were amplified in singleplex reactions using the appropriate primer pairs specific to their particular locus. These samples were run on the CE to determine amplification success, and 1uL of each sample from each locus was combined (31 alleles total) and diluted to 10 mL using sterile water. A 2uL aliquot of this combined, diluted product was taken and amplified in a second reaction, except the primer mix this time consisted of primers from all four loci. This resulted in a product consisting of completed allelic ladders for loci OheT256 V (7 alleles), OheC143 F (7 alleles), OheC186 J (8 alleles) and OheC10 B (9 alleles). A diagram to help illustrate this is in Appendix II.

The final step after trying this process with one locus from each fluorescent dye was to try combining all the loci together. A total of 109 samples needed to be amplified to make the allelic ladder. Because of this, samples were grouped into three amplification groups. Table 4 shows the loci in each group and the number of alleles in the allelic ladder for each locus.

Group 1		**Group 2**		**Group 3**	
Locus	**Number of Alleles**	**Locus**	**Number of Alleles**	**Locus**	**Number of Alleles**
OheT25g V	7	**OheT27r N**	22	**OheC186 J**	8
OheC273 M	5	**FCB193**	13	**OheC10 B**	9
OheT7 P	14	**OheC89 D**	14	**Cervid SRY**	1
OheC229a L	13	**OheC50 C**	11	**OheT159 O**	12
ZFX	1	-	-	**OheT32 Q**	22
OheC143 F	7	-	-	**OheC165 H**	8
OheT217 K	10	-	-	-	-

Table 4. Amplification groups and the number of alleles in allelic ladder for each locus

Samples were amplified in singleplex reactions using primers for their particular loci. These samples were run on the CE to determine amplification success. One difference between this time and the previous allelic ladder construction attempts is that in an effort to try and even out peak heights, RFU data was taken into consideration. Between 1-8uL of each sample was added, depending on what was needed to even out the peak heights of whichever locus it belonged. These combined samples were then diluted to 10mL and a 2uL aliquot of this product was taken and amplified in a second reaction with a primer mix containing the primers from all of the Odoplex loci, including sex markers. This final amplified Odoplex allelic ladder was run on the CE to determine amplification success.

CHAPTER III

Results

To determine the most common alleles are for all the loci in Odoplex, Odoplex STR profiles from 1529 samples from the US Fish and Wildlife Service Wildlife Forensic Lab were obtained. The majority of these samples were white-tailed deer, and cover the range of white-tailed deer populations in the United States. Appendix III consists of the breakdown of samples by species and state. Figure 2 is an example of an Odoplex STR profile.

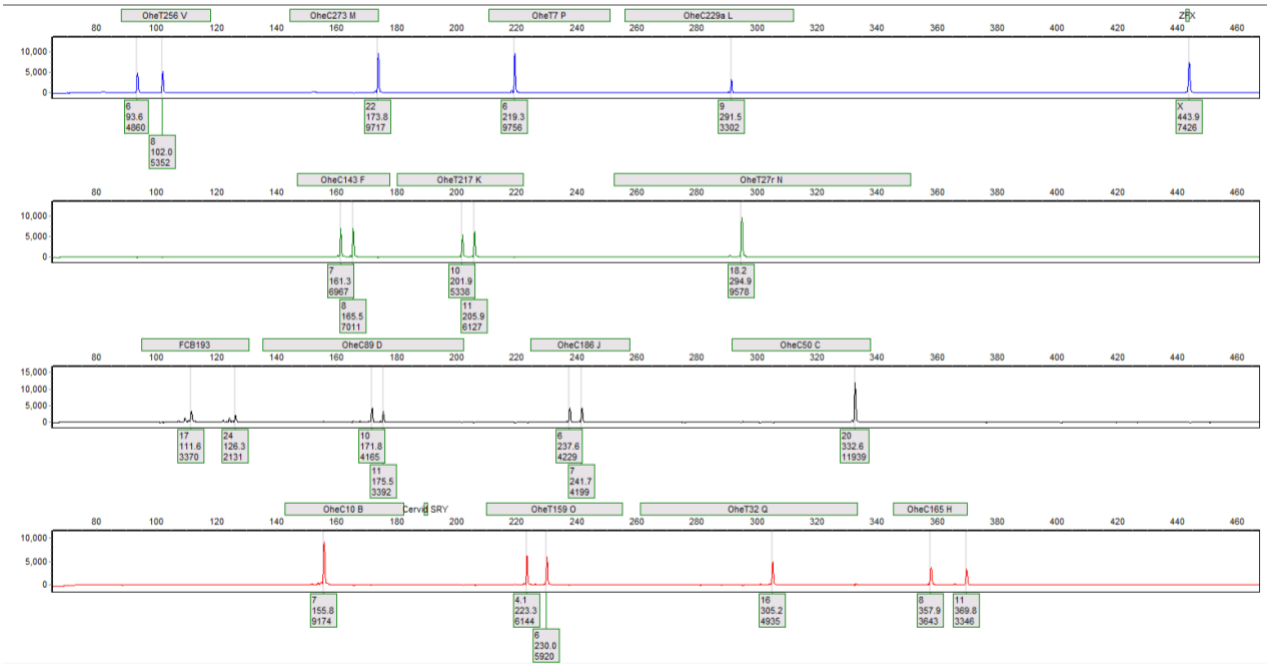


Figure 3. Electropherogram of Odoplex STR profile

Allele frequencies were calculated based on results from samples analyzed. Figures 3-18 and Tables 4-19 show the calculated allele frequencies. Allele number varied between loci with the smallest number of alleles being five at OheC273 M and the largest number of alleles being OheT27r N with 41. The most common alleles were chosen to be included in the allelic ladder, but alleles were also chosen across the range to ensure the ladder would work effectively for in

instances those alleles showed up in cases. When possible the smallest and largest alleles were chosen no matter their allele frequency to bracket the range.

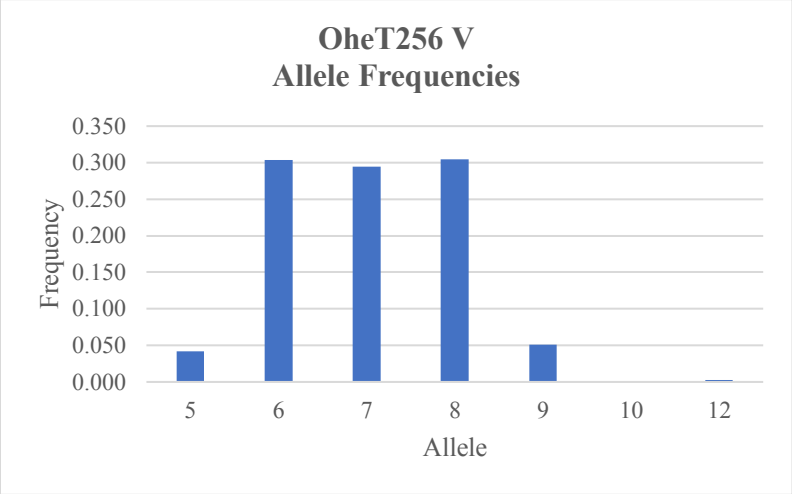


Figure 4. OheT256 V Allele Frequencies

OheT256 V

Allele	Frequency
5	*0.042*
6	*0.304*
7	*0.295*
8	*0.305*
9	*0.051*
10	0.001
12	*0.002*

Table 5. OheT256 V Allele Frequencies. Those italicized were included in allelic ladder.

For locus OheT256 V, all alleles except for allele 10 were included in allelic ladder. Alleles 6, 7 and 8 were included because they were the most frequent. Alleles 5 and 9 were included because they were the next most frequent. While allele 12 is not as frequent as other alleles, it was still included as it represented the largest allele of the locus.

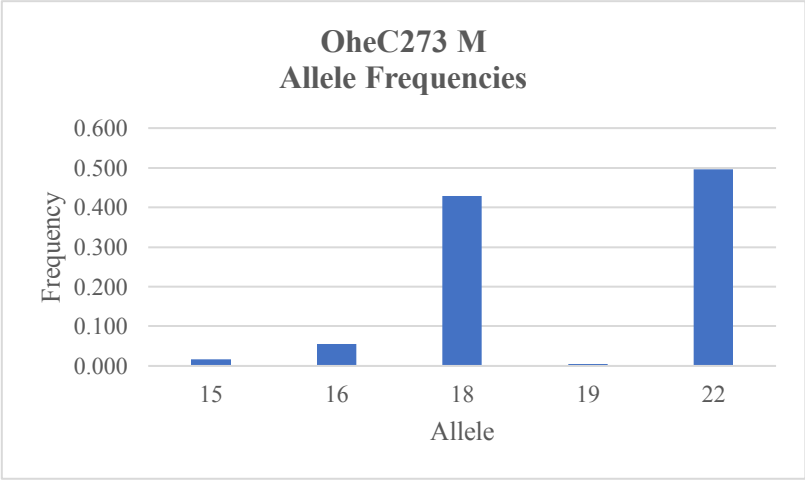


Figure 5. OheC273 M Allele Frequencies

OheC273 M

Allele	Frequency
15	*0.017*
16	*0.055*
18	*0.428*
19	0.005
22	*0.495*

Table 6. OheC273 Allele Frequencies. Those italicized were included in allelic ladder.

For locus OheC273 M, all alleles except for allele 19 were included in allelic ladder. While allele 15 is not as frequent as other alleles, it was still included as it represented the smallest allele of the locus.

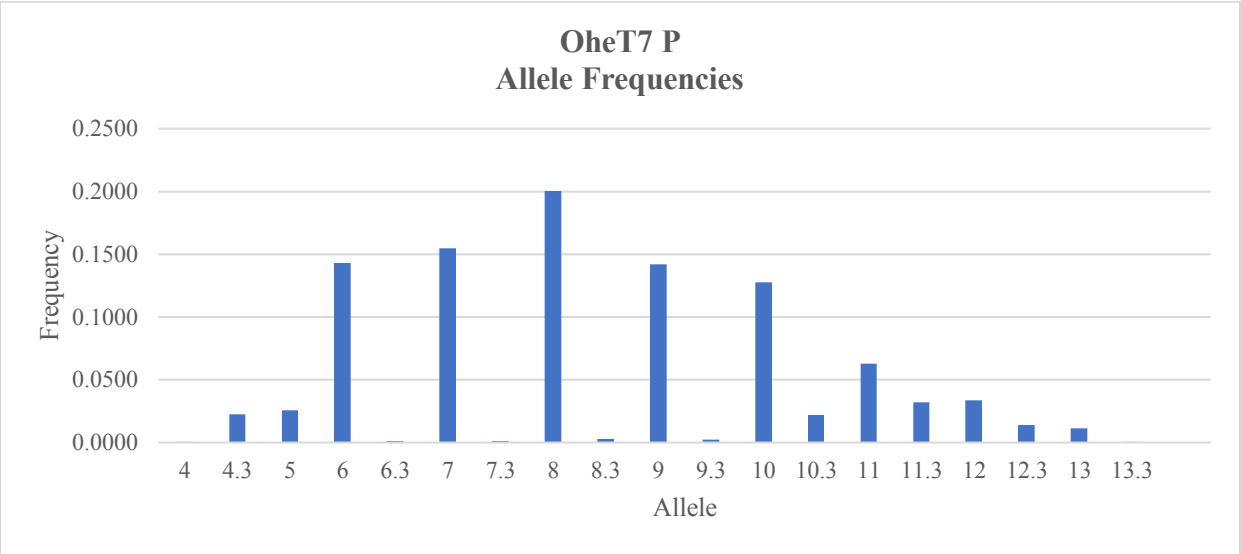


Figure 6. OheT7 P Allele Frequencies

OheT7 P

Allele	Frequency	Allele	Frequency	Allele	Frequency
4	*0.0003*	*8*	*0.2003*	***11***	*0.0626*
4.3	*0.0225*	8.3	0.0030	***11.3***	*0.0318*
5	*0.0258*	*9*	*0.1421*	***12***	*0.0338*
6	*0.1430*	**9.3**	0.0023	**12.3**	0.0142
6.3	0.0013	***10***	*0.1275*	***13***	*0.0116*
7	*0.1546*	***10.3***	*0.0219*	**13.3**	0.0003
7.3	0.0010				

Table 7. OheT7 P Allele Frequencies. Those italicized were included in allelic ladder.

For locus OheT7 P all whole tetranucleotide repeat alleles were included in the allelic ladder. Alleles 4.3, 10.3 and 11.3 were included as they were more frequent microvariants, and 13.3 was included as it represented the largest allele seen in the samples analyzed. In addition, allele 9.2 will be included due to prevalence noted in Mule Deer populations by the California Department of Fish and Wildlife Forensic Lab, though it was not seen in the USFWS dataset..

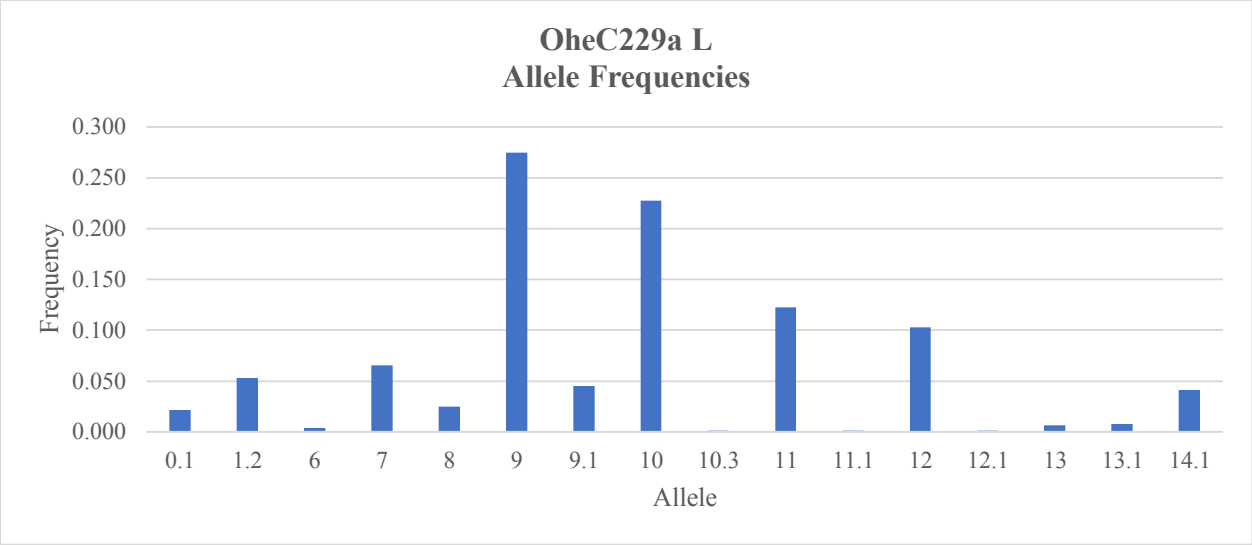


Figure 7. OheC299a L Allele Frequencies

OheC229a L					
Allele	**Frequency**	**Allele**	**Frequency**	**Allele**	**Frequency**
0.1	*0.022*	*9.1*	*0.045*	***12***	*0.103*
1.2	*0.053*	*10*	*0.228*	**12.1**	0.001
6	*0.004*	**10.3**	0.001	***13***	*0.006*
7	*0.065*	***11***	*0.122*	***13.1***	*0.007*
8	*0.025*	**11.1**	0.001	***14.1***	*0.041*
9	*0.275*				

Table 8. OheC299a L Allele Frequencies. Those italicized were included in allelic ladder.

For locus OheC299a L all whole nucleotide repeats were included. Allele 13 was included despite being infrequent as it was the largest whole nucleotide repeat. 1.2, 9.1 and 14. 1 were included for being the more frequent microvariants. Allele 0.1 was included as it represented the smallest allele and 13.1 was included to provide a second larger microvariant allele in the ladder. In addition, allele 1.2 will be included due to its prevalence as noted in Mule Deer populations by the California Department of Fish and Wildlife Forensic Lab.

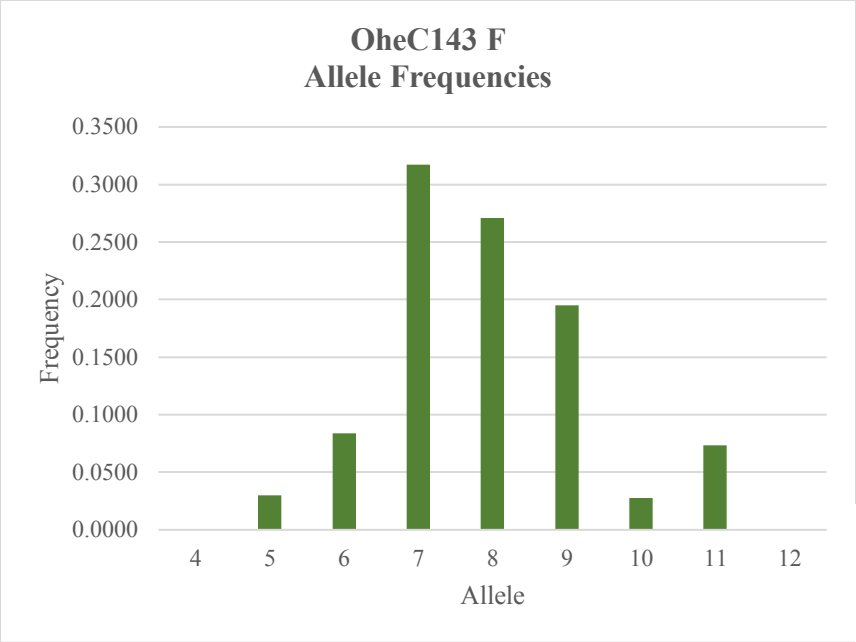


Figure 8. OheC143 F Allele Frequencies

OheC143 F

Allele	Frequency
4	0.0007
5	*0.0302*
6	*0.0841*
7	*0.3173*
8	*0.2710*
9	*0.1951*
10	*0.0276*
11	*0.0733*
12	0.0007

Table 9. OheC143 F Allele Frequencies. Those italicized were included in allelic ladder.

For locus OheC143 F all alleles were included in allelic ladder except for 1 and 12. While 4 and 12 were the largest alleles, sample selection to include alleles in allelic ladder were chosen for locus data was completely analyzed. However, because there are allele right next to them at 5 and 11, the ladder will still work.

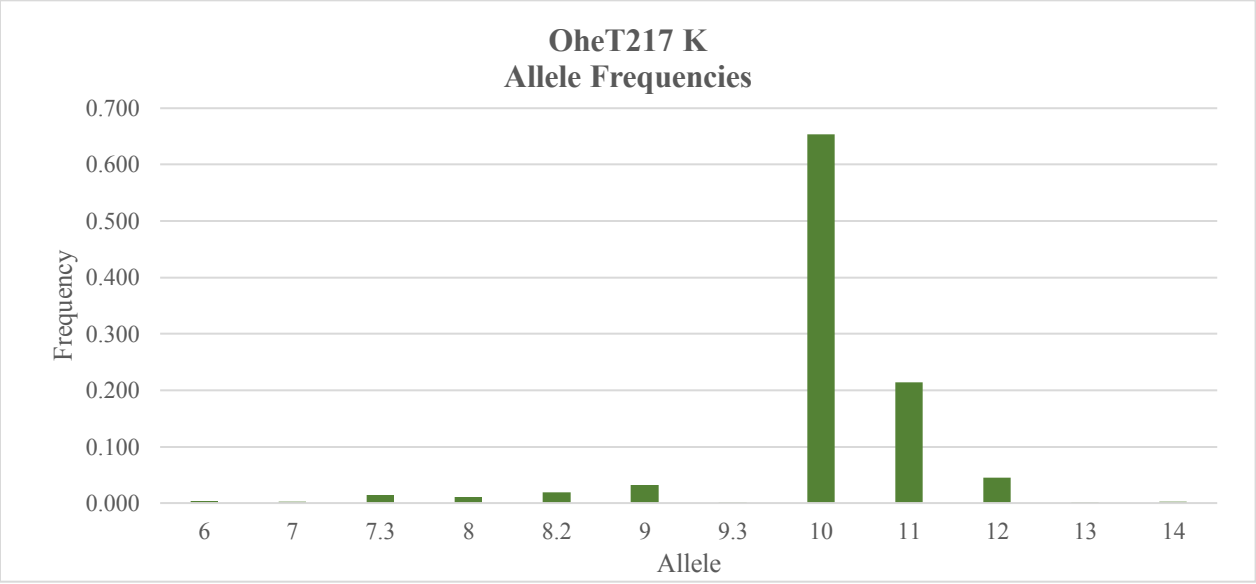


Figure 9. OheT217 K Allele Frequencies

OheT217 K					
Allele	**Frequency**	Allele	**Frequency**	Allele	**Frequency**
6	*0.0033*	8.2	0.0188	*11*	*0.2146*
7	*0.0020*	*9*	*0.0323*	*12*	*0.0445*
7.3	*0.0142*	9.3	0.0010	*13*	*0.0016*
8	*0.0106*	*10*	*0.6541*	*14*	*0.0030*

Table 10. OheT217 K Allele Frequencies. Those italicized were included in allelic ladder.

For locus OheT217 K all whole tetranucleotide repeats were included in allelic ladder. Even though 10 and 11 were much more common than the rest, the other whole tetranucleotide repeats were included to provide alleles across the entire range. Allele 7.3 was included to have a microvariant allele in the ladder.

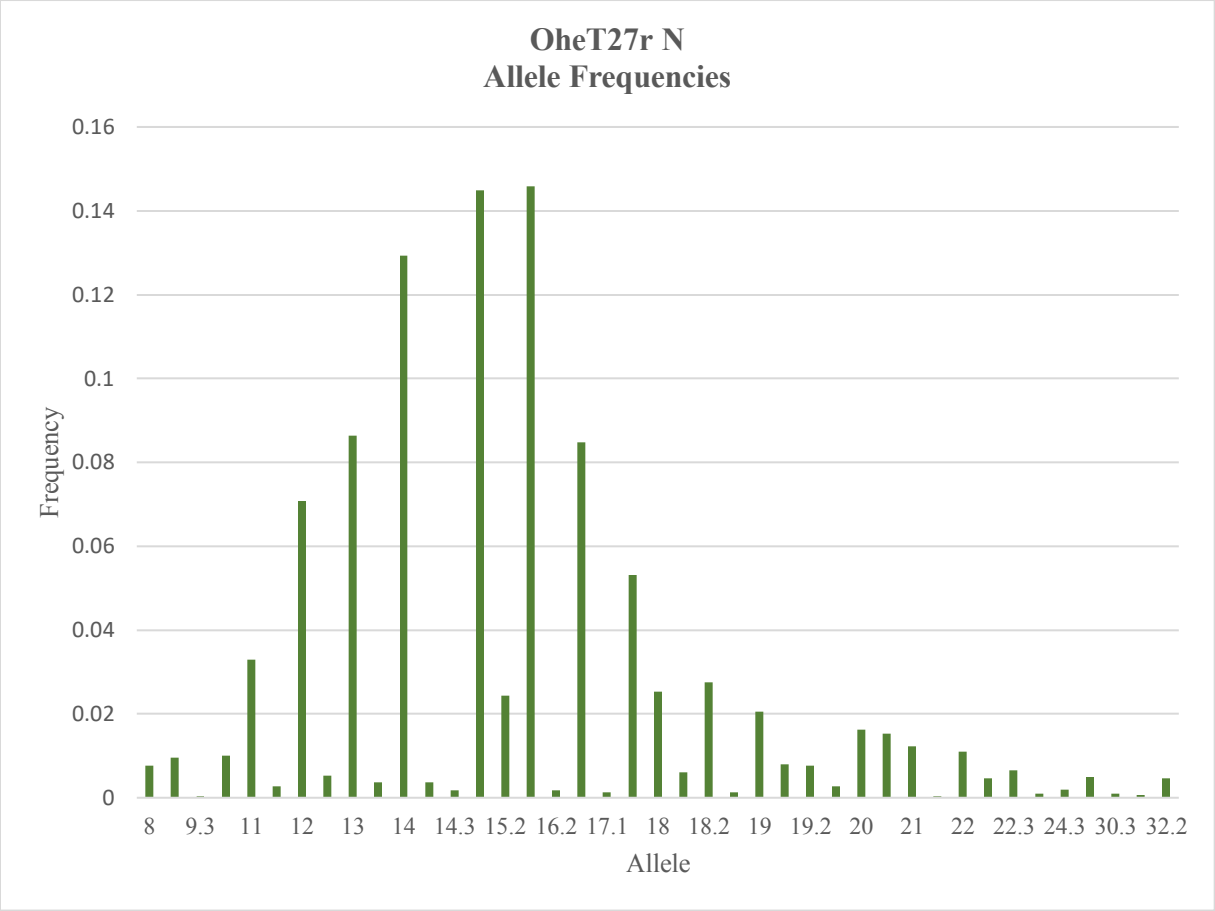


Figure 10. OheT27r N Allele Frequencies

OheT27r N					
Allele	**Frequency**	**Allele**	**Frequency**	**Allele**	**Frequency**
8	*0.0076*	**15.2**	0.0243	***20***	*0.0163*
9	*0.0096*	***16***	*0.1459*	**20.2**	0.0153
9.3	0.0003	**16.2**	0.0017	***21***	*0.0123*
10	*0.0100*	***17***	*0.0848*	**21.2**	0.0003
11	*0.0329*	**17.1**	0.0013	***22***	*0.0110*
11.2	0.0027	***17.3***	*0.0532*	**22.2**	0.0047
12	*0.0708*	***18***	*0.0253*	***22.3***	*0.0066*
12.2	0.0053	**18.1**	0.0060	**23**	0.0010
13	*0.0864*	***18.2***	*0.0276*	***24.3***	*0.0020*
13.2	0.0037	**18.3**	0.0013	***29.3***	*0.0050*
14	*0.1293*	***19***	*0.0206*	***30.3***	*0.0010*
14.2	0.0037	**19.1**	0.0080	***31.3***	*0.0007*
14.3	0.0017	**19.2**	0.0076	***32.2***	*0.0047*
15	*0.1449*	**19.3**	0.0027		

Table 11. OheT27r N Allele Frequencies. Those italicized were included in allelic ladder.

For locus OheT27r N, all the whole tetranucleotide repeat alleles were included in the allelic ladder. Alleles 17.3 and 18.2 were included to provide alleles in the ladder that were not whole tetranucleotide repeats. Alleles 22.3, 24.3, 29.3, 30.3 and 31.3 were originally included in the allelic ladder under the assumption they were whole tetranucleotide repeats. Sequencing information after the fact indicates this is not the case. Allele 32.2 was included as it represented the largest allele. In addition, allele 9.3 will be included due to prevalence noted in Mule Deer populations by the California Department of Fish and Wildlife Forensic Lab.

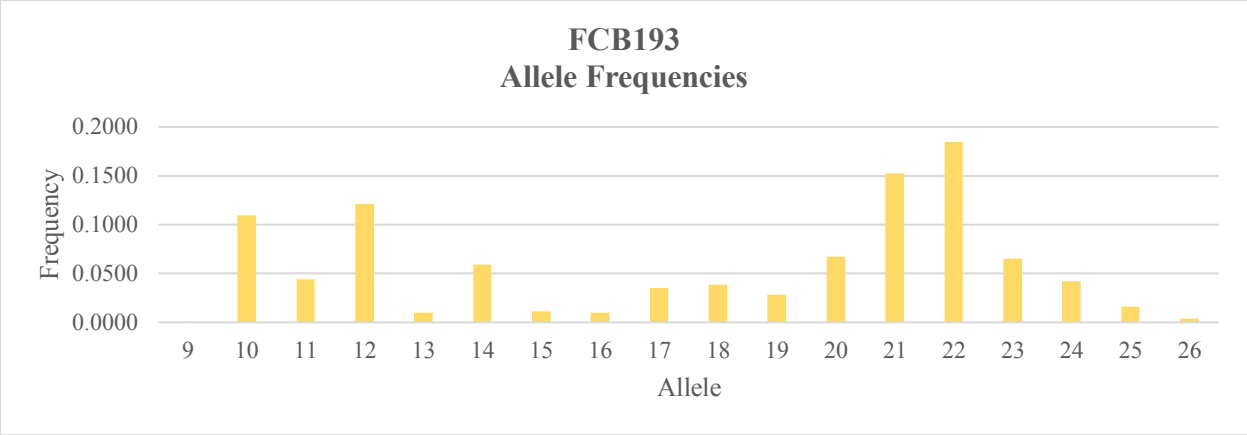


Figure 11. FCB193 Allele Frequencies

FCB193

Allele	Frequency	Allele	Frequency	Allele	Frequency
9	*0.0003*	***15***	*0.0112*	**21**	0.1527
10	*0.1097*	***16***	*0.0102*	***22***	*0.1848*
11	*0.0443*	***17***	*0.0355*	***23***	*0.0650*
12	0.1215	**18**	0.0387	**24**	0.0417
13	*0.0098*	***19***	*0.0286*	***25***	*0.0158*
14	0.0591	***20***	*0.0673*	***26***	*0.0039*

Table 12. FCB193 Allele Frequencies. Those italicized were included in allelic ladder.

For locus FCB193 all alleles were included in allelic ladder. However, due to the nature of FCB193 being a dinucleotide and therefore having a high amount of stutter, not all alleles were chosen from samples.

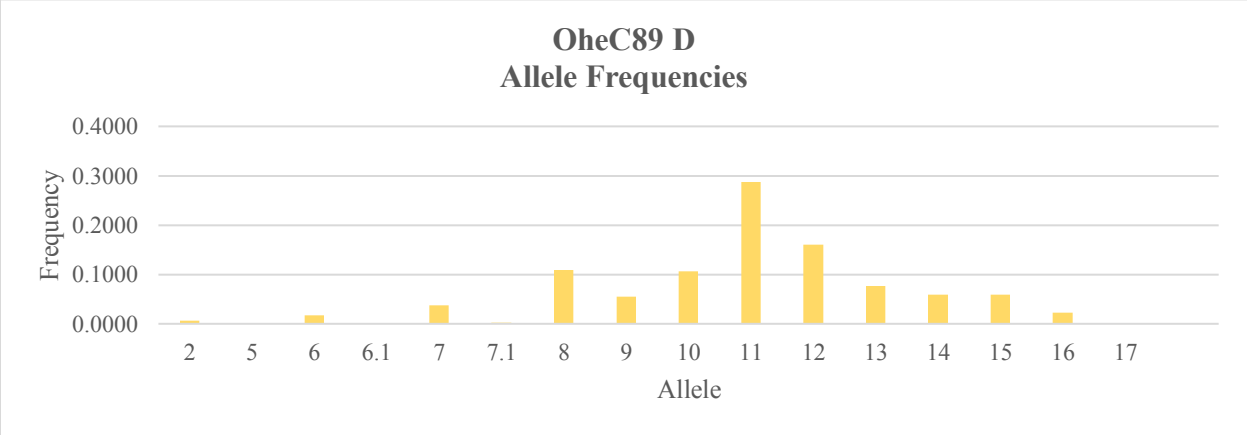


Figure 12. OheC89 D Allele Frequencies

OheC89 D

Allele	Frequency	Allele	Frequency	Allele	Frequency
2	*0.0066*	*9*	*0.0551*	***14***	*0.0591*
5	*0.0007*	***10***	*0.1059*	***15***	*0.0588*
6	*0.0179*	***11***	*0.2872*	***16***	*0.0226*
7	*0.0401*	***12***	*0.1597*	***17***	*0.0007*
8	*0.1096*	***13***	*0.0760*		

Table 13. OheC89 D Allele Frequencies. Those italicized were included in allelic ladder.

For locus OheC89 D, all alleles were included in allelic ladder.

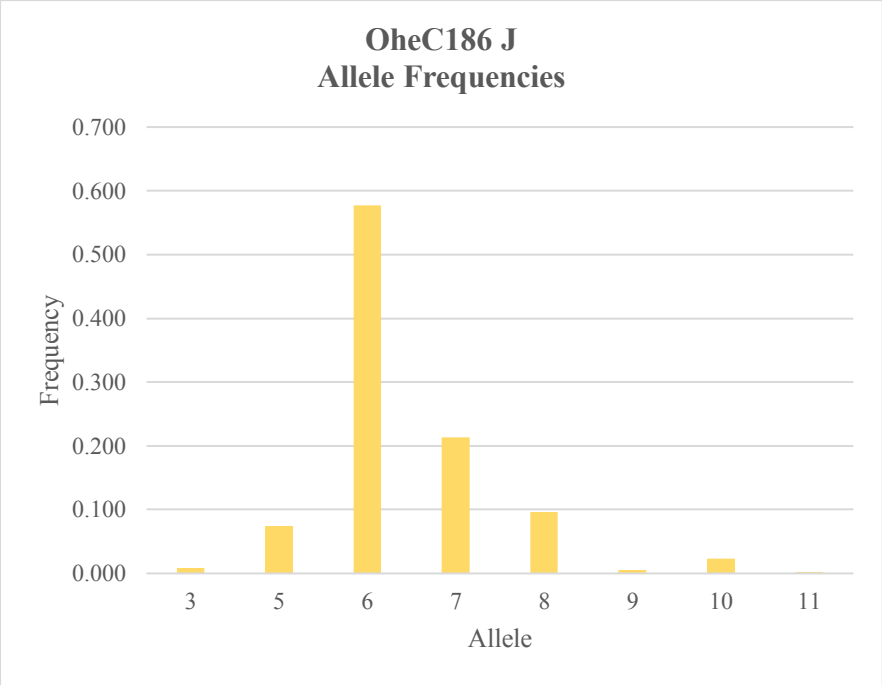


Figure 13. OheC186 J Allele Frequencies

OheC186 J

Allele	Frequency
3	*0.008*
5	*0.075*
6	*0.577*
7	*0.214*
8	*0.096*
9	*0.005*
10	*0.023*
11	*0.002*

Table 14. OheC186 J Allele Frequencies. Those italicized were included in allelic ladder.

For locus OheC186J all alleles were included in allelic ladder.

While alleles 5-8 are the most common, 3, 9, 10 and 11 were included as well to cover the whole allele range.

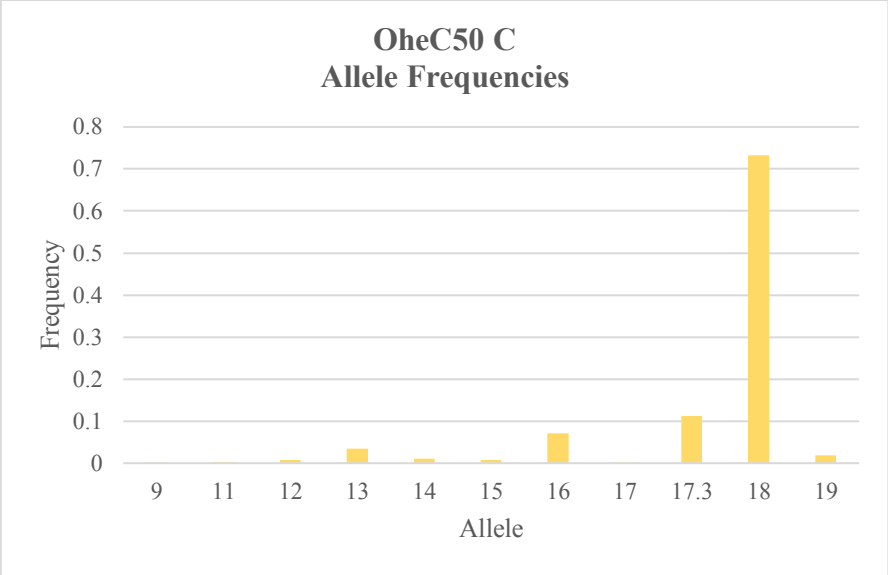


Figure 14. OheC50 C Allele Frequencies

OheC50 C

Allele	Frequency
9	*0.0017*
11	*0.0020*
12	*0.0076*
13	*0.0351*
14	*0.0106*
15	*0.0079*
16	*0.0708*
17	*0.0003*
17.3	*0.1122*
18	*0.7326*
19	*0.0192*

Table 15. OheC50 C Allele Frequencies. Those italicized were included in allelic ladder.

For locus OheC50 C, all alleles were included in the allelic ladder. While allele 18 is the most common with a frequency of 0.7326, all the other alleles were included to provide allele over the whole range. Allele 17.3 was included even though it is a microvariant because it was the second most common allele. In addition, allele 10.1 will be included due to prevalence as noted in Mule Deer populations by the California Department of Fish and Wildlife Forensic Lab.

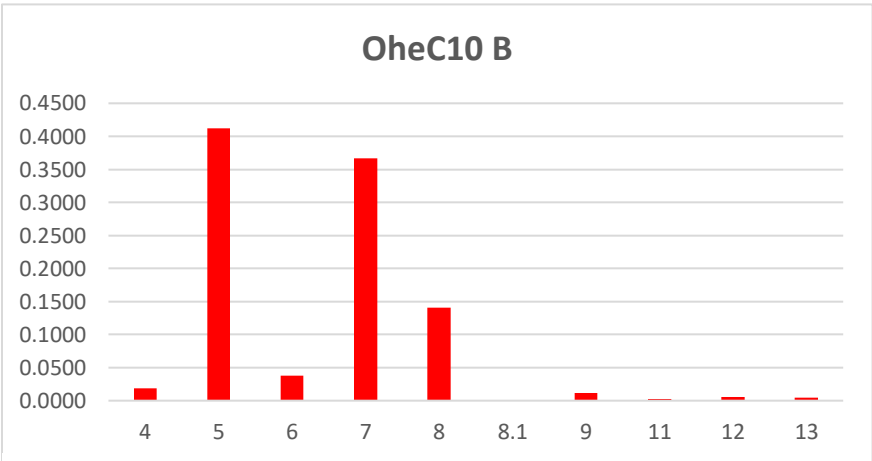


Figure 15. OheC10 B Allele Frequencies

OheC10 B

Allele	Frequency
4	*0.0188*
5	*0.4118*
6	*0.0378*
7	*0.3668*
8	*0.1405*
8.1	0.0007
9	*0.0112*
11	*0.0023*
12	*0.0056*
13	*0.0046*

Table 16. OheC10 B Allele Frequencies. Those italicized were included in allelic ladder.

For locus OheC10 B, all whole tetranucleotide repeats were included in allelic ladder. Alleles 9, 11 and 12 were the most common, and the rest were included to cover the allele range. Allele 12.1 was not included due to is being a microvariant and the least common allele.

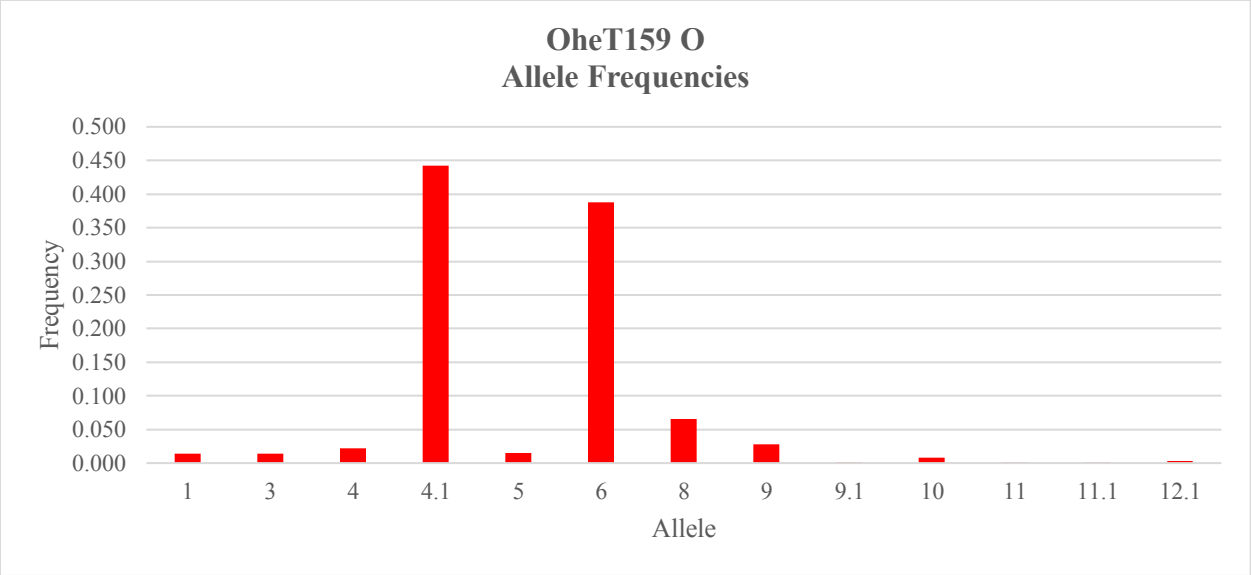


Figure 16. OheT159 O Allele Frequencies

OheT159 O

Allele	**Frequency**	Allele	**Frequency**	Allele	**Frequency**
1	*0.014*	***6***	*0.387*	***10***	*0.008*
3	*0.014*	***8***	*0.065*	***11***	*0.000*
4	*0.022*	***9***	*0.028*	***11.1***	*0.001*
4.1	*0.442*	**9.1**	0.001	***12.1***	*0.003*
5	*0.015*				

Table 17. OheT159 O Allele Frequencies. Those italicized were included in allelic ladder.

For locus OheT159 O, all alleles were included in the allelic ladder. While 4.1 and 6 were the most common, all the tetranucleotide repeats were included to cover the allele range. Despite being a microvariant allele, 4.1 was the most common so it was included. Alleles 11.1 and 12.1 were not as frequent microvariants but there were included due to being the largest alleles to bracket the range.

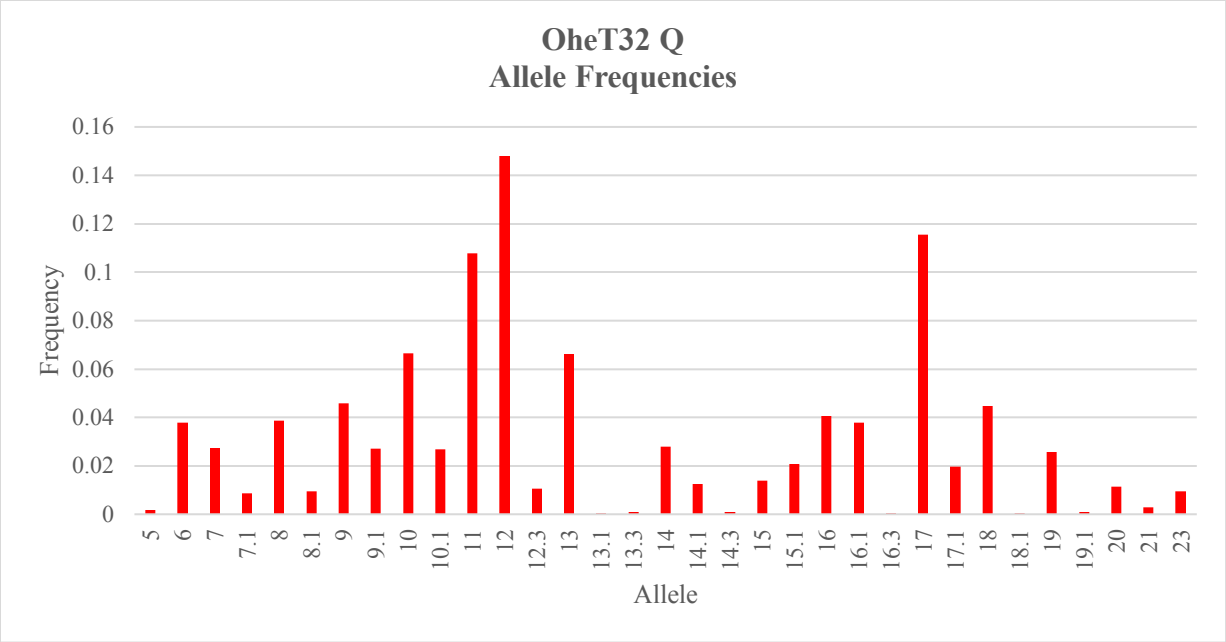


Figure 17. OheT32 Q Allele Frequencies

OheT32 Q

Allele	Frequency	Allele	Frequency	Allele	Frequency
5	*0.0017*	***12***	*0.1481*	***16.1***	*0.0379*
6	*0.0379*	**12.3**	0.0107	**16.3**	0.0003
7	*0.0275*	***13***	*0.0661*	***17***	*0.1154*
7.1	0.0086	**13.1**	0.0003	***17.1***	*0.0196*
8	*0.0386*	**13.3**	0.0010	***18***	*0.0448*
8.1	0.0096	***14***	*0.0279*	**18.1**	0.0003
9	*0.0458*	**14.1**	0.0124	***19***	*0.0258*
9.1	0.0272	**14.3**	0.0010	**19.1**	0.0010
10	*0.0665*	***15***	*0.0138*	***20***	*0.0114*
10.1	*0.0269*	***15.1***	*0.0207*	***21***	*0.0028*
11	*0.1078*	***16***	*0.0406*	***23***	*0.0096*

Table 18. OheT32 Q Allele Frequencies. Those italicized were included in allelic ladder.

For locus OheT31 Q, all whole number tetranucleotide repeats were included in allelic ladder. Alleles 9.1, 10.1, 15.1, 16.1 and 17.1 were also included due to being the most frequent of the microvariant alleles and to provide a spread of the microvariants across the range.

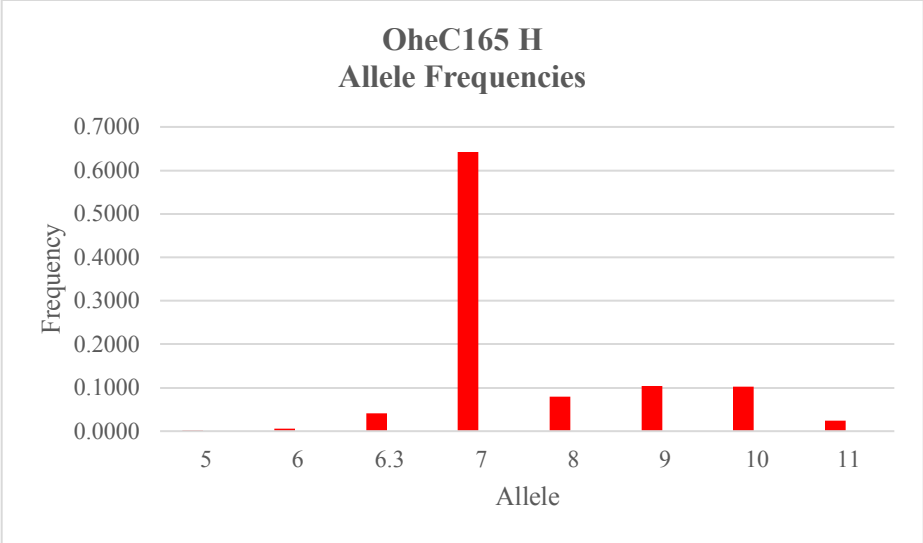


Figure 18. OheC165 H Allele Frequencies

OheC165 H

Allele	Frequency
5	*0.0003*
6	*0.0057*
6.3	*0.0415*
7	*0.6427*
8	*0.0800*
9	*0.1039*
10	*0.1022*
11	*0.0236*

Table 19. OheC165 H Allele Frequencies. Those italicized were included in allelic ladder.

For locus OheC165 H, all allele were included in allelic ladder. Allele 7 was the most common, but the other whole number tetranucleotide repeats were included to provide alleles across the whole range. Allele 6.3 was included because while it was the only microvariant, it was still more common than three of the whole tetranucleotide repeats included in the ladder.

For the construction of the allelic ladder samples were chosen for each locus that had alleles with similar RFU values. When possible, heterozygotes where chosen when possible to cover as many of the alleles of the locus as possible and homozygotes filled in any additional alleles included in the ladder. The samples chosen and corresponding RFU values can be found in Appendix IV. An allelic ladder for OheT256 V was first constructed following the protocol. A diagram to help illustrate this can be found in Appendix I. Figure 17 shows the electropherogram for the completed locus OheT256 V allelic ladder.

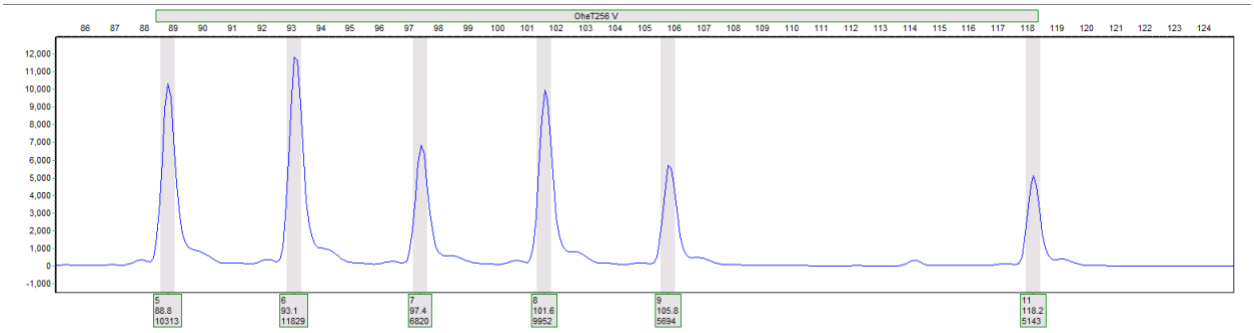


Figure 19. OheT256 V Allelic Ladder

The next step was creating an allelic ladder for four loci, one of each fluorescent dye. These four loci were OheT256 V, OheC143 F, OheC186 J, and OheC10 B. Figures 18-21 shows the electropherogram for these four completed locus allelic ladders.

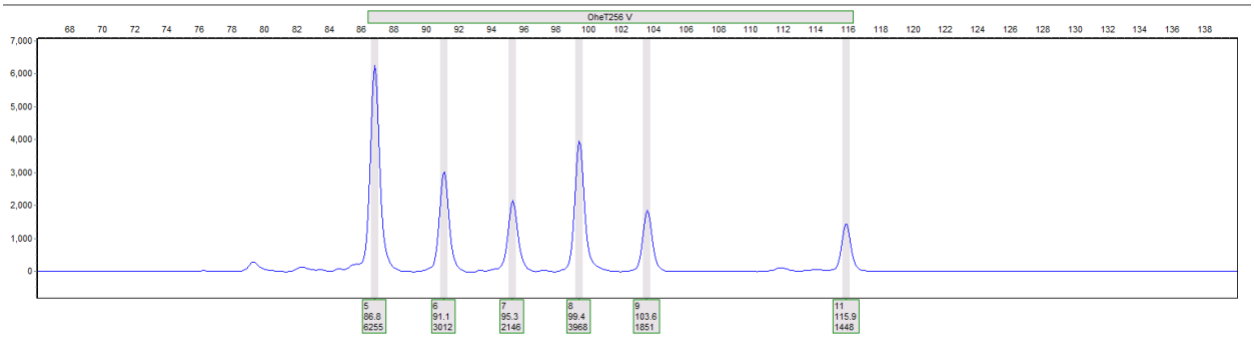


Figure 20. OheT256V Allelic Ladder from 4-Plex

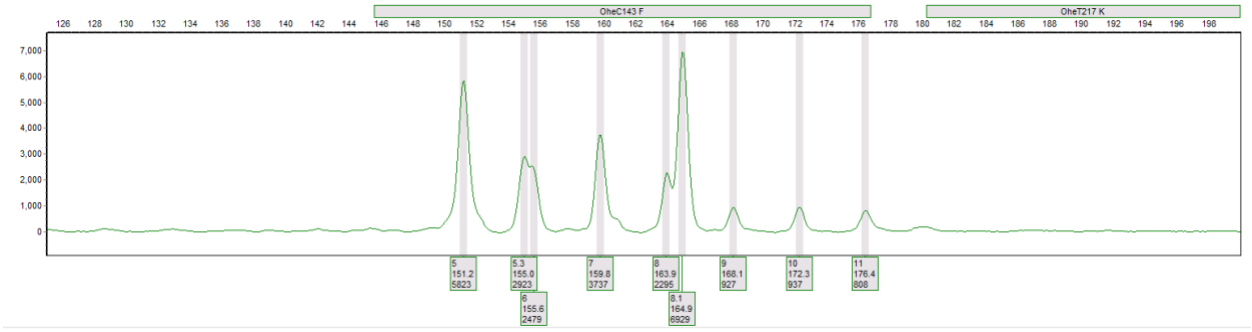


Figure 21. OheC143 F Allelic Ladder from 4-Plex

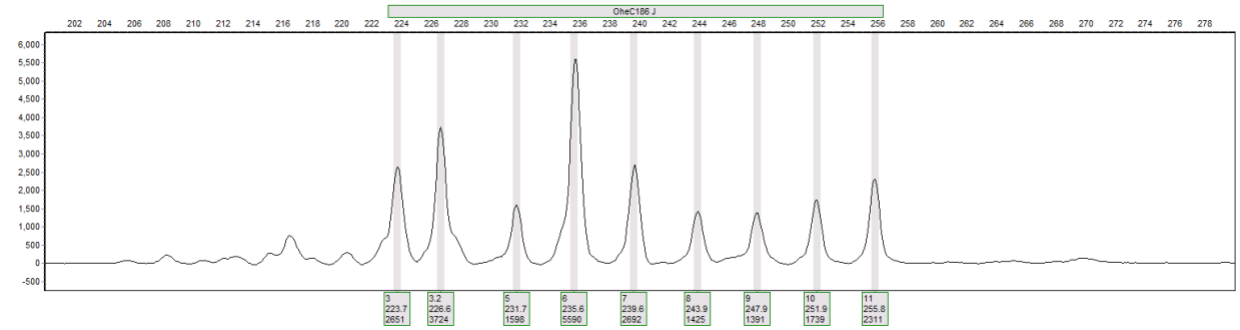


Figure 22. OheC186 J Allelic Ladder from 4-Plex

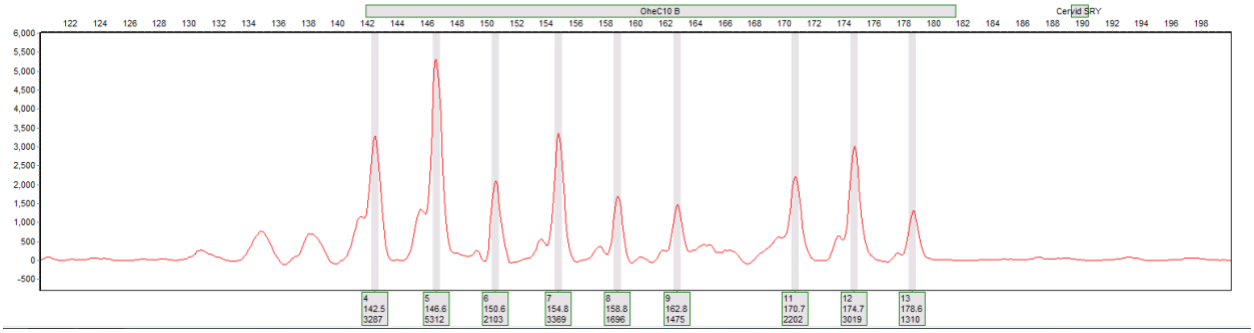


Figure 23. OheC10 B Allelic Ladder from 4-Plex

The final step was to create an allelic ladder of all the loci of Odoplex. Figures 22-25 show the electropherograms for the completed allelic ladder.

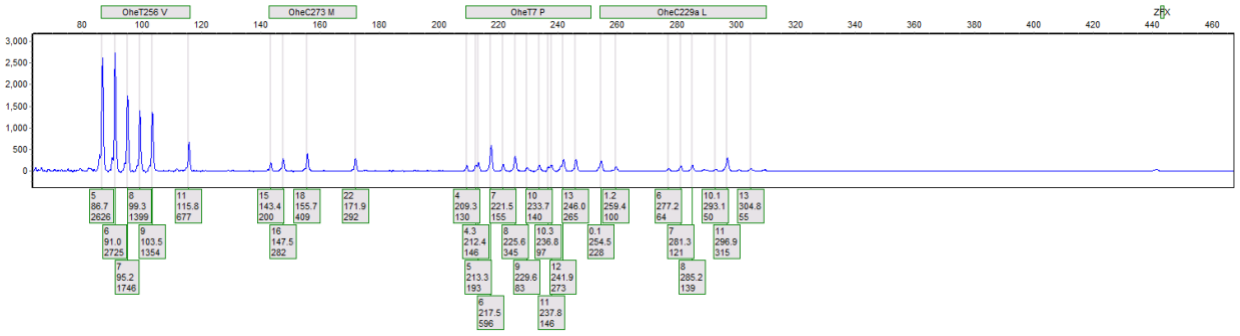


Figure 24. Allelic Ladder for 6-FAM

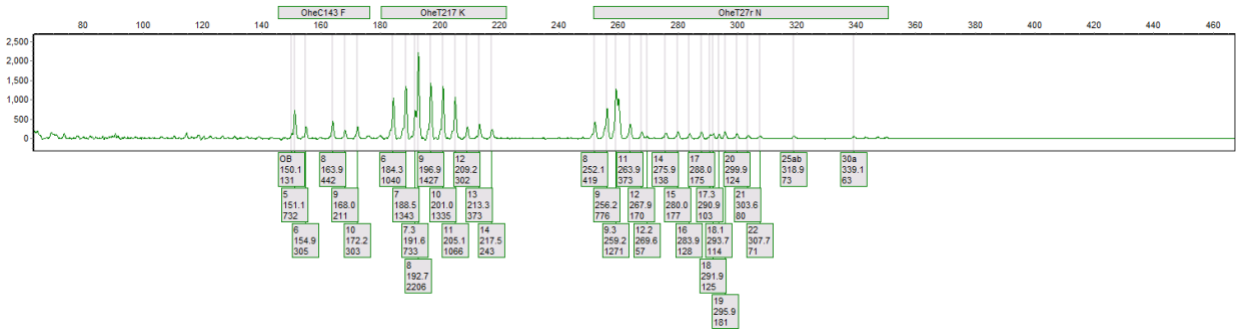


Figure 25. Allelic Ladder for VIC

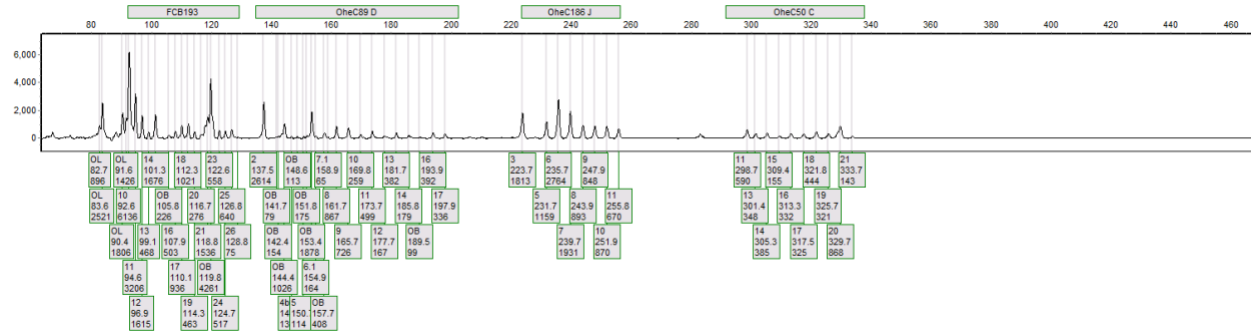


Figure 26. Allelic Ladder for NED

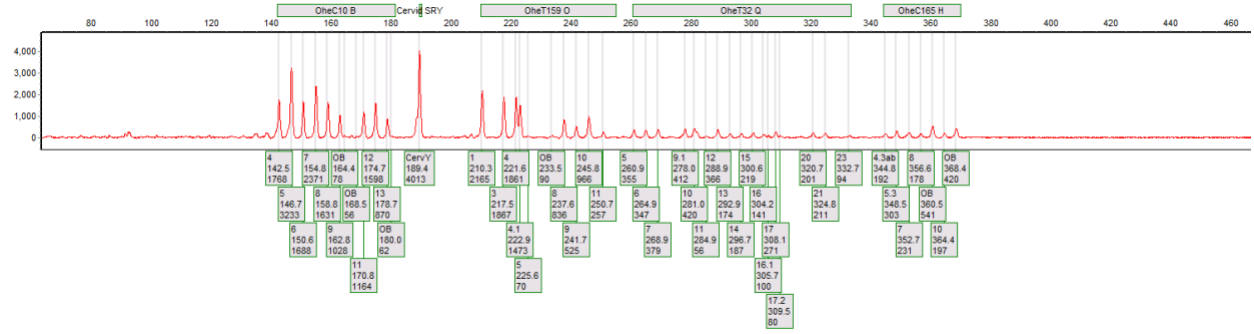


Figure 27. Allelic Ladder for PET

CHAPTER IV

Discussion

The aim of this research was not only to develop an allelic ladder to go along with Odoplex, but to use a methodology using common wildlife forensic lab techniques. A common technique used to create allelic ladders in previous research such as DogFiler (Wictum et al. 2013) and SkydancerPlex (Van Hoppe et al. 2016) was an agarose gel separation to isolate alleles, a gel purification and then some combination of dilutions and amplifications to get balanced peak heights. For the research into creating an allelic ladder for Odoplex, a 2% agarose test gel was performed. However, this attempt to isolate alleles was not successful. The gel broke apart when transferring it to the UV light box to visualize the alleles. Excising alleles with minimal exposure to the UV light of the light box was a challenge. In addition, it would be a time consuming technique. Multiple gels would have to be run and isolated would require an additional purification step.

Slab gel electrophoresis is a commonly used technique in research labs. It was a technique that was used in making the allelic ladders for multiple species both domestic in the case of DogFiler (Wictum et al. 2013) and wildlife in the case of SkydancerPlex (Van Hoppe et al. 2016). However that does not change the fact that it is not a commonly used technique in wildlife forensic labs. While it would have been possible to troubleshoot the issues of the test gel, the goal of this research was not just to create an allelic ladder, but to develop methodology that was as streamlined as possible and could easily be done in a wildlife forensic lab. Because of this, an alternative methodology that did not include slab gel electrophoresis (LaHood et al. 2002) is a better option.

The LaHood et al. methodology was the starting basis for making the Odoplex allelic ladder, however it still had potential limitations. The allelic ladders were only constructed for a single locus at a time (LaHood et al. 2002). For allelic ladder construction for Odoplex, locus OheT256 V was chosen as a starting point due to it only having seven alleles and no microvariants.

The allelic ladder construction for OheT256 V showed that LaHood's methodology could be applied to Odoplex loci. All alleles were present in the completed allelic ladder. The peak heights were not completely balanced as allele 12 had a peak height of 5143 while allele 6 had a peak height of 11829, a 43% peak height ratio. However, a peak imbalance was also present when looking at the initial singleplex amplifications of the samples, with allele 12 having a peak height of 10630 and allele 6 having a peak height of 18447, a 58% peak height ratio. A solution to this peak height imbalance would be to take RFU values into consideration before combining samples for the dilution step and putting more sample in accordingly for those alleles with lower peak heights.

LaHood et al. constructed an allelic ladder for a locus at a time. This research expanded upon what was done and amplified multiple loci at the same time to streamline the making of an allelic ladder for an entire STR panel. There were two ways this expansion was done. The first was with four loci to see if it was possible to amplify more than one loci at a time. The second was will all the Odoplex loci to see if it was possible to streamline the allelic ladder creation as much as possible.

For the four loci amplification, one locus was selected for each fluorescent dye. OheT256 V was used for the 6-FAM dye as it was already established to have worked. The same criteria that was used in selecting OheT256 V was used for selecting the loci for the other dyes. For the

remaining three fluorescent dyes, the following loci were selected: For VIC, OheC143, for NED OheC186 J and for PET OheC10 B.

The results from this four loci amplification showed that it was possible to amplify multiple loci at the same time in the second step of the methodology. All alleles for all loci were present and all alleles were in at least the 500RFU value range. However, there was a greater peak imbalance within the locus. For OheT256 V, the greatest peak imbalance was between allele 11 at 1448RFUs and allele 5 at 6255RFUs resulting in a 23% peak height ratio, a greater imbalance than when this locus was amplified by itself.

For OheC143 F, the greatest peak imbalance was between allele 8.1 at 6929RFUs and allele 11 at 808RFUs resulting in a 12% peak height ratio. It should be noted that 8.1 will not be included in the allelic ladder as it was discovered after this step that the DNA profiles containing this allele were actually elk that had been mislabeled as *Odocoileus*, but that does not change the fact that there was peak imbalance happening at this locus. Still, just like with OheT256 V there was variation in RFU values that could explain the peak imbalances.

For OheC186 J, the greatest peak imbalance was between allele 6 at 5590RFUs and allele 9 at 1391RFUs resulting in a 25% peak height ratio. In this instance, the peak imbalance is not due to the RFUs of the initial samples being greatly different, but during sample selection, two samples containing allele 6, a sample with a genotype of 6,10 and a sample with a genotype of 6,11, were chosen to be included into the allelic ladder so the not as common alleles 10 and 11 could be included. Because of this, while some of the peak imbalance happening in this locus may be corrected through adjusting how much sample goes into the dilution step, there is not much that can be down to minimize the peak of allele 6 without minimizing alleles 10 and 11

within the ladder as well. This is not a big problem however, as the ladder will still work despite having some imbalanced peaks.

For OheC10 B, the greatest peak imbalance was between allele 5 with 5312RFUs and allele 13 with 1310RFUs resulting in a 25% peak height ratio. Like with OheC186 J, two samples containing allele 5, a sample with a genotype of 5,9 and a sample with a genotype of 4,5, were chosen to be included into the allelic ladder so for this particular peak imbalance, not much can be done.

Besides peak height imbalances, another thing to note is that for OheC186 J and OheC10 B, the base lines had noise in them. For OheC186 J this noise happened in the 208-222bp range. This range is before the locus itself actually begins so it is not necessarily a huge issue, but it would still be preferable if there was a way to reduce this noise. OheC10 B is a completely different issue however. Like with OheC186 J, there is noise before the locus in the 130-140bp range, but there is also lots of noise within the locus itself. The base line has noise in the 164-168 range between the 9 and 11 alleles when all it should be is a flat baseline. In addition, all the peaks appear to have a small peak right before them. They look like they could be the result of incomplete polyadenylation, but was not further investigated. If the possible incomplete polyadenylation showed up again in the subsequent step of the complete Odoplex allelic ladder amplification it would have been investigated, but that turned out to be unnecessary.

The final step was to amplify all the samples for all the loci and combine them into a completed allelic ladder. After amplification, samples were run on CE to confirm that they amplified correctly. However, when reviewing the results, it was determined that some of the samples either did not amplify, or had different genotypes than what was expected. These samples were reamplified to determine if it was an amplification issue in the instances where the

sample did not amplify, if unexpected genotype was an incorrect sample being used in the amplification, or if the genotype was in fact correct. After amplification these samples were run on the CE to confirm their genotypes, but due to instrumentation troubleshooting and timing issues these results were not able to be obtained. Instead these samples were omitted from the final allelic ladder construction.

After the four loci amplification, samples for the remaining loci were more carefully chosen to try and select samples that had similar RFU values. However, there was still peak height differences when looking at the initial RFU values after the singleplex amplifications. To try and minimize this in the final allelic ladder, 1-8uL of a sample was added at the dilution step depending on what was needed to bring it to a similar RFU value as the rest of the alleles within a locus.

When looking at the electropherogram from this final step, while it did work, adjustments still need to be made before the ladder is ready to be used in casework. Despite adjustments in the amount of sample the went into the dilution step, there were still peak imbalance that happened within a locus. It should be noted that this could be due to the fact that the amplifications were spaced out over weeks and by the time the samples were combined for the dilution step, some of the samples had lost volume. Therefore, their DNA concentrations would have not been what was expected based on RFU values.

A bigger problem, however, is the fact that between loci there were some pretty large peak imbalances going on. Just within the 6-FAM dye, the alleles within locus OheT256 V had much greater peak heights than the alleles within locus OheC229a L. The lowest detected peak within locus OheC229a L, allele 10 only had a peak height of 50RFUs while in OheT256 V the lowest peak was at 677RFUs. In addition, OheC229a was missing alleles when it should not

have been, indicating that they did not have high enough peaks to get above the 50RFU analytical threshold when combined with the other loci.

In addition, FCB193 is the only dinucleotide repeat in the panel. Because there was no way to get around the fact that there would be stutter when any of its alleles were amplified samples were chosen in such a way to hope that stutter would fill in the missing alleles. However, this resulted in a very imbalanced locus. The smallest peak was allele 26 with 56RFUs and the greatest peak was allele 22 with 5050RFUs resulting in a peak height ratio of 1%. Due to the nature of this locus, it appears it will never have completely balanced peak heights, but at the same time they need to be much better than a 1% peak height ratio. To get a better balance at this locus, different combinations of alleles should be tried to see which works best in filling in missing gaps, but still keeping imbalanced peaks down to a minimum.

Another possibility for the peak imbalances is the fact it took over a month from the initial amplification of the first group of samples to the final combination and amplification of all the samples together. Samples were stored in the refrigerator during this time, but it is possible that sitting there could have impacted the results of the final allelic ladder. At the same time, the samples from OheT256 V were in that first amplification group and that was one of the better looking loci so it may have not made a difference. Either way, the next step that needs to be taken is for these samples to be amplified and combined again to see if there is consistency in which loci amplify better than others. If there is, then that indicates that there is something about those particular loci that make them more preferential for amplification. One possible thing to try to correct the between loci peak imbalance issue would be to adjust DNA concentrations so there is not as much of the DNA for the loci that preferentially amplify to begin with. This would give the other loci a better chance at amplification. Another possible solution would be to increase the

reaction volume from 10uL to a larger volume such as 25uL. Due to the amount of alleles being amplified in a single reaction compared to a normal profile, it could be that the components of the amplification mix are being used up too quickly. By increasing the amount in the reaction, it would allow for all the alleles to amplify properly.

Despite the major peak imbalances, this last step of combining and amplifying samples from all Odoplex loci in a single reaction shows potential. With some adjustments to create a better balance between peaks, this is a technique can be used to make an allelic ladder for an entire STR panel without having to use gel electrophoresis. This methodology provides wildlife forensic labs with a streamlined process to make the Odoplex allelic ladder, which will allow for the creation of an *Odocoileus* database.

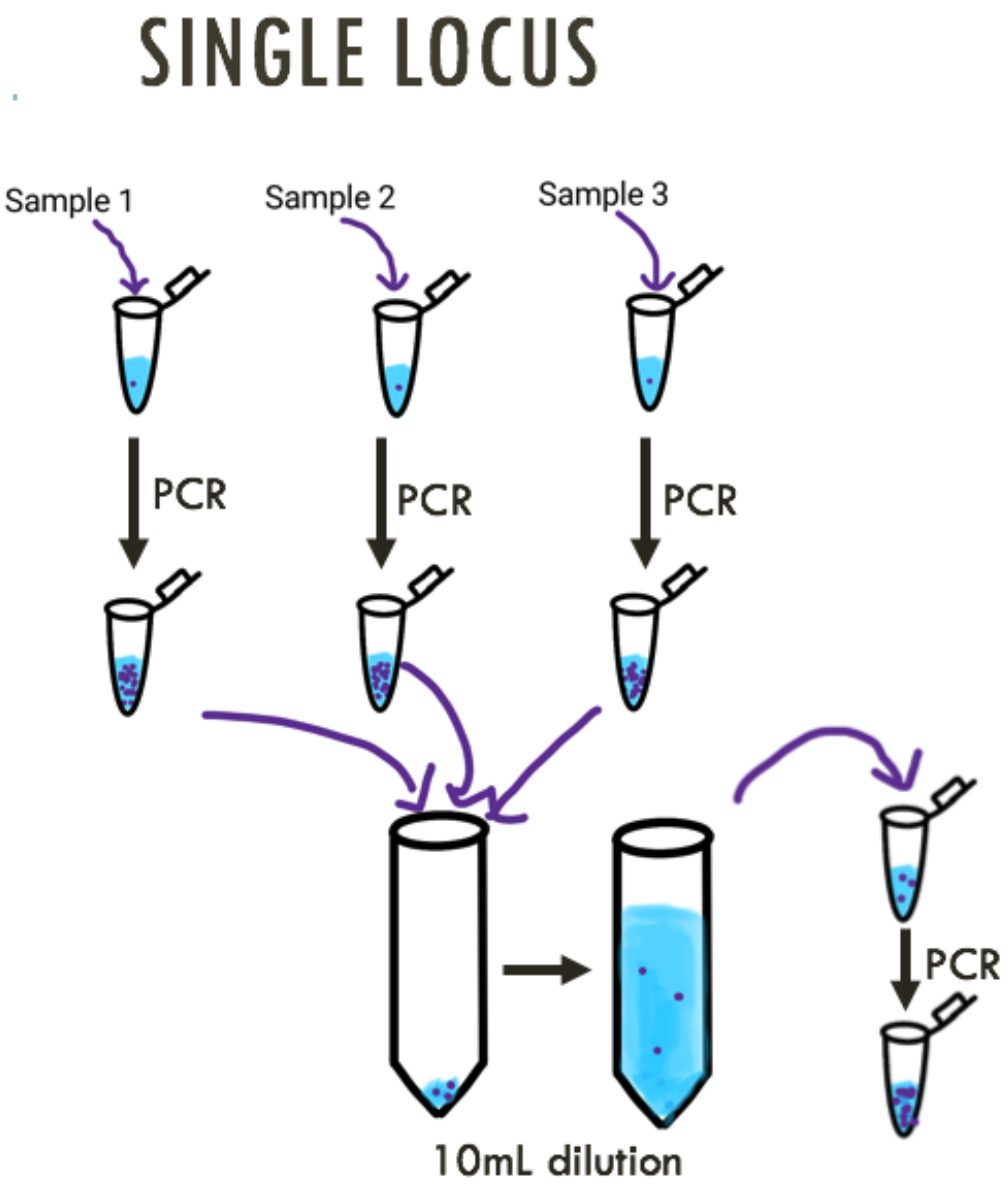
Conclusion

The purpose of this research was to not only construct and allelic ladder for the Odocoileus STR multiplex, Odoplex, but to develop as streamlined of a methodology as possible to do so. While using slab gel electrophoresis to isolate alleles is a technique that can work and has been done in many previous allelic ladder constructions (Wictum et al. 2013, Van Hoppe et al. 2016), this research was able to not only produce an allelic ladder for Odoplex, but develop a methodology that does not require slab gel electrophoresis. The methodology use also streamlined the process as much as possible by amplifying alleles for multiple loci at the same time.

Further research still needs to be done as the allelic ladder created in this research will all Odoplex loci is still not where it needs to be to be used in wildlife forensic casework. While it is possible to amplify all loci at the same time, the peak imbalance between loci is still too large. While some peak imbalance is acceptable, the difference between how well different alleles amplified is so great that some alleles were not detected. Adjustments in amount of DNA going into the reaction or the reaction volume amount are possible solutions to this issue. Once these adjustments have been made and a more balanced allelic ladder can be produced, this methodology will allow for the making of the allelic ladder necessary for an *Odocoileus* DNA database.

APPENDIX I

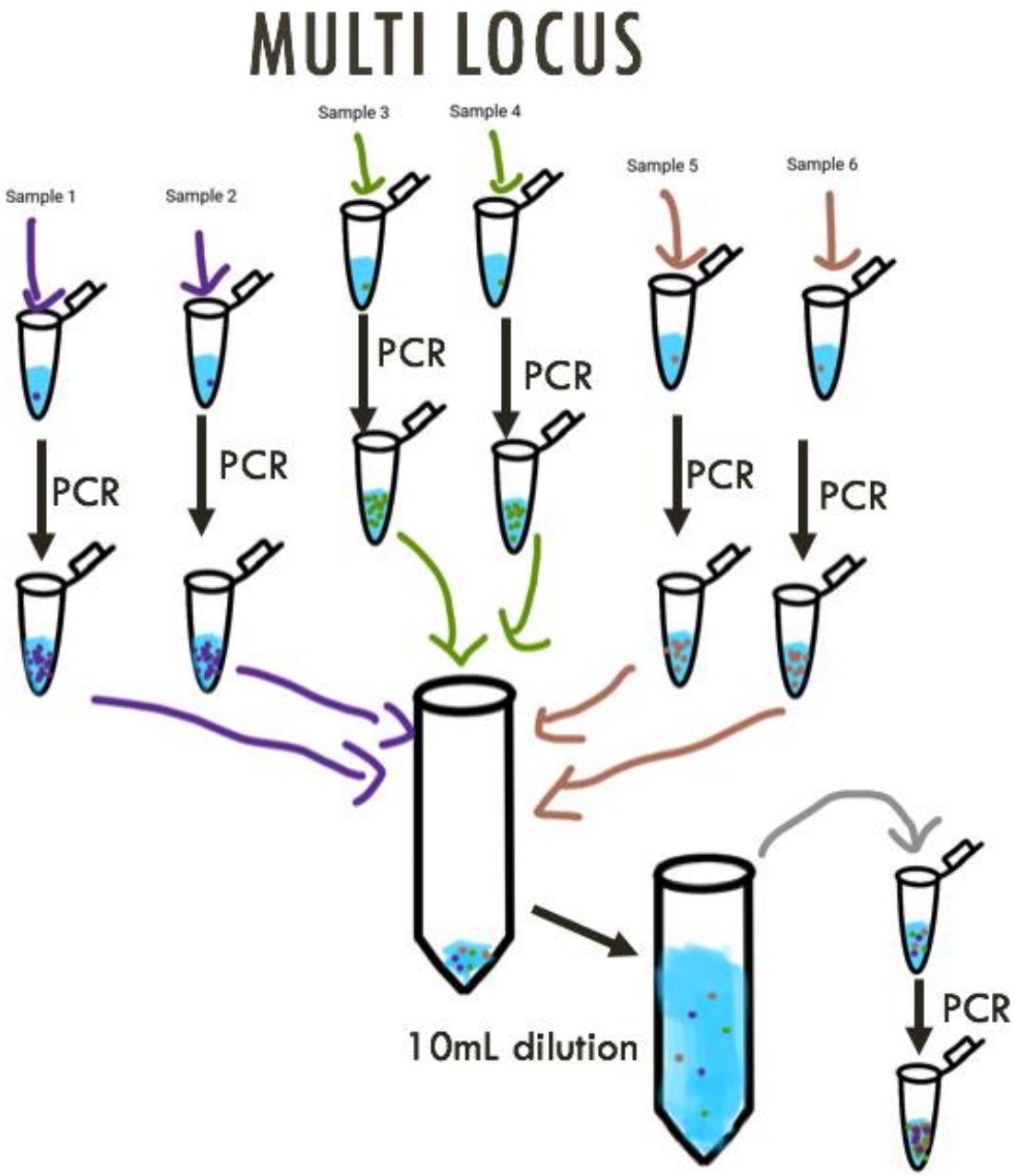
Single Sample Amplification Diagram



APPENDIX II

Multi Locus Amplification Diagram

Note: The diagram only illustrates alleles from three loci being combined, but it is the same concept with any number of loci being amplified and combined together.



APPENDIX III

Breakdown of Deer Samples by Species and State

State	Samples	State	Samples
AL	7	**NC**	47
AR	22	**ND**	21
AZ	22	**NE**	120
CO	52	**NH**	17
CT	16	**NM**	11
DE	34	**NJ**	20
FL	54	**NY**	22
GA	14	**OH**	20
ID	8	**OK**	18
IN	39	**OR**	30
KS	81	**PA**	117
LA	13	**SC**	38
MD	61	**TN**	26
MI	9	**TX**	59
MN	245	**VA**	62
MO	80	**WA**	6
MS	4	**WV**	42
MT	84	**BC**	8

Species	Sample Number
White Tailed Deer	1397
Mule Deer	89
Black Tailed Deer	43

State	Black Tailed	Mule Deer	White Tailed
OR	13	4	13
BC	8	-	-
WA	1	-	5
AZ	21	1	-
CO	-	52	-
KS	-	7	74
MT	-	11	73
NE	-	3	117
NM	-	11	-

APPENDIX IV

Samples selected and RFU values

OheT256 V

Sample	Allele 1	Allele 2	RFU 1	RFU 2
A30213	6	6	18447	18447
A40176	12	12	5976	5976
D30320	7	9	10748	10630
D30324	8	8	17607	17607
D21354	5	5	18800	18800

OheC273 M

Sample	Allele 1	Allele 2	RFU 1	RFU 2
J10464	15	16	5692	6045
H10524	18	22	7518	7965

OheT7 P

Sample	Allele 1	Allele 2	RFU 1	RFU 2
B41111	4	9	3402	2769
G41231	4.3	7	4376	6161
G21260	5	11	6575	6631
K40139	6	13	3344	3718
G41322	8	12	4864	4103
G41252	10	11.3	4561	4538
K40662	10.3	10.3	3850	3850
6351	9.2	-	-	-

OheC299a L

Sample	Allele 1	Allele 2	RFU 1	RFU 2
A40170	9	9.1	2598	2320
G41266	13	14.1	1747	1391
G41077	0.1	7	1832	1439
C20140	1.2	8	2183	1669
C10518	6	10	3381	2224
K40814	11	11	3474	3474
G11201	12	13.1	1124	1058
C2803-4	1.2	-	-	-

OheC143 F

Sample	Allele 1	Allele 2	RFU 1	RFU 2
A40167*	7	7	11810	11810
G20551	9	9	6220	6220
G30553	8	10	4025	3883
D30338	5	5.3	7130	5766
B10571	6	11	4934	3761
K40772*	7	7	5630	5630

*A40167 used in four loci amplification, but K40772 will be replacing it due to closer RFU values

OheT217 K

Sample	Allele 1	Allele 2	RFU 1	RFU 2
G30551	6	11	3763	4136
A11269	7	10	4589	3525
C30318	10	14	6261	5363
J30511	11	13	6031	5392
G41333	7.3	12	7788	5438
G30106	8	9	5147	4218

OheT27rN

Sample	Allele 1	Allele 2	RFU 1	RFU 2
C10265	32.2	32.2	3321	
B41113	17.3	31.3	2534	2730
C10162	14	30.3	8623	7216
C30522	18	29.3	4601	2291
C20720	17	24.3	3071	3552
A40166	13	22.3	6591	5480
G40364	16	22	6858	5747
C30513	12	21	5160	4588
G30853	18.2	20	3596	4401
H10308	11	19	5823	4591
J10517	9	10	4971	5021
K20835	8	15	4682	2763
1320	9.3	-	-	-

FCB193

Sample	Allele 1	Allele 2	RFU 1	RFU 2
G41343	9	22	7609	3174
G41278	11	17	4016	2898
G41328	13	25	4126	2222
K31222	16	20	3936	2539
H10338	19	23	4163	3218
K41055	15	26	7500	2879
J10509	10	10	4398	4398

OheC89 D

Sample	Allele 1	Allele 2	RFU 1	RFU 2
I11009	8	17	9452	7130
G20778	13	16	4458	5585
C20860	2	11	5824	7363
C20724	5	5	3076	3076
K40640	7	12	1887	1220
B41110	6	9	3205	2324
B11113	10	14	5813	4830
I11015	7	15	3539	2665

OheC186 J

Sample	Allele 1	Allele 2	RFU 1	RFU 2
B41119	5	9	3629	3767
C20705	3	7	4211	3667
D21340	3.2	3.2	6455	6455
C20725	7	8	4786	4447
B10578	6	10	3652	3374
B10615	6	11	4137	3459

OheC50 C

Sample	Allele 1	Allele 2	RFU 1	RFU 2
N30536	17.3	17.3	5851	5851
K40607	14	16	7172	6691
C10504	17	18	400	341
N30544	13	19	1746	1919
G30106	12	15	4885	4576
L20460	11	18	4866	4305
G21379	9	12	3916	4328
7344	10.1	16	-	-

OheC10 B

Sample	Allele 1	Allele 2	RFU 1	RFU 2
C20674	5	9	3494	3446
C20713	6	8	3388	3440
C30628	12	12	15660	15660
C20720	4	5	2744	2616
C20715	11	11	3175	3175
A40178	7	13	7732	7279

OheT159 O

Sample	Allele 1	Allele 2	RFU 1	RFU 2
C20138	12.1	12.1	5414	5414
G40123	6	11	9315	8062
A31304	9	11.1	5159	4357
G41302	4.1	10	4536	4670
G30869	1	1	5046	5046
L20458	3	4	6422	6103
C20146	5	5	3226	3226
G20969	8	8	5384	5384

OheT32 Q

Sample	Allele 1	Allele 2	RFU 1	RFU 2
G21020	14	23	3412	2496
G30116	12	21	2826	2014
G30107	10	20	3078	2676
C10176	5	7	3135	2594
B30129	9.1	17	3522	3163
G41245	16.1	17.1	2933	3031
K20812	8	19	1682	1465
A40179	11	18	3352	3249
D11202	9	15.1	2766	2591
C30963	13	16	3545	4041
K40662	6	10.1	2452	2048
C10257	15	15	3681	3681

OheC165 H

Sample	Allele 1	Allele 2	RFU 1	RFU 2
B10431	5	8	3456	3169
G41337	6	6.3	3581	3127
L20420	9	11	3346	3060
A40168	7	10	3086	2867

REFERENCES

Anderson, A. 1974. Growth and morphometry of the carcass, selected bones, organs, and glands of mule deer. Wildlife Monographs 39:3–122.

Anderson, A. E., and O. C. Wallmo. 1984. Odocoileus heminous. The American Society of Mammologists 219:1–9.

Anderson, J. D., R. L. Honeycutt, R. A. Gonzales, K. L. Gee, L. C. Skow, R. L. Gallagher, D. A. Honeycutt, and R. W. Deyoung. 2002. Development of Microsatellite DNA Markers for the Automated Genetic Characterization of White-Tailed Deer Populations. Source: The Journal of Wildlife Management 66187:67–74.

Bishop, M., M. Parsons, V. Lacaille, and S. Lotx. 1994. A genetic linkage map for cattle. Molecular Ecology 136:619–639.

Blackard, J. 1971. Restoration of the white-tailed deer to the southeastern United States. Louisiana State University.

Brezinsky, L., J. Kemp, and J. Teale. 1993. Five polymorphic bovine microsatellites. Animal Genetics 24:75.

Buchanan, F., and A. Crawford. 1993. Ovine microsatellites at the OarFCB11, OarFCB128, OarFCB193, OarFCB266 and OarFCB304 loci. Animal Genetics 24:145.

Budowle, B., T. R. Moretti, S. J. Niezgoda, and B. L. Brown. 1998. CODIS and PCR-Based Short Tandem Repeat Loci: Law Enforcement Tools. Pages 73–78 *in*. Proceedings of the Second European Symposium on Human Identification. Madison, WI.

Burgos, G., T. Restrepo, A. Ibarra, A. Gaviria, G. Machado, C. Mora, and R. Lizarazo. 2015. Easy and fast procedure to isolate, purify and immortalize DNA fragments for allelic ladders construction. Forensic Science International: Genetics Supplement Series 5:e656–

e658.

Dassmann, R. 1954. cology and social behavior of a population of the Columbian black-tailed deer. Univeisty of California.

Dawnay, N., R. Ogden, R. S. Thorpe, L. C. Pope, D. A. Dawson, and R. McEwing. 2008. A forensic STR profiling system for the Eurasian badger: A framework for developing profiling systems for wildlife species. Forensic Science International: Genetics 2:47–53.

DeYoung, R. W., S. Demarais, R. L. Honeycutt, A. P. Rooney, R. A. Gonzales, and K. L. Gee. 2003. Genetic consequences of white-tailed deer (Odocoileus virginianus) restoration in Mississippi. Molecular Ecology 12:3237–3252.

FBI. 2017. National DNA Index System (NDIS) Operational Procedures Manual Table of Contents Introduction Quality Assurance Standards - Federal DNA Identification Act Adding or Changing Information about a Laboratory.

Fujii, K., K. Sekiguchi, K. Shimizu, and K. Kasai. 2004. A new sequenced allelic ladder marker for D1S80 typing. Journal of Human Genetics 49:169–171.

Fulbright, R., and J. Ortega-S. 2006. White-tailed deer habitat: ecology and management on rangelands. A&M University Press, College Station, TX.

Geist, V. 1981. Behavior: adaptive strategies. Page 605 *in* O. Wallmo, editor. Mule and black-tailed deer of North America. University of Nebraska Press, Lincoln.

Geist, V. 1998. White-tailed deer and mule deer. In: Deer of the world: Their evolution, behaviour, and ecology. Stackpole Books, Mechanicsburg, PA.

Glock, B., D. W. M. Schwartz, E. M. Schwartz-Jungl, and W. R. Mayr. 1995. Forensic Science I Sequence determination of an allelic ladder for the STR polymorphism at the CD4 locus and application of the ladder in testing an Austrian Caucasian population sample. Forensic

Science International 78:325–30.

Guthrie, R. 1971. A new theory of mammalian rump patch evolution. Behaviour 38:132–145.

Hamlin, K., and R. Mackie. 1989. Mule deer in the Missouri River Breaks, Montana: A study of population dynamics in a fluctuating environmen. Helena, MT.

Hanley, T. 1984. Relationships between Sitka black-tailed deer and their habitat. Portland, OR.

Hauge, X. Y., and M. Litt. 1993. A study of the origin of 'shadow bands' seen when typing dinucleotide repeat polymorphisms by the PCR. Human Molecular Genetics 2:411–415. Narnia.

Van Hoppe, M. J. C., M. A. V. Dy, M. Van Den Einden, and A. Iyengar. 2016. SkydancerPlex: A novel STR multiplex validated for forensic use in the hen harrier (Circus cyaneus). Forensic Science International: Genetics 22:100–109. Elsevier Ireland Ltd.

Hsu, T. C., and K. Benirschke. 1967. An Atlas of Mammalian Chromosomes. Vol. 1. T. C. Hsu and Kurt Benirschke. Springer-Verlag,. Science. Vol 1. Springer-Verlag, New York.

Hygnstrom, S. E., S. R. Groepper, K. C. Vercauteren, C. J. Frost, J. R. Boner, S. E. ; Hygnstrom, S. R. ; Groepper, K. C. ; Vercauteren, C. J. ; Frost, J. R. ; Boner, T. C. ; Kinsell, and G. M. Clements. 2008. Literature review of mule deer and white-tailed deer movements in western and midwestern lanscapes. Great Plains Research: A Journal of Natural and Social Sciences 18:962.

J. A. DeWoody, R. L. Honeycutt, L. C. S. 1995. Microsatellite markers in white-tailed deer. Journal of Heredity 1:1994–1996.

Jones, K. C., K. F. Levine, and J. D. Banks. 2000. DNA-based genetic markers in black-tailed and mule deer for forensic applications. California Fish and Game 86:115–126. California Department of Fish and Game, Monterey.

Knapp, E. E., and P. G. Connors. 1999. Genetic consequences of a single-founder population bottleneck in Trifolium amoenum (Fabaceae). American Journal of Botany 86:124–130. Wiley.

LaHood, E., P. Moran, J. Olsen, S. Grant, and L. Park. 2002. Microsatellite allele ladders in two species of Pacific salmon : preparation and field-test results. Molecular Ecology 2:187–190.

Linacre, A., L. Gusmao, W. Hecht, A. P. Hellmann, W. R. Mayr, W. Parson, M. Prinz, P. M. Schneider, and N. Morling. 2011. ISFG: Recommendations regarding the use of non-human (animal) DNA in forensic genetic investigations. Forensic Science International: Genetics 5:501–505.

Long, E. S., D. R. Diefenbach, C. S. Rosenberry, and B. D. Wallingford. 2008. Multiple proximate and ultimate causes of natal dispersal in white-tailed deer. Behav Ecol 19:1235–1242.

Lorenzini, R. 2005. DNA forensics and the poaching of wildlife in Italy: A case study. Forensic Science International 153:218–221.

Lygo, J. E., P. E. Johnson, D. J. Holdaway, S. Woodroffe, C. P. Kimpton, P. Gill, J. P. Whitaker, and T. M. Clayton. 1994. The validation of short tandem repeat (STR) loci for use in forensic casework. International Journal of Legal Medicine 107:77–89.

Mackie, R., J. Kie, D. Pac, and K. Hamlin. 2003. Mule deer (Odocoileus hemionus). Pages 889–905 *in*. Wild mammals of North America: Biology, management, and conservation. Second edition. John Hopkins University Press, Baltimore, MD.

McShea, W., and G. Schwede. n.d. Variable acorn crops: responses of white-tailed deer and other mast consumers. Journal of Mammalogy 74:999–1006.

Moore, S., W. Barendse, T. Berger, S. Armitage, and D. Hetzel. 1992. Bovine and ovine DNA

microsatellites from the EMBL and GenBank databases. Animal Genetics 23:463–467.

Moretti, T. R., A. L. Baumstark, D. A. Defenbaugh, K. M. Keys, A. L. Brown, and B. Budowle. 2001. Validation of STR typing by capillary electrophoresis. Journal of forensic sciences 46:661–76.

Norris, R. 2017. Exonerated: A History of the Innocence Movement. New York University Press, New York.

Peakall, R., and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population software for teaching and research. Molecular Ecology Notes 6:288–295.

Peakall, R., and P. E. Smouse. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28:2537–2539.

Rogers, L., J. Mooty, and D. Dawson. 1981. Foods of white-tailed deer in the Upper Great Lakes Region -- a review. St. Paul, MN.

Sauer, P. 1984. Physical Characteristics of white-tailed deer. Pages 73–80 *in* L. Halls, editor. White-tailed deer: ecology and management. Stackpole Books, Harrisburg, PA.

Smith, W. 1991. Odocoileus virgianus. Mammalian Species 288:1–13.

Szabolcsi, Z., B. Egyed, P. Zenke, Z. Padar, A. Borsy, V. Steger, E. Pasztor, S. Csanyi, Z. Buzas, and L. Orosz. 2014. Constructing STR multiplexes for individual identification of hungarian red deer. Journal of Forensic Sciences 59:1090–1099.

Vaiman, D., R. Osta, D. Mercier, C. Grohs, and H. Levezeil. 1992. Characterization of five new bovine dinucleotide repeats. Animal Genetics 537–541.

De Valk, H. A., J. F. G. M. Meis, S. Bretagne, J. . Costa, B. A. Lasker, S. A. Balajee, A. C. Pasqualotto, M. J. Anderson, L. Alcázar-Fuoli, E. Mellado, and C. H. W. Klaassen. 2009. Interlaboratory reproducibility of a microsatellite-based typing assay for Aspergillus

fumigatus through the use of allelic ladders: proof of concept. Clinical Microbiology and Infection 15:180–187.

Wang, L., X. C. Zhao, J. Ye, J. J. Liu, T. Chen, X. Bai, J. Zhang, Y. Ou, L. Hu, B. W. Jiang, and F. Wang. 2014. Construction of a library of cloned short tandem repeat (STR) alleles as universal templates for allelic ladder preparation. Forensic Science International: Genetics 12:136–143.

Wictum, E., T. Kun, C. Lindquist, J. Malvick, D. Vankan, and B. Sacks. 2013. Developmental validation of DogFiler, a novel multiplex for canine DNA profiling in forensic casework. Forensic Science International: Genetics 7:82–91.