# DNA Fingerprinting of Red Jungle Fowl, Village Chicken and Broilers

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**ABSTRACT** : The genomic mapping of Red Jungle Fowl (*Gallus gallus*), local Village Chicken, and broiler was carried out by random amplified polymorphism DNA (RAPD) technique. Two different sets of arbitrary primers were used (Operon OPA01-20 and Genemed GM01-50). All the genomes of the three species of chickens were amplified with OPA01-20 primers. The genomes of the Red Jungle Fowl and local Village Chicken were further amplified with GM01-50 primers. Analysis of the results based on band sharing (BS) and the molecular size of individually amplified DNA fragments showed that Red Jungle Fowl and local Village Chicken shared the species similarity of 66% with Operon primers 01-20, 64% between local Village Chicken and broiler, and 63% when DNA bands between Red Jungle Fowl and broiler were compared. With GM01-50, the BS between Red Jungle Fowl and local village chicken increased to 72%. The results showed that the local village chicken is more closely related to Red Jungle Fowl than to broiler in the genetic distance. On the other hand, broiler is 1% closer in genetic distance to local village chicken than to Red Jungle Fowl. The results also indicated that primers like OPA-7, 8 and 9 can be used as species specific DNA markers for these three species of chickens. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 8 : 1040-1043*)

**Key Words** : Jungle Fowl, Village Chicken, Broiler, Genome, RAPD

## INTRODUCTION

DNA fingerprinting (DFP) technique was firstly employed by investigating a DNA fragment of a human blood protein, $\alpha$-globin (Jefferys et al., 1985). They discovered that this fragment contained a repeated sequence of bases termed as minisatellite. Later, DFP of hypervariable minisatellites was used for identification of human and animal species based on their DNA charactersitics (Jefferys, 1987). DFP has also been employed to estimate relative genetic variability in natural populations (Dunnington et al., 1991; Plotsky et al., 1995), to assess genetic distances between strains (Kuhnlein et al., 1989), to identify DNA fragments linked to quantitative trait loci (QTL) (Dunnington et al., 1991; Kuhnlein et al., 1991; Levin et al., 1994; Hans, 1997), to identify desirable genomes in gene introgression procedures (Hillel et al., 1993), and to correlate similarity in DFP patterns to degree of inbreeding (Kuhnlein et al., 1990).

The recent developments in molecular biology have also made it possible to apply DNA-based technologies for genome analysis in variety of animal species (Appa Rao et al., 1996; Jerry et al., 1997). However, the first genetic linkage mapping of chicken genomes was published by Bitgood and Somes (1990) and latter by Hillel et al. (1993). These molecular techniques such as Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphism DNA (RAPD) and Pulse Field Gel Electrophoresis (PFGE) were then employed to greatly enhance and improve the research in the area of genetic mapping of chicken genome where more than 100 genetic markers were evolved. (Bumstead and Palyga, 1992; Levin et al., 1994).

The present study was conducted for the following objectives: (*i*) to determine the genetic distances between red jungle fowl, village chicken and broiler, (*ii*) to identify suitable DNA markers unique for each species of chickens, and (*iii*) to establish genetic linkage mapping for the three species of chickens.

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## MATERIALS AND METHODS

### Experimental animals

A number of ten birds from each of three species of chickens were used. Red Jungle Fowl was obtained from Malaysian Jungle, Village Chickens were collected from Malaysia villages (*Ayam Kampung*) and broilers were obtained from one of the commercial sources. The chickens were housed in separate cages, regularly supplied with food and water before being sacrificed for tissue collection.

### PCR primers

Two sets with a total of 70 primers were used in the study. The first set of the primers contained twenty of 10-mer primers Operon (OPA01-20; 50% G+C) and the second set of the primers contained fifty of 10-mer primers Genemed (GM01-50; 60-70% G+C).

### DNA extraction and purification

DNA extraction was carried out using DNAzol, a guanidine-detergent lysing solution, which allow the

selective precipitation of DNA from a cell lysate. The protocols of DNA extraction include: tissues lysis and homogenization, centrifugation, DNA precipitation, DNA wash and DNA solubilization. Tissues of 25-50 mg from the chicken kidney or liver were pulverised using mortar and pestle in 1 ml of DNAzol by applying 5-10 strokes for complete homogenization. The homogenate was then kept for 5-10 minutes at room temperature before centrifugation. The homogenate was then centrifuged at 13,000 rpm for five minutes at 4℃ in an Eppendorf centrifuge tube and the supernatant was transferred to a new tube. Then, 0.5 ml of 100% ethanol was added to the tube containing the supernatant to precipitate DNA from cell lysate. The sample was mixed by inverting the tube 5-8 times and kept at room temperature for 1-3 minutes. The mixture was spun at 13,000 rpm for five minutes and the pellets were transferred to a new tube. The DNA pellets were washed twice with 0.8-1.0 ml of 95% ethanol. The DNA pellet was then suspended in sterile distilled water before being subjected to PCR.

**DNA amplification**

The DNA amplification protocol was carried out according to Plotsky et al. (1995) and Smith et al. (1996) with some modifications. Different amounts of DNA samples were used in different amplification process but it did not affect the results obtained. All reactions were in 50 $\mu$l which contained 10 $\mu$l of the DNA sample (template), 5 $\mu$l DNA polymerase buffer (New England Biolabs), 100 m dNTP mix (1 $\mu$l) (DyNAzymes Oy, Finland), 0.5 m of each primer (2 $\mu$l), 1.5 units of DNA polymerase enzyme (1 $\mu$l) (Deep Vent, New England Biolabs) and 31 $\mu$l sterile-distilled water. Thirty-five cycles of amplification were performed in a Perkin Elmer-Cetus DNA Thermal Cycler. Prior to cycling, the samples were heated at 95℃ for five minutes. For each cycle, samples were denatured at 94℃ for 40 seconds, annealed at 35℃ for 40 seconds and extended at 72℃ for 90 seconds. After cycling, the final extension was continued for five minutes.

**Detection of the amplified DNA product**

The detection of amplified DNA product was performed as described by Sambrook et al. (1989). Samples contaiming amplified DNA were subjected to 1% agarose gel electrophoresis. Five $\mu$l of every amplified DNA product were loaded in each well of the gel in a submarine chamber after being mixed with the mobility marker (6x bromophenol blue). Electrophoresis was carried out at 90 volts D. C. for 90 minutes. A 100 bp ladder was used with the amount of five $\mu$l per well as a marker to estimate the size of the amplified products. After electrophoresis, gels were stained by ethidium bromide, visualized under UV light transilluminator.

**Results analysis**

In order to assess the similarity of DNA fingerprint pattern (DFP) in pairwise comparisons among different species of fowls, band sharing was adopted as described by Jeffery et al. (1985). The band sharing was calculated according to the following formula:

$$BS=2(Nab)/(Na+Nb)$$

Where:
BS is the level of band sharing between lanes a and b,
Nab is the total number of bands shared by lanes a and b,
Na is the total number of bands in lane a,
Nb is the total number of bands in lane b.

Band sharing indicates degree of similarities in the genomic make-up between two species of fowls. The higher the band sharing value, the higher two species related to each other and vice versa. If the comparison between the three species of fowl, i.e. three lanes, consequently the formula would be:

$$BS=3(Nabc)/(Na+Nb+Nc) \text{ and so on.}$$

Based on banding pattern observed, if more than 90% of DNA bands at same molecular weight are observed classified as identical (I), 60-89% close (C), and <60% distinct (D).

## RESULTS AND DISCUSSION

It had been established that DNA fingerprint can be successfully used to compare genetic distances between humans (Gill et al., 1985; Jeffereys et al., 1986), domestic animals (Jeffereys and Morton, 1987) and different strains of chickens (Kuhnlein et al., 1989). In the present study, the focus was on the determination of the genetic distances between Red Jungle Fowl (JF), Local Village Chicken (VC) and broilers (B) by using RAPD technique. The band sharing (BS) levels among JF, VC and B using Operon primers OPA (01-20) and Genemed 10-MER mapping Kit 1-50 is demonstrated in table 1. The DNA bands amplified by Operon primers for JF, VC and B are much similar. However, the BS value was slightly higher between JF and VC (66%) than between JF and B (63%) or between VC and B (64%). These findings were found to be quite consistent with that of Siegel et al. (1992). They proved that the BS values between JF and domestic jungle fowl (equivalent to VC), dJF/B and JF/B were

21%, 16% and 14% respectively. However, the differences in the magnitude of BS between the two studies can be attributed to different primers used and amplification techniques adopted. Interestingly, both studies gave BS values between the three species of chicken in the same rank of order. This indicates that VC is somewhere between JF and B in the genetic distance. The degree of resemblance of DNA fragment patterns with primers OPA (01-20) among the three species of chicken is shown in table 2.

Amplification of DNA using Genemed primers (GM-10-MER) mapping kit 1-50 produce BS value of 0.72% between JF and VC. This value is 0.60 (6%) higher as compared to DNA amplified by Operon primers. The degree of resemblance of DNA fragment patterns with primers GM-10-MER (01-50) among the three species of chicken is shown in table 3. The resemblance was pronounced for most lanes except for primers GM-5, 7, 10, 19, 20, 21, 38 and 49. With GM-10-MER (01-50), 60% of these primers gave close DNA fragments patterns, 20% primers gave distinctively different patterns whereas 20% gave similar or almost identical patterns.

The high degree of resemblance of DNA bands between JF and VC with both of the primers kits used in this study is not surprising and it is in accordance with the general accepted avian evolutionary concept that JF is the progenitor of the modern chicken including the seemingly intermediate local VC (West and Zhou, 1989).

With both types of primers used in the present study the similarity between the three species of chicken was above 60%. This indicates the close genetic distances between them, although JF was found to be closer to VC than to B. As a result of this close relationship, most of the primers used in this study gave close, similar and even identical DNA pattern. Subsequently, only few primers can be used as specific markers which enable to differentiate between the three species of chickens. These primers include OPA 7, 8 and 9 which can be exploited for preparation of DNA probes.

The most salient conclusions emerging from the study include the highest similarity between JF and VC as compared with BS. This obviously indicates that the local VC is more closely related to JF than broilers as can also be seen from the appearance. These findings substantiates the previous finding that the red jungle fowl is the single ancestral form of the

**Table 1.** Band sharing levels among Red Jungle Fowl, Local Village Chicken and broilers using Operon primers OPA (01-20) and Genemed 10-MER mapping Kit (1-50)

| | Operon primer OPA (01-20) | | | Genemed 10-MER mapping Kit 1-50 | | |
|---|---|---|---|---|---|---|
| Spcies | JF | VC | B | JF | VC | B |
| JF | 1.00 | 0.66 | 0.63 | 1.00 | 0.72 | NA |
| VC | | 1.00 | 0.64 | | 1.00 | NA |
| B | | | 1.00 | | | 1.00 |

JF= Red Jungle Fowl, VC= Village Chicken, B= Broilers. NA= Not applied

**Table 2.** Degree of resemblance of DNA fragment patterns with primers OPA (01-20) among Red Jungle Fowl, Village Chicken and Broiler

| | Primers (P) | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| F/VC | I | I | C | D | C | C | D | D | D | C | C | C | I | C | C | I | I | C | C | D |
| F/B | C | C | I | D | C | C | D | D | D | D | I | C | C | I | C | I | I | I | C | C |
| C/B | C | C | C | C | I | C | C | C | C | C | C | C | C | C | I | D | C | C | C | D |

I=identical (chickens showed similar band pattern), C=close (chickens showed closer band pattern), D=distinct (DNA bands pattern is different among chicken species).
JF=Red Jungle Fowl, VC=Village Chicken, B=Broilers.

**Table 3.** Degree of resemblance of DNA fragment patterns with primers Genemed (GM-01-50) among Red Jungle Fowl, Village Chicken and Broiler

| Pattern | Primers No. | Total |
|---|---|---|
| Identical | 6*, 11, 30, 31, 32, 33, 43, 45, 46 and 50. | 10 |
| Close | 1, 2, 3, 4, 5, 8, 9, 12, 13, 14, 15, 16, 17, 20, 24, 26, 27, 28, 29, 34, 35, 36, 37, 39, 40, 41, 42, 44, 47 and 48. | 30 |
| Distinct | 7, 10, 18, 19, 21, 22, 23, 25, 38 and 49. | 10 |

* The primer number at which chickens showed identical, close or distinctly different DNA fragment pattern.

domestic fowl (West and Zhou, 1989). Our findings showed that primers designated OPA 7, 8 and 9 could be used as a basis to prepare DNA probes to distinctly differentiate the three species of chickens. The probe prepared can be used further to identify and differentiate jungle fowls from its relatives as well as for conservation work. Noteworthy, not all arbritrary primers were found to be useful to differentiate different species of chickens.

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