# Detection of Genetic Diversity through Two Poultry Breeds by using RAPD-PCR Technique

**Israa Ali Fadhil**
*Animal Genetics & Breeding, Department of Animal Production, Agricultural College, Al-Qasim Green University*
Asraar83@yahoo.com.
**Mohammed Hasan Dakheel**
*Animal Genetics & Breeding, Public Health, Veterinary Medicine, Al-Qasim Green University*
**Tamadhur Hani Hussein**
*Life Science, Animal, Department of Animal Production, Agricultural College, Al-Qasim Green University*

## Abstract

Random amplified polymorphic (RAPD) has been used to study phylogenetic relationship among two breeds of chicken like Aseel and Brahma breeds. Particular results for each breed were obtained by these methods. RAPD-PCR of 30 samples of these chicken breeds amplified with 6 primers. The six primers were used to amplify the bands of Aseel breed and Brahma breed respectively. According to this study, a total bands amplified were 24, about 66.9% of these amplified primers (17 bands) were polymorphic within Aseel breed, while a total bands amplified were 28, about 70.95% of these amplified primers (20 bands) were polymorphic within Brahma breed. Therefore, this study explains the utility of DNA testing (RAPD-PCR) in detection of genetic diversity through two poultry breeds.
**Key words:** DNA Fingerprinting "RAPD-PCR", Aseel, Brahma, Polymorphisms, Chickens, Genetic Identity, Genetic Distance.

## الخلاصة

استخدمت تقنية التضاعف العشوائي المتعدد الاشكال لسلسلة DNA (Randomly amplified polymorphic RAPD) لدراسة علاقة القرابة ما بين افراد سلالتين من الدواجن هما Aseel و Brahma. تم الحصول على نتائج الدراسة بإستخدام التقنية السابقة الذكر. تقنية التضاعف العشوائي المتعدد الاشكال و التي تعتمد على تقنية ال PCR (Polymerase Chain Reaction) لثلاثين عينه من الدواجن ضخمت بإستخدام ستة بادئات. البادئات السته استخدمت لتضخيم حزم سلالتي ال Aseel و ال Brahma على التوالي. تبعا" لهذه الدراسة، كان مجموع الحزم التي تم تضخيمها 24 من سلالة ال Aseel ، كان عدد التغايرات في نفس السلالة حوالي 17 حزمة و بنسبة 66.9% ، بينما بلغ مجموع الحزم التي تم تضخيمها من سلالة ال Brahma 28 ، كان عدد التغايرات في نفس السلالة حوالي 20 حزمة و بنسبة 70.95% . لذلك فإن الدراسة الحالية وضحت استخدام احدى اختبارات ال DNA و هي تقنية التضخيم العشوائي متعدد الاشكال (Randomly amplified polymorphic RAPD) في تنبأ التنوع الوراثي لسلالتين من الدواجن.
**الكلمات المفتاحية:** البصمة الوراثية، تقنية التضخيم العشوائي متعدد الاشكال (Randomly amplified polymorphic RAPD)، سلالة Aseel، سلالة Brahma ، دواجن، التباين الوراثي، الهوية الجينية، الابعاد الوراثية.

## Introduction

Domestic fowl are the most important type of poultry in India. Since thousand years ago, the domestication of poultry was originally started from the wild types that collect the eggs from it for hatching and rearing young birds, but later keeping the birds permanently in captivity. The term poultry is used collectively for those species of birds that have been domesticated to reproduce and grow in captivity and render product of economic value such as meat, eggs manure etc. The term poultry is applied to birds of several species like fowls or chicken, duck, turkeys etc. The fowls is the second most widely eaten form of meat globally and, along with eggs, provides

nutritionally beneficial food because it has high-quality protein with low proportion of fat. Aseel is one of their breeds of pea comb chickens are good meat sources, cock-fighting and good sitter (Prasad 2008 and 2009). Other chicken breeds are Brahma; there are amongst the biggest species of chickens, quiet chicken, pea comb and hardy in both cold and hot conditions (Taneja *et al*., 2011).

The molecular nature of the precise differences in the nucleotide sequences within gene is responsible for the variation (Stevens 1991). The genetic identity is related to difference and to check relationships between species and breeds. The discovery of polymorphic DNA regions in human and animal genome leads to the evolution of DNA typing and to study the genetic identity should be study the DNA Fingerprinting. The first time introduced this technique to identify individuals by Allec Jeffreys in 1984 (Srivastava *et al*; 2005). Four years later, the same technique was utilized to so many types of birds (Burke and Bruford 1987).

DNA profiling has a wide application in different aspects of animal, medical and veterinary sciences (Bhattacharya *et al*., 2008). It also has been widely used in the study of animal populations and has revolutionized the field of zoology (Chambers *et al*., 2014). DNA fingerprinting of an individual is the characterization of particular alleles existing at a series of polymorphic loci in gene. The locus of polymorphic is that part of genomic DNA whose sequence is various in numerous individuals (Singh 2007). One of the DNA testing methods, RAPD technique were used to study phylogenetic relationship among subspecies (Singh 2011). This technique was reported by (Williams *et al*., 1990; Li *et al*., 2006 and Zhang *et al.*, 2002b). The studies on structure of population genetic and genetic divergence by utilizing RAPD were supposed to extremely successful (Apostol *et al*., 1996 and Yu *et al*., 2004). By different authors like Farrag *et al.* (2010), they studied about the identification of poultry while, Calvo (2001) study on poultry pate by using RAPD-PCR and they explain the usefulness of RAPD profiling to identification of species in poultry pates. Therefore, it is a strong tool in DNA fingerprint analysis of different animal species, study on mapping, conservation of animal genetic resources, sex determination, analysis of population and identification of subspecies. The aim of present study is to discriminate species of two subspecies and to detection the genetic identity within and between Aseel and Brahma chicken breeds by using RAPD technique.

## Materials And Methods

### Selection of samples and DNA Extraction

By random selection of both sexes from two subspecies of Poultry viz.; Aseel and Brahma breeds, were used 30 animals, from these 30 aniamals; 14 animals from Aseel breed and 16 animals from Brahma breed. By EDTA blood collecting tubes, the blood samples were collected aseptically from: 1) wing vein and, 2) direct from the heart, by using sterile plastic syringe and the blood collected {1-2 ml} from each bird should be in a separate test tube, and punctured of the wing vein with help of sterile needle. 20-25 drops of running blood should be collected from each bird in a separate test tube. All tubes are kept in horizontal position after putting the stopper on them. Samples of sera should be transmitting in another test tube and stored in refrigerator {4$^{o}$-8$^{o}$C} and after few days, delivered to lab in this condition. The check of DNA quality occur by running DNA samples in 0.8% Agarose gel containing ethidium bromide.

**RAPD-PCR Method**

Random amplified polymorphic (RAPD-PCR) reaction mixture consist of the genomic DNA were prepared from blood using Phenol/ chloroform/ isoamyl alcohol extraction by standard methods of proteinase A digestion. Proteinase A solution was added to the lysis buffer (50 mM KCl; 10 mM Tris-HCl, pH 9; 0.1% Triton X-100) until a final concentration of 0.12 mg/ml, and the mixture was incubated overnight at 56$^{o}$C. DNA was extracted in 500 µl of phenol: chloroform: isoamyl-alcohol (25: 24: 1), precipitated with 2 volume of 100% ethanol, transferred to a sterile micro centrifuge tube, washed with 70% ethanol, centrifuged at 10,000xg and dried. The resulting pellet was dissolved in 50 µl of distilled water. The resulting pellet was dissolved in 50 µl of distilled water. By 25 cycles, the amplification was carried out with initial denaturation at 94$^{o}$C for 5 min, second denaturation for 1 min at 94$^{o}$C, annealing at 36$^{o}$C for 1 min, extension for 2 min at 72$^{o}$C and final extension at 72$^{o}$C for 5 min. By running DNA samples in 0.8% Agarose gel to test the quality of DNA. Furthermore, electrophoresis and photographed under UV illuminator (at 270, 320, 360nm) were used to estimate the quality and DNA concentration.

**Table 1: RAPD oligonucleotide primers sequence with length and Guanine-Cytosine contents.**

| Primers | oligonucleotide primers sequence (5'----------3') | Length of Primers | GC % |
|---|---|---|---|
| DF-01 | ACCGACGCCA | 10-mer | 70 |
| DF-02 | TGTCCCTGAC | 10-mer | 60 |
| DF-03 | CGTCCCACGT | 10-mer | 70 |
| DF-04 | CGGAGGCCTC | 10-mer | 80 |
| DF-05 | GTGGCAGCAT | 10-mer | 60 |
| DF-06 | ACTGCTCGGC | 10-mer | 70 |

## Statistical analysis

**1- Band Frequency (BF) and Band sharing Frequency (BSF):**

BF is the proportion of animal number's that carrying a specific band to the total number of animals screened within the population.The following formula used to calculate the Band frequency of RAPD fingerprints :

**BF = n/N**

Where: N is the number of animals total and n is the animal numbers that carrying a particular band.

While; by Gwakisa et al.,(1994) the BSF are described below:

$$\mathbf{BSF_{(between\ two\ breeds)} = 2B_{AB} / [B_A + B_B]}$$

Where: $B_{AB}$ is the bands number collective to Aseel and Brahma. The total of bands number for Aseel breed represent $B_A$. Similarly, the total of bands number for Brahma represent $B_B$.

BSF within breed were calculated according to average frequencies of band sharing within the breeds by using the following formula:

$$\mathbf{BSF_{(within\ Aseel\ breeds)} = 2B_{Ai.Aii} / [B_{Ai} + B_{Ai}]\ and}$$

$$\mathbf{BSF_{(witin\ Brahma\ breeds)} = 2B_{Bi.Bii} / [B_{Bi} + B_{Bi}]}$$

Where $B_{Ai\ .Aii}$ is BSF within a pair of Aseel chicken and $B_{Bi.Bii}$ is BSF wthin a pair of Brahma. $B_{Ai}$, $B_{Aii}$, $B_{Bi}$ and $B_{Bii}$ are the bands total showed into the breed of Aseel and Brahma respectivily.

**2-Genetic Identity (GI) and Genetics distance (D):**

The identity of genetic was estimated between two breeds according to Lynch (1990):

$$\mathbf{GI_{(between\ breeds)} = 1/N \sum [\ 2\ (F_{ix})(F_{iy})/\{(F_{ix})^2 + (F_{iy})^2\}\ ].}$$

Where, $\mathbf{F_{ix}}$ and $\mathbf{F_{iy}}$ are the occurrence frequency of $\mathbf{i^{th}}$ band in two various breeds respectively, and **N** is the band total numbers of reported by Apuya *et al*. (1988).

Whilist, by Lynch (1991) obtained the formula of genetics distance that declared below:

$$\mathbf{D_{AB} = -ln\ BSF_{AB} / \sqrt{(BSF_A\ X\ BSF_B)}}$$

Where $BFS_{AB}$ is the frequency of band sharing between Aseel and Brahma breeds of chicken. $BSF_A$ is the frequency of band sharing within Aseel breed. $BSF_B$ is the frequency of band sharing within Brahma breed.

## Results And Discussion

DNA profiling was used to analysis and interpretation of banding patterns of population genetics in poultry. The inherits of band were stable genetic characters that revealed by comparison of the banding pattern of offspring with their parents. A DNA fingerprinting technique was applied to determine the degree of inbreeding (Kuhnlein *et al*., 1990), to assess the variation of genetic range within and between lines of different origin and to build of the phylogenetic trees (Wimmers *et al*., 2000), to study the genetic variation within and between lines of fowls and determine the effects of selection on these variation (Sacharczuk *et al.,* 2005). Visible estimation of DNA revealed good concentration. Reproducibility was tested by using 30 animals from two breeds to the same electrophoresis and PCR.

**RAPD Polymorphism**

The obtained fingerprint patterns under the same controlled conditions were reproducible into the particular kinds. By subjecting 30 unrelated animals, reproducibility was tested from two species to the same electrophoresis and PCR conditions. For two species studied, Primers used appeared good and specific fingerprint patterns (Figures 1and 2).

In Aseel breed with 6 random primers, the traits of the amplification profiles are observed. Amongst the population of poultry breeds, all the 6 primers detected polymorphism. The total loci amplified were (7, 5, 2, 1, 6 and 3) at (DF-01, DF-02, DF-03, DF-04, DF-05 and DF-06 primers) respectively. The highest polymorphic loci equal (5) with DF-01 and DF-05 whilst, with DF-03 were (zero) that explain the lowest value. According to this study, a total bands amplified were 24, about 66.9% of these amplified primers (17 bands) were polymorphic within Aseel breed. The size difference average from 325 to 1325 bp. The bands are different in their size from 100 to 2250 bp (table 2). The polymorphic loci were 100% with DF-04, whereas, DF-03 produced 00% polymorphic loci .Sacharczuk *et al.* (2005) reported that the specific bands were 1 and 5 with family 1 and family 2 from Rhode Island Red (RIR) chickens.

Among the population of Brahma breed, all the 6 primers detected polymorphism. The average polymorphic loci number's from 1 to 6 and the total loci amplified were (7, 5, 3, 6, 5 and 2) at (DF-01, DF-02, DF-03, DF-04, DF-05 and DF-06 primers) respectively. The highest account of polymorphic loci with DF-01 were (6) while, recorded the lowest value with DF-03 were (1). From the 6 random primers between Brahma populations, a total amplified for 28 bands and 20 of these (about 70.95%) were found to be polymorphic and polymorphic loci of DF-06 produced

100%. The average size difference account from (145 to 1550 bp) (Table 2). The resulting amplified DNA markers of band sizes from 375 to 2090 bp are random polymorphic segments .The primers were used for two breeds studied; observed good and specific fingerprint patterns (Figure 1, 2). The polymorphic loci value equal (2) with DF-06 primer at two breeds analyzed, same results reported by (Calvo 2001) when they study about Pork pates observed a specific profile, the same size in one band in the specific band of duck pates.

**Table 2: Size variation and Size range in Aseel and Brahma breeds.**

| Primers | Size range (bp) | | Size variation (bp) | |
|---|---|---|---|---|
| | In Aseel | In Brahma | In Aseel | In Brahma |
| DF-01 | 100-425 | 375-1175 | 325 | 800 |
| DF-02 | 310-1400 | 710-1400 | 1090 | 690 |
| DF-03 | 925-2250 | 425-1975 | 1325 | 1550 |
| DF-04 | 756-1325 | 1150-2090 | 569 | 940 |
| DF-05 | 1400-1760 | 630-775 | 360 | 145 |
| DF-06 | 700-1500 | 425-1400 | 800 | 975 |

**Genetic Identity Index and BF between two Breeds:**

The values by using 6 random primers of genetic identity index between breeds are presented in table 2. The genetic identity within breed was higher in comparison to between breeds therefore the genetic identity indicating that genetic diversity was higher between breeds than within breed because the similarity genetic calculate between two breeds ranged from 0.12 with DF-05 primer to 0.59 with DF-02 primer, whereas the overall genetic similarity was 0.28 ± 0.08 . The band frequency value within Aseel breed ranged from 0.59 to 1.00, while within Brahma breed ranged from (0.43 to 0.94). The overall BF within Aseel breed were (0.80 ± 0.09), whilst, within Brahma breed were (0.28± 0.08) (Table 3).

**Table 3: Genetic Identity and resemblance within chicken breed by using 6 primers.**

| Primer Code | Band Frequency (BF) | | G.I. |
|---|---|---|---|
| | Within Aseel | Within Brahma | |
| DF-01 | 0.93 | 0.89 | 0.15 |
| DF-02 | 0.70 | 0.43 | 0.59 |
| DF-03 | 0.81 | 0.94 | 0.29 |
| DF-04 | 1.00 | 0.90 | 0.37 |
| DF-05 | 0.59 | 0.87 | 0.12 |
| DF-06 | 0.82 | 0.61 | 0.18 |
| Total : | 0.80 ± 0.09 | 0.77 ± 0.03 | 0.28± 0.08 |

**Genetic Distance and BSF between two Breeds:**

The frequency of band sharing rates within breeds in Aseel and Brahma are reported in table (4). BSF ranged within Brahma breed from (0.98 – 0.69) with two primers like (DF-03 and DF-05), while in Aseel breed, it's varied from (1.00–0.55) for primer DF-04 and DF-01 respectively. The band frequency within Brahma breed, BF varied from (0.94 – 0.61) with respect primer DF-03 and DF-06 whilst, within Aseel breed varied from (1.00 – 0.59) for primer DF-04 and DF-05 respectivly. The total

average of BSF within Aseel breed were (0.83± 0.08), as well as Brahma breed were (0.85±0.23). Similar results showed by Sacharczuk *et al*. (2005) whene they reported the BS between Line high to Family 1 and Line high to Family 2 were 0.86 inside breed (Rhode Island Red "RIR" chickens). The data of band shaing frquency revealed similar genetically between these two breeds .

The lowest genetic distance with primer DF-04 were (0.22) while the highest genetic distance with primer DF-05 were (2.15). Between the breeds, there are no more significant of distance to the genetic was found with the primers used**.**

**Table 4: The Frequency of Band Sharing (BSF) and Genetic Distance (GD) within two chicken breeds.**

| Primer Code | Band Sharing Frequency (BSF) | | G.D. |
|---|---|---|---|
| | Within Aseel | Within Brahma | |
| DF-01 | 0.55 | 0.90 | 1.60 |
| DF-02 | 0.92 | 0.79 | 1.10 |
| DF-03 | 0.83 | 0.98 | 0.63 |
| DF-04 | 1.00 | 0.83 | 0.22 |
| DF-05 | 0.95 | 0.69 | 2.15 |
| DF-06 | 0.78 | 0.91 | 0.93 |
| The overall: 0.83 ± 0.08 | | 0.85 ± 0.23 | 1.10± 0.05 |

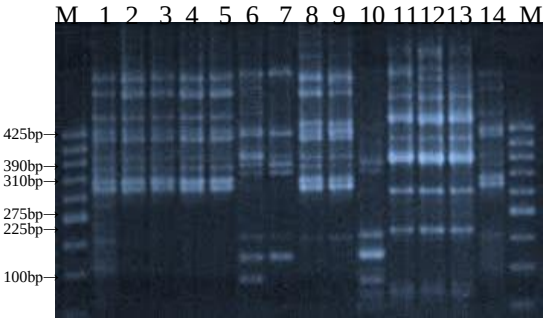


**Figure 1: Amplification profile for primer DF-01 in Aseel Chicken under UV illuminator by using RAPD fingerprinting**

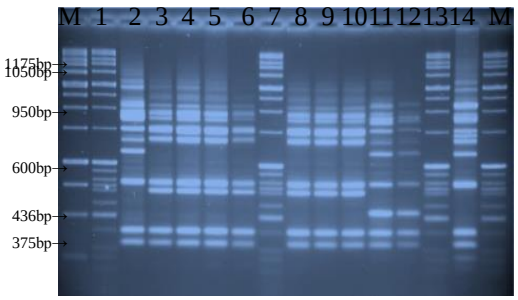


**Figure 2: Amplification profile for primer DF-01 in Brahma Chicken Under UV illuminator by using RAPD fingerprinting**

## References


Apostol, B.L.; Black, W.C.; Reiter, P.; Miller, B.R. (1996). "Population genetics with RAPD-PCR markers: the breeding structure of Aedes aegypti in Puerto Rico". *Heredity*, 76, 325-334.

Apuya, N.R.; Frazier, B.L.; Keim, P.; Roth, E.J. and Lark, K.G. (1988). "Restriction Fragment length polymorphism as genetic markers in soya bean *Glycine max (L.)* Merrill". *Theor. Applied Genet.*, 75: 889-901.

Bhattacharya, T.K.; Kumar, P. and Sharma, A. (2008). "Animal Biotechnology". *Kalyani Publishers*, New Delhi, India.

Burke, T. and Bruford, M.W. (1987). "DNA Fingerprinting in birds". *Nature J.*, 327: 149-152.

Calvo, J.H., P. Zaragoza and R. Osta, (2001). Random amplified polymorphic DNA fingerprints for identification of species in poultry pate. *Poult. Sci*., 80: 522-524.

Chambers, Geoffrey K.; Curtis, Caitlin; Millar, Craig D.; Huynen, Leon; Lambert, David M. (2014). "DNA fingerprinting in zoology: past, present, future". *Investigative Genetics* 5: 3.

Farrag, S.A.; Tanatarov, A.B. and Soltan, M.E. (2010). "Using of DNA Fingerprinting in Poultry Research". *In. J. of Pou. Sci*., 9(5): 406-416.

Gilbert, D.A.; Lehman, N.; O'Brien, S.J. & Wayne, R.K. (1990). "Genetic fingerprinting reflects population differentiation in the California channel island fox". *Nature (Lond)*, 344, 764-766.

Gwaksia, P.S.; Kemp, S.J.; and Teale, A.J. (1994). "Characterization of Zebu cattle breeds in Tanzania using random amplified polymorphic DNA markers". *Anim. Genet*. 25 (2), 89-94.

Kuhnlein, U.; Zadworny, D.; Dawe, Y.; Fairfull, R.W. and Gavora, J.S. (1990). "Assessment of Inbreeding by DNA Fingerprinting: Development of a Calibration Curve Using Defined Strains of Chickens". *The Genetic Society of America*, 125: 161-165.

Li, L.; Zhang, J.; Zhu, J.J.; Gu, S.; Sun, Q.; Zhou, G.M.; Fu, C.X.; Li, Q.; Chen, L.Y.; Li, D.X.; Liu, S.J.; Yang, Z.R. (2006). "Genetic diversity of nine populations of the Black Goat (Capra hircus) in Sichuan, PR China". *Zoology Sci.*, 23, 229-234.

Lynch, M. (1990). "The similarity index and DNA fingerprinting". *Mol. Biol. Evol.*, 7: 478-484.

Lynch, M. (1991). "Analysis of Population Genetics Structure by DNA Fingerprinting. In: DNA fingerprinting Approaches and Applications". Burke, T.G., A. Dolf, J. Jeffreys and R. Wolf (Eds.), Basel, Switzeri and, pp: 113-126.

Prasad, J. (2008). "Poultry production and Management". Kalyani Publishers, New Delhi, India.

Prasad, J. (2009). "Animal Husbandry and Dairy Science". Kalyani Publishers, New Delhi, India.

Sacharczuk, M.; Parada, R. and Jaszczak, K. (2005). DNA fingerprinting of chicken lines divergently selected for high or low incidence of skeletal malformations. Animal Science Papers and Reports vol. 23 no. 2, 119-127 Institute of Genetics and Animal Breeding, Jastrzebiec, Poland.

Singh, B.D. (2007). "Biotechnology". Kalyani Publishers, New Delhi, India.

Singh, P. (2011). "Genetics". Kalyani Publishers, New Delhi, India.

Srivastava, A.K.; Singh, R.K. and Yadav, M.P. (2005). "Animal Biotechnology". Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, India.

Taneja, V.K.; Acharya, R.M.; Swaminathan, M.S. and Pandit, A.D. (2011). "Handbook of Animal Husbandry". Directorate of Knowledge Management in Agriculture, Indian Council of Agricultural Research, New Delhi, India.

Wimmers, K.; Ponsuksili, S.; Hardge, T.; Valle-Zarate, A.; Mathur, P.K. and Horst, P. (2000). "Genetic distinctness of African, Asian and South American local chickens". Anim. G., 31:159-165.

Yu, Y.; Lian, L.S.; Wen, J.K.; Shi, X.W.; Zhu, F.X.; Nie, L.; Zhang, Y.P. (2004). "Genetic diversity and relationship of Yunnan native cattle breeds and introduced beef cattle breeds". *Biochem. Genet.*, 42, 1-9.

Yukhi, N. and O'Brien, S.J. (1990). "Variation of mammalian major histocompatibility complex reflects genomic diversity and population history". *Proceedings National Academy of Sciences, USA*, 836-840.

Zhang, X.; Leung, F.C.; Chan, D.K.O.; Yang, G.; Wu, C. (2002b). "Genetic diversity of Chinese native chicken breeds based on protein polymorphism, randomly amplified polymorphic DNA, and microsatellite polymorphism". *Poult. Sci.*, 81, 1463-1472.