A rapid and simplified procedure for isolating DNA from scat samples

The use of molecular techniques like microsatellite and mitochondrial DNA analyses has become essential for conservation of endangered species and populations. The availability of biological material for genetic analyses remains a major hurdle in the optimal application of these techniques. Scat (fecal matter) is an ideal source for collecting DNA non-invasively since it does not involve direct or indirect contact with the animals concerned[1]. However, current techniques for the isolation of DNA from scat are cumbersome, time-consuming and require a well-equipped laboratory[2–5]. Besides, the polyphenolic compounds present in the samples, which strongly inhibit PCR, are not effectively removed. We describe here a rapid and simplified technique for isolating DNA from scat samples, which takes about an hour and involves minimal handling. This procedure involves the use of polyvinyl polypyrrolidone (PVPP) to remove the polyphenols and Chelex to aid high temperature lysis. The fecal samples were dried in the sun prior to storage and use, simplifying both the collection and storage of such samples in field conditions.

0.2 g dried scat from lions and tigers was incubated in 400 µl of dung lysis buffer[6] (500 mM Tris-HCl, 16 mM EDTA, 10 mM NaCl; pH 9.0). After a brief spin, to the supernatant were added sodium dodecyl sulphate (SDS), PVPP and Chelex at a final concentration of 1%, 0.1% and 5%, respectively, and boiled for 20 min (ref. 7). DNA was extracted from the supernatant by using

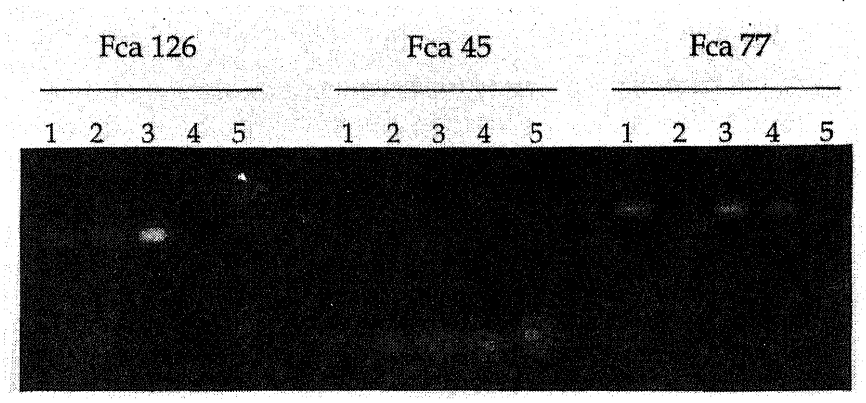

Figure 1. Amplification of 3 microsatellites from the DNA extracted from scats. Lanes 1, 6, 11 are products amplified from genomic DNA of Asiatic lions; other lanes are products amplified from 4 scat samples.

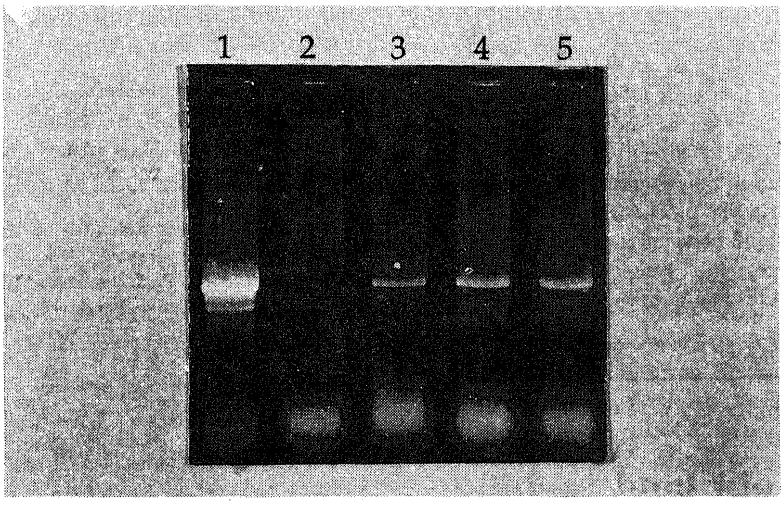

Figure 2. Amplification of mitochondrial D loop region from scats. Lane 1 is product amplified from Asiatic lion genomic DNA, lane 2 is blank reaction (no template DNA) and lanes 3–5 are products amplified from 3 scats.

Table 1. Comparison of the mitochondrial D loop region sequences from genomic and scat DNA of Asiatic lions

Blood sample	TGTTGGCGTA	TCTATAGATA	ACTGCGAACA	AGTTATGATT	TACTACTAAT	AATTGGTAAT	AATAGGGTTG	GTAAAGTTTG
Scat Sample	TGTTGGCGTA	TCTATAGATA	ACTGCGAACA	AGTTATGATT	TACTACTAAT	AATTGGTAAT	AATAGGGTTG	GTAAAGTTTG
Blood sample	TAAACGTTAA	TTCTTAGGCC	TTGTGCTTAA	ATACGGTTTA	GTCTTGTTTT	TGGGGTTTGG	CAAGACAGAA	ATAGACACGT
Scat Sample	TAAACGTTAA	TTCTTAGGCC	TTGTGCTTAA	ATACGGTTTA	GTCTTGTTTT	TGGGGTTTGG	CAAGACAGAA	ATAGACACGT
Blood sample	ATTATAATAA	GTAAGATTAA	CGGGGGGTAA	GGGGGGTTTG	TTTAAGCTAA	TTGTTTACTA	AATCAAAAAG	TTTGCATGTG
Scat Sample	ATTATAATAA	GTAAGATTAA	CGGGGGGTAA	GGGGGGTTTG	TTTAAGCTAA	TTGTTTACTA	AATCAAAAAG	TTTGCATGTG
Blood sample	TATACGTGTA	TACGTGTACG	TGTGTACGTG	TGTACGTGTG	TACGTGTGTA	CGTGTACGTG	TACGTGTACG	TGTACGTGTA
Scat Sample	TATACGTGTA	TACGTGTACG	TGTGTACGTG	TGTACGTGTG	TACGTGTGTA	CGTGTACGTG	TACGTGTACG	TGTACGTGTA
Blood sample	CGTGTACGTG	TACGTGTACG	TGTGTACGCG	TATACGTGTA	CGTGTACGTG	TGTACGTGTA	CGTGTGTACG	TGTACGTGTG
Scat Sample	CGTGTACGTG	TACGTGTACG	TGTGTACGCG	TATACGTGTA	CGTGTACGTG	TGTACGTGTA	CGTGTGTACG	TGTACGTGTG
Blood sample	TACGTGTACG	TGTGTACGTG	TACGTGTATA	CGTGTACGTG	TATACGTGTA	CGTGTATACG	TGTACGTGTA	TACGTGTACG
Scat Sample	TACGTGTACG	TGTGTACGTG	TACGTGTATA	CGTGTACGTG	TATACGTGTA	CGTGTATACG	TGTACGTGTA	TACGTGTACG
Blood sample	TGTGTACGTG	TACGTGTGTA	CGTGTACGTG					
Scat Sample	TGTGTACGTG	TACGTGTGTA	CGTGTACGTG					

Geneclean (Bio 101, Inc, USA). 1 µl of this DNA was used to perform microsatellite amplification at 3 feline CA repeat loci[8], Fca 45, Fca 77 and Fca 126. Mitochondrial D loop amplification was performed using feline-specific primers[9] and the amplified product was purified using Geneclean and directly sequenced using Cyclist manual PCR sequencing kit (Stratagene, USA).

The procedure described was effective in amplifying the feline microsatellites and mitochondrial DNA from all the scat samples tested. Three feline-specific microsatellites were amplified successfully from the DNA extracted from dried scats of Asiatic lions and Indian tigers. The PCR products matched exactly with the microsatellites amplified from blood of animals (Figure 1). Mitochondrial D loop region of approximately 1 kb was also amplified using feline-specific primers (Figure 2) and the product was directly sequenced. The sequence of DNA, including the unique repetitive stretch from scat, matched exactly with the sequence of DNA from the blood of Asiatic lions as shown in Table 1. The use of PVPP effectively removes the PCR inhibiting polyphenols by hydrogen bonding. Earlier procedures used PVPP along with high concentrations of EDTA. EDTA chelates the heavy metal ions which degrade DNA during boiling but high concentrations of it necessitate dilution for further enzymatic reactions like PCR. As the amount of animal DNA is extremely low in scat samples, dilution to this extent does not permit PCR amplification. The role of chelex is that of EDTA but as it is insoluble, effective removal is ensured. Glass milk further facilitates the removal of impurities and also reduces the loss of DNA during handling. The minimal transfer between tubes and the reduced handling time involved in this procedure prevent cross contamination and allow the processing of a large number of samples. This procedure could also be used to amplify plant DNA, using plant-specific PCR primers, which would shed light upon the dietary behaviour of the animals tested. We are planning to employ this technique for large scale sampling and genetic analysis of wild Asiatic lions and Indian tigers. This technique can also be used in the medical field for non-invasive diagnosis.

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