

# Identification of Meat Species and Meat Products by a Multiplex PCR Method

Asma Al-Tamimi, Yaquob Ashhab

Biotechnology Research Center, Palestine Polytechnic University, Hebron, Palestine

**Abstract** — Identification of animal species for industrial meat products (cooked, processed, and canned foods) is very important in respect to economical, religious, or reasons concerning public health. This service is set up in our laboratories to provide a high quality service for the food industry sector and to help in overcoming an export barrier for our processed meat. The identification of meat from different sources (cattle, goat, sheep, chicken, turkey, pig and donkey) was determined by multiplex PCR, using species-specific primers on mitochondrial DNA. This method is characterized by high efficiency and sensitivity even with very small amounts of fresh or cooked meat.

**Keywords-component;** processed meat; polymerase chain reaction; mitochondrial DNA; species identification

## I. INTRODUCTION

The identification of the origin of meat products is very important in many ways: firstly, economical level consumer need to verify that the less costly meat is not being mislabeled as meat from highly costly species. Second, at a religious level Muslims and Jews have a need for reassurance that processed meats do not contain pork.

When consumers buy fresh meat they have no problem identifying the species of this meat, but the processed and cooked meats such as sausage, canned foods and cold cut products cannot be identified by the naked eye in terms of species of origin.

The identification of the origin of meat species can be achieved using many methods such as protein based methods, including (SDS-PAGE), isoelectric focusing (IEF) and HPLC, immune based method such as ELISA (1), immune diffusion tests, sensory analysis, anatomical differences, histological differentiation of the hair that may possibly exist in the meat, properties of tissue fat, and level of glycogen in muscle tissue (2). The disadvantages of using these methods in processed meat is that these methods are tissue dependant (1, 3) and they can't differentiate between closely related species (4) and they are very expensive (5).

DNA based techniques, however, are more sensitive and specific and can be use in highly processed meat products. Some of the molecular methods used in meat species identification are DNA hybridization, PCR based methods such as sequencing of PCR products, RFLP analysis, RAPD-PCR, PCR-SSCP, and Multiplex PCR (7, reviewed in 8)

Most of literature refers to the use of mitochondrial DNA (mtDNA) rather than nuclear DNA for the identification the origin of meat products, because processed meats are likely to contain degraded DNA. (mtDNA) is more suitable than

nuclear DNA due to the high copy number of (mtDNA) per cell, which thereby increases the chance of getting good DNA from samples (6). Furthermore, the mutation rate on (mtDNA) is higher than nuclear DNA and this gives a greater chance to accumulate several point mutations, which allows the differentiation of even closely-related species. (Reviewed in 8) In this study, multiplex PCR was used to amplify DNA from processed meat samples to identify their origin, using species specific primers that were designed for (mtDNA) of the following species: (Ovis aries- NC\_001941), Goat, (Capra hircus- NC\_005044), Cattle (Bos Taurus- V00654), Pig (Sus scrofa- NC\_000845), Dog, (Canis lupus- HM048871), Donkey (Equus asinus- NC\_001788), Chicken (Gallus gallus- NC\_001323), Turkey (Milagros gallopavo- NC\_010195).

## II. MATERIAL AND METHODS

### A. primer design

T-Coffee multiple sequence alignment tool was used to compare several mitochondrial genes representing sheep, chicken, turkey, goat, pig, cattle and donkey. It was found that the D-loop sequences have a high rate of difference between species, and was used to generate species specific forward primers for use with a universal reverse primer for a highly conserved region 500bp downstream of the D-loop as in figure 1.

### B. DNA extraction from fresh, heated and processed meat

50 mg from -80°C frozen samples were ground by mortar and pestle followed by homogenization by a homogenizer in 1ml EZ DNA kit (EZ-DNA Genomic DNA Isolation Reagent (Cat.No:20-600-50)). Homogenates were then centrifuged for 10 min at 10000g at RT, and 1ml absolute ethanol was added to pellets with mix by inverting 10 times. Following storage for 3 min at RT DNA was removed by spooling with a pipette tip. Extracted DNA was then centrifuged at 5000g for 5min. The DNA pellet was washed twice with 95% ethanol and dissolved in 1X TE buffer.

### C. Simplex PCR

PCR amplification was performed in a reaction volume of 20µl containing 0.4u Taq polymerase, 2µl from 10X buffer with MgCl<sub>2</sub>, 20µM dNTPs, 0.05 µM from each primer, 0.3µg/ml from template. The thermocycler program was initial denaturation step at 94°C for 10 min followed by 35 cycles,

which were programmed as follows: 94°C for 1min, 57°C for 45 sec, 72°C for 40sec; final extension after the last cycle was at 72°C for 10 min.

**D. Multiplex PCR**

A multiplex PCR system was developed using the same primers to identify the species according to the PCR product size. The reaction was setup using eight primers on 40µl reaction, 8 µl from 2.5 µg/ µl dNTPs (sigma -DNTp10-1kT), 1µl Taq polymerase (PolyTaq, Palestine Polytechnic University), 8 µl buffer, 3 µl template DNA, 12 µl distilled water. The thermocycler program was initial denaturation step at 94°C for 3 min followed by 35 cycles as follows: 94°C for 1min, 55°C for 45 sec, 72°C for 2minutes and 20 seconds, final extension at 72°C for 10 min.

**III. RESULTS**

**A. primer design**

The primer sequences as in (table 1).

Primer sequences			
Primer	Sequences from 5' to 3'	Product size	Tm
F(Ovis aries NC 001941)	15972'tgtaggggtattaaactgcttgac'15994	961	59
F(Capra hircus-NC 005044)	15531'ccactccacaagcttacagac'15551	1428	61
F(Bos Taurus-V00654)	15941'cagaatgaattacactgcaag'15959	1052	58
F(Sus scrofa-NC 000845)	892'cattaactatgctctacacacctataa'921	604	65
F(Equus asinus-NC 001788)	16542'cgcaactgacaagcccaac'16560	464	59
F(Gallus gallus-NC 001323)	1202'caaaaccgcttctaccactat'1224	349	63
F(Milagros gallopavo-NC 010195)	16756'tgccctaacccttaagaa'16778	180	55
Reverse primer	gtggctggcagagattaccaac		67

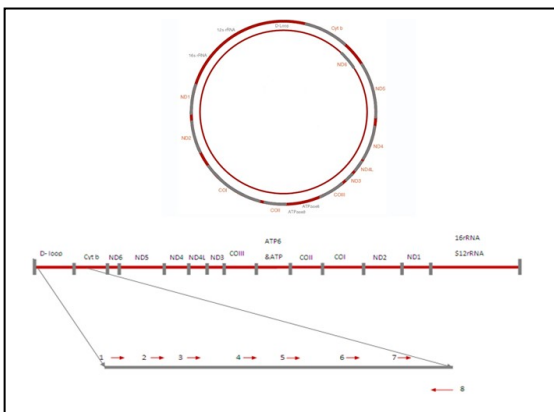


Figure1: mitochondrial DNA circular and liner, forward and reverse primers on (D-loop+500pb) region.

**B. Simplex PCR**

DNA fragments from (mtDNA) turkey -180bp, chicken-349bp, dog- 417bp, donkey -464bp, pig-604bp, sheep-961bp, cattle-1052bp and goat-1428bp were amplified (Figure 2).

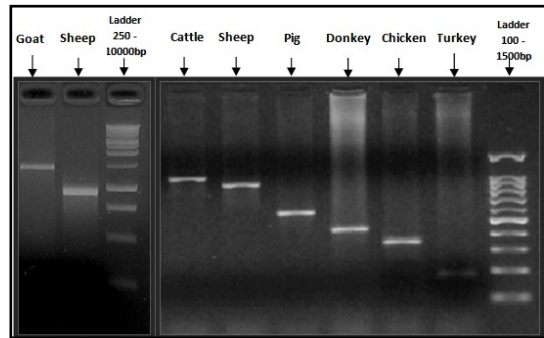


Figure2: simplex PCR for turkey, chicken, donkey, pig, sheep, cattle, goat.

**C. Multiplex PCR**

Figure 3 shows the results of the multiplex PCR reaction. The sizes of the bands and their corresponding origins are indicated by arrows.



Figure3: multiplex PCR for turkey, chicken, donkey, pig, sheep, cattle, goat.

**D. Initial market survey**

The applicability of this method to locally produced as well as to imported meat products (sausages, cold cut and ground meat) was demonstrated. A total of 30 processed meat items that are either produced locally or imported were examined. The results indicate that 15% of the samples labeled as containing pure calf meat were heavily contaminated with poultry residuals and 5% of samples were contaminated with other species, which are not in accordance with mentioned ingredients.

#### IV. DISSCUSION

Molecular methods for identify meat origin are not new approach to solve this technical problem, but using the D-loop region on mitochondrial DNA and 500 bp after this region, which are respectively exhibit a highly species diverse region followed by a highly conserved region nearby is novel. This gives our primer a better chance to be more species-specific, and makes the multiplex PCR possible with a conserved reverse primer that increases reproducibility and lowers reagent costs. The treatment of samples did not affect the result, whether the sample was from fresh meat, autoclaved, processed or lipid tissue.

Our molecular technique for testing samples is a qualitative method, but our primers can be modified based on the same principle of specific forward primers with a universal reverse primer to fit Real-Time-PCR, which is a quantitative method.

The overall mixing that was apparent from the market survey samples suggests that mixing of meat from highly costly species with less costly species may be a motive for producers. PCR of the market survey samples may provide a measure of reassurance to consumers that no taboo meats were detected.

#### V. CONCLUSION

Multiplex PCR method to identify the origin of processed meat is more specific and faster than other methods.

According to our results the Palestinian territory is not free from mislabeled local and imported processed meat. There is, therefore, a market need for follow up by specialized ministries

in order to take the legislative and executive steps to govern labeling for the local products.

#### ACKNOWLEDGMENT

The authors wish to acknowledgment veterinary doctor Mohammad Nouh and doctor Mohamad Almanasrah for reference sample collection.

#### REFERENCES

- [1] S. Ghovvati a,b\*, M.R. Nassiri a, S.Z. Mirhoseini b,c, A. Fraud identification in industrial meat products by multiplex PCR assay.
- [2] O. <rfan <LHAK\*\*, Ali ARSLAN, Identification of Meat Species by Polymerase Chain Reaction (PCR) Technique.
- [3] Andrea Pirondini, Urbana Bonas, Elena Maestri, Yield and amplificability of different DNA extraction procedures for traceability in the dairy food chain.
- [4] P. Stamoulis, C. Stamatis, T. Sarafidou, Z. Mamuris, Development and application of molecular markers for poultry meat identification in food chain,
- [5] Salah M. Abdel-Rahman, Mohamed A. El-Saadani 1 1 2Khallid M, Detection of Adulteration and Identification of Cat's, Dog's, Donkey's and Horse's Meat Using Species-Specific PCR and PCR-RFLP Techniques.
- [6] Hsing-Mei Hsieh,1 Ph.D.; Chin-Cheng Tsai,2 M.S.; Li-Chin Tsai,1 M.S, Species identification of meat products using the cytochrome b gene.
- [7] T. Matsunaga, K. Chikuni , R. Tanabe, S. Muroya, A quick and simple method for the identification of meat species and meat products by PCR assay.
- [8] A.K. Lockley\* and R.G. Bardsley, DNA-based methods for food authentication.