JS 185:
Introduction to Physical Evidence

I. **Pre-class activities**
   A. Announcements and Assignments for next week

II. **Learning Objectives-Nature of Physical Evidence**
   A. List the common types of physical evidence
   B. Explain the difference between Identification vs. Comparison
   C. Define Individual vs. Class Characteristics- Give examples
   D. Discuss the value of class evidence in criminal investigations
   E. Explain the purpose physical evidence plays in reconstructing crime
   F. List the sources of Biological Evidence
   G. Discuss proper collection of biological evidence
   H. Understanding how to avoid contamination
   I. Understanding how to detect and monitor for contamination

Announcements & Assignments

- ***Assignments***
  - ***Read Butler Chapter 1: Due Thurs Sept 5th***
  - ***Read Rudin and Inman Chapters 3 and 5: Due Sept 5th***
  - Visit the following document on collection of biological evidence- http://www.cacnews.org/wordfiles/DNA%20SampleHandling.doc
  - Summarize it in 300 words. Write a total of 3 questions and answers based on your reading. Bring hard copy to class
  - EXTRA CREDIT:
    - Find a recent (2010-2013) article on collection and storage of biological evidence. Write a brief summary (3 paragraphs) and 3 questions and 3 answers. Bring the article, your summary, questions and answers in hard copy Sept 5th to hand in.

- ***References***
  - Saferstein R. 2004. Chapter 3
  - Gary Sims- Personal communication 2003.- Powerpoint slides on contamination
  - ***http://www.cci.ca.gov/Reference/peb/peb.html***

Small Group Exercise 1

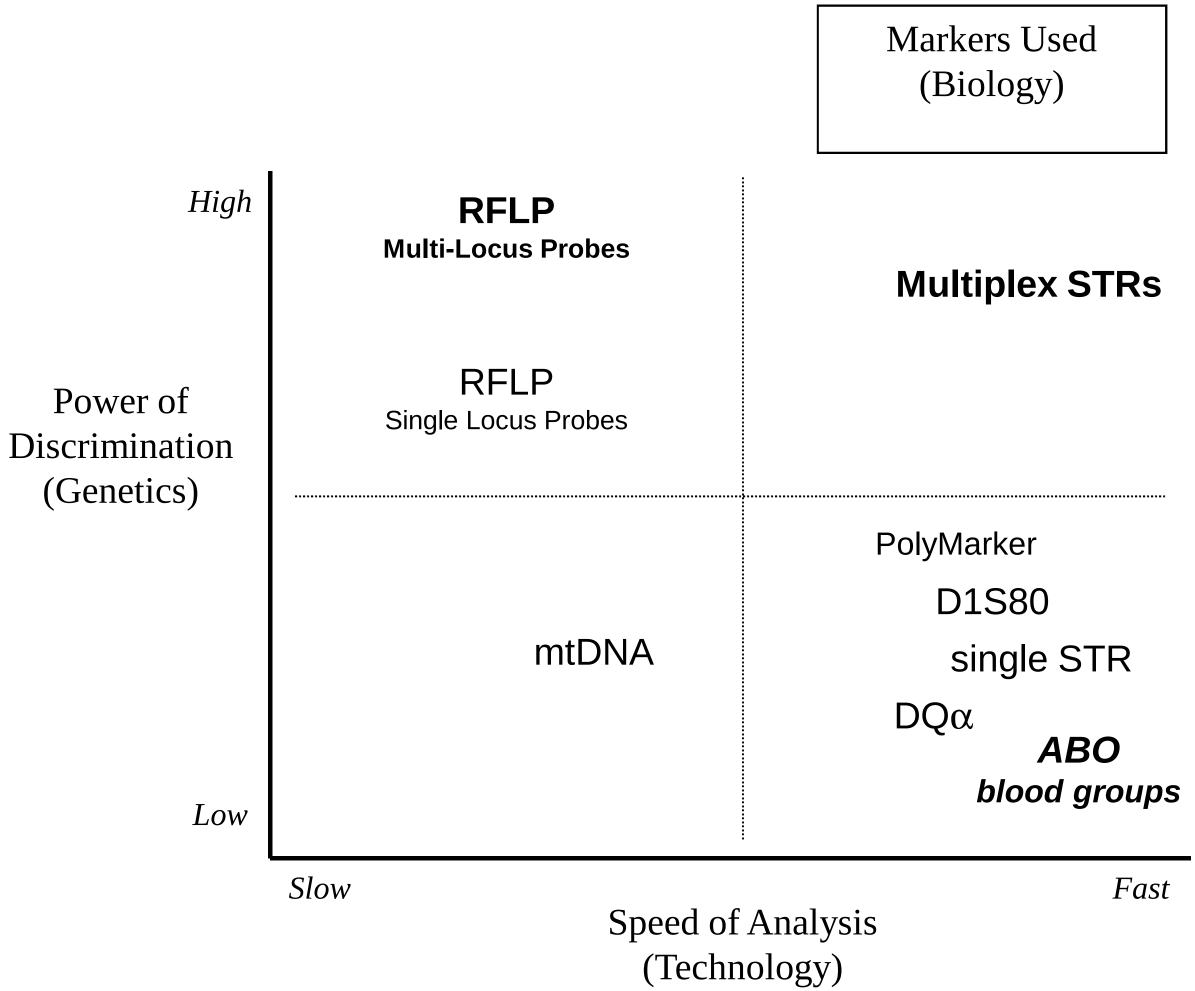
What samples provide DNA?

- DNA can be typed from a number of different types of samples and sources. You have a missing person and there are no known blood samples available as a reference.
- In your small groups, list all types of samples you believe will provide DNA typing results that may provide a reference for the missing person. Start with the ones with the highest probability of typing.
- You have 10 minutes to complete, review and edit your lists
- Be sure that all members of your group sign and print their names and submit the list

Progression of DNA Typing Markers

- RFLP
  - multilocus VNTR probes
  - single locus VNTR probes **(^{32}P and chemi)**
- PCR
  - DQ-alpha **(reverse dot blot)**
  - PolyMarker **(6 plex PCR; dots for SNPs)**
  - D1S80 **(AMP-FLPs)**
  - singleplex STRs with silver staining
  - multiplex STRs with fluorescent dyes

Comparison of DNA Typing Technologies



Overview of DNA typing

Sample Obtained from Crime Scene or Paternity Investigation

Biology

DNA Extraction → DNA Quantitation → PCR Amplification of Multiple STR markers

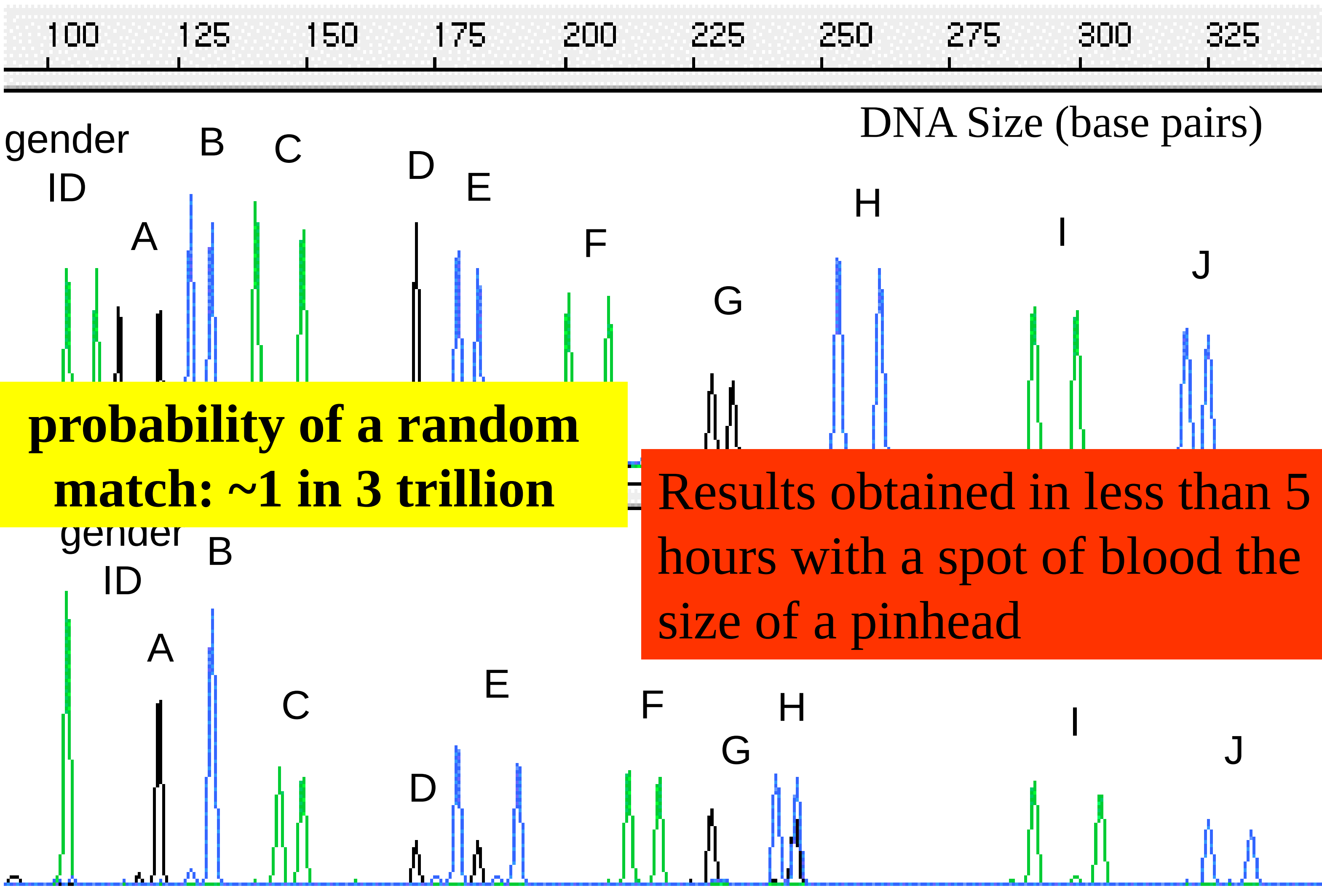
Technology

Separation and Detection of PCR Products (STR Alleles) → Sample Genotype Determination

Genetics

Comparison of Sample Genotype to Other Sample Results → If match occurs, comparison of DNA profile to population databases → Generation of Case Report with Probability of Random Match

Human Identity Testing Involves Comparing DNA Profiles



probability of a random match: ~1 in 3 trillion

Results obtained in less than 5 hours with a spot of blood the size of a pinhead

Simultaneous Analysis of 10 STRs and Gender ID

Brief History of DNA Typing

- 1980 - Ray White describes first polymorphic RFLP marker
- 1985 - Alec Jeffreys discovers multilocus VNTR probes
- 1985 - first paper on PCR
- 1988 - FBI starts DNA casework
- 1991 - first STR paper
- 1995 - FSS starts UK DNA database
- 1998 - FBI launches CODIS database

Detailed History of Serology and DNA 1

Bloodstains 384 AD
Blood groups 1888
Secretor status 1937

FORENSIC SEROLOGY AND DNA ANALYSIS TIME LINE

384	Hume II, used bloodstains to corroborate a crime or supply additional evidence.
1247	Sen-en-Roku, treatise on the mixing of blood of parties in a paternity dispute.
1853	Teichman Test, microscopic crystal test for hemoglobin using hemin crystals.
1862	J. (Izaak) Van Deen (Denmark), test for blood using guaiac, a West Indian shrub.
1863	Schönbein, blood test, ability of hemoglobin to oxidize hydrogen peroxide making it foam..
1888	Leopold Landsteiner, discovered human blood groups, Nobel Prize 1930.
1901	Dr. Paul Uhlenhuth discovered method to differentiate between human and animal blood.
1904	Oskar and Rudolf Adler developed a presumptive test for blood based on benzidine.
1912	Masaeo Takayama microscopic crystal test for hemoglobin using hemochromogen crystals.
1915	Leone Lattes antibody test for ABO blood groups.
1923	Vittorio Siracusa, absorption-elution test for ABO blood typing of stains.
1924	Bernstein, mathematician, proves that ABO blood types are in fact under genetic control.
1927	Landsteiner and Levine, M, N, and P blood factors lead to the MNSs and P typing systems.
1929	K. I. Yoshida finds serological isoantibodies in body fluids other than blood.
1931	Franz Josef Holzer developed the absorption-inhibition ABO typing technique.
1937	Holzer published the first paper on secretor status for forensic applications.
	Walter Specht developed the chemiluminescent reagent luminol to test for blood.
1940	Landsteiner and A.S. Wiener first described Rh blood groups.
1945	Frank Lundquist developed the acid phophatase test for semen.
1946	Mourant first described the Lewis blood group system. R.R. Race first described the Kell blood group system.
1950	M. Cutbush, and colleagues first described the Duffy blood group system.
1951	F.H. Allen and colleagues first described the Kidd blood grouping system.
1958	A. S. Weiner and colleagues used H-lectin to positively determine O blood type.

Detailed History of Serology and DNA 2

1958	A. S. Weiner and colleagues used H-lectin to positively determine O blood type.
1960	Maurice Muller used the Ouchterlony antibody-antigen diffusion test to determine species.
1964	N. Spencer et al. identified the polymorphic nature of red cell phophoglucomutase (PGM).
1966	Culliford and Wraxall developed the immunoelectrophoretic technique for haptoglobin typing in blood stains.
1967	Culliford initiated gel-based methods to test for isoenzymes in dried blood stains. Developed and disseminated tests for proteins and isoenzymes in blood, body fluids and secretions.
1968	Spencer et al. identify the polymorphic nature of red cell adenosine deaminase (ADA).
1971	Culliford published The Examination and Typing of Bloodstains in the Crime Laboratory.
1973	Hopkinson and colleagues first identified the polymorphic nature of esterase D (ESD).
1978	Wraxall and Storolow developed the "multisystem" method for testing the PGM, ESD, and GLO isoenzyme systems simultaneously. Developed methods for typing blood serum proteins such as haptoglobin and Gc.
1983 →	Kerry Mullis, Cetus Corp, develops polymerase chain reaction (PCR). Published in 1986.
1984 →	DNA profiling, first DNA 'fingerprint', discovered by Dr. Alec Jeffries of the Lister Institute of Leicester University, England. It involved detection of multilocus RFLP pattern. He published his findings in Nature in 1985.
1986 →	Henry Erlich, Cetus, developed PCR technique for clinical and forensic applications. Resulted in first commercial PCR typing kit, HLA DQ-alpha (DQA1), specifically for forensic use. In People vs. Pestinikas, PCR-based DNA testing (HLA DQ-alpha) used to confirm different autopsy samples are from the same person. First DNA tests accepted by U.S. civil court.
1987 →	Jeffreys used DNA profiling to identify Colin Pitchfork as the murderer of two young girls in the English Midlands. In the same case DNA exonerated an innocent suspect. RFLP used in U.S. criminal court to convict Tommy Lee Andrews of sexual assaults. New York v. Castro, admissibility of DNA challenged. Leads to quality control guidelines.
1990 →	K. Kasai et a. suggest the D1S80 locus (pMCT118) for forensic DNA analysis.
1992 →	Nat. Res. Coun. Com. on Forensic DNA publish DNA Technology in Forensic Science. Thomas Caskey et al. publish work on Short Tandem Repeats for forensic DNA analysis.
1994 →	Roche released 5 additional DNA markers to add to HLA-DQA1 for forensic DNA typing.
1996	Nat. Res. Coun. Com. on Forensic DNA publish The Evaluation of Forensic DNA Evidence. Tennessee vs Ware, mitochondrial DNA typing admitted in a U.S. court.
1998 →	FBI DNA database, enabling interstate cooperation in lining crimes, was put into practice.

"This is evidence that does not forget. It is not confused by the excitement of the moment. It is not absent because human witnesses are, it is factual evidence, physical evidence cannot be wrong, it cannot perjure itself...only its interpretation can err."

-Paul L. Kirk, 1974.

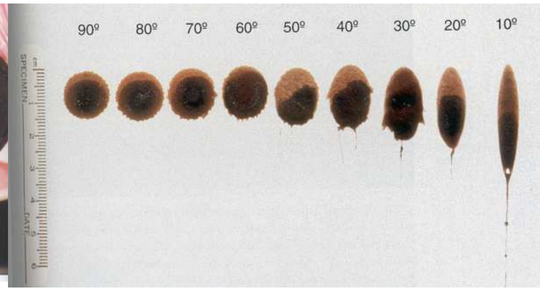
Physical Evidence

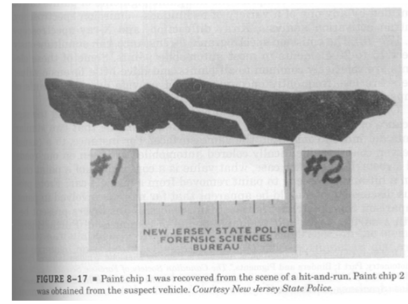
Any material either in gross or trace quantities that can establish through scientific examination and analysis that a crime has been committed.

Physical evidence utilization in other areas of forensic investigation

- Provides investigative leads for a case
- Ties one crime to a similar crime or connects one suspect with another
- Corroborates statements from witnesses to or victims of a crime

Common types of Physical evidence

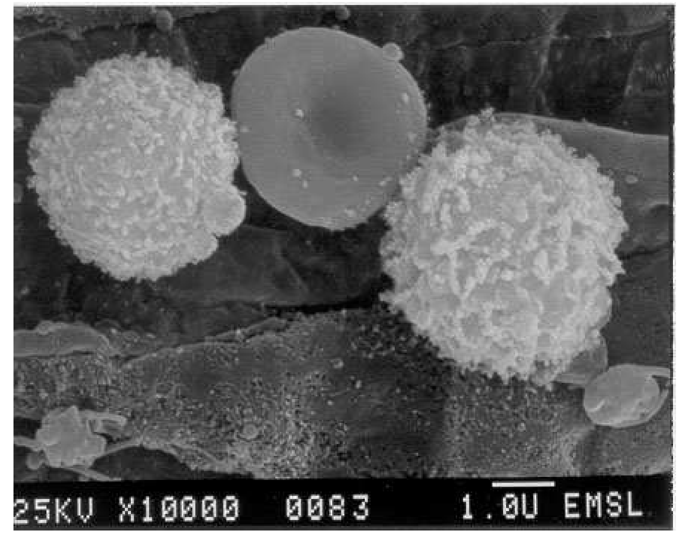




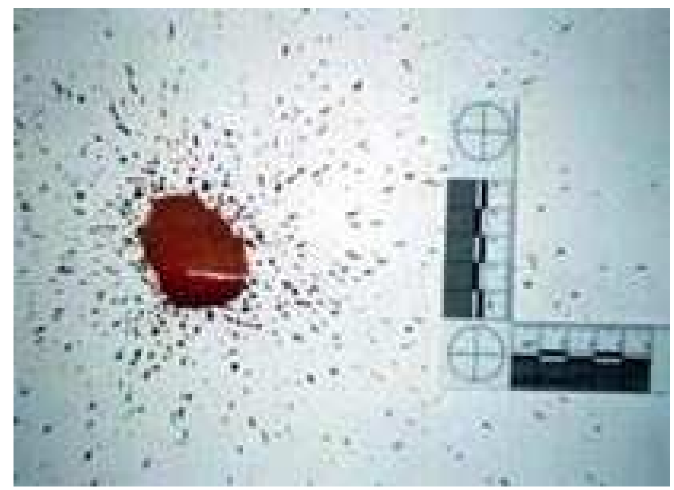
- **Blood, semen, saliva, hair, human or animal, biological samples**
- **Documents-handwriting, type, ink, indented, obliterations, burned**
- **Drugs-illegal substance-sale , manufacture, distribution, use**
- **Explosives- explosive charge material and residues**
- **Fibers, Hair, Paint**
- **Fingerprints, latent and visible**
- **Firearms and ammunition**
- **Glass-particles, fragments**
- **Impressions- tire marks, shoeprints, tracks, bite marks**
- **Organs and physiological fluids-existence of drugs or poisons, alcohol**
- **Petroleum products-e.g. gas residues, grease or oil**
- **Plastic bags-e.g. garbage bag in homicide or drug case**
- **Rubber, other polymers- remnants linked to objects recovered in suspects possession**
- **Powder residues- gun powder**
- **Serial numbers- ID numbers**
- **Soil and minerals-e.g. soil in shoes or safe insulation**
- **Tool marks-object containing impression of another object**
- **Vehicle lights- filament condition**
- **Wood and other vegetative matter- wood, sawdust, plant material, linking person or object to the crime scene**

Types of physical evidence

- **BODY FLUIDS**
- **Conventional serology:**
  - presence of blood in stains
  - species identification and ABO grouping
  - is not adequately informative to positive identify a person
- **DNA analysis** can associate victim and/or suspect with each other or with the crime scene
- **BLOODSTAIN PATTERNS**
  - additional information



SEM: erythrocytes & lymphocytes



Types of physical evidence

- **BODY TISSUES**
  - **organ samples collected at autopsy, including blood, urine and stomach contents**

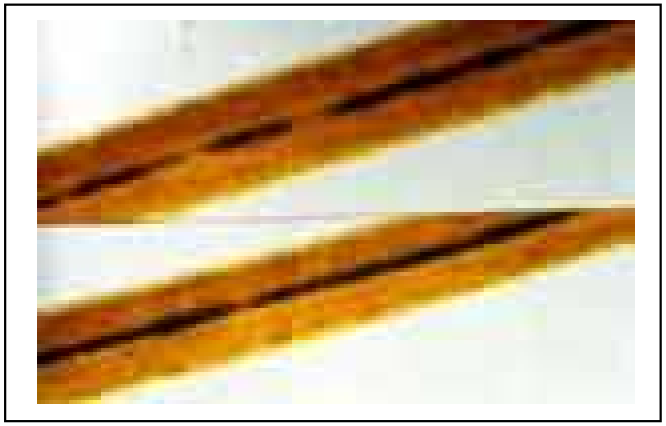
- toxicological analysis
  - volatile compounds (ethanol, methanol, isopropanol)
  - heavy metals (arsenic)
  - nonvolatile organic compounds (drugs of abuse, pharmaceuticals)
  - miscellaneous (strychnine, cyanide)

Types of physical evidence

- **HAIRS**
  - **hairs analysis can determine**
    - human/animal
    - race
    - body area
    - cosmetic treatments
    - method of removal (crushed, cut, burned, forcibly removed, fallen out naturally)
  - **morphological features**
    - can associate a hair to a person
  - **DNA analysis**
    - positive identification
  - **toxicological examination**
    - presence of drugs and poisons

Types of physical evidence

Two matching hairs identified with the comparison microscope

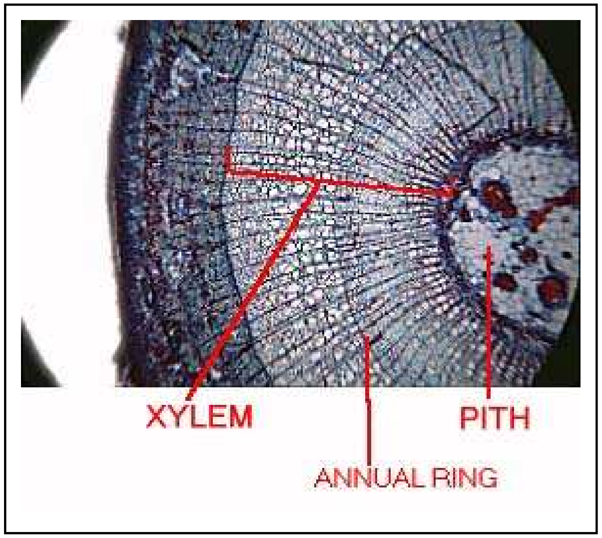


Types of physical evidence

- **SOILS & MINERALS**
  - comparison between two or more soils to determine if they share a common origin
  - color, texture, composition comparison

Layers of soil exposed at a grave site. Each layer must be sampled

- **WOOD**
  - place the suspect at the crime scene
  - side or end matching, fracture matching and species identification.

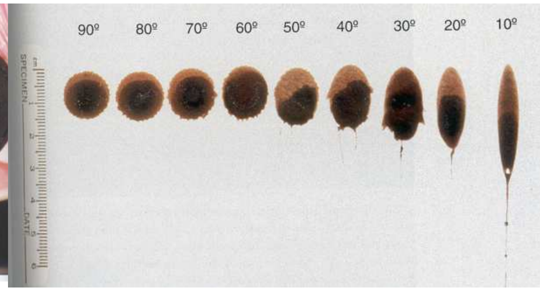


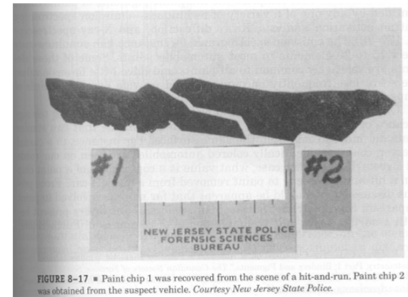
Cross-section - Xylem

Sources of Biological Evidence

Material	Reference
Blood and blood stains	Budowle 1995
Semen and semen stains	Budowle 1995
Bones	Gill 1994
Teeth	Alvarez 1996
Hair with root	Higuchi 1988
Hair shaft	Wilson 1995
Saliva (with nucleated cells)	Sweet 1997
Urine	Benecke 1996
Feces	Hopwood 1996
Debris from fingernails	Wiegand 1993
Muscle tissue	Hochmeister 1998a
Cigarette butts	Hochmeister 1991
Postage stamps	Hopkins 1994
Envelope sealing flaps	Word 1997
Dandruff	Herber 1998
Fingerprints	Van Oorschot 1997
Personal Items: Razor blade, chewing gum, wrist watch, ear wax, toothbrush	Tahir 1996

Full Service Crime Lab Services





- **Physical Science Unit-** chemistry, physics, geology on drugs, glass, paint explosives and soil
- **Biology Unit-** biologist and biochemists conduct serology and DNA testing of biological material (Fluids)
- **Firearms Unit-** Examination of firearms, discard bullets, cartridge cases, shotgun shells, ammo, and clothing for residues are performed
- **Document Examination Unit-** handwriting and typewriting studies to ascertain authenticity or source
- **Photography Unit-** Digital imaging, IR, UV X ray
- **Other units:** Toxicology, Latent Fingerprints, Polygraph, Voiceprint, and Evidence collection units

Identification vs.. Comparison

- ***Identification has as its purpose the determination of the physical or chemical identity of a substance with as near absolute certainty as existing analytical techniques will permit***
- ***Comparison analysis subjects a suspect specimen and a standard/reference specimen to the same tests and examinations for the ultimate purpose of determining whether or not they have a common origin***

Identification

- **Adoption of test or tests giving a characteristic result for a specific standard material**
- **# and types of tests completed are sufficient to exclude all other substances (e.g.. DNA typing starts with tests for heme – presumably human blood, then human DNA, then specific genetic markers, STRs)**
- **Forensic scientists need to devise specific analytical schemes**
- **Quality and quantity of specimen are not constant**
- **Therefore results and interpretation are not always simple**
- **Conclusions will be based on analysts education and experience and will have to be substantiated beyond any reasonable doubt in a court of law.**

Comparison

- Standard/reference vs.. unknown/suspect sample(s)
- Two step procedure
  1. Combinations of properties chosen from suspect and standards for comparison
  2. Conclusions must be rendered-
     1. Same source or different? If one or more properties selected do not agree then analyst will not hesitate in concluding that specimen are not the *same.
     2. All properties do agree and are indistinguishable. Does it follow that they come from the same source? Not necessarily!
- Probability-
  – The frequency of occurrence of an event- odds at which a certain event will occur
  – To comprehend the evidential value of a comparison one must appreciate probability.
  – * some exceptions to exclusions may exist :mutations in DNA

Individual characteristics

- Individual characteristics- Properties of evidence that can be attributed to a common source with an extremely high degree of certainty
- Ears- Rudin Figure 2.1
- Snowflakes- 3×10^{31}
- Fingerprints- Victor Balthazard mathematically determined the probability of two individuals having the same fingerprints is 1 in 10^{60}

Figure 2-1. Ears - a trait which probably approaches individuality.

Class characteristics

- Properties of evidence that can only be associated with a group and never with a single source
- Probability is important- Paint chip- one layer (one car model) vs.. multiple layers (one specific car)
- Blood example- Product rule- multiply the product of all frequencies = probability one individual possesses a combination of blood factors= 0.44% or 1 in 200
- DNA technology provides sufficient factors to permit individualization of biological materials to a person –However the results and interpretation are dependant on other factors

Blood Factors	Frequency
A	26%
EsD	85%
PGM 2+2−	2%

Product rule
blood factors example
0.26 x 0.85 x 0.02 = 0.00442
= 0.44%

Class characteristics

- Weakness is lack of ability to assign exact or approximate probability to most class physical evidence – (Even for DNA/blood evidence frequencies of populations are known- still requires approximations- Consider also relatives as potential suspects?)
- Rely on personal experience called upon to interpret significance
- Some evidence is subjective- e.g. eyewitness, confessions, informants
- Value of class lies in the ability to provide corroboration of events with data free of human error and bias.
- Most situations, defining significance of class evidence in exact mathematical terms is difficult to impossible
- Collective presence of more than one type of class evidence may lead to extremely high certainty that they originated from the same source

Weight or Significance of Physical Evidence

- The weight of physical evidence is left entirely to the jury of laypersons
- Scientifically evaluated evidence take on an aura of special reliability and trustworthiness-not BWAG
- Need to take proper safeguards to avoid unfairly prejudicing a case against the accused
- Can be used to exclude or exonerate – Equally as important as conviction

Physical evidence collection and documentation: Foundation for reconstruction

- Reconstruction supports a likely sequence of events by the observation and evaluation of physical evidence as well as statements made by witnesses and those involved
  - Secure- preserve evidence, safety
  - Search – Critical v Supporting v Property
  - Record – sketching, measuring, photography, videography etc
  - Reconstruct- final goal
- Team

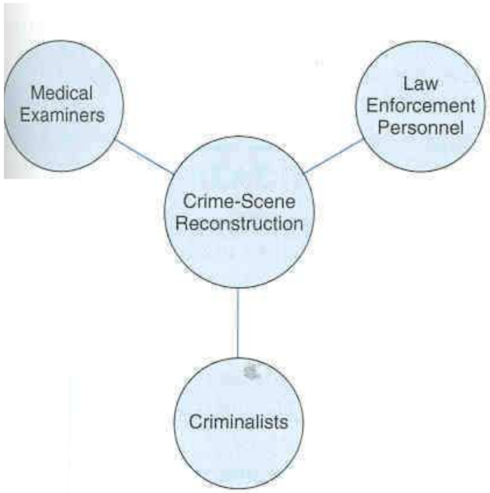


FIGURE 3–5 Crime-scene reconstruction relies on the combined efforts of medical examiners, criminalists, and law enforcement personnel to recover physical evidence and to sort out the events surrounding the occurrence of a crime.

Group Exercise 2

- Reconstruction in crime scene investigation is based in part on the analysis of biological evidence.
- Proper collection, packaging and storage are important to maintain the integrity of this evidence.
- Many different types of errors may occur. Name 5 types of errors and what consequence(s) they may have on the case outcome.
- Name 3 ways to minimize errors.

Collection of Biological Evidence at Crime Scenes

Safety First—safeguards while handling biological evidence

- Wear gloves
- Keep contaminated surface away from face—protect those mucous membranes
- Properly dispose of gloves/wash hands

Goals of biological evidence collection

- Collect as much sample as possible from a single source—keep it concentrated
- Ensure that the sample is not inadvertently mixed with other biological samples—change gloves if contaminated
- Handle the sample in a manner that minimizes deterioration—air-dry quickly (and no heat or sunlight exposure)

Further recommendations for collection of biological evidence

- Handle as little as possible—submit the item with the stain still on it
- If stain is on a large porous surface (e.g., a rug) cut out the stain area (plus unstained)
- Collect with slightly moistened (with dist. water) cotton swab—keep it concentrated, and take a control swab too

Taking care to avoid contamination

- Don’t allow one evidence stain to come into contact with other biological samples, including transfer from tools and gloves
- Don’t talk or cough over evidence stains
- Collect and package stains separately
- Clean tools (e.g., tweezers) thoroughly, with distilled water stream, dry with tissue, repeat
- May use disposable tools

Packaging biological evidence

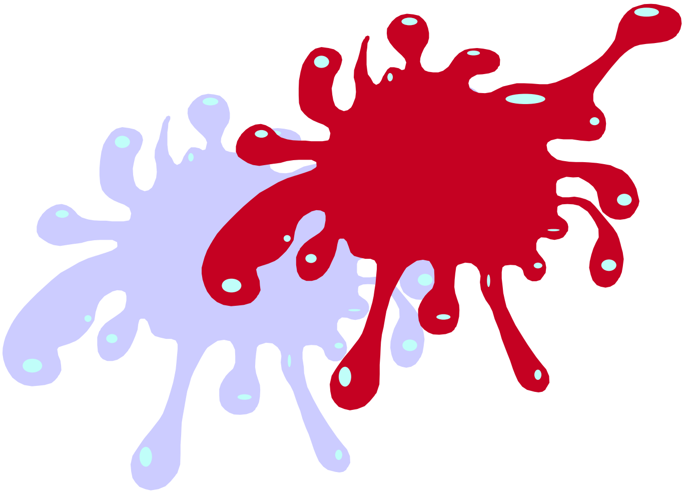
- Allow stains to air-dry as much as possible before placing in paper bag or envelope—do not use plastic
- Use separate paper containers for each item and package stains and controls separately
- Ensure that the paper container is large enough to allow circulation around the evidence item
- For garments, use clean paper to prevent different stains from contacting each other

Proper collection and procedures are critical to avoid Contamination

How else will you detect it and reduce/avoid contamination?

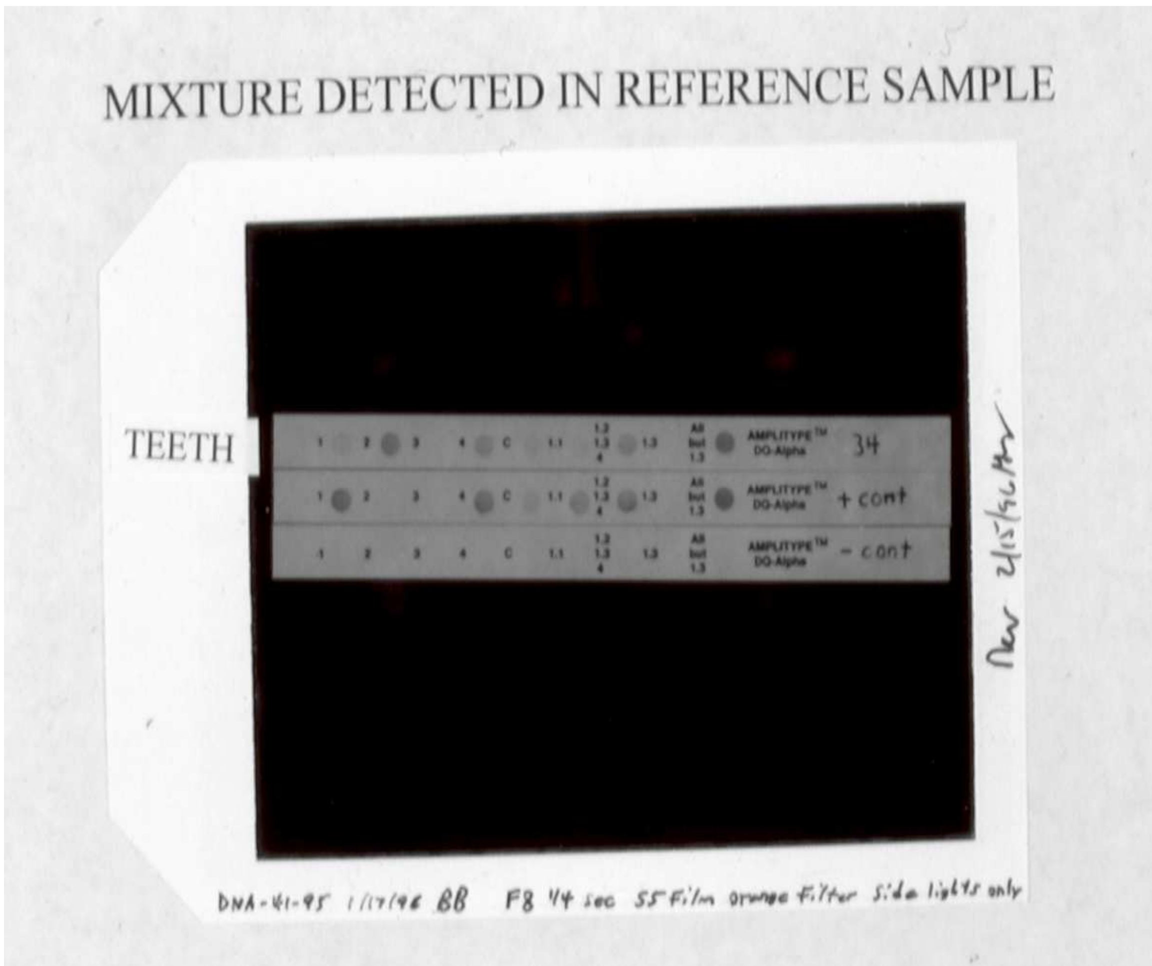
Typically, it looks like a mixture:

A combination of DNA from two/more persons

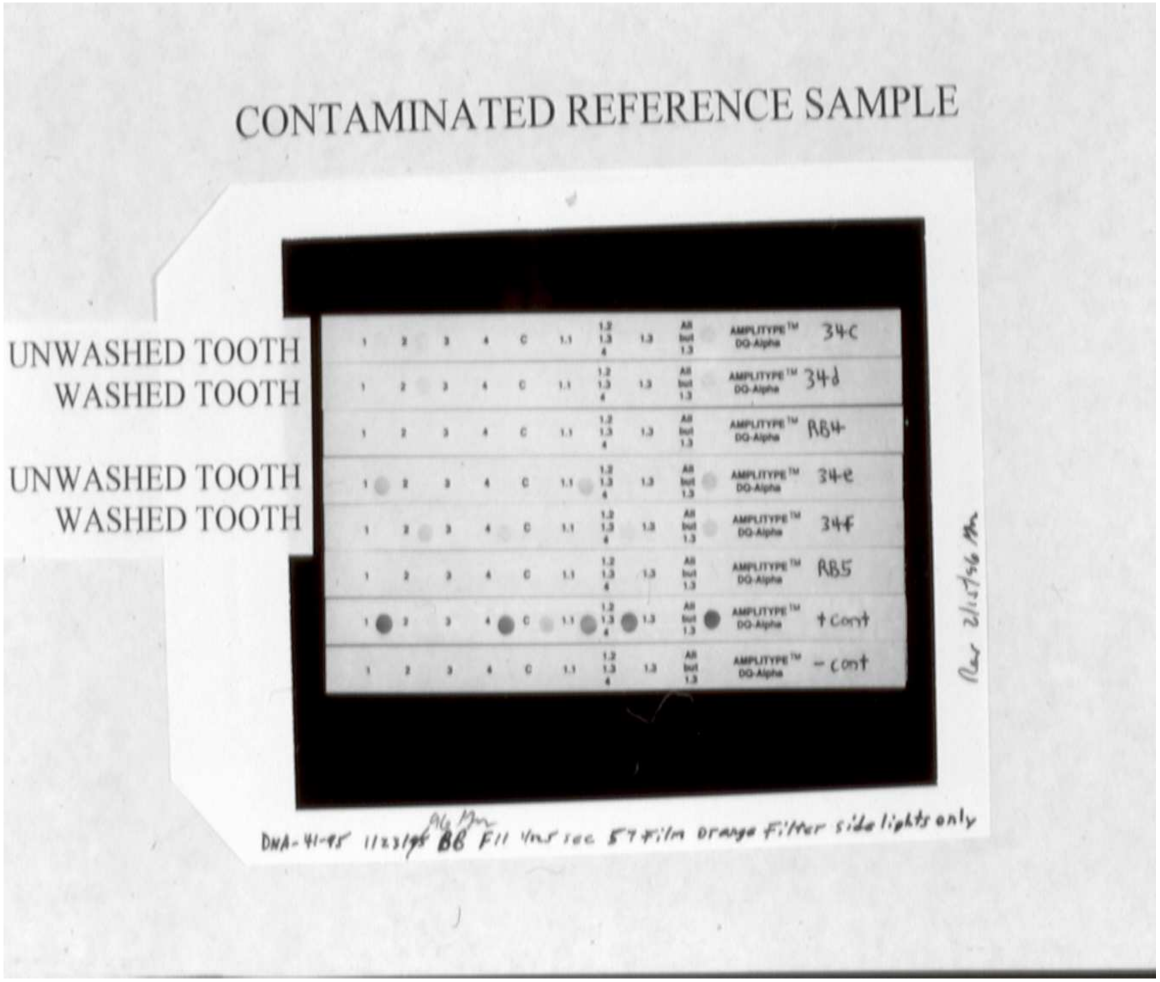


Contamination at autopsy—teeth from decomposed body

Contamination at autopsy—mixed reference

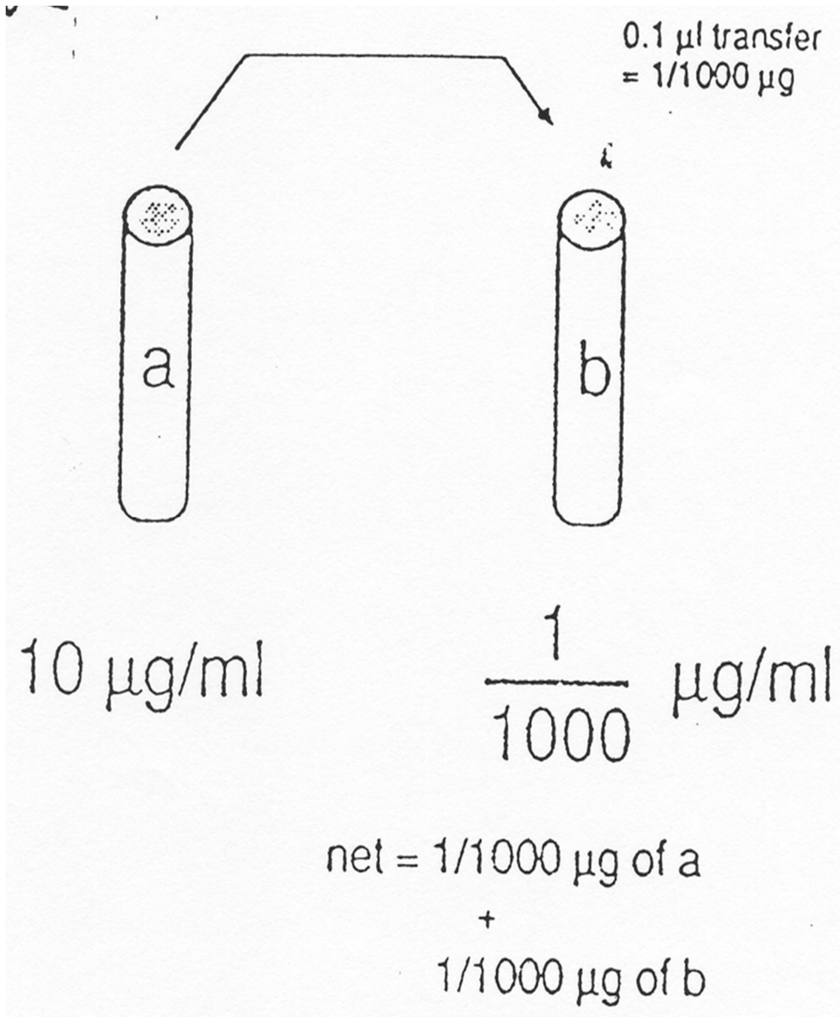


Contamination at autopsy—resolution



Contamination in the lab

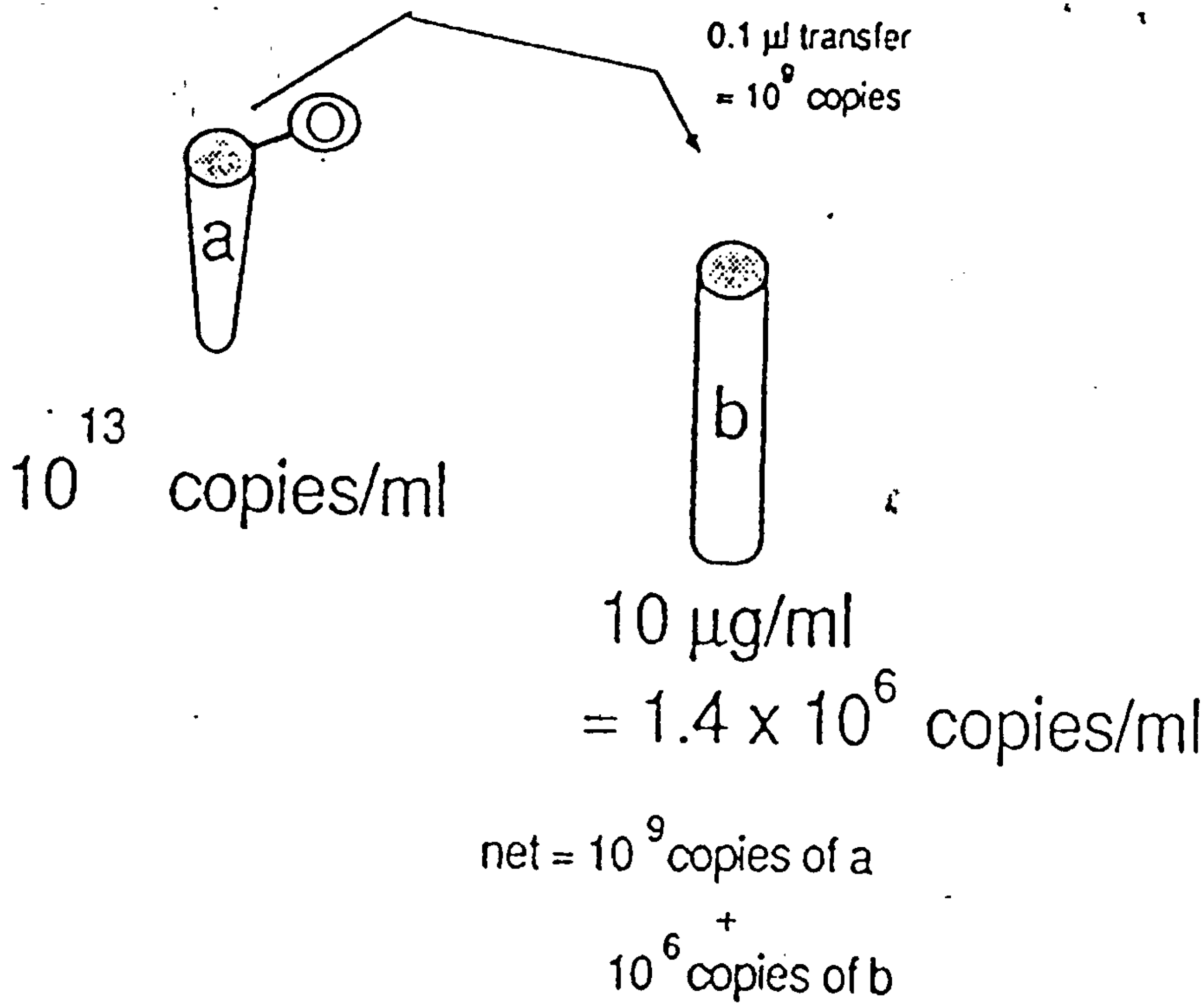
From sample with high level of DNA



To sample with low level of DNA

PCR Product Contamination—the Thousand to One Nightmare

It only takes a minuscule amount of amplified product…

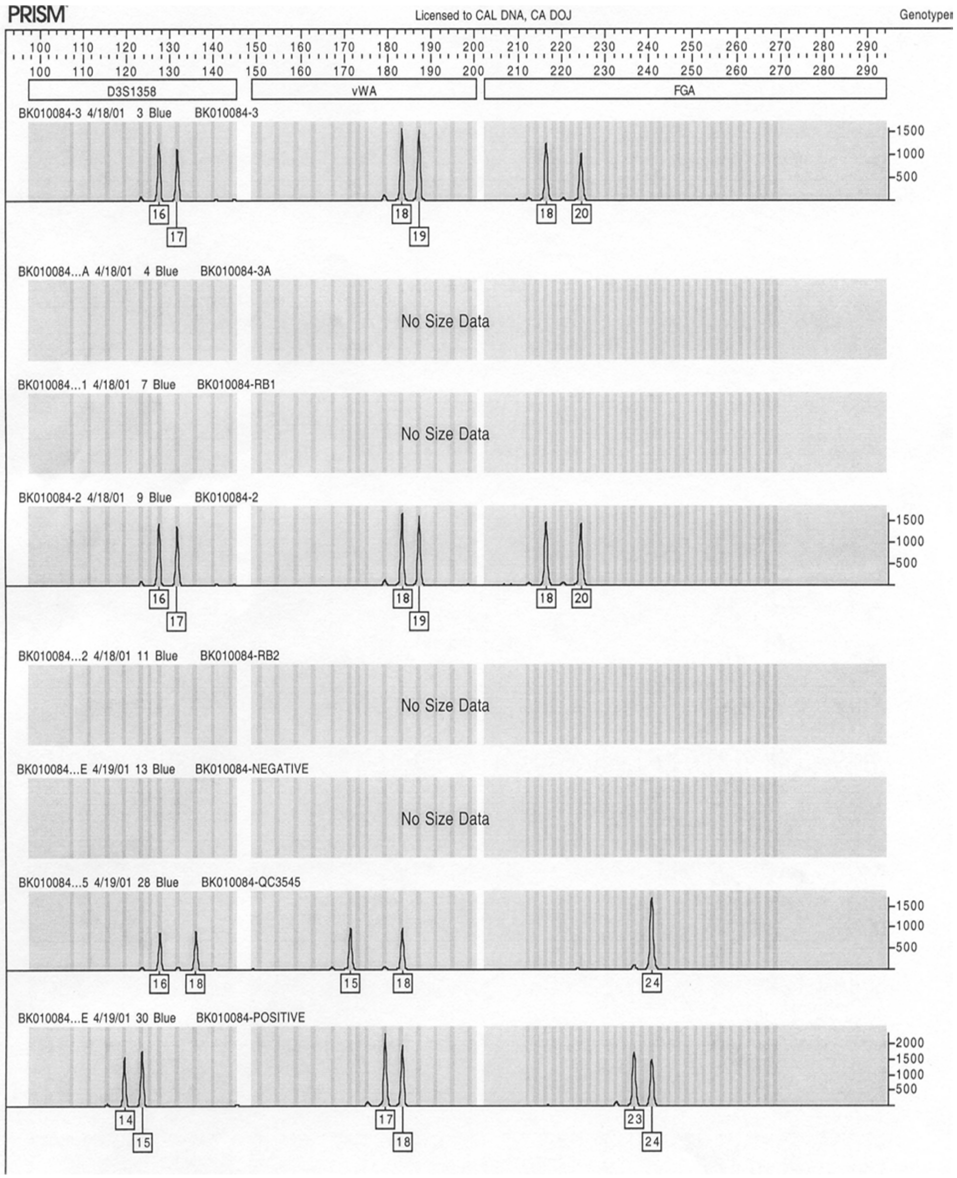


…to cause a typing disaster

Contamination—Prevention

- Sample items one-at-a-time
- Separate evidence samples from reference samples
- Use protective gear
- Separate work areas with dedicated equipment

Monitoring for Contamination—Controls ‘R’ Us



Bloodstain (Evidence)

Substrate Control

Reagent Blank—for Evidence

Victim’s Reference Sample

Reagent Blank—for References

Negative Amplification Control

Quality Control Sample

Positive Amplification Control

Summary (1)

- ***Physical evidence*** is any material either in gross or trace quantities that can establish through scientific examination and analysis that a crime has been committed.
- Examination of physical evidence conducted for ***identification or comparison* purposes**
- ***Identification*** is to determine the physical or chemical identity with as near absolute certainty as existing analytical techniques will permit
- ID requires using a test that give a characteristic result for specific substances or standard material
- Once done uses the appropriate number of tests to identify a substance and exclude all others
- ***Comparison*** analysis determines whether or not a suspect specimen and standard/reference have a common origin
- Same test used for suspect or unknown as standard or reference (e.g. databases searches)

Summary 2

- Evidence possessing ***<u>individual characteristics</u>***- can be associated to a common source with an extremely high degree of probability
  Evidence possessing ***<u>class characteristics</u>***- can be associated only to a group.
  - High diversity of class evidence makes their comparison very significant in the context of investigation
  - As the number of collective class evidence objects increases that link an individual to a crime scene, their significance increases.

- A person may be exonerated or excluded from suspicion if physical evidence is found to be different from standard or reference samples
- Evidence needs to be properly handled, collected and preserved for reconstruction
- Reconstruction relies on combined efforts of medical examiners, criminalists and law enforcement personnel

Summary (3)

- In collecting biological evidence, safety is paramount. Wear gloves- away from face- Properly dispose of gloves/wash hands
- Collect as much sample as possible - keep it concentrated- not inadvertently mixed -change gloves if contaminated
- Handle the sample in a manner that minimizes deterioration—air-dry quickly (and no heat or sunlight exposure)
- Prevent contamination- Sample items one-at-a-time- Separate evidence samples from reference samples- Use protective gear- Separate work areas with dedicated equipment- Clean the area, tools and run QC
- Monitor for contamination with controls