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COMPARATVE CHICKEN GENOME ANALYSIS OF EGYPTION LOCAL BREEDS AND DEVELOPED STRAINS 5-THE MICROSATELLITE DISCRIMINATION BETWEEN MATROUH, EL-SALAM AND BANDARAH STRAINS

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ABSTRACT

The aim of this study was to compare the genetic variation of three developed chicken strains (Matrouh, El-Salam and Bandarah). Nine highly polymorphic microsatellite markers were used in 133 birds. One hundred fifteen alleles were detected in the overall populations with a mean number of 12.78 alleles per locus. The highest number of alleles was 24 for microsatellite marker MCW 49. While, ADL171 and MCW 43 loci recorded lowest number of alleles across all populations (8). Dendrogram was generated from estimates of genetic distance among chicken populations. Mean number of alleles per strains overall loci ranged from 4.00 for Bandarah to 4.44 for Matrouh. Number of specific alleles was 8 for all strains studied and effective number of allele (ENA) ranged from 1.49 for MCW43 to 4.95 for MCW49. Averages of expected heterozygosity (H_E) were 0.597, 0.601 and 0.607 for Matrouh, El-Salam and Bandarah chicken populations, respectively. Dendogram Based Nei's genetic distance revealed that Matrouh and Bandarah chicken populations are closely related than that of El-Salam .The study revealed the existence of moderate genetic diversity in chicken populations studied and also showed that the markers used were highly informative and can be used in future studies involving breeding, management and conservation of chicken populations.

Keywords: Genotypic diversity, heterozygosity, number of alleles, effective number of alleles, microsatellite markers, local breeds.

INTRODUCTION

In recent years, animal biodiversity management has become an important issue in the international scientific community because of changes in large-scale production systems (FAO, 2007). In North America, Europe and China about 50% of documented breeds are classified as extinct, critical or endangered (Hammond, 1996) and local breeds have often been diluted by indiscriminate cross-breeding with imported stocks (FAO, 2007).The reduction in local poultry breeds due to replacement with cosmopolitan ones suggests a need for conservation of local genetic resources.

As for conservation of livestock genetic resources, if there are limitations of costs or breeding places, the population may be maintained as a core collection, which possesses as much genetic variability as possible

with appropriate population size. To develop the core collection, exact genetic evaluation for the population would be required by molecular markers. It would lead to a correct management of the population as a genetic resource (FAO, 1998).

Evaluation of genetic diversity for local breeds is becoming more challenging, and large efforts have been concentrated on maintaining minimum number of animals for each native species (FAO, 2007). Molecular marker information may provide reliable estimates of genetic diversity within and between a given set of populations. It is useful to explore genetic diversity within and between breeds or populations to analyze genetic relationships and admixtures and to provide information on evolutionary relationships and parentage within populations. Moreover, for breeds undergoing conservation, molecular data should be integrated with other information (i.e., adaptative, productive and reproductive performances; extinction probabilities) to guide decision makers (Zanatti et al., 2010).

The microsatellite marker as molecular marker is extensively used for assessing genetic structure, diversity, and relationships because of many advantages such as being numerous and ubiquitous throughout the genome, showing a higher degree of polymorphisms and codominant inheritance (Tautz, 1989). Especially, high degree of polymorphisms is considered to be greatly useful for assessing genetic diversity and relationships among closely related livestock breeds (FAO, 1998). Thus, use of microsatellite markers has become a standard method to estimate genetic diversity indices in all livestock species. However, some investigators have used microsatellites across Egyptian chicken populations (Roushdy *et al.,* 2008, 2009 and 2012 a,b ; El-Sayed et al., 2011; El-Tanany, 2011and Ramdan *et al.*, 2012).

Matrouh chicken was developed from a cross between the White Leghorn and Dokki-4 for six generations using systems of breeding coupled with selection (Mahmoud et al., 1974). The White Cornish and the Gimmizah were utilized as base population when developing Bandarah chicken for four generations. More than six years were devoted for developing this breed of chicken. This breed could be utilized as foundation stock for meat production (Mahmoud et al., 1989). El-Salam strain was designed for meat production. Originating from a cross between Nichol sires (parent line) and Mamourah females, selected for meat production, this strain is characterized by a broad breast and a keel that is carried forward (Abd El-Gawad et al.,1983).

The objective of this study was to investigate and compare genetic variance and diversity with 9 microsatellite loci in 3 Egyptian chicken populations (Matrouh, El-Salam and Bandarah).

MATERIALS AND METHODS

Chicken populations, blood sampling and DNA isolation:

Three Egyptian local strains, Matrouh, El-Salam and Bandarah chickens were assayed in the present investigation. A total of 133 individuals

were used from the three strains. Blood samples and DNA isolation were carried out as previously described by Roushdy et al. (2009).

Microsatellite loci, PCR and amplification conditions:

Nine microsatellite loci (Table 1) were selected based on the degree of polymorphism and genome coverage that have been recommended for the measurement of Domestic Animals Diversity (DAD) (FAO, 2004), for application in diversity studies. Detailed information about used microsatellites is available at the FAO website (Error! Hyperlink reference not valid.en/refer/library/guidelin/marker.pdf). The PCR reactions were carried out in a volume of 20 µl as described by Roushdy et al. (2009**).**

Microsatellite and genetic analysis:

All resulted gels were visualized and scored with Alphaimages2200 software (Version 4.0.1) All scored microsatellite data was firstly corrected to estimate each allele size according to its number of repeats. A Tandem Repeat Analyzer software package was adopted for this purpose. All possible extracted population figures were carried out employing a Arlequin 3.51 software package after data conversion using Convert program. It is common in such cases no amplicon is produced in certain samples for such primer rather than other. Thus, the absence of PCR product in these samples is manipulated as missing data. As a consequent, the analysis program accounts them as null (unknown) alleles not exceeded 0.1 of data as our default analysis.

RESULTS AND DISCUSSION

A set of nine highly polymorphic SSR markers were attended in the present investigation. Table(1) summarizes all information of the microsatellites markers used and shows locus name, gene bank NCBI accession number, genome location, microsatellite repeat type, flanking sequences, annealing temperature, reported number of alleles and sequence tagged site (STS) size in base pairs.

The genetic variability of the microsatellite loci is summarized in Figures (1and2). One hundred fifteen alleles were detected across 9 loci in three chicken populations with mean number of alleles per locus of 12.78. Locus MCW49 was highly polymorphic with 24 alleles, while ADL171 and MCW43 loci had the lowest polymorphism (8 alleles per locus).

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Results of this study indicated that most of the selected loci were reliable and informative because mean number of alleles per locus were more than four (Nassiri *et al.,*2007, 2009). Correspondingly, the standard error in the estimation of genetic distances were reduced (Nassiri *et al.*, 2007).

The mean number of alleles per locus calculated for three chicken populations was 12.78 and was nearly similar to the value of 14.00 recorded in 20 chicken breeds based on 14 markers shared with our study (Rosenberg *et al.,* 2001). Similar results were reported by Roushdy *et al.* (2012a), who investigated the value of 12 alleles for the discrimination between Dandrawi and Sinai breeds. On the other hand our value was greater than 10 reported for 52 chicken populations with 12 shared markers from a set of 22 markers (Hillel *et al.,* 2003), 10.11 reported for six South African local chicken lines based on nine markers (Van Marle-Köster et al., 2008), 10.33 reported for six Indian chicken populations based on three markers (Pirany *et al.*, 2007) and 11.4 in 64 populations of chickens from different continents by Granevitze *et al.* (2007). Lower estimates of mean number of alleles per locus were reported also by Farrag *et al.* (2013) as 5.66 in their study on genetic variation between Sinai chicken and Japanese quail and 7.5 for five subpopulations of Turkish native chicken breeds (Kaya and Yildiz, 2008). Population-specific alleles and/or allele scoring bias (allele dropout, null alleles) could explain these discrepancies in the number of alleles/locus (Nassiri *et al.,* 2007).

Effective number of alleles used to corollary the expected heterozygosity (when heterozygosity is high, ENA will be highest). The lowest ENA was 1.49 for MCW43 when H_E was 0.26 while, the highest ENA was 4.95 for MCW49 when H_E was 0.79 (Fiures 1and2). Same trend was reported by Roushdy et al. (2012b) for two populations of chicken (Gimiza and Inshas), the lowest ENA was 2.17 for MCW43 when H_E was 0.48,while the highest ENA was 10.05 for ADL176 when H_E was 0.83.

According to classification of Botstein *et al.* (1980) and Ott (2001), the highly informative markers have PIC values >0.50, the reasonably informative markers have PIC value between 0.25-0.50 and the slightly informative markers have PIC value <0.25. Six markers in the current study had highly informative PIC values of 0.55, 0.62, 0.66, 0.53, 0.54 and 0.63 for ADL136, ADL172, ADL176, ADL210, MCW49 and MCW51, respectively, and the rest of markers had reasonably informative markers. Similarly, in Turkish native chicken breeds, polymorphism information content varied from 0.426 to 0.599 (Kaya and Yildiz., 2008). Also, the investigation had done by Roushdy *et al.* (2012 b) showed that PIC values ranged from 0.3 to 0.79 and averaged 0.62 in Gimiza strain, while in Inshas ranged from 0.49 to 0.73 and averaged 0.66. Same findings obtained by Farrag *et al.* (2013), who found PIC ranged between 0.52 and 0.81 with average of 0.64 in Sinai chicken and Japanese quail, respectively. Pham *et al*. (2013) reported PIC average of 0.57 for 10 Taiwan commercial native chicken populations, two exotic breeds and one red jungle fowl population.

The H_E for all loci was > 0.50 and supported the effectiveness of the selected loci. The H_O and H_E ranged from 0.000 (MCW43) to 0.763 (ADL172) and 0.264 (MCW43) to 0.793(MCW49), respectively. For all loci, the mean H_E was higher than the mean H_O (Figure 2), which suggested sampling bias or a possible inbreeding mating system.

Table (2) summarizes the genetic variation across populations. The H_E variations were 0.597, 0.601 and 0.607 for Matrouh, El-Salam and Bandarah populations, respectively. These results are in agreement with Roushdy et al. (2012a) for Dandrawi (0.67) and Sinai (0.73)breeds, Roushdy et al. (2012b) for Gimiza (0.67) and Inshas (0.72) strains and Pham et al.(2013) for 10 Taiwan commercial native chicken populations, two exotic breeds and one red jungle fowl population (0.63). The H_O variations were 0.548, 0.310 and 0.405 for Matrouh, El-Salam and Bandarah populations, respectively.

Population fixation indices traced a 0.445 of variation referring to differences among individuals versus total variance (F_{IT}). While, among populations differences versus total variance was the lowest fixation indices (F_{ST}= 0.209) indicating low level of population differentiation. A pair wise difference among Matrouh , El-Salam and Bandarah populations was 0.299 based on among breeds F index (F_{IS}). The results are in agreement with Roushdy et al. (2012a) for Dandrawi and Sinai breeds, Roushdy et al. (2012b) for Gimiza and Inshas strains.

The genetic diversity among strains was assessed by an analysis of molecular variance (F-indices) employing Arlequin 3.51 software package as standard genetic population input data. The Wright fixation indices for F_{IS} ranged from -0.123 (ADL172) to 1.00 (MCW43), F_{ST} ranged from 0.0286 (ADL172) to 0.3299 (ADL171) and F_{IT} ranged from -0.0960 (ADL172) to 1.000 (MCW43), with means of 0.2929, 0.1593 and 0.4055, respectively (Table 6). A high positive F_{IS} indicated a high degree of observed homozygosity (MCW43) while there was excessive heterozygosity at locus ADL172, as indicated by the negative F_{IS} value. Significant deviations from HWE ($p < 0.5$) were observed across 2 populations (El-Salam, 0.002 and Bandarah, 0.04) for all loci except for Bandarah at ADL210 locus (0.42).

Table (3) showed the common and specific alleles for Matrouh, El-Salam and Bandarah populations. The specific and common alleles were 24 and 37, respectively. These specific alleles could be used as fingerprint for these chicken populatins.

Table (4) presents analysis of molecular variance (AMOVA). Variance components proved that the majority of genetic diversity obtained in the current study is represented by within individuals (55.46%) rather than others.

Cluster analysis based on Nei's genetic distance indicated that the studied populations formed two main groups (Fig. 5). The 1st group included Matrouh and Bandarah and the 2nd group harbored El-Salam.

Although genetic analyses can reveal the extent of biodiversity in chicken breeds (Nassiri *et al.,* 2007;Semik and Krawczyk, 2011) additional information on specific adaptations,distinct phenotypes, performance level, demography(including effective population size,and geographical distribution),and descriptive databases are required for adequate assessment of each breed when deciding on conservation and breeding programs (Groeneveld *et al.,* 2010).

CONCLUSION

the 9 microsatellite primers were recommended to assess molecular genetic structure of the local breeds and/or strains in Egypt. The results clearly demonstrated the genetic diversity of these chickens and would serve the appropriate managements on different levels including conservation of such genetic resources, future improvements for these breeds and/or understanding different genome arrangement and knowledge interests.

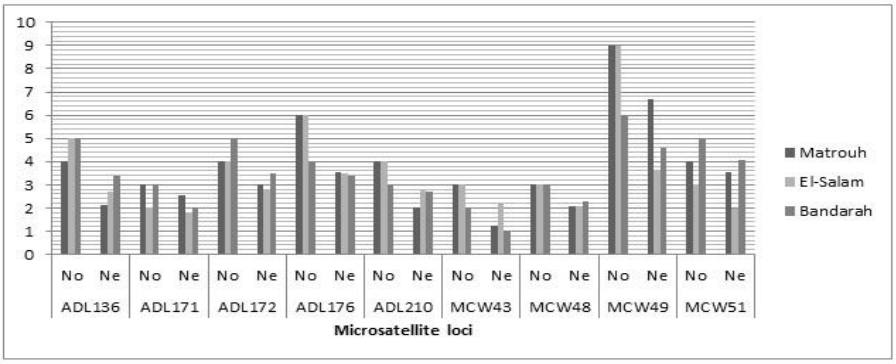


Figure (1): Number of alleles (No), effective number of alleles (Ne) estimated for each locus and strain.

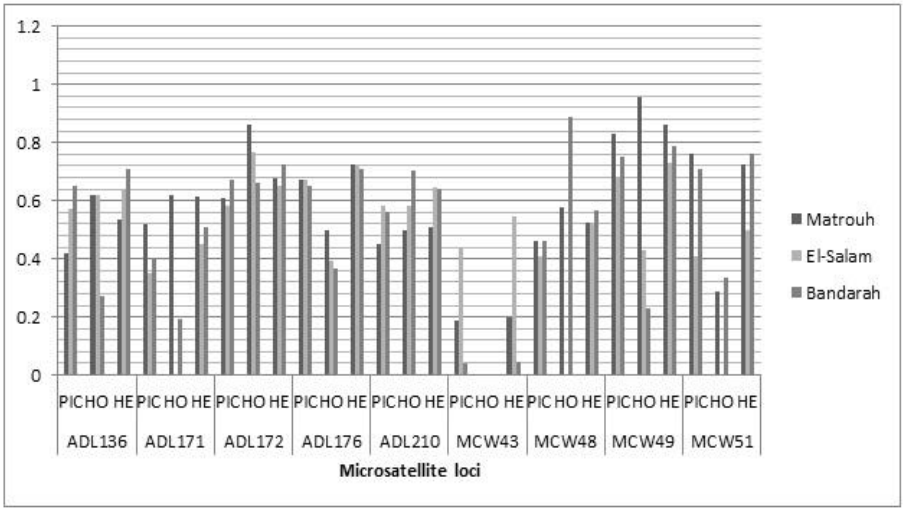


Figure (2): Polymorphism information content (PIC), observed heterozygosity

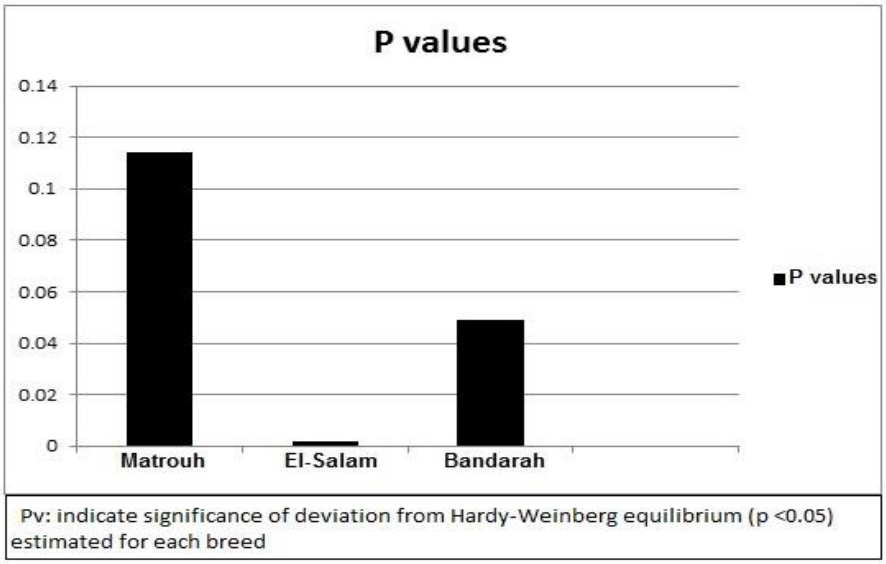


Figure (3): P values estimated for three chicken populations.

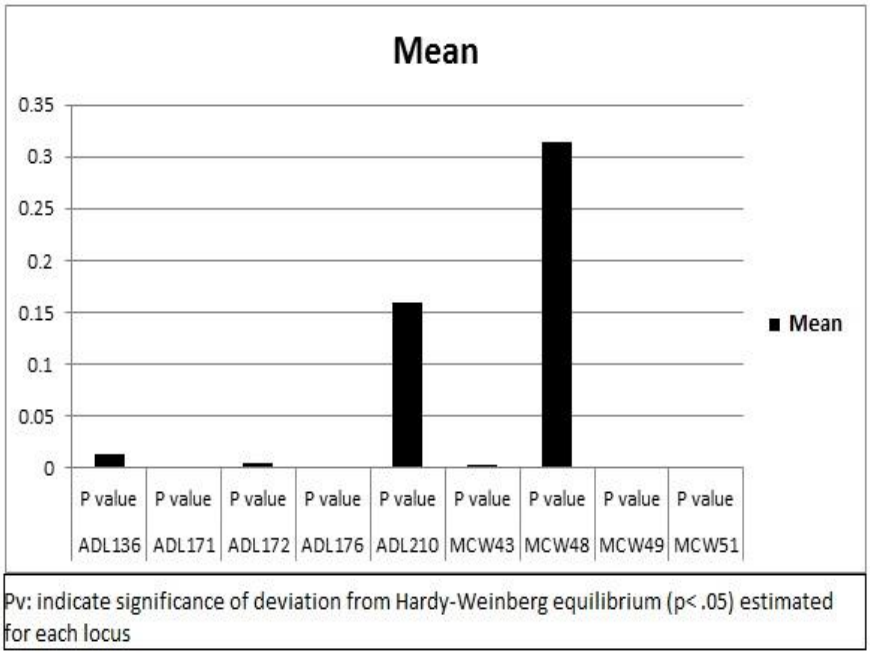


Figure (4) P values estimated for each microsatellite locus.

Table(2): Observed heterozygosity (H_O), expected heterozygosities (H_E) and their means,average population specific, F_{IS} (absolute value)estimated for each population.

	Matrouh	El-Salam	Bandarah	Mean
Mean (H_E)	0.597	0.601	0.607	0.602
Mean (H_O)	0.548	0.310	0.405	0.421
IC	0.082	0.484	0.333	
Mean F_{IS}	0.142	0.536	0.380	0.352

IC: inbreeding coefficient.($IC=(H_E. H_O)/H_E$)

Table (3): Common and specific alleles for analyzed breeds Matrouh, El-Salam and Bandarah

Locus	Common alleles bp	Specific alleles		
		Matrouh	El-Salam	Bandarah
ADL136	110,132, 154, 176,198	88	--	220
DL171	90	108,126	72	144,162, 180
ADL172	126,144,162,180	--	--	198,216
ADL176	192,204,216,228	168,180	152,240	--
ADL210	120,135,150,165	--	--	--
MCW48	190,208,226	--	82,100,118	--
MCW43	111,132,153	--	--	--
MCW49	118,130,142,154,166,178,190,202,214	226,238,250	--	106
MCW51	90,100,110,120	--	70,80	130
Total	37	8	8	8

Table (4): AMOVA analysis of Matrouh, El-Salam and Bandarah strains based on microsatellite DNA variation.

Source of variation	d.f.	S .S.	Percentage variation	Fixation indices
Among populations	2	129.65	20.91	F_{IS}=0.2987
within populations	130	446.99	23.63	F_{ST}=0.2091
Within individuals	133	247.50	55.46	F_{IT}=0.4454
Total	265	824.15	----	----

F_{IS}: Fixation indices (Among populations)
F_{ST}: Fixation indices (Among individuals within populations)
F_{IT}: Fixation indices (Within individuals)

Table(5):Nei's Original Measures of Genetic Identity and Genetic distance.

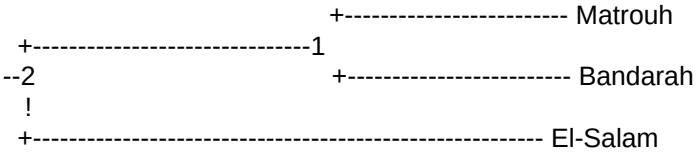
Population	Matrouh	El-Salam	Bandarah
Matroh	--	0.5547	0.6616
Salam	0.5892	--	0.5321
Bandara	0.4131	0.6309	--

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Table (6): Summary of F-Statistics and Gene Flow for All Loci

Locus	Fis	Fit	Fst
ADL136	0.1876	0.3758	0.2316
ADL171	0.4745	0.6478	0.3299
ADL172	-0.1283	-0.0960	0.0286
ADL176	0.4100	0.4317	0.0369
ADL210	-0.0049	0.0659	0.0704
MCW48	0.0848	0.3579	0.2985
MCW43	1.0000	1.0000	0.2349
MCW49	0.3134	0.3702	0.0828
MCW 51	0.6833	0.7263	0.1357
Mean	0.2929	0.4055	0.1593

Figure (5) : Dendrogram Based Nei's (1978) Genetic distance of three chicken strain produced by UPGMA clustering based on Nei's genetic distance using 9 microsatellite loci.



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مقارنه تحليليه لجينوم الدجاج المصرى المحلى والمستنبط
٥- التمييز بين سلالات الدجاج مطروح والسلام والبندره بواسطة الواسمات الوراثيه

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٢. البنك القومى للجينات – مركز البحوث الزراعيه
٣. معهد بحوث الانتاج الحيوانى– مركز البحوث الزراعيه

تهدف هذه الدراسه الى مقارنة الاختلافات الوراثيه بين ثلاثه سلالات من الدجاج (مطروح والسلام البندره). تم استخدام ٩ واسمات وراثيه مع ١٣٣ طائر. وقد اظهرت الكاشفات الجزيئيه ١١٥ أليل فى كل السلالات الثلاثه بمتوسط ١٢,٧٨ أليل للموقع. وكان اكبر عدد من الاليلات ٢٤ للكاشف الجزيئى MCW49 بينما سجلت المواقع MCW43,ADL171 اقل عدد من الاليلات فى كل السلالات (٨) . وقد تم عمل Dendogram لتقدير المسافه الوراثيه بين سلالات الدجاج الثلاثه. وكان متوسط عدد الاليلات لكل سلاله يتراوح بين ٤ للبندره الى ٤,٤٤ للمطروح وكان عدد الاليلات المحدده ٨ لكل السلالات المدروسه وعدد الاليلات الفعال تراوح بين ١,٤٩ لـــ MCW43 الى ٤,٩٥ لـــ MCW49 . وكان متوسط المتوقع من Heterozygote ٠,٥٩٧ و٠,٦١ و ٠,٦٠٧ لكل من مطروح والسلام والبندرهعلى التوالى. و قد اظهر ال Dendogram ان المسافه الوراثيه بين سلالة مطروح اقرب الى البندره منها السلام.

كشفت هذه الدراسه عن وجود تنوع وراثى فى سلالات الدجاج المدروسه واوضحت ان الكاشفات الجزيئيه المستخدمه غنيه بالمعلومات ويمكن استخدامها فى الدراسات المستقبليه فى عشائر الدجاج فى برامج التربيه والتحسين الوراثى والحفظ.

قام بتحكيم البحث

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Table(1): Summarizes all information of nine microsatellite markers used, including locus name, gene bank NCBI accession number, genome location, microsatellite repeat type, flanking sequences, annealing temperature, reported number of alleles and sequence tagged site (STS) size in base pairs.

Locus Name	Access No[1]	Location[2] Chr.No	size range	Repeat type	Forward primer sequence	Reverse primer	Ta[3]	No. of alleles[4]	STS Size[5]
ADL0136	G01561	9	107 cM	(TG)10	TGTCAAGCCCATCGTATCAC	CCACCTCCTTCTCCTGTTCA	52	10	145bp
ADL0171	G01593	8	26:35 cM	(TG)18	ACAGGATTCTTGAGATTTTT	GGTCTTAGCAGTGTTTGTTT	46	8	104bp
ADL0172	G01594	8	70:105 cM	(AC)18	CCCTACAACAAAGAGCAGTG	CTATGGAATAAAATGGAAAT	49	7	154bp
ADL0176	G01598	2	116 cM	(GT)12	TTGTGGATTCTGGTGGTAGC	TTCTCCCGTAACACTCGTCA	52	9	192bp
ADL0210	G01630	11	54 cM	(AC)15	ACAGGAGGATAGTCACACAT	GCCAAAAAGATGAATGAGTA	46	9	130bp
MCW43	D00311	1	157cM	(A)21	TGACTACTTTGATACGCATGGAGA	CACCAAGTAGACGAAAACACATTT000	55	NA	154bp
MCW48	D90071	3	270cM	(GT)18	CGTATAGGAGGGTTTCTGCAGGGA	AAGGAGGAACGCACCGCACCTTCT	55	NA	201bp
MCW49	M59361	1	418cM	(GCA)12	AGCGGCGTTGAGTGAGAGGAGCGA	TCCCCAACCCGCGGAGAGCGCTAT	55	NA	127bp
MCW51	M14230	2	358cM	(T)10	GGAACAAGCTCTTTCTTCTTCCCG	TCATGGAGGTGCTGGTACAAAGAC	50	NA	90bp

1. Gene bank accession number; www.ncbi.nlm.nih.gov/. http://www.ncbi.nlm.nih.gov/genome/sts/sts.cgi?uid=280100
2,4Locations & Number of alleles listed as reported by US chicken genome project population tester kit#9.
3.Annealing temperature, (FAO,2004)
5.STS: sequence tagged site size according to NCBI database.