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Short Communication

Buccal Swab as a Source of Noninvasive Technique for Genomic DNA Collection in Felidae

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Abstract

This study was conducted to determine whether buccal cells obtained by swabbing could be a DNA source for genetic analysis, and to compare two preservative media, distilled water and Tris-EDTA (TE), pH 8.0. Buccal cell samples were collected from 30 domestic cats (*Felis catus*), 4 captive fishing cats (*Prionailurus viverrinus*) and 8 captive tigers (*Panthera tigris*). The total concentration of DNA and purity were not statistically different between the preservative media. All samples could be graded as a minimum as high quality DNA (DNA found with A260/A280 ratio >2.0 or < 1.8). In addition, the quality of DNA extracted from both media was successfully amplified for PCR. However, when compared to whole blood samples, total DNA concentration from buccal swab was significantly lower ($p < 0.05$) .Despite the decrease in DNA yield, buccal swab could be an alternative source for obtaining DNA for genetic analyses. This non-invasive, simple and inexpensive technique eases genetic studies in this family especially in domestic cat from which whole blood samples are difficult to collect.

Keywords: buccal swab, felid, genomic DNA, non-invasive technique

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บทคัดย่อ

การเก็บเซลล์เยื่อบุกระพุ้งแก้มเพื่อเป็นแหล่งในการเก็บดีเอ็นเอในสัตว์ตระกูลแมว

จันทร์จิรา ภวภูตานนท์ [1,2,3] สุดธิษา เหล่าเปี่ยม [4] กาวิล นันท์กลาง [1] เกษกนก ศิรินฤมิตร [1] กรไชย กรแก้วรัตน์ [1]*
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การศึกษานี้มีวัตถุประสงค์เพื่อตรวจสอบเซลล์เยื่อบุกระพุ้งแก้มเพื่อนำมาใช้เป็นแหล่งของดีเอ็นเอในการตรวจวิเคราะห์ทางพันธุกรรมและเพื่อเปรียบเทียบสารละลายที่ใช้ในการเก็บรักษาดีเอ็นเอระหว่างน้ำกลั่นและ Tris-EDTA (TE), pH 8.0 ทำการเก็บตัวอย่างเซลล์เยื่อบุกระพุ้งแก้มจากแมวเลี้ยงจำนวน 30 ตัว (*Felis catus*) เสือปลาในกรงเลี้ยงจำนวน 4 ตัว (*Prionailurus viverrinus*) และเสือโคร่งในกรงเลี้ยงจำนวน 8 ตัว (*Panthera tigris*) ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติของความเข้มข้นรวมและความบริสุทธิ์ของดีเอ็นเอในสารละลายน้ำกลั่นและ TE, pH 8.0 ในทุกตัวอย่างและดีเอ็นที่สกัดได้สามารถจัดเกรดได้เป็นดีเอ็นเอที่มีคุณภาพสูง (พบดีเอ็นเอและมีค่า A260/A280 ratio > 2.0 หรือ < 1.8) นอกจากนี้คุณภาพของดีเอ็นเอที่ได้จากสารละลายทั้ง 2 ชนิดยังประสบความสำเร็จในการนำมาเพิ่มจำนวนด้วยเทคนิคพีซีอาร์ อย่างไรก็ตามเมื่อเปรียบเทียบกับตัวอย่างเลือด พบว่าความเข้มข้นของดีเอ็นเอรวมจากเซลล์เยื่อบุกระพุ้งแก้มมีค่าต่ำกว่าจากเลือดอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) ถึงแม้ว่าปริมาณดีเอ็นเอที่ได้จะมีค่าน้อยกว่าแต่การเก็บตัวอย่างจากเซลล์เยื่อบุกระพุ้งแก้มน่าจะเป็นอีกทางเลือกหนึ่งของแหล่งดีเอ็นเอที่นำมาใช้ในการศึกษาทางพันธุกรรม การเก็บเซลล์เยื่อบุกระพุ้งแก้มเป็นวิธีการที่ไม่ก่อให้เกิดอันตรายแก่ตัวสัตว์ ทำได้ง่ายและประหยัด ส่งผลให้การศึกษาทางพันธุกรรมในสัตว์วงศ์นี้ทำได้ง่ายยิ่งขึ้น โดยเฉพาะในแมวที่การเก็บตัวอย่างเลือดทำได้ยาก

คำสำคัญ: การเก็บเซลล์เยื่อบุกระพุ้งแก้ม สัตว์ตระกูลแมว จีโนมิกดีเอ็นเอ เทคนิคที่ไม่ก่อให้เกิดอันตรายแก่ตัวสัตว์

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Introduction

Among this felidae, tiger (*Panthera tigris*) and fishing cat (*Prionailurus viverrinus*) which are endangered large and medium-sized wild cats (IUCN 2012 status-EN) are of interest to researchers for genetic study (Luo et al., 2004; Xua et al., 2005; Bhagavatula and Lalji, 2006). Owing to their dwindling number, it is difficult to get their DNA samples in the wild. DNA samples used for genetic studies are usually obtained from whole blood or tissue in order to get large amount of DNA. However, the method to handle wild or young animals and obtain blood samples is difficult especially animal in the felid family. Therefore, captive felids' are alternative sources of DNA samples and because they are easily inclined to feel stressed non-invasive technique is recommended.

Non-invasive samples from feces, urine or shed hair are widely used in wildlife or threatened animal. However, these samples encounter problems of contamination, degradation, low amounts of DNA and PCR inhibitor (Bellemain and Taberlet, 2004; Cheng et al., 2010). Buccal swab or mouthwash is an alternative technique to collect exfoliated buccal epithelial cells in human. There are different types of buccal swab techniques, e.g. cotton swabs and cytobrushes that could obtain the same DNA yields and PCR success rate (Garcia-Closas et al., 2001). Hence, buccal swab technique could be applied to felids with acceptance of their owners. There are many commercial buccal DNA test kits such as Buccal DNA Collection and Preservation Kit (NorgenBiotek Corporation), Isohelix DNA Buccal Swabs and Isolation Kits (Cell Projects Ltd). These test kits contain all components required for collecting, preserving and transporting a DNA sample. They are easy and convenient to use, however they are considerably expensive. Alternative preservative media are distilled water and Tris-EDTA. Tris-EDTA can solubilize and protect DNA from degradation and it is widely used as diluent for long-term storage of

DNA samples (Smith and Morin, 2005).

The purpose of this study was to compare buccal swab in distilled water and TE buffer preservative media and blood for DNA extraction by using domestic cats and wild felids as models.

Materials and Methods

Sample collection

Buccal cell samples: Experiment I: Thirty domestic cats (*Felis catus*), 4 captive fishing cats (*Prionailurus viverrinus*) and 8 captive tigers (*Panthera tigris*) were used as animal models for sample collection. The domestic cats came from 3 cat kennels, the fishing cats and tigers came from a zoo. Buccal swab and blood samples of the domestic cats were collected without sedation. The fishing cats and tigers were anesthetized with 15 mg/kg of ketamine-HCl (Katamil, Troy Lab, Smithfield, NSW, Australia) and 0.5 mg/kg of xylazine-HCl (Ilium xylaxil, Troy Laboratories) and maintained as necessary with isoflurane inhalant anesthesia (Aerane, Baxter Health Care Corp., Deerfield IL, USA; 1-2%, v/v). One sterile swab was rubbed against the inside left cheek of the individual's mouth and then the tip of the cotton swab put in a 2 ml eppendrof that contained 1 ml of distilled water preservative medium solution. Then, the cotton tip was stirred in preservative medium for a few minutes and thrown away. In order to compare different preservative media, the right cheek followed the same process but the cotton tip was put into 1 ml of Tris-EDTA preservative medium, pH 8.0. All the samples were kept at -20^{0}C until DNA extraction (3-4 months of storage).

Whole blood samples

Experiment II: In order to evaluate DNA quality of buccal DNA, samples in the suitable preservative medium were further compared with samples from whole blood DNA samples. These samples was composed of 172 buccal samples (30 samples from the first experiment and 142 new samples) and whole blood samples were collected by venipuncture from cephalic vein of 111 domestic cats. One milliliter of blood sample was put in a 2.0 ml BD Vacutainer® EDTA blood collection tube (BD Franklin Lakes NJ, USA) and stored at -20^{0}C for as long as experiment I.

DNA extraction method: Two buccal swabs per animal were isolated using EZNA® Tissue DNA Kit (Omega Bio-Tek, Inc) following the manufacturer's instructions with slight modification. After centrifugation at 13,000 rpm for 5 min, 0.2 ml of buccal sediment sample within preservative medium was used for each DNA extraction. The protocol started by adding 200 µl of TL buffer and 25 µl of OB protease into 0.2 ml of buccal sediment sample, vortexing to mix the sample and incubating the sample at 55^{0}C to complete cell lysis. The sample was vortexed every 5-10 min, average lysis time was 1 hour. The sample was centrifuged at 13,000x g for 5 min and the supernatant was carefully aspirated and transferred to a sterile microfuge tube. Then, the sample was added with 220 µl of BL buffer and incubated at 70^{0}C for 10 min. DNA was precipitated with 220 µl of absolute ethanol and the sample was transferred the sample into the HiBind DNA Mini Column and washed 2 times with 700 µl of DNA wash buffer. The sample was centrifuged at 13,000x g for 30 sec and the supernatant was discarded. The HiBind DNA Mini Column was placed on the collection tube. Then, the sample was added with 30 µl of elution buffer and centrifuged at 13,000x g for 1 min to elute DNA from the column.

DNA was isolated from white blood cells of 0.1 ml whole blood sample in EDTA tube using standard phenol-chloroform which is a conventional method for DNA extraction (Sambrook and Russell, 2001). One microliter of extracted DNA was determined for purification and concentration by using the NanoDrop 2000™ Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). The final volume of DNA extraction from buccal swab and whole blood samples were adjusted to 30 µl and stored at -20^{0}C until used.

DNA concentration and purity determination: Total DNA concentration was determined by spectrophotometer at 260 nm. DNA quality was evaluated by spectrophotometer at A260/A280 ratio (Cheng et al., 2010). DNA quality of samples were divided into 3 grades: grade 1, the highest quality DNA, DNA found with A260/A280 ratio between 1.8-2.0; grade 2, high quality DNA, DNA found with A260/A280 ratio < 1.8 or > 2.0; and grade 3, the poor quality DNA, no DNA found.

PCR amplification success rate: Extracted DNA from every buccal swabs and blood samples was used as a template for PCR amplification of two cat microsatellite markers (F124 and FCA298). Amplification reaction was performed in a 20 µl volume using 100 ng of genomic DNA. The PCR condition was amplified with a thermal cycle program; initial denaturation for 3 min at 93^{0}C followed by 10 cycles of denaturation for 15 sec at 94^{0}C, annealing for 15 sec at 55^{0}C, and extension for 30 sec at 72^{0}C, followed by 20 cycles of denaturation for 15 sec at 89^{0}C, annealing for 15 sec at 55^{0}C, and extension for 30 sec at 72^{0}C and final extension for 30 min at 72^{0}C. The amplified DNA fragments were analyzed in 1.5% agarose gel electrophoresis and DNA bands were visualized with GelStar® Nucleic Acid Gel Stain (Cambrex Bio Science Rockland, Inc). The results of the PCR were scored as positive if there was amplification or negative if there was no amplification.

Results and Discussion

Buccal cells DNA concentration and purity: DNA was successfully extracted from all buccal samples. The DNA concentration and purity from the domestic cats, fishing cats and tigers are shown in Table 1. The DNA concentration obtained from buccal swab in the domestic cats ranged from 13.0-67.9 ng/µl and 12.4-66.1 ng/µl in distilled water and TE preservative medium, respectively. The DNA purity of domestic cats was in the highest grade (grade 1) and high

quality DNA (grade 2), the value of A260/A280 ratio ranged from 1.51-2.84 in distilled water and 1.54-3.02 in TE (Table 1). The range of DNA concentration was 36.2-51.2 ng/µl (distilled water) and 30.6-59.7 ng/µl (TE) in the fishing cats and 42.0-158.9 ng/µl (distilled water) and 53.7- 97.8 ng/µl (TE) in the tigers. Their DNA purity was lower than that of the domestic cats and was graded as only high quality DNA (grade 2).

From all the samples, 50% (15/30) of the domestic cat DNA preserved in distilled water were in grade 1 and 50% (15/30) in grade 2. Similarly 53.33% (16/30) of the cells preserved in TE were in grade 1 and 46.67% (14/30) in grade 2. In contrast, all of the DNA samples of the fishing cats and tigers from both preservative media were in grade 2. The mean purity ratio of buccal DNA of the domestic cats, fishing cats and tigers was 1.97-1.98, 1.14-1.16 and 1.18-1.25, respectively (Table 1). The total concentration and purity of DNA were not statistically different between the preservative media ($p > 0.05$). Therefore, distilled water was chosen as the preservative medium to compare with whole blood samples in the second experiment.

To determine the quality of DNA preserved in the chosen medium, 172 buccal swab samples preserved in distilled water, 30 samples from the first experiment and 142 new samples, were analyzed with 111 DNA samples from whole blood. When compared DNA purity from buccal swab with whole blood, less than 50% (29/111) of the samples from whole blood were in grade 1. *T*-test showed that total DNA concentration from the whole blood yielded significantly higher DNA concentration than the buccal swab ($p < 0.05$). The concentration values ranged from 1.20-8.45 µg in the whole blood and 0.56-5.21 µg in the buccal swab samples (Table 2). While neither of the DNA samples from these 2 sources had poor quality (grade 3) as shown in Fig 1, there was no statistical difference in purity of the DNA extracted from these 2 methods.

There was more distribution of total DNA concentration from the whole blood than the buccal swab samples. The highest yield of the buccal swab samples was 5-6 µg compared to 8-9 µg of the whole blood samples. More than 80% of the buccal swab samples had DNA concentration 1-2 µg. The adequacy and quality of DNA were assessed by the success rate of amplifying DNA fragment of two microsatellite markers. PCR amplification success was observed in both 111 whole blood and 172 buccal swab samples.

From the first experiment, the DNA yield preserved in distilled water obtained per swab in this study was 0.39-2.04 µg (domestic cats), 1.09-1.54 µg (fishing cats) and 1.26-4.77 µg (tigers). Compared to buccal DNA concentration from domestic dog (0.15-1.69 µg) and human (0.54-1.05 µg), the amount of DNA per swab was in the same range (Woo et al., 2007; Mitsouras and Erica, 2009). In the second experiment, when the DNA concentration from whole blood and buccal swab samples were compared, the distribution of whole blood DNA concentration per animal varied more than buccal swab DNA. This difference may come from the amount of white blood

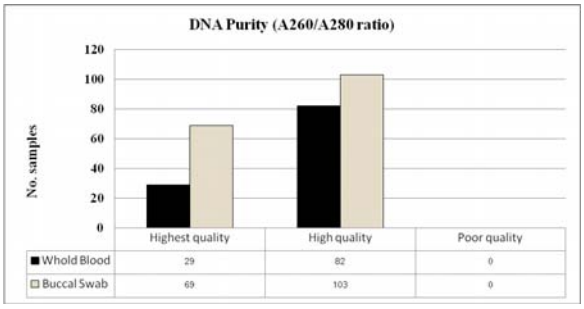

Figure 1 DNA quality of 111 whole blood and 172 buccal swab from domestic cat samples were divided into 3 grades: grade 1, the highest quality DNA, DNA found with A260/A280 ratio 1.8-2.0; grade 2, high quality DNA, DNA found with A260/A280 ratio < 1.8 or > 2.0; and grade 3, the poor quality DNA, no DNA found.

Table 1 Concentration and purity ratio (A260/A280) of DNA from domestic cats, fishing cats and tigers preserved in distilled water (H_2O) and TE

Species	Distilled H_2O			
	Total DNA conc. (µg)		A260/A280	
	Mean±SD	Range	Mean±SD	Range
Domestic cat (n = 30)	1.08±0.46	0.39–2.04	1.98±0.29	1.51–2.84
Fishing cat (n = 4)	1.31±0.21	1.09–1.54	1.16±0.12	1.02–1.29
Tiger (n= 8)	2.65±1.21	1.26–4.77	1.25±0.09	1.12–1.38
Species	**TE**			
	Total DNA conc. (µg)		**A260/A280**	
	Mean±SD	Range	Mean±SD	Range
Domestic cat (n = 30)	0.92±0.37	0.37–1.98	1.97±0.29	1.54–3.02
Fishing cat (n = 4)	1.36±0.39	0.92–1.79	1.14±0.08	1.04–1.23
Tiger (n = 8)	2.29±0.50	1.61–2.93	1.18±0.08	1.11–1.34

Table 2 Compared mean concentration and purity of DNA from 111 whole blood and 172 buccal swab samples preserved in distilled water of domestic cats

Method of collection	Spectrophotometry					
	Total DNA conc. (µg)		A260/A280		DNA grade	
	Mean±SD	Range	Mean±SD	Range	1	2
Whole blood (n = 111)	4.25±1.64	1.20 – 8.45	1.7±0.16	1.28 – 2.09	29	82
Buccal swab (n = 172)	1.67±0.88	0.56 – 5.21	2.0±0.17	1.55 – 2.50	69	103

cells and buccal cells in each sample. A study in human indicated that the protein contamination of buccal DNA was more than blood DNA. In human buccal DNA, the average purity of A260/A280 ratio was 1.3 (Livy et al., 2012) and 1.6-2.0 (Satia-Abouta et al., 2002). We found the low A260/A280 ratio result in buccal DNA from the fishing cats and tigers, which indicated protein contamination. However, in the domestic cats the average purity was 2.0 (A260/280 ratio), which was in the range of good DNA purity (1.8-2.0 A260/A280 ratio) (Wang et al., 2011). The different ratios may result from different plasma protein concentration in different species that contaminates the purity of DNA.

The result revealed that both distilled water and TE could be used as preservative media of buccal cells. DNA samples from buccal swab preserved in distilled water provided enough quantity and purity for PCR reactions that identified short DNA fragment revealed by 100% of PCR amplification successful rate. When compared the cost, distilled water should be more suitable than TE because it is less expensive. PCR reaction in this laboratory used 100 ng of genomic DNA. When we observed the yield of genomic DNA from buccal swab technique preserved in distilled water of domestic cats, fishing cats and tigers, this technique provided sufficient DNA for an estimation of 4-20 PCR reactions in domestic cats, 11-15 PCR reactions in fishing cats and 12-47 PCR reactions in tigers. This amount of DNA is enough for diagnosis of genetic disease such as polycystic kidney disease in domestic cat or blood parasite detection by using PCR technique. However, genetic study such as DNA fingerprint, parentage testing identified more than 10 loci for the analysis. Therefore, it is necessary to collect more than one cotton swab per animal. Awareness of buccal cell samples collection is food intake and milk sucking in young animals. The particle of food or milk remaining in the mouth might be contaminated with DNA of other animal. To avoid this problem, we recommend the same protocol with human to wait at least 45 minutes after smoking, drinking or eating before sample collection (Cheng et al., 2010). These animals should rinse their mouth after eating and wait at least 45-60 minutes prior to DNA sample collection.

The advantage of buccal cells over blood is the method to get the samples especially in young or very small animal. It is easy and decreases the risk of anesthesia or stress to handle the animal. In captive felid such as tiger and lion, zoo keeper can take the buccal samples without sedating the animals. This non-invasive source of DNA is widely accepted by patients for genetic studies and clinical disease diagnosis (Cheng et al., 2010). Moreover, samples stored in a freezer at -20^{0}C for up to 4 months still contain genomic DNA as found in this study. Therefore, it should be suitable for sample collection in field study where laboratory is not available for sample processing immediately after sample collection. However, there was a report on storage duration that affected the quality of DNA in human DNA (Nedel et al., 2009). Degradation of DNA occurred after storing the buccal samples. Therefore, processing the samples as soon as possible is the better way to receive good yield and quality of DNA samples.

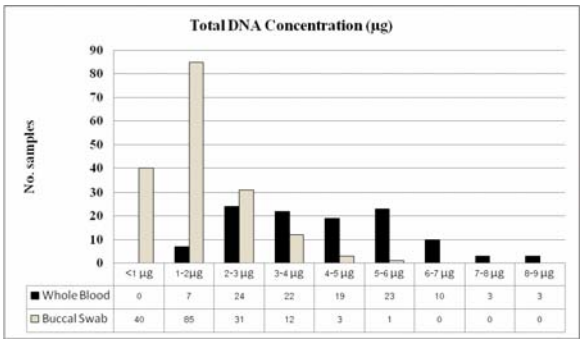

Figure 2 Distribution of total DNA concentration from whole blood and buccal swab samples

Researchers are always in search of appropriate noninvasive techniques to get DNA samples for their studies. Whole blood is the ideal samples but it is not suitable for some studies especially in the case of wildlife, very small or young animals. The result of this study revealed that the collection of DNA from buccal swab preserved in distilled water is a cost-effective and practical method in felid species. Even though the yield of DNA is lower than peripheral blood, the quality, concentration of genomic DNA and success rate of PCR amplification are sufficient for genetic studies. The success of research studies is not only on the quality of DNA, but also on the number of participants in the project. This method is noninvasive and decreases stress of captive animal, therefore it encourages owners to participate in a research and increases participant samples in the field study.

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References

Bhagavatula J and Lalji S 2006. Genotyping faecal samples of Bengal tiger *Panthera tigris* for population estimation: A pilot study. BMC Genet. 7: 48.

Bellemain E and Taberlet P 2004. Improved noninvasive genotyping method: Application to brown bear (*Ursusar ctos*) faeces. Mol. Ecol. Notes 4: 519-522.

Cheng TH, Sheng-Pyng C, Tzu-Chuan L, Wen-Chi C, Jenn-Shing S, and Yi-Shing S 2010. Optimal DNA extraction from buccal swab samples. J Med Sci 30(4): 149-154.

Garcia-Closas M, Kathleen ME, Jeannine A, Polly AN, Linda TE, Tracie F, Patrick KB, Jeanne CB, Loïc LM, Annette L, Michael A, Richard BH, Joni R, Kenneth B, Louise AB, and Nathaniel R 2001. Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and mouth wash. Cancer Epidem Biomar & Prev. 10: 687-696.

Livy A, Sayhean L, Chahil KJ, Nurul H, Velapasamy S, Lian WL and Bagali P 2012. Evaluation of quality of DNA extracted from buccal swabs for microarray based genotyping. Ind J Clin Biochem. 27(1): 28-33.

Luo SJ, Jae-Heup K, Warren EJ, Joelle van der W, Janice M, Naoya Y, Dale GM, Olga U, John MG, Howard BQ, Ronald T, Gerald B, Paolo M, Vellayan S, Charles M, Sun H, Shi-Qiang H, Wenshi P, Ullas KK, Melvin S, James LDS and Stephen OB 2004. Phylogeography and genetic ancestry of tigers (*Panthera tigris*). PLoS Biol. 2(12): e442.

Mitsouras K and Erica AF 2009. Saliva as an alternative source of high yield canine genomic DNA for genotyping studies. BMC Res. Notes 2: 219.

Nedel F, André DA, de Oliveira IO, Tarquinio SB, Demarco FF 2009. Buccal cells submitted to three different storage conditions before DNA extraction. J Appl Oral Sci. 17: 113-115.

Sambrook J and Russell DW 2001. Preparation and analysis of eukaryotic genomic DNA. In: Molecular Cloning: A Laboratory Manual. 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. 6.1-6.62.

Satia-Abouta IBK, Jessie SA, Mark DT, Jeannette B, Ruth EP, Alan RK, Ann LS, John DP and Emily W 2002. Buccal cell DNA yield, quality, and collection costs: Comparison of methods for large-scale studies. Cancer Epidem Biomar & Prev. 11: 1130-1133.

Smith S and Morin PA 2005 Optimal storage conditions for highly dilute DNA samples: A role for trehalose as a preserving agent. J Forensic Sci. 50(5): 1-8.

Wang TY, Wang L, Zhang JH and Dong WH 2011. A simplified universal genomic DNA extraction protocol suitable for PCR. Genet Mol Res 10(1): 519-525.

Woo JG, Guangyun S, Mary H, Subbarao I, Lisa JM, Joseph PB, Ranjan D and Daniel W 2007. Quality assessment of buccal versus blood genomic DNA using the Affymetrix 500 K Gene Chip. BMC Genet. 8: 79.

Xua YC, Bo L, Wan SL, Su YB, Yu J, Xiao PL, Ming BG, Song YJ and Wei Z 2005. Individualization of tiger by using microsatellites. Forensic Sci Int. 151: 45-51.