Conservation Genet Resour
DOI 10.1007/s12686-016-0572-5

METHODS AND RESOURCES ARTICLE

Improved DNA profiles from aged horse feces using pressure cycling technology

K. Deshpande[1,2] · **M. Villarreal**[1,2] · **D. K. Mills**[1,2]

Received: 2 October 2015 / Accepted: 5 July 2016

Abstract Feces represent an easily available, non-invasive source of DNA. Often DNA extraction from feces is compounded with contamination, dietary inhibitors and primarily low quantity and poor quality of host DNA. In an effort to improve host DNA recovery from fecal samples, a new DNA extraction methods was developed. The objectives of this project was to use non-invasive sampling techniques of feces from domestic horses, provide a viable protocol for an improved DNA extraction, and genotype using six equine microsatellite on feces aged for 6 days. The technique used a modified Qiagen® DNA Stool Mini Kit protocol (Q) with pressure cycling technology (PCT). The DNA yield using PCT extraction was five fold greater as compared to the Q. The PCT mediated Qiagen (Q + PCT) technique yielded complete (six loci) equine DNA profiles of 100 % samples ≤2 days old, 90 % at 4 days and 60 % at 6 days post defecation. The results indicated that the Q + PCT increased DNA yield, and thereby increased the likelihood of obtaining an equine DNA profile from aged fecal samples. Using ML-RELATE analyses, the profiles obtained from the six equine loci could also be used for kinship analysis. Samples aged up to Day 6 as well as pasture samples with unknown days since defecation were successful in individualization of the contributors and could also be used to analyze kinship. The Q + PCT method should prove to be an extremely useful and reliable method for conservation or forensic cases where fecal matter may be the only sample available for analyses.

Keywords Non-invasive sampling · Fecal matter · Pressure cycling technology · Equine microsatellites

Introduction

Fecal matter sampling has received much attention from molecular biologists as a source of host DNA. Feces contain hosts cells shed from epithelial lining of the digestive system plus a complex mixture of gut microorganisms, food particles, digestive enzymes, bile salts and mucus (Kohn and Wayne 1997; Fernando et al. 2003; Piggott and Taylor 2003; Wehausen et al. 2004), many of which act as inhibitors for the polymerase chain reaction (Kohn and Wayne 1997). These factors plus the age of the feces at collection time often make it difficult to obtain a complete genetic profile of the contributing individual.

Feces is a less considered but potentially significant item of evidence that can be encountered at crime scenes (Brettell et al. 2009) and can be used to prove the defendant had been present at the scene of the crime. For instance, there are reported cases where DNA has been extracted from fecal stains found on the clothing of rape victims, feces recovered from places that have been burglarized and dog feces found on the sole of the suspect sneakers as well as clothing have helped link suspects to the crime (Norris and Bock 2000; Johnson et al. 2005; https://www.vgl.ucdavis.edu/forensics/links.php). There is also an increase in forensic use of animal DNA to determine the identity of samples to species or assigning samples to an individual or

✉ D. K. Mills
millsd@fiu.edu

[1] Department of Biological Sciences, Florida International University, OE 167, 11200 SW 8th Street, Miami, FL 33199, USA

[2] International Forensic Research Institute, Florida International University, 11200 SW 8th Street, Miami, FL 33199, USA

Published online: 12 July 2016

to kin (Linacre et al. 2011). Apart from its forensic application, fecal sampling is commonly used in wildlife studies for identification of species, assigning genotypes to an individual, population, or geographic origin, gender, estimate population size, study diet and feeding patterns, and to conduct disease surveys (Waits and Paetkau 2005). Also, fecal matter (scat) provides a non-invasive sample when capture of an animal is not possible.

There are, however, some disadvantages of using fecal samples. These include: (1) inefficient recovery of host cells and low-quality, degraded host DNA (Bonin et al. 2004; McKelvey and Schwartz 2004); (2) samples are highly contaminated by non-target DNA of the food source and gut microbes (Bradley and Vigilant 2002; Pompanon et al. 2005); and (3) complex mixtures of inhibitors which can lead to no or low amplification rates and result in the introduction of artifacts and genotyping errors such as allelic drop-out or drop-in. Even with these disadvantages, fecal matter may provide the only sample available from which to obtain a genetic profile (Kohn 2010).

Numerous commercial extraction kits have been evaluated for their cell lysis efficiency and high-yield of clean DNA in order to increase PCR performance (Nechvatal et al. 2008; Yu and Morrison 2004; Anderson and Lebepe-Mazur 2003). The commercial QIAamp® Stool Kit by Qiagen has been shown to have high extraction efficiencies and a relatively high DNA yield that works well in downstream applications (Nechvatal et al. 2008).

Pressure cycling technology (PCT) is a cell lysis method that employs hydrostatic pressure in rapid succession of low ambient and ultra-high pressures to induce mechanical stress on cells and disrupt biomolecular interactions (Gross et al. 2008a; Tao et al. 2003). The high-pressure destabilizes the cell membrane, rupturing and releasing cellular components including DNA, RNA, proteins, and lipids (Gross et al. 2008a). PCT has been used successfully for diverse sample types including blood, bone (Yuan et al. 2011), animal and plant tissue (Harrington et al. 2004; Okubara et al. 2007), insects, small organisms and microbes (Tao et al. 2003; Smejkal et al. 2006; Garrett et al. 2002) and cell lines (Gross et al. 2008b). More recently, it has been shown to improve differential DNA extractions from cellular mixtures, which are commonly encountered in forensic casework (Nori and McCord 2015). In the present study, the ability of pressure cycling to differentially disrupt the epithelial cells was crucial in obtaining increased host DNA from the horse fecal samples.

The objectives of this study were: (1) to use PCT to improve the differential host cell lysis and increase DNA yield from fecal matter; (2) to obtain DNA profiles of individual horses from aged fecal samples using this PCT optimized method; and (3) to test the methods on fecal matter gathered from a pasture as representative samples, where the time of deposition and contributor of the feces were unknown and to "match" the sample with the horse of origin.

Materials and methods

Sources of fecal samples

Ten domestic horses that had previously been DNA typed using hair samples provided the DNA reference profiles. Fecal samples were collected from each horse's stall within an hour of defecation. The outside of fecal boluses were sampled with three separate cotton swabs moistened with 1× Phosphate buffered saline (PBS), and all swabs were extracted within an hour of collection. After the initial (fresh) sampling on day zero, the same fecal samples were placed in air permeable containers and kept at an average ambient outdoor temperature of 17 °C. The same swab collection protocol was used for each aged sample on days 2, 4 and 6 (N = 30 per time point) in order to determine the temporal span within which a fecal sample could be sampled and still provide a useful DNA profile.

Pasture study

To test whether a DNA profile could be obtained from an unknown contributor where days since defecation were not known and be matched to that individual, five random samples from a nearby pasture were collected. Swabs were collected in triplicate (N = 15) as described previously and DNA was extracted within an hour of collection. The horses that had access to that pasture were a subset of the ten horses used in this study and each individual had a known reference genotype. The physical condition and texture of each fecal sample from the field were recorded (Table 1).

Cell lysis and DNA extraction

Samples were extracted using the following protocol for all swabs (N = 135). To assess if pressure cycling could enhance host cell lysis and DNA yields from fecal samples, a comparison was first made to a modified Qiagen QIAmp® DNA Stool Mini Kit (Qiagen, Valencia, CA) extraction. The modifications to the kit protocol were as follows: Each swab was placed in a 2 mL microfuge tube and incubated overnight in 1 mL of Buffer ASL with 25 µL of proteinase K at 55 °C in a thermo-shaker (Eppendorf AG, Hamburg, Germany) set at 300 rpm (Archie et al. 2003). After overnight incubation, only half of an InhibitEX tablet was added to the tube instead of a full tablet (Renan et al. 2012). Since proteinase K was added to the overnight digestion, it was omitted during sample digestion in

Table 1 DNA quantitation and amplified alleles for five unknown pasture samples

Unknown samples (UNK)	Sample description	Average DNA yield (ng/μL)	# Alleles amplified	% Expected alleles amplified
UNK 1	Semi dry exterior, moist inside	19.40 ± 2.4	12	100
UNK 2	Dry exterior with moist patches	12.03 ± 3.1	11	92
UNK 3	Moist bolus but disintegrating	15.90 ± 3.3	11	100
UNK 4	Dried hard bolus, lighter in weight	3.30 ± 0.7	4	36
UNK 5	Extremely dry bolus both exterior and interior	5.90 ± 1.9	6	50

At the time of fecal bolus swabbing on sampling days the average ambient outside temperature was 17 °C

Buffer AL (Archie et al. 2003). To enhance the binding of DNA to the Qiagen column, 1 μL of carrier RNA along with Buffer AL was added prior to incubation at 70 °C. Finally, the DNA was eluted in 50 μL of Buffer AE after incubation at room temperature for 5 min prior to the final centrifugation step.

Pressure cycling technique (PCT)

To assess if pressure cycling could enhance host cell lysis, a PCT step was incorporated into the extraction protocol described above. After the overnight incubation step, the swab (including the Buffer ASL that was incubated overnight with swab) was placed into a no-disk PULSE tube (ND-PULSE tubes, Pressure Biosciences Inc., South Easton, MA). The tube was sealed with a cap on one end of the PULSE tube and a moveable ram on the other end. Each swab was subjected to 50 rapid, repeated pressure cycles of 15,000 psi in the Barocycler NEP2320 (Pressure Biosciences Inc., South Easton, MA). Each cycle provided 20 s of high pressure followed by 10 s of atmospheric pressure. The purpose of a lower pressure (15 k psi) was to optimize lysis of the horse epithelial cells and minimize the lysis of the plant and microbial cells. After the PCT procedure, the samples were extracted according to the modifications listed above. The recovered DNA was quantified using Qubit™ 2.0 Fluorometer (Table 2) to provide a comparison of the DNA yield between the two extraction methods, even though it was recognized that the DNA represented a mixture of genomic DNA from horse, plant and microbes. Quantification data were used to normalize extracted DNA concentrations to 1 ng for PCR amplification.

PCR amplification and fragment analysis

Using fluorescently labeled forward primers, we amplified six equine loci (all dinucleotides; VHL20, HTG4, HTG6, HMS6, HTG7, and HMS3 (Guérin et al. 1994; Ellegren et al. 1992; Marklund et al. 1994; van Haeringen et al.

Table 2 A comparison of the two DNA extraction methods based on the average DNA yield for Day 0 samples using the Qubit™ 2.0 Fluorometer

Sample	Average yield in ng/μL	
	Modified QIAmp® DNA Stool Mini Kit (Q) ± SE	Modified QIAmp® DNA Stool Mini Kit with PCT (Q + PCT) ± SE
1	4.30 ± 1.8	22.72 ± 1.6
2	5.78 ± 0.4	28.67 ± 1.3
3	4.31 ± 1.6	27.29 ± 1.3
4	7.77 ± 0.2	29.98 ± 1.2
5	5.98 ± 0.4	20.81 ± 1.1
6	4.76 ± 1.4	26.20 ± 1.5
7	5.04 ± 3.3	25.18 ± 1.7
8	4.93 ± 0.4	22.82 ± 1.3
9	6.68 ± 1.9	27.37 ± 2.6
10	5.01 ± 2.5	22.15 ± 1.5
Unknown 1	4.16 ± 0.1	19.40 ± 2.4
Unknown 2	3.70 ± 0.7	12.03 ± 3.1
Unknown 3	3.78 ± 1.7	15.90 ± 3.3
Unknown 4	0.46 ± 0.9	3.30 ± 0.7
Unknown 5	1.97 ± 2.2	5.90 ± 1.9

1994) in one multiplex PCR after optimizing relative primer concentrations (Table 3). The six markers were a subset from the markers included in the StockMarks® for Equine 17-plex Genotyping Kit. They were selected not only because they were shorter markers that are best suited for degraded DNA but also to correctly compare sample profiles with the commercially available equine genotyping kit. The final reaction mix contained: 1× PCR buffer, 2.5 mM $MgCl_2$, 0.25 mM dNTPs, 4 µL of 6-plex primer mix (Table 3), 1.0 Unit AmpliTaq Gold® polymerase, 1 ng DNA and DEPC water to volume (15 µl). PCR cycling conditions were: 95 °C for 10 min for one cycle, then 30 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 60 s with a final extension at 72 °C for 60 min (Dimsoski 2003). One microliter of the PCR product was added to 11.5 µL Hi-Di™ Formamide and 0.5 µL GeneScan™ 600 LIZ® Size Standard, denatured at 95 °C for 3 min and then immediately placed on ice for 3 min. The samples were separated on ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA) using a 36 cm capillary array, POP-7 polymer (Applied Biosystems), 1× electrophoresis running buffer with EDTA (Applied Biosystems) with a 36 cm well to read distance (WTR), using Module DS33, filter G5v2. Samples were analyzed using GeneMapper® 3.7 software (Applied Biosystems, Foster City, CA). Analysis parameters were set to local Southern size calling and the minimum analytical threshold was set to 50 relative fluorescent units (RFUs).

At present, there is no allelic ladder for the equine STR profiling, so allele calls are based upon the internal size standard and known positive controls. The StockMarks® for Equine 17-plex Genotyping Kit equine control DNA was used as a positive control for each run. In addition, each profile from the fecal samples was compared to the reference genotype for each horse. Since the horses in this study were Quarter Horses, most had been DNA typed at an external laboratory according to the American Quarter Horse Association (AQHA) registration requirements and these external DNA typing profiles were used to verify all allele calls for this study. Allele sizes were converted to allele repeat numbers based on latest equine typing standardization (Van de Goor et al. 2009).

Analysis

Two-tailed *t* test assuming unequal variance was used to analyze corresponding peak heights of the expected alleles for method comparison and were performed using Excel (Fig. 1). To compare the extraction efficiency, the average peak heights in RFUs for each allele and standard deviation were calculated (Fig. 2). The averaged percent of amplified alleles compared to the known number of alleles for each horse was calculated for all alleles obtained on Days 0, 2, 4

Table 3 Primer sequences and concentrations per reaction for the equine 6-plex STRs and their size range

Locus	Fluorochrome	Primer sequence (5′–3′)	Final conc. (µM)	Size range (bp)	Reference
VHL20	6-FAM	F: CAAGTCCTCTTACTTGAAGACTAG	0.25	87–105	van Haeringen et al. 1994
		R: AACTCAGGGAGAATCTTCCTCAG			
HTG4	6-FAM	F: CTATCTCAGTCTTGATTGCAGGAC	0.07	127–139	Ellegren et al. 1992
		R: CTCCCTCCCTCCCTCTGTTCTC			
HTG6	VIC	F: GTTCACTGAATGTCAAATTCTGCT	0.17	84–102	Ellegren et al. 1992
		R: CCTGCTTGGAGGCTGTGATAAGAT			
HMS6	VIC	F: GAAGCTGCCAGTATTCAACCATTG	0.19	151–169	Guérin et al. 1994
		R: CTCCATCTTGTGAAGTGTAACTCA			
HTG7	NED	F: CCTGAAGCAGAACATCCCTCCTTG	0.22	118–128	Marklund et al. 1994
		R: ATAAAGTGTCTGGGCAGAGCTGCT			
HMS3	NED	F: CCATCCTCACTTTTTCACTTTGTT	0.13	148–170	Guérin et al. 1994
		R: CCAACTCTTTGTCACATAACAAGA			

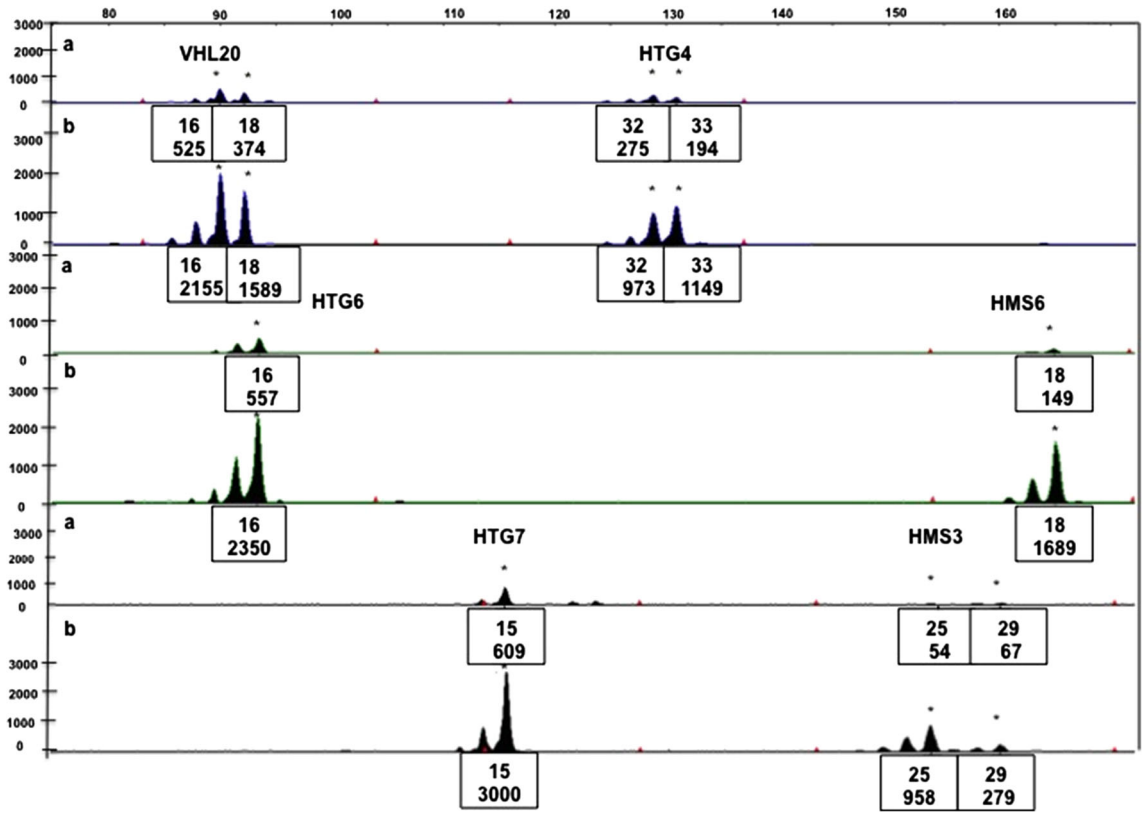

Fig. 1 An example of an equine STR profile in which the DNA was extracted with *a* Modified Qiagen QIAmp® DNA Stool Mini Kit protocol only, and *b* Modified Qiagen QIAmp® DNA Stool Mini Kit protocol with PCT. All nine alleles amplified matched the reference sample. The true peak is denoted by *asterisk*, while the repeat number and peak intensities are shown in the *boxes* below each peak

and 6 (Table 4). ML-Relate software (Kalinowski et al. 2006) was also used to check if the six loci could identify kinship for the ten domestic horses (Table 5). ML-RELATE calculates maximum likelihood to estimate relatedness and assigns relationship from co-dominant genetic data, e.g. microsatellites. Profiles obtained on Days 0, 2, 4 and 6 were used for kinship analysis (Fig. 3).

Results

Extraction comparison

Fecal samples from ten horse and five from the pasture samples were tested using the modified Qiagen QIAamp® stool kit protocol (Q) and Qiagen protocol modified plus PCT (Q + PCT) protocol. The Q + PCT method yielded the greatest concentrations of DNA from fresh and aged samples ($p < 0.001$) (Table 2).

Profile comparison

There was a 100 % success rate obtained for full horse DNA profiles from fresh (Day 0) and fecal samples aged up to Day 2.

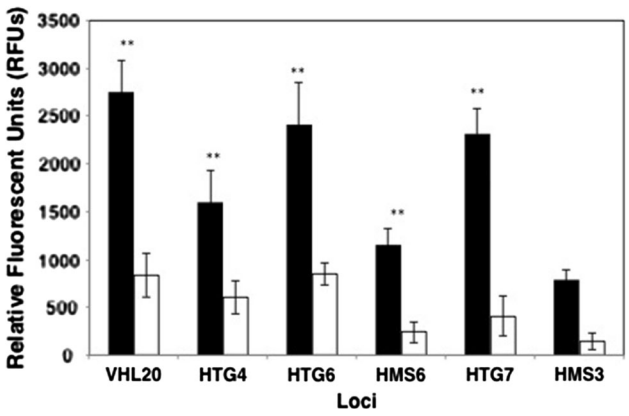

Fig. 2 An example of a successful amplification of DNA extracted from fecal sample collected on *a* Day 0 and *b* Day 6. All 11 alleles amplified matched the reference sample. The true peak is denoted by *asterisk*, while the repeat number and peak intensities are shown in the *boxes* below

Additionally, the profiles were identical to the reference samples verifying that extraction and purification methods were reliable and reproducible (Table 2). For 90 % of the samples, full horse profiles were obtained for Day 4 and 60 % of the samples

Table 4 DNA quantitation and number of amplified alleles for ten horses on days 0, 2, 4 and 6

Sample	Days	Average DNA yield (ng/μL)	Amplified # of alleles	% of expected alleles amplified (%)	Alleles not amplified
Horse 1	0	22.72 ± 1.6	12	100	
	2	20.05 ± 1.1	12	100	
	4	18.60 ± 1.7	10	83	HMS3
	6	11.40 ± 1.2	10	83	HMS3
Horse 2	0	28.67 ± 1.3	12	100	
	2	24.99 ± 2.1	12	100	
	4	11.00 ± 1.5	11	92	HMS6 (1 allele)
	6	10.52 ± 0.9	5	42	HMS6, HMS3, VHL20 (1 allele), HTG7 (1 allele)
Horse 3	0	27.29 ± 1.3	9	100	
	2	26.85 ± 0.9	9	100	
	4	23.25 ± 0.8	9	100	
	6	11.37 ± 1.0	9	100	
Horse 4	0	29.98 ± 1.2	12	100	
	2	23.03 ± 1.0	12	100	
	4	11.31 ± 0.9	8	67	HMS6, HMS3
	6	10.78 ± 0.7	8	67	HMS6, HMS3
Horse 5	0	20.81 ± 1.1	11	100	
	2	20.20 ± 0.4	11	100	
	4	10.17 ± 1.1	11	100	
	6	6.60 ± 1.6	11	100	
Horse 6	0	26.20 ± 1.5	12	100	
	2	22.46 ± 1.9	12	100	
	4	14.58 ± 0.7	12	100	
	6	14.10 ± 1.9	12	100	
Horse 7	0	25.18 ± 1.7	12	100	
	2	18.20 ± 0.8	12	100	
	4	11.56 ± 2.4	12	100	
	6	10.90 ± 2.2	12	100	
Horse 8	0	22.82 ± 1.3	12	100	
	2	19.64 ± 1.1	10	83	HMS3
	4	9.90 ± 1.4	8	67	HMS6, HMS3
	6	6.80 ± 0.9	8	67	HMS6, HMS3
Horse 9	0	27.37 ± 2.6	11	100	
	2	20.20 ± 0.7	11	100	
	4	15.80 ± 0.6	10	91	HMS3 (1 allele)
	6	7.40 ± 1.9	10	91	HMS6 (1 allele)
Horse 10	0	22.15 ± 1.5	12	100	
	2	19.53 ± 1.4	11	92	HMS6 (1 allele)
	4	11.24 ± 1.0	10	83	HMS6, HMS3
	6	10.60 ± 0.8	7	58	HMS6, HMS3, VHL20 (1 allele)

At the time of fecal bolus swabbing on sampling days the average ambient outside temperature was 17 °C

produced full profiles even 6 days post defecation (Fig. 2). It was not unexpected that the percent of amplified alleles would decrease as the age of samples increased (Table 4).

For the full profiles obtained at Day 4, 100 % of them could be used to assess kinship while 60 % of the profiles obtained on Day 6 could still be used (Table 5). The M–L Relate data

Table 5 Kinship analysis for three unknown pasture samples based on the obtained genotype

Domestic horse	UNK 1	UNK 2	UNK 3
Horse 1	HS	U	U
Horse 2	Self	HS	HS
Horse 3	U	U	U
Horse 4	U	U	U
Horse 5	PO	PO	PO
Horse 6	U	U	U
Horse 7	HS	HS	HS
Horse 8	HS	Self	Self
Horse 9	U	U	U
Horse 10	FS	HS	HS

Partial profiles obtained from unknown pasture samples 4 and 5 were not used for kinship analysis. The five unknown fecal samples belonged to two horses (Horse 2 and Horse 8). Unknown fecal samples are listed here as in table as UNK 1, UNK 2 and UNK 3

The kinship abbreviations are *HS* half sib, *FS* full sib, *PO* parent offspring, *U* unrelated, *Self* matched

were designated as parent offspring, half or full sibling and unrelated. Profiles obtained from unknown pasture samples were determined to be fecal samples that matched horse 2 (Unknown 1, 3 and 5) and horse 8 (Unknown 2, 4) (Table 5). Using these "unknown" profiles, relationships of parent offspring and half sibs could be determined for unknown samples 1, 2 and 3. However, since only partial profiles were obtained for unknown pasture samples 4 and 5, relationship determination could not be done using the kinship software.

Discussion

Conventional methods or commercial kits for isolating DNA from feces extract total metagenomic DNA from the sample. However, an accurate individual genotype can be derived only when host cells are efficiently lysed and host DNA is not "diluted out" by non-target organismal DNA in the sample. Preferably, this would involve selective disruption of host cells over other cell types in the sample and would release more target DNA versus non-host DNA. PCT has previously been compared to other standard DNA extraction methods for its ability to differentially lyse cells by utilizing pressure variation (Bradley et al. 2000; Okubara et al. 2007; Gross et al. 2008b). Gross et al. (2008b) applied PCT to selectively release mitochondria from rat kidney and skeletal

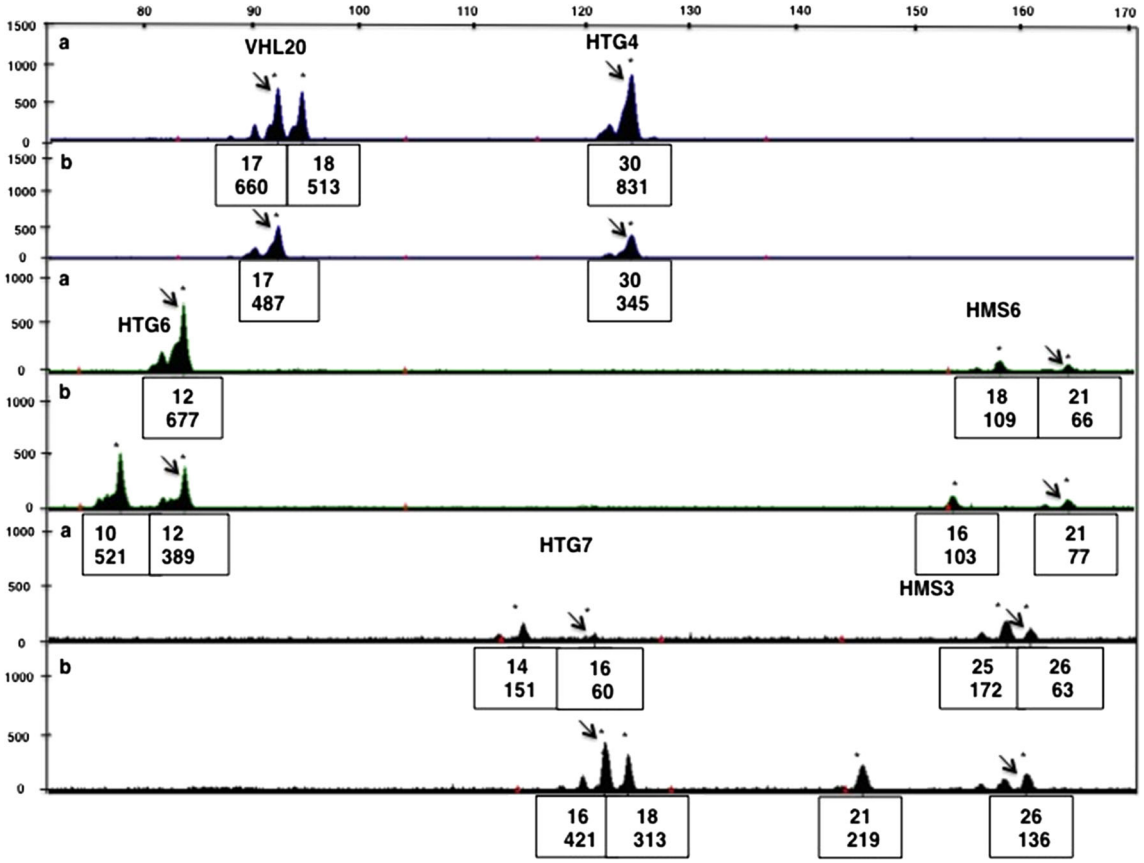

Fig. 3 Equine profile showing relationship. *a* Dam *b* Offspring. The true peak is denoted by *asterisk*, while represents the common alleles used to determine relationship. The repeat number and peak intensities are shown in the *boxes* below each peak

muscle tissue without rupturing membrane protein complexes in the outer cellular membrane. Recently, differential extraction of sperm and vaginal epithelial cells was carried out using alkaline lysis combined with PCT. This method successfully separated male and female DNA from a mixture without compromising DNA recovery (Nori and McCord 2015). In this study, the sloughed, intact horse epithelial cells were more readily recovered from the surface of the feces with wetted swabs than from within fecal bolus, making surface swab sampling more amenable for host cell recovery.

The DNA extraction from fecal swabs using Q + PCT in this study dramatically increased the host DNA yield compared to only the modified Qiagen protocol as indicated in the three-fold increase of relative peak height intensities in the subsequent DNA profiles. The higher host DNA yield using Q + PCT extraction technique provided 100 % amplification success for fresh as well as fecal samples aged up to 4 days and still useful partial profiles on Day 6 post defecation. PCT's ability to differentially lyse host cells provided a higher yield of horse DNA with minimal interference from other cellular macromolecules and microbial and plant DNA.

Past studies using various DNA extraction techniques have demonstrated amplification success rates ranging from 70 to 80 % of brown bear scat samples (Bellemain et al. 2005), 27–77 % profiles from wild boar samples (Kolodziej et al. 2012), and 40 % of wolf scat (Creel et al. 2003) on samples with unknown age of defecation. The success of complete genotypes was after numerous replicates. While these studies generated individual DNA profiles, a significant loss of alleles or the addition of erroneous alleles in the sampled population often proved to be a problem requiring multiple replicates and limiting the information on individualization as well as number of individuals (Waits and Paetkau 2005). In the current study using Q + PCT, 100 % of the profiles for Day 0, 2 and 90 % at Day 4 showed no allelic dropouts or erroneous alleles. Only four of the samples on Day 6 showed a decrease in allele amplification of the larger amplicons (HTG4, HTG6 and HMS3) with some peaks dropping below the analytical threshold but no erroneous alleles were observed. These results were far better than those reported by Piggott (2005) and Santini et al. (2007) where allele amplification success of fecal samples from the brush-tailed rock-wallaby (*Petrogale penicillata*) and the red fox (*Vulpes vulpes*) (Piggott 2005) and wolf (*Canis lupus*) (Santini et al. 2007) declined rapidly within 3 days post-defecation and genotyping was not reliable or possible beyond 1 week. Ultimately, successful genotyping from complex samples depends on maximizing the concentration of host DNA, and minimizing non-target animal (food remnants) and plant DNA (Deuter et al. 1995; Flagstad et al. 1999; Fernando et al. 2003; Wehausen et al. 2004) and that goal was achieved in this current study using the Q + PCT extraction technique.

The use of genotypes obtained on Day 4 and 6 for successfully identifying relationship is important in cases where the days since defecation are unknown. The profiles obtained from the five pasture fecal samples of unknown age were able to be matched to two of the reference horses. The ability to extract DNA and obtain complete or partial profiles from dry, a disintegrating fecal bolus would be crucial in the sampling of feces in the wild. Not only were the profiles from pasture samples able to identify the individual contributors but also three out of five profiles were also useful in determining kinship. In wild animal population studies, individualization would be crucial for population census. These DNA profiles could also provide valuable information on reproductive success, inbreeding, and genetic diversity and the data would be extremely helpful in formulating strategies in conservation and management.

In this study the Q + PCT method increased the DNA yield, which, in turn, improved PCR results and reduced common artifacts. In addition, it increased the ability to identify individuals even from fecal samples as old as 6 days post defecation. The method provided genotypes that could be used to identify the individual contributor and kinship from randomly chosen pasture samples. The future application of this optimized method could prove very useful in wildlife or domestic animal forensics cases where fecal matter may be the only available source of host DNA.

Acknowledgments The authors thank the Forensic DNA Profiling Facility, International Forensic Research Institute, Florida International University for their instrumental support and Beatrice Kallifatidis, MSFS, for her support and guidance.

Compliance with ethical standards

Conflict of interest The authors have declared no conflict of interest.

References

Anderson KL, Lebepe-Mazur S (2003) Comparison of rapid methods for the extraction of bacterial DNA from colonic and caecal lumen contents of the pig. J Appl Microbiol 94(6):988–993

Archie EA, Moss CJ, Alberts SC (2003) Characterization of tetranucleotide microsatellite loci in the African savannah elephant (*Loxodonta africana africana)*. Mol Ecol Notes 3(2):244–246

Bellemain EVA, Swenson JE, Tallmon D, Brunberg S, Taberlet P (2005) Estimating population size of elusive animals with DNA from hunter-collected feces: four methods for brown bears. Conserv Biol 19(1):150–161

Bonin A, Bellemain E, Bronken Eidesen P, Pompanon F, Brochmann C, Taberlet P (2004) How to track and assess genotyping errors in population genetics studies. Mol Ecol 13(11):3261–3273

Bradley BJ, Vigilant L (2002) False alleles derived from microbial DNA pose a potential source of error in microsatellite genotyping of DNA from faeces. Mol Ecol Notes 2(4):602–605

Bradley DW, Hess RA, Tao F, Sciaba-Lentz L, Remaley AT, Laugharn JA, Manak M (2000) Pressure cycling technology: a novel approach to virus inactivation in plasma. Transfusion 40(2):193–200

Brettell TA, Butler JM, Almirall JR (2009) Forensic science. Anal Chem 81(12):4695–4711

Creel S, Spong G, Sands JL et al (2003) Population size estimation in Yellowstone wolves with error-prone noninvasive microsatellite genotypes. Mol Ecol 12(7):2003–2009

Deuter R, Pietsch S, Hertel S, Müller O (1995) A method for preparation of fecal DNA suitable for PCR. Nucleic Acids Res 23(18):3800–3801

Dimsoski P (2003) Development of a 17-plex microsatellite polymerase chain reaction kit for genotyping horses. Croatian Med J 44(3):332–335

Ellegren H, Johansson M, Sandberg K, Andersson L (1992) Cloning of highly polymorphic microsatellites in the horse. Anim Genet 23:133–142

Fernando P, Vidya TNC, Payne J et al (2003) DNA analysis indicates that Asian elephants are native to Borneo and are therefore a high priority for conservation. PLoS Biol 1(1):110–115

Flagstad Ø, Røed K, Stacy JE, Jakobsen KS (1999) Reliable noninvasive genotyping based on excremental PCR of nuclear DNA purified with a magnetic bead protocol. Mol Ecol 8(5):879–883

Garrett PE, Tao F, Lawrence N, Ji J, Schumacher RT, Manak MM (2002) Tired of the same old grind in the new genomics and proteomics era? Targets 1(5):156–162

Gross V, Carlson G, Kwan AT, Smejkal G, Freeman E, Ivanov AR, Lazarev A (2008a) Tissue fractionation by hydrostatic pressure cycling technology: the unified sample preparation technique for systems biology studies. J Biomol Tech 19(3):189

Gross V, Lazarev AV, Lawrence NP, Schumacher RT (2008b) Isolation of mitochondria from cell cultures by PCT for proteomic analysis. Biotechniques 45(1):99

Guérin G, Bertaud M, Amigues Y (1994) Characterization of seven new horse microsatellites: HMS1, HMS2, HMS3, HMS5, HMS6, HMS7 and HMS8. Anim Genet 25(S2):62

Harrington S, McCouch S, Tao F, Lawrence N, Schumacher RT (2004) Use of pressure cycling technology (pct) for the release of dna from plants. In: Annual plant, animal and microbes genomics conference. San Diego, CA

Johnson DJ, Martin LR, Roberts KA (2005) STR-typing of human DNA from human fecal matter using the QIAGEN QIAamp® stool mini kit. J Forensic Sci 50(4):802

Kalinowski ST, Wagner AP, Taper ML (2006) ML-Relate: a computer program for maximum likelihood estimation of relatedness and relationship. Mol Ecol Notes 6(2):576–579

Kohn MH (2010) Noninvasive genome sampling in chimpanzees. Mol Ecol 19(24):5328–5331

Kohn MH, Wayne RK (1997) Facts from feces revisited. Trends Ecol Evol 12(6):223–227

Kolodziej K, Theissinger K, Brün J, Schulz HK, Schulz R (2012) Determination of the minimum number of microsatellite markers for individual genotyping in wild boar (*Sus scrofa*) using a test with close relatives. Eur J Wildl Res 58(3):621–628

Linacre A, Gusmao L, Hecht W et al (2011) ISFG: recommendations regarding the use of non-human (animal) DNA in forensic genetic investigations. Forensic Sci Int Genet 5(5):501–505

Marklund S, Ellegren H, Eriksson S, Sandberg K, Andersson L (1994) Parentage testing and linkage analysis in the horse using a set of highly polymorphic microsatellites. Anim Genet 25(S2):19–23

McKelvey KS, Schwartz MK (2004) Genetic errors associated with population estimation using non-invasive molecular tagging: problems and new solutions. J Wildl Manag 68(3):439–448

Nechvatal JM, Ram JL, Basson MD, Namprachan P, Niec SR, Badsha KZ, Kato I et al (2008) Fecal collection, ambient preservation, and DNA extraction for PCR amplification of bacterial and human markers from human feces. J Microbiol Methods 72(2):124–132

Nori DV, McCord BR (2015) The application of alkaline lysis and pressure cycling technology in the differential extraction of DNA from sperm and epithelial cells recovered from cotton swabs. Anal Bioanal Chem 407(23):6975–6984

Norris DO, Bock JH (2000) Use of fecal material to associate a suspect with a crime scene: report of two cases. J Forensic Sci 45(1):184–187

Okubara PA, Schroeder KL, Li C, Schumacher RT, Lawrence NP (2007) Improved extraction of *Rhizoctonia* and *Pythium* DNA from wheat roots and soil samples using pressure cycling technology. Can J Plant Pathol 29(3):304–310

Piggott MP (2005) Effect of sample age and season of collection on the reliability of microsatellite genotyping of faecal DNA. Wildl Res 31(5):485–493

Piggott MP, Taylor AC (2003) Remote collection of animal DNA and its applications in conservation management and understanding the population biology of rare and cryptic species. Wildl Res 30(1):1–13

Pompanon F, Bonin A, Bellemain E, Taberlet P (2005) Genotyping errors: causes, consequences and solutions. Nat Rev Genet 6(11):846–847

Renan S, Speyer E, Shahar N, Gueta T, Templeton AR, Bar-David S (2012) A factoral design experiment as a pilot study for noninvasive genetic sampling. Mol Ecol Res 12(6):1040–1047

Santini A, Lucchini V, Fabbri E, Randi E (2007) Ageing and environmental factors affect PCR success in wolf (*Canis lupus*) excremental DNA samples. Mol Ecol Notes 7(6):955–961

Smejkal GB, Robinson MH, Lawrence NP, Tao F, Saravis CA, Schumacher RT (2006) Increased protein yields from *Escherichia coli* using pressure-cycling technology. J Biomol Tech 17(2):173

Tao F, Lawrence NP, Miller W et al (2003) Biological sample preparation system using pressure cycling technology (PCT). In: Winter R (ed) Advances in high pressure bioscience and biotechnology II. Springer, Berlin, pp 413–417

Van de Goor LHP, Panneman H, Van Haeringen WA (2009) A proposal for standardization in forensic equine DNA typing: allele nomenclature of 17 equine-specific STR loci. Anim Genet 41:122–127

van Haeringen H, Bowling AT, Stott ML, Lenstra JA, Zwaagstra KA (1994) A highly polymorphic horse microsatellite locus: VHL20. Anim Genet 25(3):207

Waits LP, Paetkau D (2005) Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. J Wildl Manag 69(4):1419–1433

Wehausen JD, Ramey RR, Epps CW (2004) Experiments in DNA extraction and PCR amplification from bighorn sheep feces: the importance of DNA extraction method. J Hered 95(6):503–509

Yu Z, Morrison M (2004) Improved extraction of PCR-quality community DNA from digesta and fecal samples. Biotechniques 36(5):808–813

Yuan MQ, Tu Z, Li CX (2011) A rapid and effective bone DNA extraction method using pressure cycling technology. Forensic Sci Technol 4:012