Touch DNA and DNA Recovery

H. Miller Coyle

What is the link between cell biology & forensic science?

- Cells are the trace substances left behind that can identify an individual.
- Cells contain DNA.
- There are two forms of DNA – nuclear and mitochondrial.
- Most forensic science laboratories are accredited or certified to perform nuclear DNA tests – "fragment length analysis".
- A fewer number of forensic laboratories are accredited for the more specialized and time consuming mitochondrial DNA tests – "sequencing".

Question #1: How many cells are there in a crime scene sample?

This is a comparative exercise that can be performed with basic microscopy skills and just a few chemicals to visualize what is being collected at a crime scene or from reference samples for DNA.

A basic human cell

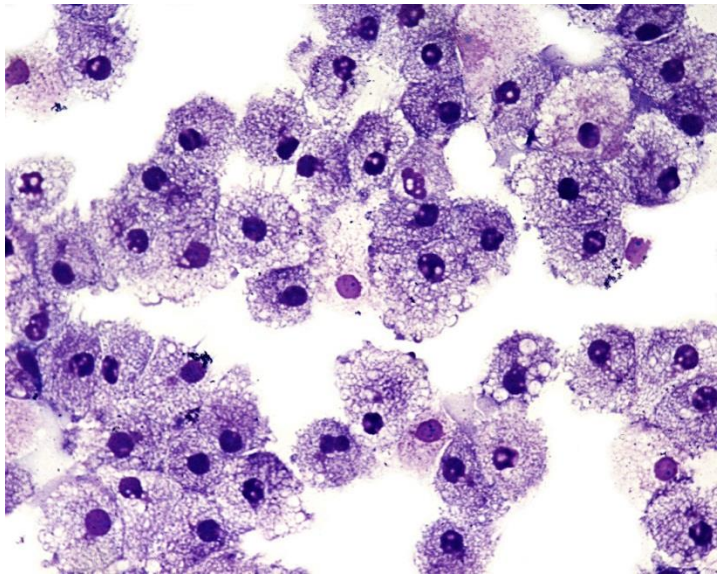
- A membrane-bound compartment of proteins, lipids, carbohydrates, DNA, RNA, and water.
- Carefully balanced to create an individually functioning unit that can also integrate and function as tissues and organs.
- Tissues and organs make up the organism.

Forensic sampling

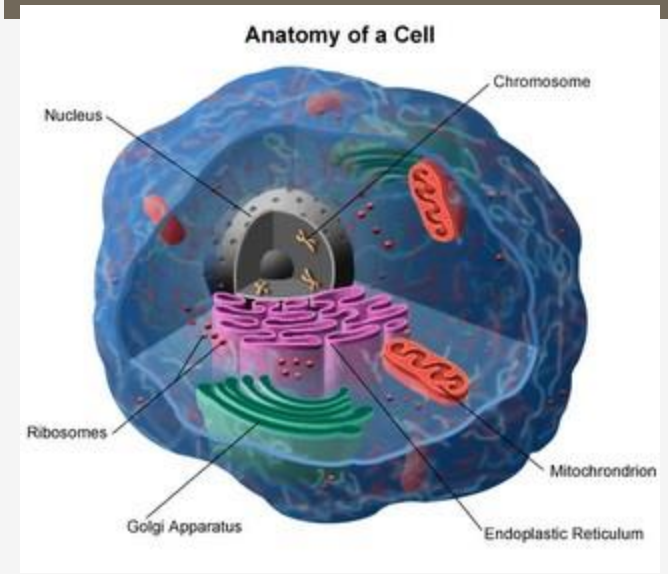
- DNA is the same in most all cells and tissues collected from the same individual.
- Rare tissue specific mutations can occur but these are rare events.
- Trace biological evidence can be cells from touching objects, shed or forcibly removed hairs, body fluids and all will have the same DNA profile.
- The difficulty with conclusively identifying an individual by DNA occurs when partial results or complex DNA mixtures are obtained.

What factors affect DNA results?

- At a crime scene, there are environmental factors beyond the control of the laboratory.
- These factors include: bacteria, sunlight, humidity, temperature, drying time, pressure applied during deposit, DNase activity etc. all of which will ultimately determine the quantity and quality of DNA recovered.
- These factors result in either no profiles, full DNA profiles or partial DNA profiles being obtained.



Example – buccal reference samples (>50 cells); these are the types of samples collected for crime scene comparison and DNA databases.

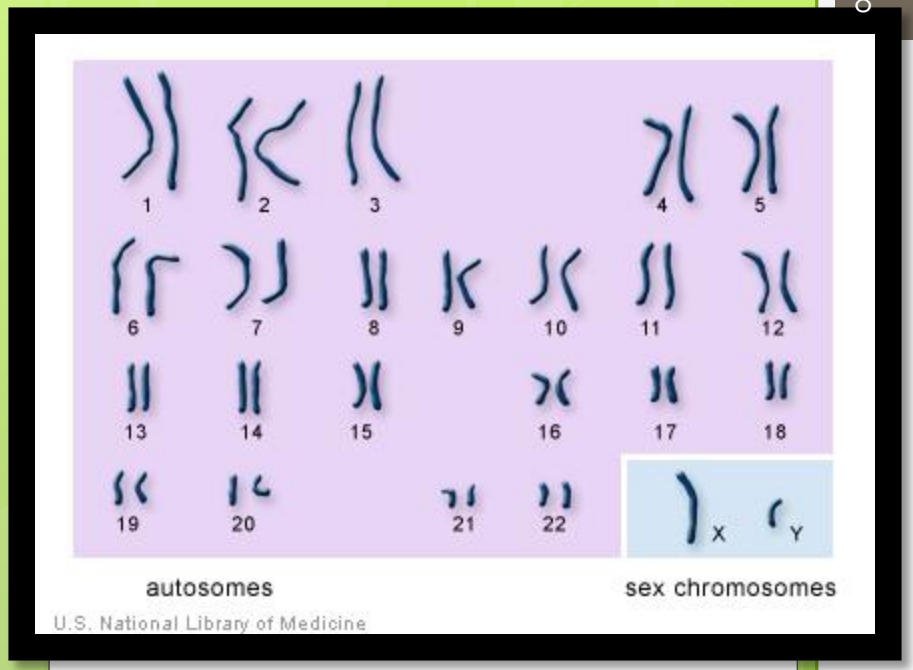


Human Cell

Cells stained on a glass slide

- Membrane
- Cytoplasm
- Nucleus
- Mitochondria (not visible)

H. Miller Coyle

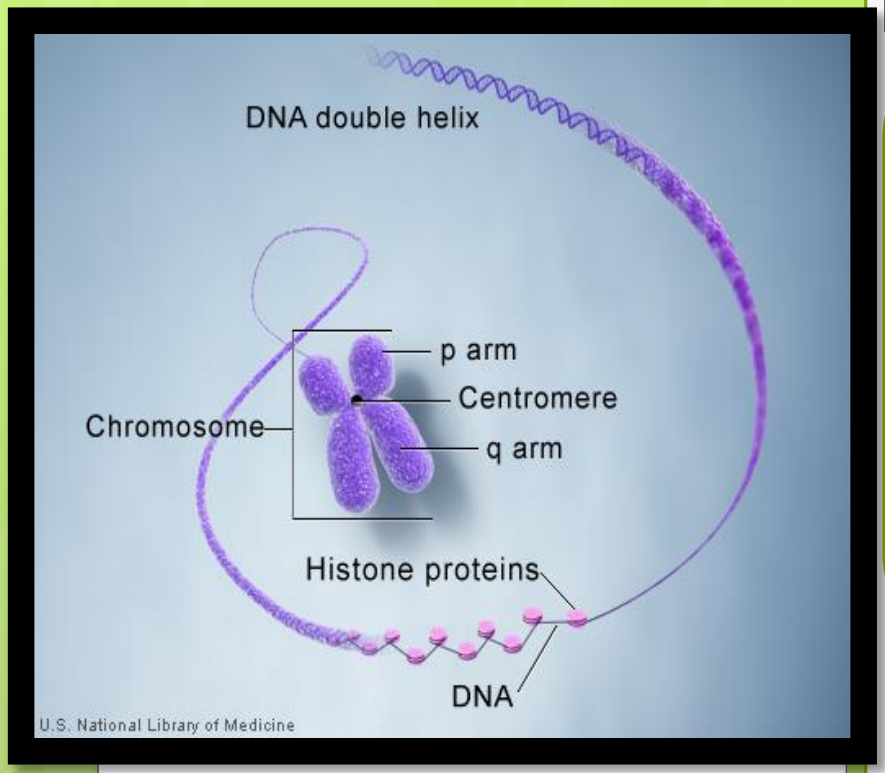


Karyotype – digitized image of the chromosomes from a single cell; each fragment of DNA is on a separate chromosome.

Human DNA

Packaged into 23 pairs of chromosomes inside the nucleus. In DNA extraction methods, both the cell and nuclear membrane need to be ruptured to release the DNA for purification.

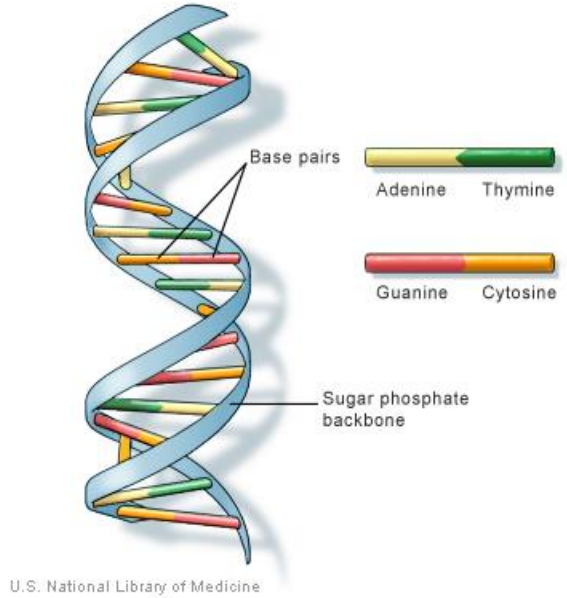
H. Miller Coyle

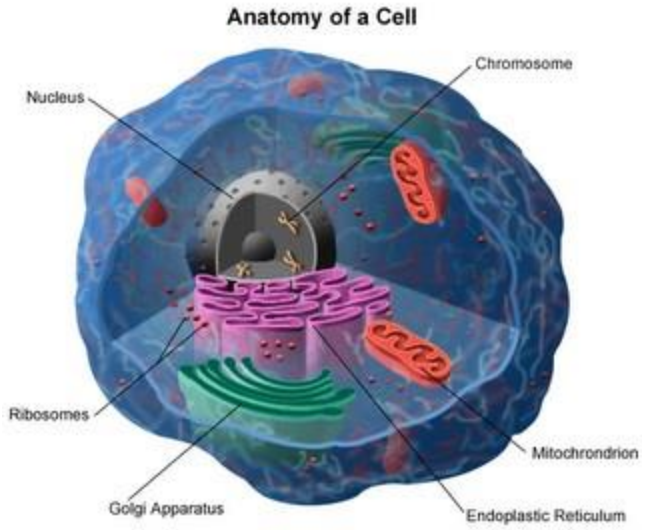


Chromosomes are not visible in the cell's nucleus—not even under a microscope—when the cell is not dividing. However, the DNA that makes up chromosomes becomes more tightly packed during cell division and is then visible under a microscope.

Human DNA

Wrapped around histone proteins and folded into these structures.





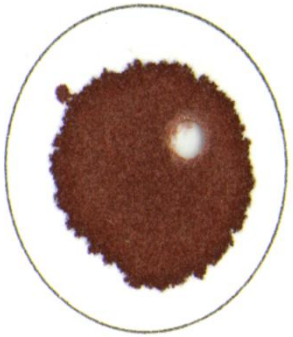
Nuclear DNA

Targeting different points of DNA (deoxyribonucleic acid)on those 23 pairs of chromosomes.

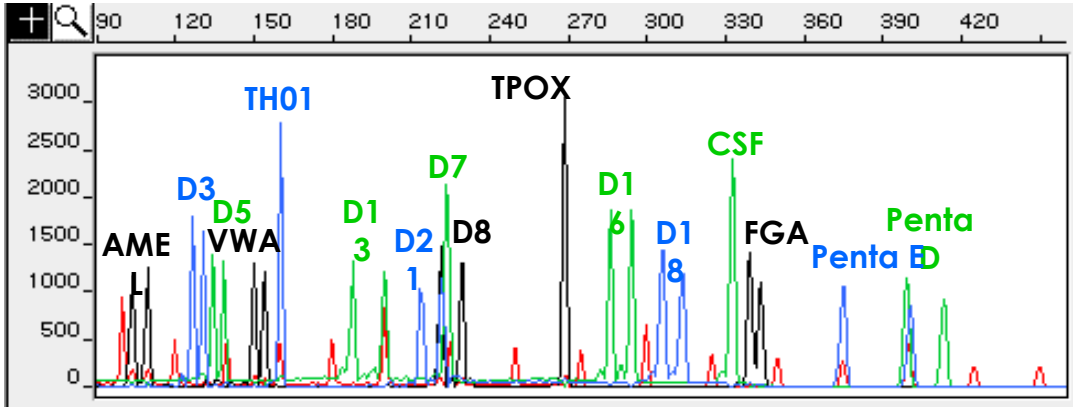
Sugar-phosphate backbone (blue ribbons) linked by nucleotide bases (A-T; G-C)

H. Miller Coyle

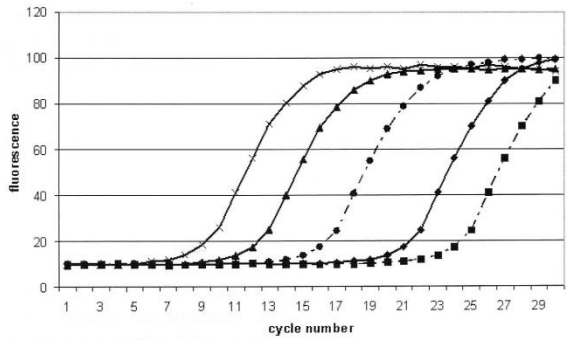
Overview of Steps Involved in DNA Typing



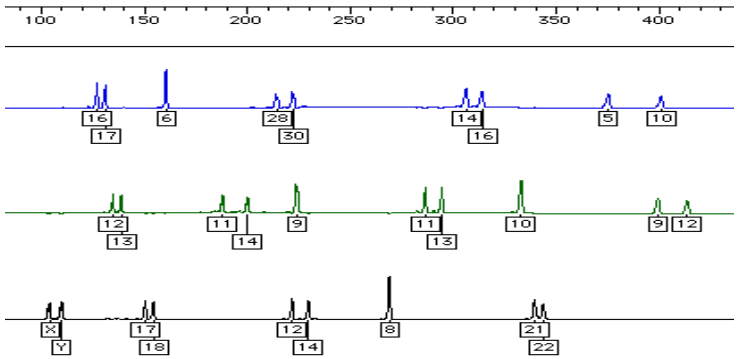
Blood Stain



PCR Amplification with Fluorescent STR Kits and Separation with Capillary Electrophoresis



DNA Quantitation using Real-Time PCR



Genotyping

H. Miller Coyle

Sampling has changed in the past 10 years for DNA

- A bloodstain the size of a quarter used to be required for older methods such as RFLP (restriction fragment length polymorphisms).
- Current methods use fluorescence, a more sensitive detection method, and can process DNA from 1-20 cells. This process is STR (short tandem repeat analysis).

Sufficient quantity & quality DNA – example:

15 locus profile suitable for CODIS database entry which only requires 13 core loci

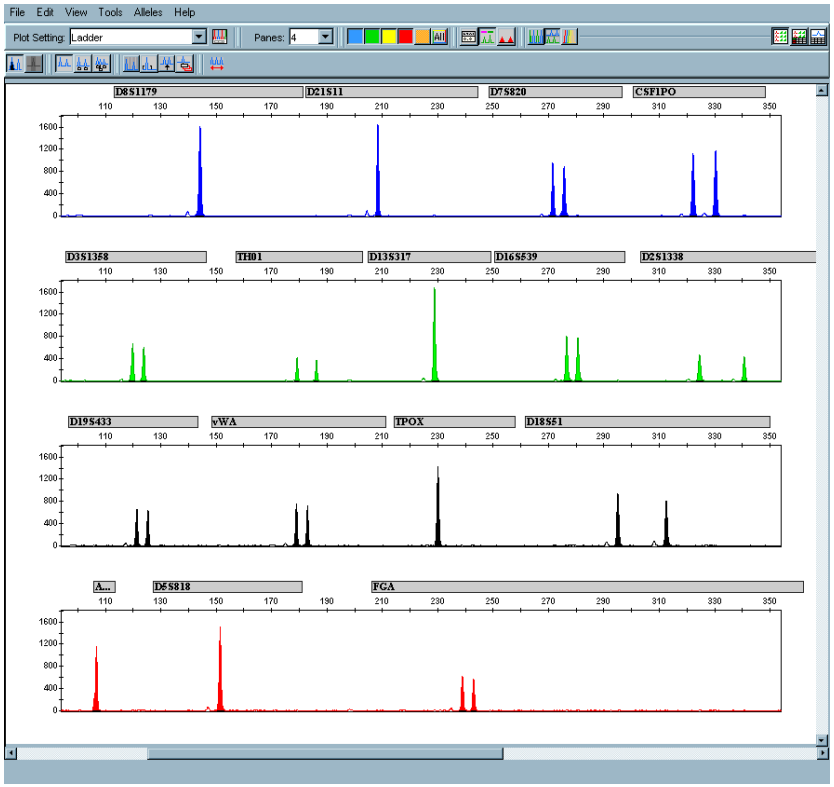


Figure 1: GeneScan® software electropherogram showing AmpFLSTR® Identifiler® PCR Amplification Kit results for 15 STR loci and the Amelogenin locus analyzed on the ABI PRISM® 310 Genetic Analyzer. DNA fragments are labeled in 6-FAM™ dye (blue), VIC™ dye (green), NED™ dye (yellow, depicted in black), and PET™ dye (red). The GeneScan™-500 size standard is labeled with LIZ™ dye (orange). Partial profiles are similar but missing information at some of these sites.

H. Miller Coyle

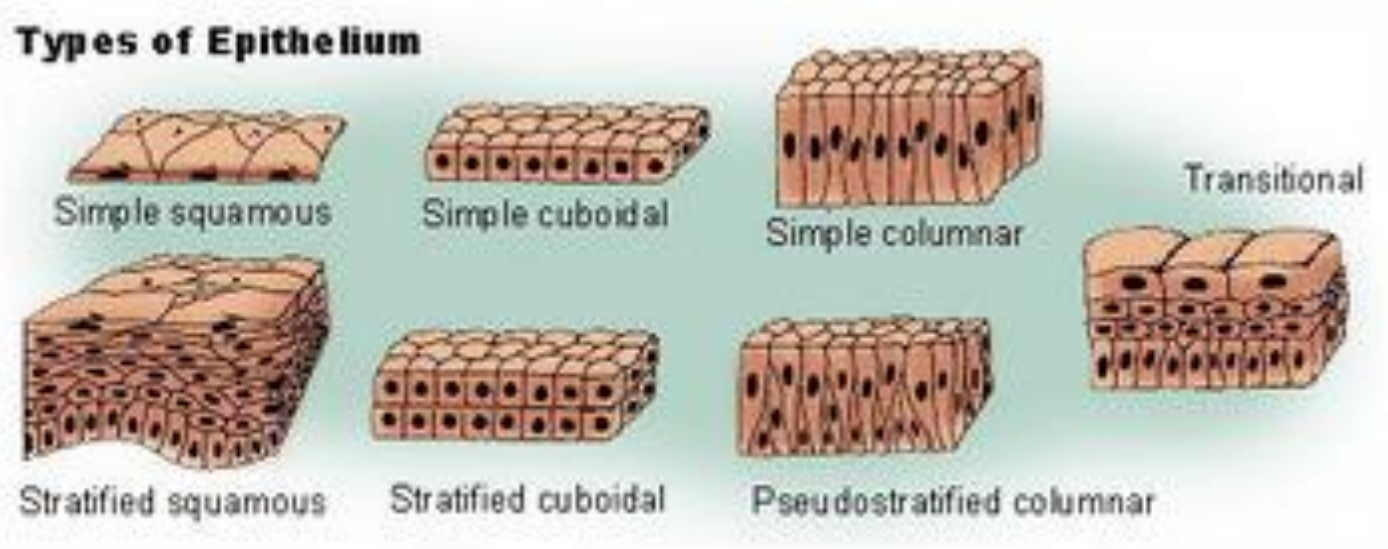
Standard cotton swab

- Sterile.
- Cost-effective.
- Available at any pharmacy.
- Insert in mouth and rub inside of cheek area 10-20 times.
- Air dry 5-10 minutes.

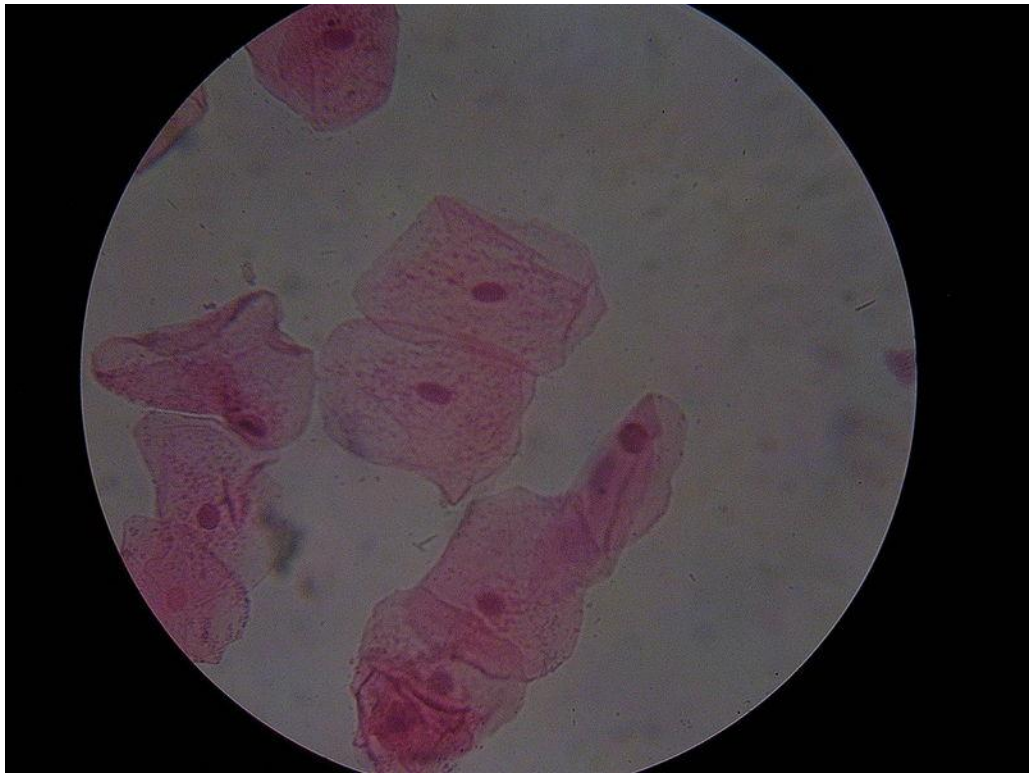
Create a slide smear

- Rehydrate the swab with 2-5 drops of sterile water
- Place swab in a clean plastic tube
- Add more water to cover the tip of the swab (cotton area)
- Gently agitate the swab so cells are released into the water solution
- An optional centrifugation step can concentrate the cells into a pellet at bottom of tube
- Plastic pipette can be used to transfer sample to slide
- Air dry and view under microscopy

Epithelial cells are avascular therefore they rely on cells underneath to nourish them; they are shed from skin surfaces on a continuous basis while the nucleus is also breaking down. Number of nucleated cells recovered will depend on where you sample from (cheek vs. fingerprint).



Cheek cells (non-keratinized stratified squamous epithelium) at 500x magnification; nuclear fast red stain.



H. Miller Coyle

Epithelial cells or epithelium

- Epithelial tissues line the surfaces of structures throughout the body, and also form many glands.
- Functions of epithelial cells include secretion, selective absorption, protection, transcellular transport and detection of sensation.

Traditional cell biology

Purpose:

- To allow the student to consider the biology behind what we do in evidence collection.

Swabs are used for:

- Blood
- Urine
- Semen
- Saliva
- Touch DNA (cells in fingerprints or from handled items)

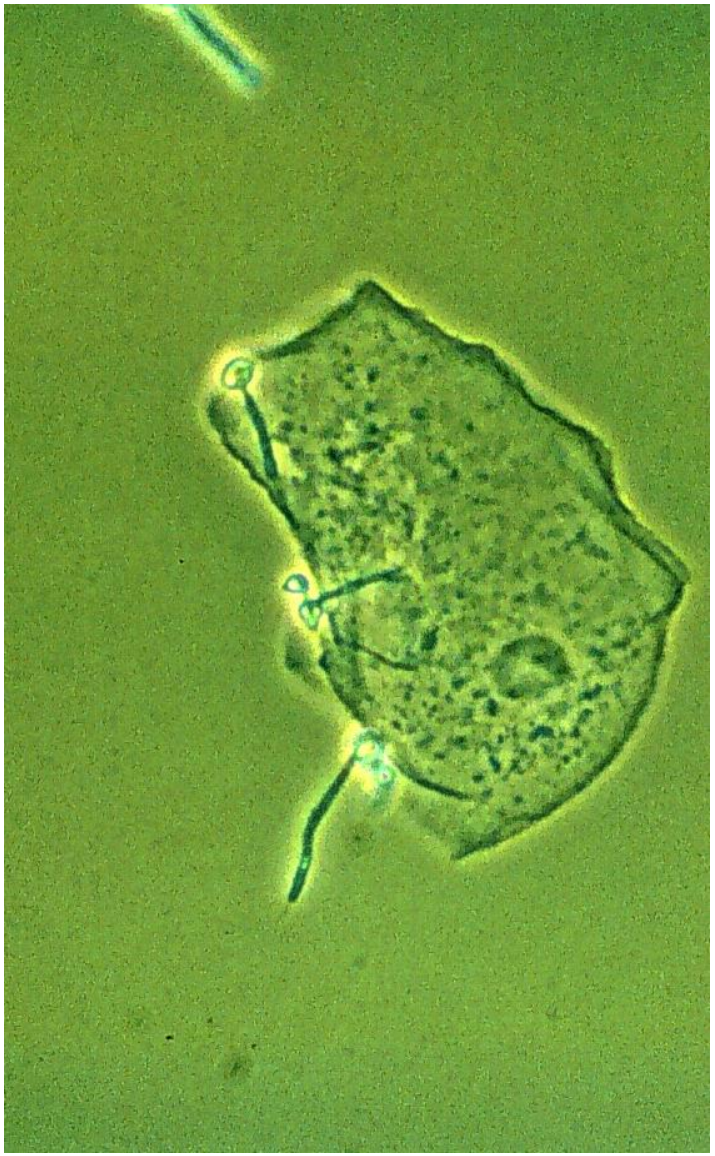
Factors affecting cell recovery

Before collection

- Total number of cells available initially.
- Environment can affect preservation.
- Genetic differences.

After collection

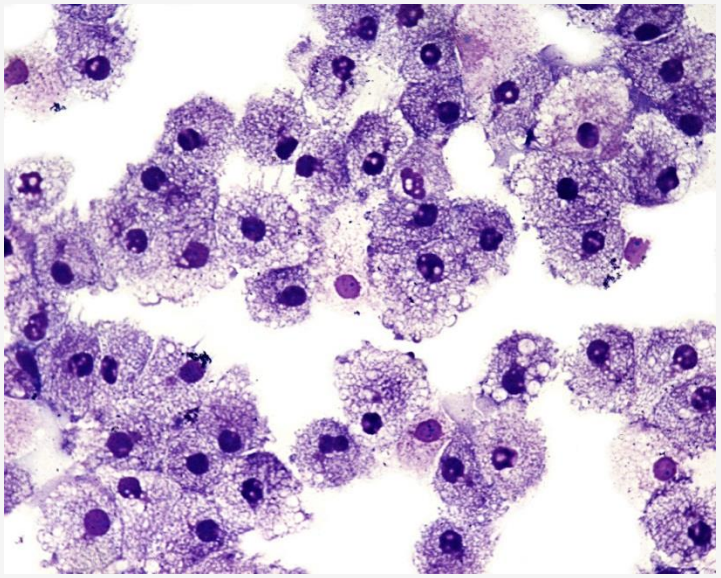
- Drying time can affect quality of cell recovery.
- Rehydration step can affect cell recovery.



Buccal epithelial cell

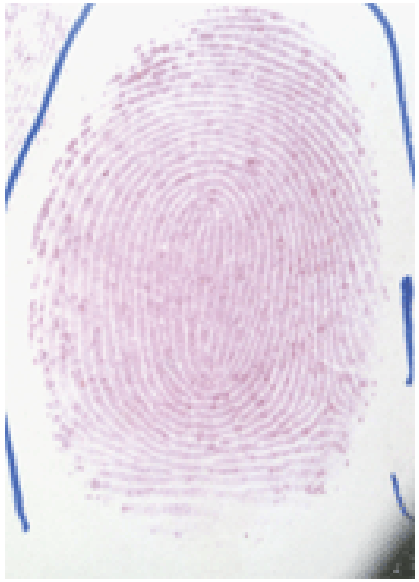
- Nucleus is present
- Outer membrane is clear, slightly folded on one side
- Granular cytoplasm is clear
- C. albicans (yeast) is also present (thats not typical) as thin tube-like structures
- Bacteria are visible

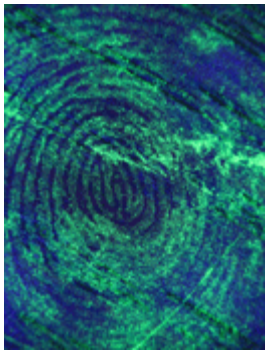
H. Miller Coyle



- This is typically straight forward for cell recovery from buccal samples
- Sometimes beautiful cell slides are the outcome; other times, cells are present but ‘crumpled’ due to dehydration, mechanical pressure during slide preparation or over-rinsing.

H. Miller Coyle





Cells from fingerprints

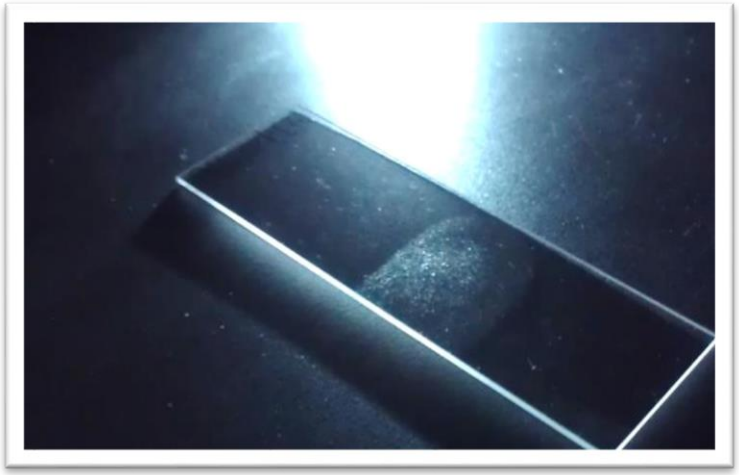
- "Touch DNA"
- Cells or free DNA
- Perspiration
- Trace amount of biological material
- You can place a print on a slide and survey for oils and transferred cells

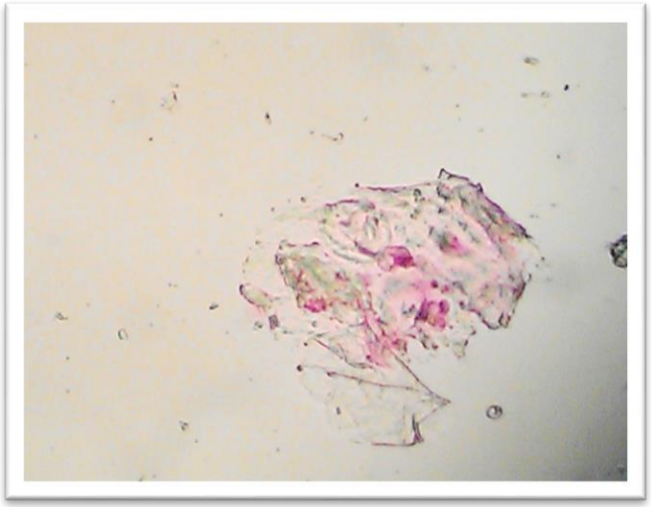
H. Miller Coyle

Example of fingerprint on glass slide

- Cells will be visible after staining but may not contain a nucleus.
- Cell counts may vary from 1-2 cells - >100 cells (non shedder vs. shedder status).
- Factors affecting shedding: lotion, genetics, exercise, time of day, etc. (varied).

Single thumbprint on a slide



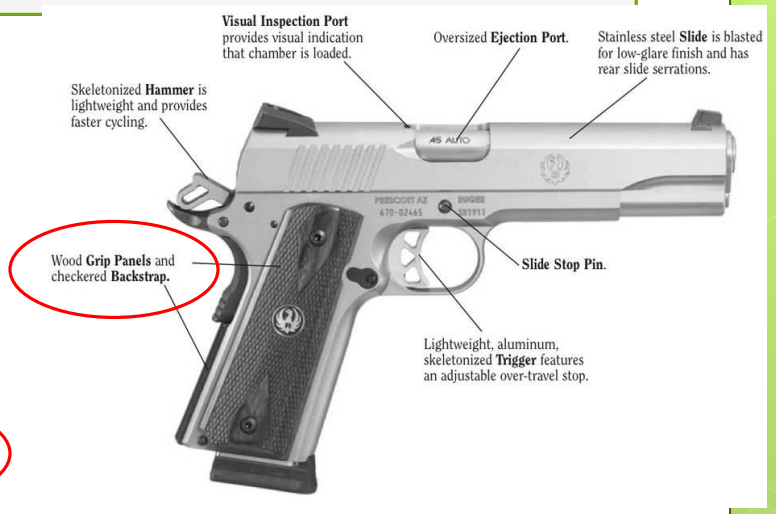


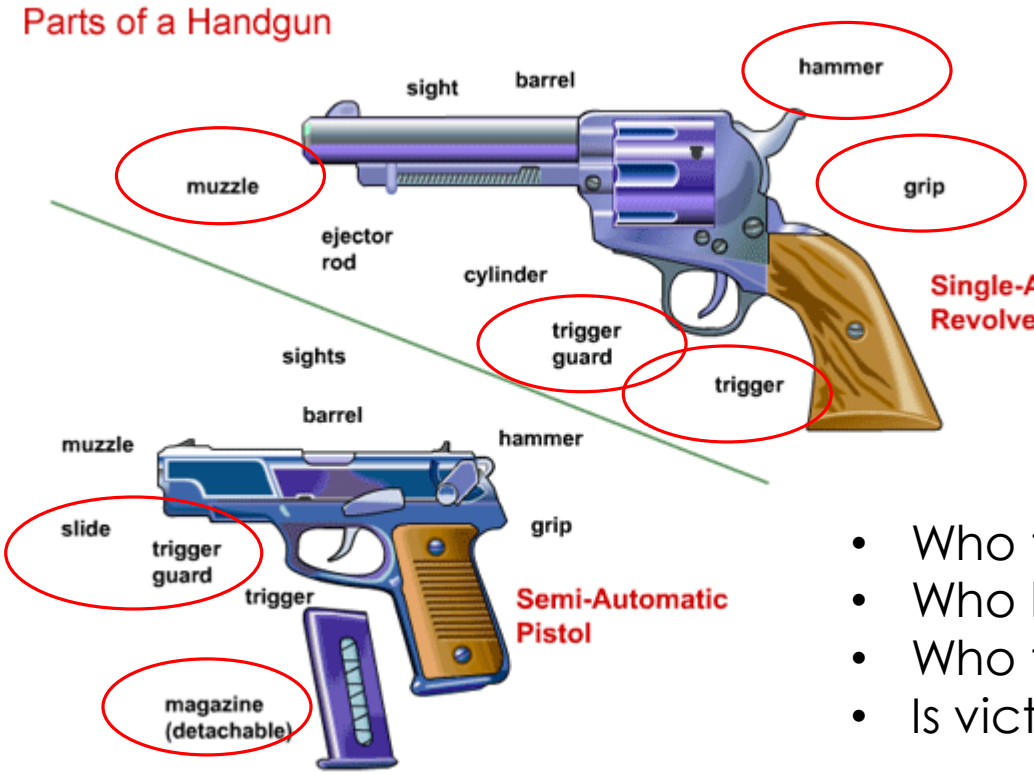
H. Miller Coyle

Some Applications to Forensic Science

- Homicides – clothing, bodies
- Property Crimes –
  - Stolen items (e.g. wallets; jewelry)
  - Disposed of items (e.g. frisking policies; pawned items)
  - Weapons (knives, ammunition, firearms)
    - Swabs of handles, blades
    - Swabs of cartridges
    - Swabs of trigger, grip, end of barrel, backstrap

Firearms





- Who touched or handled?
- Who loaded weapon?
- Who fired weapon?
- Is victim detected on weapon?

H. Miller Coyle

Knives

- Who handled knife? (touch DNA)
- Any sign of victim on knife? (handle, hilt, blade)

Some questions that need to be addressed for the courts:

- Whose DNA is detected?
- At what locations on item?
- Are multiple individuals (DNA mixtures) detected?
- How long would one have to handle the object for transfer?
- Is DNA likely from direct or indirect transfer?
- What can be determined about circumstances based on DNA?