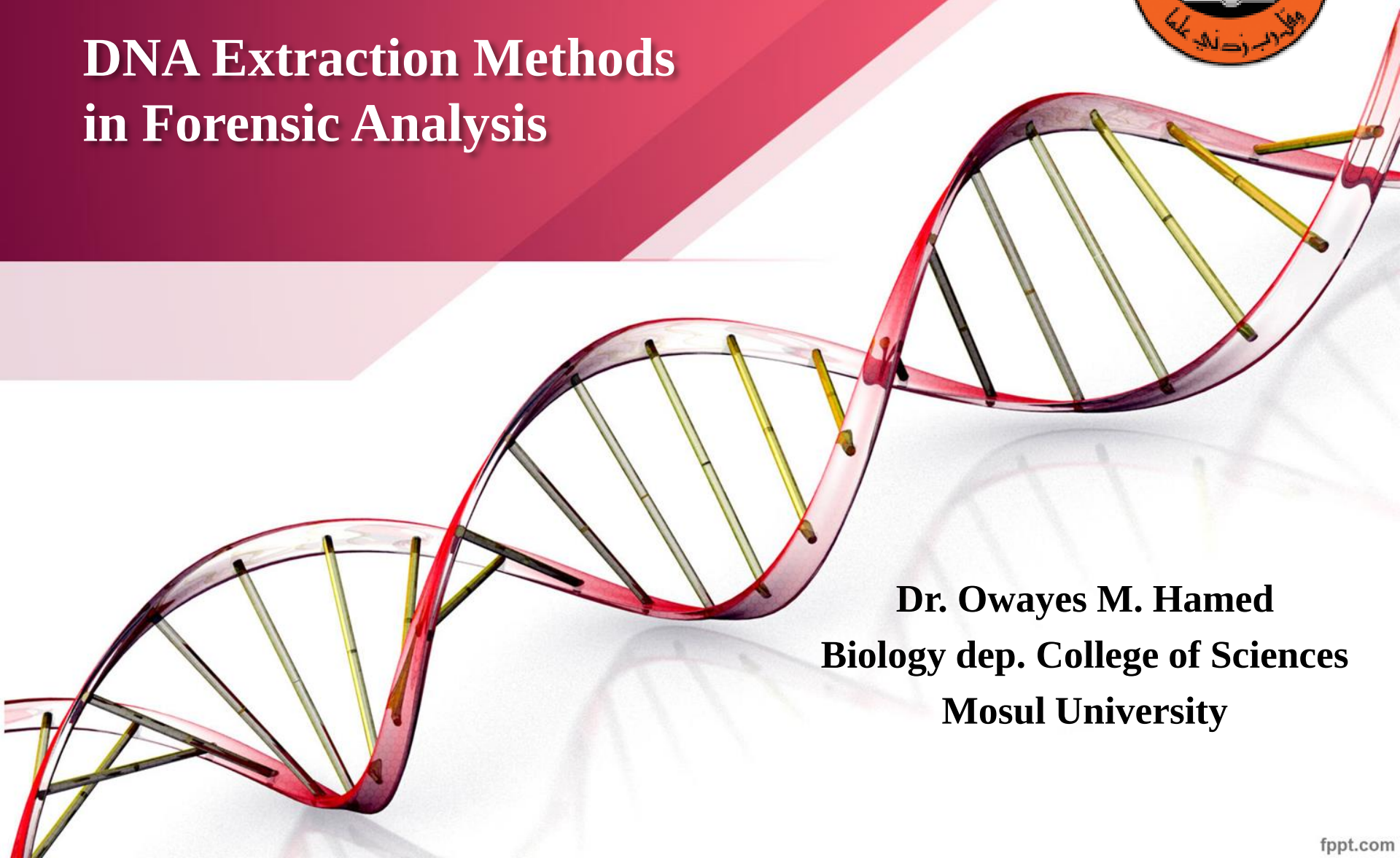
DNA Extraction Methods in Forensic Analysis



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Introduction

- Forensic biological evidence encompasses a diverse conglomerate of samples containing genetic material found in a variety of biological fluids and tissues such as human blood, semen, saliva, epithelial cells, hair, bone, teeth, fingernails, and putrefied tissues. In addition to human samples, nonhuman samples from plant, animal, bacteria, and fungi may also need to be processed. The samples and body fluids may be present as dried stains on an assortment of substrates, often mixed with PCR (polymerase chain reaction) inhibitors, exposed to environmental conditions and uncontrolled degradation, and are often present in limited quantities.
- they can also be grouped by their extrinsic characteristics such as the substrate (e.g. swab, clothing) and nature of the crime (e.g. sexual assault, burglary, homicide) from which they are derived.

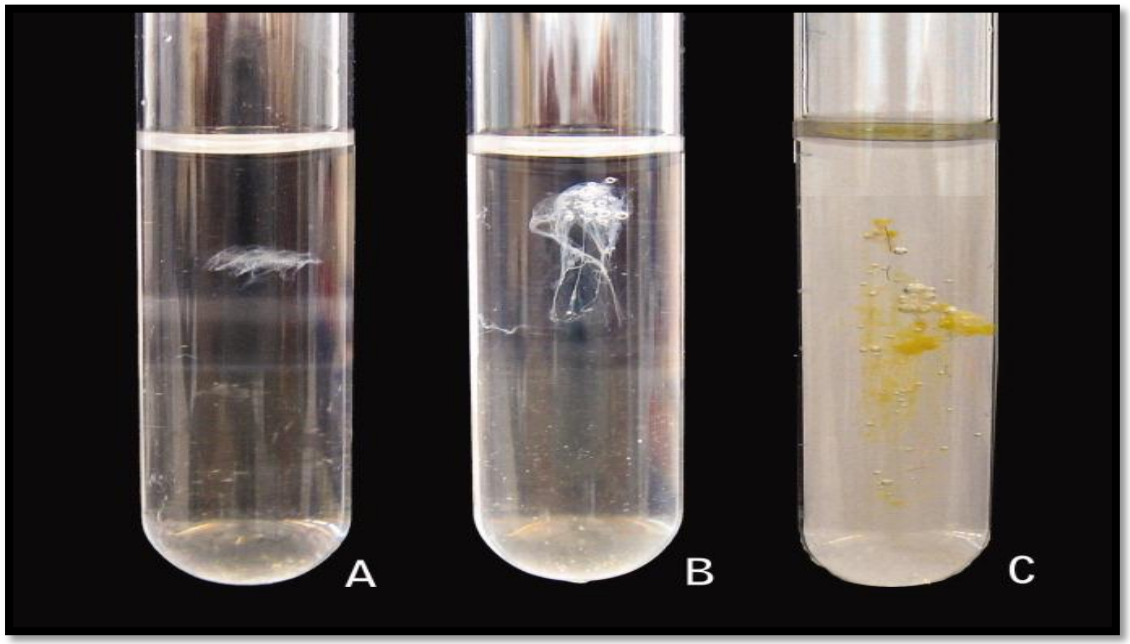
The Aim of DNA extraction

- The ultimate goal of DNA analysis is to obtain interpretable genotype results (profiles). Therefore, the DNA extraction chemistries are evaluated for their abilities to obtain the maximum amount of DNA from samples containing small quantities of biological material, while simultaneously resulting in high-quality DNA free of detectable PCR inhibitors.

Introduction to Forensic Deoxyribonucleic Acid Extraction

- different methods used in forensic laboratories for the extraction of DNA are classified into two groups:

1. a single step wherein the biological sample is lysed to generate PCR compatible lysate
2. two steps wherein samples are lysed and DNA is purified



Extraction of Deoxyribonucleic Acid from Single-source Samples

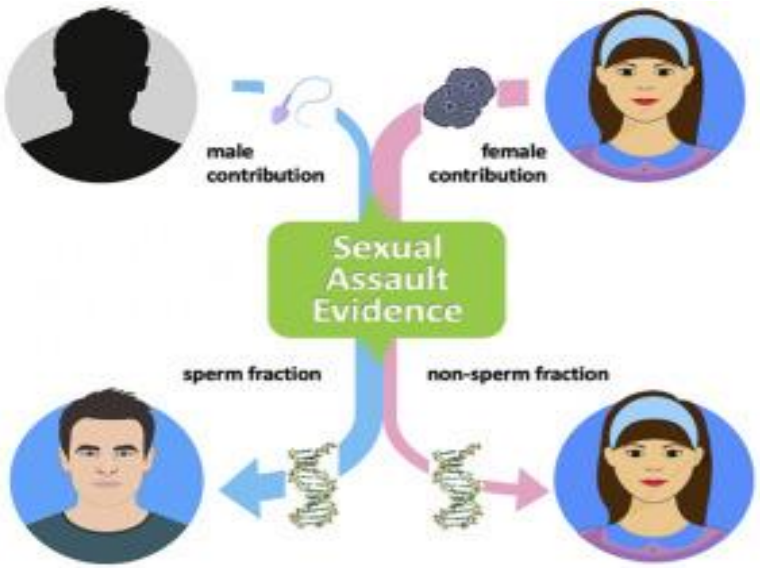
- Examples wherein biological samples are collected from a single donor include paternity testing, DNA database generation, reference samples in casework, and relationship testing
- Single-source samples have certain advantages in that they contain ample quantity of biological material, are collected and stored in relatively controlled environments, are less likely to be degraded, As these samples contain high quantities of DNA and relatively lower amounts of PCR inhibitors

Extraction of Deoxyribonucleic Acid from Evidence Samples

- Extraction of DNA from an evidence sample is the most critical step as a better quality and higher quantity of DNA leads to higher quality interpretable genotype. However, the amount of DNA isolated from a forensic evidence sample cannot be predicted. This is because forensic biological samples submitted for analysis are highly variable and unpredictable. Challenges in processing an evidence sample are due to variation in the source of biological samples (e.g. body fluids, tissue, hair, bone, and tooth), deposition of body fluids on a wide range of substrates, exposure to varying environmental conditions, uncontrolled degradation, contamination with inhibitors of PCR, and that these samples are often present in limited quantities.

Extraction of Deoxyribonucleic Acid from Sexual Assault Cases

- Samples from sexual assault evidence may contain mixtures of male spermatozoa in semen and female vaginal epithelial cells. Typical sexual assault evidence includes a vaginal swab from the victim. Naturally, such a sample comprises many epithelial cells from the victim and few sperms from the perpetrator deposited during a sexual assault. The quantity of epithelial cells from the victim is, therefore, far greater than the number of sperms from the perpetrator, the genotype of which is of primary importance in resolving the case. Thus, it is required that DNA from epithelial cells and sperm are isolated separately for obtaining independent genotypes – each for victim and perpetrator.

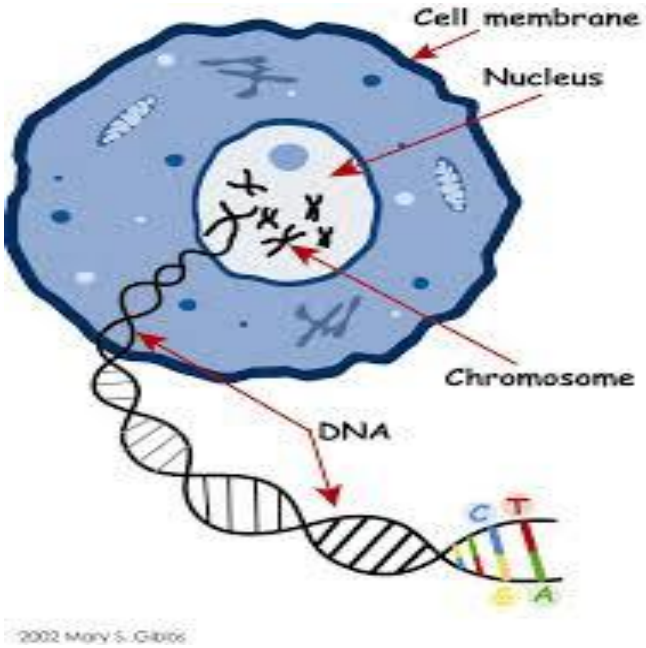


Bone and Teeth Extraction

- Bone and tooth are preferred samples for cases such as ancient, mass disaster, missing persons, and other compromised and severely degraded case scenarios. Skeletal elements often survive when all other tissues have vanished due to catastrophic circumstances or the end result of natural decomposition. Several publications testify to the success of obtaining interpretable genotypes from bone and teeth sample types.
- The success of obtaining genotypes from such ancient and compromised samples is due to their inherent resilience and structural stability and also because the cellular material in bone and teeth is complexed with matrix components such as hydroxyapatite, collagen, osteocalcin, and minerals, resulting in better preservation in adverse conditions than for softer, non-complexed tissues.

Considerations for an Effective Deoxyribonucleic Acid Extraction Method

- achieves extraction of DNA from a variety of biological samples;
- reduces loss of available DNA from a sample resulting in the highest DNA recovery of sufficient quality for downstream analysis;
- enables isolation of DNA from samples that contain small quantities of biological material;
- isolates DNA at a high concentration so that the volume of extract used for genotyping is minimal and provides for future DNA testing;
- removes substances that interfere with PCR;
- does not introduce any contaminants;
- does not introduce inhibitors of PCR;
- maintains sample integrity: does not degrade the DNA;
- is rapid;
- is amenable for automation;
- reduces hazardous waste; and
- can be used to co-extract RNA.



Forensic Deoxyribonucleic Acid Extraction Methods

- There are several different methods of DNA extraction that can be classified into two basic categories:

(i) those that simply release the DNA without purification and

(ii) methods that both release and purify the DNA.

- Each DNA extraction method contains one or more of the following steps:

1. Lyse the cells: Disruption of the cell membranes

2. Isolation of the DNA from cellular materials and other chemicals and debris is usually accomplished by physiochemical methods.

3. Collection and concentration of the DNA.

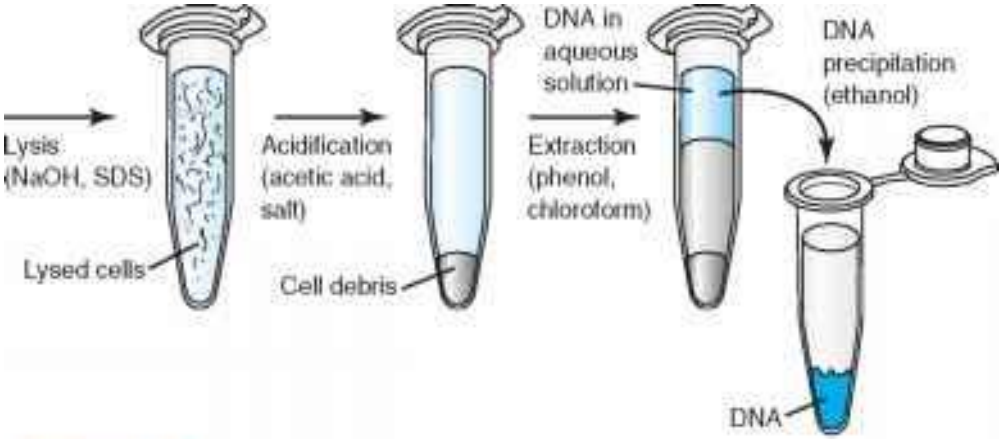


Figure 4-1 General scheme of organic DNA isolation.

Forensic DNA Extraction Kit

- A wide variety of DNA extraction kits are now available from manufacturers worldwide. Some examples are QIAamp Micro DNA kit, QIAamp DNA Investigator Kit, EZ1 DNA Investigator Kit, and QIAsymphony DNA Investigator Kit (Qiagen, Valencia, CA); DNA IQ™ System and DNA IQ Reference Sample Kit for MaxwellR 16, and DNA IQ Casework Sample Kit for Maxwell 16 (Promega Corporation, Madison, WI); PrepFiler™ Forensic DNA Extraction Kit.

Thank you for Listening