Forensic DNA Technology- Saving lives with DNA

Learning Objectives

a. Understand why scientists study DNA- Who cares and why?
b. Basic Review of DNA – What is DNA? Organized? Inherited?
    a. DNA Structure and Function - Base Pairing AT, GC
    b. Limerick structure/function review
c. Basic Steps of Forensic DNA Analysis
    a. Screen, Extract, Quantify, Distinguish
    b. Blood, saliva and semen screening.
d. Laboratories
    a. Screening:
        a. Hands-on Presumptive test for Blood- and Questions
        b. Hands-on Presumptive test for Semen- and Questions
    b. DNA Extraction and Questions

Define cell, nucleus, chromosome, DNA, central dogma, bases and base pairs of DNA, alleles, homozygous vs heterozygous

Who Cares?

- **Law Enforcement**
  - **Criminal Investigation- Casework, Databanks**
  - **Reuniting immigrant families- Paternity**
  - **Missing persons, Exonerating the Innocent**
- **Evolutionary, Agricultural and Zoological applications**
  - **Assessing genetic diversity**
  - **Fingerprinting endangered species and pathogens**
  - **Assessing unrelatedness to breed for increasing genetic diversity**
  - **Assessing relationships for all biological predictions**
  - **Ancient DNA analyses for reconstructing history (how we populated the globe)**
- **Other Human Applications**
  - **Making sense of the Human Genome project results- Bioinformatics**
  - **Developing rapid medical diagnostics such as those associated with triplet repeat diseases (STRs)- (Moxon et al. 1999 Sci Amer. 280:94)**
  - **Understanding the molecular basis of development, disease and aging**
  - **Screening candidates for bone marrow/organ transplants and grafts**

WE ALL DO!

Human Identity Testing

- Forensic cases **-- matching suspect with evidence**
- Exonerate persons wrongly accused of crimes**--freeing the innocent**
- Establish paternity and other family relationships**—identifying dad**
- Historical investigations**–DNA testing of human remains**
- Missing persons investigations
- Mass disasters **-- putting pieces back together**
- Military DNA “dog tag”**– Missing soldier ID**
- Identify endangered and protected species as an aid to wildlife officials (could be used for prosecuting poachers)**- Wildlife forensics**
- Authenticating consumables**- e.g. caviar or wine**
- Detect bacteria and other organisms that may pollute air, water, soil, and food or that may be used in bioterrorism- **Homeland security**
- Convicted felon DNA databases

DNA Facts and Jargon

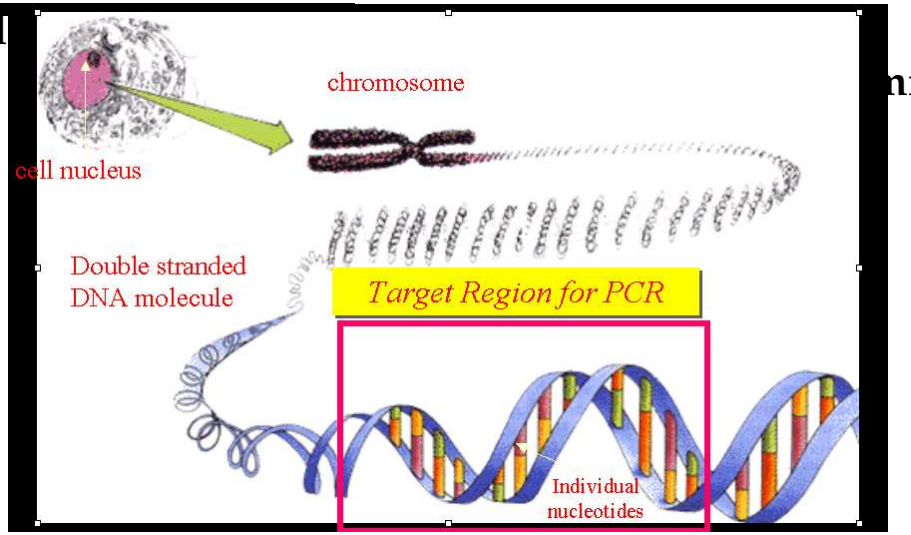
Where is it? How is it stored?

DNA is found in every *cell= basic unit of life (inside the nucleus)

Inside nuclei (organization center for the cell containing DNA, RNA and proteins) and mitochondria (ATP powerhouse of the cell) & chloroplasts for plants- (making our food via photosynthesis)

Nuclei are not found

In white blood cells,

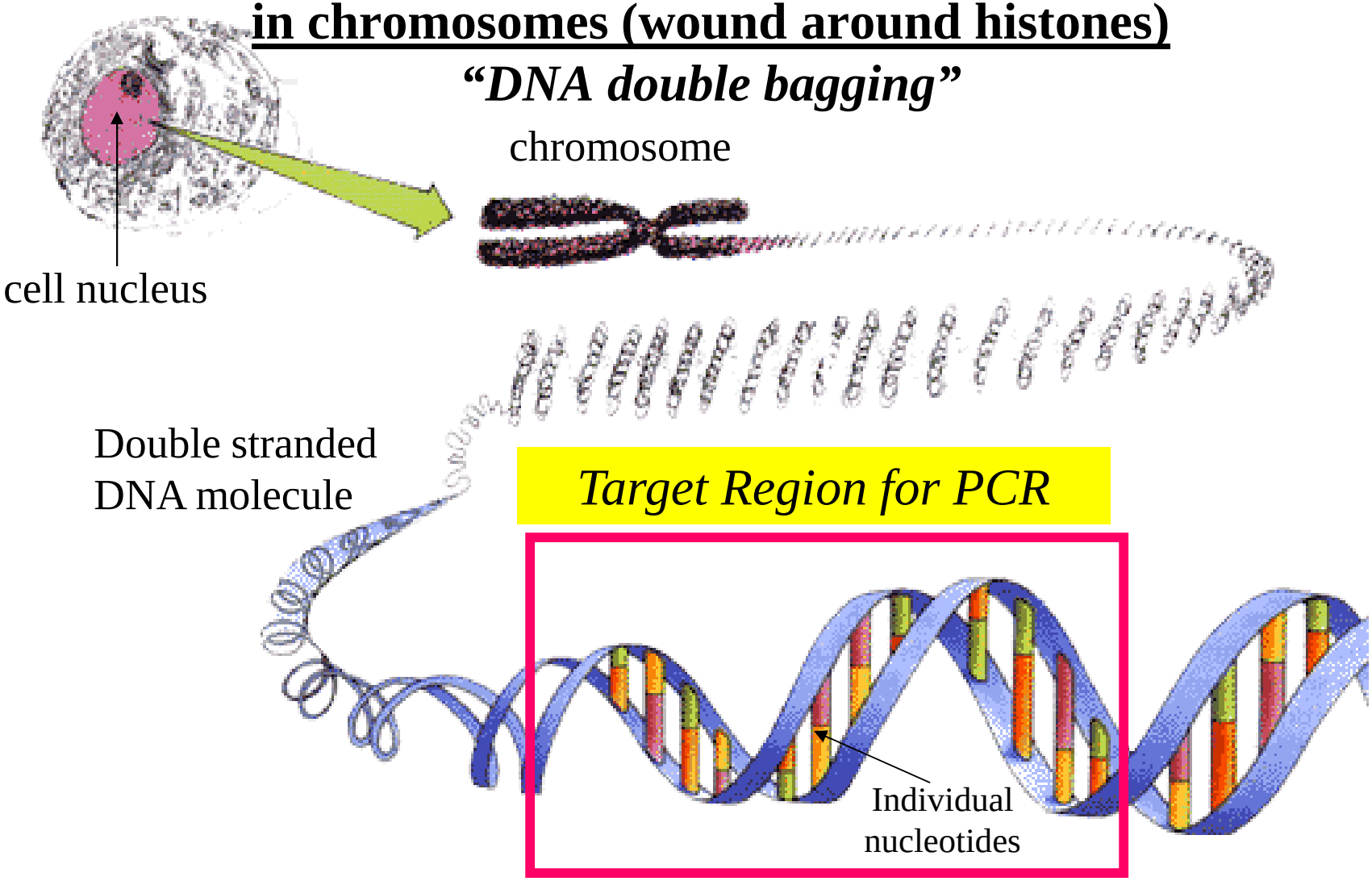


nitus, earwax etc.

DNA in the Cell

In nuclei, mitochondria and chloroplasts (plants) **organized in chromosomes (wound around histones)**

"DNA double bagging"



I. Intro to DNA : Facts and Jargon

DNA: Deoxyribonucleic acid

Different in every *individual

The same in every **cell of an individual's body

*except for identical twins that have the same DNA - "The time honored method of cloning humans"

** diseased individuals may be mosaics

DNA function

What's it do?

DeoxyriboNucleic Acid : blueprints of life

Replication, Information Storage and Mutation

Central Dogma

information flow---------->

DNA------->RNA------>protein

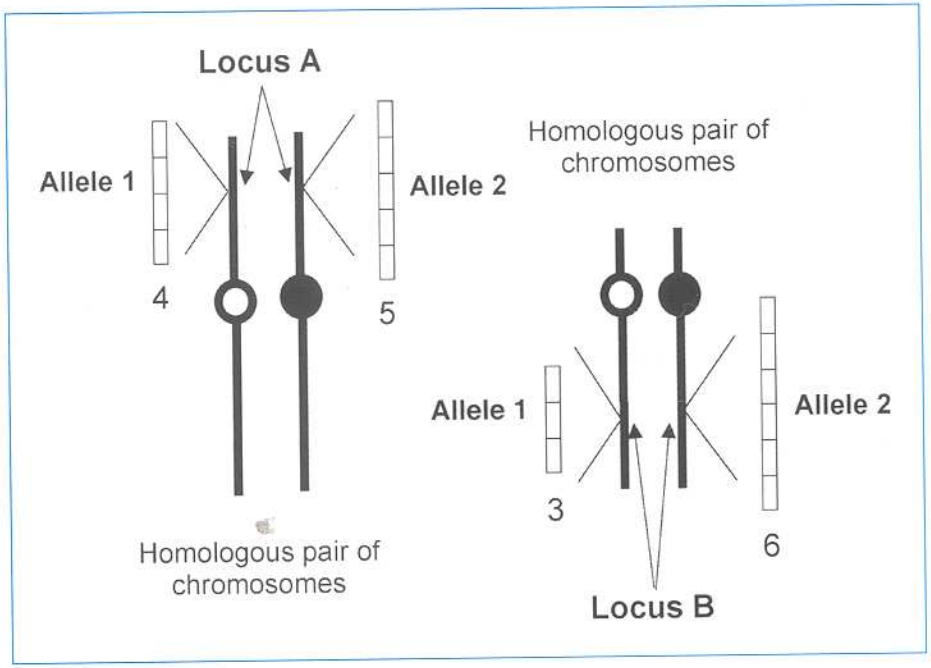
transcription translation

DNA Organization and Inheritance

Human Genome Contains 23 Pairs of Chromosomes

It is inherited from your mom and dad

Definitions of Locus and Allele



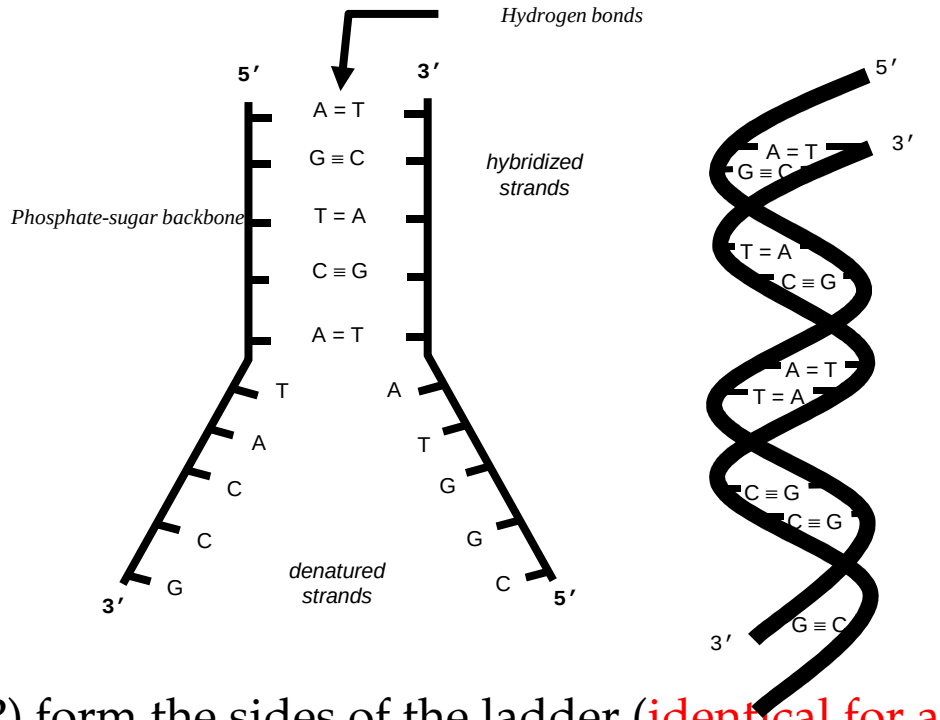
- 2 pairs of Homologous chromosomes (white from dad, dark from mom)
- ***Locus (singular) or Loci (plural)*** are defined locations where specific genes or markers are found
- ***Alleles*** are different forms of the same gene or marker
- When alleles have the same form on a locus they are said to be ***homozygous***. When different they are ***heterozygous***

DNA Structure

What is it?

Bases (AGCT) form the stairs of the ladder, are faithfully paired and exhibit differences.

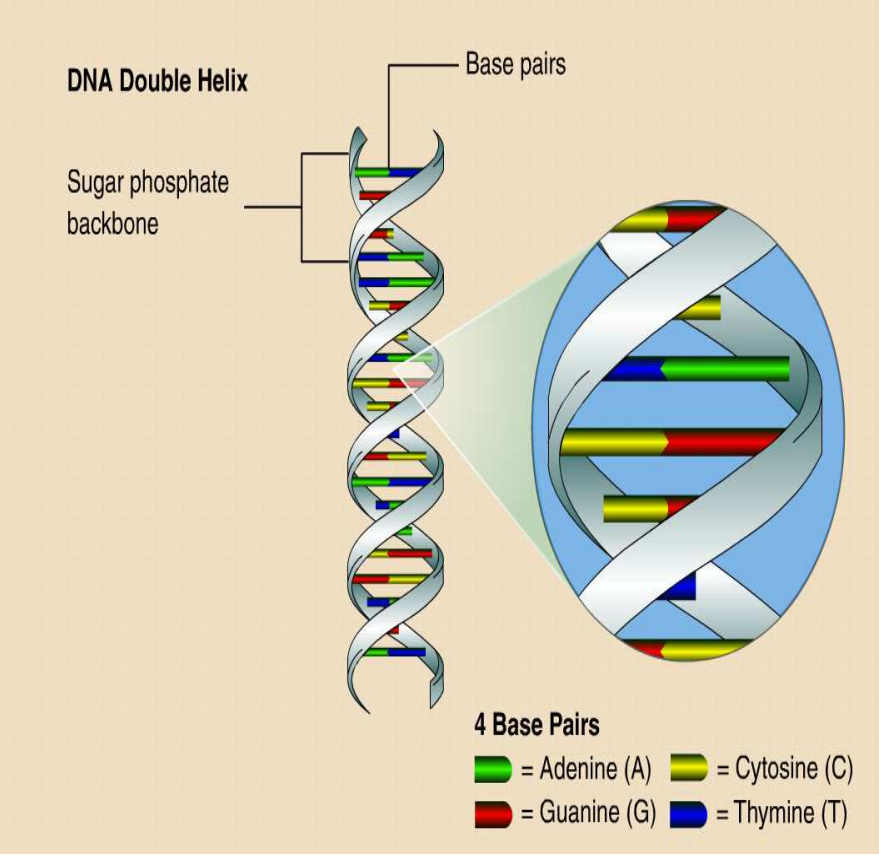
P
S-A : T-S
P P
S-G : C-S
P P
S-A : T-S
P P
S-G : C-S
P



Sugars (S) and phosphates (P) form the sides of the ladder (identical for all DNA).

Bases (AGCT) form the stairs of the ladder, are faithfully paired by hydrogen bonds and exhibit differences. **A : T and G : C**

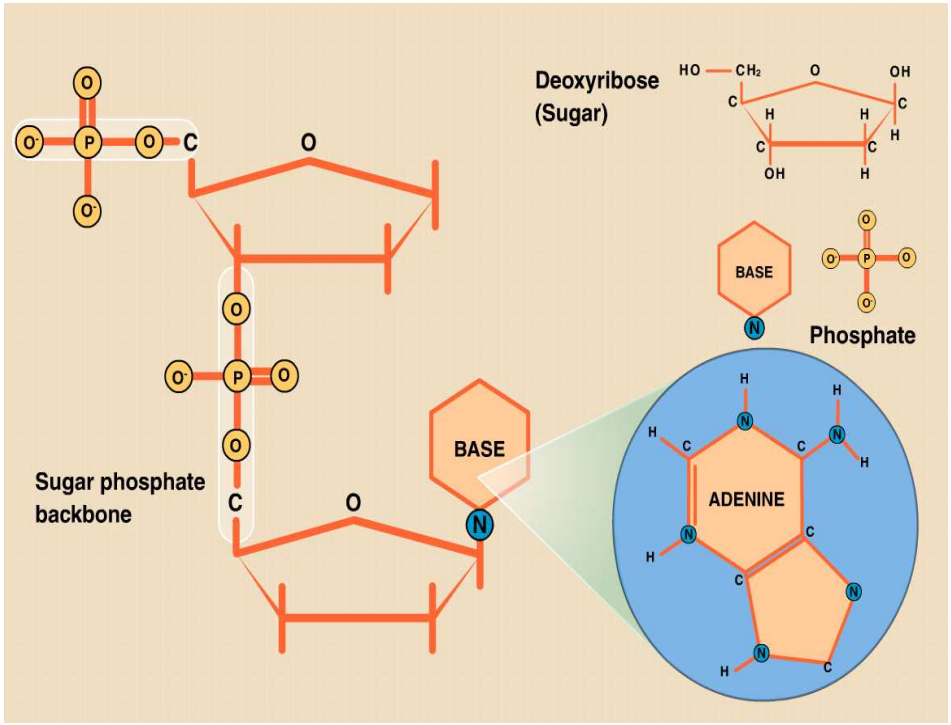
DNA Structure



- Primary genetic material is composed of two complementary strands
- Form a double helix or twisted ladder
- Sides are sugar phosphate and the steps are base pairs
- Four Bases- 2 Purines – Adenine and Guanine and 2 Pyrimidines- Cytosine and Thymine
- Asian Guys are Pure!

DNA Structure

Nucleotides are the building blocks themselves composed of PBS



Nucleotides-PBS

- Phosphate (negative charge)
- Base (AGCT-Asian Guys Can Teach)
- Sugar (deoxyribose-5C)

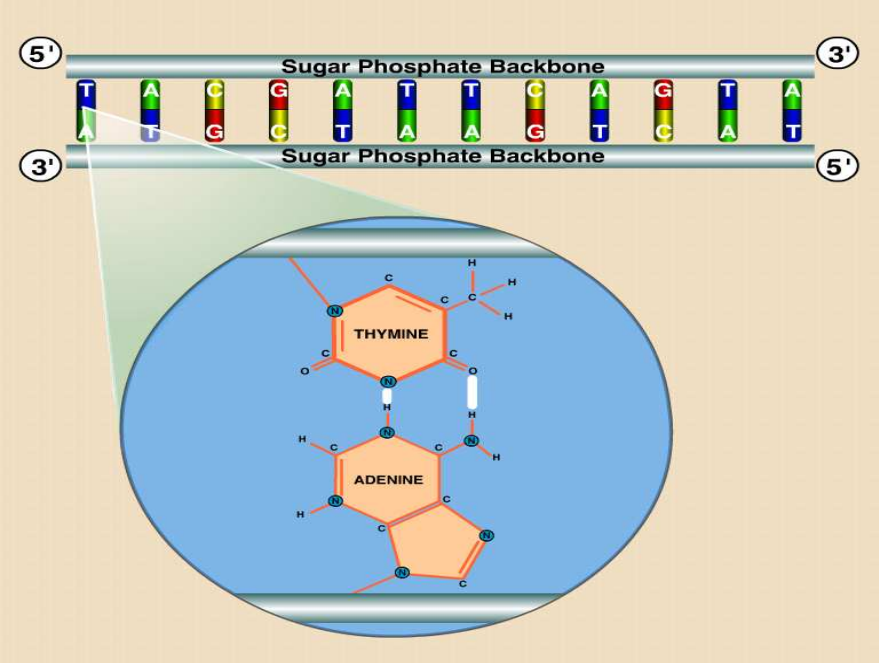
Phosphate-Sugars

- Connected by phosphodiester linkages

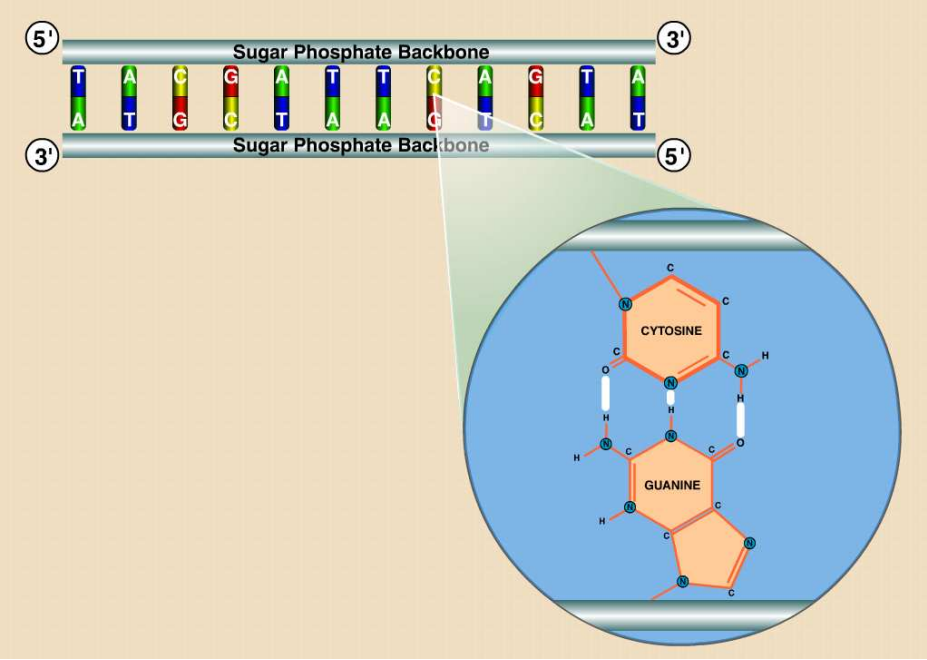
DNA Structure

2 Complimentary,
Antiparallel Strands
held together by Base Pairs- H Bonds

A:T held with 2 H Bonds



G⁝C held with 3 H Bonds



DNA St. Patricks Day Salute to the Molecule of Heredity
From Biology 110- UNC 1993 Steve Lee

The molecular structure today
Is heredity's DNA
With nucleotides
completely comprised
of a sugar and phosphate and base

The bases you see are so keen
They include thymine and adenine
Cytosine and one more
with guanine can store
all the info with rungs in between

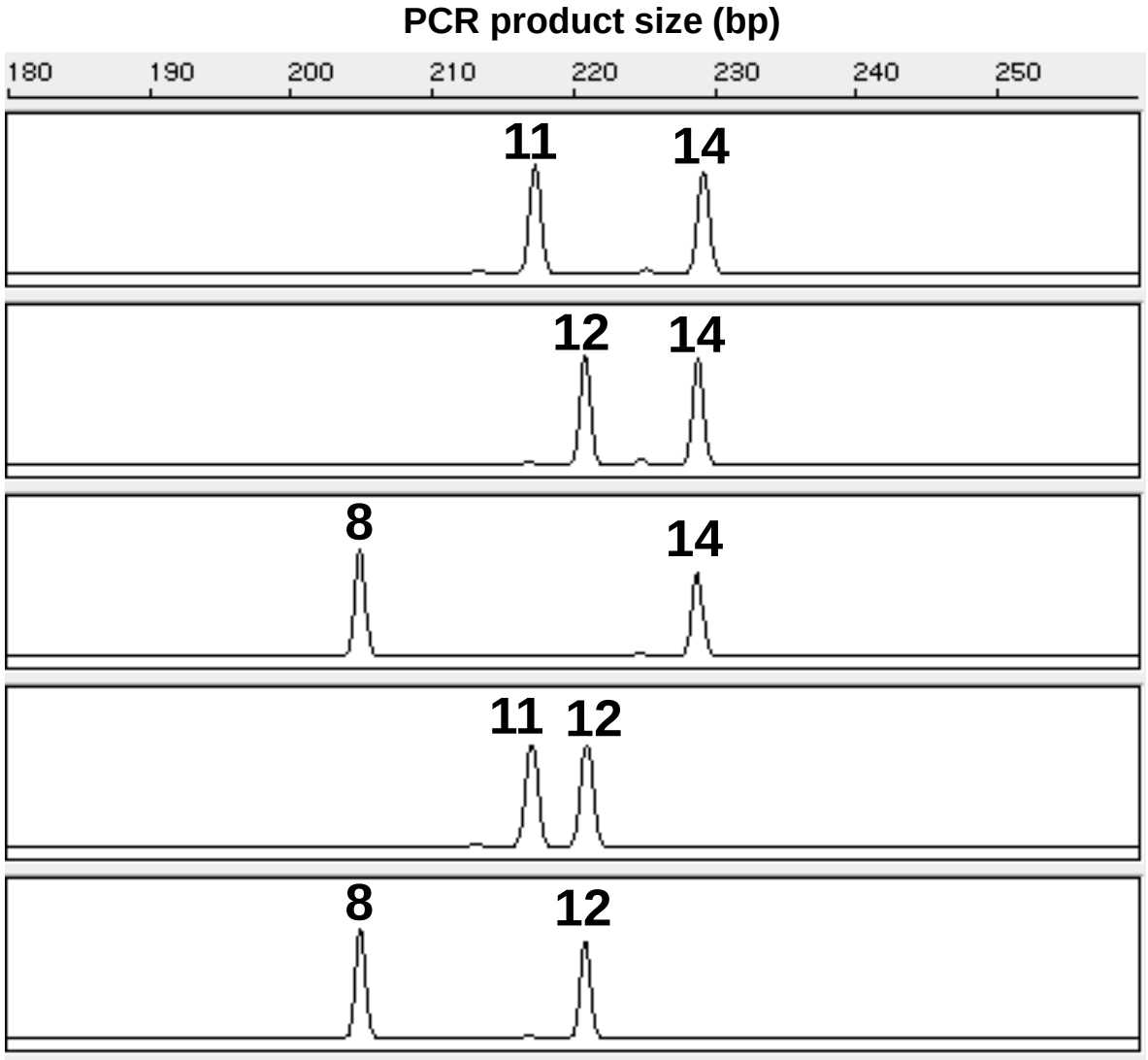
The sides of the ladder you know,
are sugar and phosphate which show
that Franklin was right
double helix is tight
ten base pairs per turn in a row

Adenine and thymine can base pair
Forming two hydrogen bonds for one stair
Cytosine and guanine
pair with three in between
and are equal in size when compared

DNA strands are just not the same
One is coding and one is called lame (anticoding)
They are opposite
in direction and this
is called antiparallel in name

Complimentary nature of strands
lets replication proceed just as planned
with A paring to T
and G pairing to C
the fidelity is precise and quite grand

Team Exercise 2: Where's Daddy?

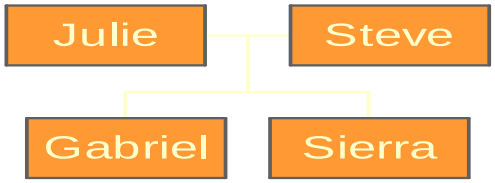


Review- Mendelian Genetics

Law of Independent Segregation-
Big D and little d will evenly segregate into the next generation
And results in equal inheritance from mom and dad

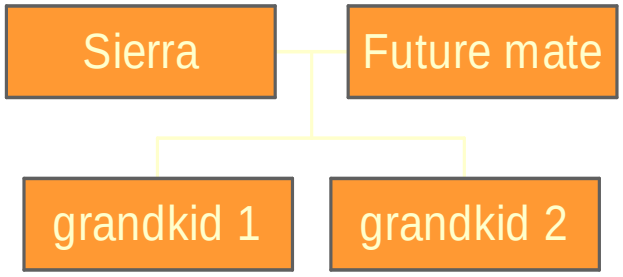
Lee family pedigree
Hair color
D = Dark
d = blonde

Name	Genotype	Gametes possible
Steve	D,D	D
Julie	d,d	d



•	d	d
•D	•Dd	Dd
•D	•Dd	Dd

Punnett Square



Basic Steps of Forensic DNA

- **Screening**- Is it there?
  - Detect biological samples-blood, semen, saliva
  - Presumptive and Confirmatory Tests
- **Extraction**- Can you get the DNA out?
  - Isolate DNA from other cellular materials
  - Uses mechanical disruption and chemicals
- **Quantification**- How much and how good is it?
  - Evaluate quantity and quality
  - Can be done by DNA gel electrophoresis (see sizes)
- **Distinguish**- What is the DNA type?
  - Using RFLP or PCR compare to suspects and victims
- **Interpret**- How powerful is the result?
  - Estimate statistical significance with population genetics

Review: DNA is organized inside the cell nucleus and mitochondria

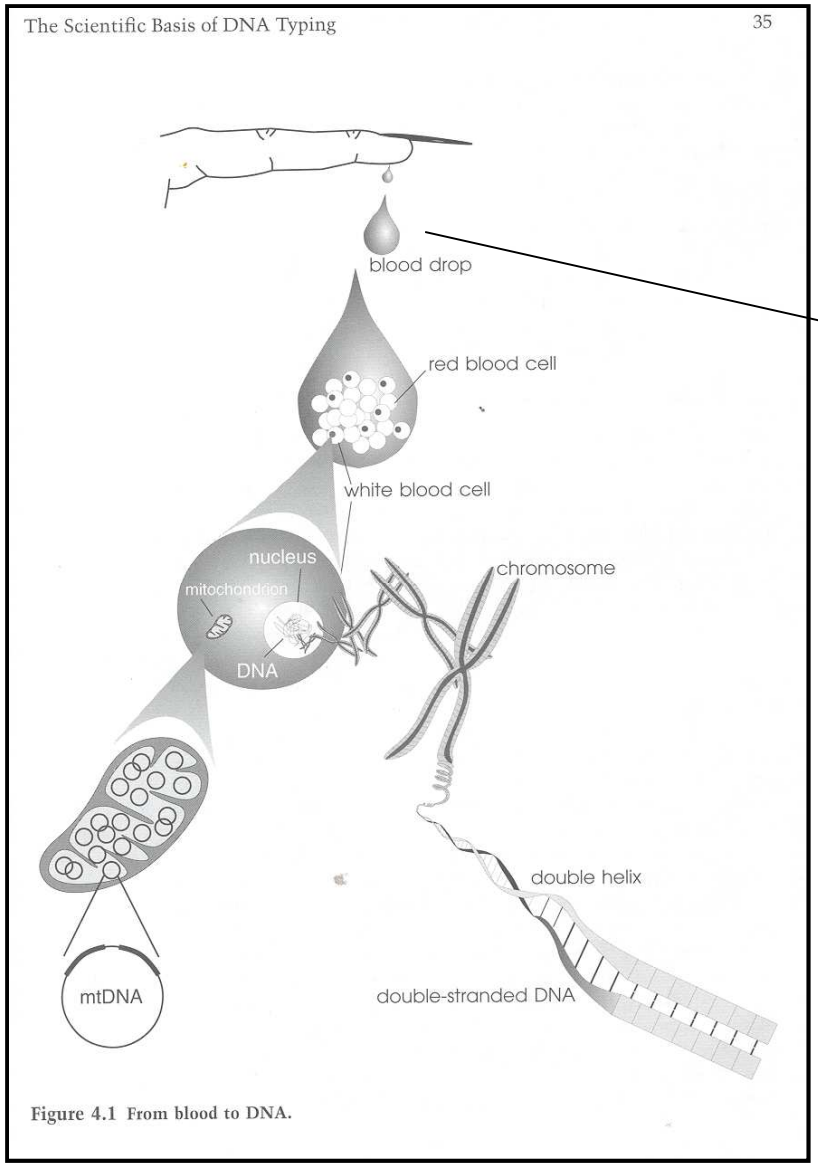


Figure 4.1 From blood to DNA.

Biological Fluids ?

- What are they?
- Forensic Value ?
  - Cells
- Most commonly analyzed

BODY FLUIDS	Cell Types
Blood	White Blood Cells
Semen	Spermatozoa
Saliva	Skin Cell

Blood as Physical Evidence

- **Occurrence of a blood stain in a certain place**
  - on an item may substantiate an account of a crime

- **Bloodstain Pattern Interpretation:**
  - Shape, position, size or intensity of a bloodstain
  - may support a particular sequence of events

- **DNA typing** analysis can be used to eliminate
- whole groups of people as suspects

Forensic Identification of Blood

Two categories of identification tests:

- **Presumptive or preliminary test**
  - Used for screening specimens that might contain the substance or material of interest
  - Both false positive and false negative results may be obtained
- **Confirmatory test**
  - Are tests which are entirely specific for the substance or material for which it is intended
  - A positive confirmatory test is interpreted as an unequivocal demonstration that the specimen contains the substance or material

Shape,

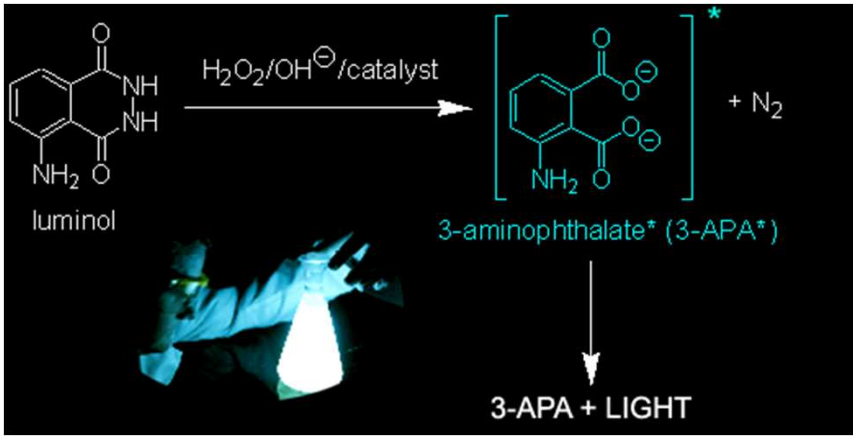
Position,

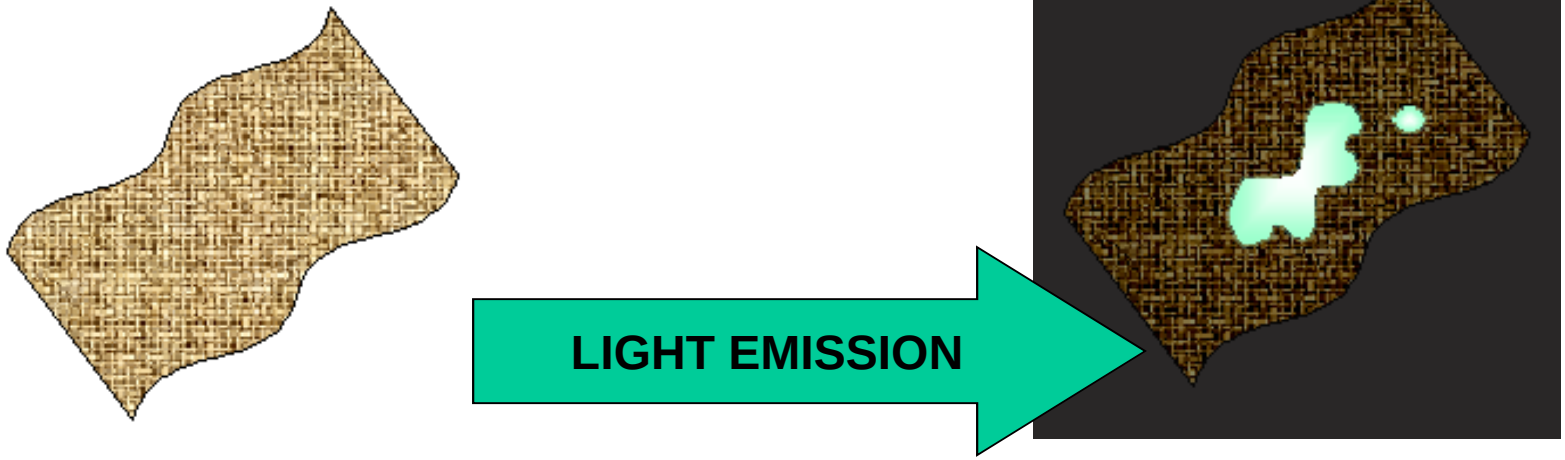
Size ?

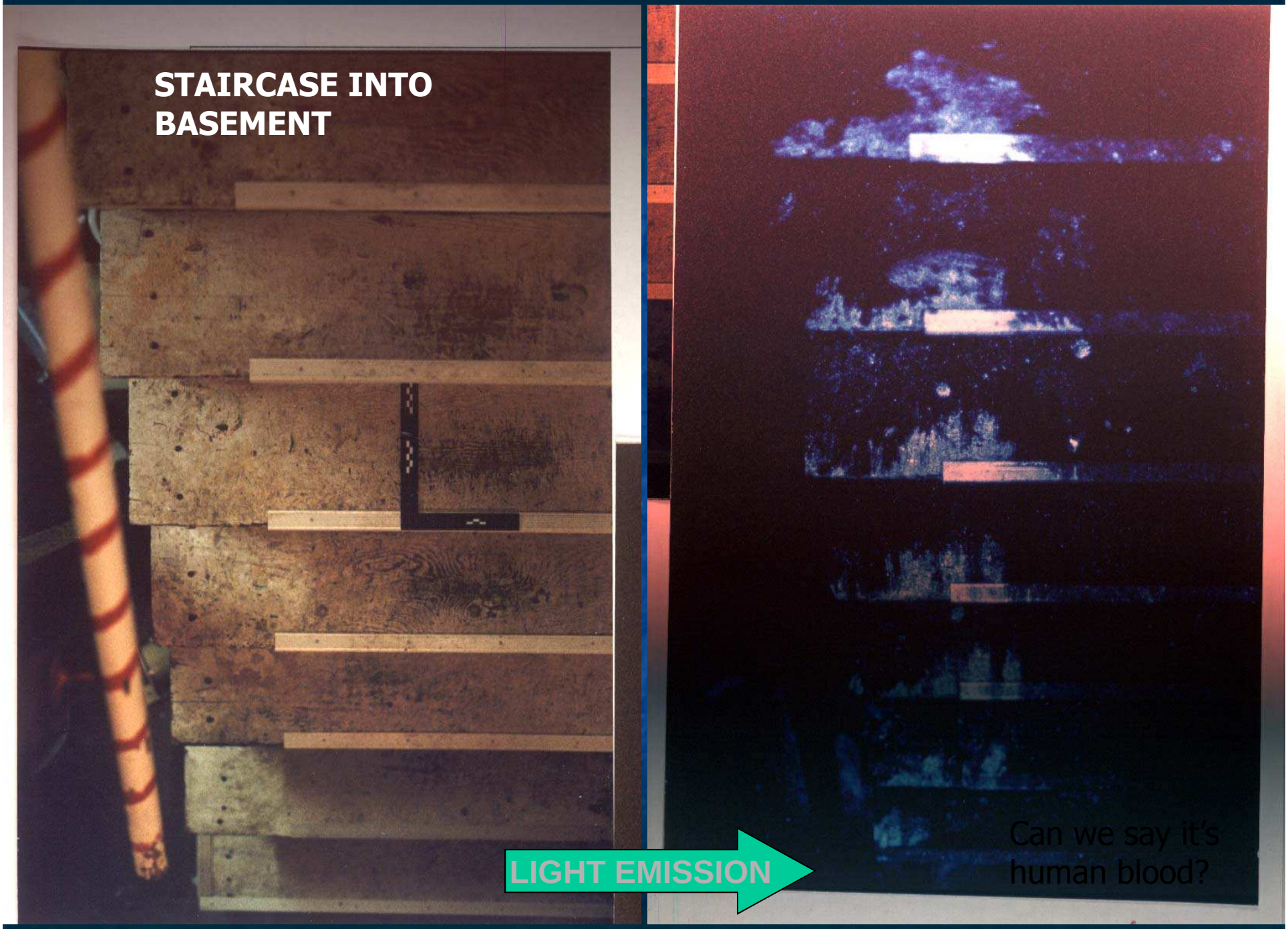
Neck incisions (scene)

Forensic Characterization of Bloodstains cont'd

- **Luminol Test**
- Produces light rather than color
  - Typically sprayed onto suspected stains to reveal stains & patterns

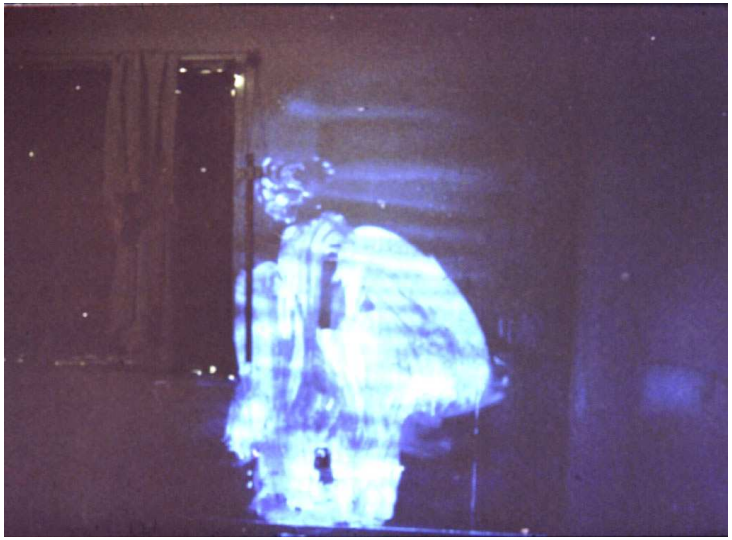





STAIRCASE INTO BASEMENT
LIGHT EMISSION
Can we say it's human blood?

Presumptive Test for Blood
Case Example : Homicide Scene

Natural Light
No treatment



Darkened Room
Luminol Treated

Forensic Characterization of Bloodstains using Digital Imaging

- Bloodstains that are on an item that's dark or has a complex pattern can be difficult to see
- A digital imaging system with an infrared filter attached can make bloodstains more visible by filtering out the background color


仰勇男戦赤
仰勇男戦赤
龍

VI. Forensic Identification of Blood

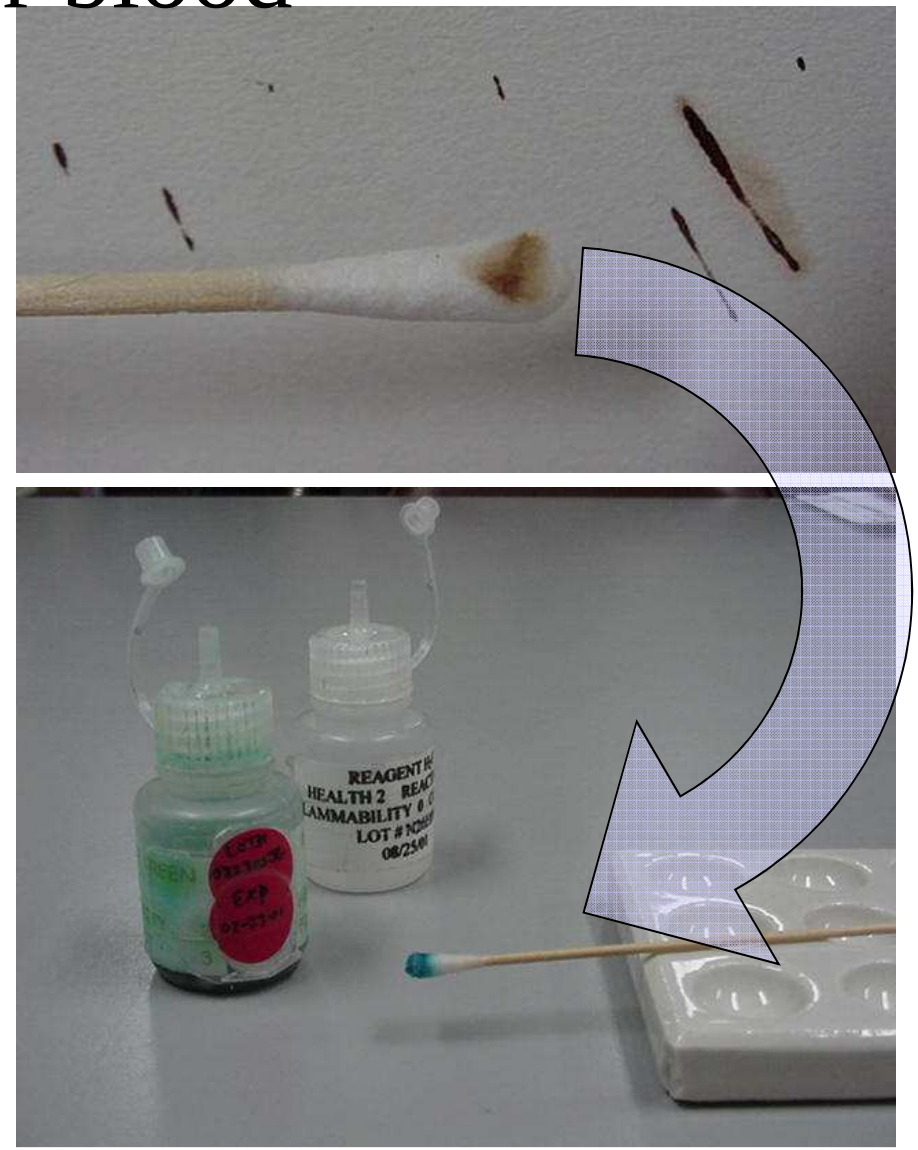
Presumptive Tests for Blood:

- Presumptive blood tests are used to screen evidence for the possible presence of blood
- Most are **color tests and are based on the peroxidase-like activity of hemoglobin**
- Peroxidase catalyzes the following reaction
- Reduced Dye + peroxide --> Oxidized dye + water
- The presence of hemoglobin catalyzes the reaction, forming a colored dye product
- Positive presumptive tests do not prove that blood is present

Presumptive Test for blood

Is This Blood??

- **Chemical color tests**
  - Based on hemoglobin's peroxidase-like activity (Peroxidase: enzyme that oxidizes organic compounds)
  - Ex: **Lab**/**phenolphthalein**, O-tolidine, **Crime Scene**/Hemastix
  - *Advantage*: very sensitive
  - *Disadvantage*:
    - false positive rxn
      - Potato/Horseradish
      - Strong oxidizers like bleach

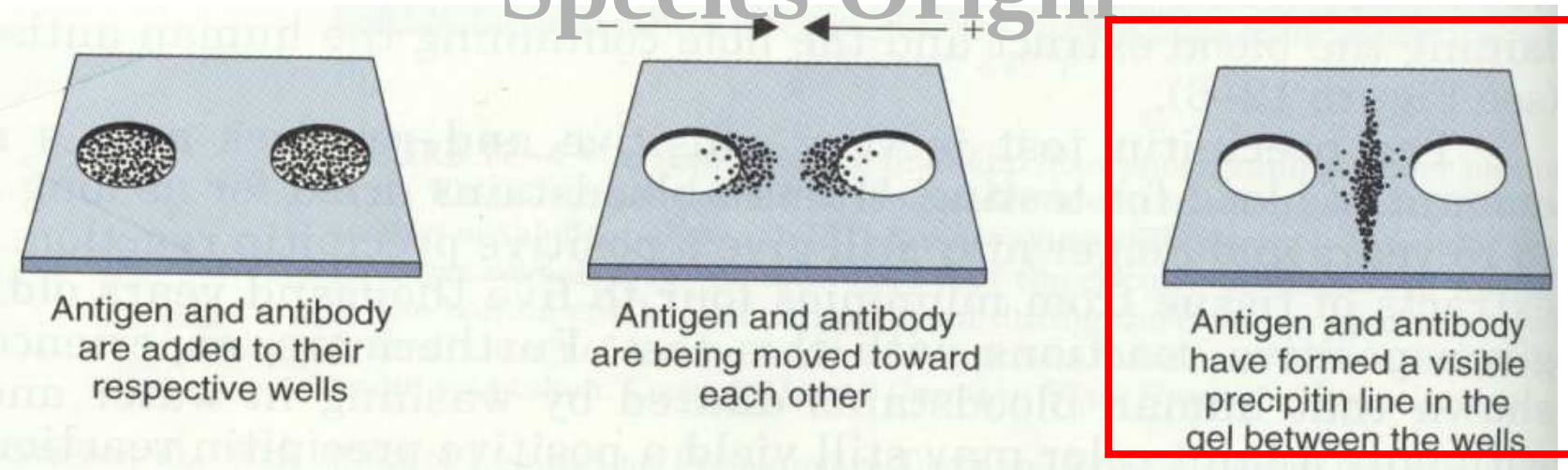


Confirmatory Tests for Bloodstains

- **Micro Crystal Tests**
  - Takayama
  - Tiechman
- *Advantages*
  - More specific then chemical test
- *Disadvantages*
  - Not as sensitive
  - More susceptible to interference

Confirmatory Test - Bloodstains:

Species Origin



A stain is used to visualize precipitin band

Hands-on- Phenolphthalein

- Presumptive blood test
- Chemical indicator phenolphthalein is used to detect the possible presence of hemoglobin.
- **Peroxidase-like activity of hemoglobin in blood to catalyze the oxidation of phenolphthalin (the colorless reduced form of phenolphthalein) into phenolphthalein, which is visible as a bright pink color.**

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

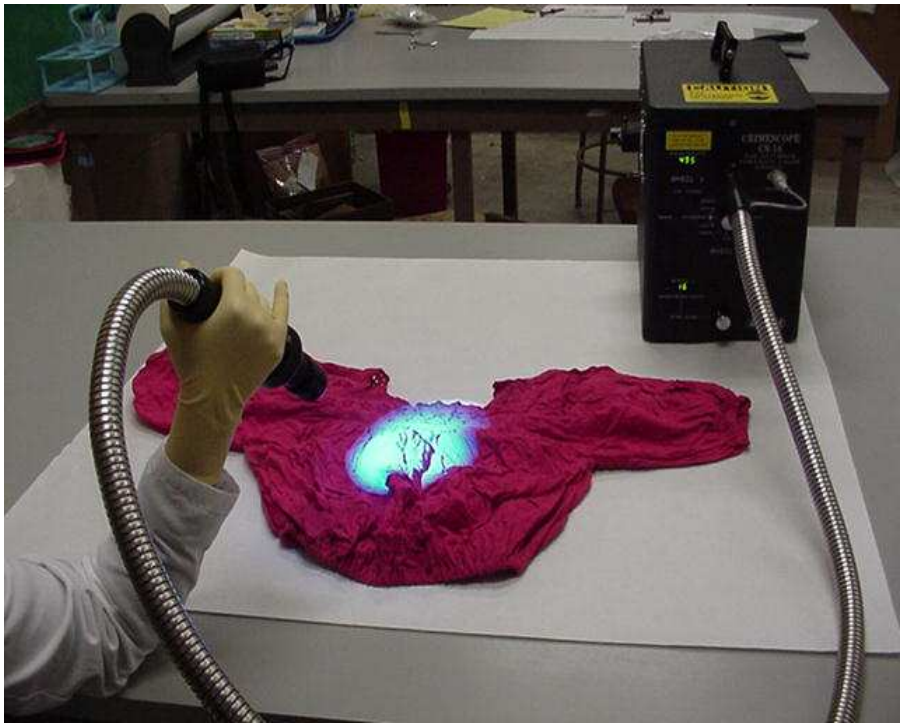
Blood Test

- Put on gloves
- Take out your kit droppers
- Alcohol, Phenolphthalein and Peroxidase
- Conduct your negative control first
- Add 1 drop alcohol
- Add 1 drop Phenolphthalen
- Add 1 drop Peroxidase
- Record any color change you observe
- Repeat for negative control
- Repeat for your crime scene stain- need to swab with water first

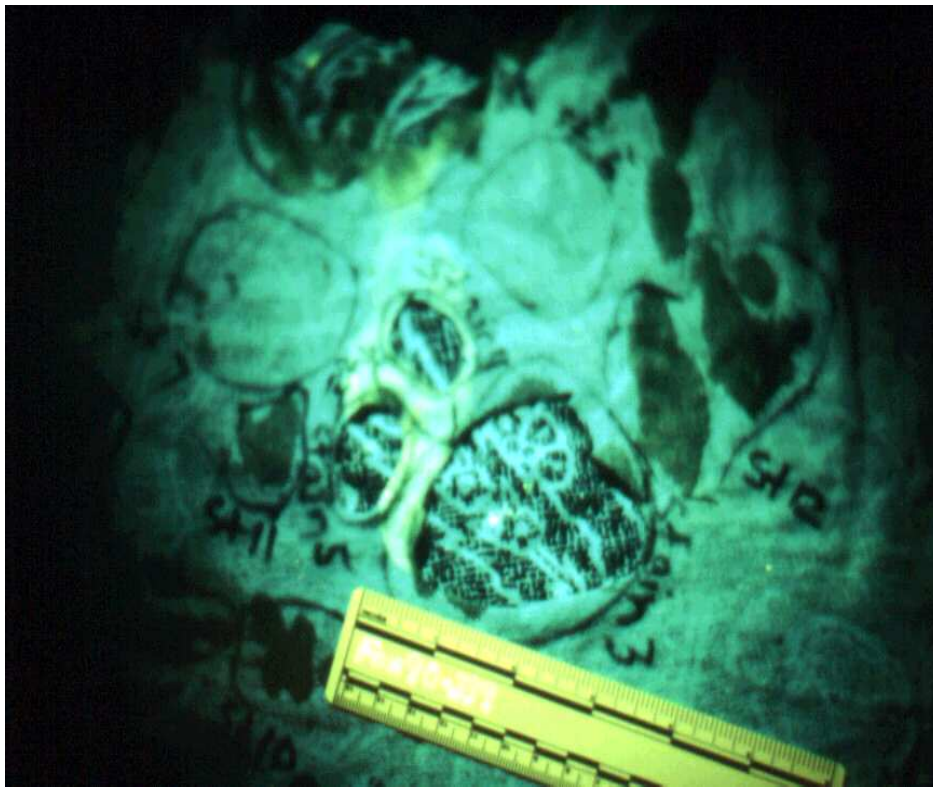
Questions for Blood Presumptive

- 1. Why do we run a positive control? Negative control?
- 2. Would you trust the results if your positive control did not work? Why?
- 3. Would you trust your results if your negative control did not work? Why?
- 4. Was your crime scene stain positive or negative? Put your results up on the board.
- 5. Did all teams have their positive control work? If not, what are some possible explanations for the unexpected result?
- 6. For one explanation, design an experiment to test your explanation. Use controls.

Forensic Characterization of Semen: 1. VISUALIZATION



MANY BODY FLUIDS FLUORESCE WITH ALTERNATE LIGHTING SOURCES

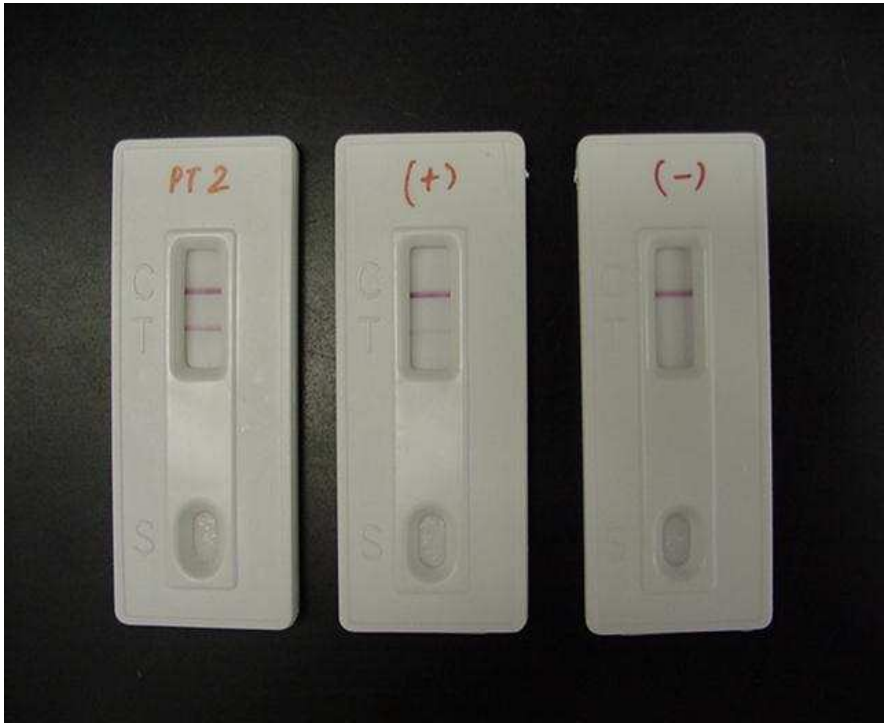


Forensic Characterization of Semen: 2. PRESUMPTIVE TESTING

ACID PHOSPHATASE ENZYME / FOUND IN LARGE CONCENTRATIONS IN SEMEN

Forensic Characterization of Semen: 3. CONFIRMATION

A. MICROSCOPIC IDENTIFICATION OF SPERM



B. DETECTION OF P30, A MALE PROSTATE PROTEIN. USEFULL FOR VASECTOMIZED MALES

Hands-on Demo

- Fluorescence Test – Demo by instructor

  Controls- Positive, Negative, Stain
- Be sure you put on the safety glasses as UV is dangerous and should not be looked at directly with the naked eye.
- One by one, each team will come up to visualize the stains with the instructor. In teams determine if you detect ‘semen’ on the crime scene stain.
- Sketch and record the stain or stains you detect.
- If you see UV fluorescence it is consistent with detection of semen indicating the crime may be rape-homicide

Questions for Semen Presumptive

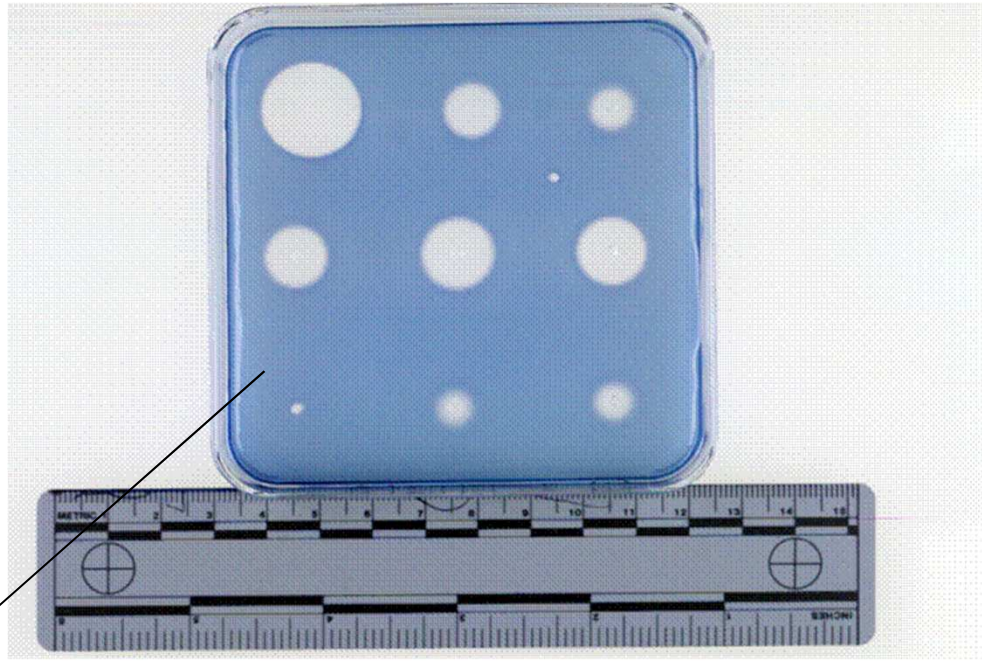
- 1) Does this indicate with 100% certainty that there was semen at the crime scene? Why or why not?
- 2) What are some other reasons you may see fluorescence?

Forensic Characterization of Saliva

- Evidence commonly tested for the presence of saliva includes:
  - Cigarette butts
  - Envelope flaps
  - Swabs taken from the body of sexual assault victims
  - Bottles, cans, & straws

Detection of Amylase

(breaks down starch in gel)



Gel contains starch that is broken down (circles) in the presence of Amylase enzyme

Summary 1

- **Why study DNA**
  - **Law enforcement, evolution, agricultural, and human applications-medical diagnostics**

- **DNA Biology and Genetics**
  - DNA is contained in **cells** –the basic unit of life
  - Found in **nuclei, mitochondria** and chloroplasts
  - Organized in **chromosomes**. Located at positions called **loci** and come in different forms or **alleles**.
  - **Homozygous** if the same, **heterozygous** if different

- **DNA Function and Structure**
  - **D**eoxyribo**N**ucleic **A**cid : blueprints of life
    Replication, Information storage and mutation RIM
  - **Central Dogma**
    DNA------->RNA------>protein
    transcription translation

Summary 2

- **DNA Structure and Function continued**:
  - **Bases of DNA** are Adenine, Guanine, Cytosine and Thymine- Asian Guys Can Teach: **AGCT**
  - **Base pairing is A to T and G to C**- DNA is where its AT
  - Sequence of Bases Store information- Like the sequence of numbers in a Phone Number
  - **Nucleotides** are the building blocks (**dNTPs)** themselves made of phosphate base and sugar= PBS- The only station Sierra and Gabriel can watch
  - DNA base pairs- DNA velcro (David Letterman

Forensic DNA Technology- Saving lives with DNA

I. Summary of DNA structure, function, forensic DNA extraction

II. Learning Objectives

a. Basic Steps of Forensic DNA Analysis
   a. Screen, Extract, Quantify, Distinguish by RFLP vs PCR
b. Laboratories
   a. Hands-on DNA – Who done it? DNA Gel Electrophoresis

Define cell, nucleus, chromosome, DNA, central dogma, bases and base pairs of DNA, alleles, homozygous vs heterozygous

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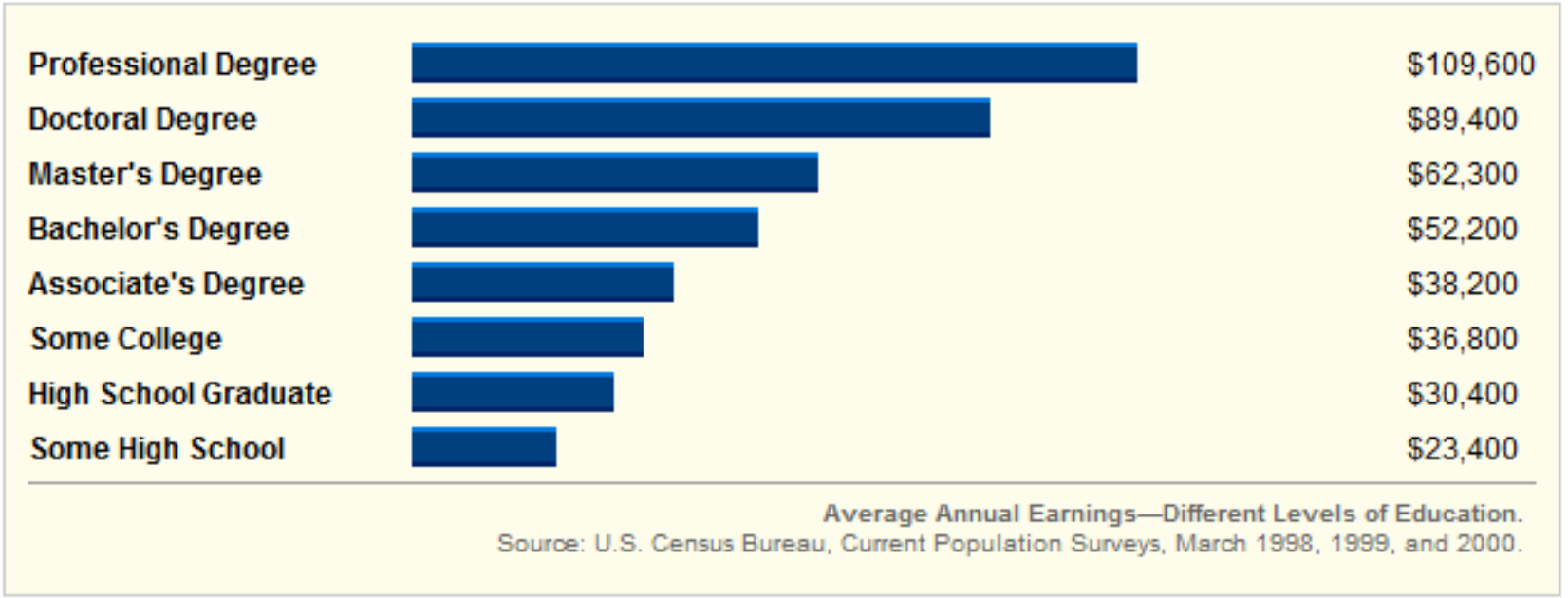
  - DNA base pairs- DNA velcro (David Letterman
- **Uses of DNA- Casework, Agriculture, Medicine, History, Business, Diagnostics**
- **Forensic DNA Steps- SEQ DI**
- **Screening**
  - **Presumptive Tests vs Confirmatory Tests**
  - **Blood Presumptive tests- Luminol, PT (phenolphthalein- aka Kastle-Meyer)**
  - **Semen Presumptive tests- UV, stain, Acid Phosphatase**
  - **Saliva Presumptive test- Amylase**
- **Extraction- Mechanically open cells, add detergent to lyse membranes, isolate and purify with alcohol**

Average Salary by Education Level

http://www.earnmydegree.com/online-education/learning-center/education-value.html

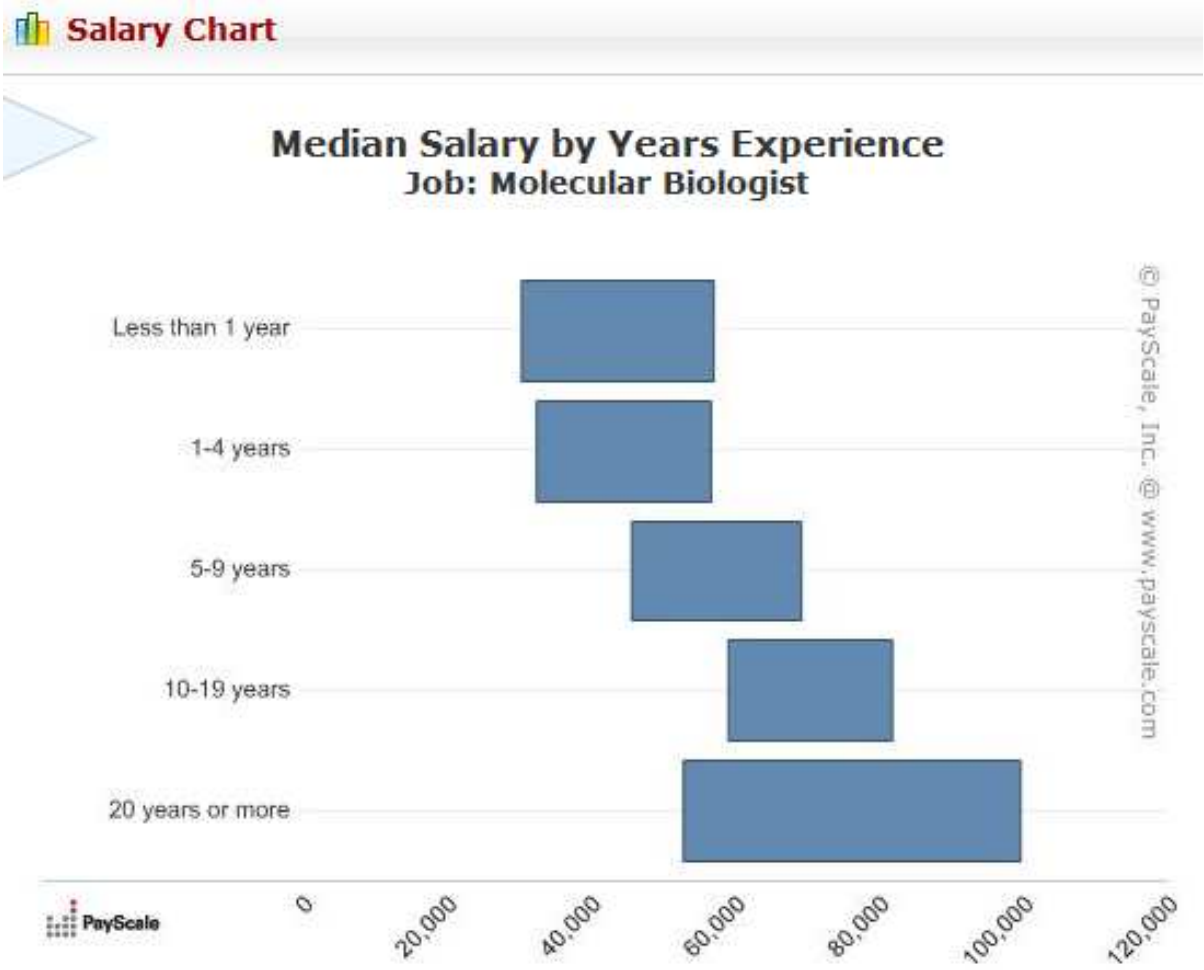
Today, a formal, focused education is an essential ingredient. Employers have increasingly used diplomas and degrees as a way to screen applicants. And once you've landed the job you want, your salary will reflect your credentials. On average, a person with a Master's degree **earns $31,900 more per year** than a high school graduate—a difference of as much as 105%!

Average Annual Earnings for College Graduates and Non-Graduates



Average Annual Earnings—Different Levels of Education.
Source: U.S. Census Bureau, Current Population Surveys, March 1998, 1999, and 2000.

Average Salary – Molecular Biologist – Forensic Biologist



Types of DNA Variation

- Length Variation

(GATA)(GATA)(GATA)(GATA)

  - short tandem repeats (STRs)

(CA)(CA)(CA)(CA(CA)(CA)

    - microsatellites
    - simple sequence repeats (SSRs)
  - minisatellites (VNTRs)- RFLP

(G/A)

- Sequence Variation
  - single nucleotide polymorphisms (SNPs)
  - insertions/deletions

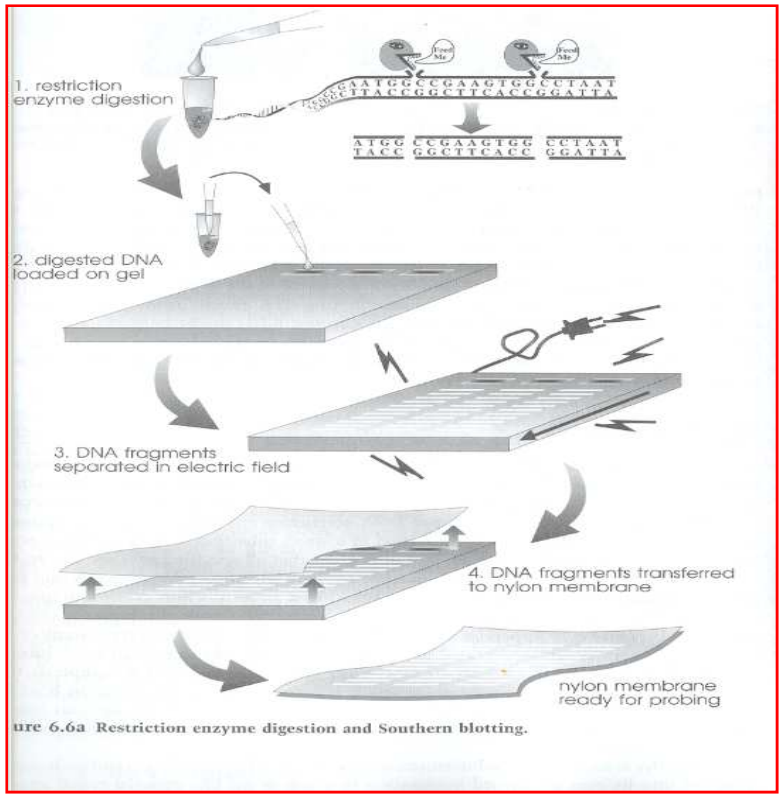
Many different forms (alleles) of a particular gene location (locus) is one criterion for forensic DNA markers

You have *A* better CHANCE of distinguishing *individuals with*

many types

Total # genotypes- $\frac{n\,(n+1)}{2}$

RFLP- Rudin and Inman



ure 6.6a Restriction enzyme digestion and Southern blotting.

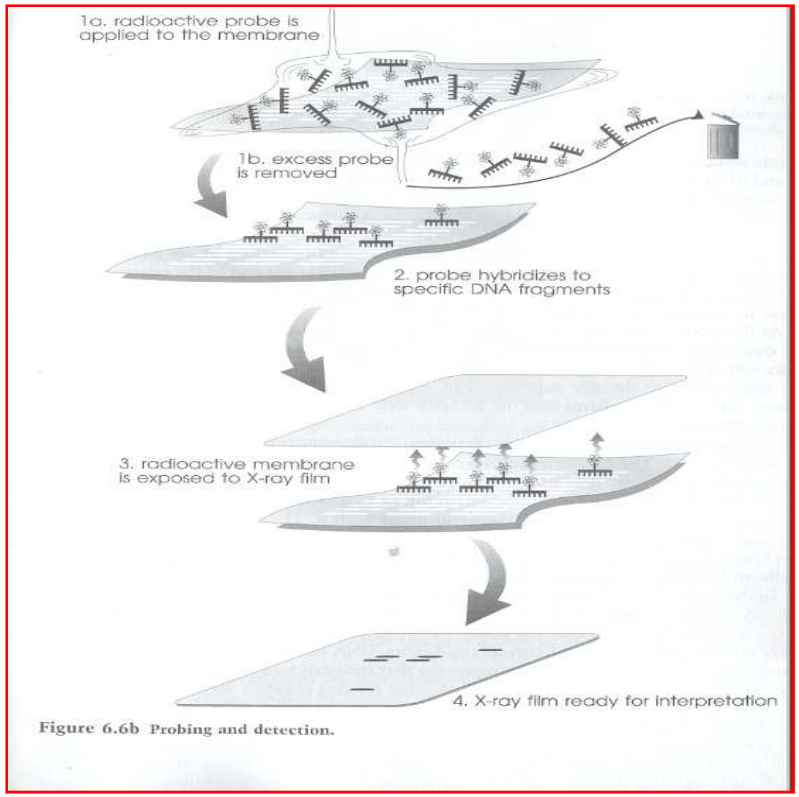


Figure 6.6b Probing and detection.

DNA Methods

1) Extract
2) Quantitate
3) Distinguish
   - Size
   - Content

RFLP : Restriction Fragment Length Polymorphisms
PCR: Polymerase Chain Reaction

RFLP methods require large amounts of undegraded DNA and the process takes 1-2 weeks.
PCR methods require only small amounts of DNA, are useful on degraded DNA and require much less time (as little as 1-2 days in some cases).

- The base sequence can exhibit differences in length and content between individuals.

Christopher Drew	... AAAGAAAGAAGAAAC...
Ben Gibbard	... AAAGAAAGAAGA...
Tyson Ritters	... AAAGAAAGAAGT...
Billy Idol	... AAAGAAAGAAGA...
Stevie Nicks	... AAAGAAAGAA...
Hayley Williams	... AAAGAAAGA...
Kid Cudi	... AAAGAAAGC...
Bill Whithers	... AAAGAAAGT...
Too Short	... AAAGAAAG…

- **Although different between individuals* DNA is identical in every cell of an individuals body** Some exceptions*identical twins**diseased individuals, mtDNA** (sport analogs)

Restriction Fragment Length Polymorphisms (RFLP)

CD	...GG|CCAAAGAAAGAAAGG|CC...	15
BG	...GG|CCAAAGAAACGG|CC...	12
SL	...GG|CCAAAGAATGG|CC...	11

GGTTTCTT : Probe

1) Cut DNA with Restriction Enzymes that recognize specific sequences : GGCC.

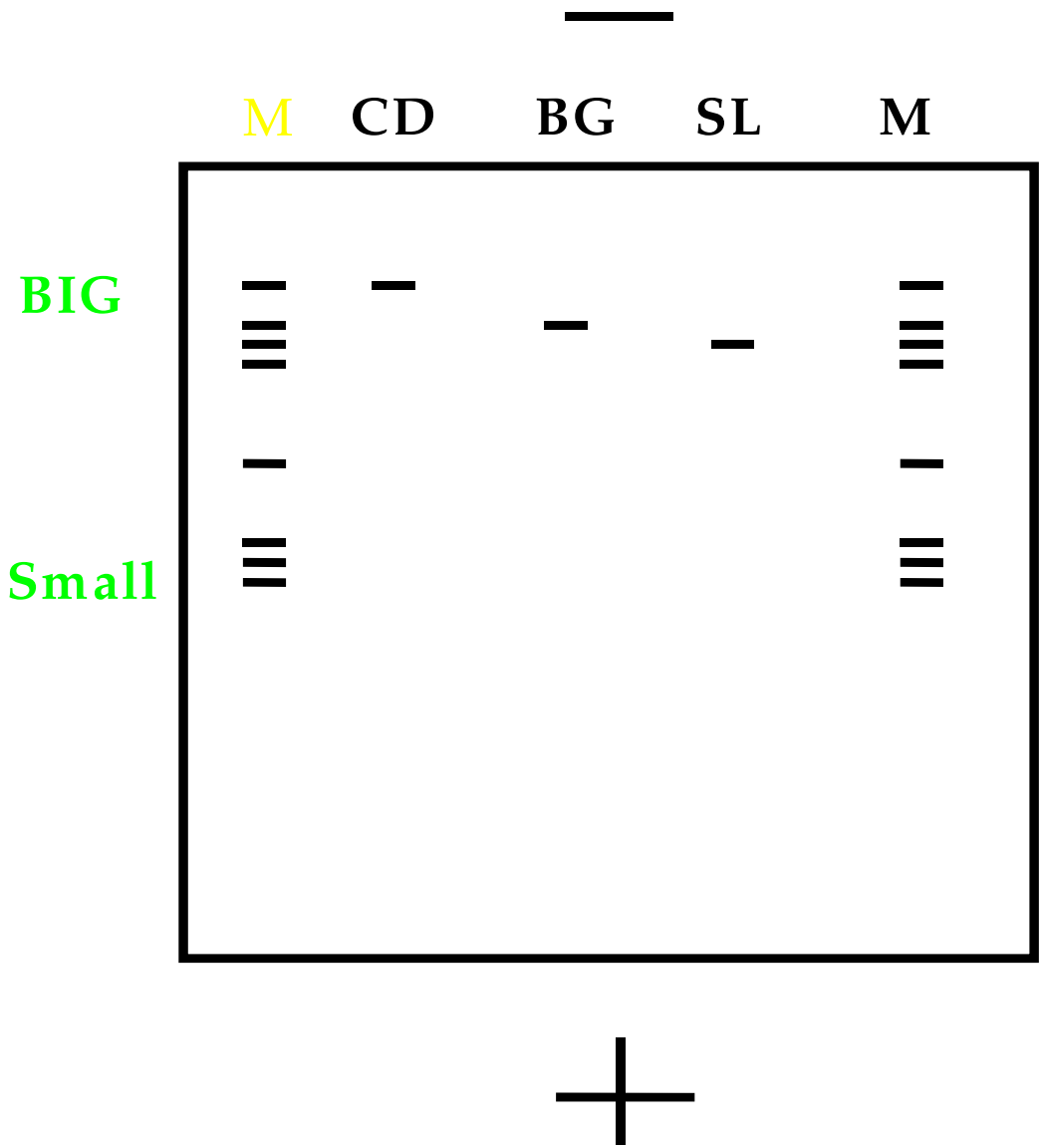
2) Separate the many fragments produced by gel electrophoresis. The fragments represent a wide range of sizes.

3) Blot and probe the fragments with specific DNA sequences that base pair only with those that contain the sequence of interest.

RFLP

Gel Electrophoresis separates fragments by size

DNA Probe base pairs with specific fragments



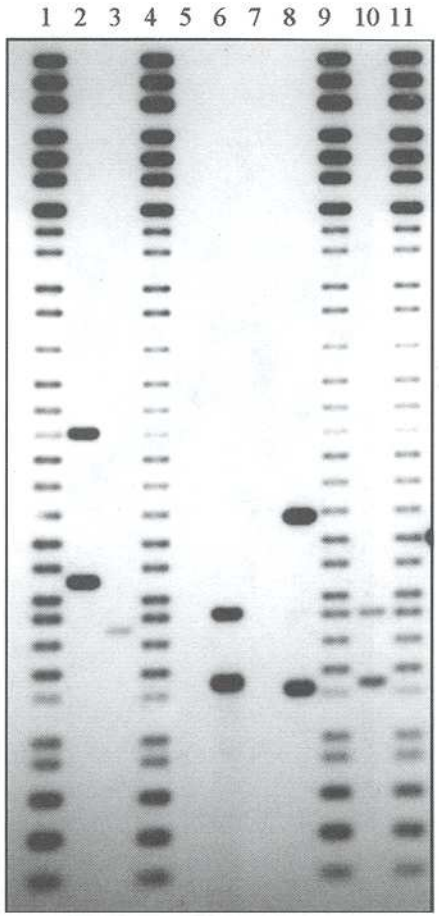
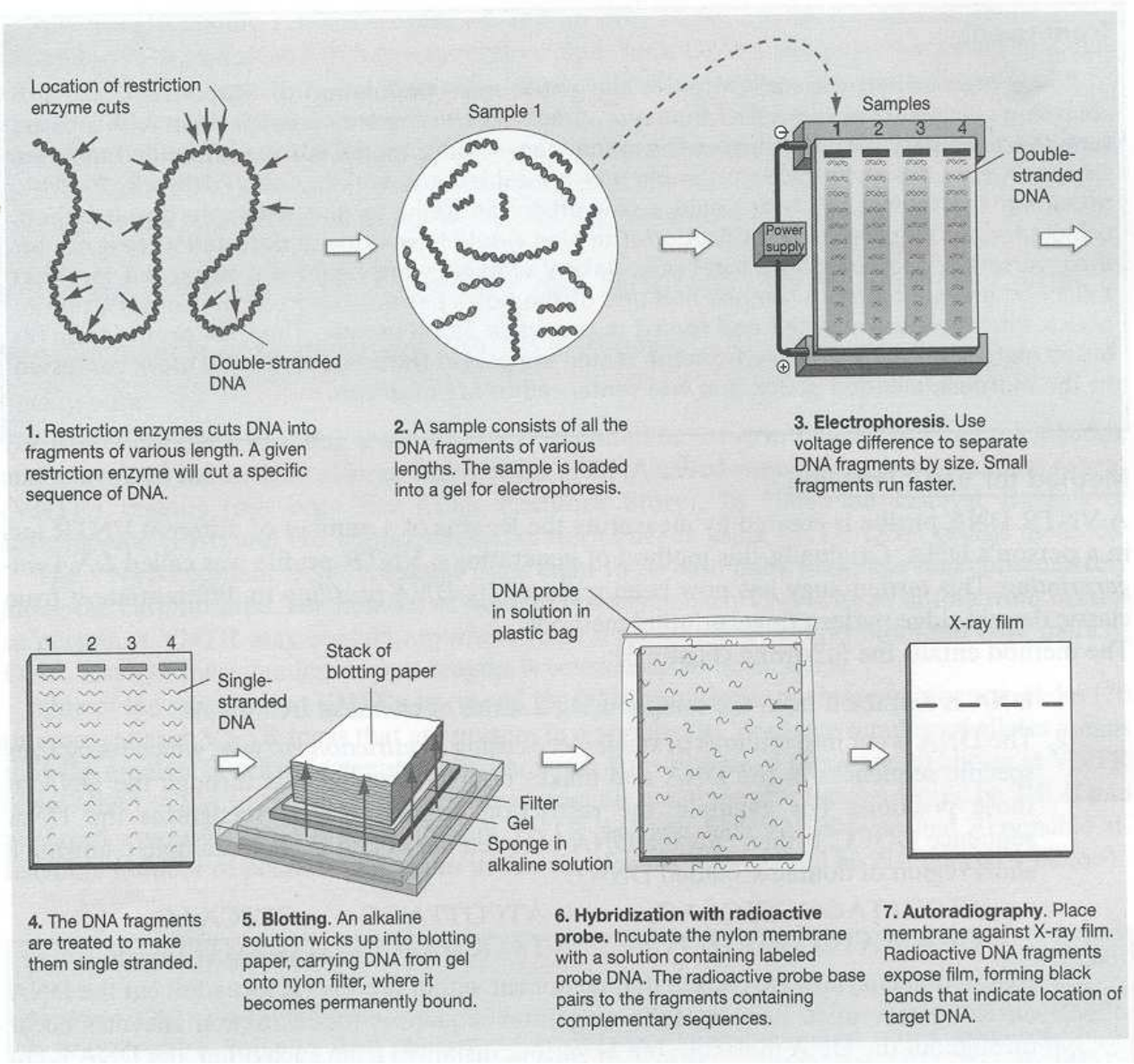


Figure 6.9 An RFLP autorad. The locus probed is D2S44. Lanes 1, 4, 9, and 11 contain molecular ladders. Lanes 5 and 7 contain no sample. The pattern of bands in lanes 6 and 10 appear indistinguishable. All other samples show different patterns, both from these two and from each other.

RFLP Spencer



Restriction Fragment Length Polymorphism Demo

1) Take a sheet of the colored paper
2) A= Red T= Blue G= Green C= Yellow
2) DNA sequence is 5’ GGCCATGATCATGTCAAG
3) Need enzyme = Hae III restriction enzyme cut –GG/CC
4) Power supply= person to turn on and off the gel
5) We will use the classroom desks as the agarose in the gel that is the classroom itself.
6) Run the gel and observe the migration of fragments
7) DNA is negative and runs to the positive electrode

Comparison of RFLP and PCR

Characteristic	RFLP Methods	PCR Methods
Time required to obtain results	6-8 weeks with radioactive probes; ~1 week with chemiluminescent probes	1-2 days
Amount of DNA needed	50-500 ng	0.1-1 ng
Condition of DNA needed	high molecular weight, intact DNA	may be highly degraded
Capable of handling sample mixtures	Yes (single locus probes)	Yes
Allele identification	Binning required	Discrete alleles obtained
Power of Discrimination	~1 in 1 billion with 6 loci	~1 in 1 billion with 8-13 loci (requires more loci)

Questions for DNA Gel Electrophoresis – Who Done it?

1) From the photograph, visually assess the DNA types of the suspects 1 to 5.
2) Can you include any suspect as the donor of the blood at the crime scene?
3) Can you exclude others with certainty?
4) If you were to be asked to go to court and testify that your selected suspect was the murderer, would you do it under oath? Why of why not?
5) Why did some fragments move further in the gel?
6) What sample is missing from the gel that would help to provide a size to the fragments?

Your turn to develop a 2 minute poem, chant, skit, rap etc. to summarize a topic from the lectures/labs.

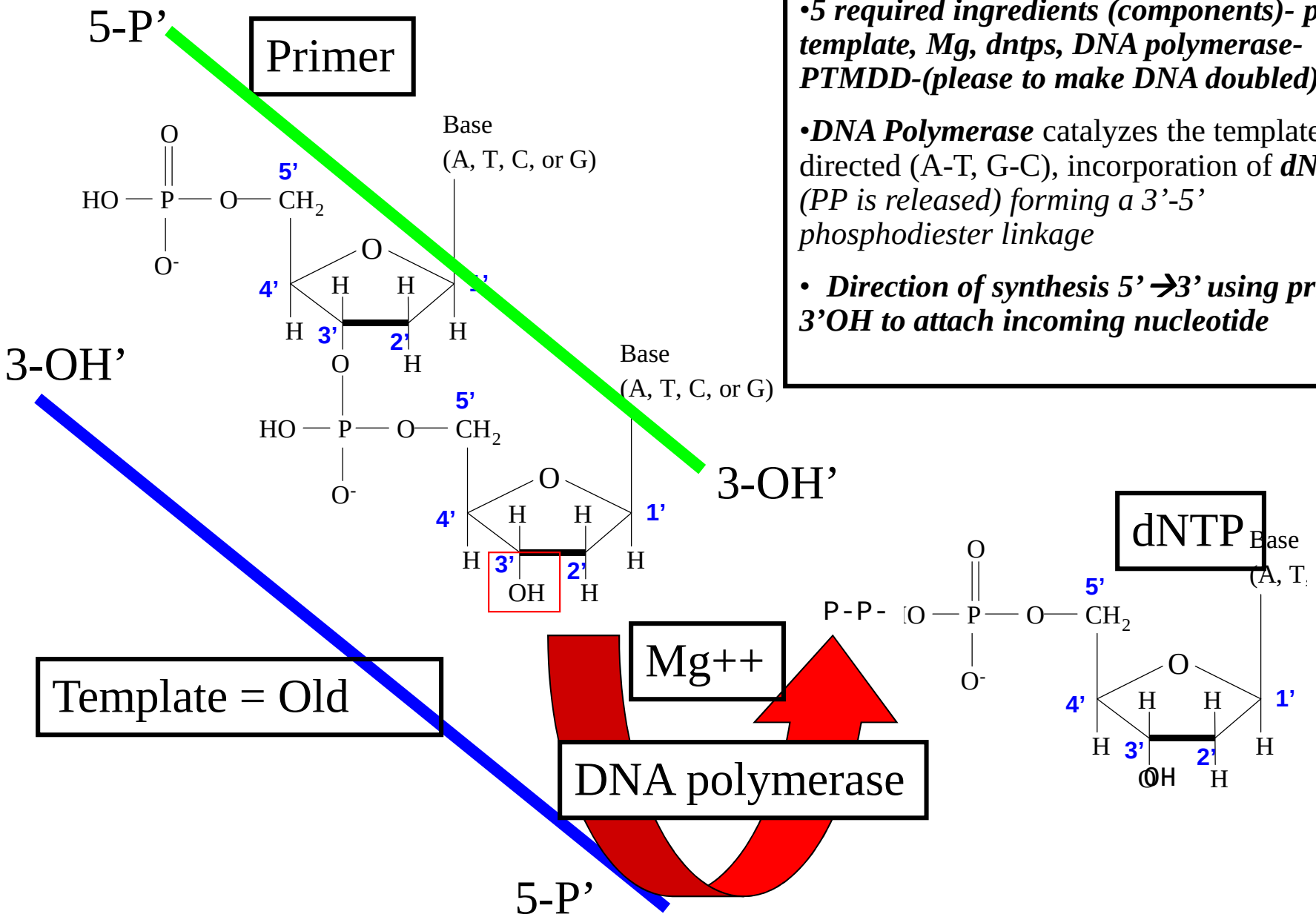
Polymerase Chain Reaction:
PCR is simply repeated rounds of DNA replication

PCR based systems are rapid,
require less material than RFLP
and less time for typing

- Molecular xeroxing
- Calvin and Hobbes example

PCR: repeated rounds of DNA Replication

- ***5 required ingredients (components)- primer, template, Mg, dntps, DNA polymerase- PTMDD-(please to make DNA doubled)***
- ***DNA Polymerase*** catalyzes the template directed (A-T, G-C), incorporation of ***dNTPs*** *(PP is released) forming a 3'-5' phosphodiester linkage*
- ***Direction of synthesis 5'→3' using primer 3'OH to attach incoming nucleotide***



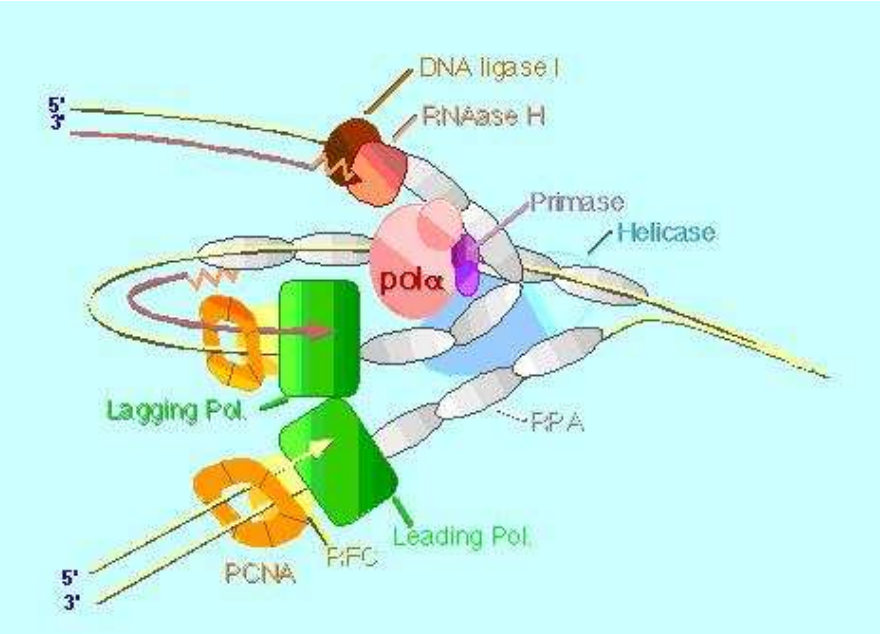
Replication of DNA is Semi Conservative
One old and one new

http://dir.niehs.nih.gov/dirlmg/repl.html

Enzymes of Replication

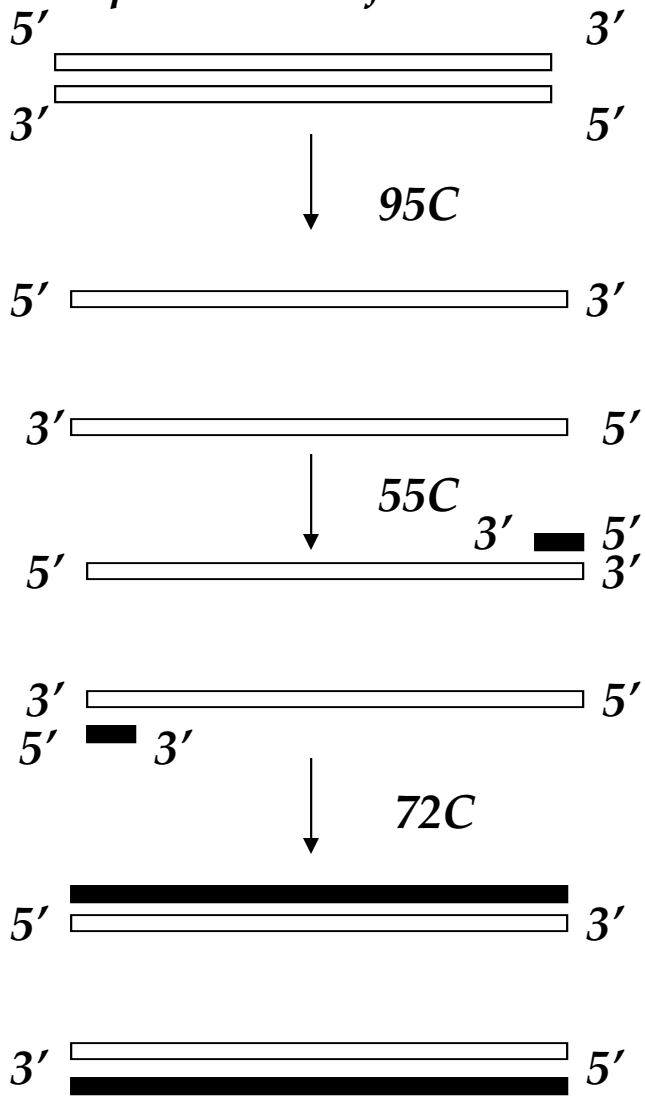
DNA is replicated or copied in our cells. When completed, the new double strands consist of one old template and one newly made strand- This is called semi conservative replication.

There are many enzymes that are required. They include unwinding (helicases, gyrases), priming (primases), copying (DNA polymerases) and touch up enzymes (DNA ligases).



PCR is simply repeated rounds of DNA replication

Template- DNA from blood etc.



Step 1: Denature
Separate H bonds with heat at 95C

Step 2: Anneal
Primers bind at lower temp 55C

Step 3: Extend

Taq polymerase extends primer 3′OH

at 72C (dNTPs and Mg++)

Step 4: Repeated 28-30 rounds of D, A, E

PCR Number of Target Molecules Created

Cycle Number	Number of Double-stranded Target Molecules
1	0
2	0
3	2
4	4
5	8
6	16
7	32
8	64
9	128
10	256
11	512
12	1024
13	2048
14	4096
15	8192
16	16,384
17	32,768
18	65,536
19	131,072
20	262,144
21	524,288
22	1,048,576
23	2,097,152
24	4,194,304
25	8,388,608
26	16,777,216
27	33,544,432
28	67,108,864
29	134,217,728
30	268,435,456
31	536,870,912
32	1,073,741,824

- Bank account paying 100% interest every 5 minutes
- Swimming pool - 10 drops

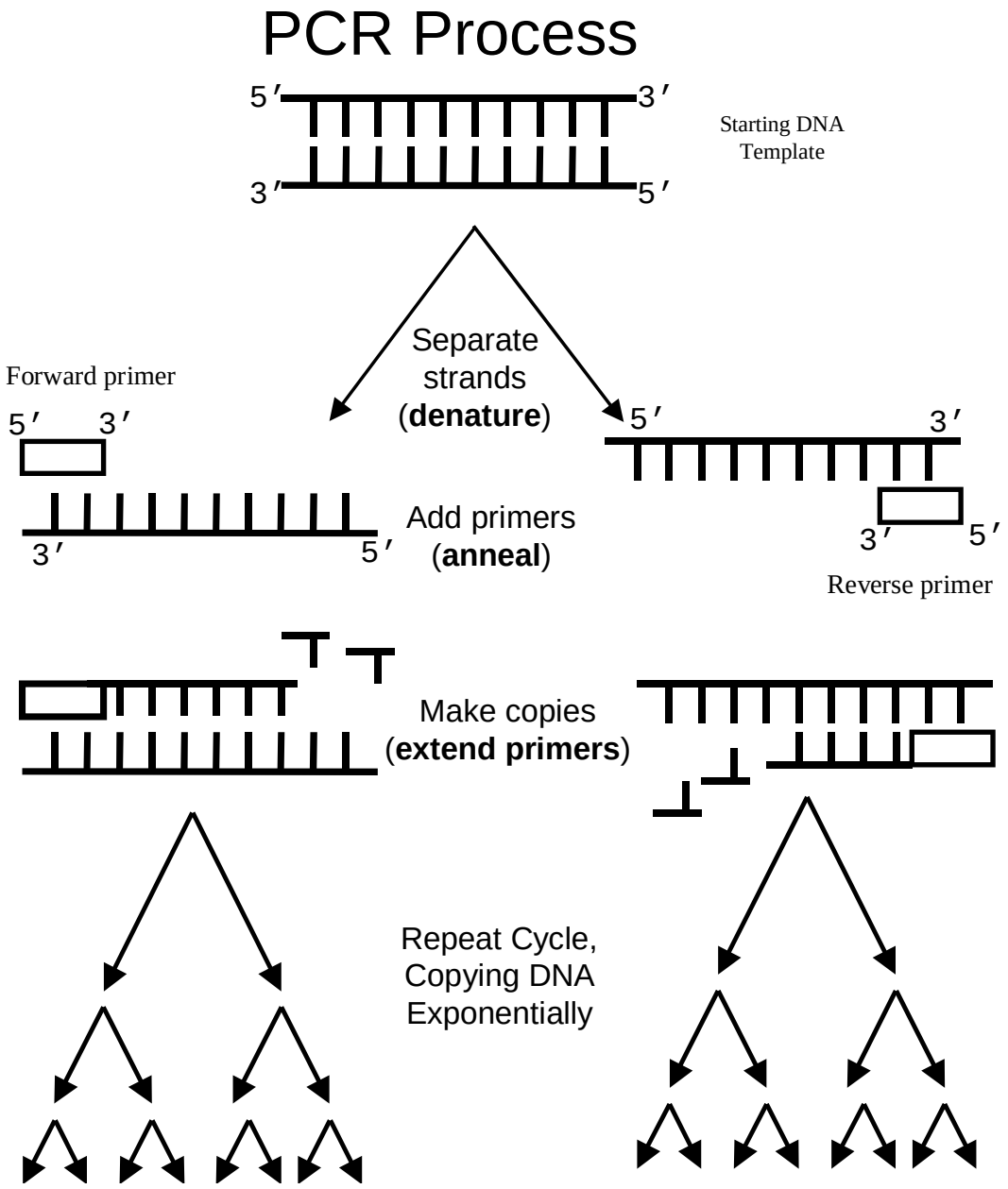
PCR Contamination Prevention

Contamination prevention

- **Separation of pre and post PCR areas**
- **Use of dedicated equipment**
- **Aerosol pipette tips**
- **Controls: Negative, Positive, Stochastic**
- **Process one sample at a time,**
- **Separate reference samples from evidence**
- **Avoid splashing**
- **Wear protective gear and reagent prep care**
- **Do not move from PCR area into non PCR area without decontamination**

•*PCR: Primers, Template, Mg,dNTPs,Taq*

•Please to make DNA twice



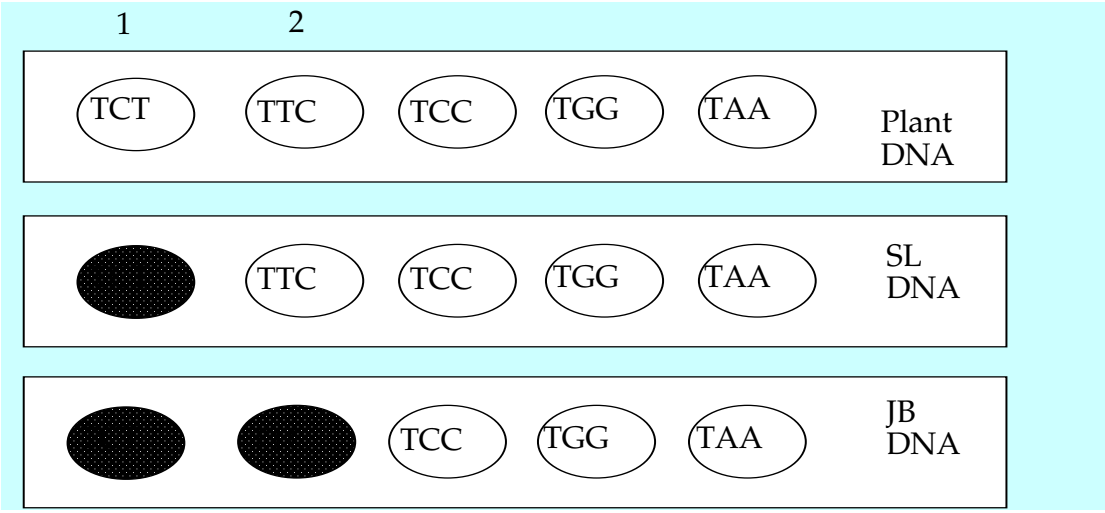
PCR ‘quiz’

- Template =
- **5′GGACTCCTATGTATGTATGCTTTAAGGCA 3′**
  3′CCTGAGGATACATACATACGAAATTCCGT 5′
- ***Design two primers*** (five bases long): Remember-the 3’ OH end will be extended and DNA is antiparallel
- Make the primers 5 bases long on each side.
- Be sure to amplify the entire template.
- ***List the other required components***, ***materials and procedure*** needed to conduct a successful PCR reaction
-

Once amplified detection can be done by DNA battleship

DNA probes can detect specific fragments by base pairing (complementation:hybridization)

```
                1    2
PROBE         TCT  TTC
JB      AAAGAAAGCCAG
JM      AAAGAAGGCC
MW      AAAGAAACCC
LG       AAAGAACCC
SL       AAAGAATT
Plant    AAATTCCC
```



Dot blot hybridization or macroarray

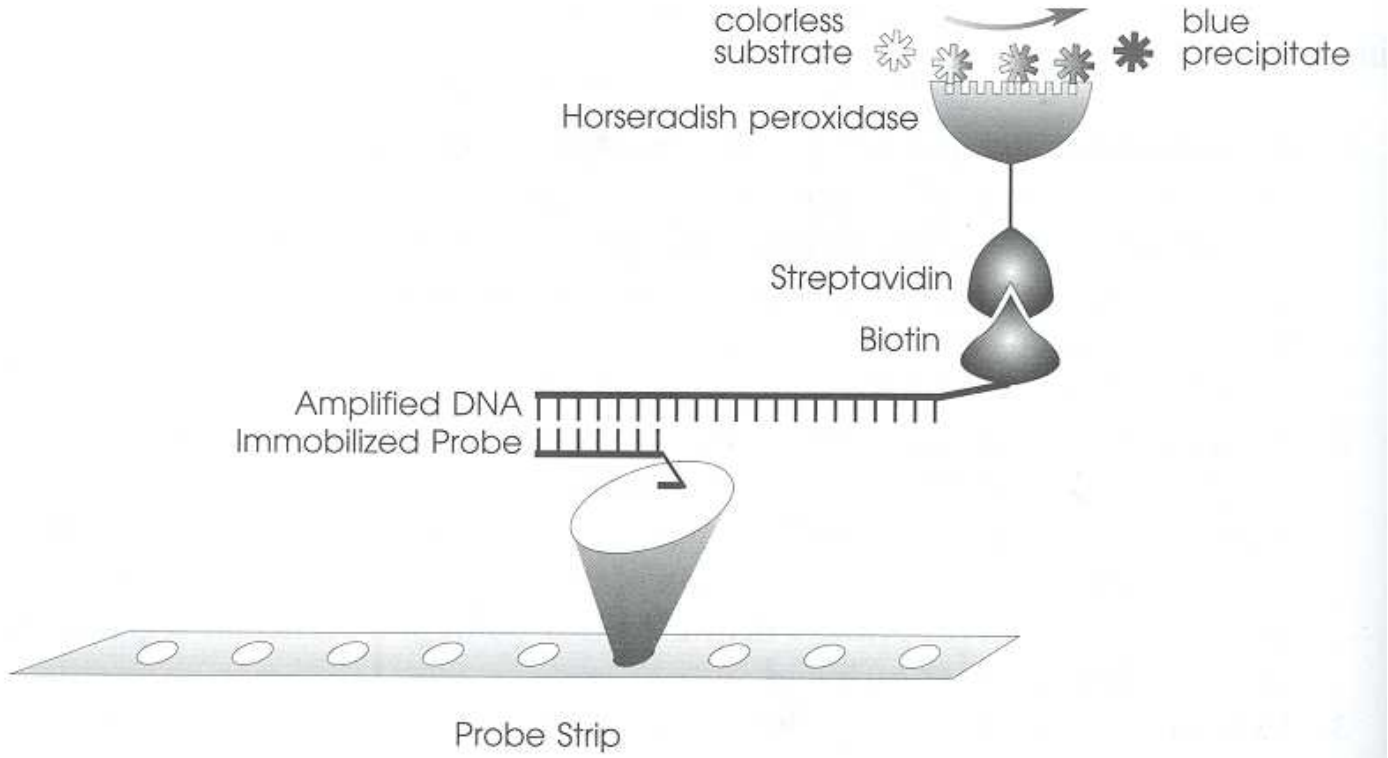


Figure 6.10 Detection of PCR product on a reverse dot blot.

Relative power of tests

Test type		time	power
RFLP-VNTR		weeks	+++ *
PCR:			
DQAlpha-	macroarray	1 day	+
PM -	macroarray	1 day	++
D1S80	- gel- VNTR	2 days	++
STRs	**-gel,CE, arrays**	**2 days**	**+++**
mtDNA-	gel, CE, arrays	2 days	+
alu	-gel, CE, arrays	2 days	++

- * not useful on degraded DNA

Comparison of RFLP and PCR

Characteristic	RFLP Methods	PCR Methods
Time required to obtain results	6-8 weeks with radioactive probes; ~1 week with chemiluminescent probes	1-2 days
Amount of DNA needed	50-500 ng	0.1-1 ng
Condition of DNA needed	high molecular weight, intact DNA	may be highly degraded
Capable of handling sample mixtures	Yes (single locus probes)	Yes
Allele identification	Binning required	Discrete alleles obtained
Power of Discrimination	~1 in 1 billion with 6 loci	~1 in 1 billion with 8-13 loci (requires more loci)

Summary

- Quantification of DNA follows DNA extraction in most forensic DNA laboratories. Goals are to determine the quantity and quality of the extracted DNA as different methods require different amounts of DNA
- RFLP (old) required approximately 50ng of DNA at a minimum. PCR requires as little at 500pg or 100 times less!
- Methods for quantification include yield gel, slot blots, UV spec, pico green plate assays, Alu repeats and real time PCR assays. Most forensic laboratories conduct slot blot assays to determine the amount of human DNA

Summary 2

- Length and sequence variation have been used in forensic DNA typing. Short tandem repeats are the current form of variation being analyzed in most forensic DNA laboratories.
- The greater the number of forms (alleles) the greater the power of the test.
- One method to examine variation of variable number of tandem repeats (VNTRs) is RFLP= restriction fragment length polymorphisms
- RFLP requires many steps, undegraded DNA and takes days to weeks to complete

Summary 3

- In contrast, typing of STRs using PCR can be performed on very small amounts of degraded DNA and takes hours to a day to complete.
- PCR is polymerase chain reaction and is repeated rounds of DNA synthesis.
- There are 5 components needed, PTMDD.
- Other markers that have been used in forensic PCR assays include, dot blot assays of DQ alpha, polymarker, and D1S80.
- Mitochondrial DNA sequencing and Y chromosome STR markers are also being used.

DNA Chant

The subject of the course today (me)
Is simply stated DNA (you)
Sugar-Phosphate backbone chains (me)
Hold the base pairs heres their names (you)
Chorus: AT(me)- AT(you)
GC(me)- GC(you)
ATGC, ATGC (together)
RFLP holy grail
Put bad guys away in jail
PCR can lend a hand
Amplifying those weak bands ---------->Chorus

Blood, saliva, semen too,
Can be used as crucial clues
Fingernails and skin and hair
DNA is everywhere ---------->Chorus