



Genetic Diversity of Egg-Type Guinea Fowl Varieties (*Numida meleagris*) in Nigeria Based on Band Frequencies

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Abstract –This research investigated the Genetic diversity of egg-type helmeted guinea fowls varieties in Nigeria based on band frequencies. The experimental varieties were Pearl (Sake), Lavender (Hurudu), and Black (Angulu). A base population comprising a total of 120 adult females and 60 males sourced from Zaria, Kaduna state were used to generate F1 keets. The selected 144 F1 keets comprised 48 birds per variety, and each variety was randomized into three replicates containing 16 birds each in C.R.D experiment. Ten day-old pullets of domestic fowl (Isa Brown) were used as control. The entire experimental animals were managed till the first laying phase. At the middle of lay, blood samples were collected and pooled from 5 animals per replicate for DNA analysis. 14.5 mg of DNA was extracted with a purity value of 1.7 using QIAgen DNeasy® blood and tissue DNA extraction protocol (2006). Polymerase chain reaction (PCR) was carried out afterwards using three SNP primers: GHR424F, GHRe5; NYPmap9, NYPmap10; GnRHRmap5 and GnRHRmap8. At the end of the PCR, the PCR product was treated with two restriction enzymes: ECOR1 and ECOR5 to cut the DNA into characteristic band sizes. The enzyme-treated DNA samples were subjected to agarose gel electrophoresis. The genetic data were treated statistically. The Black expressed the highest band frequency compared with the control, whereas the Lavender had the least frequency of the bands studied. The band sharing frequency (bsf) shows that without the control, Pearl X Lavender (bsf 0.834) are the most related, whereas the Black X Lavender (bsf 0.389) were the most diverged. The same result was obtained when the varieties were compared to the control. Altogether the three varieties are moderately related (bsf 0.400 and 0.231). The higher the band sharing frequency, the greater the genetic relatedness among the guinea fowl varieties studied. This implies that the Pearl and Lavender variety showed the highest genetic relationship with respect to the genes studied for egg production. However, the Black and Pearl varieties are recommended for more genetic improvement and commercial egg production.

Keywords - Band Frequency, Egg-Type Guinea Fowl, Genetic Diversity.

I. INTRODUCTION

Guinea fowl (*Numida meleagris*) are indigenous to West-Africa, where there is an estimated population of about 4.7 million [1]. According to [2], the northernmost part of Nigeria has larger population of the birds, and this calls for attention to attempt to develop the birds in the south (rainforest zone). The eggs and to a lesser extent the meat of guinea fowl are widely eaten by Nigerians because of the distinctive flavour they produce [3]; [4]. The good

keeping quality of guinea fowl eggs and the hardy disease resistant nature of the stock contribute to the prominent position of this species in Nigeria. Among domestic types which the peasant farmers have long identified and given local names based on their coloration are Pearl (Sake), Lavender (Hurudu), Black (Angulu) and White (Faren Zabi), [5]. The Pearl is the most common and probably the first developed from the Wild West Africa birds.

Association studies are often carried out between egg production traits and genetic parameters using genetic markers. This often reveals differences among different varieties of a stock as may be evidenced in the differences in their allelic or band frequencies. There is limited information on the genetic diversity in varieties of the birds. Thus, the ability to detect and track the heritability of these genes using marker genes that mark “spot” on the genome will permit efficient selective breeding for improved meat and egg production.

A number of molecular genetics techniques exist that can now be used to effectively characterize genetic stocks and studies involving them can lead to the discovery of genes or germplasma that require conservation. These techniques include: Restriction fragment length polymorphism [6]; polymerase chain reaction (PCR)[7] probing and hybridization, are technologies which study the molecular structure of genes and provide a molecular explanation of their functions[8]. It is important therefore, to develop egg-laying guinea fowl strains through its genotype characterization and subsequent informed intensive selection for better performance or genic manipulation for improvement using biotechnological techniques. The objective of this study was therefore to identify their band frequency differences and genetic relationships.

II. MATERIALS AND METHODS

Location of study

This study was carried out in Michael Okpara University of Agriculture, Umudike, located at about ten kilometers from Umuahia, the Abia State capital. Umudike bears the coordinate of 5°28' North and 7°32' East, and lies at an altitude of 122 meters above sea level. The environment of study was situated within the tropical rainforest zone and is characterized by an annual rainfall of about 2177 mm. The relative humidity during the rainy season is well over 72 %. Temperature ranged from 22 °C-36 °C with March being the warmest month, while July to

October represents the coolest period with a temperature range of 22 °C – 30 °C.

Acquisition and Mating of Base Population

A base population comprising a total of 120 adult females and 60 males sourced from Zaria, Kaduna state, Nigeria, were used to generate F1 keets. These adults were quarantined for two weeks. A mating ratio of 1 male: 3 females were maintained and the mating scheme adopted was as shown below:

- Pearl male X Pearl female - Homozygous Pearl variant main cross
- Lavender male X Lavender female - Homozygous Lavender variant main cross.
- Black male X Black female - Homozygous Black variant main cross

Experimental Animals and Management

The eggs laid by the base population were set and hatched at Kanem Hatcheries off Aba-Owerri Road, Aba, Abia state Nigeria. The F₁ was generated from the successfully hatched keets. The keets were brooded for six weeks and subsequently reared until the 28th week when they started laying eggs. The keets were sexed by visualizing the vent and listening to the cry of the birds. The male hatches were culled leaving only F₁ female keets which were used for the experiment. At the 28th week, 144 adult females were randomly selected and wing-barded. The 144 adult females consisted of 48 females of Pearl, Lavender and Black each. Each variety was replicated three times, which gave a total of 9 replicates for all the varieties, with 16 females per replicate. The guinea fowl varieties were raised on the deep litter pens under natural daylight. Feed and water was provided *ad-libitum*. During the laying phase, layers mash containing 2, 900 kcal/kgME and 20.5% CP according to [9] was introduced to the guinea fowl varieties. The nutrient composition of the layers diet is shown in table 3.1 below:

Table 1: The Nutrient Composition of the Layers Diet

Ingredient	Percent Composition
Maize	54.9
Groundnut cake	21.4
Wheat offal	8.60
Fish meal	1.73
Soybean meal	3.40
Limestone	2.40
Bone meal	7.00
Salt	0.50
Vitamin premix	0.27
Total	100

Vitamin/mineral premix composition: Vit A – 10,000,000 IU, Vit D3 – 2,200,000 IU, Vit.E – 10,000 mg, Vit.K3 – 2,000 mg, Vit.B2 – 5,000 mg, Folic acid – 500 mg, Niacin – 15,000 mg, Calpan – 5,000 mg, Vit.B12 – 1,500 mg, Vit.B1 – 1,500 mg, Vit.B6 – 1,500 mg, Biotin – 20 mg, Antioxidant – 125,000 mg; Selenium – 200 mg, Iodine – 1,000 mg, Iron – 40,000 mg, Cobalt – 200 mg, Manganese – 7,000 mg, Copper – 4,000 mg, Zinc – 50,000 mg, Choline chloride – 150,000 mg. Calculated composition: Ca – 3.50, P – 1.11. Energy level 2900 kcal/g; Protein level (20.5 %CP).

Experimental Control

About ten-day old egg laying type females of Isa Brown used as control were purchased from a reputable farm in Nigeria. They were managed just like the guinea fowls and during the laying phase their blood was collected for DNA analysis. Poultry species are more genetically related than other vertebrates, and given that the chicken genome has been sequenced, the adult hen served as a good control for comparing the guinea fowl varieties for their genetic relationships with respect to egg production traits. The DNA ladder was also used as a second control.

DNA Data Collection and Analysis

Blood sample was collected once by brachial venipuncture into micro tubes containing anticoagulant. The DNA extraction of the sample was performed using QIAgen DNeasy® Blood and Tissue DNA extraction protocol (2006). At least three pooled samples were collected for each guinea fowl variety, and one for the chicken breed. The extracted DNA was subjected to polymerase chain reaction, PCR. Multiplex PCR machine and Single Nucleotide Polymorphism (SNP) primers were used for the PCR analysis. The primer pairs used for the PCR analysis include GHR424F, GHRex5, NYPmap9, NYPmap10, GnRHRmap5 and GnRHRmap8. The sequences of the primers are:

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NYPmap9 F   TCTCAGAGCTCCAACGTATGA
NYPmap10 R  ATATTTCTGTGCCTGAACAACA
GnRHRmap5 F  GGTGTCTGAGGCTCATTCA
GnRHRmap8 R  TAGCAATCGCTTGCCAGAG
GHR424 F    TTTATCCCCTGTTCTCTTGACA
GHRex5R     ACGAAAAGTGTTTCAGTGTTGA
  
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These primers were actually required to hybridize and amplify similar DNA sequences in the blood samples of the different DNA samples using polymerase enzymes to the extent that they can produce reasonable bands during gel electrophoresis. The SNP primers were mixed together for the amplification process given the fact that polygenes interact interdependently to influence the expression of egg traits. The normal PCR process was observed using Bioneer Accupower (R) TLA PCR premix protocol. A total of ten pooled samples representing the nine replicates (P1, P2, P3, B1, B2, B3, L1, L2, and L3) and the control (C) were used for the DNA analysis. Each replicate sample contained a mixture of blood pooled from 5 animals chosen at random from each replicate. All other necessary conditions were observed for the PCR analysis.

The DNA extracted yielded 14.5 mg and the purity value was 1.7 in buffer AE of the QIAgen extraction kit at an absorbance level of 260 nm. After the PCR analysis, the PCR products were treated with restriction enzymes, EcoR1 and EcoR5. These restriction enzymes were used purposely to cut the amplified DNA samples into characteristic band sizes. The agarose gel was prepared and then the restricted PCR product was passed through it in an electrophoresis machine for 30 minutes at 100V. Each of the nine samples was placed in different lanes in the electrophoresis machine. A 100 bp DNA ladder protocol which contained a controlled amount and is known to produce characteristic bands in chickens was

used as a standard of comparison. The 100 bp DNA ladder ranged from 100 bp to 1517 bp. The DNA ladder protocol contained blue gel loading dye. The dye made of bromophenol blue moved from one electrode to the other with the passage of current. The passage of current also made the DNA samples in the restricted PCR products to move from the negative to the positive electrode as DNA itself is negatively charged. The movement of these samples varied in the three varieties and as such produced different bands. After the gel electrophoresis, the products were then placed in a scanning and photographic machine connected to a Video Display Unit, where the DNA bands of each variety was viewed.

III. RESULTS AND DISCUSSION

Band Frequencies in Three Varieties of Helmeted Guinea Fowl Using ECOR1

Table 1: Band Frequencies in Three Varieties of Helmeted Guinea Fowl Using ECOR1

Bands (bp)	All varieties		Black		Lavender		Pearl	
	N	Frq	N	Frq	N	Frq	N	Frq
900	1	0.11	1	0.33	0	0	0	0
850	5	0.55	0	0	3	1.00	2	0.66
830	2	0.22	2	0.66	0	0	0	0
800	6	0.66	3	1.00	2	0.66	1	0.33
750	3	0.33	0	0	1	0.33	2	0.66
700	1	0.11	0	0	0	0	1	0.33
650	2	0.22	2	0.66	0	0	0	0
400	3	0.33	3	1.00	0	0	0	0
300	4	0.44	3	1.00	0	0	1	0.33
250	2	0.22	0	0.00	0	0	2	0.66
200	6	0.66	3	1.00	0	0	3	1.00
100	9	1.00	3	1.00	3	1.00	3	1.00
Total observed	44		20		9		15	
Expected	108		36		36		36	
Total Band frq = Total observed/total expected	0.41		0.56		0.25		0.42	

N= number, Frq. = Frequency, bp= Base pair, ECOR1= Restriction enzyme

Plate 1 shows the agarose gel display of the DNA bands. The frequency of the genes studied shows that Black had the highest frequency followed by Pearl, and then the Lavender. These differences in frequencies may be connected with factors that causes change in gene frequency such as mutation, migration, random drift and selection [10],[11]. The differences in band expression show polymorphism of the SNP primers in the three varieties which probably may influence their individual egg production or egg quality characteristics. According to [12]), genetic differences among populations, breeds and species are largely due to differences in gene frequencies.

Band Frequencies in Three Varieties of Helmeted Guinea Fowl Using ECOR5

The band frequencies in three varieties of helmeted guinea fowl using ECOR5 is shown in table 2. It can be observed that using the ECOR5, the three varieties of helmeted guinea fowls produced 7 different bands with a band frequency of 0.46 in the three populations. From the

The band frequencies in three varieties of helmeted guinea fowl using ECOR1 is shown in table 1. The three varieties of helmeted guinea fowl produced 12 different bands with a band frequency of 0.41 in the sampled population. From the table, the major bands characterizing helmeted guinea fowls are 800 bp and 100 bp, followed by the 200 bp and 850 bp bands. The distinguishing bands in the Black variety include the 900 bp, 830 bp, 650 bp, and the 400 bp. In the Pearl variety, the 250 bp is the distinguishing band. No distinguishing band was noticed in the Lavender variety. However, the Lavender variety was highly limited in the number of bands produced by the restriction enzyme. The Black and Pearl varieties produced 8 bands with a higher band frequency in the Black (0.56) than in the Pearl population (0.42). The Lavender variety produced only 4 bands with a band frequency of 0.25.

result, it was again noticed that the major band that commonly characterizes the three varieties of helmeted guinea fowls using ECOR5 include the 800 bp and the 100 bp, followed by the 700 bp band. The distinguishing bands in the Black variety are 750 bp, 720 bp, 400 bp and 300 bp. The Lavender and Pearl varieties had a band 700 bp in common, which is not present in the Black. The Black variety produced the highest number of bands (6 bands) with a band frequency of 0.62. The Pearl and Lavender variety both produced 3 bands.

However, the Lavender variety had a higher band frequency of 0.43 than the Pearl variety (0.33). Plate .2 shows the agarose gel display of the DNA bands.

Band Frequencies Common to the Three Varieties of Helmeted Guinea Fowl and the Control (Isa Brown) Using ECOR1

The band frequencies common to the three varieties of helmeted guinea fowl and the control (Isa Brown) is shown in table 3.

Table 2: Band Frequencies in Three Varieties of Helmeted Guinea Fowl Using ECOR5

Bands (bp)	All varieties		Black		Lavender		Pearl	
	N	Freq.	N	Freq.	N	Freq.	N	Freq.
800	7	0.77	3	1.00	3	1.00	1	0.33
750	2	0.22	2	0.66	0	0	0	0
720	1	0.11	1	0.33	0	0	0	0
700	6	0.66	0	0	3	1.00	3	1.00
400	2	0.22	2	0.66	0	0	0	0
300	2	0.22	2	0.66	0	0	0	0
100	9	1.00	3	1.00	3	1.00	3	1.00
Total observed	29		13		9		7	
Expected	63		21		21		21	
Total Band freq. = Total Observed/Expected	0.46		0.62		0.43		0.33	

N = number, Freq. = Frequency, bp= Base pair, ECOR1= Restriction enzyme

Table 3: Band Frequencies Common to the Three Varieties of Helmeted Guinea Fowl and the Control (Domestic Fowl) Using ECOR1

Chicken Bands (bp)	Black		Lavender		Pearl	
	N	Freq.	N	Freq.	N	Freq.
850	0	0.00	3	1.00	2	0.66
650	2	0.66	0	0	0	0
400	3	1.00	0	0	0	0
300	3	1.00	0	0	3	1.00
200	3	1.00	0	0	3	1.00
100	3	1.00	0	0	3	1.00
Total observed	14		3		11	
Expected	18		18		18	
Total Band freq. = Total Observed/Expected	0.78		0.17		0.61	

N= number, Freq. = Frequency, bp= Base pair, ECOR1= Restriction enzyme

The result showed that the Black variety had the highest number of bands (5) in common with the control with a band frequency of 0.78, followed by the Pearl (4) with a band density of 0.61, and the least was the Lavender variety which had only one band in common with the control with a band density of 0.17.

The Black and Pearl varieties were similar in their relationship to the control except in the bands, 850 bp found in Pearl but not in Black, and 650 bp found in Black but not in Pearl. The Pearl and Lavender variety shared one band (850 bp) in common in relationship to the chicken. The Lavender and the Black variety shared no band in common in relationship to the control.

[13], has shown that genetic divergence is least between Pearl and Lavender varieties of guinea fowl and maximum divergence between Lavender and commercial broiler. His report is consistent with the findings of this study as the Lavender variety showed on average very few bands in common with the control (Isa Brown). The Black variety however showed the least divergence and closest relationship to the control in this study. Of interest is the presence of the band 850 in Lavender (freq. 1.000) and Pearl (freq. 0.667) but not in Black (freq. 0.000). This band which is common in the two but not in Black, may be exploited. Other additional bands present in the Pearl but not in the Lavender may be connected with additive X additive interaction [8]. The difference in the band expression of the three varieties of guinea fowl in relation

to the control again shows polymorphism in the band expression which probably may also influenced egg production. Workers like [14], [15], and [16], have found associations for polymorphisms in the putative candidate genes IGF-1, GH, and GHR in the growth hormone endocrine pathway and egg production and egg shell quality. Other workers such as [17], have studied the use of chicken genetic markers in studying genetic polymorphism in guinea fowls.

Band Frequencies Common to the Three Varieties of Helmeted Guinea Fowl and the Control (Isa Brown) Using ECOR5

The band frequencies common to the three varieties of helmeted guinea fowls and the control (Isa Brown) using ECOR5 is shown in table 3. It can be observed that the Black variety had the highest number of bands (5) in common with the control with a band frequency of 0.67. The Lavender and Pearl variety had three bands in common with the control. However, the Lavender variety had a higher band density of 0.50 than the Pearl variety, 0.39.

Based on their relationship to the control, the Black variety differed from the Pearl and Lavender variety in the following bands: 750 bp, 700 bp, 400 bp, and 300 bp. The Pearl and Lavender variety shared the band size 700 bp similar with the control which was not present in the Black variety.

Table 3: Band Frequencies Common to the Three Varieties of Helmeted Guinea Fowl and the Control (Domestic Fowl) Using ECOR5

Chicken Bands (bp)	Black		Lavender		Pearl	
	N	Freq.	N	Freq.	N	Freq.
800	3	1.00	3	1.00	1	0.33
750	2	0.66	0	0	0	0
700	0	0	3	1.00	3	1.00
400	2	0.66	0	0	0	0
300	2	0.66	0	0	0	0
100	3	1.00	3	1.00	3	1.00
Total Observed	12		9		7	
Expected	18		18		18	
Total Band freq. = Total Observed/Expected	0.67		0.50		0.39	

N= number, Freq. = Frequency, bp= Base pair, ECOR1= Restriction enzyme

[12], has shown that genetic divergence is least between Pearl and Lavender varieties of guinea fowl and maximum divergence between Lavender and commercial broiler. His report is consistent with the findings of this study as the Lavender and the Pearl variety both showed on average very few bands in common with the control. The Black variety however showed the least divergence and closest relationship to the control in this study. It is expected that the high frequency in Black should correspond to high egg production being very close to the control. The presence of the band 700 bp in Lavender (freq. 1.000) and Pearl (freq. 1.000) but not in Black (freq. 0.000) should be noted. The difference in the band expression of the three varieties of guinea fowl in relation to the control again shows polymorphism in the band expression which probably also influenced egg production. Other workers such as, [17] have studied the use of chicken genetic markers in studying genetic polymorphism in guinea fowls.

Average Band Sharing Frequency in Three Varieties of Helmeted Guinea Fowl

The average band sharing frequency in three varieties of helmeted guinea fowl is shown in table 4. It can be observed that the three varieties showed a band sharing frequency of 0.300 and 0.500 using ECOR1 and ECOR5 respectively with an average band sharing frequency of 0.400 using the DNA ladder as the control. This result implies that generally, the three varieties had a 30 % and 50 % (average of 40 %) genetic similarity, and 70 % and 50 % (average of 60 %) genetic dissimilarity using ECOR1 and ECOR5 respectively.

The Lavender and Pearl varieties showed the closest relationship when the two restriction enzymes are considered with an average band sharing frequency of 0.834 using only the DNA ladder as the control. This was followed by the Black X Pearl combination with an average band sharing frequency of 0.472. The Black X Lavender combination showed the least relationship with an average band sharing frequency of 0.389 using only the DNA ladder as the control.

Table 4. Average Band Sharing Frequency in Three Varieties of Helmeted Guinea Fowl

Treatment	Variety	Band Sharing Frequency		Average Band
				Sharing Frequency
		ECOR1	ECOR5	ECOR1 +ECOR5/2
Without control	Black X Lavender X Pearl	0.30	0.50	0.40
	Black x Lavender	0.33	0.44	0.38
	Black x Pearl	0.50	0.44	0.47
	Lavender x Pearl	0.66	1.00	0.83
With control	(Black + Lavender + Pearl) x (Control)	0.00	0.46	0.23
	Black x Control	0.62	0.83	0.72
	Lavender x Control	0.25	0.66	0.45
	Pearl x Control	0.57	0.66	0.57

From table 4 using Isa Brown as the control, it can be observed again that the three varieties of helmeted guinea fowls showed a band sharing frequency of 0.000 and 0.462 using ECOR1 and ECOR5 respectively with an average band sharing frequency of 0.231. This implies that the helmeted guinea fowls studied had a 0 % and 46.2 % (average of 23.1 %) genetic similarity and 100 % and 53.8 % (average of 76.9 %) genetic dissimilarity or divergence

with the control using ECOR1 and ECOR5 respectively based on the bands studied.

The Black variety showed the closest relationship to the control (Isa Brown) with a band sharing frequency of 0.625 and 0.833 using ECOR1 and ECOR5 respectively with an average band sharing frequency of 0.729. The Black variety was followed by the Pearl variety which had a band sharing frequency of 0.571 and 0.667 using

ECOR1 and ECOR5 respectively with an average band sharing frequency of 0.619. The Lavender variety showed the least relationship to the control with a band sharing frequency of 0.250 and 0.667 using ECOR1 and ECOR5 respectively with an average band sharing frequency of 0.459.

The percentage genetic dissimilarity or divergence of the helmeted varieties from the control (Isa Brown) based on the band frequencies of the genes studied were 27.1 %, 38.1 %, and 54.1 % in Black, Pearl and Lavender respectively. This result still shows that the Black is the least diverged and thus most related, while Lavender is the most diverged and thus less related to the control (Isa Brown). According to [17], the higher the band sharing frequency, the greater the genetic relatedness among the guinea fowl varieties studied. This implies that the Pearl and Lavender variety showed the highest genetic relationship with respect to their higher band sharing frequencies. This was followed by Pearl x Black combination.

IV. CONCLUSION

The SNP markers used in this study actually showed polymorphisms in the bands displayed from the agarose gel electrophoresis. However, these polymorphisms can best be deduced through DNA sequencing. The Black and Pearl varieties can therefore be utilized for exploitation of genetic improvement purposes for commercial egg production in the guinea fowl.

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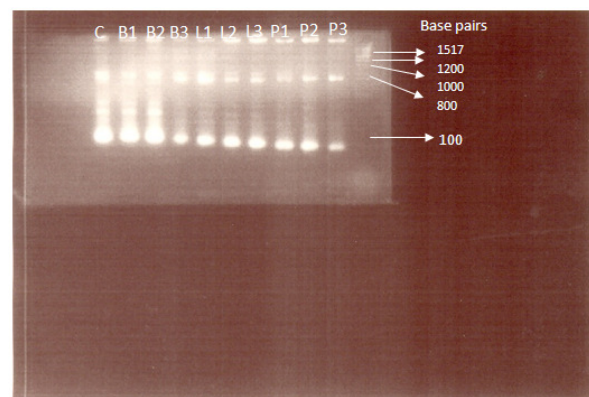


Plate 1: Agarose gel product showing the restricted band sizes using 3 DNA primers: GHR424F and GHRex5, NYPmap9 and NYPmap10, GnRHRmap5 and GnRHRmap8 using the restriction enzyme ECOR5

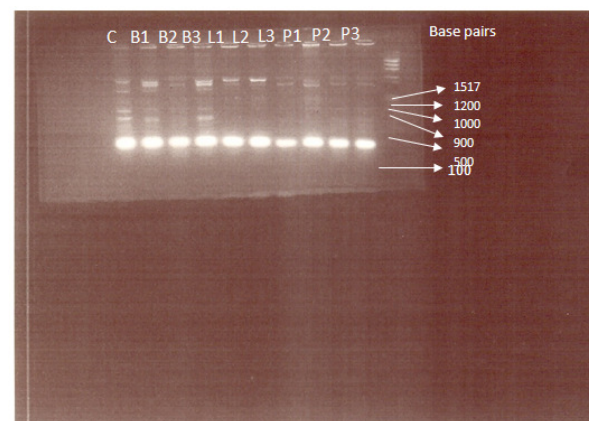


Plate 2: Agarose gel product showing the restricted band sizes using 3 DNA primers: GHR424F and GHRex5, NYPmap9 and NYPmap10, GnRHRmap5 and GnRHRmap8 using the restriction enzyme ECOR1