

**USE OF MOLECULAR GENETICS TECHNIQUE  
FOR DIFFERENTIATION OF MEAT  
SPECIES AND DETECTION OF  
MEAT ADULTERATION**

**BY**

**MOHAMMED ALI MOHAMMED ABDELFAH**

B.Sc. Agric. Sc. (Animal Production), Ain Shams University, 2003

M. Sc. Agric. Sc. (Animal Breeding), Ain Shams University, 2010

**A thesis submitted in partial fulfillment**

**of**

**the requirements for the degree of**

**DOCTOR OF PHILOSOPHY**

**in**

**Agriculture Sciences  
(Animal Breeding)**

**Department of Animal Production  
Faculty of Agriculture  
Ain Shams University**

**2016**

**Approval Sheet**

**USE OF MOLECULAR GENETICS TECHNIQUE  
FOR DIFFERENTIATION OF MEAT  
SPECIES AND DETECTION OF  
MEAT ADULTERATION**

BY

**MOHAMMED ALI MOHAMMED ABDELFAH**

B.Sc. Agric. Sc. (Animal Production), Ain Shams University, 2003

M. Sc. Agric. Sc. (Animal Breeding), Ain Shams University, 2010

**This thesis for Ph. D. degree has been approved by:**

**Dr. Samy Abou-bakr Mahmoud** .....

Prof. of Animal Breeding, Faculty of Agriculture, Cairo University

**Dr. Ahmed Ragheb Shemeis** .....

Prof. of Animal Breeding, Faculty of Agriculture, Ain Shams  
University

**Dr. Mohammed Abdelsalam Rashed** .....

Prof. Emeritus of Genetics, Faculty of Agriculture, Ain Shams  
University

**Dr. Omar Yousry Abdallah** .....

Prof. Emeritus of Animal Breeding, Faculty of Agriculture, Ain Shams  
University

**Date of Examination: 17 / 7 / 2016**

# **USE OF MOLECULAR GENETICS TECHNIQUE FOR DIFFERENTIATION OF MEAT SPECIES AND DETECTION OF MEAT ADULTERATION**

BY

**MOHAMMED ALI MOHAMMED ABDELFATTAH**

B.Sc. Agric. Sc. (Animal Production), Ain Shams University, 2003

M. Sc. Agric. Sc. (Animal Breeding), Ain Shams University, 2010

**Under the supervision of:**

**Dr. Omar Yousry Abdallah**

Prof. Emeritus of Animal Breeding, Department of Animal  
Production, Faculty of Agriculture, Ain Shams University (Principal  
Supervisor)

**Dr. Mohammed Abdelsalam Rashed**

Prof. Emeritus of Genetics, Department of Genetics, Faculty of  
Agriculture, Ain Shams University

## ABSTRACT

**Mohammed Ali Mohammed Abdelfattah. The Use of Molecular Genetics Technique for Differentiation of Meat Species and Detection of Meat Adulteration. Unpublished Doctor of Philosophy Dissertation, University of Ain Shams, Faculty of Agriculture, Department of Animal Production, 2016.**

This work described a simplex and a multiplex polymerase chain reaction (PCR) assays for the accurate identification of two meat kinds forbidden in Islamic foods (pig and donkey meats) and five meat kinds commonly marketed in Egypt (goat, sheep, cattle, camel and buffalo meats). Meat samples from the seven investigated species were used for molecular analysis of each species as per standard method. *Cytochrome-b* gene was amplified by PCR using a common forward oligonucleotide primer. By mixing species specific reverse oligonucleotide primers in the appropriate ratio, DNA-fragments could be identified by only one multiplex PCR. PCR products were resolved by agarose gel electrophoresis and characteristic band pattern was observed for each species. The PCR products showed amplicons of 290, 370, 480, 580, 700, 800, 1000 bp from goat, sheep, pig, cattle, camel, buffalo and donkey meats, respectively. Simplex PCR assay was applied to detect adulteration in luncheon, burger and hotdog manufactured by three reputed companies. Following genomic DNA extraction from raw products which were claimed to be made of beef, PCR was performed and detected buffalo (*Bubalus bubalis*) meat as an adulterant. The oligonucleotide primers amplified mitochondrial DNA sequences and revealed specific 580 and 800 bp for cattle and buffalo, respectively. The sequel of this study suggests that the method of detection used can be applied by quality control laboratories and inspection services to determine adulteration of different kinds of meats and meat products. Linear and nonlinear types of regression were developed to

construct a curve that has the best fit to a series of data points of Y= DNA percentage versus X= PCR amplicon concentration (ng/μl). With coefficients of determination ranging between 0.78 and 0.96, the linear model appeared the most statistically appropriate to be used for estimating DNA percentage for all the seven investigated species, since only for the linear model the regression coefficient was significantly different from zero ( $P < 0.05$ ) for all the investigated species.

**Keywords:** Meat adulteration, Meat origin species, Species identification, *Cyt-b* gene, Simplex and multiplex PCR assays.

## **ACKNOWLEDGEMENT**

I dedicate this work to the spirit of the deceased Dr. Karima A. Shahin, Professor of Animal Breeding, Faculty of Agriculture, Ain Shams University, who inspired me to work on this subject under her supervision.

I would like to express my sincere gratitude to my advisor Dr. Omar Y. Abdallah, Professor of Animal Breeding, Department of Animal Production, Faculty of Agriculture, Ain Shams University for the continuous support of my Ph.D study and related research, for his patience, motivation and immense knowledge. His guidance helped me in all the time of research and writing of the thesis. I could not have imagined having a better advisor and mentor for my Ph.D study. My sincere gratitude also goes to my co-advisor Dr. Mohammed A. Rashed, Professor of Genetics, Department of Genetics, Faculty of Agriculture, Ain Shams University who provided me an opportunity to join his team, and also gave me access to his laboratory and research facilities. Without his precious support it would not be possible to conduct this research.

Dr. Mahmoud Magdy, Lecturer of genetics, Department of Genetics, Faculty of Agriculture, Ain Shams University has been always there to listen and give advice. I am deeply grateful to him for the long discussions that helped me sort out the technical details of my work. My deepest gratitude is to Dr. Gouda F. Gouda, Lecturer of Animal Breeding, Department of Animal Production, Faculty of Agriculture, Ain Shams University. His insightful comments, constructive criticisms and valuable suggestions at different stages of my research were thought-provoking and they helped me focus my ideas. I am also indebted to the members of Department of Animal Production and Department of Genetics, Faculty of Agriculture, Ain Shams University with whom I have interacted during the course of my graduate studies.

My immediate family, to whom this dissertation is dedicated to, has been a constant source of love, support and strength all these years.

# **CONTENTS**

	<b>Page</b>
LIST OF TABLES	iv
LIST OF FIGURES	vi
ABBREVIATIONS, INITIALISMS AND ACRONYMS	ix
INTRODUCTION	1
REVIEW OF LITERATURE	4
2-1 Overview of non DNA-based techniques	4
2-1-1 Physical and anatomical techniques	4
2-1-2 Histological technique	5
2-1-3 Chemical technique	5
2-1-4 Biological techniques	6
2-1-4-1 Precipitation Test (PT)	6
2-1-4-2 Complement Fixation Test (CFT)	7
2-1-4-3 Enzyme-Linked Immunosorbent Assay (ELISA)	7
2-1-5 Protein electrophoresis technique	8
2-2 Review of DNA-based techniques	8
2-2-1 Random Amplified Polymorphic DNA (RAPD-PCR)	9
2-2-2 Restriction Fragment Length Polymorphism (RFLP)	11
2-2-3 Species-specific PCR	18
2-2-4 Real time-PCR	24
MATERIAL AND METHODS	28
3-1 Raw meat sample collection and storage	28
3-2 Raw meat sample preparation	28
3-3 Retrieving the whole genome sequences of the target species	28
3-4 Oligonucleotide primers designing	34
3-5 DNA extraction and evaluation of its adequacy for PCR amplification	34
3-6 Ingredients and performing steps for conventional PCR amplification assays	34
3-7 Checking the purity of the conventional PCR reaction mixture from contaminating DNA	34

	<b>Page</b>
3-8 Checking the specificity of the oligonucleotide primers using the simplex PCR assay with raw meats	39
3-9 Checking the specificity of the oligonucleotide primers using the multiplex PCR with raw meats	39
3-10 The relationship between DNA percentage and PCR products concentration using simplex PCR assay	39
3-11 Checking the declared species origin in commercially labelled manufactured meats using simplex PCR assay	42
3-12 Resolving amplimers resulting from conventional PCR amplification assays	42
<b>RESULTS</b>	<b>43</b>
4-1 Adequacy of extracted DNA for species-specific conventional PCR amplification	43
4-2 Checking the purity of conventional PCR reactions contaminating DNAs	43
4-3 Simple PCR specificity of DNA isolated from raw meats	43
4-4 Multiplex PCR specificity of DNA isolated from raw meats	43
4-5 Checking the relationship between DNA percentage and PCR products concentration with simplex PCR assay	56
4-6 Identification of undeclared species in commercially labelled manufactured meat using simplex PCR assay	56
<b>DISCUSSION</b>	<b>73</b>
Preference for DNA biomarker rather than lipid or protein biomarkers	73
Preference for DNA mitochondrial DNA rather than nuclear DNA	75
Preference for <i>Cytochrome-b</i> gene as agenetic marker rather than other mitochondrial genes	76
Preference for conventional PCR assay	76



	<b>Page</b>
Preference for conventional PCR technique utilizing species-specific oligonucleotide primers rather than utilizing other identifiers	77
Preference for fulfilling strict requirements for species-specific oligonucleotide primer designing	77
Preference for multiplex PCR	79
Intra-animal species variation in amplicon molecular length resulted from M-PCR assay	81
Limitations of DNA testing as it can detect but not quantify the presence of a species in a meat sample	81
Misleading statements and images on meat labels to communicate information about species origin	83
Use of simplex PCR to identify the most used species in commercially labelled meat products	84
SUMMARY	87
CONCLUSION	92
REFERENCES`	93
ARABIC SUMMARY	

## LIST OF TABLES

No.	Page
(1) Species origin of meats-related terms distinctions to draw	2
(2) Number and source of meat samples collected from the seven species investigated in this study	29
(3) Meat samples used in the present study	31
(4) DNA samples used in the sensitivity analysis, and quantitative detection of meat adulteration	32
(5) Accessions used to retrieve <i>mtCyt-b</i> gene sequences for the seven species investigated in this study	33
(6) Sequence, length, melting temperature and GC-content of the oligonucleotide primers designed for the seven species investigated in this study	35
(7) Steps performed for DNA extraction in this study	36
(8) Amount and source of mix components for species-specific conventional PCR assays in this study	37
(9) Steps and parameters for performing species-specific conventional PCR assays in this study	38
(10) Design for performing each set of reverse oligonucleotide primer with a non-target species (not underlined) and a target species (underlined) in a species-specific simplex PCR, and final extension given for each of the five primers used in the study	40
(11) A design for performing a mix of reverse oligonucleotide primers with a mix of DNA target species in a triplex, quadruplex and heptuplex species-specific PCRs	41
(12) Ranges of concentration and purity of DNA of samples from the seven investigated species	44
(13) References depicting animal species with different/same amplicon molecular lengths resulted from PCR assays using mitochondrial genes	80

(14) Linear relationship serving quantitative and sensitivity tests	82
(15) Results of examining luncheon, burger and hotdog products of three local processing companies with regard to species declared on the label vs species identified by species-specific simplex PCR	85

## LIST OF FIGURES

<b>No.</b>	<b>Page</b>
(1) Degrees of zoological relatedness between the seven species investigated in this study	30
(2) Agarose gel electrophoreses of PCR products from meats of the three species of small animal (goat, sheep and pig)	45
(3) Agarose gel electrophoresis of simplex PCR products from meats of the four species of large animals (cattle, camel, buffalo and donkey)	46
(4a) Agarose gel electrophoreses of simplex PCR products from five samples taken for goat meat	47
(4b) Agarose gel electrophoreses of simplex PCR products from five samples taken for sheep meat	48
(4c) Agarose gel electrophoreses of simplex PCR products from five samples taken for pig meat	49
(4d) Agarose gel electrophoreses of simplex PCR products from five samples taken for cattle meat	50
(4e) Agarose gel electrophoreses of simplex PCR products from five samples taken for camel meat	4951
(4f) Agarose gel electrophoreses of simplex PCR products from five samples taken for buffalo meat	52
(4g) Agarose gel electrophoreses of simplex PCR products from five samples taken for donkey meat	53
(5) Agarose gel electrophoresis of simplex PCR products from meats of the seven species	54
(6) Agarose gel electrophoresis of triplex, tetraplex and heptuplex PCRs products from meats of the seven species	55
(7) Estimation of DNA percentage (Y) using PCR amplicon concentration (X) in goat samples using four regression models (linear, logarithmic, quadratic and cubic)	57

<b>No.</b>	<b>Page</b>
(8) Estimation of DNA percentage (Y) using PCR amplicon concentration (X) in sheep samples using four regression models (linear, logarithmic, quadratic and cubic)	58
(9) Estimation of DNA percentage (Y) using PCR amplicon concentration (X) in pig samples using four regression models (linear, logarithmic, quadratic and cubic)	59
(10) Estimation of DNA percentage (Y) using PCR amplicon concentration (X) in cattle samples using four regression models (linear, logarithmic, quadratic and cubic)	60
(11) Estimation of DNA percentage (Y) using PCR amplicon concentration (X) in camel samples using four regression models (linear, logarithmic, quadratic and cubic)	61
(12) Estimation of DNA percentage (Y) using PCR amplicon concentration (X) in buffalo samples using four regression models (linear, logarithmic, quadratic and cubic)	62
(13) Estimation of DNA percentage (Y) using PCR amplicon concentration (X) in donkey samples using four regression models (linear, logarithmic, quadratic and cubic)	63
(14a) Label of luncheon manufactured by company "X", communicating that it had been made of only cattle meat (beef)	64
(14b) Agarose gel electrophoresis of simplex PCR products amplified from luncheon of company "X"	64
(15a) Label of luncheon manufactured by company "Y", claiming that it had been made of only cattle meat (beef)	65
(15b) Agarose gel electrophoresis of simplex PCR products amplified from luncheon of company "Y"	65
(16a) Label of luncheon manufactured by company "Z", claiming that it had been made of only cattle meat (beef)	66
(16b) Agarose gel electrophoresis of simplex PCR products amplified from luncheon of company "Z"	66

<b>No.</b>	<b>Page</b>
(17a) Label of burger manufactured by company "X", claiming that it had been made of only cattle meat (beef)	67
(17b) Agarose gel electrophoresis of simplex PCR products amplified from burger of company "X"	67
(18a) Label of burger manufactured by company "Y", claiming that it had been made of only cattle meat (beef)	68
(18b) Agarose gel electrophoresis of simplex PCR products amplified from burger of company "Y"	68
(19a) Label of burger manufactured by company "Z", communicating that it had been made of only cattle meat (beef)	69
(19b) Agarose gel electrophoresis of simplex PCR products amplified from burger of company "Z"	69
(20a) Label of hotdog manufactured by company "X", claiming that it had been made of only cattle meat (beef)	70
(20b) Agarose gel electrophoresis of simplex PCR products amplified from hotdog of company "X"	70
(21a) Label of hotdog manufactured by company "Y", communicating that it had been made of only cattle meat (beef)	71
(21b) Agarose gel electrophoresis of simplex PCR products amplified from hotdog of company "Y"	71
(22a) Label of hotdog manufactured by company "Z", communicating that it had been made of only cattle meat (beef)	72
(22b) Agarose gel electrophoresis of simplex PCR products amplified from hotdog of company "Z"	72
(23) A step-wise presentation of the discussion chapter topics related to the material, methods and results of the present study and previous research	74

