Species Identification Of Some Non-Domestic Animals Using PCR-RFLP Analysis Of Cytochrome b Gene

Abo-Hadid AH, Alkelch AM , Mervat H Ghoneim and Mayada R Awad-allah
Dept. of Forensic Medicine and Toxicology , Faculty of Vet. Medicine, Zagazig University, Egypt.

ABSTRACT

PCR and PCR-RFLP methods were used to discriminate between some non domestic animals (american black bear, blue nile monkey, Barbary sheep, bacterian camel and llama) and human. DNA from hair was extracted to amplify a mitochondrial cytochrome b gene segment (358 bp) using universal primer followed by agarose gel electrophoresis. The total cyt b amplicon was digested with 4 restriction endonucleases (*Alu* I , *Hae*III, *Hinf*I and *Taq* I) and the resulting fragments were resolved through electrophoresis. The different specific electrophoretic patterns and total restriction fragments were clearly observed among the studied species.

INTRODUCTION

Mitochondrial DNA (mtDNA) has been the most widely studied region of eukaryotic genomes and has played a critical role in development of population and evolutionary genetics. Mitochondrial genome has several advantages over nuclear genome for diagnostic studies of animals because of greater abundance in sample extracts and consequently has thousands of copies per cell. Thus amplification of mtDNA segment is a relatively sensitive procedure and the identification of the species can be based on mutations of the amplification products *(1-3)*.

Cyt b gene is used as a tool in studies of legal medicine and molecular evolution *(4-6)*. Cytochrome b gene has been completely sequenced *(3,7-9)* or partially sequenced [10-13] in many species of mammals , birds *(14-16)*, reptiles, amphibian and fishes *(17-20)*and also some invertebrates *(21,22)*.

Species identification studies based on cyt b gene have ranged from both short (< 400 base pair) to long (> 900 base pair) PCR-RFLP DNA sequencing and variable size species specific multiplex PCR *(23-26)*.

An alternative DNA detection system is based on the polymerase chain reaction (PCR) and the amplification of a segment of the mitochondrial cyt b gene *(27-29)*.Subsequent cleavage by a restriction enzyme which gives rise to species specific pattern.

One of the most important issues in forensic medicine is to identify sample's origin and discriminate wether they descend from human or non human origins so in this study we used a technique based on PCR-RFLP analysis of 358 bp fragment encoding the mitochondrial cyt b gene which enabled identification of human, American black bear, blue nile monkey, Barbary sheep, Lama and Bacterian camel. This technique required equipments and cost than sequencing.

MATERIALS AND METHODS

DNA sources and extraction procedures

Plucked hairs from animals mentioned in Table 1 were collected and used for DNA extraction. Ten basal hair segments of approximately 15 mm length including roots were used to extract DNA using *QIAamp® tissue kit (QIAGEN GmbH, Hilden, Germany)*, according to the manufacture's instructions.

Table 1 The number of animal's hair samples tested in this study (n=10)

Common name	Order	Family	Scientific name
Human	primates	Hominidae	*Homo sapiens*
American black bear	carnivora	ursidae	*Urus americanus*
Blue nile monkey	primates	ceropithecidae	*Ceropithecus mitis*
Barbary sheep	Artiodactyla	Bovidae	*Amotracus lervia*
Bacterian camel	Artiodactyla	camelidae	*Camelus bactrianus*
Lama	Artiodactyla	camelidae	*Lama glama*

DNA purification

After extracting the DNA using the last mentioned kits , the concentration and purity of samples were measured by NanoDrop® ND-1000 (Full-Spectrum UV Spectrophotometer at 260/280 nm) as shown in Table 2

Table 2. The concentration and purity of DNA extracted from hair samples

The sample	Concentration (ng/ µl)	Purity (at 260/280nm)
Human	22.93	1.6
Bear	1050.7	1.8
Monkey	20.00	1.6
Barbary sheep	69.55	1.7
Lama	188.77	1.7
Bacterian camel	43	1.3

PCR primers and amplification

Primer 1 (L 14816) = P1 : conc. 0.25 µM (5´- CCA TCC AAC ATC TCA GCA TGA TGA AA - 3´)

Primer 2 (H15173)(5´ -CCC CTC AGA ATG ATA TTT GTC CTC A- 3´) = P2 : conc. 0.25 µM *(Metabion international AG, Deutschland).*

PCR Master mix (RED Taq ReadyMix PCR Reaction Mix, with $MgCl_2$) : (total volume 25 µl) contaning (20 mM Tris- HCl, Ph 8.3, with 100 mM KCl , 3 mM $MgCl_2$, 0.002% gelatin, 0.4 mM dNTP mix (dATP, dCTP, dGTP, TTP) , stabilizers, and 0.06 unit/ µl of Taq DNA polymerase) *(Sigma, catalog number R2648).*

PCR water *(Sigma, cataloge no W1754).*

Table 3 The amount of PCR mix, primers, extracted DNA and PCR water used in amplification of cyt b gene fragment.

Ingredient / Species	Human	Bear	monkey	Barbary sheep	Lama	Bacterian camel
RED Taq readymix (µl)	12.5	12.5	12.5	12.5	12.5	12.5
Forward primer(µl)	1	1	1	1	1	1
Reverse primer(µl)	1	1	1	1	1	1
DNA template (sample) (µl)	5	1	4.5	1.5	1	2.5
PCR reaction water (µl)	5.5	9.5	6.0	9.0	9.5	8.0
Total volume (µl)	25	25	25	25	25	25

For amplification 5 µl of the extract was used for PCR in a 9600 GeneAmp (Perkin Elmer) and performed according to the described protocol *(1)* in a total volume of 25 µl consisting of 1X PCR reaction buffer (20 mM Tris- HCl, pH 8.3, with 100 mM KCl , 3 mM $MgCl_2$), 0.4 mM dNTP , 0.25 µM each primer and 0.06 unit/ µl of Taq DNA polymerase. (Perkin Elmer) for 35 cycles at 94 ^{O}C for 30 seconds , 50 ^{O}C for 45 seconds and 72 ^{O}C for 45 seconds including one initial denaturation step at 95 ^{O}C for 11 minutes.

Gel electrophoresis and RFLP analysis

Samples were electrophoresed on 1.6% agarose gel that dissolved in 0.5 X TBE buffer. The separation has been achieved at 100-150 Ma for about 30-45 minutes. Visualization of DNA fragments was done in UV cabinet unit and photographed with a Polaroid camera.

PCR products (10ul) were directly digested by 5 U of each one of restriction endonucleases, *Alu* I , *Hae*III, *Hinf*I and *Taq* I *(Roche Diagnostics, Roche applied science, Mannheim Germany).* For 2 hours at 37 ^{O}C . Digestion products were separated by electrophoresis on 3% agarose gels. and visualized under UV illumination and photographed with a Polaroid camera . The length of restriction fragments was compared with the DNA standard 100 bp DNA Ladder (100 to 1000 bp scale) *(Jena Bioscience, GmbH, Germany).*

RESULTS

The universal primers of *L14816* and *H15173* were used to amplify part of the cyt b gene (358 bp) from the six species listed in Table (1). The PCR produced a single amplification product for each genomic template. The size of all PCR products from the animals tested shown no obvious differences when separated on 1.6 % agarose gel with the expected size (358 bp) as shown in Fig. (1).

The size of bands produced by electrophoresis after digestion with the 4 restriction endonucleases (*Alu* I , *Hae*III, *Hinf*I and *Taq* I) were identified by comparison of standard size marker using graphical methods. The cleavage patterns including band numbers and sizes are shown in Table (4)

The RFLP of cyt b region in the different species showed high specificity that even there was no common fragments shared among them. Restriction endonucleases digestion showed specific patterns as follows:

1.The restriction enzyme *Alu* I

The restriction enzyme *Alu* I yielded 2 fragmens of different sizes in all animal species (240+118 bp, 250+108 bp, 190+168 bp, 310+48 bp and 300+58 bp) for Bear, Monkey, Barbary sheep, Lama and Bacterian camel respectively, whereas no cleavage was observed in human (Fig. 2).

2.The restriction enzyme *Hae*III

*Hae*III restriction enzyme revealed 2 restriction fragments patterns of sizes (233+125 bp, 284+74 bp and 200+158 bp) in human , Barbary sheep and Bacterian camel respectively, and 3 fragments in both Monkey and Lama of sizes (200+100+58 bp and 150+120+88 bp) respectively, while there was no digest observed in Bear Fig (3).

3.The restriction enzyme *Hinf*I

Using of *Hinf*I restriction enzyme produced 2 fragments of different sizes for each of human (196+162), Bear (290+68), Bacterian camel (303+55) and Barbary sheep (150+208) and 3 fragments in Monkey (190+128+40)while no fragmentation enerated for Lama by this enzyme Fig (4).

4.The restriction enzyme *Taq* I

When the amplified cyt b segment was digested by *Taq* I restriction enzyme it gave 2 bands differe in their sizes between all species except Barbary sheep where no digest was observed, the sized of segments in different species where (230+128, 154+204, 183+175, 100+258 and 270+88) in Human, Bear, Monkey, Lama and Bacterian camel respectively Fig (5).

Table 4. The number and size of electrophoretic bands of cyt b gene fragments (bp) after treatment with 4 different restriction endonucleases.

Animal species	Restriction endonuclease cleavage pattern			
	Alu I	*Hae*III	*Hinf*I	*Taq* I
Human	1 (358)	2 (233+125)	2 (196+162)	2 (230+128)
American black bear	2 (240+118)	1 (358)	2 (290+68)	2 (154+204)
Blue nile monkey	2 (250+108)	3 (200+100+58)	3 (190+12+40)	2 (183+175)
Barbary sheep	2 (190+168)	2 (284+74)	2 (208+150)	1 (358)
Lama	2 (310+48)	3 (150+120+88)	1 (358)	2 (258+100)
Bacterian camel	2 (300+58)	2 (200+158)	2 (303+55)	2 (270+88)

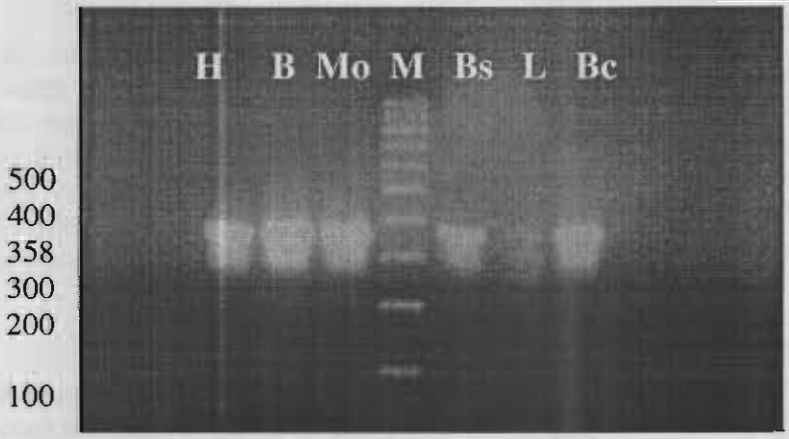

Fig 1. Agarose gel electrophoresis of PCR products (358 bp) of cytochrome b gene generated by primers ((L 14816) and (H15173). Lane H is human, lane B is bear, lane Mo is monkey, lane Bs is barbary sheep, lane L is lama, lane Bc is bacterian camel and lane M is a molecular weight marker (100bp)

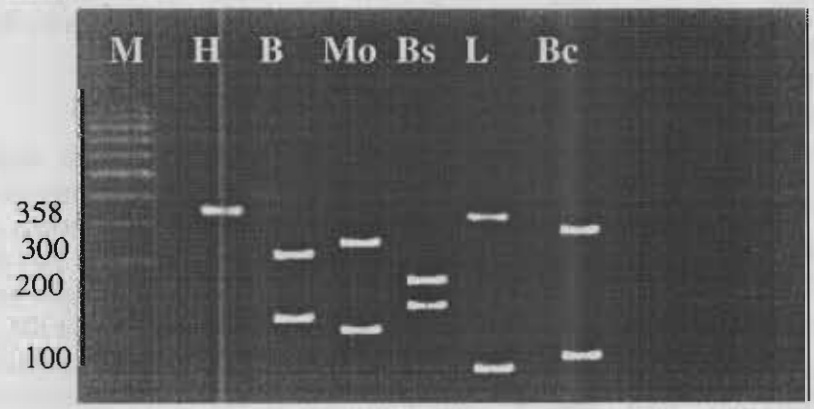

Fig 2. Agarose gel electrophoresis of amplified cyt b gene fragment following digestion with *AluI*. Lane H is human (358 bp), lane B is bear (240+118 bp), lane Mo is monkey(250+108 bp), lane Bs is barbary sheep (190+186 bp), lane L is lama(310+48 bp), lane Bc is bacterian camel (300+58 bp), and lane M is a molecular weight marker (100bp).

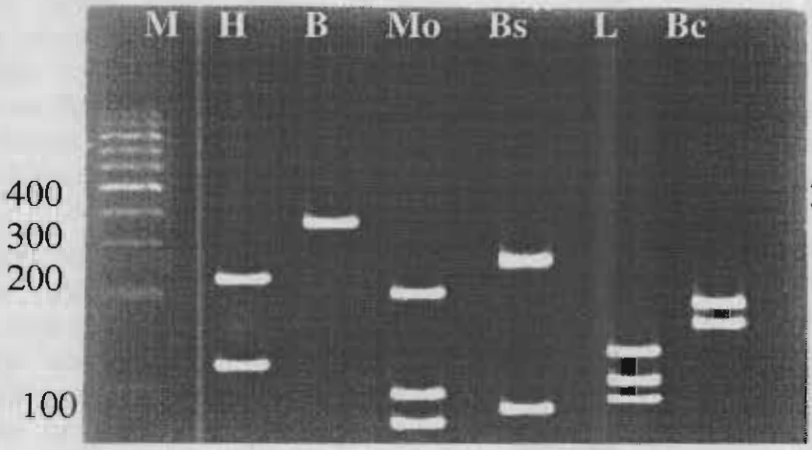

Fig 3. Agarose gel electrophoresis of amplified cyt b gene fragment following digestion with *Hae*III. Lane H is human (233+125 bp), lane B is bear (358 bp), lane Mo is monkey(200+100+58 bp), lane Bs is barbary sheep (284+74 bp), lane L is lama(150+120+88 bp), lane Bc is bacterian camel (200+158 bp), and lane M is a molecular weight marker (100bp).

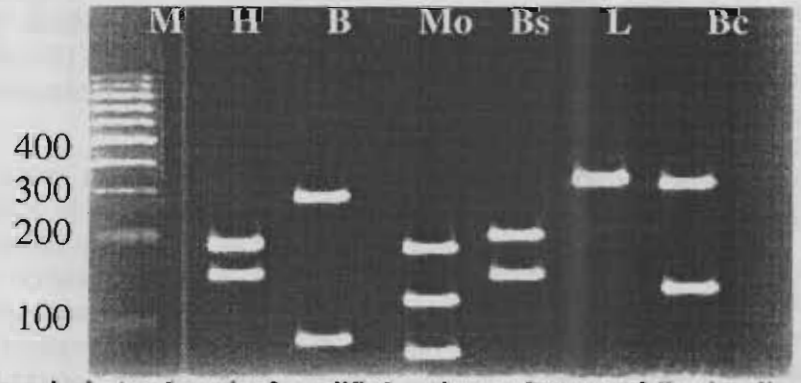

Fig 4. Agarose gel electrophoresis of amplified cyt b gene fragment following digestion with *Hinf*I. Lane H is human (196+162 bp), lane B is bear (290+68 bp), lane Mo is monkey(190+128+40 bp), lane Bs is barbary sheep (150+208bp), lane L is lama(358 bp), lane Bc is bacterian camel (303+55bp), and lane M is a molecular weight marker (100bp).

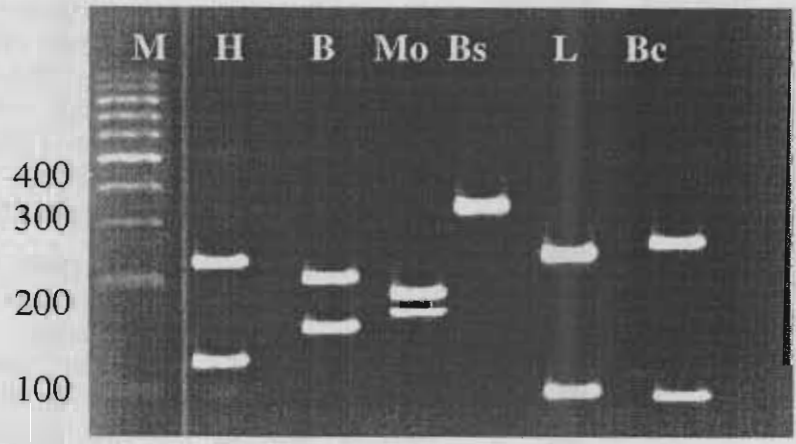

Fig 5 . Agarose gel electrophoresis of amplified cyt b gene fragment following digestion with *Taq* I. Lane H is human (230+128bp), lane B is bear (154+204bp), lane Mo is monkey(183+175bp), lane Bs is barbary sheep (358bp), lane L is lama(100+258 bp), lane Bc is bacterian camel (270+88bp), and lane M is a molecular weight marker (100bp).

DISCUSSION

DNA identification methods generally give better resolution than the traditional morphological or protein identification methods, DNA profiling is one of the most useful tools for determining animal species in commercial foods *(30)*. When analysis is undertaken it usually involves sequencing all or part of the mitochondrial genome which is then compared to known sequences on Gene Bank. This technique is accepted, but has many problems including, being cost, time and labour intensive due to the extra step of sequencing products, mixtures can not be separated and generated samples may not generate sufficient sequence data *(31)*. More modern techniques now allow the identification of species specific markers or repeats (SSR), mitochondrial DNA restricted fragment length polymorphism (mtDNA-RFLP), and random amplified polymorphic PCR (RAPD-PCR) which has the major advantages over protein analysis. The samples heated to 120 ^{o}C can still be analyzed and discriminated between species *(32-34)*.

In this study a short fragment (358 bp) of the mitochondrial cyt b gene was investigated for species identification because this fragment has by far the widest taxonomic representation in nucleotide databases *(3,15,35, 36)*. The resulting fragments were digested with four restriction endonucleases (*Alu* I , *Hae*III, *Hinf*I and *Taq* I) using electrophoresis. Selection of these enzymes for further experiments was based on their putative ability o discriminate between the species in polyacrylamide gels.

The RFLP of cyt b gene region in the different species showed high specificity that even there was no common fragment shared among them.the current study showed that restriction endonucleases digestion had specific patterns for each species. *Alu* I , *Hae*III, *Hinf*I and *Taq* I restriction enzymes were used to screen five horse breeds in Greek and these enzymes have restriction sites (recognition sites on cyt b gene and were able to identify polymorphism and classify the horse breed studied *(37)*. Our results confirmed the study which reported that the PCR- RFLP method allow to identify cattle, horse, donkey, pig, sheep, dog, cat, rabbit, chicken, and human through the different restriction patterns of the enzymes *Alu* I , *Hae*III, *Hinf*I on cyt b gene fragment (358 bp) *(15)*, The authers concluded that this method may be useful as a first assessment in forensic evidence.

Alu I enzyme generated different digestion patterns enabling identification of cod fish imported into Japan and it has been found that the PCR-RFLP methods were sufficient for rapidly screening cod products *(30)*. Species specific primers were used to identify cat's, dog's, donkey's and horse's meat, could be differentiate between cat's, dog's, and horse's meat but the same PCR amplification size of horse (221bp) was obtained for donkey and to differentiate between the two species restriction enzyme AluI was used which yielded three fragments in horse's meat (189,96 and 74 bp) whereas no fragments were obtained in donkey's meat *(29)* .

To identify Bos gaurus origin from DNA recovered from other possible ruminant species by PCR-RFLP of cyt b gene using *Hae*III, this method could give conclusive results *(38)*. Similarly the PCR-RFLP was proved to be a rapid, reliable and simple method that enabled the identification of six commercial filefish proceesed species, where a fragment with molecular weight (465bp of cyt b gene) from processed filefish meats was amplified , the obtained fragments were subjected to digestion by HaeIII enzyme which could differentiate the species of A. monoceros (285+180bp), A. scriptus (180+159+126 bp), M. chinensis (424+41bp), T. hypergyrus (180+159+94+32 bp), T. modestus (229+180+56 bp) and C. penicilligerus (285+139+41bp) *(39)* .

PCR-RFLP of cyt b gene was used to differentiate Tiger panthera tigris and Leopard panthera pardus with using *Hinf*I which appeared more likely to produce easily distinguishable differences in RFLP banding patterns between the two species and these results are in consistent with the current study concerning using *Hinf*I enzyme for identification of animal species *(40)*.

Our investigation concerning using *Taq* I enzyme was proved by previous study in which a segment of cyt b gene (359 bp) was amplified and exposed to digestion by TaqI restriction enzyme to discriminate between buffalo's and cattle's meat which generate two fragments (191 and 168 bp) in buffalo, whereas the 359 bp of cyt b not digested in cattle because using of species specific primers couldn't differentiate between the two species. The results showed that cytochrome b gene PCR-RFLP analysis provide a rapid and effective methods to detect meat species *(41)* .

Conclusion

From the obtained results we concluded that the use of RFLP products of mtDNA cyt b gene could be considered as a simple, quick and cheap method in forensic identification of different animal species.

REFERENCES

1. ***Bartlett S E and Davidson W S (1992):*** FINS (forensically informative nucleotide sequencing): A procedure for identifying the animal origin of biological specimens. *BioTechniques.*; *12: 408–11*
2. ***Zehner R, Zimmermann S and Mebs D (1998):*** RFLP and sequence analysis of the cytochrome *b* gene of selected animals and man: methodology and forensic application. *Int J Legal Med.*; **111**: 323–7.
3. ***Parson W , Pegoraro K, Niederstatter H, Foger M and Steinlechner M (2000) :*** Species identification by means of the cytochrome *b* gene. *Int J Legal Med.*; **114**: 23–8
4. ***Kocher T D , Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca FX, and Wilson AC (1989):*** Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primes. *Proc Natl Acad Sci USA.*; **86**: 6196–200.
5. ***Montgelard C, Catzeflis FM and Douzery E (1997):*** Phylogenetic relationships of artiodactyls and cetaceans as deduced from comparison of cytochrome *b* and 12S rRNA mitochondrial sequences. *Mol Biol Evol.*; **14**: 550–9
6. ***Prusak B, Grzybowski G and Zieba G (2004):*** Taxonomic position of *Bison bison* (Linnaeus, 1758) and *Bison bonasus* (Linnaeus, 1758) based on analysis of *cytb* gene. *Anim Sc Pap Rep.*; **22**: 27–35.
7. ***Bing S U , Wang YX, Lan H, Wang W and Zjang Y (1999):*** phlogenetic study of complete cytochrome b genes in Musk deer (Genus Moschus) using Museum samples. J. Molecular Phylogenetics and Evolution. Vol. 12 No. 3 pp 241-249.
8. ***Zhang YP and Ryder OA (1998) :*** mitochondrial cytochrome b gene sequences of old world monkeys: with special reference on evolution of asian colobines. J. Primates, 39(1): 39-49.
9. ***Branicki W, Kupiec T and Pawlowski R (2003):*** Validation of cytochrome b sequence analysis as a method of species identification. J. Forensic Sci. **48** (2003), pp. 83-87.
10. ***Cook CE, Ying W and George S (1999) :*** A mitochondrial control region and cytochrome b phlogeny of sika deer (cervus Nippon) and Report of tandem repeats in the control region. J. Molecular Phylogenetics and Evolution. Vol. 12 No. 1 pp 47-56.
11. ***Hsieh HM, Chiang HL, Tasi LC, Lai SY, Huang NE, Linacre A and Lee JCI (2001):*** Cytochrome b gene for species identification of the conservation animals , Forensic Sci. Int. **122** (2001) , pp. 7-18.
12. ***Pfeiffer I, Burger J and Brenig B (2004):*** Diagnostic polymorphisms in the mitochondrial cytochrome b gene allow discrimination between cattle, sheep, goat, roe buck and deer by PCR-RFLP. *BMC Genetics* **5**:30 pp. 1-5
13. ***Prusak B, Grzybowski T and Bednarek J (2005):*** Cytochrome b gene (*cytb*) in analysis of anonymous biological traces and its application in veterinary diagnostics and

animal conservation. *Animal Science Papers and Reports* vol. **23** (2005) no. **4**, 229-236

14. ***Shen, X J , Suzuki H and Tsudzuki M (1999):*** Comparison Of Cytochrome B Region Among Chinese Painted Quail, Wild Strain Quail, And White Broiler Chicken Based On PCRRFLP Analysis . Jpn. Poult. Sci. 36: 287-294.

15. ***Bravi CM, Liron J P , Mirol P M, Ripoli M V, Garcia P P and Giovambattista G (2004):*** A simple method for domestic animal identification in Argentina using PCR-RFLP analysis of cytochrome b gene. J. Legal medicine vol 6 246-251

16. ***Andrzej D and Kamila K (2005)*** : Anew PCR-RFLP with in the domestic pigeon (Columba livia var, domestica) cytochrome b (MTCYB) gene . J. Appl. Genet 46 (3), pp. 315-317

17. ***Chow S, Clarke ME and Walsh PJ (1993):*** PCR-RFLP analysis on thirteen western Atlantic snappers (subfamily Lutjaninae): A simple method forspecies and stock identification. Fish Bull **30**:207–224.

18. ***Ram J L, Ram M L and Baidoun F F, (1996)*** : Authentication of canned tuna and bonito by sequence and restriction site analysis of polymerase chain reaction products of mitochondrial DNA. J Agric Food Chem **44**: 2460–2467.

19. ***Quinteiro J, Sotelo CG, Rehbein H, Pryde SE, Perez-Martin RI, Rey-Mendez M and Mackie IM (1998):*** Use of mtDNA direct polymerase chainreaction (PCR) sequencing and PCR-restriction fragment length polymorphism methodologies in species identification of canned tuna. J Agric Food Chem **46**: 1662–1669.

20. ***Lindstrom D P (1999):*** Molecular species identification of newly hatched Hawaiian *amphidromous gobioid* larvae. Mar Biotech **1**: 167–174.

21. ***Wang L, Yokoyama K, Takahasi H, Kase N, Hanaya Y, Yashiro K, Miyaji M and Nishimura K (2001):*** identification of species in Aspergillus flavi based on sequencing of the mitochondrial cytochrome b gene . International Journal OF Food Microbiology 71:75-86.

22. ***Lee J C , Tasi L C, Liao S P, Linacrre A and Hsieh H M (2009 a)*** : Species identification using the cytochrome b gene of commercial turtle shells. J. Forensic Science International : Genetics 3 67-73.

23. ***Bellis C, Ashton K J, Freney L, Blair B and Griffiths L R (2003):*** A molecular genetic approach for forensic animal species identification. Forensic Sci Int 2003; **134**:99-108.

24. ***Bottero M T , Civera T, Nucera D, Rosati S, Sacchi P and Turi R M (2003):*** A multiplex polymerase chain reaction for the identification of cows, goats, and sheep's milk in dairy products. Int. Dairy J 2003; **13**:277-82.

25. ***Aranishi F , Okimoto T and Izumi S (2005)*** : Identification of gadoid species (Pisces, Gadidae) by PCR-RFLP analysis. J. Appl. Genet. 46 (1), pp. 69-73.

26. ***Lee J C , Hsieh H M, Huang L H, Kuo Y C, Wu J H, Chin S C, Lee A Linacrre H and Tasi L C (2009 b)*** : Ivory identification by DNA profiling of cytochrome b gene . International Journal of Legal MedicineVolume 123, Number 2 pp. 117-121.

27. ***Murray B W, McClymonts R A and Strobeck V (1995):*** forensic identification of ungulate species using restriction digestion of PCR-amplified DNA. J. For. SCI. , 40: 943-951.

28. ***Abdel-Rahman S M and Ahmed M M M (2007):*** Rapid and sensitive identification of buffalos, cattles, and sheeps milk using species specific PCR and PCR-RFLP techniques. Food Control, 18: 1246-1249.

29. ***Abdel-Rahman S M , El-Saadani M A, Ashry K M and Amany S Haggag (2009):*** Detection of adulteration and identification of cats, dogs, donkeys and horses meat using species specific PCR and PCR-RFLP

techniques. Austuralin journal of basic and applied sciences, 3 (3): 1716-1719.

30. ***Akasaki T , Yanagimoro T, Yamakami K, Tomonaga H and Sato S (2006):*** species identification and PCR-RFLP analysis of cytochrome b gene in cod fish (Order Gadiformes) products. J. Food Science. Vol. 71 No. 3 pp. 190-195.

31. ***Lenstra J A , Buntjer J B and Janssen F W (2001):*** On the origin of meat-DNA techniques for species identification of meat products. *J. Vet. Sci. Tomrrow, 2 :1-15.*

32. ***Ahmed M M M (2005):*** PCR amplification of species specific repeat for meat DNA identification via genetic markers in cattle and sheep. Biotechnol. Anim. Husbandry, 21:1-11.

33. ***Abdel-Rahman S M (2006) :*** Evidences reveal that cattle and buffalo are evolutionary derived from the same ancestor based on cytogenetic and molecular markers. Biotechnol. Anim. Husbandry, 22:1-10.

34. ***Tobe S S and Linacre A (2008):*** A method to identify a large number of mammalian species in the UK from trace samples and mixtures without the use of sequencing J. forensic Science International : Genetic supplement series 1 pp 625-627.

35. ***Pancorbo M M D, Casro A, Fernandez I F and Cuevas N (2004):*** cytochrome *b* for identification of animal species in processed food . international congress series 1261, PP 592-594.

36. ***Guo X , He S, and Zhang Y (2005):*** phylogeny and biogeography of Chinese sisorid catfishes re-exmined using mitochondrial cytochrome b and 16S rRNA gene sequences. J. Molecular Phylogenetic and Evolution 35: 344-362.

37. ***Apostolidis A P, Alifakiotis T, Mamuris Z and Karkavelia E (2000):*** PCR-RFLP analysis of mitochondrial cytochrome b gene among Greek horse breeds. Ital. J. Zool., 67: 195-162.

38. ***Viseshakul N and Sarikaputi M (2003):*** Detection of the cytochrome b gene and its RFLP pattern from Bos gaurus specimen as a forensic application. The 11th International Symposium of the World Association of Veterinary Laboratory Diagnosticians and OIE Seminar on Biotechnology, November 9-13.

39. ***Jung Wu Y , Hsieh C H, Chen H M and Hwang D F (2008):*** identification of six common species of processed filefish using cutochrome b gene sequence and PCR-RFLP analysis. The Rraffles Bulletin of Zoology supplement No. 19 : 151-158.

40. ***Nagata J , Aramilev V V, Belozor A, Sugimoto T and McCullough D R (2005):*** fecal genetic analysis using PCR-RFLP of cytochrome b to identify sympatric carnivores, the tiger panthera tigris and leopard panthera pardus, in far eastern Russia. Conservation Genetics 6: 863-865.

41. ***Ahmed M M M , Abdel-Rahman S M and El-Hanafy A A (2007):*** application of species specific polymerase chain reaction and cytochrome b gene for different meat species authentication . Biotechnology 6 (3): 426-430.

الملخص العربي

استخدام جين السيتوكروم بي في الاستعراف علي بعض الحيوانات الغير مستانسة عن طريق تقنية PCR-RFLP

علي حيدر ابو حديد ، القلش مصطفي القلش، مرفت حسن غنيم، ميادة رجب فرج عوض الله
قسم الطب الشرعي والسموم- كلية الطب البيطري- جامعة الزقازيق

أجريت هذه الدراسة بغرض الاستعراف علي بعض الحيوانات الغير مستأنسة وهي الدب الامريكي الاسود، قرد النيل الازرق، الكبش الاروي، اللاما والجمل ذو السنامين وكذلك الانسان حيث تم تجميع عينات من شعر تلك الحيوانات والانسان لاستخلاص الحمض النووي الديؤكسي ريبوزي عن طريق تقنية PCR-RFLP وتم استخدام بادئ عام للحصول علي قطعة من جين السيتوكروم بي (358bp)وتم تفريدالقطع الناتجة علي الاجار جيل بواسطة تقنية الفصل الكهربي. ثم عرضت القطع الناتجة للهضم بواسطة اربعة من انزيمات القطع وهي*Taq* I*Hinf*I ,*Hae*III, *Alu* I وقد قامت هذه الانزيمات بتقطيع الجين الي مجموعة من القطع حيث اختلفت اعداد تلك القطع واطوالها بالنسبة لكل حيوان . من ذلك نستخلص انه يمكن استخدام هذه التقنية كطريقة سهلة وبسيطة في الاستعراف علي الحيوانات المختلفة.