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Genetic Diversity Among Saudi Sheep Breeds As Detected by Random Amplified Polymorphic DNA Marker

[1,2]Amr A. El-Hanafy, [1,3]Ayman I. ElKady, [1]J. Sabir and [1]M. Mutawakil

[1]Department of Biological Sciences, Faculty of Science, P. O. Box: 80203, King Abdulaziz University, Jeddah, 21589, Saudi Arabia
[2]Department of Nucleic Acid Research, Genetic Engineering and Biotechnology Research Institute, City for Scientific Research &Technology Applications, Borg EL-Arab, P.O. Box: 21934, Alexandria, Egypt
[3]Department of Zoology, Faculty of Sciences, Alexandria University, Alexandria, Egypt

Abstract: Phylogeny analysis using Random Amplified Polymorphic DNA (RAPD) markers was performed for studying genetic variation in three Saudi sheep breeds, namely: Harri, Nagdi and Sawakni. Blood samples were collected from animals and leukocytes were isolated and subjected to RAPD-PCR assay using four 10-b random primers. The results showed that Harri and Sawakni sheep breeds lay in the same group and share about 95 % genetic similarity. On the other hand, Nagdi breed is more distant from both other breeds showing about 75% genetic similarity with both of them.

Key words: Sheep · Saudi breeds · RAPD · Genetic similarity

INTRODUCTION

Mutton is the most favorite meat to Saudi citizens followed by chicken, camel, fish and beef. Therefore, sheep and goats occupy a special niche in Saudi Arabian agribusiness [1, 2]. The number of sheep in Saudi Arabia is about 5.2 million head, according to Saudi Ministry of Agriculture, 2010. Saudi government began intensive efforts for encouragement of investments in the field of agribusiness since 1980s [2]. There is worldwide decline in biodiversity, where 20% of the domestic animal was reported to be at risk of extinction [3- 7]. This decline in biodiversity is critical because the lose of genetic diversity is in-compensable [4]. This loss of biodiversity might be due to economic and/or socio-political reasons [8]. It is estimated that 180 sheep breeds (14%) are extinct [5, 6]. Thus, conservation and maintenance of animal genetic biodiversity of local breeds will facilitate the effective management of farm animal genetic resources. Indigenous sheep breeds are reported to be a valuable source of genetic material due to adaptation to local, sometimes, harsh environmental conditions, nutritional fluctuations and resistances to diseases and parasites [2, 9-12]. Several studies were conducted for characterization and evaluation of genetic diversity in sheep and goat which represent first step in the conservation and utilization of indigenous sheep and goat breeds [2, 13, 14].

Breed characterization requires the knowledge of genetic variation that can be effectively measured within and between populations [15]. Genetic markers may provide useful information at different levels: Population structure, levels of gene flow, phylogenetic relationships, patterns of historical biogeography and the analysis of parentage and relatedness [16].

During the last few years, the great strides of molecular biology virtually gave access to the entire genome, but their complexity and high cost limited their use to precisely targeted projects in population biology. Interestingly, the polymerase chain reaction (PCR) opened a new era for genetic variations studies [17- 19]. The PCR technique is basically a primer extension reaction for amplifying specific nucleic acids in vitro. The use of a thermostable polymerase referred to as "Taq" allows a short stretch of DNA to be amplified to about a million fold so that one can determine its size, nucleotide sequence, etc. In addition, the extensive genetic polymorphism revealed by DNA markers could be used as

Corresponding Author: Ayman I. El-Kady, Department of Biological n Sciences, Faculty of Science, P.O. Box: 80203, King Abdulaziz University, Jeddah, 21589, Saudi Arabia. E-mail: aielkady@yahoo.co.uk.

an advantage to resolve genetic difference of even closely related individuals. The breakthrough of population biology is that it is now possible to work with a very small initial amount of DNA (virtually, one cell is sufficient).

Application of the random amplified polymorphic DNA technique has greatly increased the ability to understand the genetic relationships within species at the molecular level. Information on genetic relationships in livestock within and between species has several important applications for genetic improvement and in breeding programs [20]. The RAPD technique has also been used for constructing trees in other animals such as buffalo, cattle, goat and sheep [2,20, 13], tilapia fish [21], bacteria [22] and date palm [23]. Domestic animal genetic diversity will meet current and future production needs under various environments for allowing sustainable genetic improvement and facilitating rapid adaptation to change breeding purpose [24, 25].

Since little information are available about the genetic relationships between Saudi local sheep, the aim of this research is to study genetic diversity among these breeds in an attempt to have a clear image about phylogeny and genetic relations between these local adapted breeds, which will aid in the future for genetic improvement of these local valuable genetic resources.

MATERIALS AND METHODS

Blood samples were collected from three sheep breeds, Harri, Nagdi and Sawakni, living in Jeddah and Riyadh provinces, Saudi Arabia. Blood samples were taken randomly from individuals from each breed. Approximately, 10ml venous blood was collected from each animal using 0.5 ml of 2.7% EDTA as an anticoagulant. Genomic DNA was isolated from blood using DNA extraction kit (GF-1, Vivantis) according to the manufacturer's instructions. The quality of DNA was checked by spectrophotometry taking ratio of optical density (OD) value at 260 and 280 nm. Good quality DNA having OD ratio between 1.7 and 1.9 was used for further work. The poor quality DNA was re-extracted with a mixture of phenol: chloroform.

Ten blood samples from each breed were run with four 10 b RAPD primers using RAPD- PCR technique as previously described [26]. The PCR was carried out in a 25 µL reaction mixture containing: 100–150 ng genomic DNA, 0.5 µM of each primer, 1.00 U of Taq DNA Polymerase, 2. 5 µL of 10 × PCR assay buffer (1.5 mM MgCl2), dNTPs each at 100 µM. The amplification was carried out using a pre-programmed thermal cycler (Eppendorf Mastercycler). The sequence of the four primers and their annealing temperature are shown in Table (1).

Table 1: Sequence of primers and annealing temperature used in RAPD study

Primer	Sequence	Annealing Temperature °C
1	5`- GTG GGC TGAC -3`	35
2	5`- GTC CAT GCCA- 3`	35
3	5`- ACA TCG CCCA- 3`	35
4	5`-GTAGACCCGT- 3`	35

The thermal cycle profile was as follow: 4 min initial denaturation at 95°C, 45 cycles of 1 min at 95°C, 1 min at 35°C, 1 min at 72°C, followed by a final extension at 72°C for 10 min. PCR product were analyzed in 2% agarose gel stained with ethidium bromide. Gels were photographed by Gel Documentation system (Syngene). PCR products were scored across the lanes as variables. The data entry was done into a binary data matrix as discrete variables. Jaccard's coefficient of similarity was measured and a dendrogram based on similarity coefficients was generated by using Unweighted Pair Group Method with Arithmatic Mean (UPGMA). Data were scored on bases of the presence or absence of bands. If band was present in a genotype it was designated as 1, in case of the absence of certain band in a genotype, it was designated as 0. Using Statistical Package for Social Science (SPSS) computer program, phylogenetic relationships were calculated

RESULTS AND DISCUSSION

To ensure that the amplified DNA bands originated from genomic DNA and not primer artifacts, negative control was carried out with each of the four primers and no amplification was detected in control reactions. All amplification products were found to be reproducible when reactions were repeated using the same reaction conditions. All of the four primers used in this study were successfully amplified polymorphic bands among the individuals three sheep breeds studied as is shown in (Figures 1-4). DNA polymorphism as revealed by highly polymorphic 4 primers is described in Table 2 of the 687 bands scored 512 were polymorphic and the rest monomorphic. The highest percentage of polymorphism (82.03%) was obtained with primers 2.

RAPD analysis was used for constructing parsimony tree depicting relationships among the three sheep breeds studied (Figure 5). Data presented in Figure 5 showed that

Table 2: List of primers code, number of produced bands, polymorphic, monomorphic bands among four primers used

Primer code	Total no. of amplified fragments	No. of monomorphic fragments	No. of polymorphic fragments	Polymorphism %	Fragments size range (bp)
1	177	60	117	66.10	250-1200
2	167	30	137	82.03	400-1400
3	205	70	135	65.85	300-1300
4	138	15	123	67.21	150-1200
Total	687	175	512	74.52	

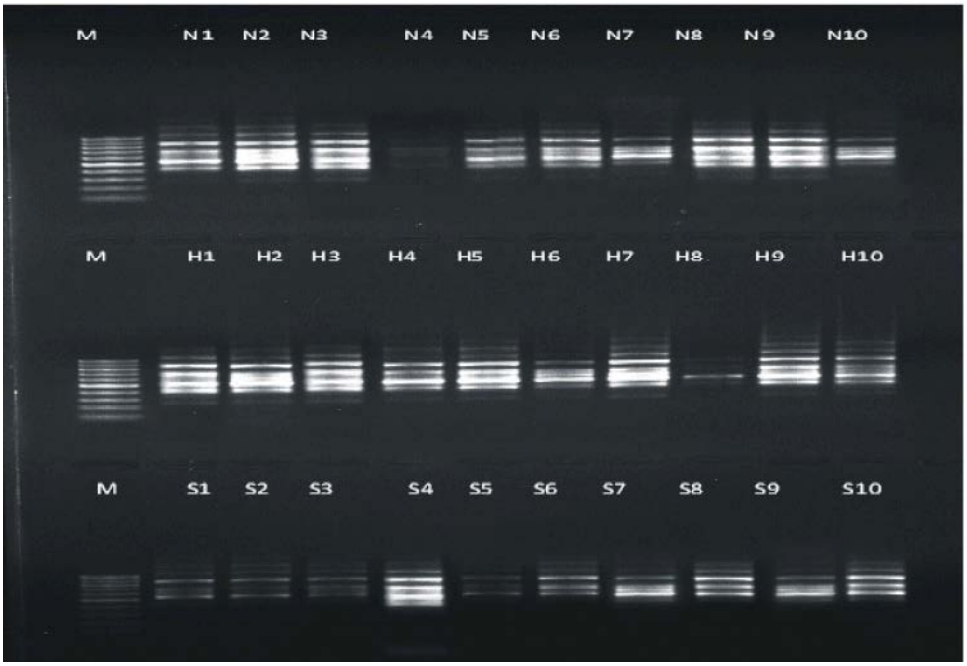

Fig. 1: RAPD profiles obtained with primer 1 from the DNA of Nagdi (N1-N10),Harri (H1-H10), Sawakni (S1-S10), Lane 1 M: Molecular size marker(100 bp DNA ladder)

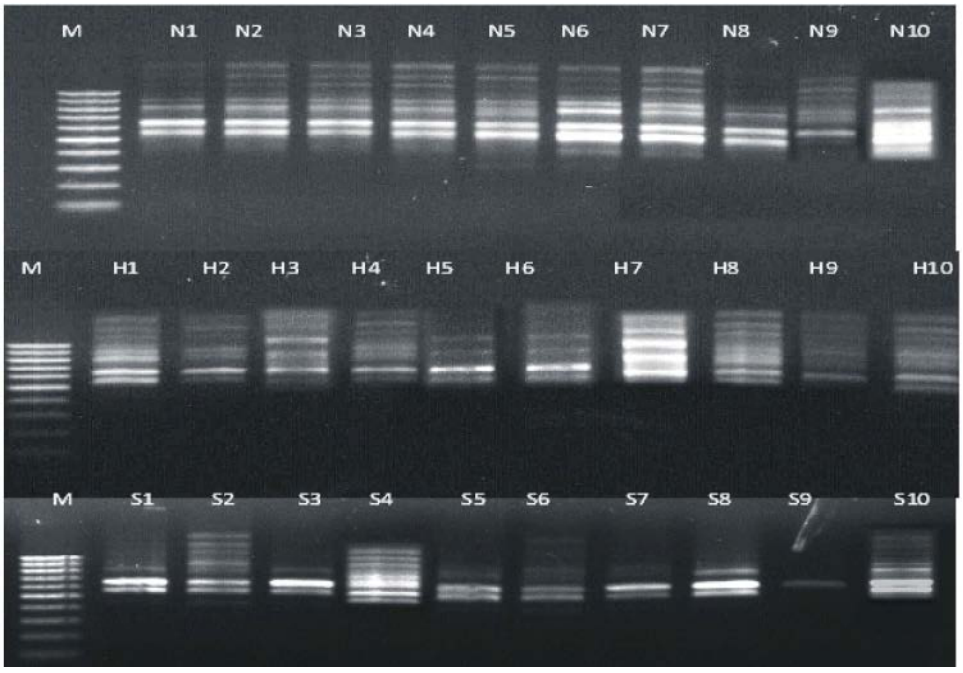

Fig. 2: RAPD profiles obtained with primer 2 from the DNA of Nagdi (N1-N10),Harri (H1-H10), Sawakni (S1-S10), Lane 1 M: Molecular size marker(100 bp DNA ladder)

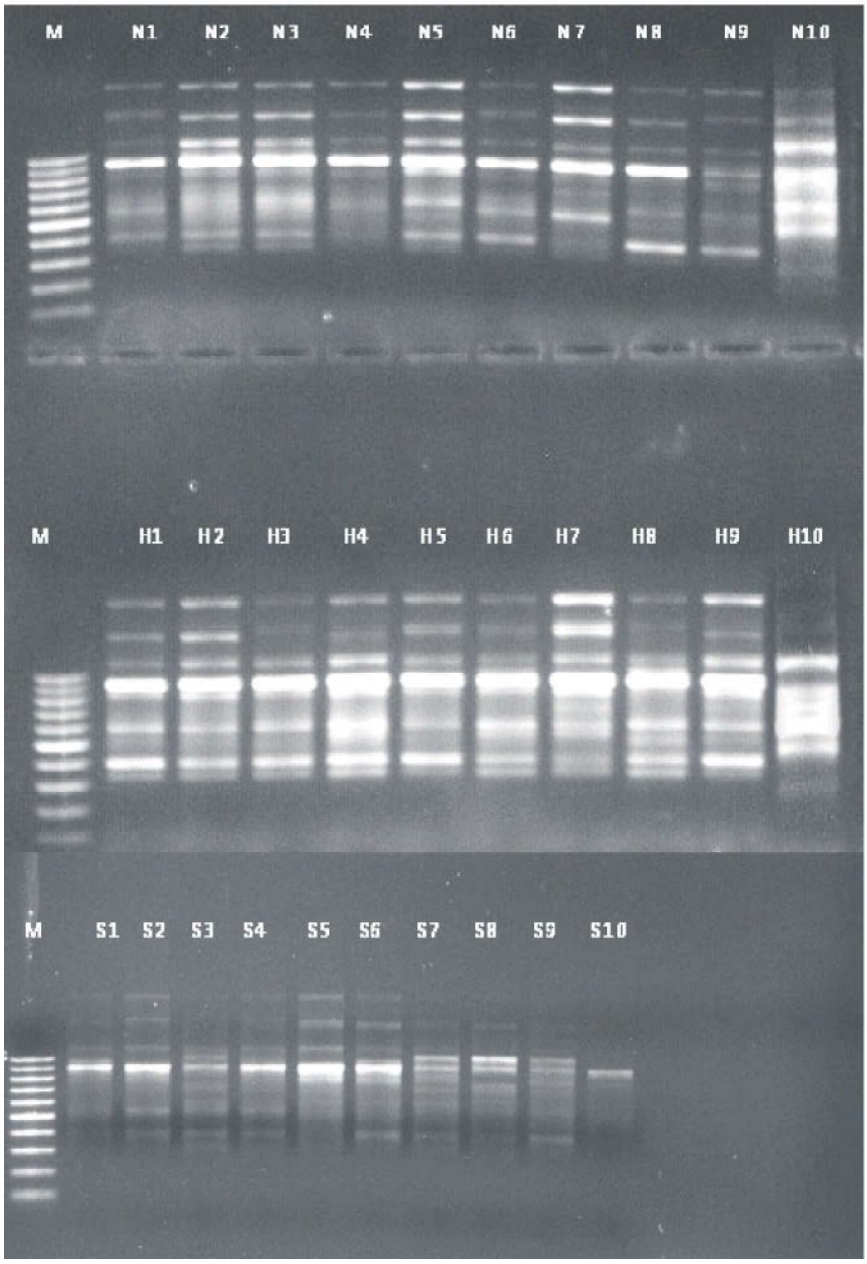

Fig. 3: RAPD profiles obtained with primer 3 from the DNA of Nagdi (N1-N10),Harri (H1-H10), Sawakni (S1-S10), Lane 1 M: Molecular size marker(100 bp DNA ladder)

both Harri and Sawakni breeds share about 75% of genetic similarity with Nagdi breed, while Harri and Sawkni breeds share high genetic similarity to each other. This result can be explained on the basis that origin of Nagdi breed in the middle of Saudi Arabia is far away from the other two breed which found mainly in the western area of the kingdom. In addition, Sawkni breed was imported to Saudi Arabia from Sudan which is close to western area of Saudi Arabia.

Several studies were conducted for the conservation of indigenous genetic resources including sheep breeds [27, 28]. Estimation of the genetic distance between the indigenous domestic breeds represents an important step toward conservation of these indigenous breeds, hence improving the genetic performance of these breeds [29, 30]. Regarding the situation in Saudi Arabia, little genetic information is available about domestic sheep and goat breeds and there are few studies which conducted

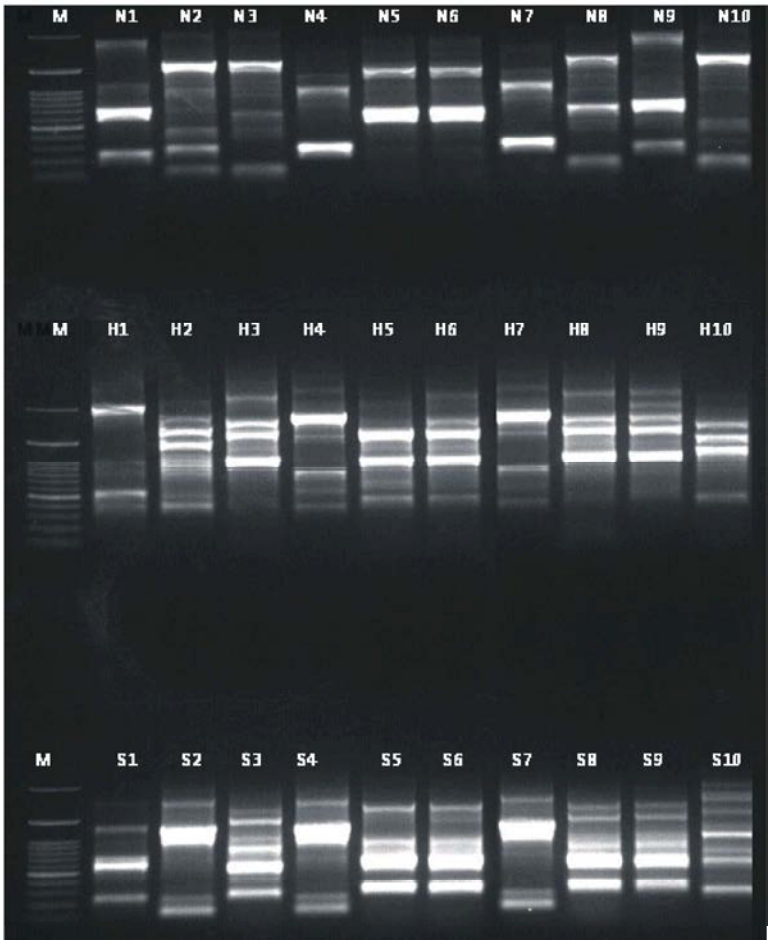

Fig. 4: RAPD profiles obtained with primer 4 from the DNA of Nagdi (N1-N10),Harri (H1-H10), Sawakni (S1-S10), Lane 1 M: Molecular size marker (100 bp DNA ladder)

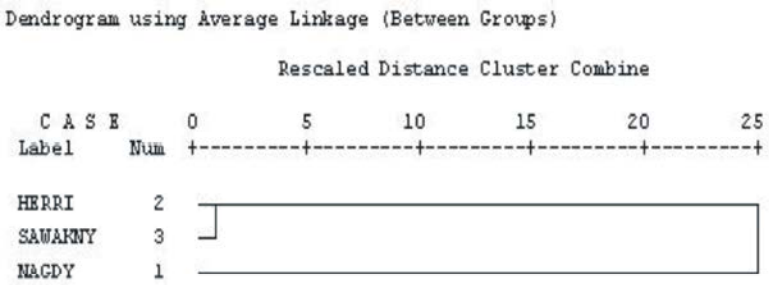

Fig. 5: Dendrogram using Average Linkage (Between Groups) based on RAPD data analysis among the three breeds of sheep

with Saudi breeds to estimate the genetic similarity between these breeds [13, 2]. This study considers primary step for clarify the image about genetic diversity of these local Saudi sheep breeds and should be followed by further studies using large number of animals from different geographic region in Kingdom to get precious estimation about the phylogeny of these local genetic resources and even within each breed. With further experimentations, the RAPD profile generated for each breed can be effectively used as a supporting marker for taxonomic identification. In taxonomic and molecular systematic, species-specific RAPD markers could be an invaluable tool for species variation and establishing the status of organisms and its evolution [20, 31, 32].

CONCLUSION

In conclusion, the genetic diversity exist among the three Saudi sheep breeds and demonstrate the usefulness of the RAPD approach for detecting DNA polymorphism in sheep and establishing the relationships within and among different Saudi sheep breeds, especially few information are available until nowadays about these genetic relationships.

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REFERENCES

1. Abouheif, A., G. Abdo, M. Basmaeil and A. Al Sobayel, 1989. Identification of the preference patterns of different breeds of sheep for consumption in Saudi Arabia. Asian Australian Journal of Animal Science, 2: 129-132.
2. Sabir, J.M., N.S. Awad, E.E. Alhallous and M.H.Z. Mutawakil, 2013. Assessment of genetic diversity among Saudi sheep breeds for characterization and conservation purposes. Global Veterinaria, 11: 634-639.
3. FAO, 2000[a]. Domestic Animal Diversity Information System (DAD-IS 2.0): http://dad.fao.org/dad-is/home.htm.
4. FAO, 2000[b]. In: Scherf, B. (ed), World Watch List for Domestic Animal Diversity, 3[d] ed., Rome.
5. Cardellino, A., 2004. Conservation of farm animal genetic resources a global view. Farm Animals Genetic Resources, 30: 1-14.
6. FAO, 2007. The State of the Worlds Animal Genetic Resources for Food and agriculture. FAO, Rome, Italy.
7. Kunene, W., C. Bezuidenhoutb and V. Nsahlaic, 2009. Genetic and phenotypic diversity in Zulu sheep populations: Implications for exploitation and conservation. Small Ruminant Research, 84: 100-107.
8. Taberlet, P., A. Valentini, R. Rezaei, S. Naderi, F. Pompanon, R. Negrini and A. Marsani, 2008. Are cattle, sheep and goats endangered species. Mol. Ecol., 17: 275-284.
9. Nsoso, J., B. Podisi, E. Otsogile, S. Mokhutshwane and B. Ahmadu, 2004. Phenotypic characterization of indigenous Tswana goats and sheep breeds in Botswana: continuous traits. Tropical Animal Health and Production, 36: 789-800.
10. Kunene, W. and A. Fossey, 2006. A survey on livestock production in some traditional areas of Northern KwaZulu-Natal in South Africa. Livestock Research for Rural Development, 18: 108-113.
11. Kosgey, S. and O. Mwai, 2007. Genetic improvement of small ruminants in low input, small-holder production systems: technical and infrastructural issues. Small Ruminant Research, 70: 76-88.
12. Galal, S., O. Gürsoy and I. Shaat, 2008. Awassi sheep as a genetic resource and efforts for their genetic improvement review. Small Ruminant Research, 79: 99-108.
13. Sabir, J.S.M., M.H.Z. Mutawakil, A.A. El-Hanafy and M.M.M. Ahmed, 2012. Genetic similarity among four breeds of goat in Saudi Arabia detected by random amplified polymorphic DNA marker. Afr. J. Biotechnol., 11: 3958-3963.
14. Ali, B.A., 2003. Genetics similarity among four breeds of sheep in Egypt detected by random amplified polymorphic DNA Markers. Afr. J. Biotechnol., 2: 194-197.
15. Hetzel, D.J.S. and R.D. Drinkwater, 1992. The use of DNA technologies for the conservation and improvement of animal genetic resources. FAO Expert Consultation on Management of Global Animal Genetic Resources. Rome, April.
16. Feral, J.P., 2002. How useful are the genetic markers in attempts to understand and manage marine biodiversity. J. Exp. Mar. Biol. Ecol., 268: 121-145.
17. Mulis, K.B. and F.A. Faloona, 1987. Specific synthesis of DNA in vitro via polymerase catalyzed chain reaction. Methods Enzymol., 155: 335-350.
18. Sakai, R.K., D.H. Delfet, S. Stoffel, S.J. Sharf, R. Higushi, G.T. Horn, K.B. Mulis and H.A. Ehrlich, 1988. Primer directed amplification of DNA with a thermostable DNA polymerase. Science, 239: 487-491.
19. Erlich, H.A., 1989. PCR technology. Stockton Press, New York.
20. Rao, K.B., K.V. Bhat and S.M. Totey, 1996. Detection of species specific genetic markers in farm animals through random amplified polymorphic DNA (RAPD). Genetic analysis: Bimolecular Engineering, 13: 135-38.

21. Baradakci, F. and D.O.F. Skibinski, 1994. Application of the RAPD technique in tilapia fish: species and subspecies identification. Heredity, 73: 117-123.
22. EL-Hanafy, A.A., H.E. Abd –Elsalam and E.E. Hafez 2007. Fingerprinting for the lignin degrading bacteria from the soil. Journal of Applied Sciences Research, 3: 470-475.
23. Soliman, S.S., B.A. Ali and M.M. Ahmed, 2003. Genetic comparisons of Egyptian date palm cultivars (Phoenix dactylifera L.) by RAPD-PCR. Afr. J. Biotechnol., 2: 86-87.
24. El-Hanafy, A.A. and M.A. El-Saadani, 2009. Fingerprinting of fecb gene in five Egyptian sheep breeds. Biotechnol. Anim. Husbandry, 25: 205-212.
25. El-Hanafy, A.A., M.A. El-Saadani, M. Eissa, G.M. Maharem and Z.A Khalifa, 2010. Polymorphism of β-lacto globulin gene in Barki and Damascus and their cross bred goats in relation to milk yield. Biotechnology Anim. Husbandry, 26: 1-12.
26. Williams, J.G.K., A.R. Kubelic, K.J. Livak, J.A. Rafalski and S.V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Res., 18: 6531-6535.
27. Dovie, K., C. Shackleton and E. Witkowski, 2006. Valuation of communal area livestock benefits, rural livelihoods and related policy issues. Land Use Policy, 23(3): 260-271.
28. Du Toit, J., 2008. The indigenous livestock of Southern Africa. http://www.thepdfportal.com/parisroundtable_125309.pdf
29. Bjornstad, G. and H. Roed, 2001. Breed demarcation and potential for breed allocation of horses assessed by microsatellite markers. Animal Genetics, 32: 59-65.
30. Notter, R., 1999. The importance of genetic diversity in livestock populations of the future. Anim. Sci., 77: 61-69.
31. Allard, M.W., M.M. Miyamoto, Jarecki F. Kraus and M.R. Tennant, 1992. DNA systematics and evolution of the artiodactyl family Bovidae. Proc. Natl. Acad. Sci. USA, 18: 3972-3976.
32. Dinesh, K.R., T.M. Lim, W.K. Chan and V.P.E. Phang, 1996. Genetic variation inferred from RAPD fingerprint in three species of tilapia. Aquaculture International, 4: 19-30.