Molecular characterization by two types of DNA markers for indigenous ducks in Kurdistan

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Abstract:
This experiment was carried out at the Poultry Breeding Unit of the Teaching and Research Farm of the Animal Science Department at College of Agricultural Engineering Sciences, University of Sulaimani, from February,1,2022 to June,20,2022.
Fifty ducklings were collected from different locations in Suleimani at one week age, The housing was divided into (4) floor cages. The average of the studied samples across all populations was (10.000). All 15 markers used to investigate the genetic diversity of local ducks produced (0.695) alleles on average, of which (1.137) were determined to be effective. The mean of Shannon diversity index was (0.147), and the mean of diversity was (0.091). Finally, the mean of unbiased diversity was (0.101).
The Result of dendrogram clustering confirmed the result of PCoA. The Main samples indicated three main classes. The results of the analysis of molecular variance (AMOVA) showed that among populations accounted for 25% of the genetic variance. Within populations, 75% of the genetic variation was detected.

Keywords: Local Duck, ISSR and URP marker.

Introduction

Ducks are members of the order Anseriformes, family Anatidae, and diverged from the rest chickens (Galliformes) a long time ago. Ducks, along with ostriches, emus, peacocks, turkeys, quail, and other avian, play an important role in bird evolution research (Ata *et al.*, 2019). Domestic duck plays an important role in animal protein provision and is said to have a high genetic diversity. Ducks are the second most important poultry species raised for egg and meat production, Indigenous ducks have long been regarded as a hardy breed with superior disease resistance (Pal *et al.*, 2022). Advances in molecular genetics have resulted in the identification of multiple genes or genetic markers associated with genes that affect quantitative traits over the last few decades. This has created opportunities to improve response to selection for traits that are difficult to improve through conventional selection (low heritability or traits for which phenotype measurement is difficult, expensive, only possible late in life, or not possible on selection candidates). A molecular marker (genetic marker) is a Genetic loci of DNA
That linked to a specific region of the genome. A marker is typically thought of as a constituent that determine the function of a structure. The discovery of the PCR had a significant on eukaryotic genome research and contributed to the development and application of various DNA markers. Molecular impact markers derived from polymerase chain reaction (PCR)

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amplification of genomic DNA are a critical component of evolutionary geneticists' work (Wakchaure et al., 2015). The aim of this study was to determine genetic diversity of local duck by using two molecular DNA markers techniques (ISSR & URP) in addition to estimate genetic distance among local duck individuals)

he genetic diversity of local ducks using molecular techniques. Specifically, using inter simple sequence repeat (ISSR) and universal rice primer (URP) as DNA markers to estimate the genetic distance between individuals and the diversity in local duck.

Materials and methods

Fifty ducklings were collected from different locations in Suleimani at one week age, The housing was divided into (4) floor cages. Each floor cage had a dimension of 2 by 1.5 meters. Throughout the experimental period, the ducklings received food and water at their discretion from separate feeders and drinkers. Feeders and drinkers were manually filled. The birds were fed *ad libitum* during the experiment.

Blood Sampling

A Blood sample was collected an amount of 3 ml from the brachial wing vein of each bird using a 3 ml syringe and then transferred into 5 ml tubes containing Ethylenediamine Tetra-Acetic Acid (EDTA) as an anticoagulant. All tubes were shaken for 10 seconds and stored at −20 °C for further use after transferring to the laboratory.

DNA Extraction

HiPurA Blood Genomic DNA Miniprep Purification Kit procedure was carried out for DNA extraction**.**

PCR

The protocol of the PCR was: add 3 µl of de-ionized water to the PCR tube, then 10 µl of master mix, then 2 µl of primer, then 5 µl of extracted DNA, and (20) primers were used for (50) samples. Amplification was carried out in thermal cycler with the following condition: initial denaturation at 94°C for 9 minutes, followed by 35 cycles (denaturation at 94°C for 1 minute, annealing (was according to primer's annealing temperature) for 1 minute, extension at 72°C for 2 minutes) and 9 minutes at 72°C for final extension.

Table 1 PCR components

Ingredients	Amount
2x PCR master mix	10 µL
Primer	2 µL
DNA	5 µL
Double distilled water	3 µL

Agarose Gel Electrophoresis

Agarose gel electrophoresis was used for PCR product visualization. To prepare the gel (1) gm agarose powder was added to (100 ml) TBE 1x buffer, for DNA result and (1.5) gm agarose powder was added to (100 ml) TBE 1x buffer for PCR products, then the mixture heated in microwave to get a pure liquid after cooling (10 µl) of ethidium bromide was added, then the solution was poured to the mold. When the gel was ready the amount of (10 µl) of PCR product was loaded in each well and the DNA ladder in the first well and then run with an applied voltage of 50 for 30 minutes. The result was documented by the gel documentation machine.

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Data Analysis

The result of fragment analysis was imported into GenAlEx 6.5 (Peakall and Smouse 2006) software and analyzed using the microsatellite plugin. All ISSR and URP loci were defined according to the allele range in bp by using PyElph software application system for gel image analysis and phylogenetics. ISSR and URP alleles were exported in Microsoft Excel format and used for further analysis.

Table 2 List of primers

Primer name	Sequence	Annealing temp. C/Time	Type of Primer
(CT)8GC	5'-CTCTCTCTCTCTCTCTGC-3' (18mer)	56	ISSR
URP-13R	5'-TACATCGCAAGTGACACAGG-3'(20mer)	59	URP
(GA)8GG	5'-GAGAGAGAGAGAGAGAGG-3' (18mer)	56.1	ISSR
URP-30F	5'-GGACAAGAAGAGGATGTGGA-3'(20mer)	60	URP
URP-25F	5'-GATGTGTTCTTGGAGCCTGT-3'(20mer)	59.7	URP
URP-1F	5'-ATCCAAGGTCCGAGACAACC-3'(20mer)	63.1	URP
(AG)8G	5'-AGAGAGAGAGAGAGAGG-3' (17mer)	54.2	ISSR
URP-38F	5'-AAGAGGCATTCTACCACCAC-3'(20mer)	59.3	URP
(AC)8GA	5'-ACACACACACACACACGA-3'(18mer)	58.4	ISSR
(AC)8CA	5'-ACACACACACACACACCA-3'(18mer)	58.8	ISSR
(CA)8AGC	5'-CACACACACACACACAAGC-3' (19mer)	58.5	ISSR
(CA)8G	5'-CACACACACACACACAG-3' (17mer)	55.3	ISSR
URP-9F	5'-ATGTGTGCGATCAGTTGCTG-3'(20mer)	59.2	URP
URP-4R	5'-AGGACTCGATAACAGGCTCC-3'(20mer)	61.5	URP
(GGGGT)3G	5'-GGGGTGGGGTGGGGTG-3'(16mer)	73.3	ISSR
(CAG)5	5'-CAGCAGCAGCAGCAG-3'(15mer)	57.6	ISSR
URP-17R	5'-AATGTGGGCAAGCTGGTGGT-3'(20mer)	64.6	URP
(GA)8CC	5'-GAGAGAGAGAGAGAGACC-3'(18mer)	56.3	ISSR
(TG)8A	5'-TGTGTGTGTGTGTGTGA-3'(17mer)	55.7	ISSR
URP-2F	5'-GTGTGCGATCAGTTGCTGGG-3'(20mer)	63.8	URP

Result and discussion

The results showed in the (table 1) indicate that the mean of allele numbers produced by the studied markers was 0.695 with a standard error of 0.028. The highest and lowest number of produced alleles (Na)with 0.735 and 0.636 alleles were observed in Pop1 and Pop4, respectively. Consequently, these two populations also possessed the highest and lowest effective number of alleles (Ne) combined, which was 1.155 and 1.115, respectively. Additionally, these two-population showed, respectively, the highest and lowest Shannon diversity indices (I) with 0.163 and 0.130. furthermore, the highest and lowest Diversity (h) and Unbiased Diversity (uh) also indicated by Pop1 and Pop4.

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Table 3 The summery of the results of genetic analysis of ISSR and URP markers for studied ducks

Pop		N	Na	Ne	I	H	Uh
Pop1	**Mean**	10.000	0.753	1.155	0.163	0.102	0.113
	SE	0.000	0.064	0.017	0.015	0.010	0.011
Pop2	**Mean**	10.000	0.710	1.137	0.148	0.091	0.102
	SE	0.000	0.063	0.016	0.014	0.009	0.010
Pop3	**Mean**	10.000	0.667	1.133	0.141	0.088	0.097
	SE	0.000	0.062	0.016	0.014	0.009	0.010
Pop4	**Mean**	10.000	0.636	1.115	0.130	0.080	0.089
	SE	0.000	0.061	0.013	0.013	0.009	0.009
Pop5	**Mean**	10.000	0.710	1.144	0.153	0.095	0.106
	SE	0.000	0.063	0.016	0.015	0.010	0.011
Total	**Mean**	10.000	0.695	1.137	0.147	0.091	0.101
	SE	0.000	0.028	0.007	0.006	0.004	0.005

Na: Number of alleles; Ne: Number of effective alleles; I: Shannon's Information Index; H: Diversity; UH: Unbiased Diversity.

To sum up, the average of the studied samples across all populations was 10.000. All 15 markers used to investigate the genetic diversity of local ducks produced 0.695 alleles on average, of which 1.137 were determined to be effective. The mean of Shannon diversity index was 0.147, and the mean of diversity was 0.091. Finally, the mean of unbiased diversity was 0.101.

in the study to look into the genetic composition of several domestic duck populations from Turkey's Central Anatolia region's Kirsehir and Yozgat provinces by the effective number of alleles (Ne), was calculated to be 1.26. The values of Shannon's information index (I) and gene diversity (H) were computed to be 0.198 and 0.331, respectively (Tunca et al. 2015).

Nei's genetic distance

Nei's genetic distance is a helpful method for examining population diversity, it's crucial to remember that Nei's genetic distance does not account for how genetic variations between populations are impacted by natural selection, gene flow, or genetic drift. In addition, variables including the quantity and kind of genetic markers utilized, the sampling plan, and the statistical techniques applied can have an impact on how genetic distances are interpreted (Nei, 1972).

A pairwise matrix of Nei's genetic distance for studied local ducks breeds is cleared in (table 2). Based on the results, population 1 and population 2 had the lowest genetic distance, with a value of 0.017. demonstrating a high degree of similarity between these groups. When Nei's genetic distances between pop1 and pop3, pop1 and pop4, pop1 and pop5 was (0.064, 0.070, 0.068) respectively, and Nei's genetic distances for pop2 and pop3, pop2 and pop4, pop2 and pop5 was (0.051, 0.056, 0.053) respectively. Also, Nei's genetic distances between pop3 and pop4, pop3 and pop5, pop4 and pop5 were (0.021, 0.040, 0.039) respectively. In summary, the examined groups showed low to moderate levels of genetic differentiation or divergence, indicating some genetic differences but not a significant degree of distinctness or full

separation across the populations. The results demonstrate that although there is some genetic variety or variation, there is also a significant level of genetic similarity amongst the groups.

according to the results reported by Su Ying et al., (2008) The smallest genetic distance (0.195) was measured between the Jianchang and Gaoyou duck populations, according on Nei's genetic distances between populations and the means of the similarities. The egg-type duck showed little variation, with Nei distances ranging from 0.514 to 0.633. The ducks from Gaoyou and Jinding had the highest mean similarities, followed by those from Gaoyou and Jianchang. The ducks from Beijing and Liancheng shared the lowest similarities. Tunca et al., (2015) reported that the populations with the greatest genetic distance were L3 (samples from Dulkadirli in Kirsehir) and L4 (samples from Saray in Yozgat) (0.0991), whereas the populations with the least genetic distance were L1 (Seyfe lake region) and L2 (near Hirfanli Dam) (0.0157).

Table 3 Pairwise Nei genetic distance among Population

Pop1	Pop2	Pop3	Pop4	Pop5	
0.000					Pop1
0.017	0.000				Pop2
0.064	0.051	0.000			Pop3
0.070	0.056	0.021	0.000		Pop4
0.068	0.053	0.040	0.039	0.000	Pop5

Percentage of Polymorphic Loci

According to the results explained in (table 4), the percentage of polymorphic loci mean for the all 5 populations is (34.55%). The highest percentage of polymorphism is (37.23%) in the population number (1) and the lowest percentage of polymorphism is (31.60%) in the population number (4). The population (5,2, and 3) have a (35.50%, 35.06%, and 33.33%) respectively.

Table 4 Percentage of Polymorphic Loci

Population	**%P**
Pop1	37.23%
Pop2	35.06%
Pop3	33.33%
Pop4	31.60%
Pop5	35.50%
Mean	34.55%
SE	0.96%

Band Patterns for Binary (Haploid) Data:

Banding pattern (Figure 1) for allelic data depicted that among all populations, number of bands and its frequency for the population 4 was the least by (74), and the highest number was (88) in population 1, and it was (82, 83, 77) for population 5, 2, 3 respectively. As well as number of private or unique band was found also the highest number was in the population 1 which was (29), and population 2,3 showed the same number of private bands which was the lowest number among populations by (19). And the number was (23,22) in the population 5, 4 respectively.

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While number of locally common bands (<=25%) was zero for all populations. But number of locally common bands (<=50%) was (31, 28, 26, 24, 23) in the population 2, 5, 4, 3, 1 respectively from highest to lowest. The overall population banding pattern showed that the highest and the lowest gene diversity(h) mean was (0.102, 0.080) in the population 1,4 respectively. The results also showed that the highest and the lowest unbiased diversity (uh) mean indicated by population 1,4 by (0.113, 0.089) respectively.

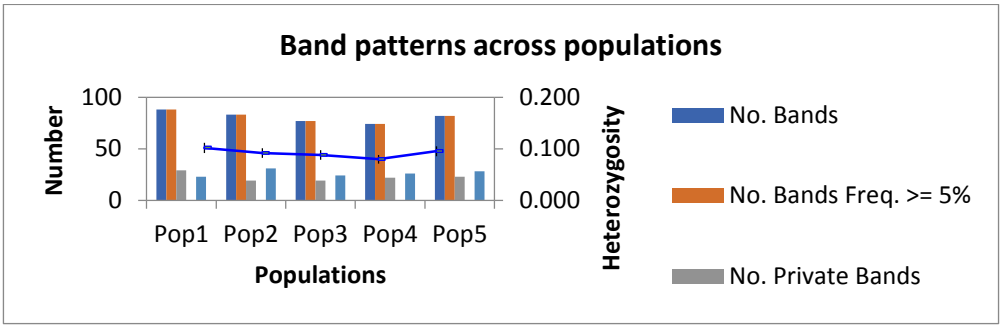

Figure 1 Band patterns across populations

Analysis of molecular variance (AMOVA)

Within-individual variation is significant in the setting of population genetics because it can impact estimates of genetic diversity and organization (Hedrick, 2005). For instance, based on a limited sample size, it could be challenging to accurately estimate the genetic diversity of a population if there is a high level of within-individual variation. The majority of population genetic analyses, however, make the assumption that variation within and between individuals is much greater than variation within and between populations (Hedrick, 2005). This presumption stems from the reality that the majority of genetic markers utilized in population genetics, like microsatellites or single nucleotide polymorphisms (SNPs), are assumed to be fixed within an individual and are inherited in a Mendelian manner (Hedrick, 2005). As a result, population genetic analyses like AMOVA, which divide the total genetic diversity into various components based on variance across individuals within populations, among populations, and among groups, typically do not incorporate within-individual variation. But in order to reduce its influence, it's critical to be aware of the possible implications of within-individual variation on estimates of genetic diversity and to choose suitable sampling techniques (Hedrick, 2005).

The results of the analysis of molecular variance are presented in (Figure 2). AMOVA gives results that shed light on the distribution of genetic variation at various levels. The study's results showed that among populations accounted for 25% of the genetic variance. Within populations, 75% of the genetic variation was detected. This suggests that every individual has a significant amount of genetic variety, most likely due to differences in their specific genetic composition, According to these findings, most genetic variation is found within individuals, but there may be some genetic heterogeneity among populations. This information can be valuable for understanding the genetic structure and diversity of the studied individuals.

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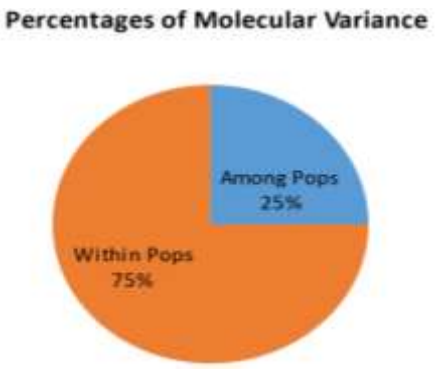


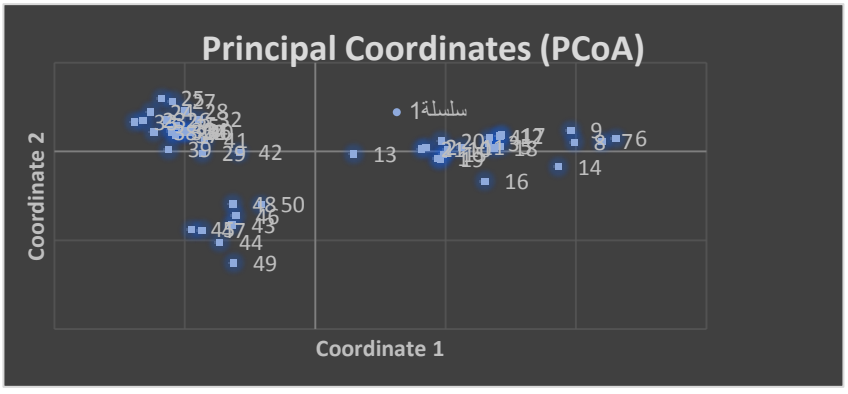
Figure 2 Analysis of molecular variance of all studies population

According to the results indicated by Lai et al., (2020) The majority of variation in the examination of all studied populations was discovered Within individual (76%) and then within populations (19%) and (5%) of the variation was explained by the differences between individuals within populations. Similar to the results of the present study, Paramasivam et al., (2017) found that the results of the analysis of molecular variance (AMOVA) show that there is 24.88% variation between populations and 75.12% variation within populations.

Principal Coordinates Analysis (PCoA)

Principal coordinates analysis, frequently referred to as classical scaling, is a metric multidimensional scaling technique based on projection. It approximates a matrix of distances/dissimilarities by the distances between a collection of points in a small number of dimensions via spectral decomposition. Visualizations can make use of the points (Gower, 2014).

The results of the principal coordinate analysis of ISSR and URP markers for the studied individuals (figure 3), according to the bands we purchased the birds divided into 3 main classes. Each class contain the birds that showed the same band or the ones who are relatively correlated together.



The numbers (1-50) refer to the bird's number

Figure 3 Principal coordinates analysis of ISSR and URP markers for studied populations

Genetic relationships among studied populations

A dendrogram of genetic similarity between studied birds explained in (figure 4). The result of dendrogram clustering confirmed the result of PCoA. main samples indicated three clusters. The first cluster consist of duck number

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(13,1,2,16,14,9,12,20,21,15,3,19,10,11,4,5,17, 18,8,6,7). The second cluster consisted of duck number (43,44,46,47,45,49,48,50), and the third cluster consisted of duck number (29,41,25,27,28,24,26,35,34,36,33,40,39,22,23,30,31,37,38,32,42). According to this result the birds were divided to five populations based on the dissimilarity between them.

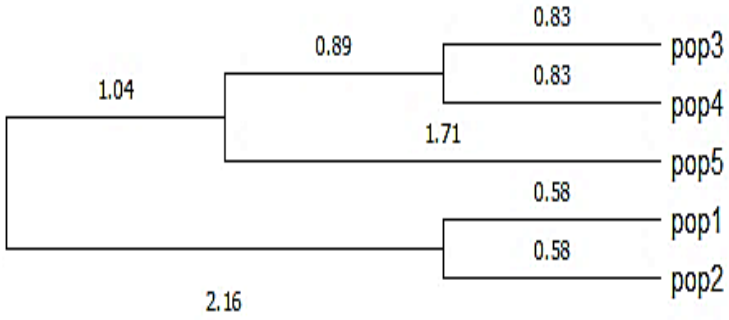

Figure 4 Hierarchical clustering dendrogram for studied genetic groups populations

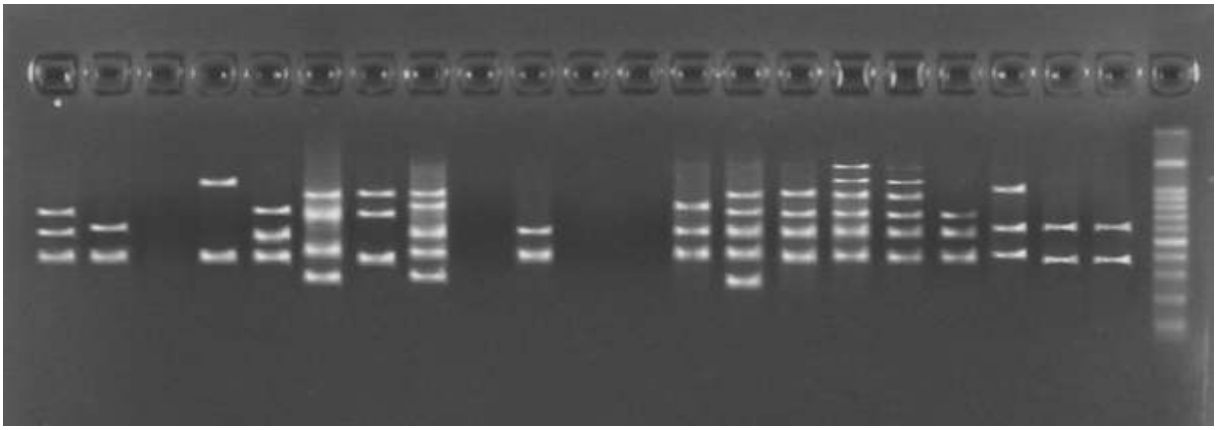

Figure 5 results of primer (3) samples from 1-21

Figure 6 results of primer (3) samples from 22-42

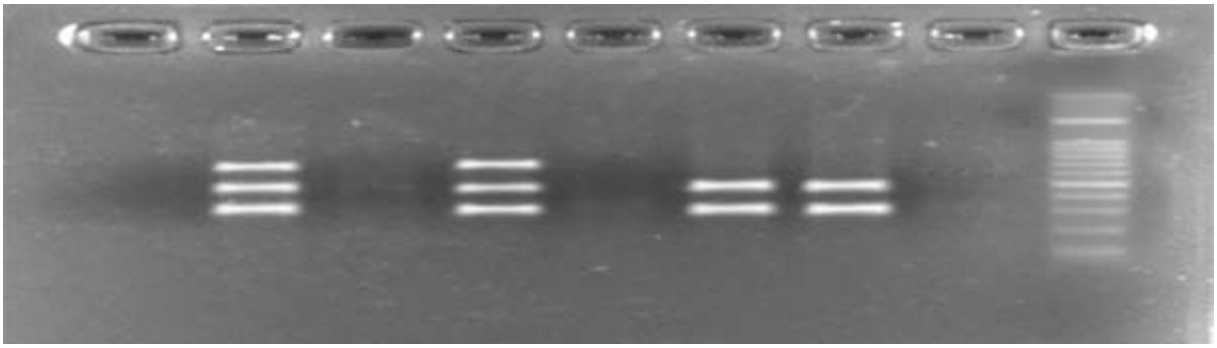

Figure 7 results of primer (3) samples from 43-5

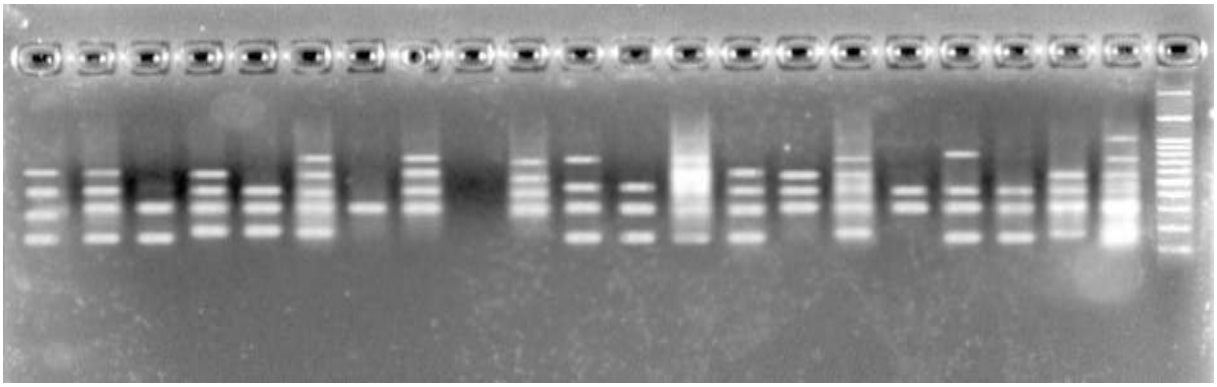

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Figure 8 results of primer (7) samples from 1-21

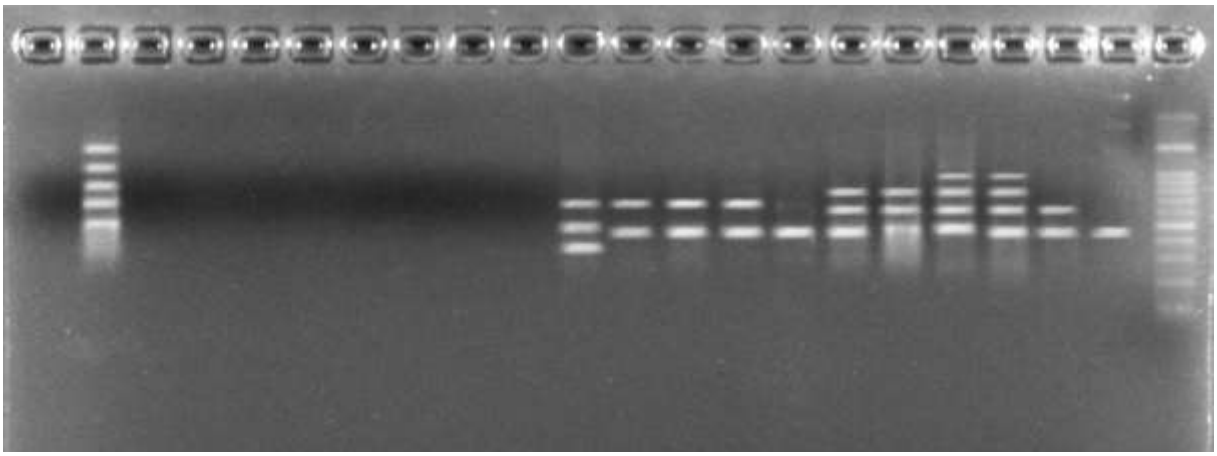

Figure 9 results of primer (7) samples from 22-42

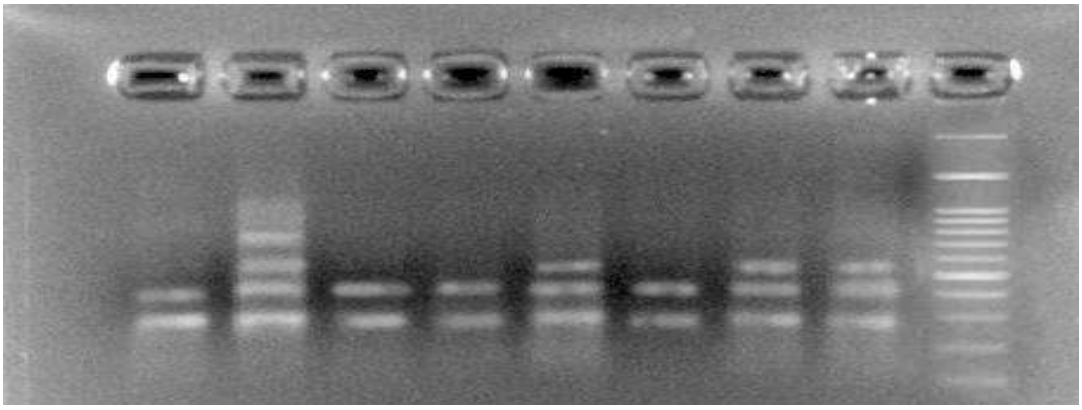

Figure 10 results of primer (7) samples from 43-50

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