Biological Stain Analysis

BARRY SAVILLE PH.D., MARCH 23, 2020

FORENSIC SCIENCE PROGRAM

TRENT UNIVERSITY

Overview

Blood Characteristics
- Detecting blood at a crime scene
- Tests on blood

Seminal Stains, detection and confirmation

Rape evidence

DNA Analysis
- DNA Structure and typing
- Polymerase Chain Reaction (PCR)
- Capillary electrophoresis
- SNPs
- Massively Parallel Sequencing

Nature of Blood

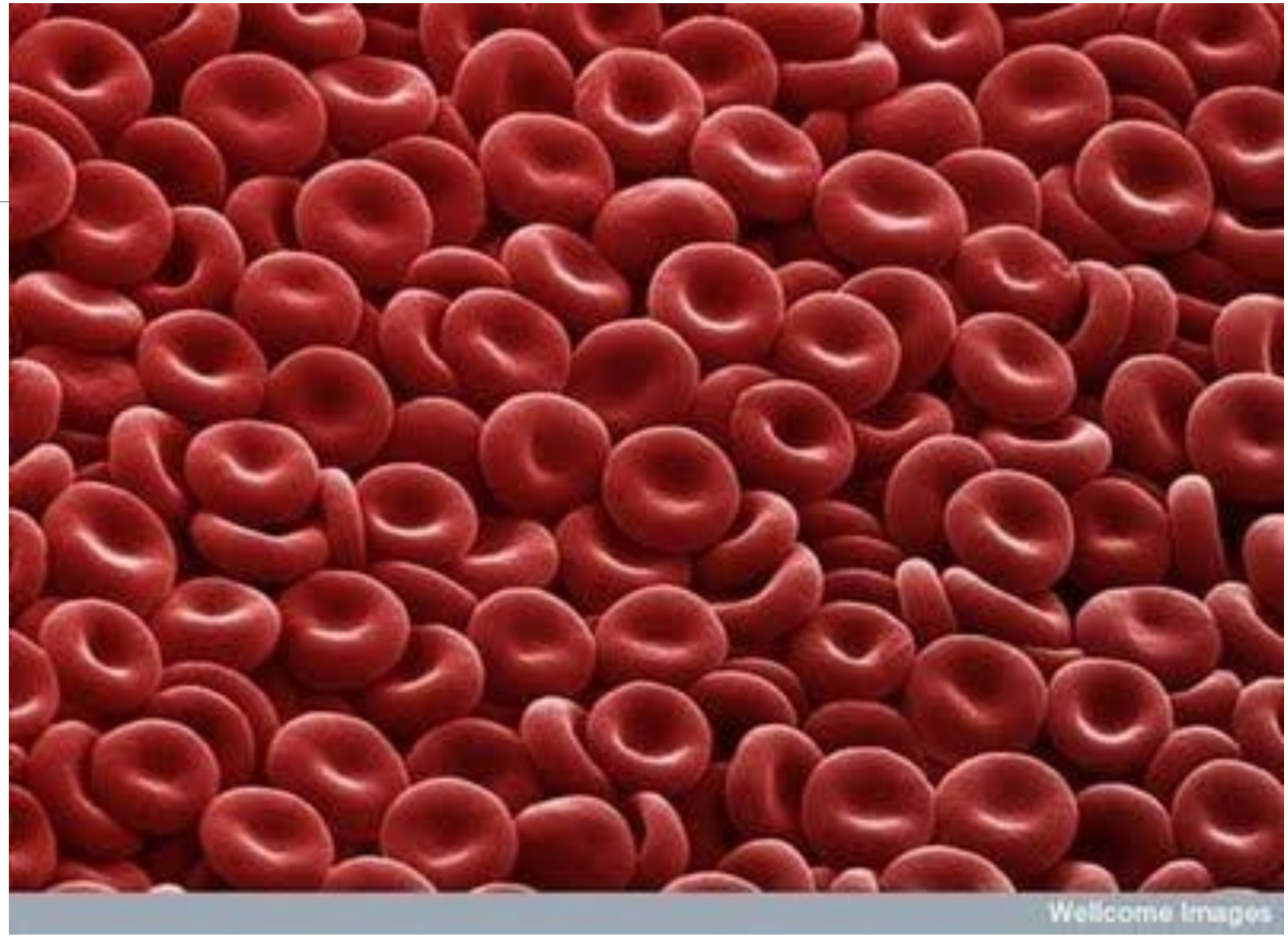
The word blood refers to a highly complex mixture of cells, enzymes, proteins, and inorganic substances.

Plasma, which is the fluid portion of blood, is composed principally of water.

Red blood cells (erythrocytes), white blood cells (leukocytes), and platelets are the solid materials suspended in plasma.

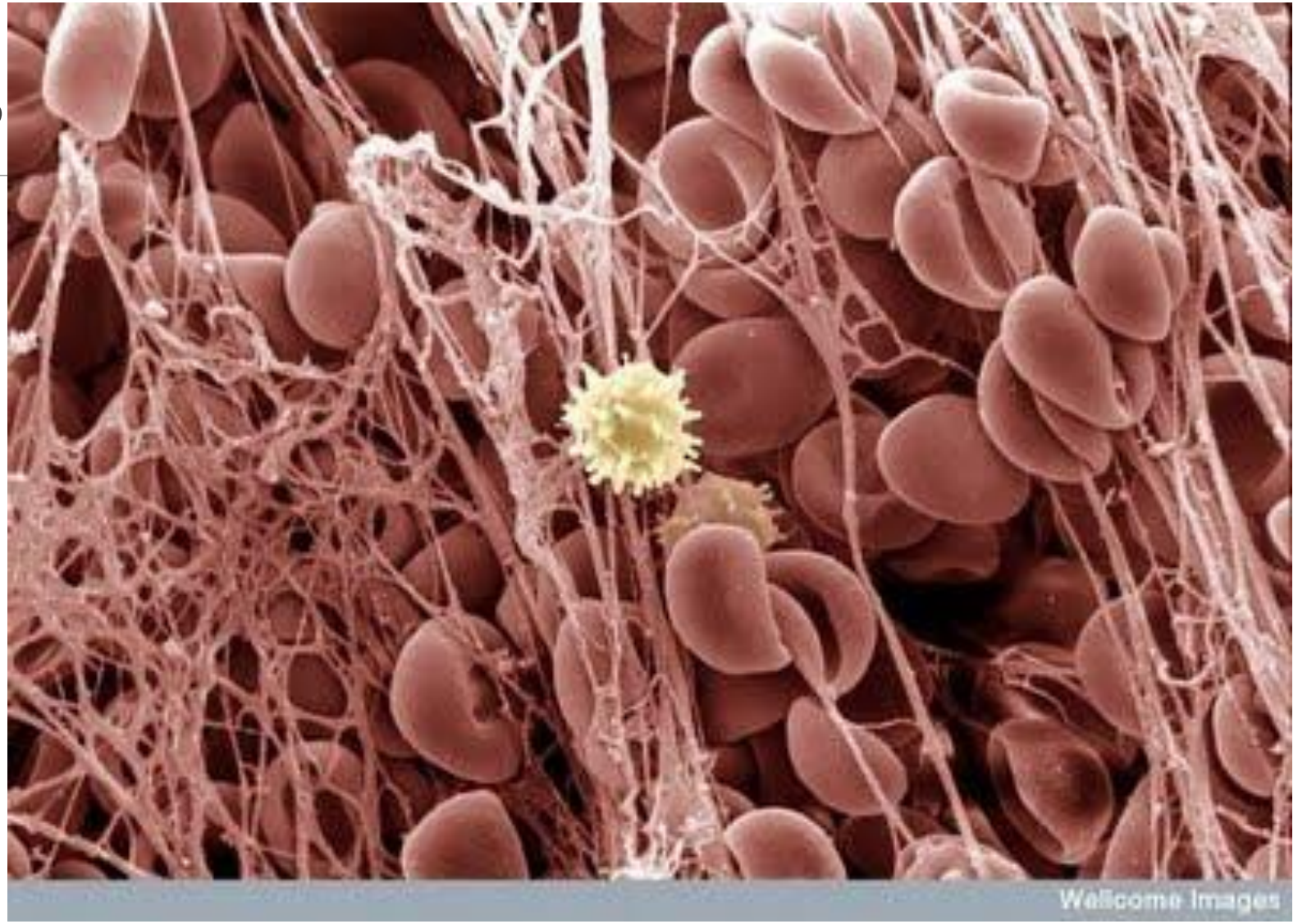
Antigens, usually proteins, are located on the surface of red blood cells and are responsible for blood-type characteristics.

RBCs



http://www.jtirregulars.com/2009/11/this-is-amazing-photography-think-about.html

Clotting RBCs



http://www.jtirregulars.com/2009/11/this-is-amazing-photography-think-about.html

Blood Typing

More than 15 blood antigen systems have been identified, but the A-B-O and Rh systems are the most relevant to forensics.

An individual that is type A has A antigens on their red blood cells, type B has B antigens, AB has both A and B antigens, and type O has neither A nor B antigens.

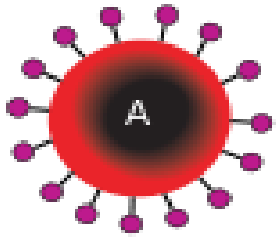
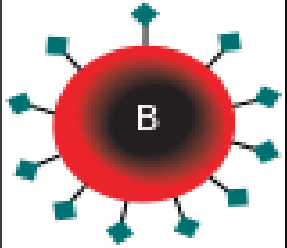
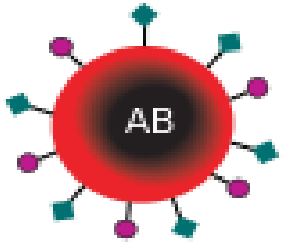
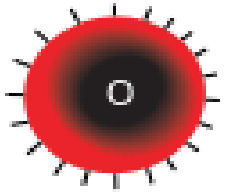
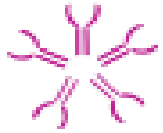
Rh factor is determined by the presence of another antigen, the D antigen.

People having the D antigen are Rh positive; those not having the antigen are Rh negative.

For every antigen there is a specific antibody that will react with it to form clumps known as agglutination.

Thus, if serum containing anti-B is added to red blood cells carrying B antigen, they will immediately react.

ABO Blood Groups

	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies in Plasma	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens in Red Blood Cell	 A antigen	 B antigen	 A and B antigens	None

http://en.wikipedia.org/wiki/ABO_blood_group_system

Forensics of Blood

The forensic scientist must be prepared to answer the following questions when examining dried blood:

1. Is it blood?
2. From what species did the blood originate?
3. If the blood is of human origin, how closely can it be associated to a particular individual?

The determination of blood is best made by means of a preliminary color test.

Blood Tests

A positive result from the Kastle-Meyer color test is highly suggestive of blood.

- Hemoglobin causes a deep pink color.

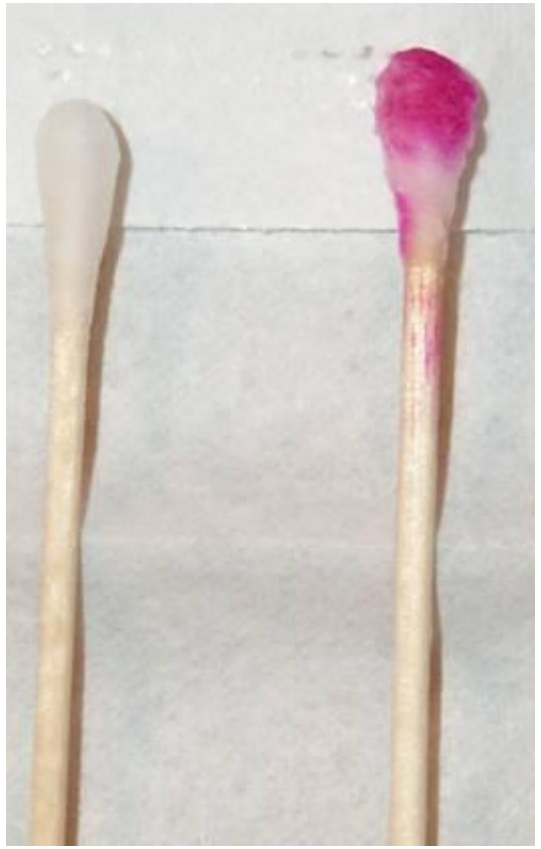
Alternatively, the luminol test is used to search out trace amounts of blood located at crime scenes.

- Produces light (luminescence) in a darkened area.

Bluestar

Microcrystalline tests, such as the *Takayama* and *Teichmann* tests, depend on the addition of specific chemicals to the blood so that characteristic crystals will be formed.

Kastle-Myer Test



+ve

http://site.utah.gov/dps/biology-ser-testing.htm

Luminol

http://ecrimescenechemistrymiller.wikispaces.com/Serology

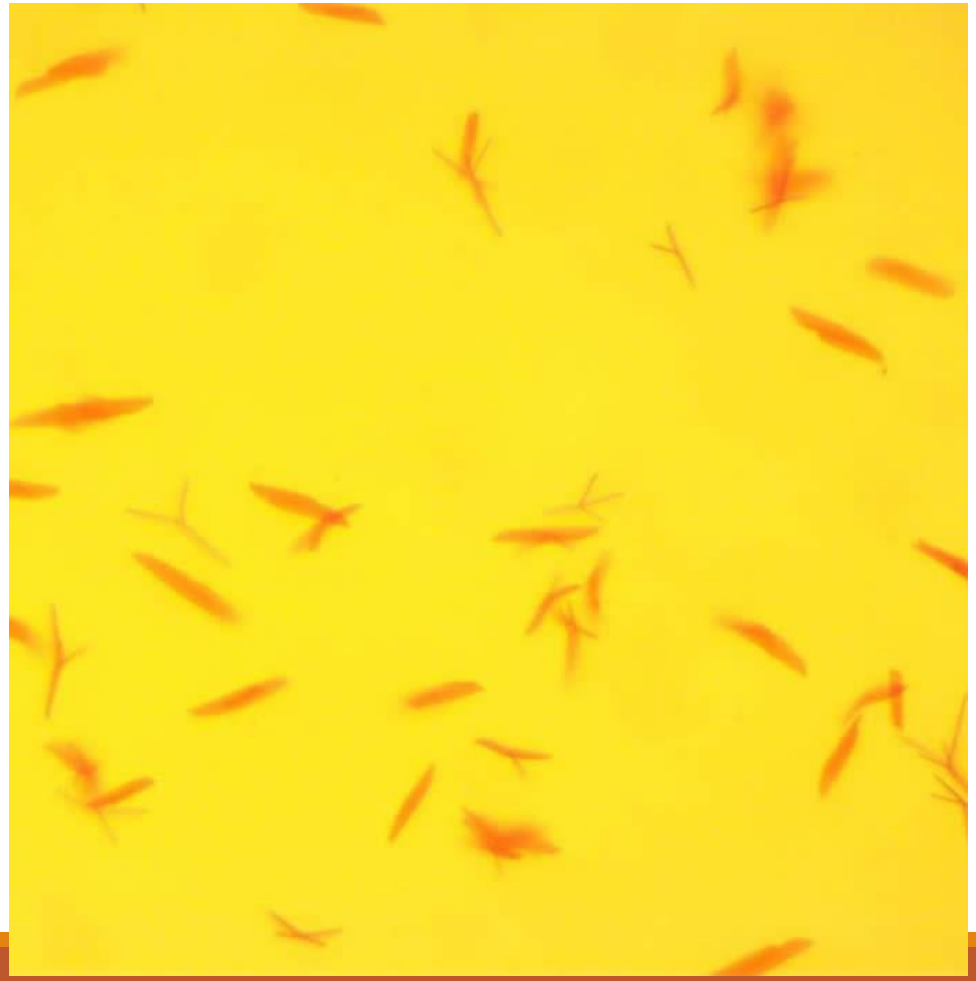
Bluestar

http://www.bluestar-forensic.com/

Takayama Test

pyridine + blood = characteristic shaped crystal
If the crystals appear and they are the predicted shape, then it is blood if the shape is different it is not confirmed as blood by this test.

positive test →



http://site.utah.gov/dps/biology-ser-testing.htm

Blood Origin Testing

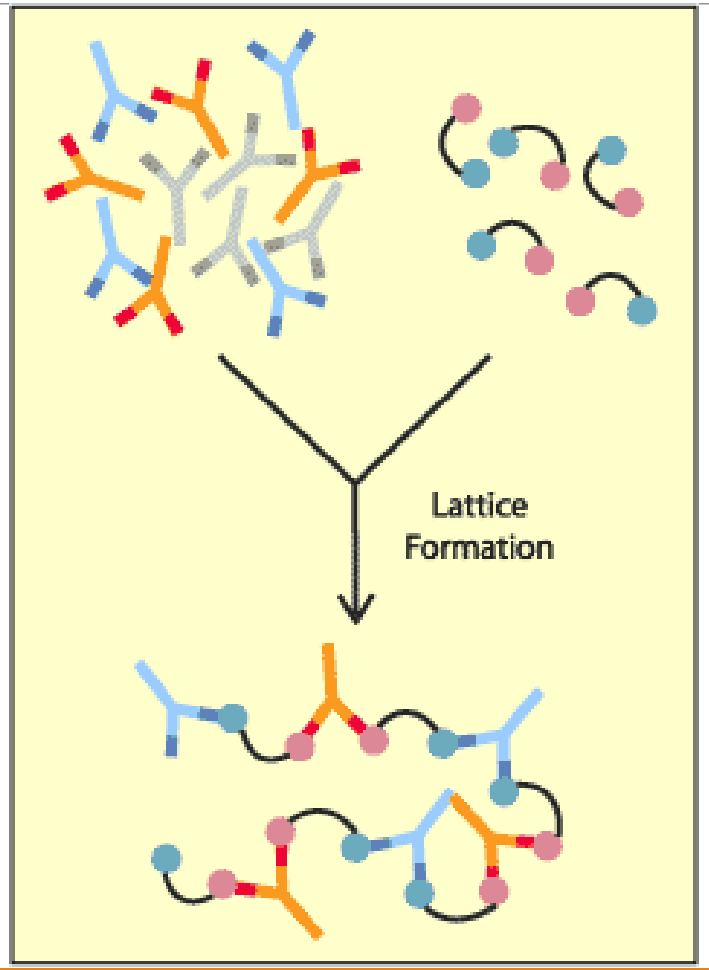
Once the stain has been characterized as blood, the precipitin test will determine whether the stain is of human or animal origin.

The precipitin test uses antisera normally derived from rabbits that have been injected with the blood of a known animal to determine the species origin of a questioned bloodstain.

The technique of *gel diffusion* takes advantage of the fact that antibodies and antigens diffuse or move toward one another on an agar plate.

The extracted bloodstain and the human antiserum are placed in separate holes opposite each other on the gel. If the blood is human, a line of precipitation forms where the antigens and antibodies meet.

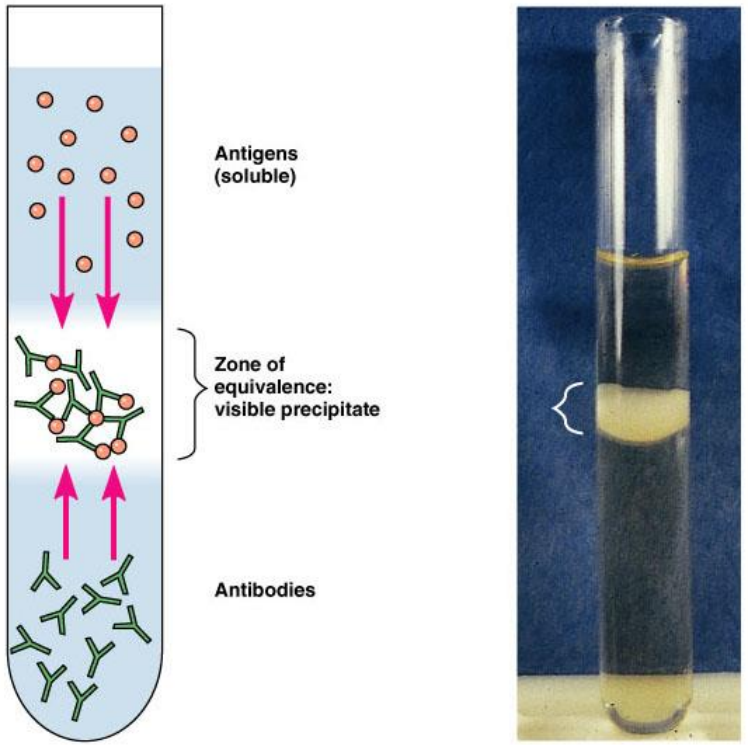
Antigen-Antibody complexes



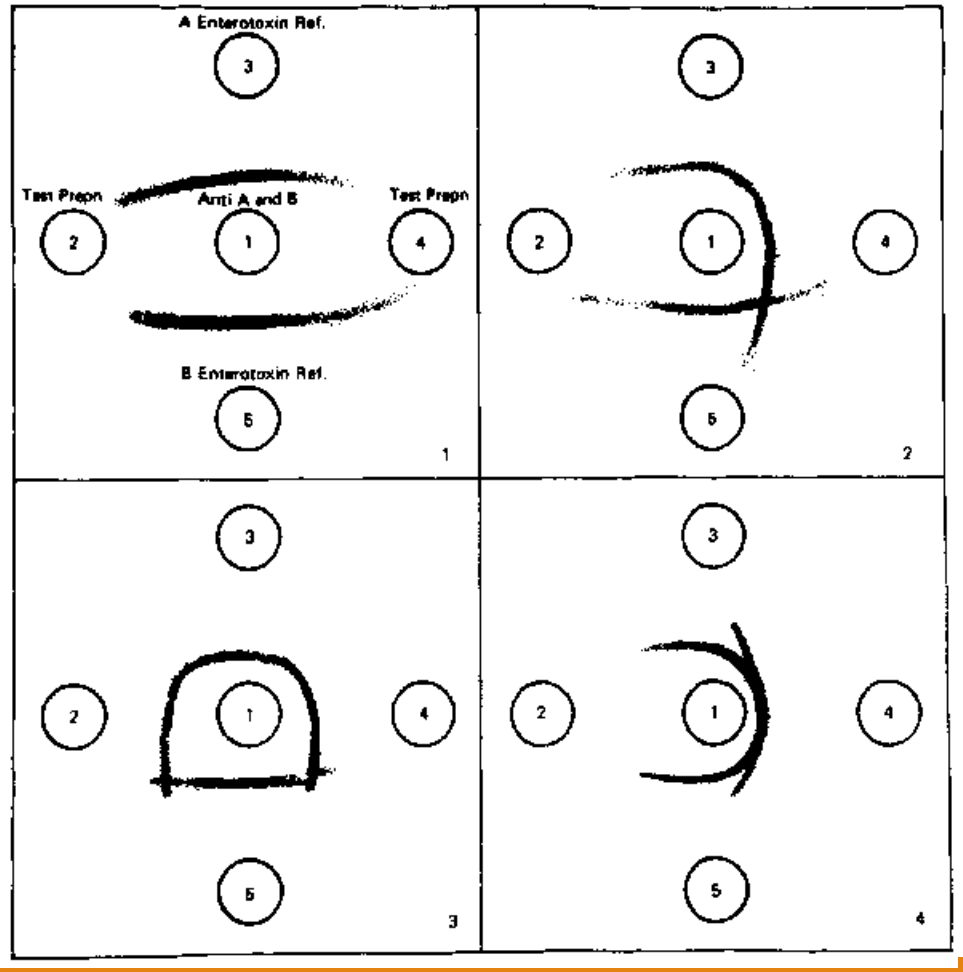
- Formed in precipitin tests, gel diffusion, and immunoelectrophoresis
- Yields a precipitate.

http://toolboxes.flexiblelearning.net.au/demosites/series4/412/laboratory/studynotes/snImmunIdentTechMOrg.htm

Precipitin



Gel Diffusion (bivalent test)



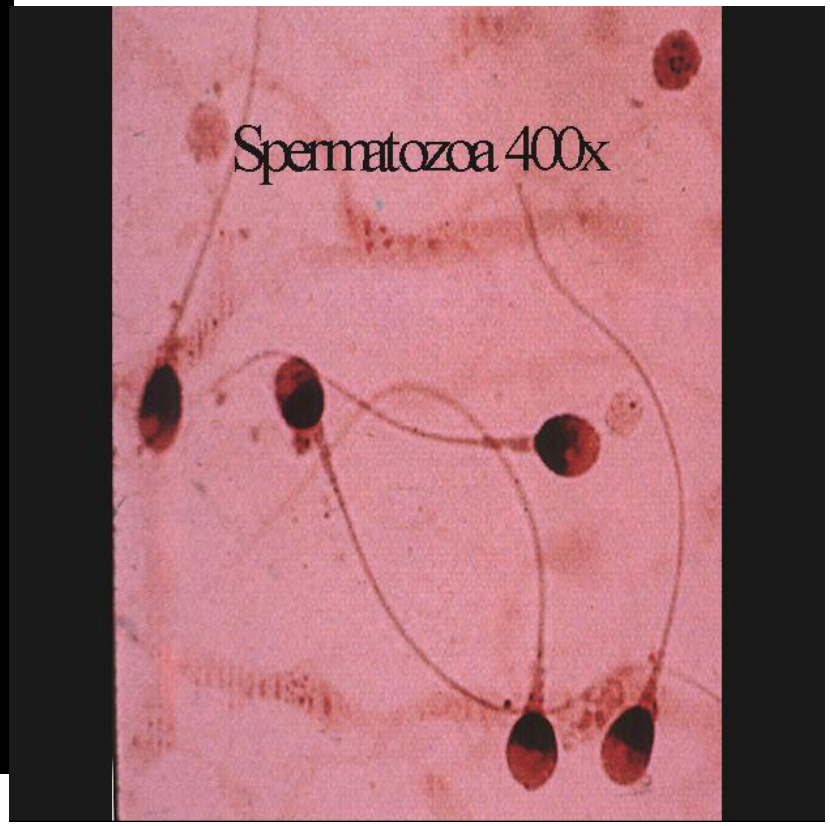
Individualizing Bloodstains

Once it has been determined that the bloodstain is of human origin, an effort must be made to associate or dissociate the stain with a particular individual.

DNA analysis may allow forensic scientists to associate blood to a single individual.

Testing for Seminal Stains

- Many of the cases sent to a forensic laboratory involve sexual offenses, making it necessary to examine exhibits for the presence of seminal stains.
- The best way to locate and at the same time characterize a seminal stain is to perform the acid phosphatase color test.
  - Acid phosphatase is an enzyme secreted into seminal fluid
  - A purple color indicates the presence of acid phosphatase enzyme.
- Semen can be unequivocally identified by either the presence of spermatozoa or of p30, a protein unique to seminal plasma.
- Forensic scientists can successfully link seminal material to an individual by DNA typing.



Acid Phosphatase Test, pink colouration indicates positive presumptive test for semen, http://site.utah.gov/dps/biology-ser-testing.htm

https://www.spyguy.com/products/checkmate-semen-detection-kit

http://www.semen-detection.com

Rape Evidence

- The rape victim must undergo a medical examination as soon as possible after the assault.
- At that time, the appropriate items of physical evidence including clothing, hairs, and vaginal and rectal swabs can be collected for subsequent laboratory examination.
- All outer and undergarments should be carefully removed and packaged separately in paper (not plastic) bags.
- Bedding, or the object upon which the assault took place, may also be carefully collected.

Sex Crime Evidence Collection

TX100 CONTENTS:

1- TX1001 Kit Box, 10.5” x 7” x 3” (26.7cm x 17.8cm x 7.6cm)
1- TX1002 Evidence Collection Instructions
1- TX101 Request for Medical Exam & Release of Medical Records Form, 3-Part
1- TX102AC Examination & Forensics Report Form, Pages 1-3, 3-Part NCR
1- TX102EL Body Diagram Forms, Various, 3-Part NCR
1- TX103 Oral Swabs & Smear Envelope
1- TX104A Changing Paper Bag with 23” x 45” (58.4cm x 114.3cm) Paper Sheet
1- TX104B Undergarments/Diaper Bag
2- TX104C Outer Clothing Bag
1- TX106 Pulled Head Hair Envelope
1- TX105 Head Hair Combings Envelope w/Towel & Plastic Comb
1- TX107 Debris Collection Envelope w/Paper Bindle, Sterile Swabs & Box
1- TX108 Anal Swabs & Smear Envelope
1- TX109 Vaginal/Penile Swabs & Smear Envelope
1- TX110 Pubic Hair Combings Envelope w/Towel & Plastic Comb
1- TX111 Pulled Pubic Hair Envelope
1- TX112 Fingernail Scrapings/Swabbings Envelope w/2 ea. Paper Bindles & Scrapers, Swabs & Swab Box
1- TX113 Known Blood Sample Blood Collection Tube, 7ml w/EDTA
1- TX114 Known Saliva Sample Envelope w/Sterile Swabs & Box
1- TX115 Receipt of Information Form, 3-Part NCR
1- TX116 Authorization to Assign Payment Form, 3-Part NCR
1- 999311 FDA Manufacturer’s Insert
1- KCP206 Biohazard Label, 1” x 1.25” (2.5cm x 3.2cm), Black on Orange
1- KCP214 Pencil, Sharpened
2- KCP164 Evidence Seals, Red, “Warning! Police Seal, Do Not Remove”
http://store.sirchie.com/Southwestern-Sexual-Assault-Evidence-Collection-Kit-P1926.aspx

Rape Evidence

- If a suspect is apprehended within 24 hours of the assault, it may be possible to detect the victim's DNA on the male's underwear or on a penile swab of the suspect.
- Items routinely collected from the suspect include all clothing, pubic hair, head hair, penile swab, and a blood sample or buccal swab for DNA typing.
- The forceful physical contact between victim and assailant may result in a transfer of physical evidence such as blood, semen, saliva, hairs, and fibers.

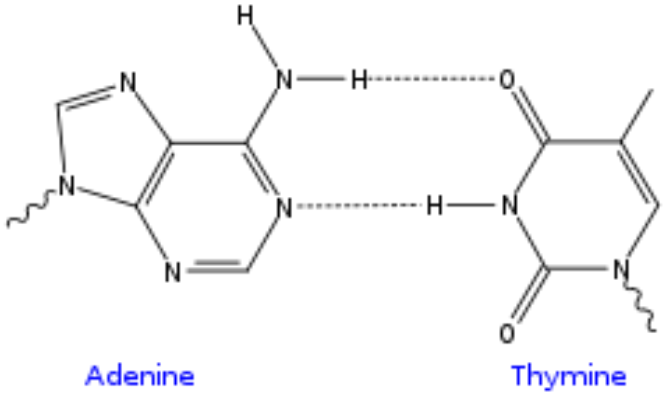
DNA Analysis

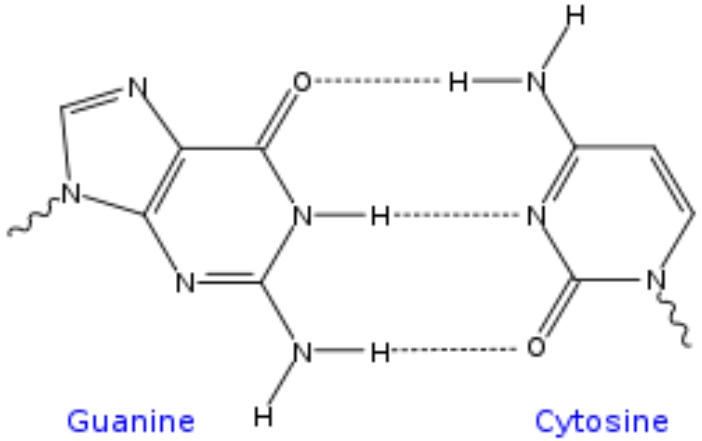
- DNA Structure - reminder
- DNA typing
- Polymerase Chain Reaction (PCR)
- Capillary electrophoresis
- SNPs
- Massively Parallel Sequencing

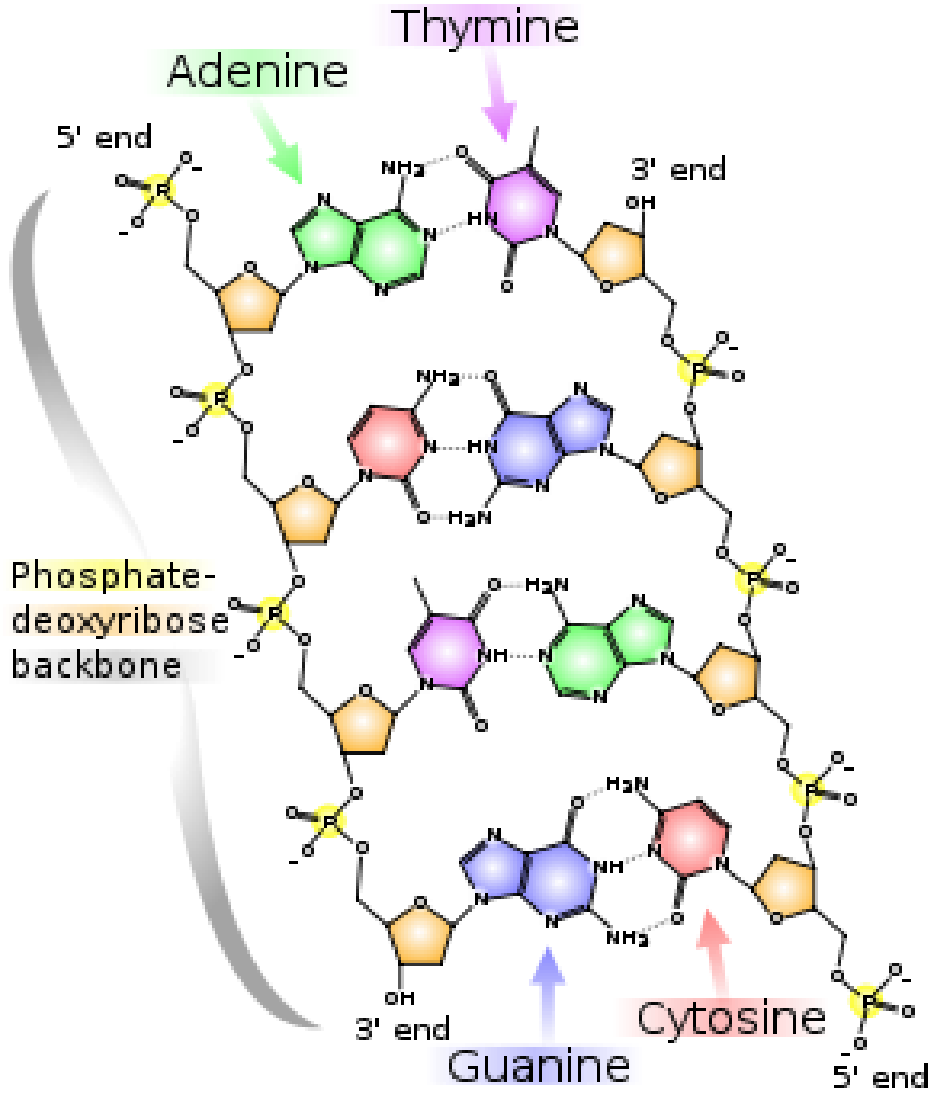
The Structure of DNA

- DNA is a very large molecule composed of linked repeating units called nucleotides.
- A nucleotide is composed of a sugar, a phosphorous-containing group, and a nitrogen-containing molecule called a base.
- Four types of bases are associated with the DNA structure: adenine (A), guanine (G), cytosine (C), and thymine (T).
- The bases on each strand can align in a double-helix configuration, which brings the two strands of DNA together in a coil.
- As a result, adenine pairs with thymine (A=T) and guanine pairs with cytosine (G≡C).
- This concept is known as base pairing.

DNA Structure







http://en.wikipedia.org/wiki/DNA

DNA Typing

- Portions of the DNA molecule contain sequences of bases that are repeated numerous times, known as tandem repeats.
- To a forensic scientist, these tandem repeats offer a means of distinguishing one individual from another through DNA typing.
- Tandem repeats seem to act as filler or spacers between the coding regions of DNA.
- What is important to understand is that all humans have the same type of repeats, but there is tremendous variation in the number of repeats each of us have.

Short Tandem Repeats (STRs)

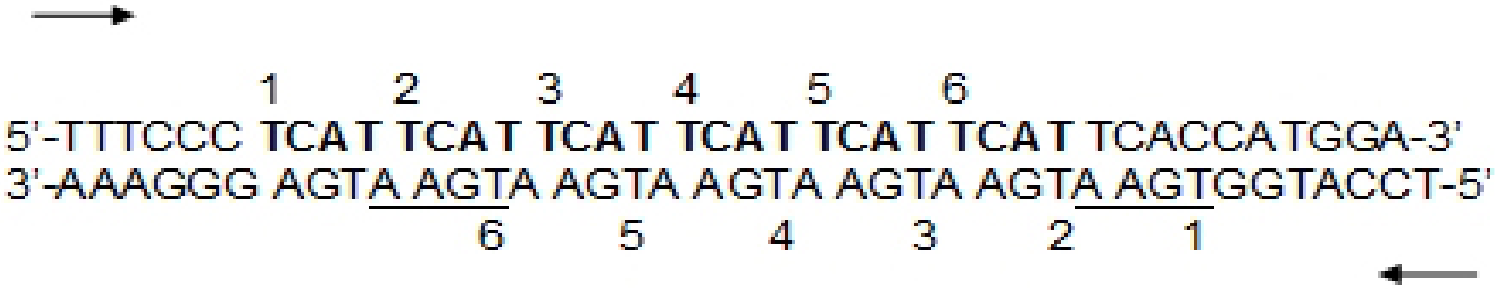
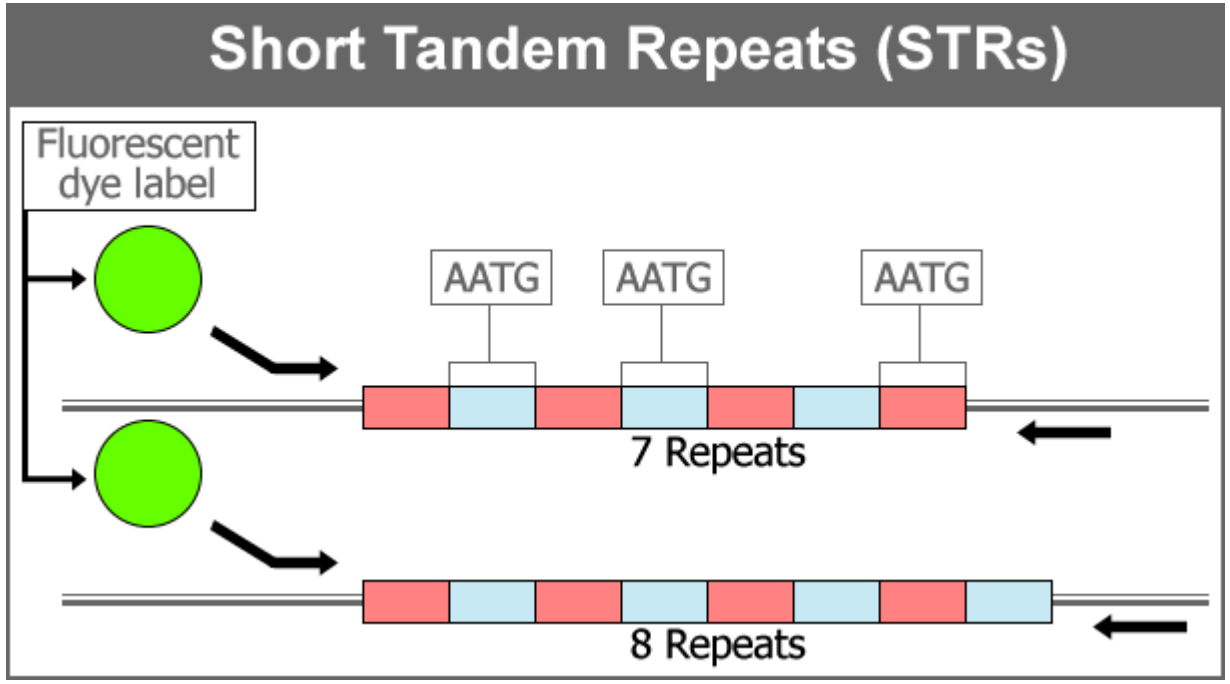


Figure 5.2, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

Detecting STRs



http://www.nfstc.org/pdi/Subject04/pdi_s04_m02_00.htm

NOTE DETECTION IS BASED ON SIZE OF THE PCR PRODUCT

The Power of STR

- What makes STRs so attractive to forensic scientists is that hundreds of different types of STRs are found in human genes.
- The more STRs one can characterize, the smaller will be the percentage of the population from which a particular combination of STRs can originate.
- Using the technology of PCR, one can simultaneously extract and amplify a combination of different STRs.
- This process is referred to as multiplexing.

Multiplex Kits

Standardizing STR Testing

- Currently, U.S. crime laboratories have standardized on 13 STRs for entry into a national database (CODIS = Combined DNA Index System).
- A high degree of discrimination and even individualization can be attained by analyzing a combination of STRs (multiplexing) and determining the product of their frequencies.
- With STRs, as little as 125 picograms (10^{-12} of a gram) of DNA is required for analysis.

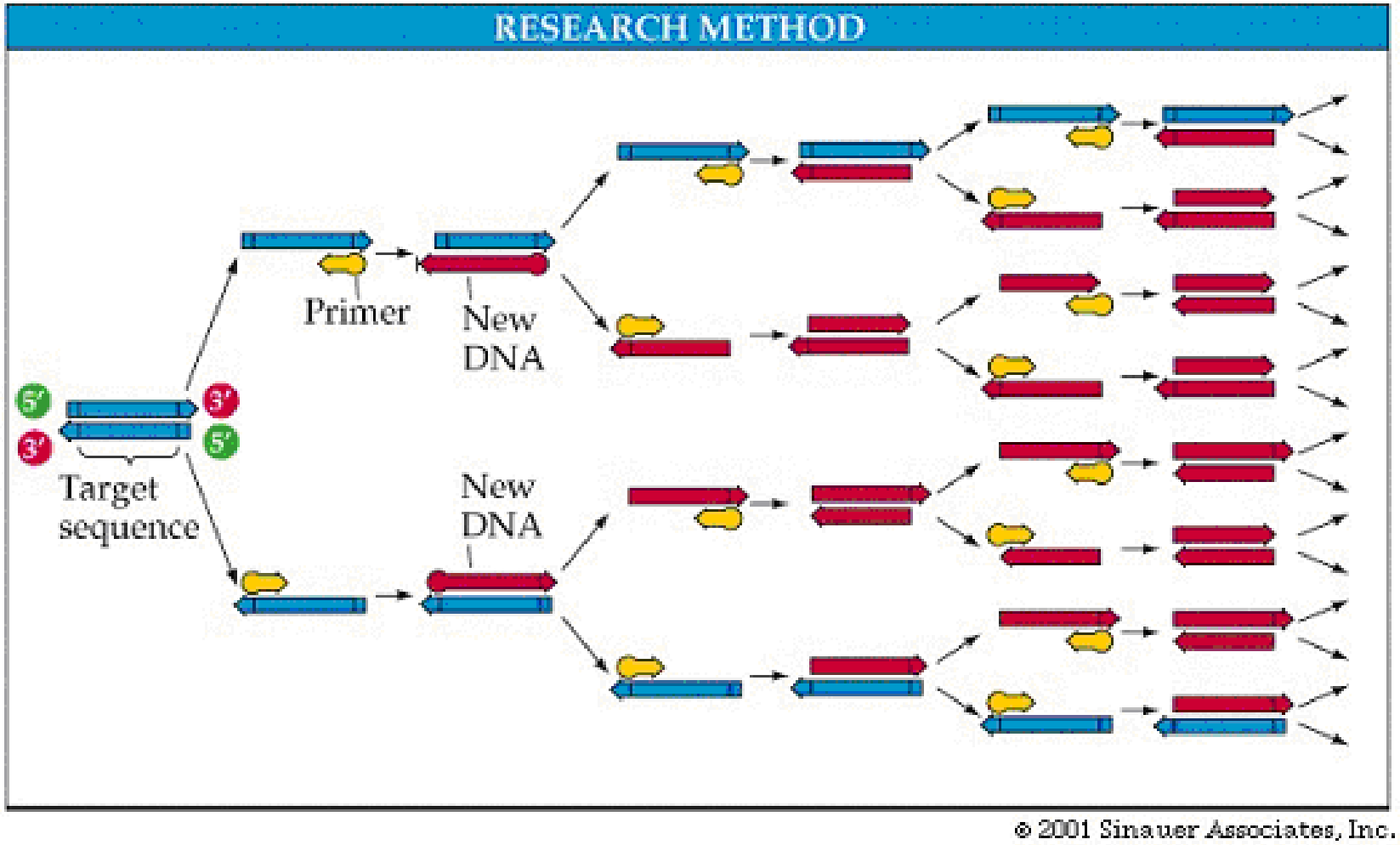
PCR Testing

- Polymerase chain reaction (PCR) is a technique for replicating small quantities of DNA or broken pieces of DNA found at a crime scene, outside a living cell.
- The ability to multiply small bits of DNA by PCR now means that sample size is no longer a limitation in characterizing DNA recovered at a crime scene.
- For the forensic scientist, PCR offers a distinct advantage in that it can amplify minute quantities of DNA many millions of times.

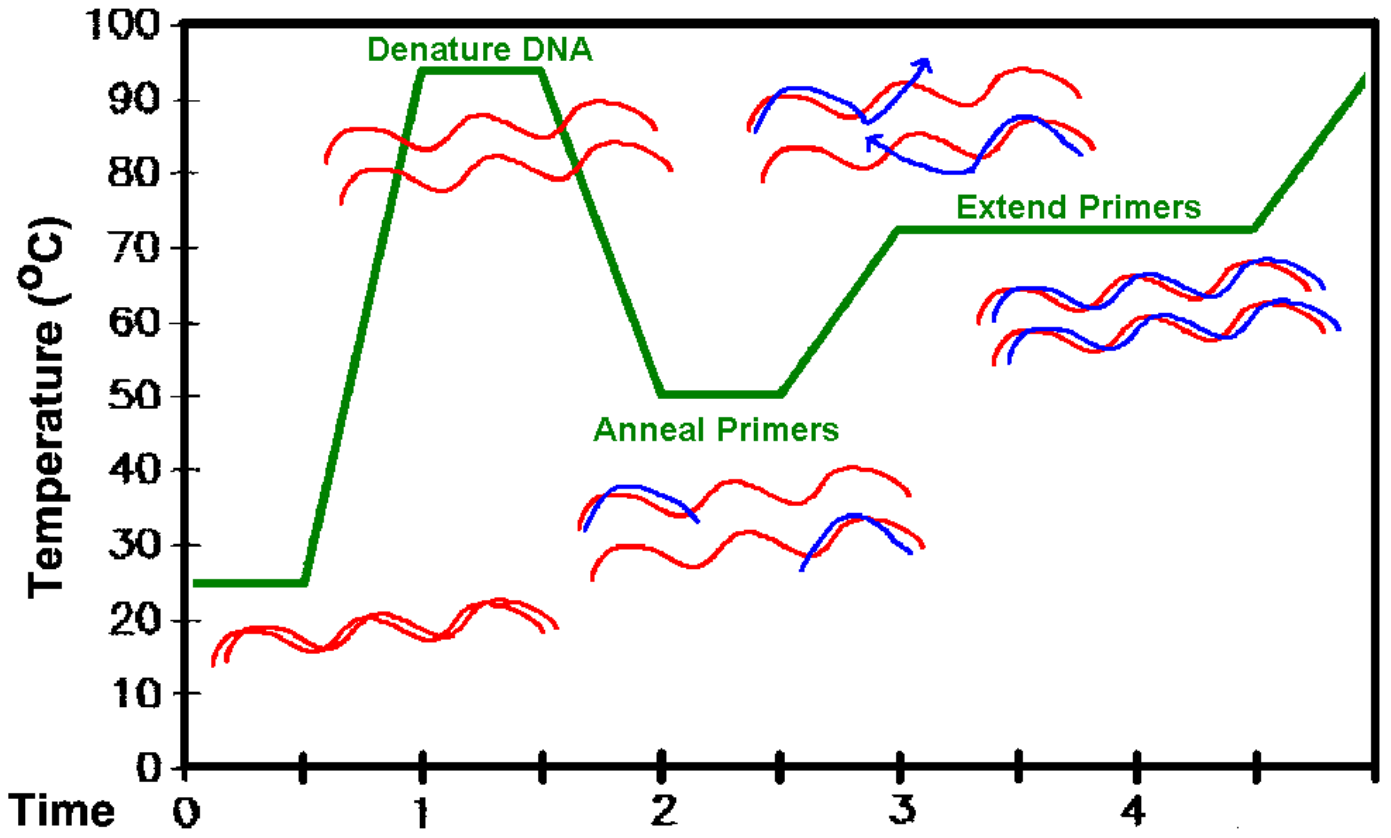
Steps in PCR

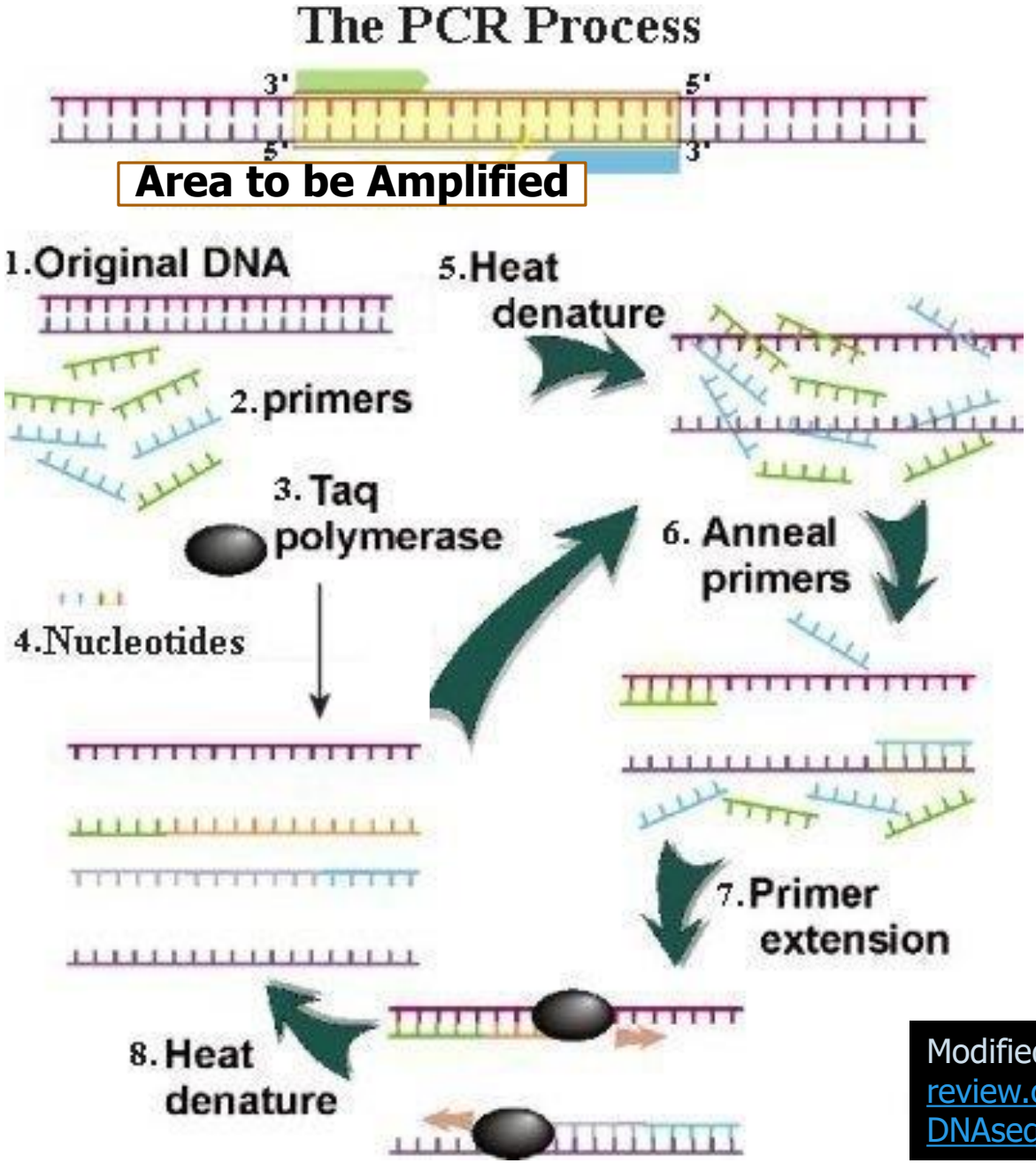
- Denaturation: First, the DNA is heated to separate the strands.
- Primer Annealing: Second, primers (short strands of DNA used to target specific regions of DNA for replication) are added, which hybridize with the separated DNA strands.
- Extension: Third, DNA polymerase and free nucleotides are added to rebuild each of the separated strands.
- This process is repeated 25, 30 or more times.

PCR


RESEARCH METHOD
Primer
New DNA
5′
3′
3′
5′
Target sequence
New DNA
© 2001 Sinauer Associates, Inc.

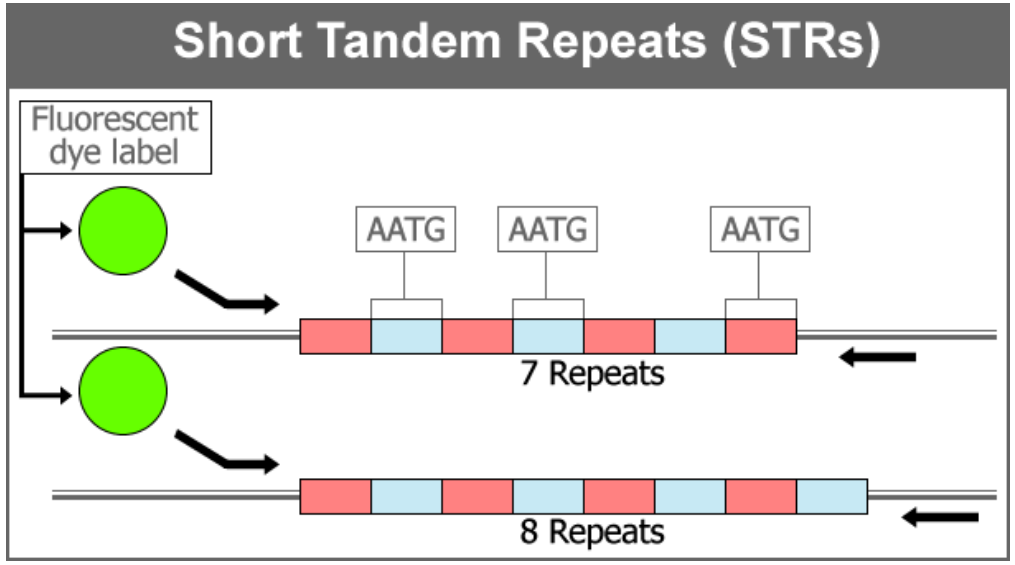
PCR STEPS


Denature DNA
Extend Primers
Anneal Primers
Temperature (°C)
100
90
80
70
60
50
40
30
20
10
0
Time
0
1
2
3
4



Modified from: http://universe-review.ca/R11-16-DNAsequencing.htm

Detecting STRs



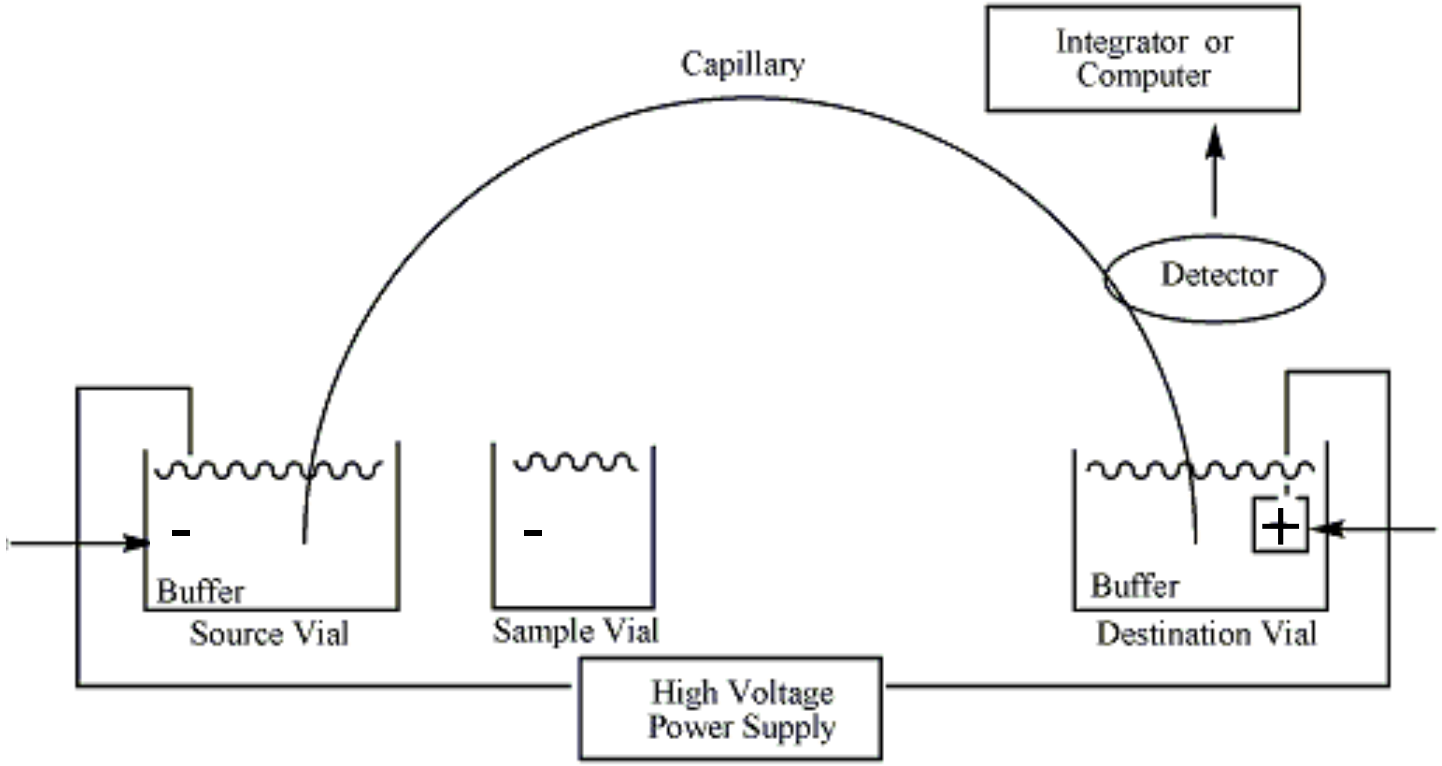
http://www.nfstc.org/pdi/Subject04/pdi_s04_m02_00.htm

NOTE: DETECTION IS BASED ON SIZE OF THE PCR PRODUCTS

Capillary Electrophoresis

- Capillary electrophoresis separates DNA fragments in a thin glass column filled with a gel that allows fragments of different size to move at different rates .
- Each end of the column is immersed in a reservoir of buffer liquid with electrodes.
- The DNA-containing sample solution is electro-injected into one end of the column.
- The STR fragments move through the electrified column at a speed that is related to the length of the STR fragments.
- As the DNA passes through the detector, they are recorded as a peak on a display known as an *electropherogram.*

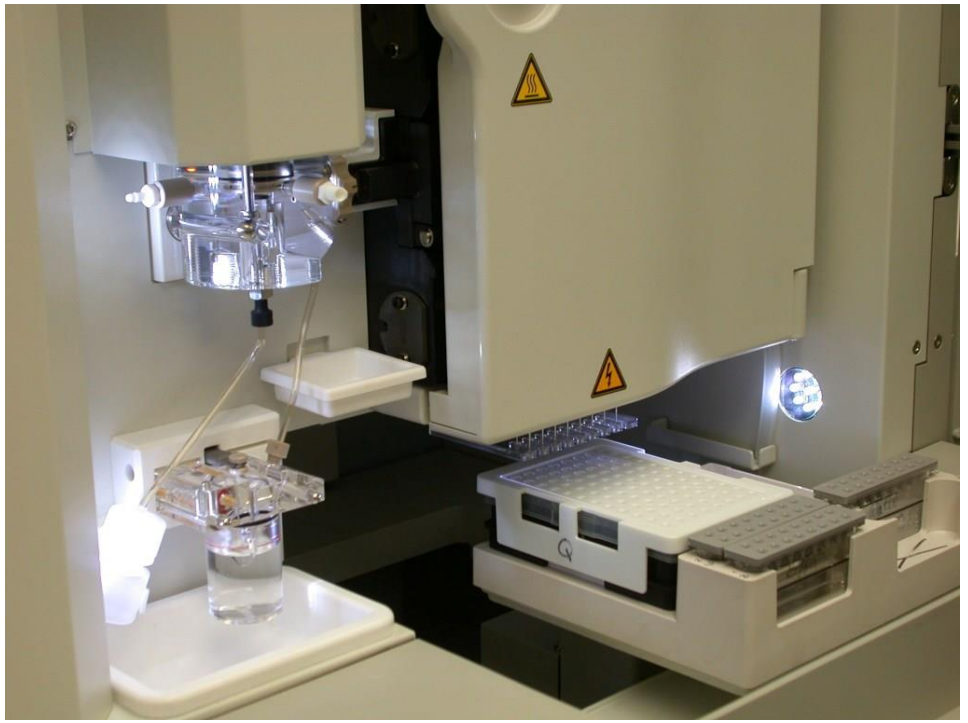
Capillary Electrophoresis



http://en.wikipedia.org/wiki/Capillary_electrophoresis

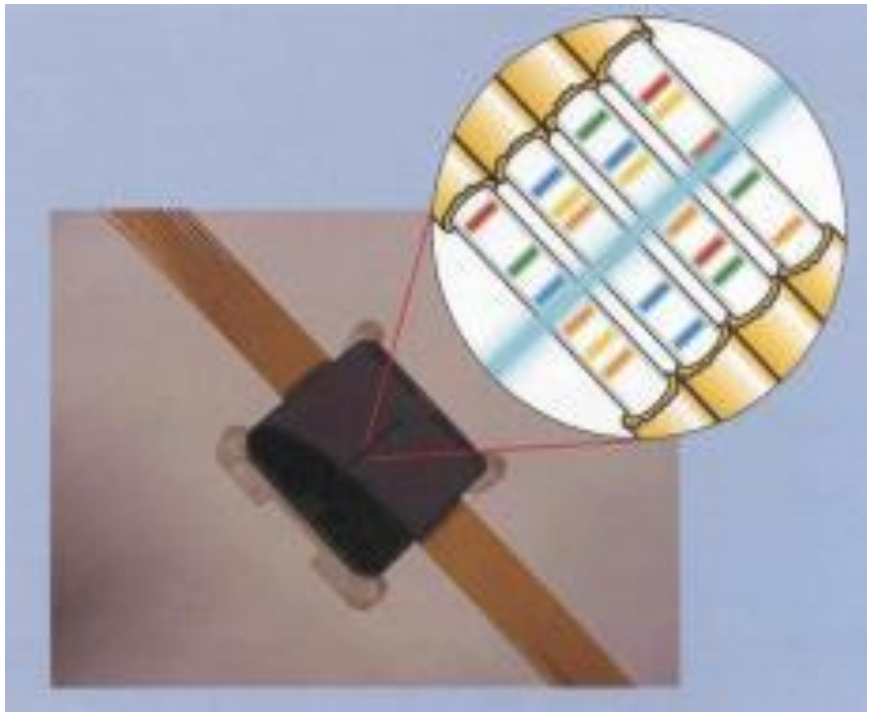
ABI 3100 16 Cap-older version

http://biology200.gsu.edu/core/instrumentation/equipment/abi3100.html

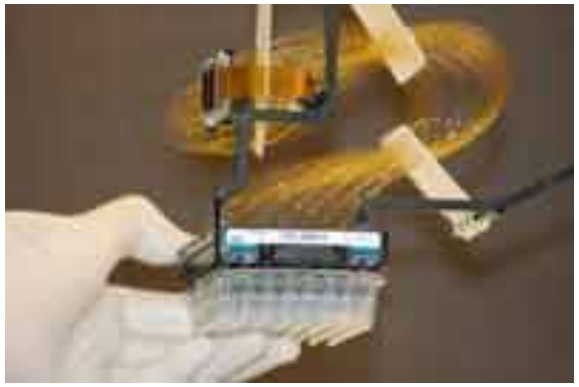


http://dps.alaska.gov/CrimeLab/DNA.aspx

Capillary Arrays

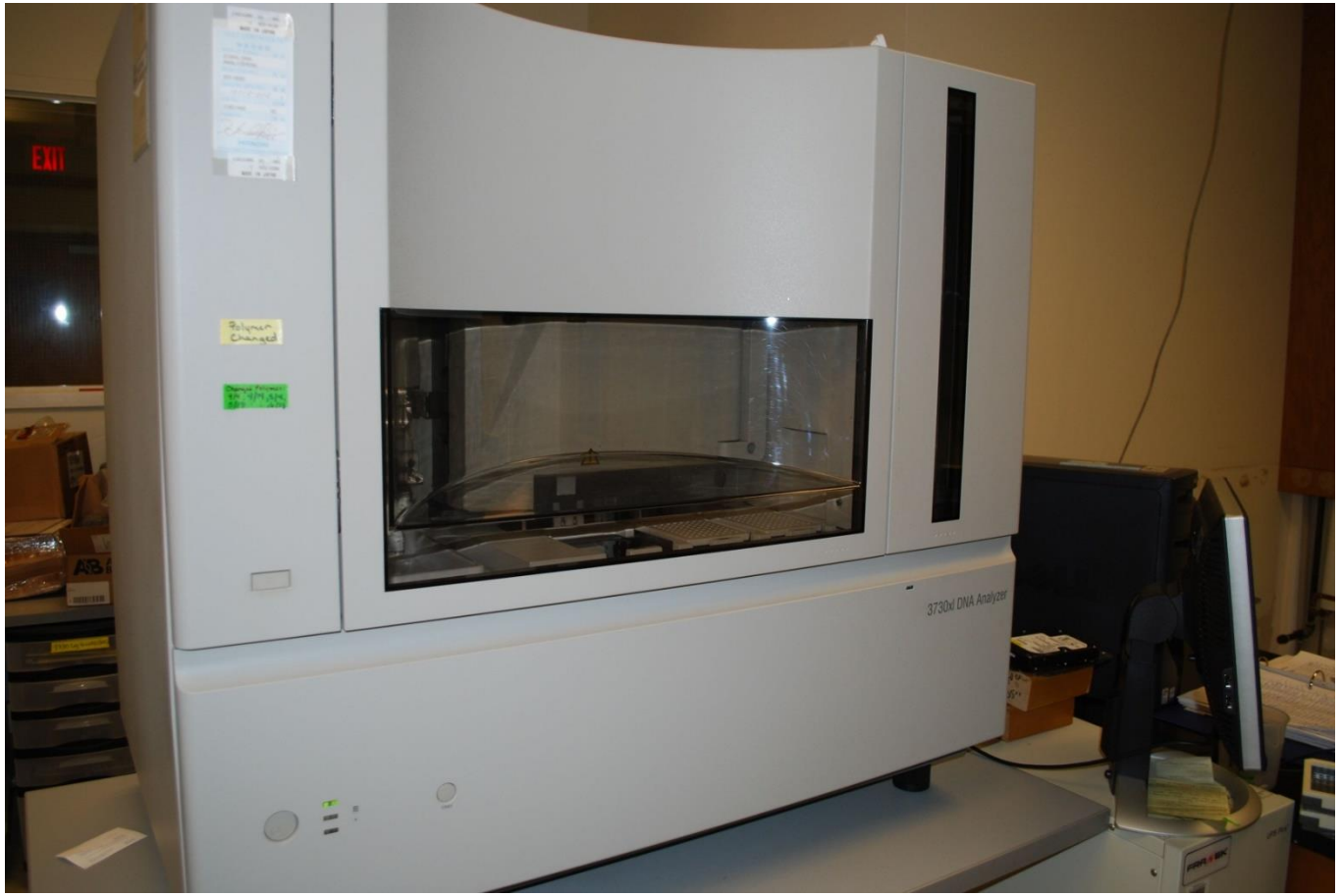


http://www.ucalgary.ca/dnalab/sequencing/3730xl



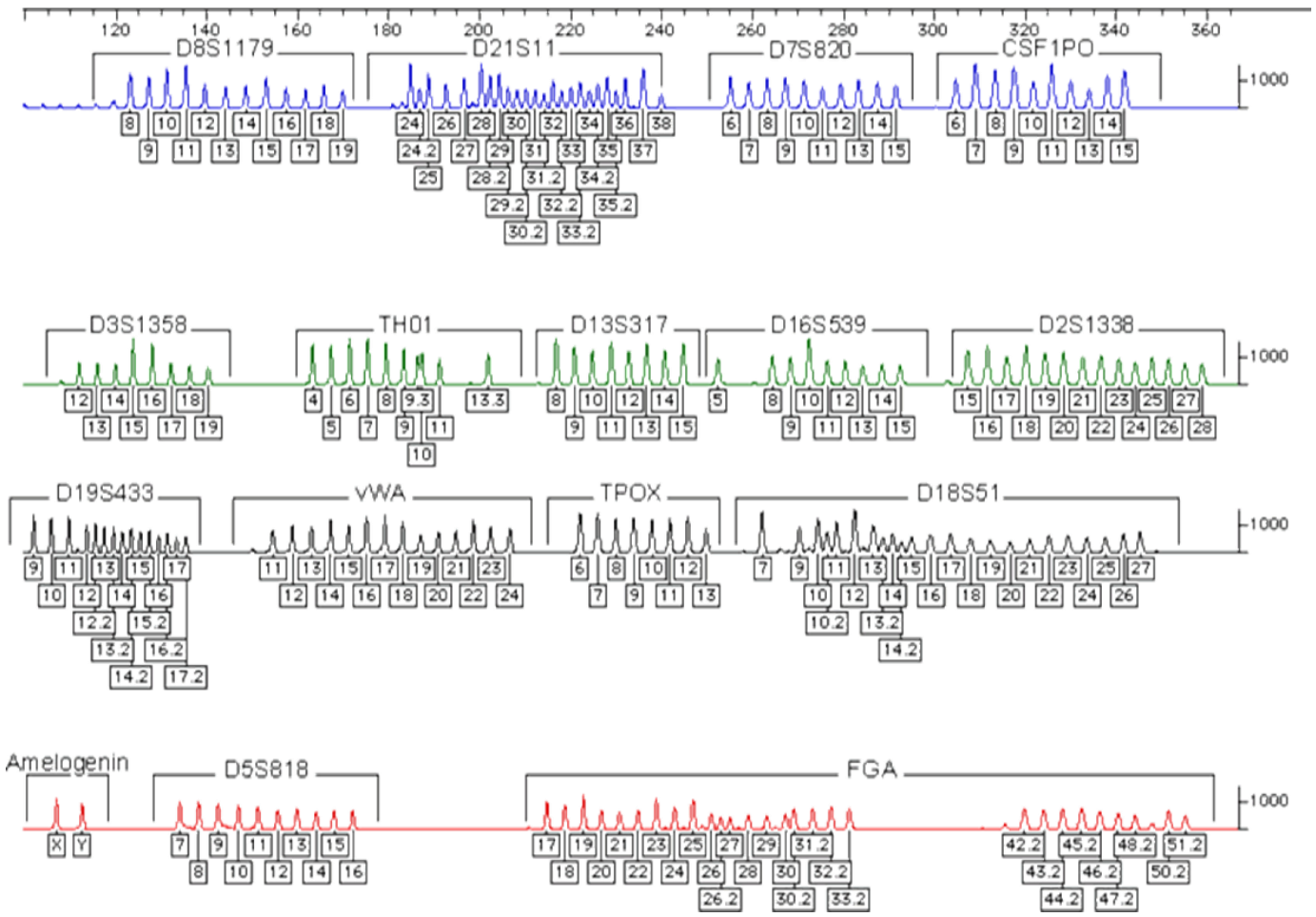
http://www2.webscientific.co.uk/acatalog/ABI3730.html

ABI 3730 XL



http://gsl.cals.ncsu.edu/sequencing/

Identifiler™ Allelic Ladders



http://www.nfstc.org/pdi/Subject05/pdi_s05_m02_01_f.htm

CODIS

- Perhaps the most significant tool to arise from DNA typing is the ability to compare DNA types recovered from crime scene evidence to those of convicted sex offenders and other convicted criminals.

- CODIS (Combined DNA Index System) is a computer software program developed by the FBI that maintains local, state, and national databases of DNA profiles from convicted offenders, unsolved crime scene evidence, and profiles of missing persons.

Canadian Forensic Databases

National DNA Data Bank is responsible for two principal indices:

- The **Convicted Offender Index** (COI) is the electronic index that has been developed from DNA profiles collected from offenders convicted of designated primary and secondary offences identified in section 487.04 of the Criminal Code

- The **Crime Scene Index** (CSI) is a separate electronic index composed of DNA profiles obtained from crime scene investigations of the same designated offences addressed in the Act.

Locus Name	Chromosomal Location	Physical Position [a]	Repeat Motif ISFG Format [b]	GenBank Accession [c]	GenBank Allele	Allele Range [d]	Number of Alleles Seen [e]
CSF1PO	5q33.1 c-fms proto-oncogene, 6th intron	Chr 5 149.484 Mb	TAGA	X14720	12	5–16	20
FGA	4q31.3 alpha fibrinogen, 3rd intron	Chr 4 156.086 Mb	CTTT	M64982	21	12.2–51.2	80
TH01	11p15.5 tyrosine hydroxylase, 1st intron	Chr 11 2.156 Mb	TCAT	D00269	9	3–14	20
TPOX	2p25.3 thyroid peroxidase, 10th intron	Chr 2 1.436 Mb	GAAT	M68651	11	4–16	15
VWA	12p13.31 von Willebrand Factor, 40th intron	Chr 12 19.826 Mb	[TCTG][TCTA]	M25858	18	10–25	28
D3S1358	3p21.31	Chr 3 45.543 Mb	[TCTG][TCTA]	NT_005997	18	8–21	24
D5S818	5q23.2	Chr 5 123.187 Mb	AGAT	G08446	11	7–18	15
D7S820	7q21.11	Chr 7 83.401 Mb	GATA	G08616	12	5–16	30
D8S1179	8q24.13	Chr 8 125.863 Mb	[TCTA][TCTG]	G08710	12	7–20	17
D13S317	13q31.1	Chr 13 80.52 Mb	TATC	G09017	13	5–16	17
D16S539	16q24.1	Chr 16 86.168 Mb	GATA	G07925	11	5–16	19
D18S51	18q21.33	Chr 18 59.098 Mb	AGAA	L18333	13	7–39.2	51
D21S11	21q21.1	Chr 21 19.476 Mb	Complex [TCTA][TCTG]	AP000433	29	12–41.2	82

[a] Physical positions and chromosomal locations determined on July 2003 human genome reference sequence (NCBI build 34) using hgBLAT (http://genome.ucsc.edu).
[b] The DNA Commission of the International Society of Forensic Genetics (ISFG) has published several papers encouraging standardization in STR allele nomenclature (see Bar *et al.* 1994, 1997). STR repeats should be called on the strand sequence originally described in the first public database entry using the first 5′-nucleotides that can define a repeat motif.
[c] GenBank sequence information for a particular STR locus may be accessed at (http://www.ncbi.nlm.nih.gov/GenBank) by entering the accession number shown here. Reference sequences are also available at http://www.cstl.nist.gov/biotech/strbase/seq_ref.htm.
[d] Numbers in this column refer to the number of repeat units present in the alleles. More detail on alleles that have been observed and their PCR products with commercially available STR kits may be found in Appendix I.
[e] See Appendix I.

http://str.ausbio.com/index.php?title=COMMONLY_USED_SHORT_TANDEM_REPEAT_MARKERS%E3%80%80AND_COMMERCIAL_KITS

Beyond STRs - Variation Linked to Traits

SNPs = Single nucleotide Polymorphisms

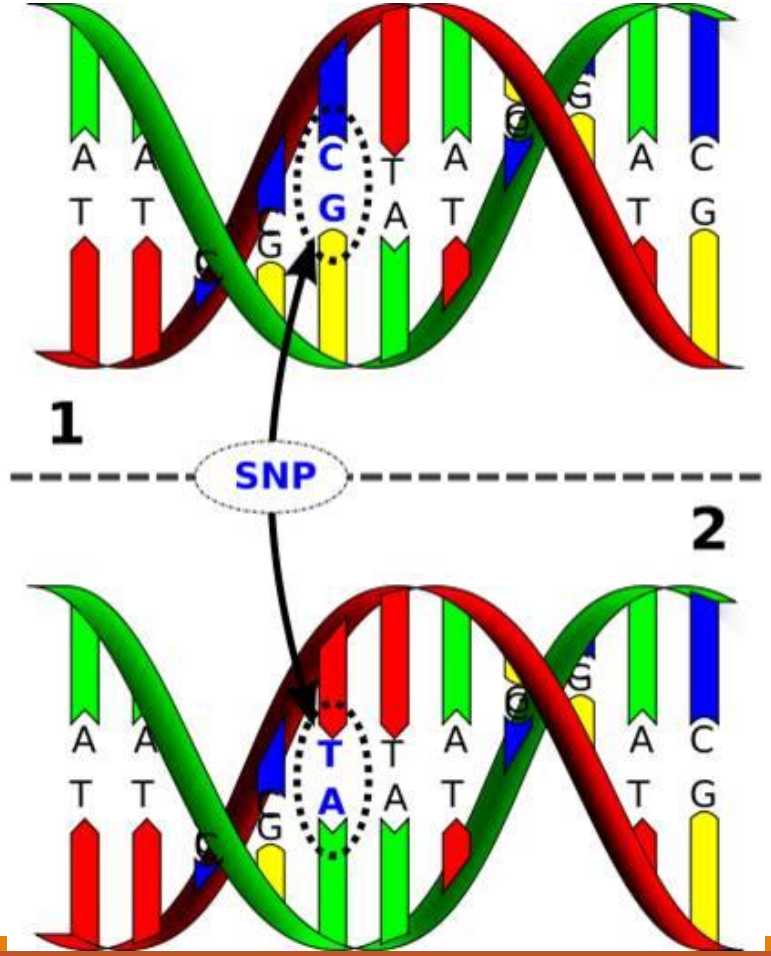
Obtained from Genome Sequence Level analyses

Single Nucleotide Polymorphisms

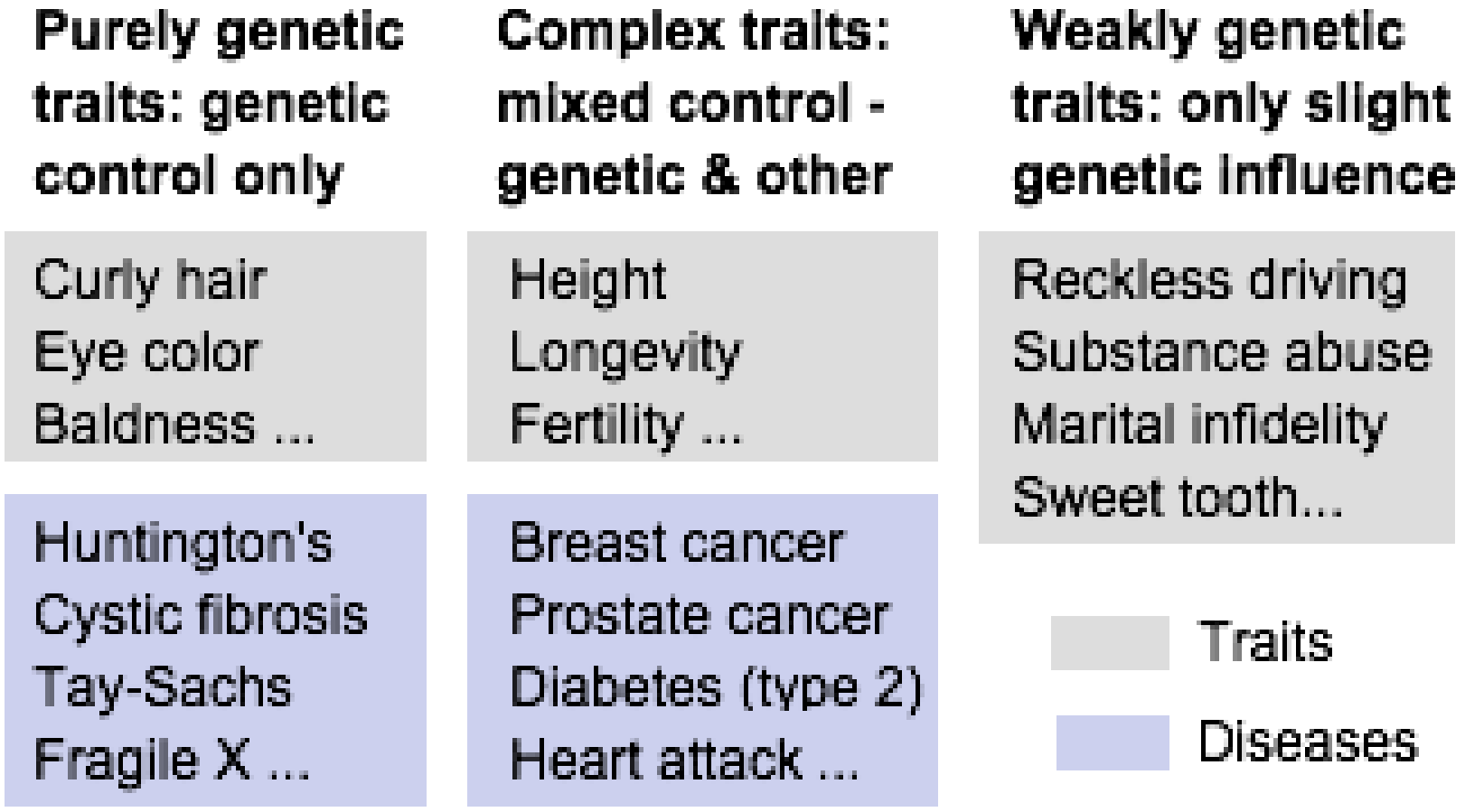
More subtle change

More abundant than the STRs

Therefore they can be correlated with human traits



Examples of human traits and diseases



http://www.snpsandchips.com/tutorial_a2.html

SNPs and Forensics

- Imagine collecting DNA at the scene of a crime and from that being able to determine facial characteristics, physical parameters and personality traits of the perpetrator

Massively Parallel Sequencing(MPS) / Next Generation Sequencing (NGS)

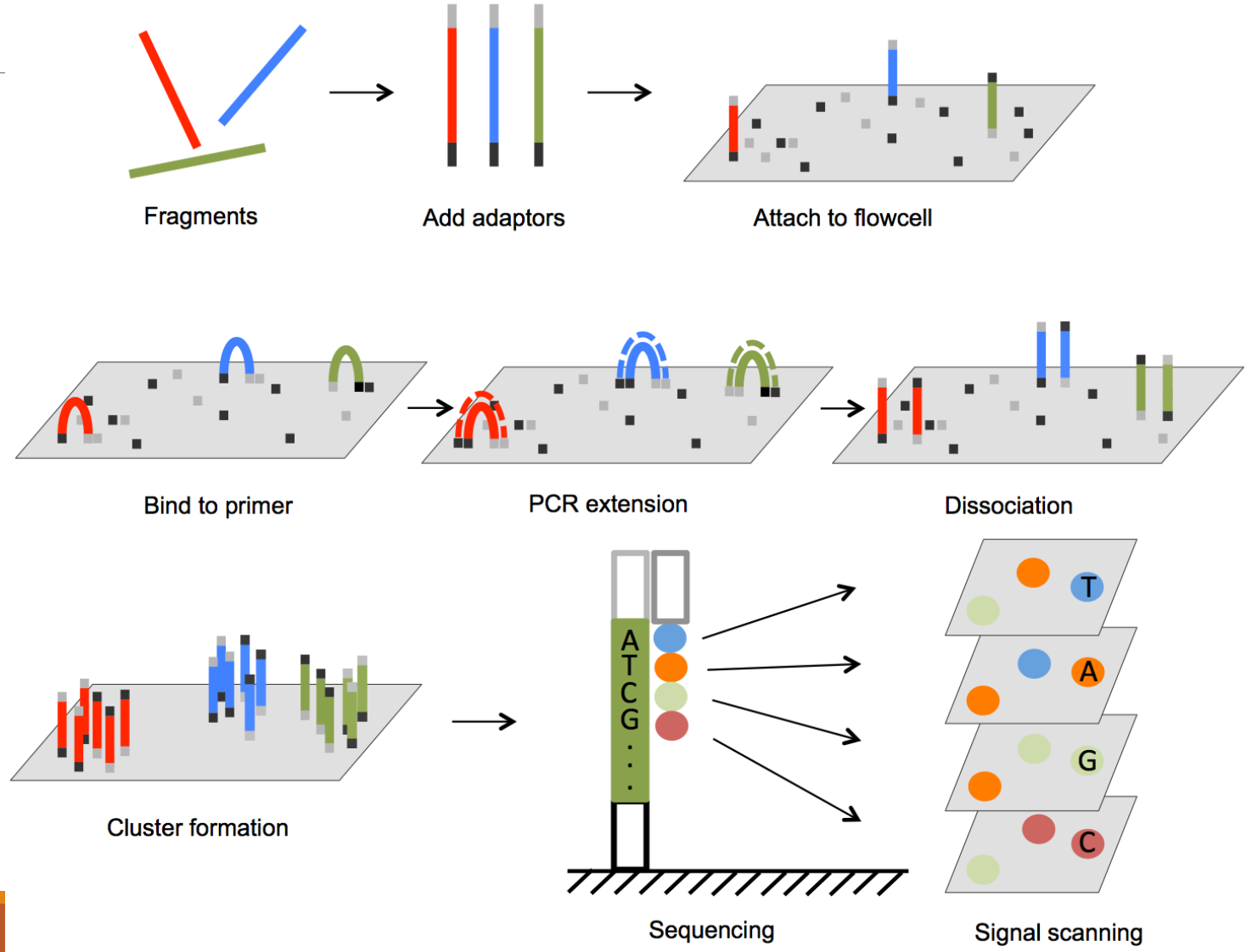
- This is the technology used currently to sequence whole genomes
- In forensic applications we use targeted sequencing and two separate platforms are being developed:
  - MiSeq FGx™ (Illumina) system with ForenSeq™, a multiplex kit consisting of more than 200 markers including 27 autosomal STRs, 24 Y-STRs, 7 X-STRs, 94 identity SNPs, 56 ancestry SNPs, 24 phenotypic SNPs and Amelogenin.
  - HID-Ion Personal Genome Machine (PGM)™(Thermo Fisher Scientific) with HID-Ion AmpliSeq™ Identity Panel kit consisting of 124 markers including 34 upper Y-Clade SNPs and 90 autosomal SNPs
  - 83 SNP markers in common

Process of implementing MPS/NGS

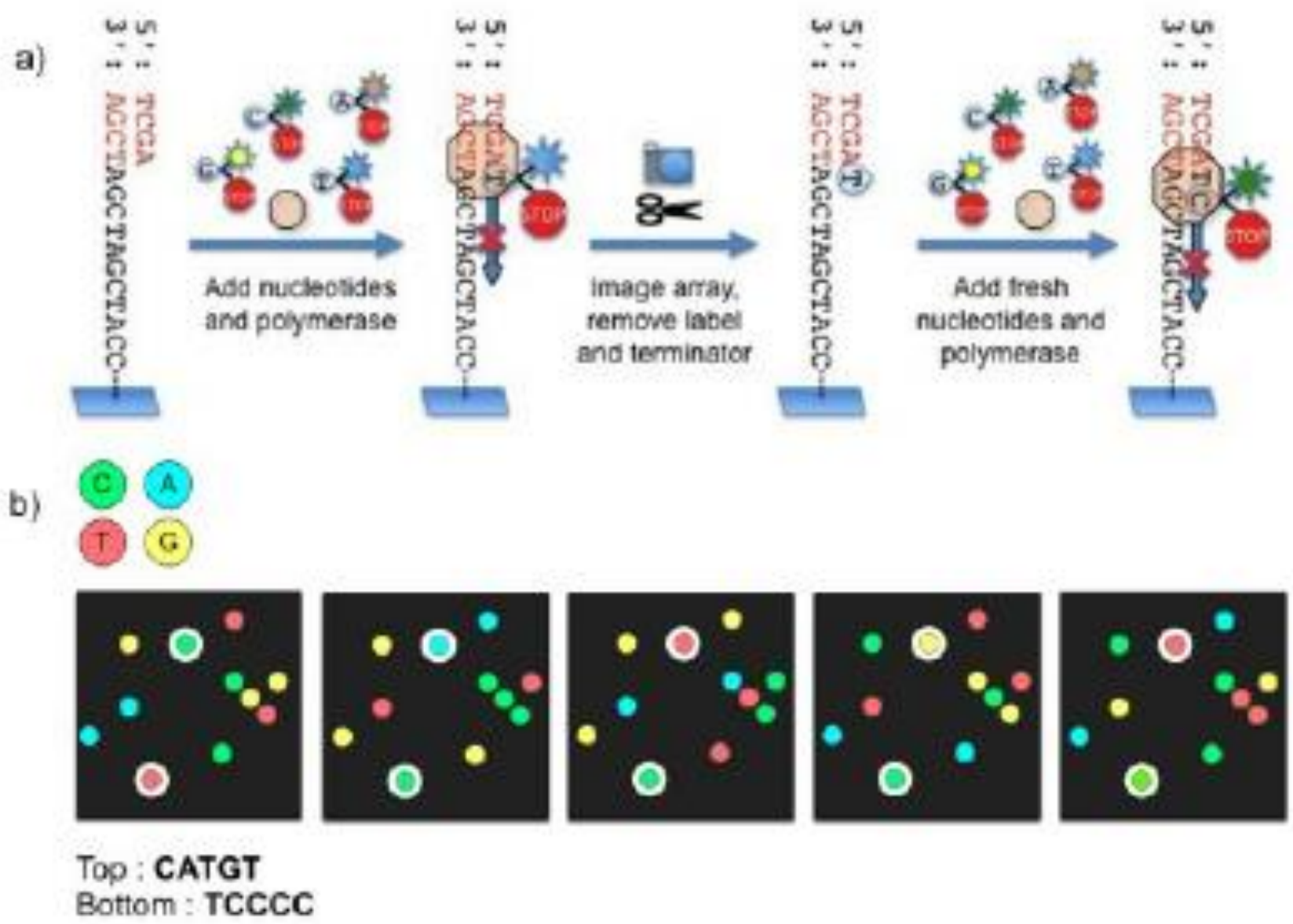
- Has it been validated
  - Yes in France and The Netherlands, underway in USA for ForenSeq™
- Have data bases been set up
  - Yes in France and the Netherlands
- Can it be used on low quantities of DNA
  - Yes ng or even pg levels of DNA can be analyzed
  - Amplicons are short allowing these to be used on degrade DNA

Illumina Sequencing

- Sample Prep
- Bridge Amplification – cluster generation
- Sequencing



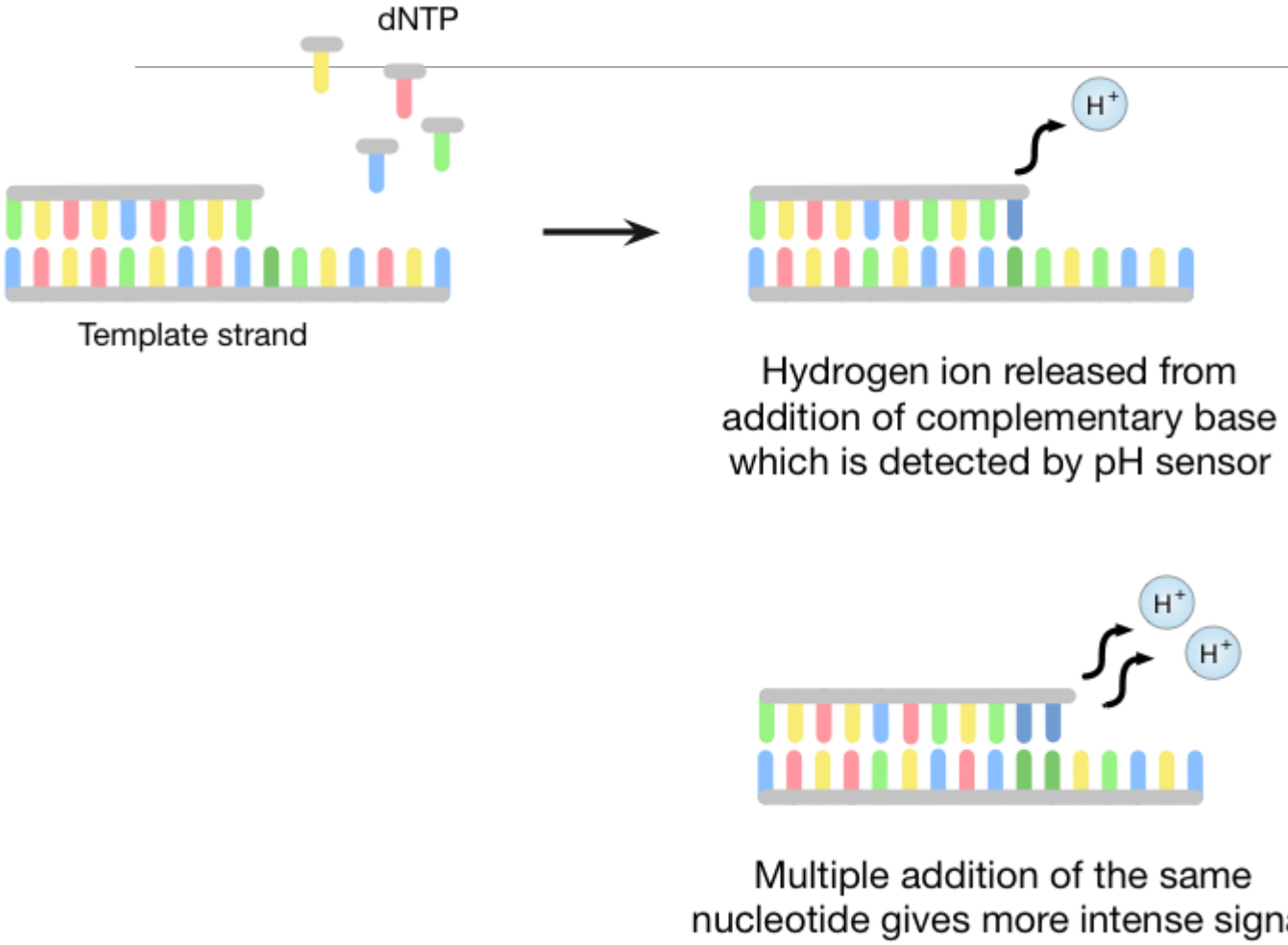
Illumina Sequencing



Illumina

https://www.youtube.com/watch?v=fCd6B5HRaZ8

Ion semiconductor sequencing: Ion Torrent



Ion torrent by Life Technologies

https://www.youtube.com/watch?v=WYBzbxIfuKs

Review Biological Stain Analysis

Blood Characteristics

Detecting blood at a crime scene

Tests on blood

Seminal Stains, detection and confirmation

Rape evidence

DNA introduction – structure, STRs, PCR, capillary electrophoresis, SNPs and next Gen Sequencing