# PCR-BASED DETECTION OF CHICKEN AND CATTLE MEAT TARGETING *CYTOCHROME B* GENE AS AFFECTED BY HEATING AND MIXING OF MEAT

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## ABSTRACT

**This study was conducted to examine the effects of heating and mixing of cattle and chicken meat on their identification by PCR targeting cytochrome b gene using species-specific primers. In addition to simplex PCR, multiplex-PCR was used for detection in meat mixtures.**

**Five samples of cattle meat and five samples of chicken meat were subjected to 80, 90, 100, 110 and 120 C for 20 minutes. The results of PCR targeting cytochrome b gene showed that all heat treatment groups of cattle and chicken meat were identical.**

**After mixing equal quantities of cattle and chicken, ten samples were selected from different locations in the mixture. Results showed that 50 % of samples were present both cattle and chicken, whereas, only 30 % of samples were present cattle only, in addition, only 20 % of samples were present chicken only.**

**The results of this study confirm that DNA was unaffected by heating up to 120 C for 20 minutes and refer to the importance of increasing sample numbers and sites of tested meat mixtures.**

## INTRODUCTION

Meat adulteration in ground and comminuted products has been a wide spread problem in retail markets. Identification of the species origin in meat samples is relevant to consumers for several reasons: (a) possible economic loss from fraudulent substitutions or adulterations, (b) medical requirements of individuals who might have specific allergies, and (c) religious reasons (Miguel *et al.,* 2004). There is a call for the availability of reliable and rapid methods to identify animal species in food. Polymerase chain reaction (PCR) is the most commonly used technique in many fields of molecular biology owing to its sensitivity, specificity and capability to detect even a single copy of DNA sequence from a single cell sample (Chikuni *et al.*, 1994).DNA molecules have been used as target compounds for species identification due to their high stability and unique variability which allow the differentiation of closely related species. Among DNA-based methods, Polymerase Chain Reaction (PCR) is an effective technique that is highly accurate and relatively fast. Most of literature (Brown et al., 1982; Hopwood et al.,1999; Prado et al.,2002) 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 (Hsieh *et al*., 2005). Mitochondria are small granular or filamentous intracellular bodies, which are referred to as 'powerhouse of the cell'. The number of mitochondria in a cell depends upon its metabolic activity. On average, around 800–1000 mitochondria have been found per cell in animals. Each mitochondrion contains two to six circular DNA molecules with a size of about 16,500 bp..Each DNA molecule contains 22 tRNAs, 2 rRNAs and 13 protein coding genes (Gardner & Snustad, 1984).

The study was carried out with the following objectives. (1) To standardize individual and cattle –chicken mixed meat species identification by PCR amplification of cytochrome b gene. (2) To identify the effect of exposing cattle and chicken meat to different temperature up to 120 C for 20 minutes on meat identification by PCR.

## MATERIALS AND METHODS

The fresh meat samples from cattle and chicken were collected from local market in Baghdad. The collected samples were transported to the laboratory under refrigeration and were stored frozen at -20 C prior to analysis.

Cattle and chicken meat were used to examine the effect of exposing meat to different temperatures (80 .90, 100,110 and 120***°C***) for 20 minutes on detection of meat species.

Equal quantities of cattle and chicken meat were comminuted and mixed well. Then ten samples were selected from different location and subjected to DNA extraction. Also, DNA samples of both cattle and chicken were mixed in different quantities as follows*:*

| species | Mixtures | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Cattle | 50 ng | 60 ng | 70 ng | 80 ng | 90 ng |
| Chicken | 50 ng | 40 ng | 30 ng | 20 ng | 10 ng |

DNA was extracted from 25 mg of meat samples using DNeasy® tissue kit (Qiagen, Hilden, Germany) as per the Manufacturer's instructions. One hundred and eighty microliters of ATL buffer and 20 µl proteinase K were added and vortexed. The mixture was incubated at 56°C in a water bath, to disperse the sample, until the tissue was completely lysed. The mixture was vortexed for 15 s; 200 µl of AL buffer was added to the sample and vortexed thoroughly. 200 µl of ethanol (96 %) was added to the mixture and it was vortexed to yield a homogenous solution. The homogenous solution was transferred into the DNeasy® mini column in a 2 ml collection tube. The homogenous solution was centrifuged at 8000 rpm for 1 min. The flow-through and collection tubes were discarded and the DNeasy® mini column was put in a new 2 ml collection tube. 500 µl of AW1 buffer was added and spun at 8000 rpm for 1 min. The flow-through and collection tubes were discarded and the DNeasy® mini column was placed in another 2 ml collection tube. 500 µl of AW2 buffer was added and centrifuged at 14,000 rpm for 3 min to dry the DNeasy membrane and then, the flow-through and collection tube were removed. The DNeasy® mini column was placed in a clean 1.5 ml micro centrifuge tube. 200 µl of AE buffer was transferred directly onto the DNeasy® membrane and incubated at room temperature at 1 min followed by spinning at 8000 rpm for 1 min to elute it. Elution was repeated to increase the final DNA concentration. The concentration of DNA was measured by spectrophotometer the DNA solutions were stored at -20°C.

The DNA concentration was accounted by the following formula:

DNA concentration = OD260 x extinction coefficient (50 µg/ml) x dilution factor

A spectrophotometer device (Cecil, France) was used to determine the concentration of DNA in the solution. The samples were exposed to ultraviolet light at 260 and 280 nm. A 260:280 ratio was determined as the qualification of nucleic acids.

A set of primers specific to *cytochrome b* gene family (Matsunaga *et al.,* 1999) was custom synthesized at Cinagen. The primer sequences were derived from the *cytochrome b* gene sequences from cattle and chicken species.

| **Name** | **Primer** | **Sequences (5' – 3')** | **No. of Bases** |
|---|---|---|---|
| common | F | 5'-GACCTCCCAGCTCCATCAAACATCTCATCTTGATGAAA-3' | 38bp |
| CHICKEN | R | 5'-AAGATACAGATGAAGAAGAATGAGGCG-3' | 27bp |
| CATTLE | R | 5'-CTAGAAAAGTGTAAGACCCGTAATATAAG-3' | 29bp |

Polymerase chain reaction amplification was performed in a final volume of 25 µl, containing 12.5 µl master mix, 1 µl of 10 x primer forward, 1 µl of 10 x primer reverse, 2 µl of DNA template, 8.5 µl of RNase-free water. Amplification was performed in a thermocycler (Applied Biosystem, USA), with the following cycling conditions: denaturation 1 at 95 C for 3 min., 35 cycles were programmed as follows: at 94°C for 30 s, annealing at 62°C for 30 s, extension at 72°C for 30 s and final extension at 72°C for 10 min.

As for the simplex PCR, multiplex PCR amplification was performed in a final volume of 50 µl containing 25 µl of master mix, 15 µl of 10x primer mix, 3 µl of DNA template ( mix the two species), 7 µl of RNase-free water. Thermal

cycling was programmed following the same procedure used for simplex PCR.

Electrophoresis was run on agarose gel (1.5%) at 50 V for 1 h on a 10-µl portion of the amplified DNA fragments. The resulting gel was stained with ethidiumbromide (0.5 µg/ml), visualized using a UV transilluminator, and photographed with a Polaroid camera. The experiments were conducted in triplicate.

## RESULTS AND DISCUSSION

### Heat treatment

Five samples of chicken meat (50 g each) were subjected to 80, 90, 100, 110 and 120 °C for 20 minutes. The results of PCR targeting *cyt b* gene indicate that all heat treatment groups of chicken meat were identical (Fig.1). Also, five samples of cattle meat (50 g each) were subjected to the same heat treatments as chicken meat. The results of PCR targeting *cyt b* gene indicate that all heat treatment groups of cattle meat were identical (Fig.2).These results confirm that DNA in heat treatment groups was unaffected by heat processing , thus , we can conclude that the effect of heat depend on the degree of temperature and time .

Heat treatmentsare those steps, which mainly affect the quality of DNA causing its degradation into small size fragments(Dias Neto *et al* ,1994;Martinez and Yman ,1998). For this reason ,meat samples were cooked in the present study at 80,90,100,110 and 120 °C for 20 minutes to stimulate cooking .As noted from the results obtained in this study , DNA was unaffected by heating up to 120°C for 20 minutes in cattle and chicken meat .

Meyer *et al* (1994) reported that pig meat was identified at a ratio of less than 2% in beef products that were subjected to heat treatment at 121 °C for 10 minutes, using a specific primer. Also, Hopwood *et al* (1999) reported the identification of chicken meat heated to the temperature of 120 °C for 30 minutes**.** Arslan *et al* (2006)reported considerable resistance to high temperature (cooking at 97.5 °C for 140, 200 and 230 minutes) of DNA derived from beef meat.

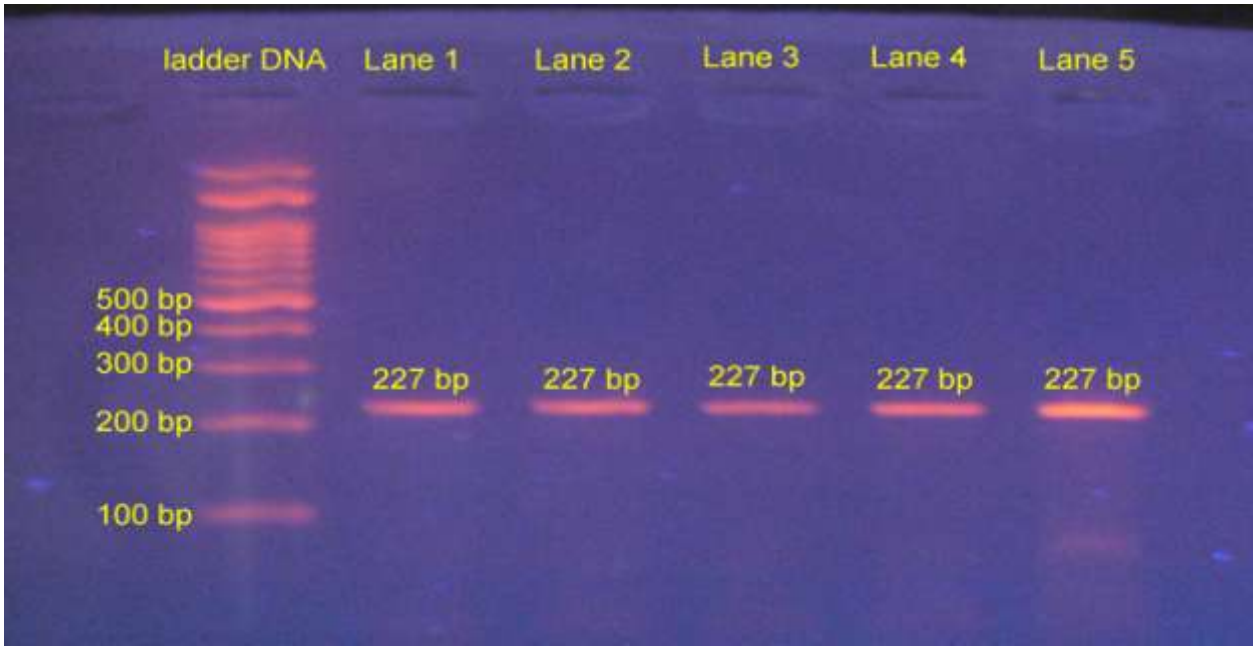


**Figure 1.** PCR product results of chicken meat subjected to heat for 20 min (1) 80°C, (2) 90°C,( 3) 100°C ,(4) 110°C and (5) 120°C.M100 :100 bp ladder DNA.

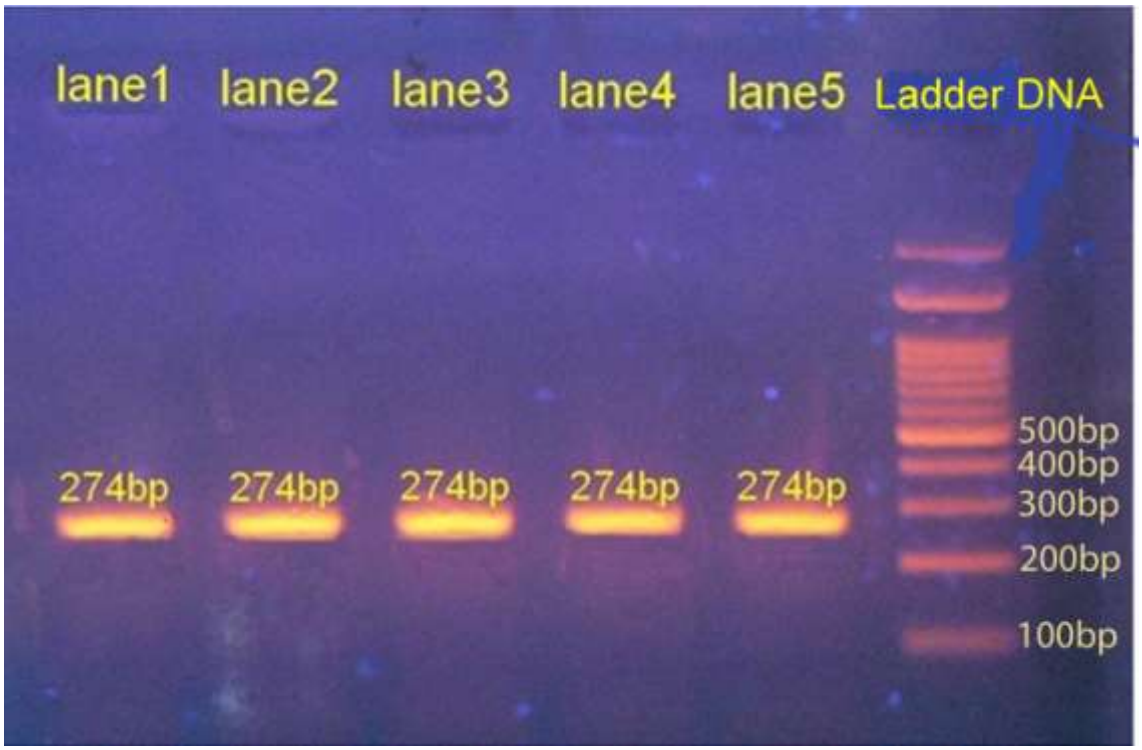


**Figure 2.** PCR product results of cattle meat subjected to heat for 20 min (1) 80°C,(2) 90°C,(3) 100°C,(4) 110°C and (5) 120°C.M100 :100 bp ladder DNA**.**

**Meat and DNA mixtures**

Equal quantities of cattle and chicken were comminuted and mixed, well. Then ten samples were selected from different locations. Results showed that 50% of samples (n =5) were present both cattle and chicken, whereas, only 30% of samples (n =3) were present cattle only, also, only 20% of samples (n=2) were present chicken only (Fig. 3). These results demonstrate the importance of increasing the number of tested samples. These different results for the same mixture may be attributed to degree of homogenizing of meat mixture. Figure 4 show that all DNA mixtures present both cattle and chicken This result may be attributed to the great number of mitochondrial DNA copies that may found in 10 ng of DNA (Bai *et al* ., 2009) .

Using primers designed on the basis of the sequence coding the titin PEVK region for chicken DNA,Spychaj *et al* (2009) detected 1% chicken meat in the mixture consisting of chicken and beef and chicken and pork.

Moreover ,targeting *12SrRNA* gene, the results of Rodriguez *et al* (2004) revealed possibilities of detecting pork meat at the level of 0.5% in pork –beef mixtures subjected to sterilization (heating at 121C for 20 minutes).

The results of this study confirm that DNA was unaffected by heating up to 120 C for 20 minutes and refer to the importance of increasing sample numbers and sites of tested meat mixtures.

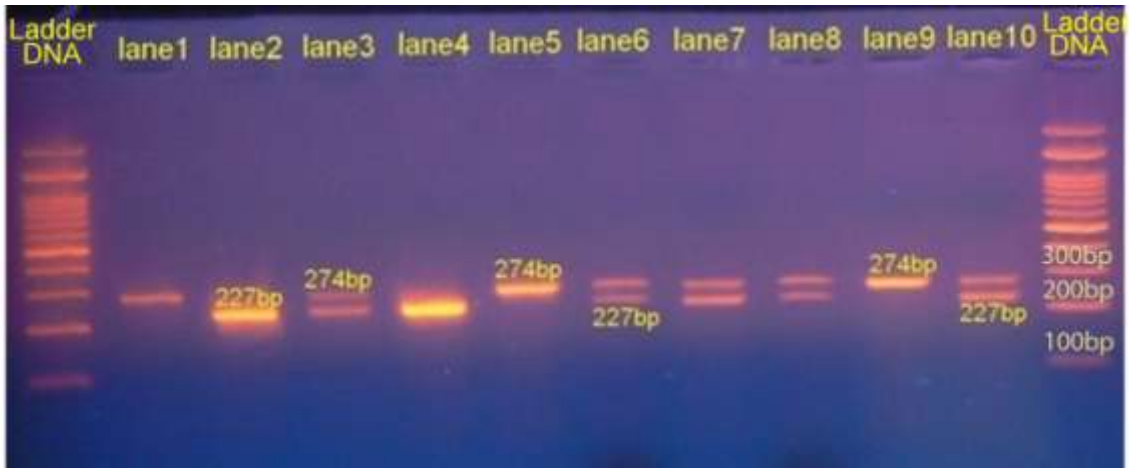


**Figure 3.** Results of PCR products sizes of cattle: chicken mixture (50:50). (1,5,9) beef , (2,4) chicken , (3,6,7,8,10) beef plus chicken.M100 :100 bp ladder DNA.

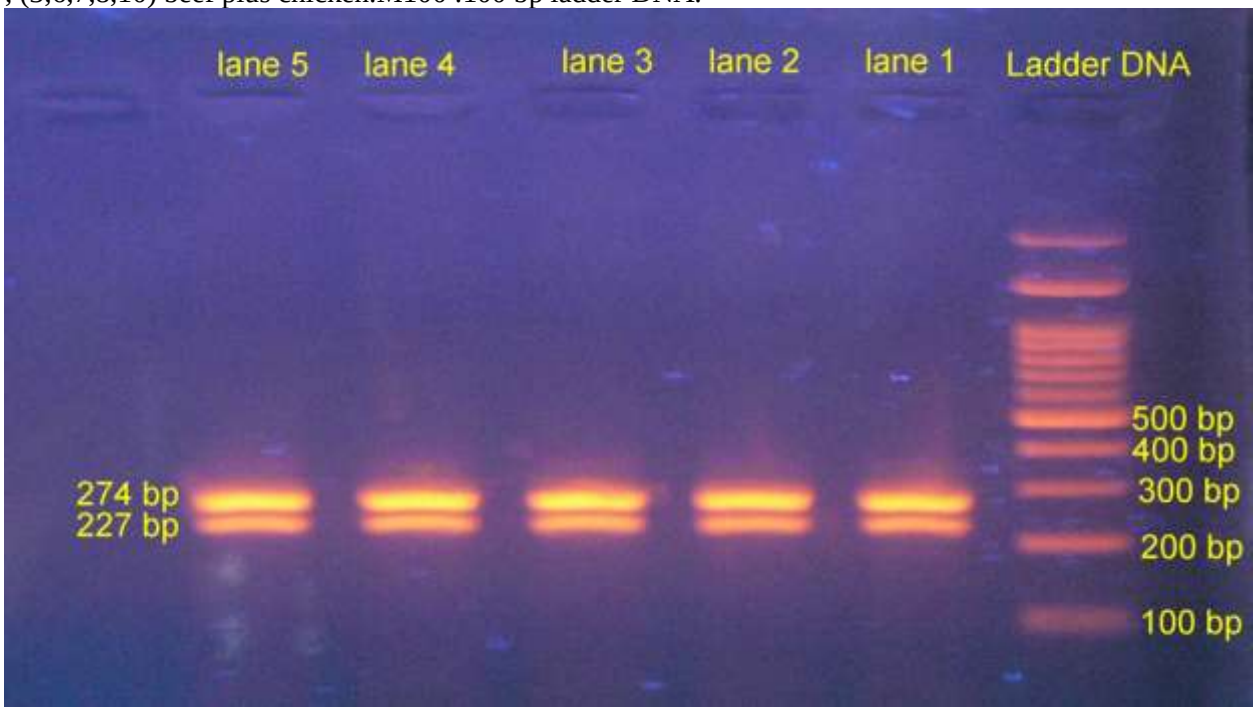


**Figure 4.** Results of PCR products sizes of DNA template mixtures (cattle: chicken) (1) 50:50 ng ; (2) 60:40 ng ; (3) 70:30 ng ; (4) 80:20 ng ; (5) 90:10 ng .M100 :100 bp ladder DNA.

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## الكشف المعتمد على تفاعل البلمرة التسلسلي PCR للحوم الدجاج والابقار باستهداف جين السايتوكروم–ب تحت ظروف الحرارة وخلط اللحوم

**الملخص**

اجريت هذه الدراسة لاختبار تأثيرات الحرارة وخلط لحوم الدجاج والابقار في تشخيص هذه اللحوم باستخدام تفاعل البلمرة التسلسلي PCR باستهداف جين السايتوكروم – ب باستخدام بادئات متخصصة بالنوع .وبالاضافة لاستخدام PCR الفردي استخدم تفاعل البلمرة التسلسلي المتعدد multiplex PCR لغرض كشف انواع اللحوم في مخاليط اللحم .

تم تعريض 5 عينات من لحم الابقار و5 عينات من لحم الدجاج لدرجات حرارة 80 و 90 و100 و 110 و 120 درجة مئوية لمدة 20 دقيقة . اظهرت النتائج بأن جميع مجاميع المعاملة بالحرارة للحوم الدجاج والابقار كانت متماثلة .

بعد خلط كميات متساوية من لحوم الدجاج والابقار ، تم انتخاب 10 عينات من مواقع مختلفة في الخليط . واظهرت النتائج ان 50 % من العينات اظهرت كل من الدجاج والابقار في حين 30 % من العينات اظهرت ابقار فقط و 20 % من العينات اظهرت دجاج فقط .

اكدت نتائج هذه الدراسة على ان الحامض النووي منقوص الاوكسجين DNA لم يتأثر بالحرارة لغاية 120 درجة مئوية لمدة 20 دقيقة كما اشارت النتائج الى اهمية زيادة عدد ومواقع العينات لمخاليط اللحم المفحوصة .