CHAPTER I
INTRODUCTION

Deoxyribonucleic acid is a long molecule that contains our unique genetic code. Like a recipe book it holds the instructions for making all the proteins. DNA is the hereditary material in humans and almost all other organisms. Nearly every cell in a person body has the same DNA. Most DNA is located in the cell nucleus, but a small amount of DNA can also be found in the mitochondria. Mitochondria are structures within cells that convert the energy from food into a form that cells can use.The information in DNA is stored as a code made up of four chemical bases: adenine (A), guanine (G), cytosine (C), and thymine (T). Human DNA consists of about 3 billion bases, and more than 99 percent of those bases are the same in all people. The order, or sequence, of these bases determines the information available for building and maintaining an organism, similar to the way in which letters of the alphabet appear in a certain order to form words and sentences.[12]

Each base is also attached to a sugar molecule and a phosphate molecule. Together, a base, sugar, and phosphate are called a nucleotide. Nucleotides are arranged in two long strands that form a spiral called a double helix. The structure of the double helix is somewhat like a ladder, with the base pairs forming the ladders rungs and the sugar and phosphate molecules forming the vertical sidepieces of the ladder. with hydrogen bonds to make double-stranded DNA. The complementary nitrogenous bases are divided into two groups, pyrimidines and purines. In DNA, the pyrimidines are thymine and cytosine; the purines are adenine and guanine.Both strands of double-stranded DNA store the same biological information.[17]

This information is replicated as and when the two strands separate. A large part of DNA (more than 98% for humans) is non-coding, meaning that these sections do not serve as patterns for protein sequences. The two strands of DNA run in opposite directions to each other and are thus antiparallel. Attached to each sugar is

one of four types of nucleobases . It is the sequence of these four nucleobases along the backbone that encodes genetic information.[6]

DNA is organized into long structures called chromosomes. These chromosomes are duplicated in the process of DNA replication, providing a complete set of chromosomes for each daughter cell.[10]

Eukaryotic organisms (animals, birds, plants, fungi and protists) store most of their DNA inside the cell nucleus as nuclear DNA, and some in the mitochondria as mitochondrial DNA or in chloroplasts as chloroplast DNA.In contrast, prokaryotes (bacteria and archaea) store their DNA only in the cytoplasm, in circular chromosomes. Within eukaryotic chromosomes, chromatin proteins, such as histones, compact and organize DNA. These compacting structures guide the interactions between DNA and other proteins. Information is replicated as and when the two strands separate.[15]

A large part of DNA (more than 98% for humans) is non-coding, meaning that these sections do not serve as patterns for protein sequences. The two strands of DNA run in opposite directions to each other and are thus antiparallel. Attached to each sugar is one of four types of nucleobase. Both strands of double-stranded DNA store the same biological information. It is the sequence of the four nucleobases along the backbone that encodes genetic information. Under the genetic code, these RNA strands specify the sequence of amino acids within proteins in a process called translation.Within eukaryotic cells, DNA is organized into long structures called chromosomes. Before typical cell division, these chromosomes are duplicated in the process of DNA replication, providing a complete set of chromosomes for each daughter cell. Eukaryotic organisms (animals, plants, fungi and protists) store most of their DNA inside the cell nucleus as nuclear DNA, and some in the mitochondria as mitochondrial DNA or in chloroplasts as chloroplast DNA.[19]

In contrast, prokaryotes (bacteria and archaea) store their DNA only in the cytoplasm, in circular chromosomes. Within eukaryotic chromosomes, chromatin

proteins, such as histones, compact and organize DNA. These compacting structures guide the interactions between DNA and other proteins, helping control which parts of the DNA are translation.[7]

The first in a sequence of pages leading on to how DNA replicates (makes copies of) itself, and then to how information stored in DNA is used to make protein molecules.

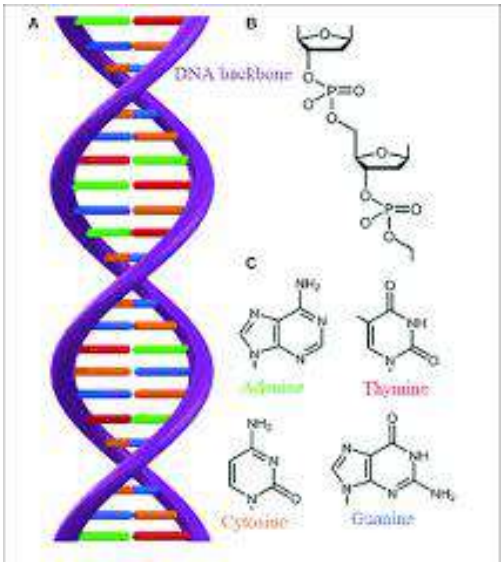

Fig: 1 DNA structure[23]

DNA is very important in forensic science. Have to profiling and also called DNA fingerprinting is the process of determining an individual's DNA characteristics. DNA analysis intended to identify a species, rather than an individual, is called DNA barcoding.DNA profiling is a forensic technique in criminal investigations, comparing criminal suspects' profiles to DNA evidence so as to assess the likelihood of their involvement in the crime. It is also used in parentage testing, to establish immigration eligibility, and in genealogical and medical research. DNA profiling has also been used in the study of bird populations in the fields of zoology, botany, and agriculture. It included Wildlife forensics also. The wildlife forensics relatively new field of

Criminal investigation. Its goals are to use scientific procedures to examine, identify, and compare evidence from crime scenes, and to link this evidence with a suspect and a victim, which is specifically bird. Killing wild bird that are protected from hunting by laws, also called poaching, is one of the most serious crimes investigated by wildlife forensic scientists.[1]

Other crimes against wildlife include buying and selling protected bird and buying and selling products made from protected bird. The international organization that monitors trade in wild animals and plants is the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), which was established in 1963 and, as of 2004, includes 167 member countries. In the United States, the Endangered Species Act, which was authorized in 1973, protects endangered and threatened species and the U.S. Department of Fish and Wildlife has the authority to prosecute violations against protected species. Trent University in Ontario, Canada houses one of the largest wildlife DNA forensics departments in North America, incorporating an extremely active research facility. The types of evidence analyzed by a wildlife forensics lab include any part of an bird including blood and tissue samples, carcasses, hair, teeth, bones, claws, talons, tusks, hides, fur, feathers, or stomach contents. Wildlife forensic scientists may also investigate materials used to kill or harm animals and bird such as poisons, pesticides, projectiles, weapons and medicine. Products that are made from animals are also of interest, including leather goods and medicines, especially those from Asia. One of the most critical problems facing wildlife forensic scientists is identifying a particular species from crime scene evidence. For example, wildlife forensic scientists may have to distinguish if a piece of leather on a watchband is made from a protected bird, like peacock, Siberian crane, Amur falcon, demoiselle crane or blue throat. They must be able to determine if a medicinal powder contains the pulverized remains of a protected bird. They must be able to differentiate between the roe of protected fish from farm-raised caviar. A variety of scientific techniques allow wildlife forensic scientists to answer these types of questions. Techniques similar to those used in a police crime

lab are used to identify and analyze parts of animals as well as bullets, shot casings, paint chips, soil, and fibers found at the crime scene. Experts in fingerprinting, ballistics, soil analysis, and hair comparisons examine evidence visually and with microscopic techniques. Pathologists examine carcasses for wounds in order to determine how the animal died and to distinguish natural death from human killing. Experts in the morphology, or the form, of animals can identify the species, and sometimes subspecies, of bird found at crime scenes. They can often determine the age and sex of bird as well as the time-since-death by careful observations of feathers, skulls and skeletons. Chemists may be asked to identify poisons and pesticides, characterize the contents of Asian medicines, and provide species identification, when possible. Molecular biologists use protein and DNA analyses to provide information about the identity of a sample. Genetics can be particularly useful when the sample is very small or unidentifiable from its morphology. Some answers that genetic tests may provide include identification of species, characterization of the familial relationships between bird, and evaluation of two different samples in order to determine if they originated from the same individual. In addition, geneticists may be able to provide environmental information about bird.[3]

Feathers, the trade mark of Aves, provide the insulation necessary to maintain a high" Engine" (body) temperature, ranging from 107 to113˚F across species. Additionally, the long feathers of the wings acts as air foils which helps to generate the lift necessary for the flight. Well-developed pectoral muscle power the flapping motion of the wings. They are light but very strong, and they are flexible but very tough. Birds take a lot of time caring for their feathers. This is called preening. They use their feet and beaks to arrange their feathers. Avians are warm-blooded. This means that their body temperature remains the same even in differing temperatures. There are 8600 species of birds in the world today. They play a vital role in balancing the nature. They nibble each feather from the base of the tip. Feathers grow only in certain areas in birds called "Feather Tracks". A streamlined body shape and a light

weight skeleton composed of hollow bones minimize air resistance and reduce the amount of energy necessary to become and remain airborne.[5]

All birds have wings, but not all birds fly. Feathers, protect the bird's skin and insulate them. Feathers can be fluffed up in the winter or squeezed down in the summer. Feathers are also used to line nests. Feathers are made of a tough and a flexible material called ‘Keratin’. The spine down the middle, called ‘Shaft’ which is hollow. The vanes are the two halves of the feather.The vanes are made of thousands of branches called ‘Barbs’. Feather grows from a bump in the skin Growth . When a bird hatches, the tip separates and looks like fuzz on the baby bird. This is soft towards the down. The tip forms a tough, protective sheath. As the tip continues to grow, the downy fuzz is pushed ahead of it. Each feather is rolled inside the sheather. It is called a pin feather at this stage. Feathers are used for pillows, quilts and clothing. A Birds survival depends upon the condition of its feather. Birds have between 1,000 - 25,000 feathers. The bird's wing is the basic structure for flight. It is the shape of the wing that allows a bird to fly the use of feathers in veterinary clinical practice simplifies the sampling of avian genomic DNA, especially when blood extraction is difficult because of the age or the size of the bird.[15]

Purification of the genomic DNA is performed with phenol: chloroform: isoamyl alcohol extraction and phenol precipitation. This protocol consistently provided significant amounts of high-quality genomic DNA from more than 800 birds belonging to 120 different species. Genomic DNA isolated with this method was used for southern blotting and also in several polymerase chain reaction systems devoted to sex determination and paternity testing. Previously , chicken sexing has been an integral part in the breeder, broiler and layer industry since 1935. There are several primers used both in the case of cranes and chicken for sex determination. Feathers can be divided into six major categories are Contour Feathers , Semiplume Feathers , Down Feathers , Filoplume feathers, Bristle Feathers , Powder-Down Feathers Not all birds have all the types of feathers. It depends on what type of bird it is. But all birds have feathers on their wings. The shape of the wing is made by the feathers. Feathers are a difficult material to isolate DNA from, since only the feather tip contains DNA.

Attached to the outside are old skin cells. While inside are old blood cells, from when the feather was still growing. [11]

However, feathers often advantages compared to the blood samples because they can be collected much earlier from young chicks and DNA analysis can be performed at a very early stage. Isolation of DNA is done from two different bird feathers such as Emu, Quail

The Emu (Dromaius novaehollandiae) is the largest bird native to Austraslia and the only extant member of the genus Dromaius. It's the Third- largest extant bird in the world by height, after its ratitie relative. , the Ostrich and Quail is a collective name for several genera of mid-sized birds generally placed in the order Galliformes. Old World Quail are placed in the family Phasianidae , and New world Quail are placed in the family Odontophoridae. The species of buttonquail are named for their superficial resemblance to quail, and from the family Turnicidae in the order Charadriiformes. The king Quail, an Old World quail, often is sold in the pet trade, and within this trade is commonly, though mistakenly, referred to as a "button quail". Many of the common larger species are farm-raised for table food or egg consumption, and are hunted on game farms or in the wild, where they may be released to supplement the wild population, or extend into areas outside their natural range. In 2007, 40 million quail were produced in the U.S.[12]

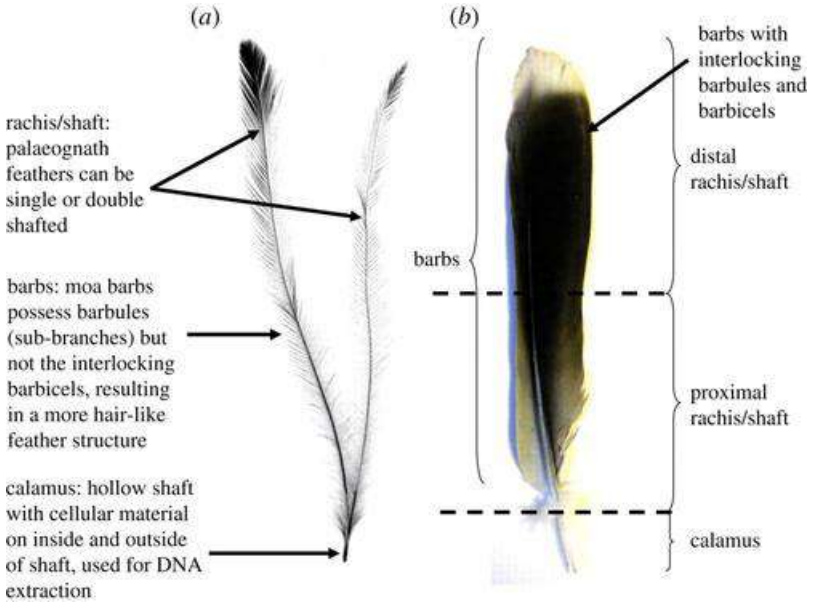

Fig: 2 EMU: (Dromaius Novaehollandiae) Bird Feathers[9]

Fig: 3 QUAIL: (Coturnix Ypsilophora) Bird Feathers[8]

EMU: (Dromaius Novaehollandiae)

Kingdom	Animalia
Phylum	Chordate
Class	Aves
Order	Struthioniforome (casuariforms)
Family	Casuaridae
Genus	Dromaius
Species	Dromaius novaehollandiae
	Dromaius novaehollandiae (Lantham,1790)

QUAIL: (Coturnix Ypsilophora)

Kingdom	Animalia
Phylum	Chordata
Class	Aves
Order	Galliformes
Family	Phasianidae
Genus	Coturnix
Species	C.conturnix

CHAPTER II

LITERATURE REVIEW

V.Costantini[a]et.al DNA sexing in Humboldt Penguins (*Spheniscus humboldti*) from feather samples (2007) - Humboldt Penguins (*Spheniscus humboldti*) show little sexual dimorphism, and although males are usually heavier and larger than females, sexing by direct observation may be difficult, especially in young subjects. In this paper they evaluated the utility of the molecular approach, for sexing impuberal Humboldt Penguins from feathers. Firstly, a PCR test was used employing primers that amplify the homologous region of the CHD-W gene, unique in female, and the CHD-Z gene, occurring in the two sexes. The analysis of the PCR products showed a band of 370 bp in males and two bands of 370 and 380 bp in females. Additionally, to confirm these results, the PCR products were digested with HaeIII and Asp700 for RFLP analysis. Male PCR products showed two bands (310 and 60 bp) after digestion with HaeIII, and a unique band (370 bp) using Asp700, while all fragments obtained from females resolved into three bands using both HaeIII (380, 310 and 60 bp) and Asp700 (370, 270 and 110 bp), confirming the previous PCR sex determination. Results from these two different DNA-based tests were in accordance, in all cases, with sexes checked by preliminary tests. Thus, it was found that the PCR method from feather samples alone is sufficient, reliable and without any risks for a rapid sexing in Humboldt Penguin. This non-invasive sexing technique can be useful at any age to verify the sex ratio in field populations and for gender identification in *ex situ* conservation programs.

Edward I. Patterson - Differential expression of two isolates of beak and feather disease virus capsid protein in *Escherichia coli* (2013) - Expression of recombinant beak and feather disease virus (BFDV) capsid-associated protein (Cap) has relied on inefficient techniques that typically produce low yields or use specialized expression systems, which greatly increase the cost and expertise required for mass production. An *Escherichia coli* system was used to express recombinant BFDV Cap derived from two isolates of

BFDV, from a Long-billed Corella (*Cacatua tenuirostris*) and an Orange-bellied parrot (OBP; *Neophema chrysogaster*). Purification by affinity and size exclusion chromatography was optimized through an iterative process involving screening and modification of buffer constituents and pH. A buffer containing glycerol, β-mercaptoethanol, Triton X-100, and a high concentration of NaCl at pH 8 was used to increase solubility of the protein. The final concentration of the corella-isolated BFDV protein was fifteen- to twenty-fold greater than that produced in previous publications using *E. coli* expression systems. Immunoassays were used to confirm the specific antigenicity of recombinant Cap, verifying its validity for use in continued experimentation as a potential vaccine, a reagent in diagnostic assays, and as a concentrated sample for biological discoveries.

F.iraqid.robinson - A Restriction Enzyme Map of the Sex-Linked Late-Feathering Locus of Chickens (1995) - A 34-kb restriction endonuclease map of the region associated with an endogenous virus integration site and *K,* the gene that confers sex-linked late-feathering (LF) in chickens, is presented. Hybridizations of genomic blots of DNA from early-feathering and LF White Leghorns indicated that the region also contains additional repetitive elements upstream from a chicken repetitive (CR1) element. This extended map and the probes described should be useful in identifying the molecular alterations associated with this locus.

Pedro SilveiraRamos et.al - Polymerase chain reaction-single strand conformation polymorphism applied to sex identification of *Accipiter cooperii* (2009) - Determination of sex in birds is valuable for studying population dynamics and structure, habitat use, behavior and mating systems. The purpose of the present study was to optimize a DNA-based methodology to allow the sex identification in *Accipiter cooperii* nestlings. Chromo-helicase-DNA-binding (*CHD1*) gene was used in this work as a marker for sex identification. CHD-W and CHD-Z sequences should present length and/or sequence differences providing a way to identify gender. We used a non-invasive method for DNA extraction from feathers and performed polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) method. The length difference between CHD-W and CHD-Z amplified fragments observed by electrophoresis in conventional agarose

gel was not enough to provide a clear differentiation between males and females. However, patterns obtained by PCR-SSCP differentiated undoubtedly males and females in *A. cooperii*. This tool provides a precise gender identification assay and will be applied to confirm and refine morphometrically based sexing techniques used in the field.

Li Zhang - MicroRNA profile analysis on duck feather follicle and skin with high-throughput sequencing technology(2013) -Skin acts an important protection role in animal survival and it evolves with the animal divergence. We identified the conserved miRNA families of skin among duck and other species. Cluster analysis showed that the species with similar skin characteristics were clustered into the same group, indicating miRNAs are important in skin function and skin evolution. The miRNA profiles demonstrated that different miRNA regulation mechanism may exist in contour feather follicles (with the surrounding skin) and down feather follicles (with the surrounding skin). Comparing the highly abundant miRNAs with those of mammalian hair follicles and skins, different miRNAs and miRNA families were found, suggesting the different ways in feather follicles and mammalian hair follicles. Bioinformatics prediction indicated that seven miRNAs probably targeted the genes of Wnt/β-catenin, Shh/BMP and Notch pathways which were important in feather morphogenesis. Further analysis should be conducted to experimentally validate the relationships between miRNAs and their predicted target genes because the target genes were based exclusively upon the bioinformatics.

Camilla F.Speller- Identifying the sex of archaeological turkey remains using ancient DNA techniques(2016) - Accurate sex identification of archaeological turkey remains is important for deciphering hunting and husbandry practices in pre-contact North America, particularly in the Southwest United States and Mesoamerica where domestic turkeys were raised. Although the sexual dimorphism of turkeys means that relatively complete elements can be distinguished using osteometric approaches, sexing fragmentary or juvenile remains is challenging. Here, we propose a simple and highly-sensitive co-amplification approach which targets highly-repetitive DNA (hrDNA) sequences on the turkey W chromosome. This technique simultaneously co-amplifies both hrDNA and mitochondrial DNA (mtDNA) fragments: the amplification of the W chromosome identifies the heterogametic sex

(females), while the mtDNA fragment acts as an internal positive control to monitor for false negative results. To demonstrate the sensitivity and accuracy of this technique, we applied it to 20 modern turkeys and 117 archaeological turkey bones from 25 sites (ca. AD700–1700), including 32 samples from Sand Canyon Pueblo (AD1250–1300). We amplified ancient DNA from 86% of the ancient remains, demonstrating the sensitivity of the technique for targeting nuclear DNA. The correspondence between morphological size and the genetic sex identification for 100% of the complete skeletal elements demonstrates the accuracy and robusticity of this approach. Although within the larger regional assemblage, more males than females were identified (61% vs 39%), the site-specific analysis at Sand Canyon Pueblo suggests that adult male and female turkeys were present in a relatively even ratio.

Natalia Bello et.al - Isolation of genomic DNA from feathers(2001) - The use of feathers in veterinary clinical practice simplifies the sampling of avian genomic DNA, especially when blood extraction is difficult because of the age or the size of the bird. A rapid and accurate protocol was used to isolate high-quality genomic DNA from feathers. The technique includes a lysis step of the feather quill, which differs in temperature and time of incubation depending on the feather size. Purification of genomic DNA is performed with phenol : chloroform : isoamyl alcohol extraction and ethanol precipitation. This protocol consistently provided significant amounts of high-quality genomic DNA from more than 800 birds belonging to 120 different species. Genomic DNA isolated with this method was used for Southern blotting and also in several polymerase chain reaction systems devoted to sex determination and paternity testing.

Laboratoire d'Ecologie et.al - A Single Plucked Feather as a Source of DNA for Bird Genetic Studies(1991) -Our studies require only very small amounts of DNA, and we developed a nondestructive sampling technique. The technique requires that a few feathers are plucked, and then preserved in 70% ethanol at room temperature. One advantage of this procedure is the ease with which samples can be collected and sent by mail. Growing feathers plucked from nestlings of P. caeruleus contain microgram amounts of tissue and are easily used for DNA extraction. Freshly plucked nongrowing feathers contain only a few nanograms of pulp cells. Enough DNA can be extracted from mature feathers to

permit multiple amplification via the polymerase chain reaction. We developed a fast and simple procedure of tissue sampling and extraction that will facilitate the study of DNA sequence variation in natural bird populations.

Philip A Morin et.al - DNA extraction, amplification, and direct sequencing from hornbill feathers(1999) – Two methods for extracting DNA from feathers for non invasive multilocus genotyping of are described. DNA was amplified by the polymerase chain reaction and the double stranded products were sequenced directly without the need for cloning. One method uses a single extraction step with Chelex as the extraction medium, thus reducing opportunities for contamination, and eliminating the need to use organic solvents and proteinases. It constitutes a simple, fast and relatively birds inexpensive method of DNA-level genotyping.

CHAPTER III

AIM AND OBJECTIVES

AIM:

To differentiate between the DNA of EMU And QUAIL obtaining from their feathers.

OBJECTIVES:

- To extract the DNA from feathers.
- To determine the species of birds from DNA.

CHAPTER IV
MATERIALS AND METHODOLOGY

Material Required

I. DNase/RNase- free water, Deionised water for dilution of DNA prior to reading.
II. One sterile 1.5 ml microcentrifuge tube for each sample. Plastic (for concentrated samples, > 50µg ml-1) or quartz (for low concentration samples, <50 µg ml-1) cuvettes.
III. Spectrophotometer.
IV. UV light.
V. Microcentrifuge tubes(1.5 milliliters) .
VI. Gloves.
VII. Micropipettes and tips.
VIII. Vortex.
IX. Incubator
X. Eletrophoresis Chamber.
XI. Volumetric flask.
XII. Beaker.
XIII. Distilled Water
XIV. Reagent:

- Sodium Dodecyl Sulphate.
- Ammonium Acetate.
- Phenol:Chloroform:Isoamyl Alcohol.
- Sodium Chloride.
- Ethanol, Iso Propanol.
- Sodium Carbonate.
- EDTA.

- TE buffer.
- Saturated Butanol.
- 1X TAE.
- Ethidium Bromide.
- Sodium Acetate.

Protocol:

The feathers of two different kinds of birds were collected from a Zoo. The feathers are collected in such a manner that the tip is not damaged in either way. Feathers were plucked so that some cells / tissue from the feather follicle remained attached to the tip (Calamus). The tip of the feathers are been diced up and 5 gram of the sample is taken in a mortar and pestle and grind them with 5 microlitre of Extraction buffer.Transfer the mixture to a centrifuge tube (1.5ml) and incubate at -20 degree Celsius for 10-15 minutes.Centrifuge the tube at 6,000 rpm for 5 minutes.Discard the supernatant and to the pellet add 500 microlitres of Suspension buffer and 20 microlitres of 20% Sodium dodecyl sulphate, invert the tube and mix well. Incubate at 62-65 degree Celsius for 20-30 minutes. Then add 200 microlitres of 7.5M ammonium acetate and keep it at -20 degree Celsius for 10 minutes. Centrifuge at 10,000 rpm for 10 minutes. Transfer the supernatant to a new microcentrifuge tube and add equal amount of Phenol: Chloroform : Isoamyl alcohol (25:24:1) to precipitate the nucleic acid. Mix well and centrifuge for 15 minutes at 1000 rpm.Then transfer the aqeuous layer to a new centrifuge tube and add 2M Sodium chloride in equal amount. Then centrifuge at 12,000 rpm for 10 minutes. Wash the pellet with 75% ethanol, air dry and resuspend in 1X TE buffer. To remove RNA 5 microlitres of DNAase free RNase A (10 milligram/ millilitre) was added to DNA.

Dialysis is a process by which small molecules are selectively removed from a sample containing mixture of both small and large molecules. Dialysis is effectively accomplished using a special type of membrane known as semi-permeable membrane.Cut the membrane tube into pieces (3-4cm length wise) Boil for 10 minutes

in 2% Sodium carbonate and 10mM EDTA. Wash both sides with sterile double distilled water. Keep in 40% Ethanol and store it in refridgerator

Electroelution:

Wash the bag in TE buffer thoroughly (do not let it dry). Put the clip on one side of the bag. Put the "Cut gel piece" inside, add some TE buffer, put the clip on the other side of the bag (air bubble should not be trapped). Put the assembly in gel tank, run for one hour at 100V(tank buffer- 1X TAE buffer) Run at 100V, reverse polarity, for 1 minute to release the DNA from the wall of the bag.Open one side of the bag, drain (pipette out) the buffer containing the DNA. Give a water saturated Butanol wash (add equal amount, invert 5-10 times, keep undisturbed for 3 minutes) remove the upper layer containing Ethidium Bromide. Give Chloroform and take upper aqueous layer in new Eppendorf. Precipitate using 1/10th volume of 3M Sodium acetate and Iso propanol. Keep it in -20˚Celsius for O/N precipitation. Centrifuge at 12,000 rpm for 10 minutes at 4˚Celsius. Wash the pellet with 70% Ethanol. Dissolve the dried pellet in 20 microlitre TEbuffer.

DNA quantification:

Preparing DNA samples:

Remove the sample from the freeze and thaw them on ice or in the refrigerator.mix them by tapping the side of the tube with a finger.Do not vortex to mix. In a separate sterile 1.5 milliliters microcentrifuge tube for each sample, mix 10 microliters of DNA with 990 microliters of deionised water. Vortex to mix. Let this solution stand for 10 minutes to ensure the complete diffusion of DNA throughout the solution. This represents a 1:100 dilution of DNA samples.

Agarose Gel Electrophoresis:

Agarose gel electrophoresis is a simple and effective method of separating and purifying nucleic acid fragments.it can be used both as an analytical and

preparative tool. The voltage applied at the ends of an agarose gel generates an electric field with the strength defined by the length of the gel and potential difference (v/cm) at the ends. Nucleic acid molecules migrates toward anode in the electric field due to negatively charged phosphate group along the back bone. due to similar charge to mass ratio of nucleic acid molecules of different lengths, it is the nucleic acid that determines the role of movement. Molecules of linear duplex DNA travel through gel matrices at a rate that is inversely proportional to their molecular weight. The molecular weight of a fragment can thus be determined by comparing its mobility to the mobility of fragments of known size (markers). This is the most valuable feature of agarose gel electrophoresis as it provides a reproducible and accurate means of characteristics of nucleic acid fragments by size. The DNA isolated was run and separated by electrophoresis in 1.4 to 1.8% agarose gel and visualised by staining with Ethidium Bromide and viewed under UV light.

Prepare the gel tray by placing in the casting tray place the comb and level the tray. Boil and prepare 1.4% agarose gel in1X TAE buffer. Cool it to 60˚C and add ethidium bromide of appropriate volume. Pour the gel into the tray and avoid air bubbles. Allow to set for 30-40 minutes. Place the gel in electrophoresis tank, fill it with 1X TAE buffer, remove the comb carefully . Pour the buffer till the gel is fully immersed.Load the samples carefully. Connect the leads and start electrophoresis, run at constant 60V. Stop the run when bromophenol blue dye has travelled less than 2/3rd of the length of gel. View the gel under UV light and photograph the gel.

CHAPTER V
OBSERVATION

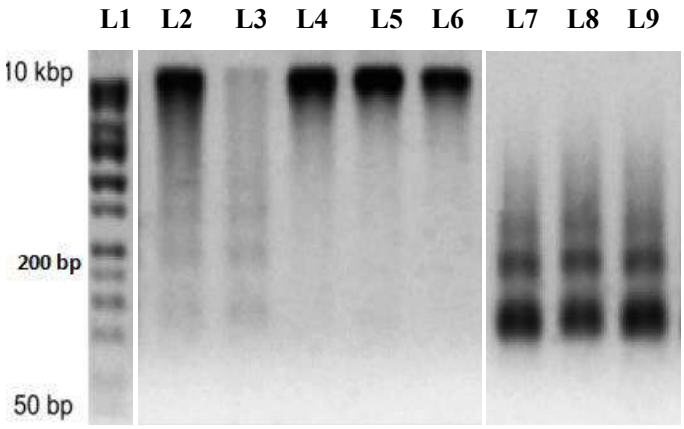

Fig4:Agarose Gel Eetrophoresis

According to observation, in agarose gel electrophoresis, the emu feathers ran upto 10kbp while the quail feathers ran upto 200bp. A molecular weight size marker, also referred to as a protein ladder, is a set of standards that are used to identify the approximate size of a molecule run on a gel during electrophoresis, using the principle that molecular weight is inversely proportional to migration rate through a gel matrix. Therefore, when used in gel electrophoresis, markers effectively provide a logarithmic scale by which to estimate the size of the other fragments (providing the fragment sizes of the marker are known). Protein, DNA, and RNA markers with pre determined fragment sizes and concentrations are commercially available. These can be run in either agarose or polyacrylamide gels. The markers are loaded in lanes adjacent to sample lanes before the commencement of the run.

Sl .No	Lane	Band obtained
1.	Lane 2(L2)	Emu
2.	Lane 3(L3)	Emu
3.	Lane 4(L4)	Emu
4.	Lane 5(L5)	Emu
5.	Lane 6(L6)	Emu
6.	Lane 7(L7)	Quail
7.	Lane 8(L8)	Quail
8.	Lane 9(L9)	Quail

CHAPTER VI
RESULTS AND CONCLUSION

RESULT:

DNA has been isolated with the usual SDS protocol. The DNA isolated was run and separated by electrophoresis in 1.4 to 1.8% agarose gel and visualized by staining with Ethidium Bromide and viewed under UV light.

CONCLUSION:

The DNA can be extracted from feathers and separated through electrophoresis. Feathers are known to contain amplifiable DNA at their base and it provide an important genetic source for various specimens.

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