A STR Multiplex for Columbian Black-Tailed Deer

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INTRODUCTION:

Noninvasive genetic sampling is a powerful approach to obtaining DNA through a variety of sources such as hair, saliva, shed skin, and feces, without requiring disturbance of wildlife. Throughout the nuclear genome, short tandem repeat (STR) markers (also known as "microsatellites") are abundant and are often sufficiently polymorphic to perform individual identification, parentage, relatedness, gene mapping, and other applications requiring high-resolution, Mendelian genetic profiles (Butler 2005). Noninvasively collected samples typically yield low-quantity and highly fragmented DNA, requiring assays that make efficient use of available genetic material. Time and cost are limiting factors, which necessitates development of a multi-marker assay ("multiplex") with all loci amplified in a single polymerase chain reaction (PCR). The multiplex assay must be composed of a suite of markers with sufficiently high polymorphism to resolve identity between different individuals, even closely related ones. Inclusion of Y-chromosome markers also can enable sex determination. We created and optimized such a multiplex assay to identify individual Columbian black-tailed Deer (*Odocoileus hemionus columbianus*) from noninvasively collected DNA. This assay is to be used for genetic capture-mark–recapture studies to estimate herd sizes based on deer pellets collected along transects (see Lounsberry et al. poster).

METHODS:

Our approach was (1) use high-quality DNA extracted from deer tissue to screen markers for polymorphism, (2) design the multiplex, (3) conduct efficacy tests on DNA diluted to a range of known concentrations, (3) test the multiplex on DNA derived from scat, and (4) quantify genotyping error and assay performance.

RESULTS:

Multiplex panel design

We initially screened 24 STR primer pairs originally developed for cattle, domestic sheep, deer (*Odocoileus* spp.), or other cervids. From these, we selected 10 loci and a sex marker based on successful amplification, size distribution of the alleles, polymorphism, and primer compatibility according to predictions of the Autodimer program (Ruitberg 2001). Fluorescent tags were added to the 5' ends of the forward primers to distinguish loci with overlapping allele size ranges (Figure. 1). Observed numbers of alleles ranged from 2 (ADCYC) to 12 (SBTD04 & ETH152) per locus in the 32 deer that were sampled.

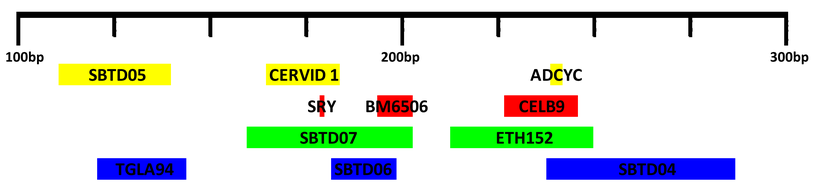

Figure 1. Fluorescent dye label colors, blue, (FAM), green (VIC), red (PET), and yellow (NED), relative to PCR product size ranges, illustrating how 10 STR and 1 sex marker were combined in a single multiplex reaction.

DNA samples were amplified using a Qiagen Multiplex PCR Kit. Products were electrophoretically separated using an ABI 3730 Genetic Analyzer and analyzed using the software STRand (Veterinary Genetics Laboratory, UC Davis) to determine genotypes (Figure 2). Allele sizes are called relative to the Genescan 500-LIZ size standard that is incorporated into the genotyping process.

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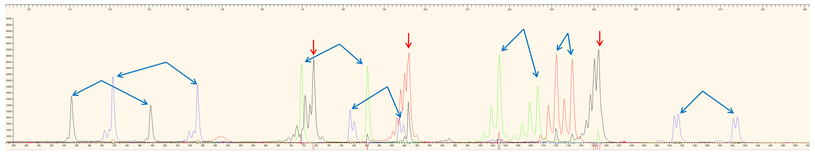

Figure 2. Electropherogram of the multiplex genotype of a (female) Columbian black-tailed deer. The X-axis measures the allele size in base pairs. The Y-axis measures the intensity of fluorescent peaks in relative fluorescent units. Arrows indicate homozygous (red) and heterozygous (blue) loci.

Multiplex Panel Performance Evaluation:

Tissue and scat DNA samples were quantified using real-time qPCR (Lindquist et al, 2011). In a prototypic multiplex, we saw that majority of the scat samples amplified at ≥8 loci at DNA template concentrations greater than 200pg/ul (Figure 3).

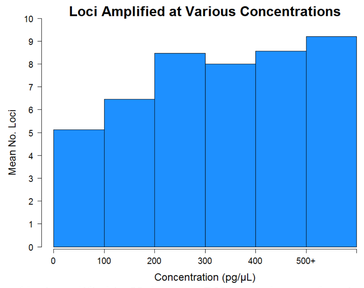

Figure 3. Number of loci amplified in 60 deer scat DNA samples as a function of DNA concentration

We used serially diluted tissue-extracted DNA to test the sensitivity of the multiplex at lower template DNA concentrations. Full genotyping profiles were attainable with DNA concentrations as low as 100 pg/ul of high quality DNA(Figure 4). The multiplex was designed to target 60-85% of the concentrations found within a representative sample of deer fecal DNA samples.

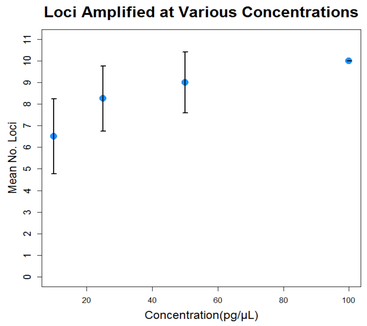

Figure 4. Multiplex sensitivity in terms of average (+/-1 SD) number of loci amplified among 4 DNA samples.

The sex marker (SRY) was detectable at concentrations as low as 10 pg/µl and was among the last loci to drop out in our sensitivity studies. This marker was able to correctly distinguish between all known male and female individuals in which the males displayed a 179 bp fragment while females did not (Figure 5). Depending on the sex ratio, including the SRY sex marker would increase the discriminatory power of this multiplex panel up to 2x.

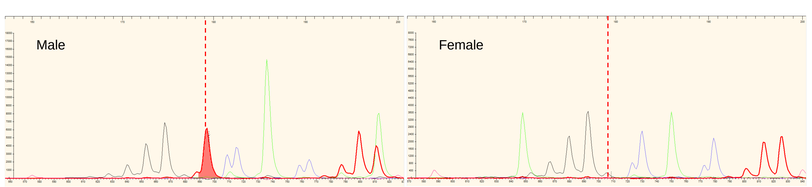

Figure 5. Partial view of multiplex STR genotypes, indicating SRY amplification in male (left) but not female (right) deer. A vertical dashed line indicates location where peak (SRY) is expected in males but not females.

Genetic Diversity and Genotyping Error Rates:

Scat samples were replicated to assess genotyping error rates for each STR locus (Table 1). Errors attributed to false alleles (FA) or allelic dropouts (AD) were estimated based on consensus genotypes. Genetic diversity and probability of identity [P(ID)] of the STR DNA loci were evaluated with Microsatellite Toolkit (Park 2001) and Genecap (Wilberg 2004), respectively. With the Hardy-Weinberg probability of identity [HW P(ID)] being 3×10^{-11}, and more conservatively, the P(ID)Sibs (Waits et al, 2001) values being 0.0001, this assay can be used to identify individuals with high confidence. Generally, P(ID)Sibs values between 0.001-0.0001 are sufficiently low and can be applied to forensic applications in natural populations (Waits et al 2001). Examination of pairwise linkage disequilibrium revealed no significant deviations from linkage equilibrium among the 10 STR loci after applying the sequential Bonferroni test (Rice 1989). Only two loci were found to deviate significantly from Hardy-Weinberg Equilibrium, CERVID1 and SBTD05, with an F_{IS} (inbreeding coefficient) of 0.358 and 0.469 respectively. Both loci showed large heterozygote deficiencies (Table 1), but low AD rates (i.e., nonspecific allelic dropout), suggesting the possible presence of null alleles (consistent dropout of particular alleles) or substructure within the sample. Nevertheless, these highly polymorphic loci can be used to distinguish between even closely related deer in the population.

Table 1. Characteristics of the 10 STR markers. Genotyping error rates based on replicate (n =4) genotyping of 47 scat samples. Genetic Diversity evaluated using 32 Columbian black-tailed deer tissue samples (25 F, 7 M).

	Genotyping Error Rate		Genetic Diversity					
Locus	AD	FA	P(ID)Sibs	HW P(ID)	H_e	H_o	P-Value	PIC
ADCYC±	0.017	0.000	0.594	0.375	0.507	0.531	1.000	0.375
BM6506+	0.025	0.004	0.449	0.158	0.692	0.781	0.488	0.625
CELB9^	0.030	0.000	0.364	0.061	0.816	0.656	0.022	0.781
CERVID1§	0.020	0.000	0.372	0.069	0.804	0.516	0.000	0.765
ETH152"	0.028	0.000	0.320	0.029	0.888	0.719	0.038	0.861
SBTD04*	0.020	0.000	0.321	0.029	0.887	0.906	0.330	0.860
SBTD05*	0.011	0.004	0.360	0.062	0.824	0.438	0.000	0.785
SBTD06*	0.004	0.000	0.451	0.159	0.689	0.563	0.194	0.623
SBTD07*	0.018	0.000	0.346	0.049	0.846	0.969	0.908	0.812
TGLA94^	0.004	0.000	0.546	0.297	0.565	0.531	0.229	0.456
Mean	0.018	0.001	0.0001	3.00E-11	0.752	0.661		0.694

Although genotyping errors were common in a single replicate of a multilocus genotype, consensus genotypes based on ≥3 replicates are expected to have very low error. For example, 3 replicates ensures a probability < 0.0005 of 2 samples from the same individual being falsely considered to be two distinct individuals (Figure 6). Thus, a replicated assay well suited for individual identification.

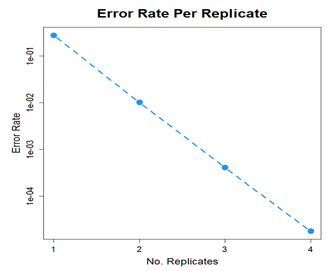

Figure 6. Frequency of incorrectly genotyping scat DNA as a function of PCR replications.

CONCLUSION:

This 5 dye STR multiplex system is to be used for individualizing Columbian black-tailed deer. This multiplex was designed to fit 10 polymorphic markers and a sex marker in a single reaction tube. Primer pairs successfully produced amplicons ranging from 100 – 300 base pairs in length and the sex marker was able to correctly identify male and female individuals. This suite of STR markers is sufficient to differentiate between closely related individuals, enabling use in genetic mark-recapture estimation of population abundance. This assay can serve as an important tool for monitoring ungulate populations where obtaining population sizes through direct observation is challenging. Moreover, the use of a standard panel to genotype deer throughout the state will provide reference population data that can be used in assignment analyses to determine geographic origins of particular deer in forensic investigations.

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