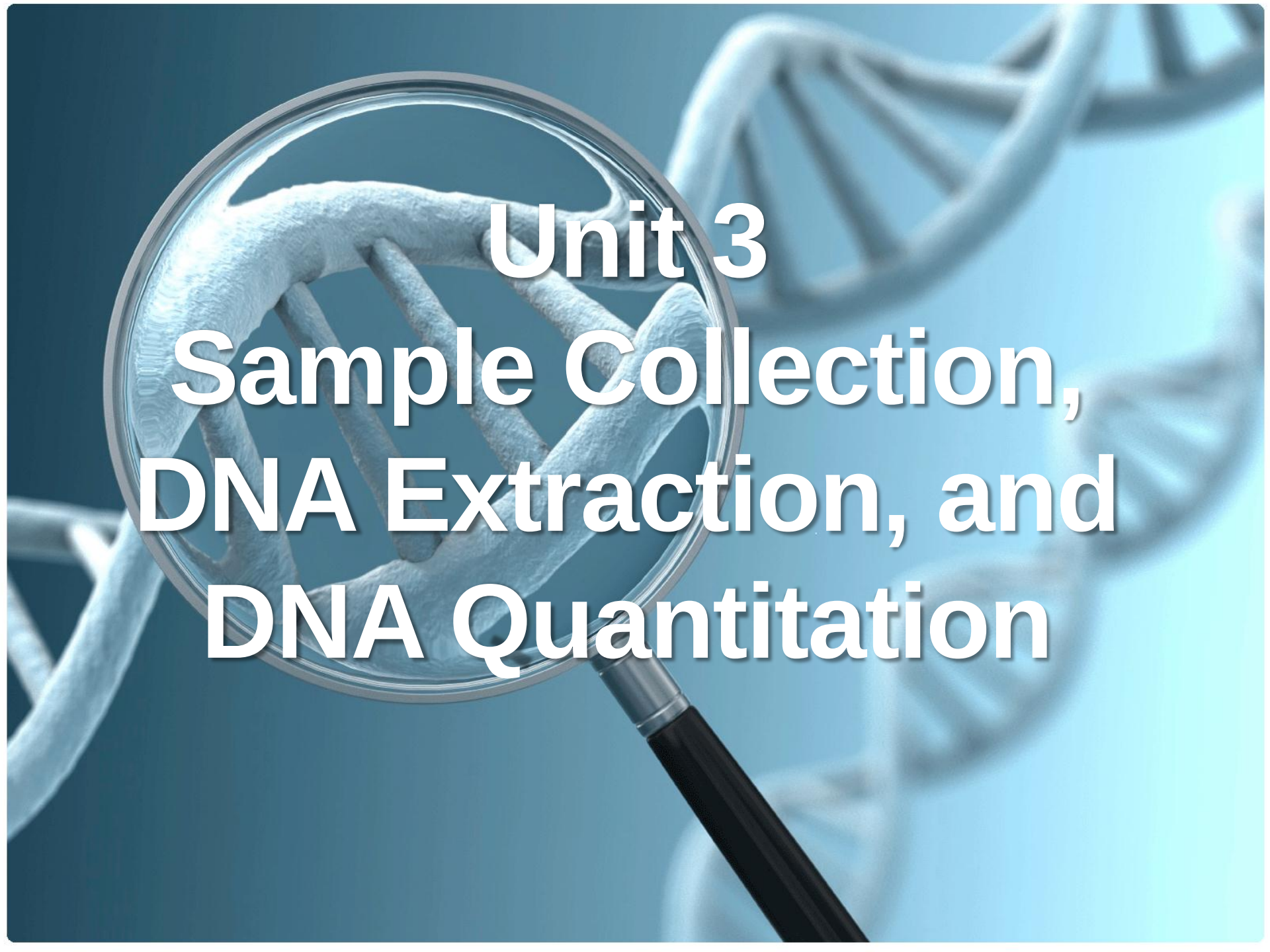
Module 7B

DNA Collection, Extraction, and Analysis

Forensic Science Teacher Professional Development



Unit 3
Sample Collection, DNA Extraction, and DNA Quantitation

Part 1 Sample Collection

- DNA can be found in any nucleated cell, so it is present in any biological material found at a crime scene.
- DNA can be extracted from many different biological materials such as those listed in Figure 12.
- Since the discovery of the polymerase chain reaction, the spectrum of samples for analysis has been further extended.
- The most typical materials analyzed by forensic DNA laboratories are blood and semen.

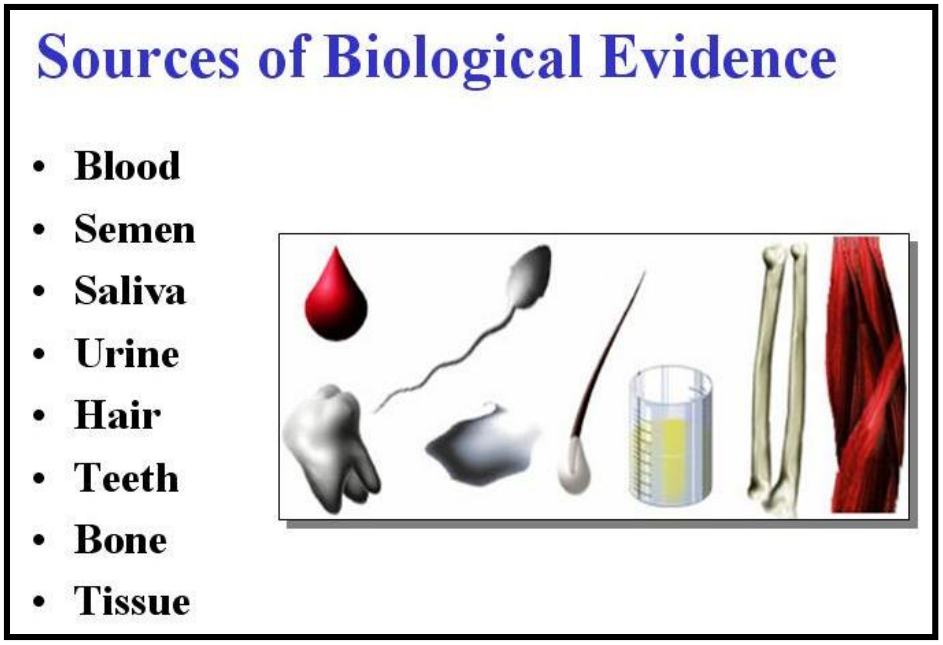


Figure 12
Sources of biological materials for DNA analysis

Part 1 Sample Collection

- Any biological material can be used to include or exclude a suspect of being connected with a crime scene.
- DNA transfer from one subject to another or to an object may link a suspect to a crime scene.
- DNA collection at the crime scene must be performed very carefully.
- A chain of custody must be maintained to guarantee legally accepted DNA results in court.
- Strict protocols need to be followed for the collection, preservation, storage, and transportation of DNA evidence before the evidence is analyzed.

Part 1 Sample Collection

IDENTIFYING DNA EVIDENCE

Evidence	Possible Location of DNA on the Evidence	Source of DNA
Bandanna, hat, mask	Anywhere (inside or outside)	Dandruff, hair, saliva, sweat
Baseball bat or similar weapon	End, handle	Blood, hair, skin, sweat, tissue
Bite mark	Clothing, skin	Saliva
Blanket, pillow, sheet	Surface area	Blood, hair, saliva, semen, sweat, urine
Bottle, can, glass	Mouthpiece, rim, sides	Saliva, sweat
Cotton swab, facial tissue	Surface area	Blood, ear wax, mucus, semen, sweat
Dirty laundry	Anywhere	Blood, semen, sweat
Envelope, stamp	Licked area	Saliva
Eyeglasses	Ear- or nosepiece, lens	Hair, skin, sweat
Fingernail, partial fingernail	Scrapings	Blood, sweat, tissue
Ligature, tape	Inside/outside surface	Blood, skin, sweat
"Through and through" bullet	Outside surface	Blood, tissue
Toothpick	Tips	Saliva
Used cigarette	Cigarette butt	Saliva
Used condom	Inside/outside surface	Rectal or vaginal cells, semen

Figure 13
Examples of DNA evidence

Part 1 Sample Collection

SAFEGUARD DNA EVIDENCE AND YOURSELF

Biological material may contain hazardous pathogens, such as the hepatitis A virus, which can lead to potentially lethal diseases. At the same time, such material can easily become contaminated. To protect both the integrity of the evidence and the health and safety of law enforcement personnel, officers should:

- Wear gloves and change them often.
- Use disposable instruments or clean them thoroughly before and after handling each sample.
- Avoid touching any area where DNA might exist.
- Avoid talking, sneezing, or coughing over evidence.
- Avoid touching one's own nose, mouth, and face when collecting and packaging evidence.
- Air-dry evidence thoroughly before packaging.
- Put evidence into new paper bags or envelopes. Do not place evidence in plastic bags or use staples.

https://www.ncjrs.gov/pdffiles1/jr000249c.pdf

Figure 14 Evidence collection and preservation guidelines

Part 1 Sample Collection

- DNA is a comparative test. After a DNA profile is obtained from evidence samples, reference samples need to be collected for comparison purposes.
- In the early days, blood samples were drawn from suspects or convicted felons, but today a buccal swab, from the inside of the cheek area of the mouth, is the preferred method.
- After collection, the buccal swab sample should be dried before being placed in an envelope for transportation.
- All the wet biological samples collected at the crime scene (saliva, blood, semen) should be dried and packaged in a paper envelope or bag prior to transportation.
- Once the sample is in the laboratory, it can be stored at 4°C, -20°C, or -80°C depending on the storage term.

Part 2 Presumptive Tests for Most Common Biological Fluids (Serology)

- Before DNA extraction, all the materials containing biological samples submitted to the forensic laboratory should be screened for the presence of
  - blood,
  - semen, and
  - saliva.

Part 2 Presumptive Tests for Most Common Biological Fluids (Serology)

Blood

- The target for the analysis of blood is the hemoglobin.
- In the case of the detection of human hemoglobin, various immunological tests are available.
- These tests are usually very sensitive and accurate.

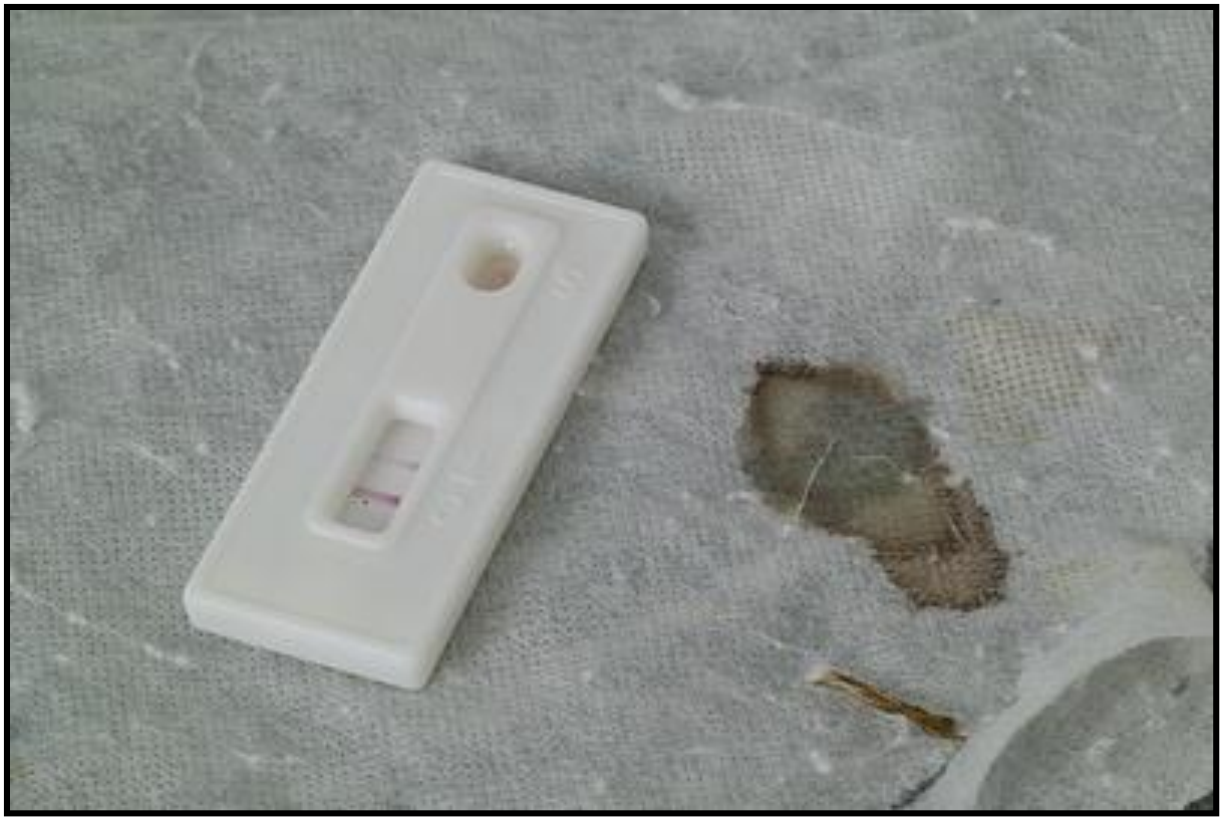


Figure 15
Example of an immunological test for human blood detection

Part 2 Presumptive Tests for Most Common Biological Fluids (Serology)

Blood, continued

- Luminol is another test used to detect blood.
- Using the oxidative properties of the hemoglobin, the reaction generates a blue luminescence that can be seen only in dark conditions.
- This reaction is very sensitive; it can detect blood diluted up to 10 million times.
- It can be applied in cases where the floor has been cleaned after the crime or even in washed clothes.
- The advantage of the use of luminol is that it will not interfere with posterior DNA analysis.

Figure 16
Luminol reaction at a crime scene

For additional information on how luminol works, see the website below:

http://science.howstuffworks.com/luminol1.htm

Part 2 Presumptive Tests for Most Common Biological Fluids (Serology)

Semen

- Semen can be detected through acid phosphatase (AP), prostate specific antigen (PSA or P30), or direct visualization of stained material (“Christmas tree” stain).
- Acid phosphatase is a prostatic enzyme found in semen, and its concentration is up to 400 times higher than in any other fluids.
- A purple color indicates the presence of semen.
- Prostate specific antigen or p30 is a protein that is found in semen at concentrations of 300-4200ng/mL.
- It should be noted that this protein is also found in other human fluids like breast milk, but at much lower concentrations.
- Abacus Diagnostics (West Hills, CA) also provides a p30 immunological test.

Part 2 Presumptive Tests for Most Common Biological Fluids (Serology)

Semen, continued

- Many laboratories screen the presence of semen by staining the semen sample with “Christmas tree” stain and viewing under a microscope. The sperm’s head is stained red and the sperm’s tail green. See Figure 17.

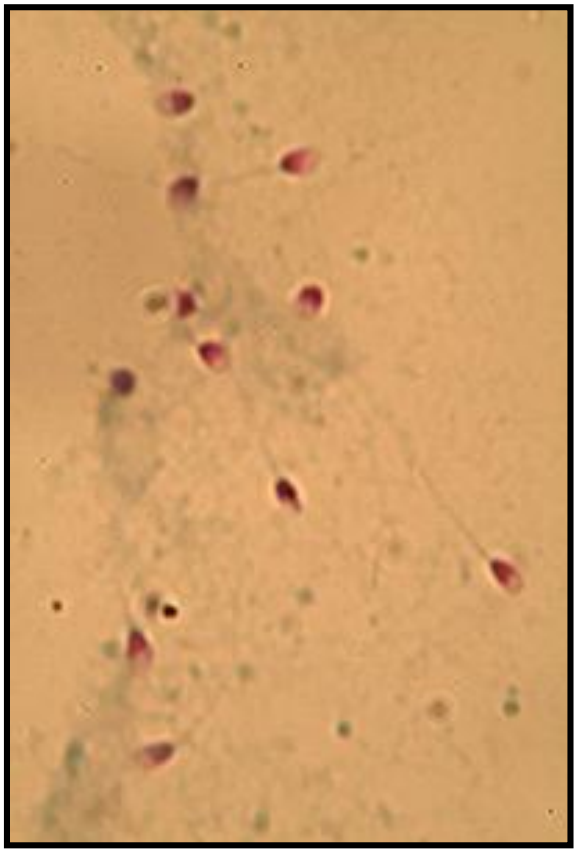


Figure 17
Christmas tree staining for the presence of sperm

Part 2 Presumptive Tests for Most Common Biological Fluids (Serology)

Saliva

- The target enzyme for saliva is amylase, an enzyme present in saliva at high concentrations.
- There are two tests to confirm the presence of saliva in forensic samples: the Phadebas test and the starch iodine radial diffusion test.

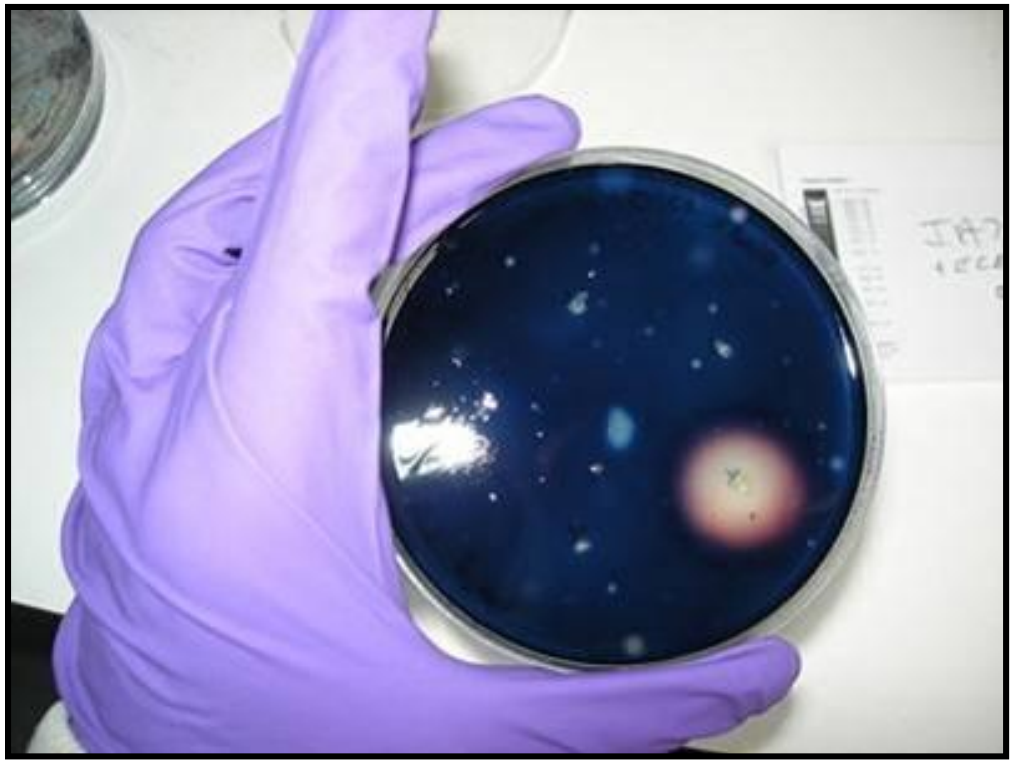


Figure 18
Starch iodine radial diffusion test
The white circle indicates the presence of amylase (saliva).

Part 3 DNA Extraction

- DNA is extracted to separate it from proteins that protect it in the nucleus of the cell and other materials.
- Chemicals are added to digest the protecting molecules and produce “naked” DNA molecules.
- The quality and quantity of DNA need to be measured before further analysis.
- The most common techniques used in forensic laboratories for DNA extraction are
  - organic extraction,
  - chelex extraction,
  - solid-phase extraction, and
  - differential extraction.

Part 3 DNA Extraction

Organic extraction

- This method requires the use of a detergent sodium dodecyl sulfate (SDS) to dissolve the cell walls, a protease to break down proteins, and proteinase K and dithiothreitol (DTT) to break disulfide bonds.
- After a digestion with these chemicals, an organic extraction with phenol, chloroform, and isoamyl alcohol is performed to separate proteins and fat from the DNA.
- The DNA is finally recovered in the aqueous phase. The DNA is then concentrated and desalted by filtration.

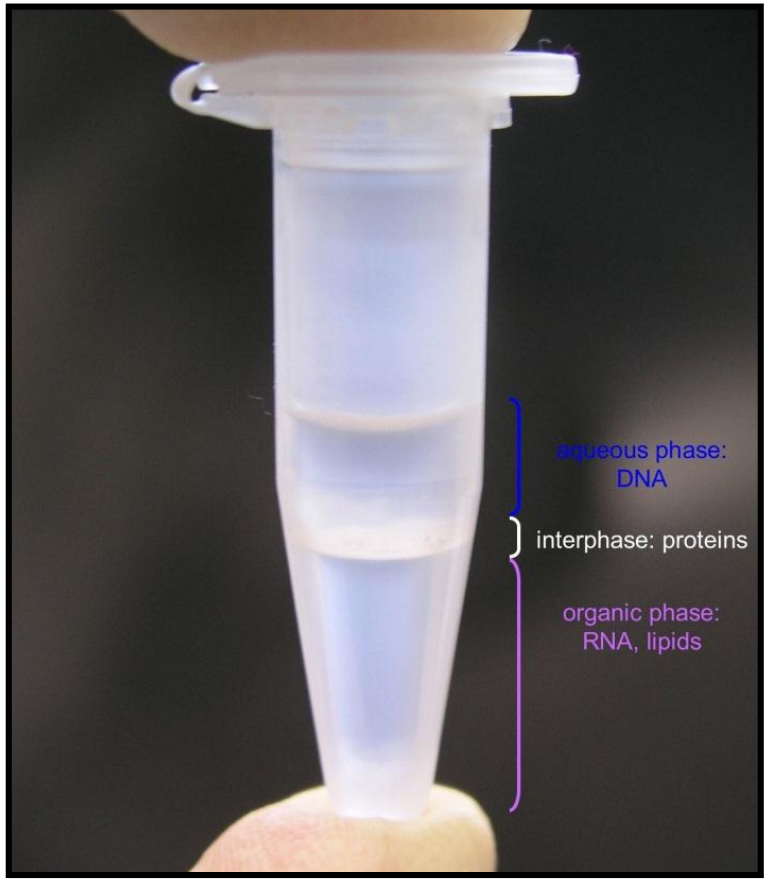


Figure 19
Organic extraction of DNA

Part 3 DNA Extraction

Chelex extraction

- This is faster than organic extraction since it requires fewer steps and is a single-tube extraction reducing potential contamination.
- The extraction process is the most critical step related to contamination.
- This is why evidence and reference samples are processed at different times and even at different locations.
- Once the DNA is extracted it should be stored at -20°C or -80°C depending on the planned time of storage.
- If DNA is extracted from blood, it should be collected in tubes containing EDTA.
- EDTA chelates magnesium and in this way prevents the action of specific enzymes known as nucleases that degrade DNA using magnesium as a co-factor.

Part 3 DNA Extraction

Chelex extraction, continued

- Chelex is an aqueous suspension of an ion-exchange resin composed of chelating groups.
- These groups bind to any polyvalent metal ions like magnesium.
- The biological sample is mixed with the suspension and then boiled for 8 minutes to disrupt the cell membranes, destroy proteins, and release the DNA from the cell.

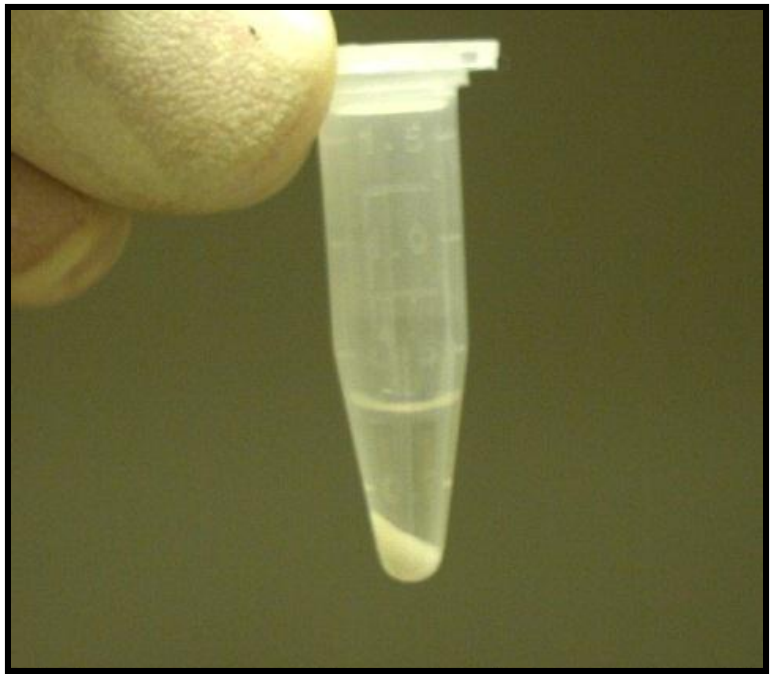


Figure 20
Chelex extraction of DNA

Part 3 DNA Extraction

Solid-phase extraction

- This method uses silica as a matrix and chaotropic salts to selectively absorb the DNA at pH 7.5.
- The chaotropic salts stabilize denatured proteins and DNA allowing the binding of DNA to the silica.
- The DNA is then washed and eluted at basic pH.
- Since this method can be automated, it enables high-throughput DNA extractions.

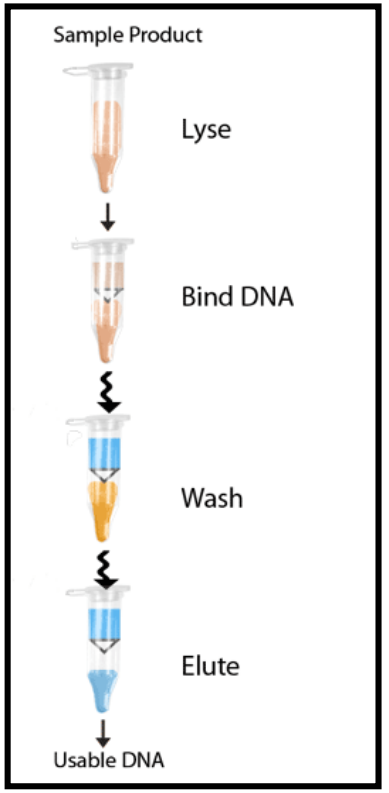


Figure 21
Silica-based extraction method for DNA

Part 3 DNA Extraction

Differential extraction

- This is the method used to separate sperm DNA and vaginal cells DNA in sexual assault samples.
- It is based on the organic extraction method. This method has two steps.
- During the first step the vaginal cells are lysed with proteinase K and SDS.
- The sperm remain intact during this step.
- After washing the non-digested sperm, these cells are then digested with a combination of proteinase K, SDS, and DTT.
- Since DTT is able to break down the sperm nuclear membranes, the DNA is released after this treatment.
- Finally, two fractions are obtained: the female fraction containing the vaginal cells DNA, and the male fraction containing the sperm DNA.

Part 4 DNA quantitation

- The DNA Advisory Board standard 9.3 requires a human-specific DNA quantitation method to ensure that the DNA extracted is from human origin.
- The quality and quantity of DNA should be assessed because most PCR-based assays work in a narrow range of DNA concentration.
- Usually the optimal amount of DNA template for a PCR reaction is between 1 and 2.5 ng.

Part 4 DNA Quantitation

- Excess DNA will result in split peaks or off-scale peaks.
- Too little DNA will result in drop-out alleles.

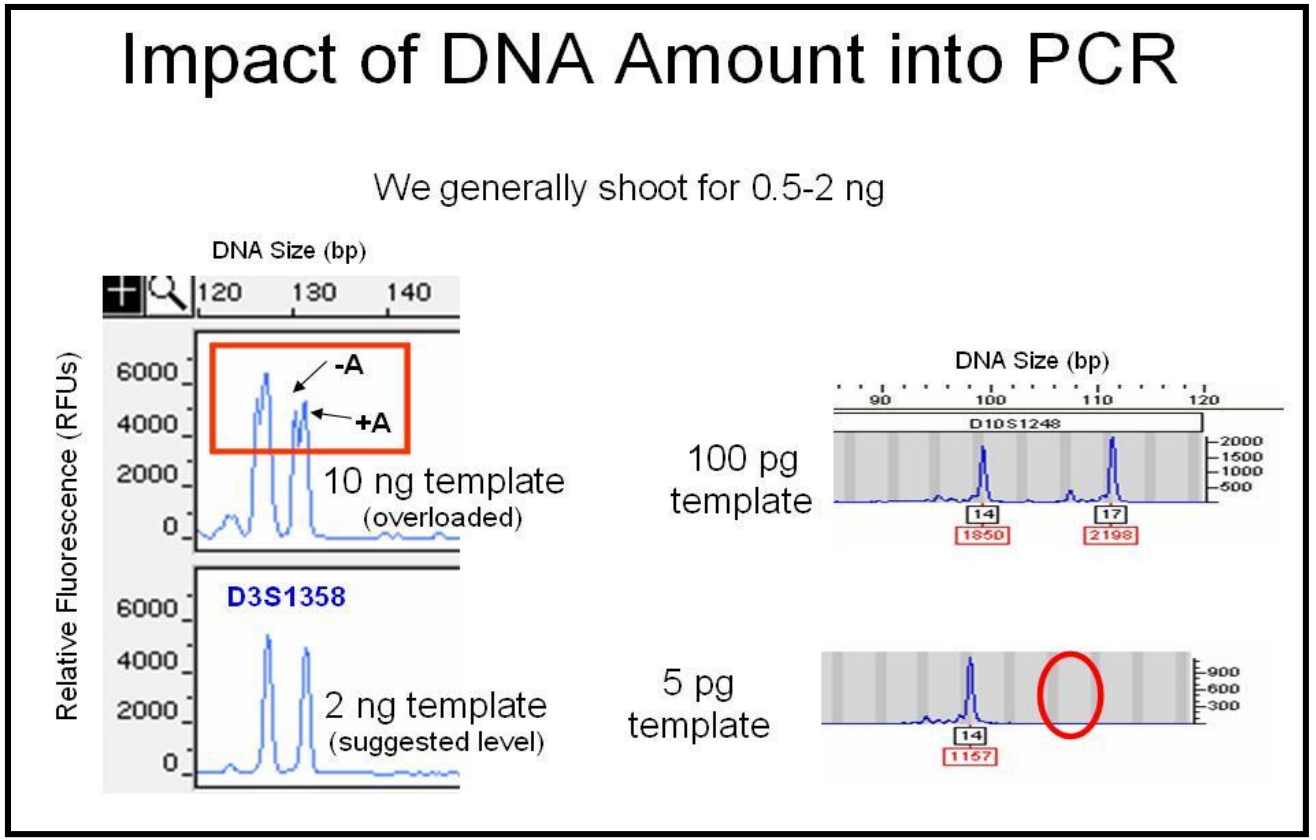


Figure 22
Effect of DNA amount in PCR products
Excess DNA will generate split peaks and low DNA will result in drop-out alleles.

Part 4 DNA Quantitation

- Classical methods to quantify DNA include the measurement of UV absorbance at 260nm and agarose gels stained with ethidium bromide.
- These methods are neither specific for human DNA nor sensitive.

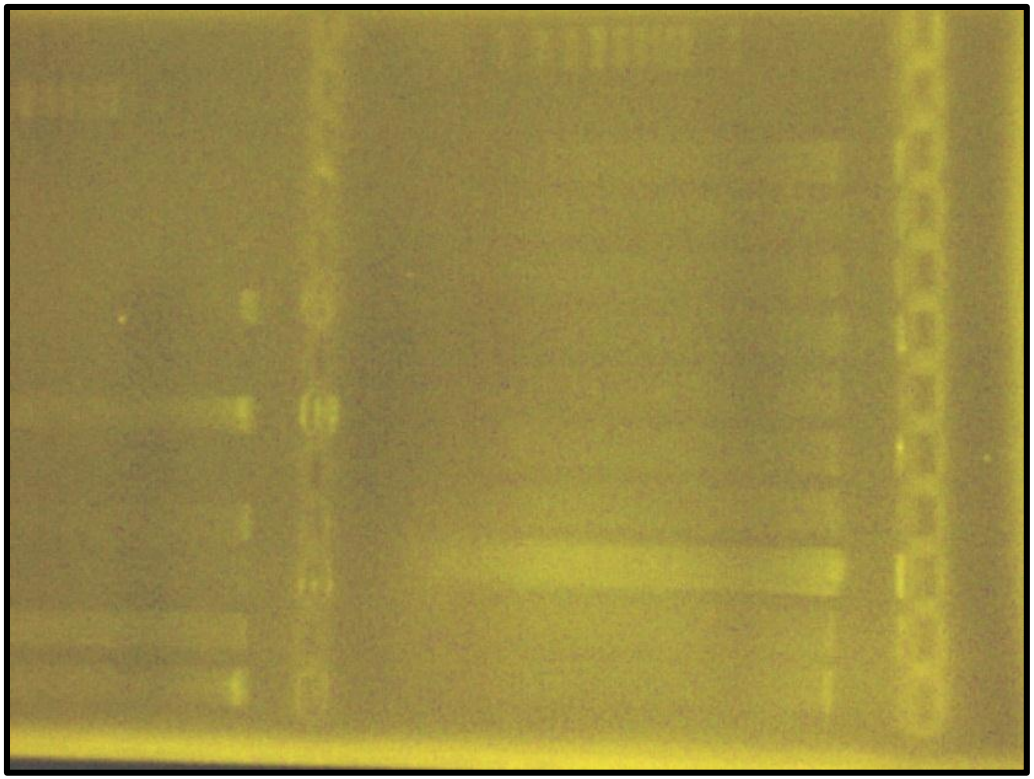


Figure 23
Agarose gel stained with ethidium bromide

Part 4 DNA Quantitation

- Newer methods include slot blot, Picogreen® microtiter assay, AluQuant™ human DNA quantitation system, and real-time PCR or quantitative PCR.
- The slot blot method uses a probe specific for human and primate and colorimetric detection.
- The detection limit is about 200 pg of DNA.
- The disadvantage to slot blot is that the comparison against standards of known concentrations is visual and therefore inaccurate.
- Picogreen® is a fluorescent dye that intercalates to double-stranded DNA, enhancing its fluorescence.

Part 4 DNA Quantitation

- The assay is very sensitive and can be automated for high throughput, but it is not specific to human DNA.
- The AluQuant™ assay uses *Alu* repeat probes that are very abundant in the human genome, providing a great sensitivity (0.1-50 ng of DNA).
- This system uses luciferin that produces light as a result of a series of enzymatic reactions.
- The amount of light emitted is proportional to the original DNA amount.

Part 4 DNA Quantitation

- The golden standard for forensic DNA quantitation of human DNA is the real-time PCR or quantitative PCR.
- The goal of performing DNA quantification is to assess the amount of “amplifiable” DNA.
- The quality and quantity of DNA should be assessed to verify the presence of PCR inhibitors, degraded DNA, or an insufficient amount of DNA.
- All these factors can be detected by real-time PCR assay.
- The most common real-time PCR techniques are SYBR® Green and TaqMan® assays.

End of Module 7B

Forensic Science Teacher Professional Development