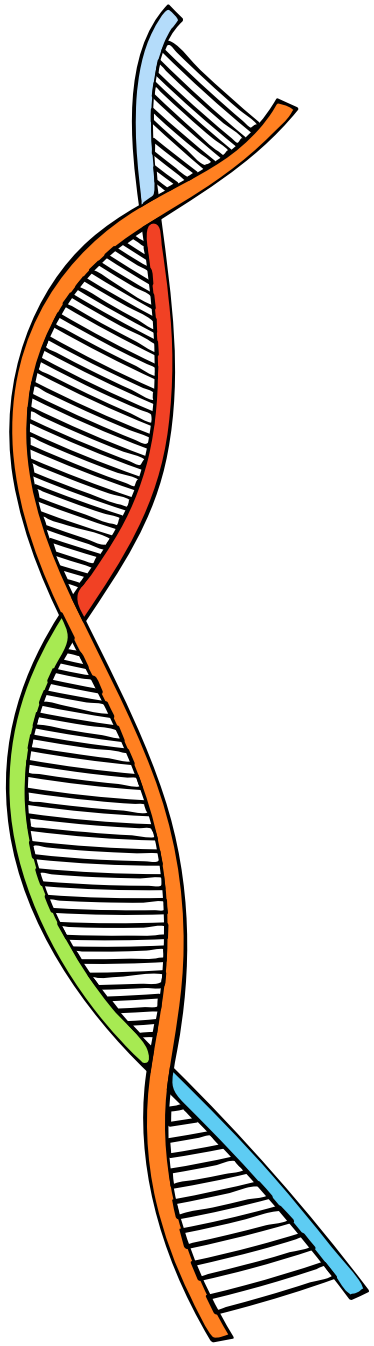
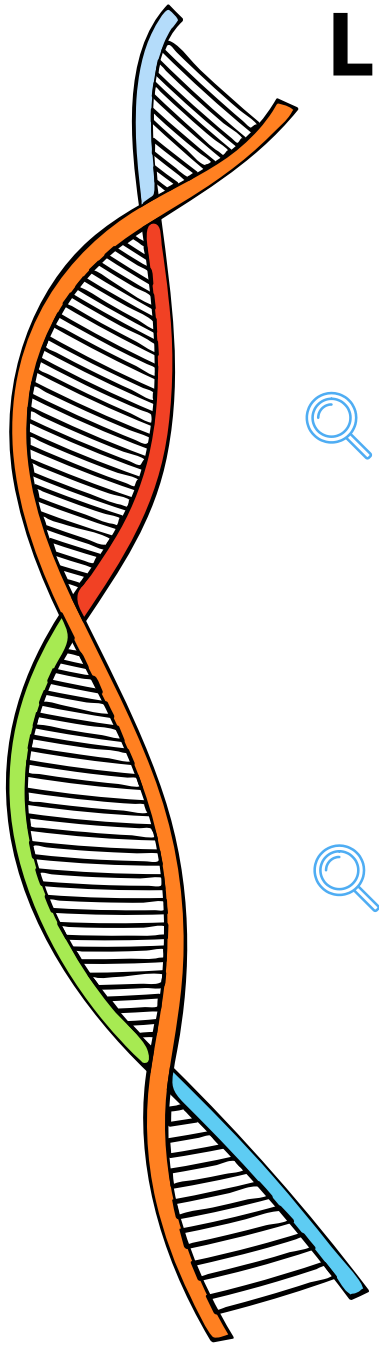


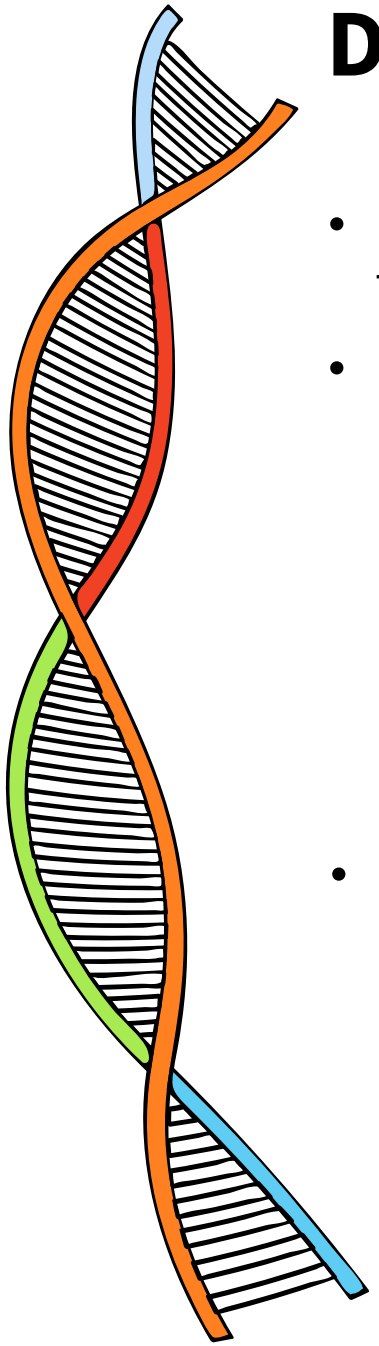
Lecture 4: DNA Fingerprinting

Summer 2026



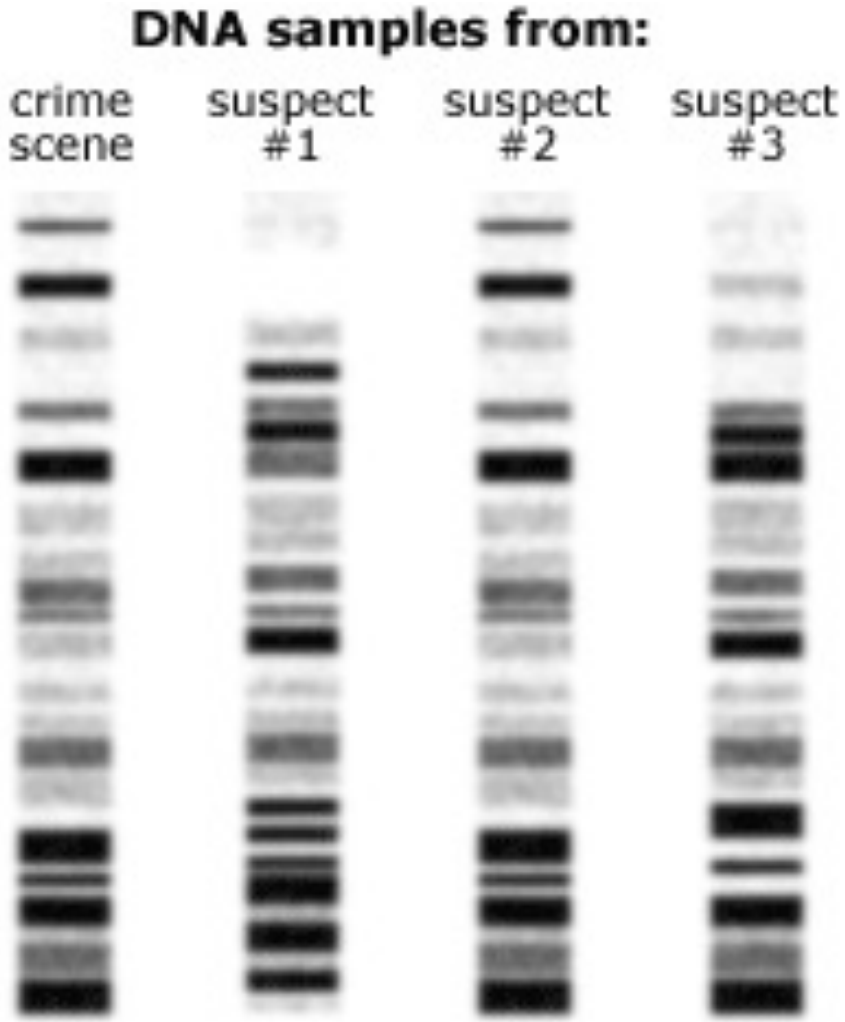
Learning objectives

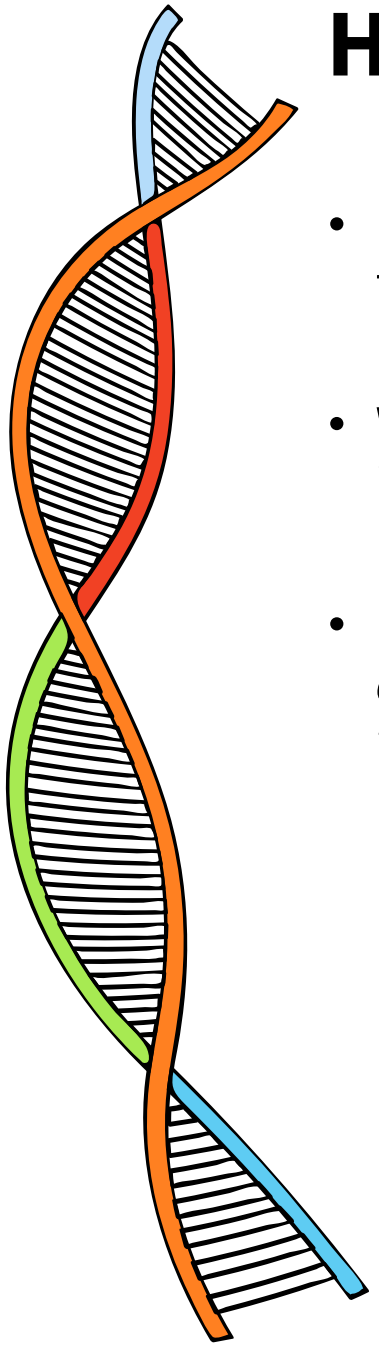
- Understand the role of genetics in DNA fingerprinting
- List the differences between mini and micro-satellites
- Define heterozygous and homozygous
- Explain step by step the method of DNA fingerprinting
- Describe the process of differential extraction
- Analyse a DNA fingerprint to determine potential suspects or paternity



DNA fingerprinting

- Until 1986, there was no good forensics method that allowed for individualization
- Remember that evidence must be:
  - Testable
  - Have acceptable error rates
  - Be accepted by the scientific field
- In 1987, DNA fingerprinting came along and revolutionized forensic science





History of DNA fingerprinting

- Dr. Alec Jefferys was the father of DNA fingerprinting with publication in 1998 on minisatellites.
- Working to develop genetics markers more informative than single nucleotide polymorphisms (SNPs)
- DNA fingerprinting came about by "*Pure accident and academic curiosity, simple as that*"- Dr Jefferys

Alec Jeffreys, the father of forensic DNA analysis

History of DNA fingerprinting

- In Australia, we would call this “pulling a Bradbury”
- Steven Bradbury won the first Winter Olympics gold medal in 2002, winning the race when a corner pileup took everyone else out.

“Pulling a Bradbury”

Cell, Vol. 18, 1–10, September 1979, Copyright © 1979 by MIT

DNA Sequence Variants in the $^{G}\gamma$–, $^{A}\gamma$–, δ– and β–Globin Genes of Man

Alec J. Jeffreys
Genetics Department
University of Leicester
University Road
Leicester LE1 7RH, England

Restriction Fragment Length Polymorphisms seen in the human globin loci using Southern blots and hybridization with radioactive probes.

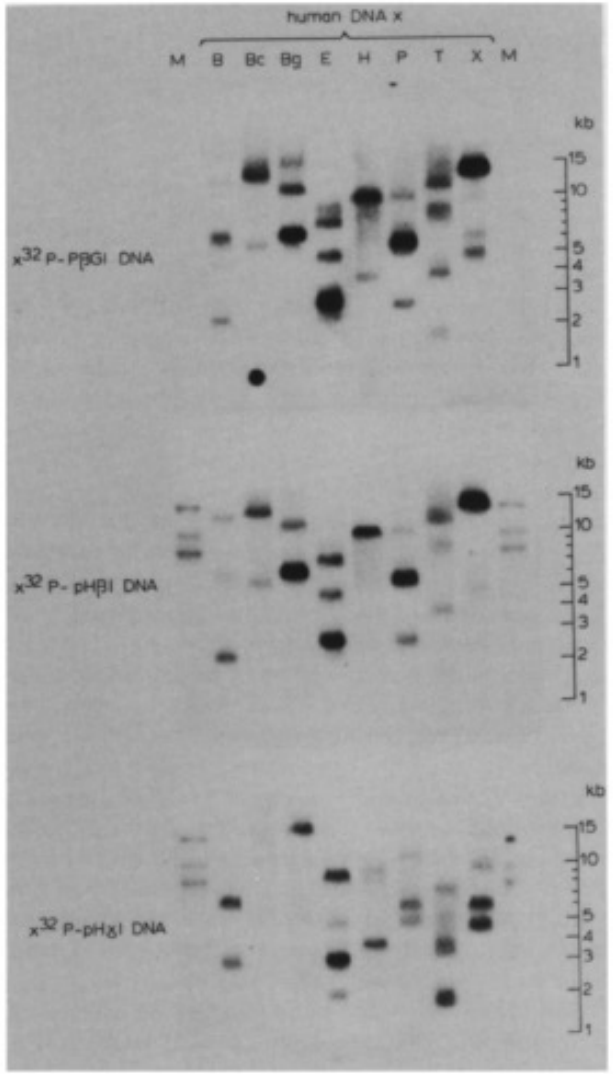


Figure 1. Comparison of Human Globin DNA Fragments Detected by ^{32}P-Labeled PβG1 DNA, pHβG1 DNA and pHγG1 DNA

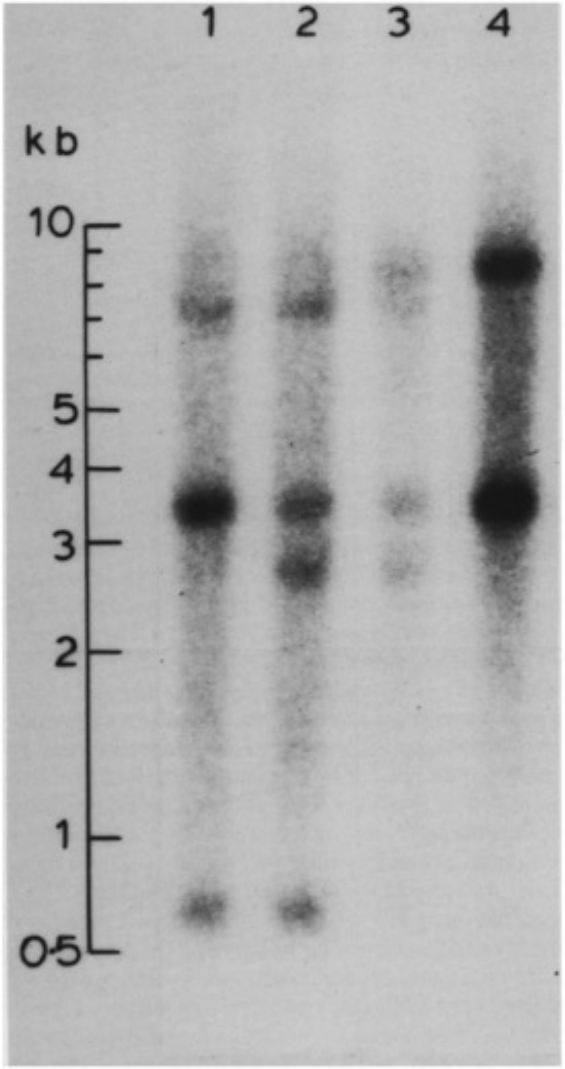
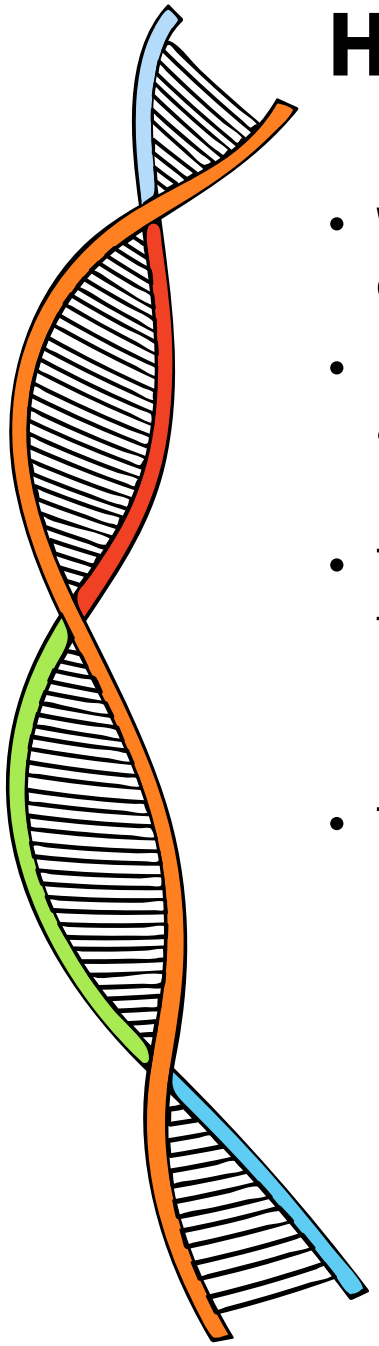


Figure 5. Variant Patterns of γ-Globin DNA Fragments Produced by Cleaving Human DNA with Endonuclease Hind III: the $^{G}\gamma$H ± Polymorphism

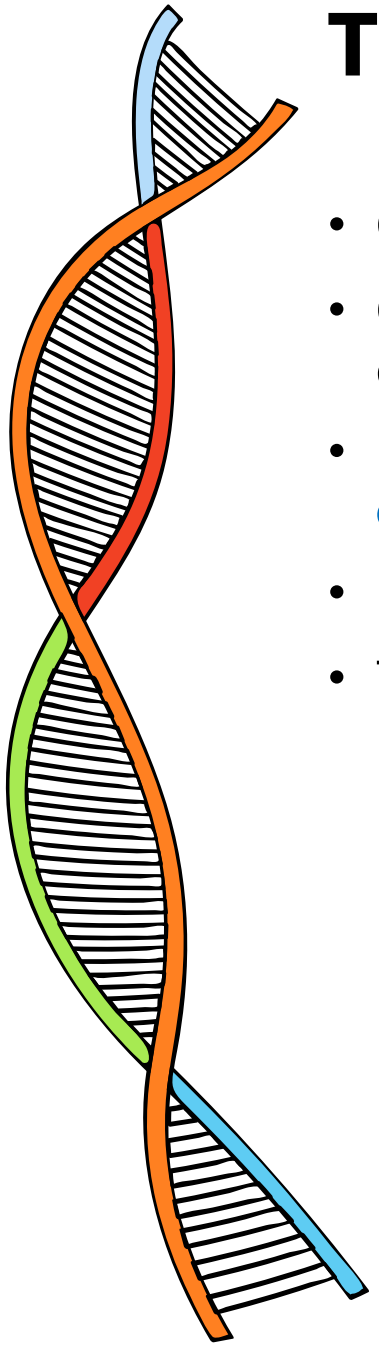


History of DNA fingerprinting

- We discussed the first ever conviction made based on DNA fingerprinting evidence in lecture 2
- 1987: Colin Pitchfork was arrested for the sexual assault and murder of two young girls in the Leicestershire area, UK
- The DNA first exonerated him, but then it was found that his friend had used his DNA to cover for pitchfork

- To understand DNA fingerprinting, we need to understand the genome and genetics

The Colin Pitchfork case – lecture 2



The genome

- Genomes contain the blueprints of life
- Consist of DNA which carry genes that determine the functionality of the organism
- Each human genome is unique – **can you think of an exception?**
- Uniqueness is conferred by **polymorphisms**
- The flow of genetic information in the human body is determined by **the central dogma**

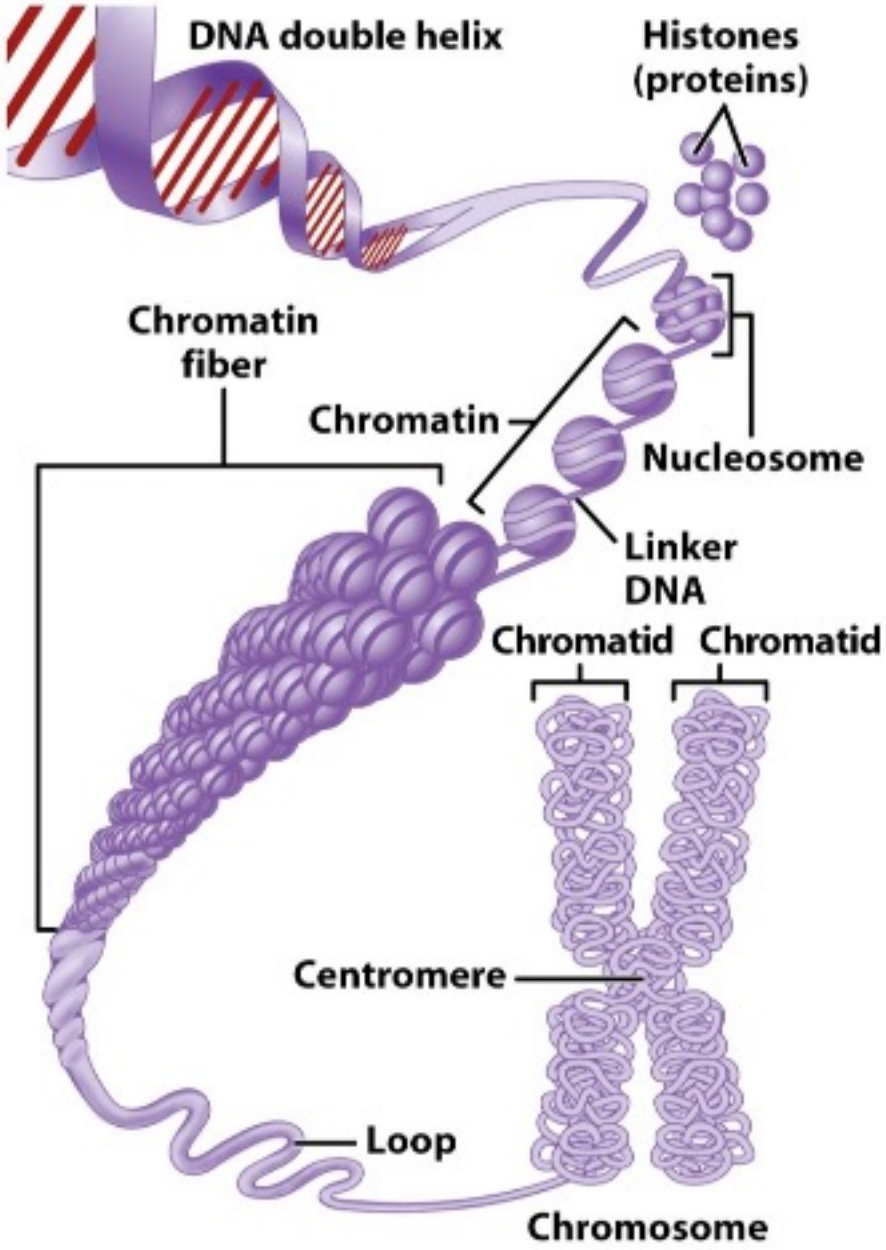


Figure 3-23 Principles of Anatomy and Physiology, 11/e
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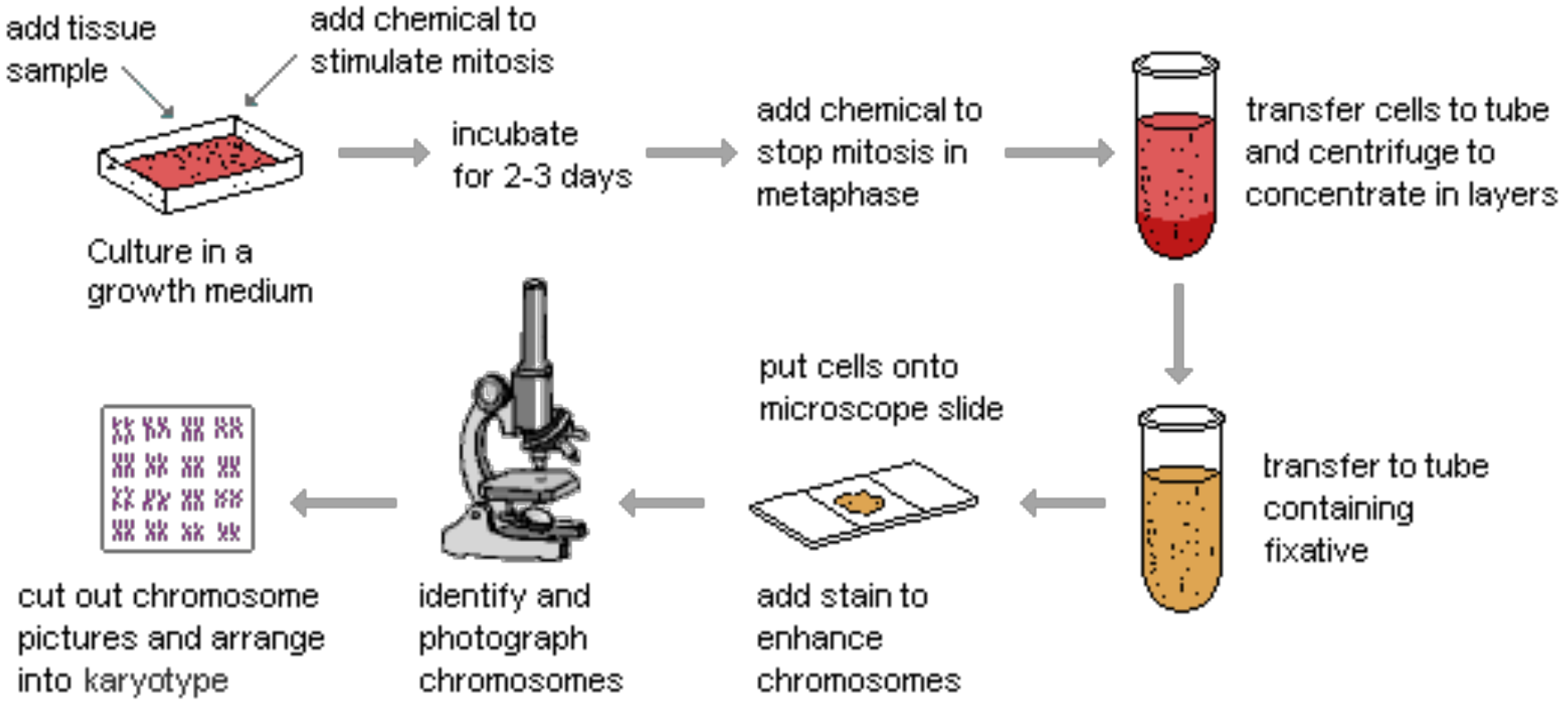
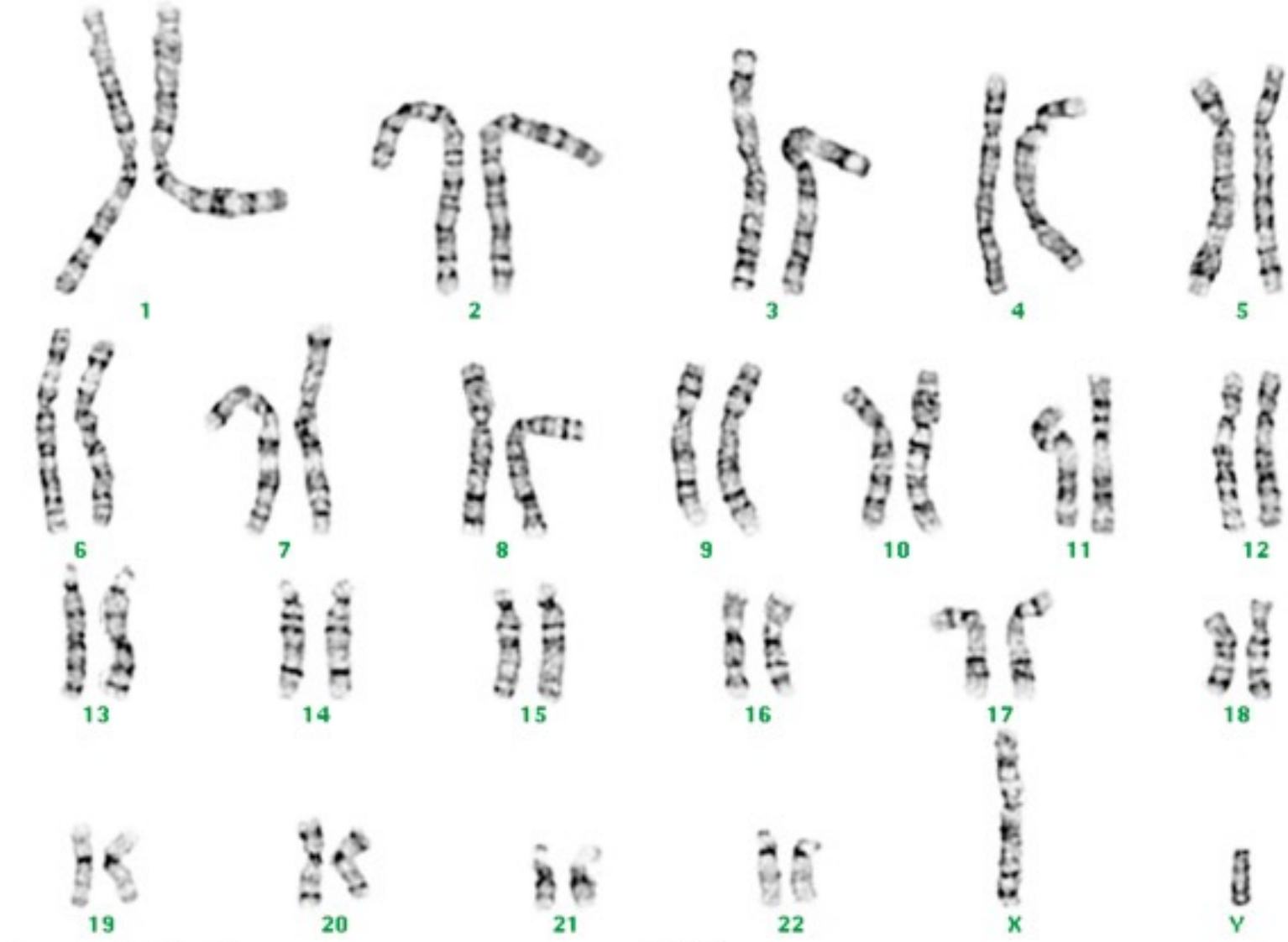
Karyotyping

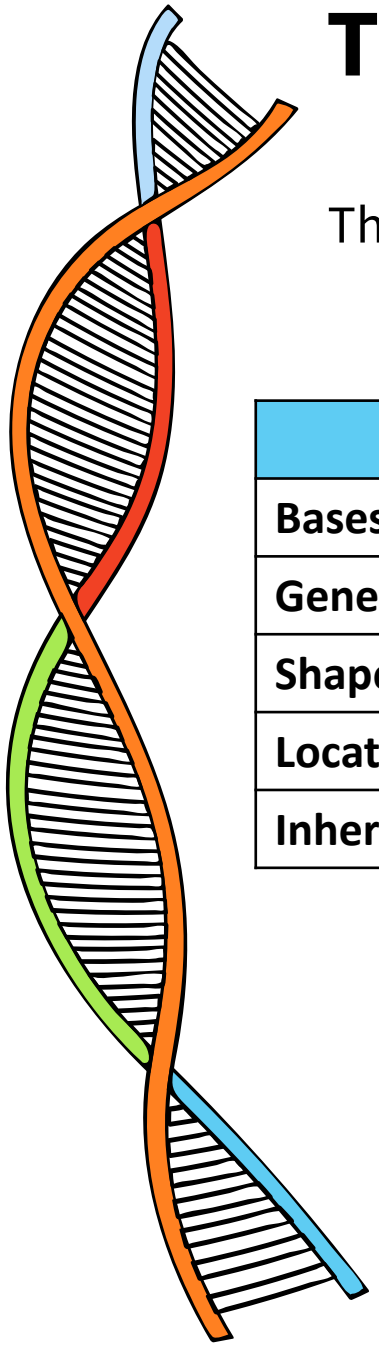
- Until 1956, it was thought that we had 48 chromosomes in our cells - just like the other great apes
- However, through karyotyping, it was determined we only have 46



The human karyotype



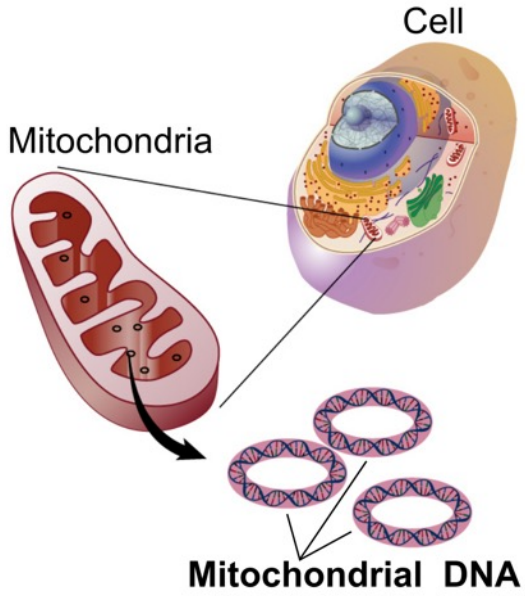
Normal Male Karyotype 46,XY

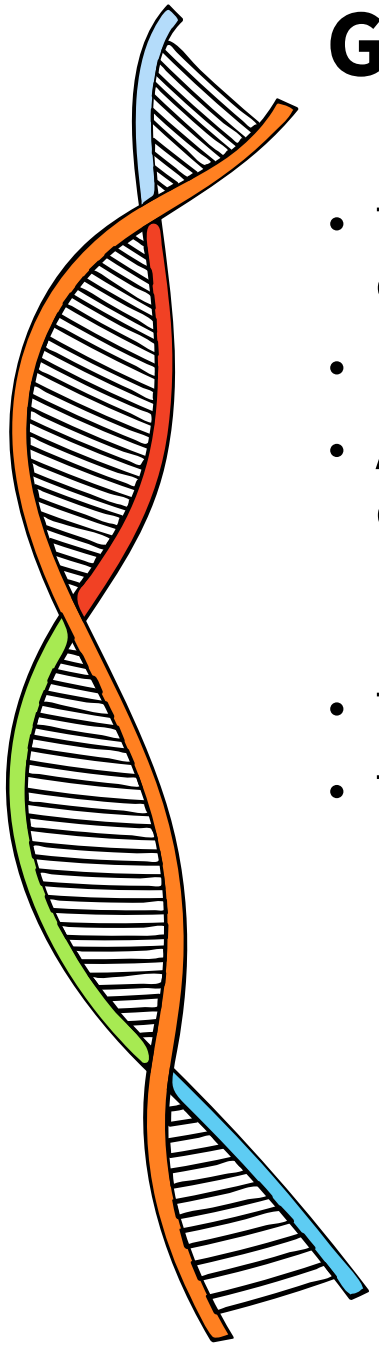


Types of DNA

There are 2 types of DNA in humans: **nuclear DNA** and **mitochondrial DNA**

	Nuclear DNA	Mitochondrial DNA
Bases	3.2 billion	16,569
Genes	25,000	37
Shape	Linear	Circular
Location	Nucleus	Mitochondria
Inheritance	Mendelian	Maternal

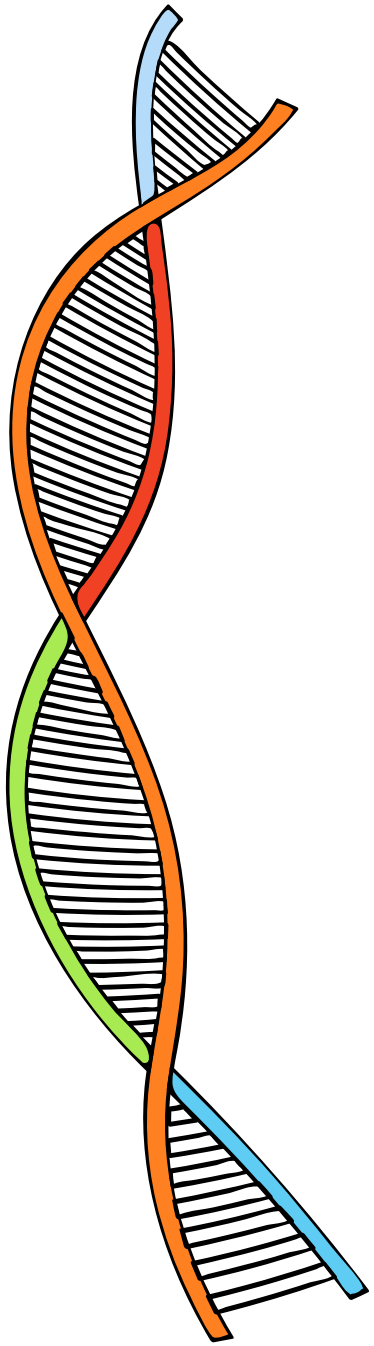


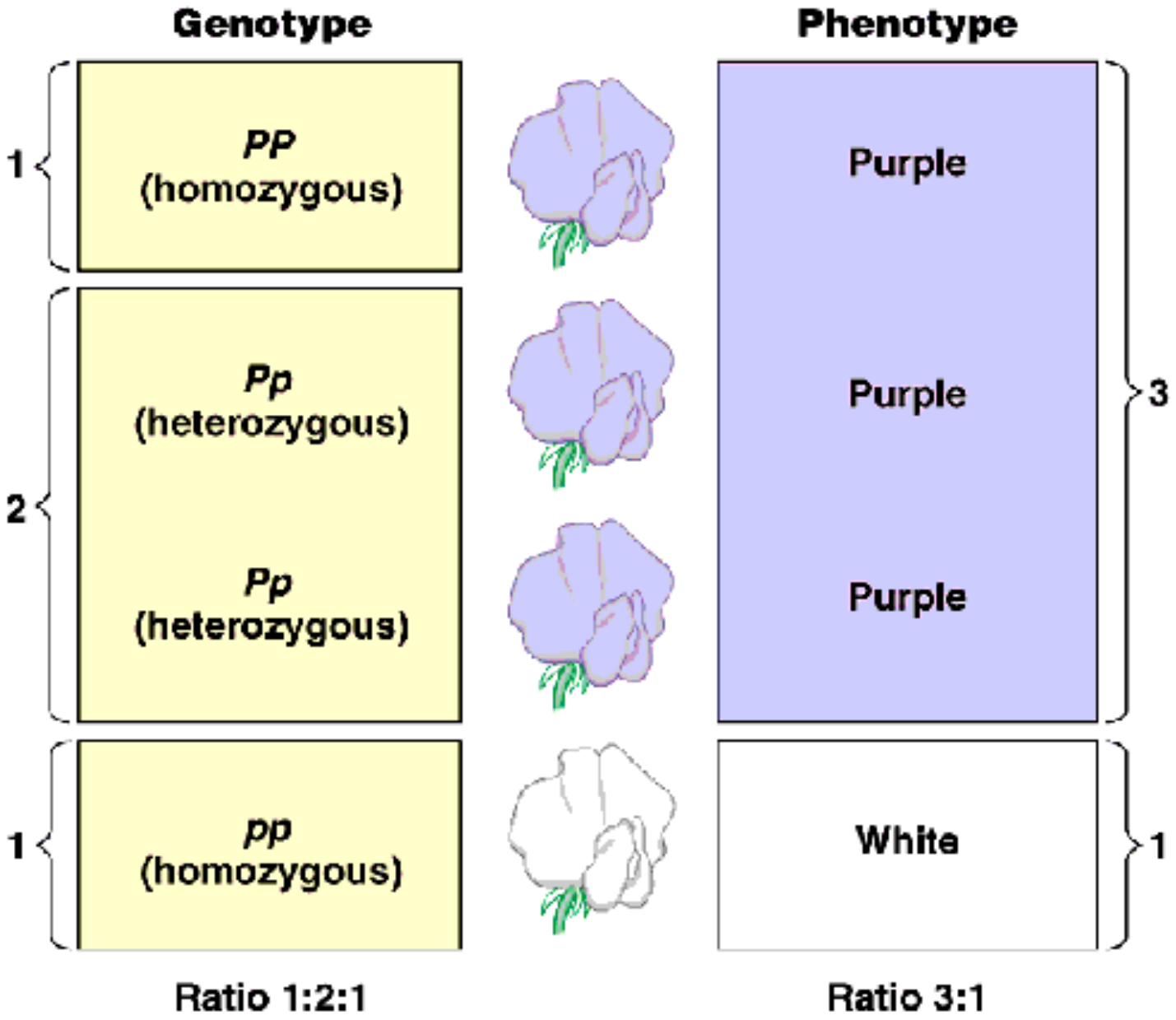


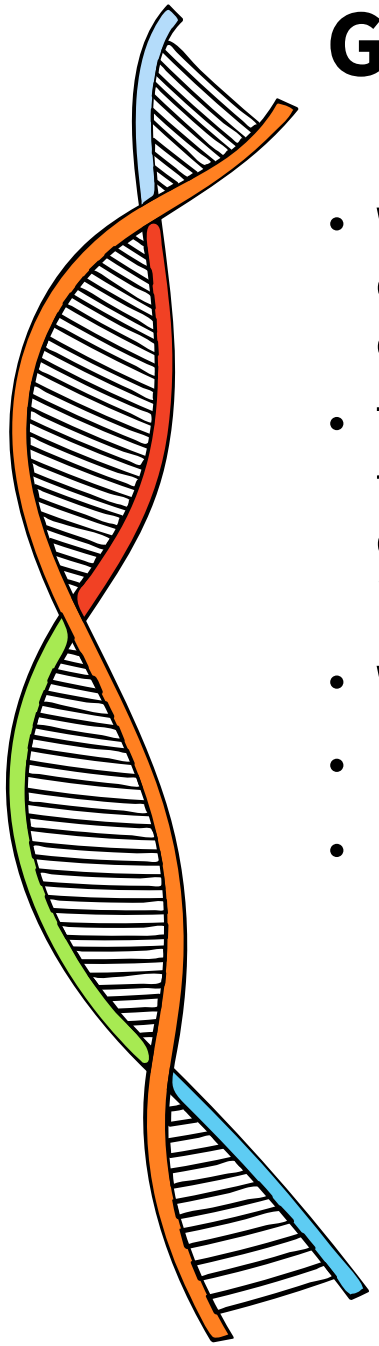
Genotype vs. phenotype

- The **genotype** is the genetic information that is responsible for a particular physical characteristic
- In a diploid organism, a genotype consists of 2 alleles
- A genotype can be **homozygous** (having the same 2 alleles) or **heterozygous** (having 2 different alleles)

- The **phenotype** is the observable expression of the physical trait
- The phenotype depends upon the genotype

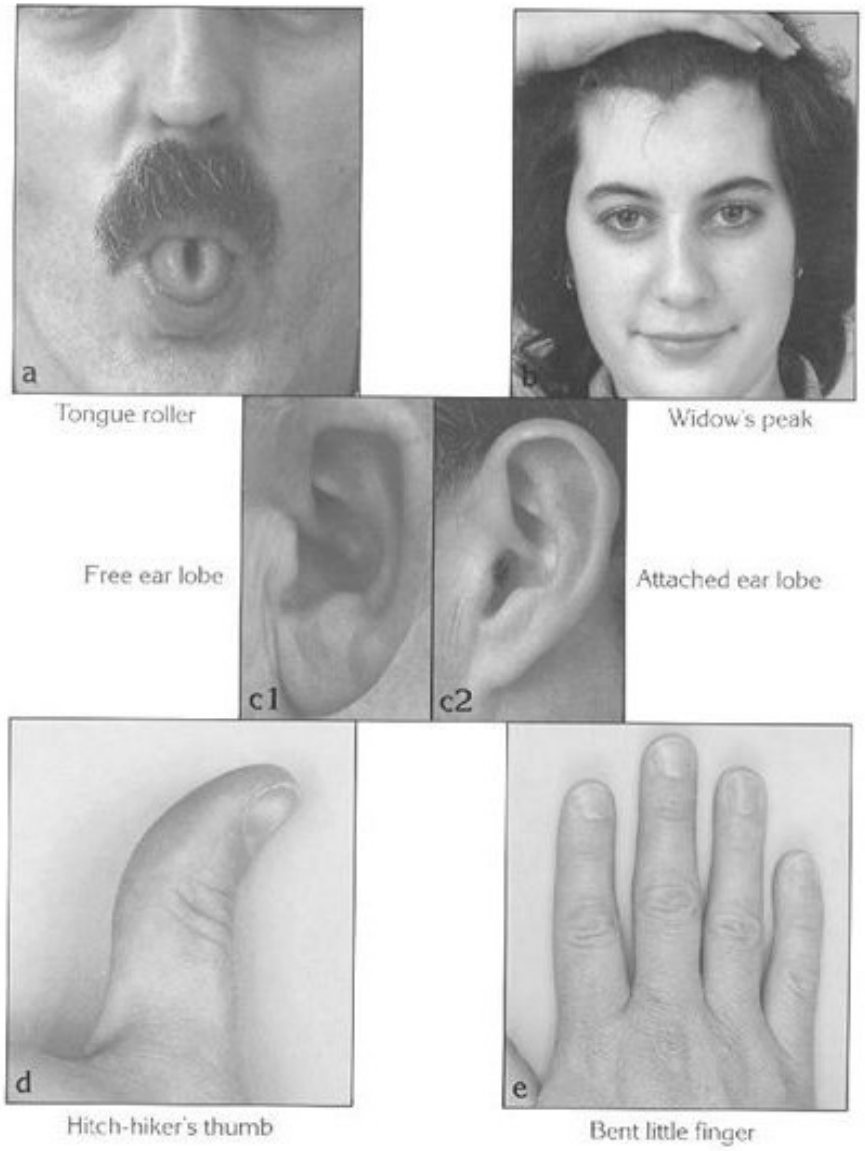


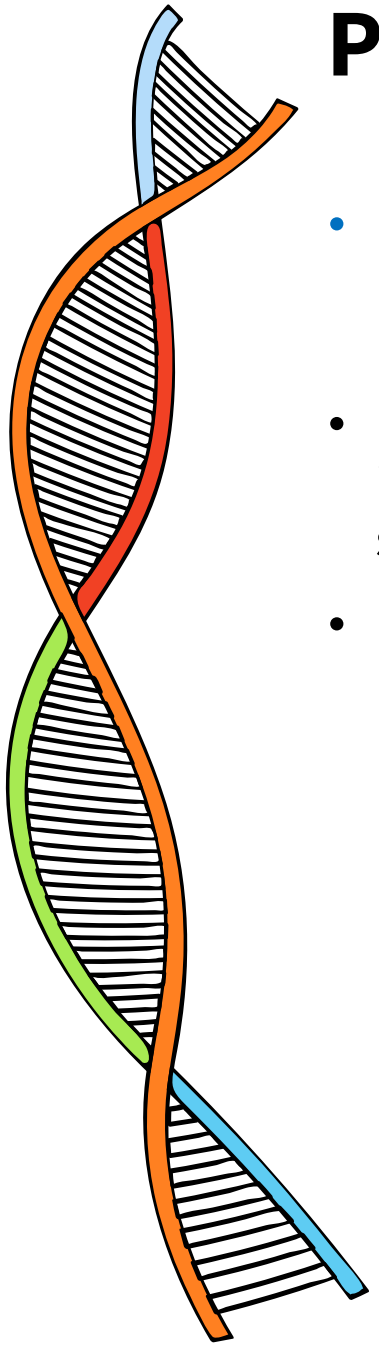




Genes are units of heredity

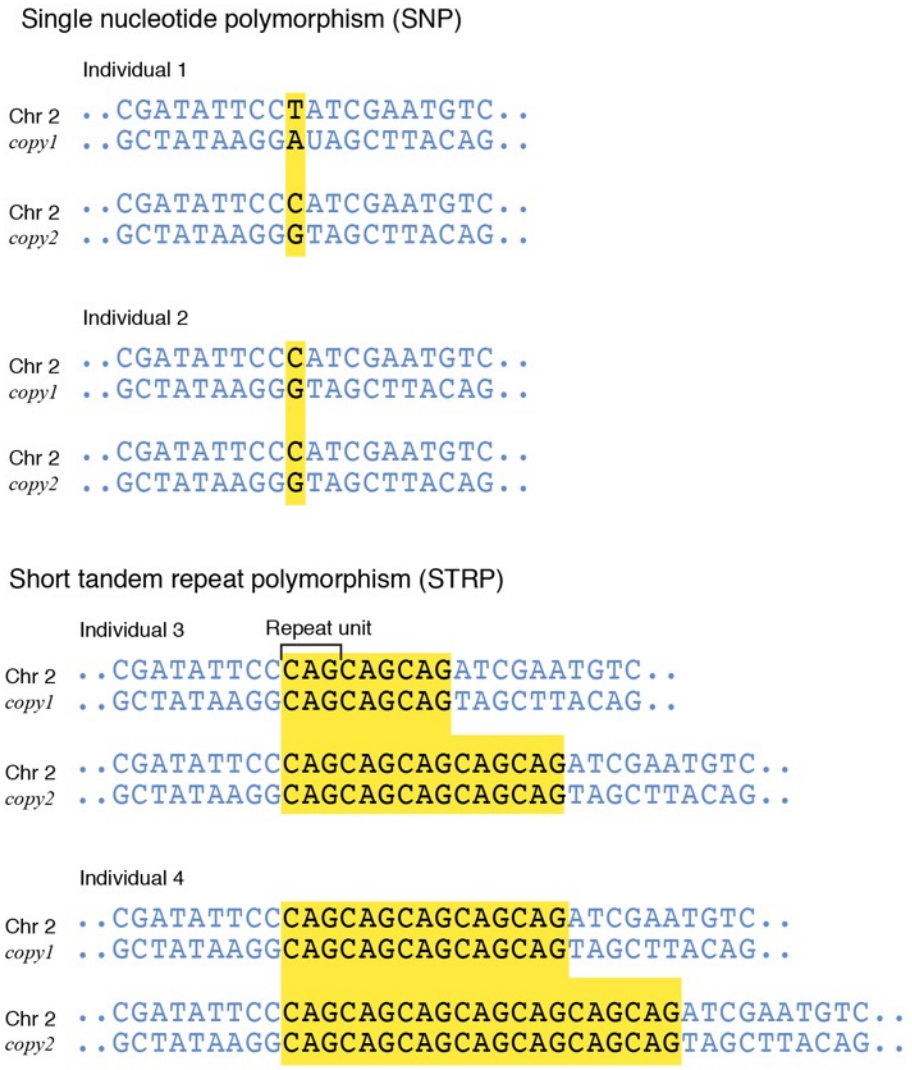
- We inherit our genes from our parents – one copy from each, except for the genes on the sex chromosomes
- The high level of variability that we see from person to person is due to small differences within genes that our inherited
- Who here can roll their tongue?
- Do you have an attached ear lobe?
- Do you have hitch-hikers thumb?

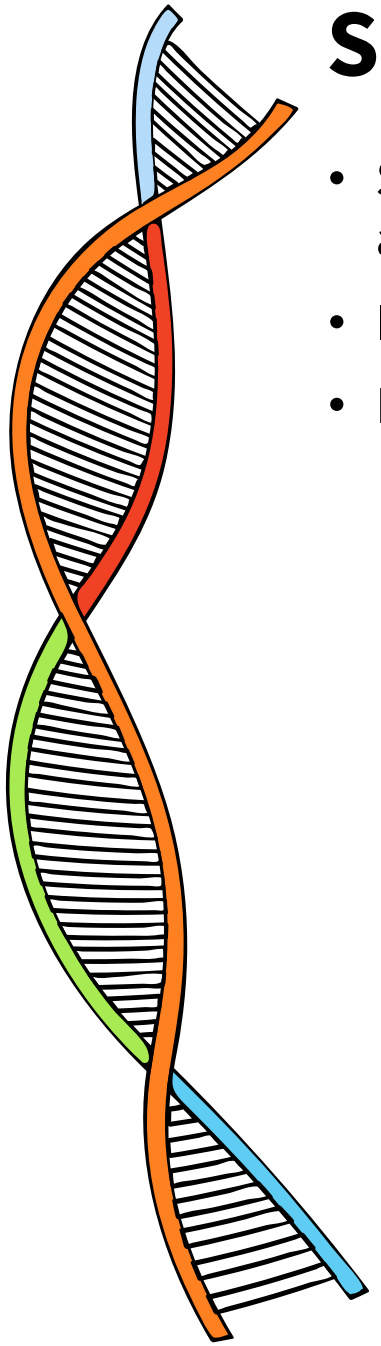




Polymorphisms

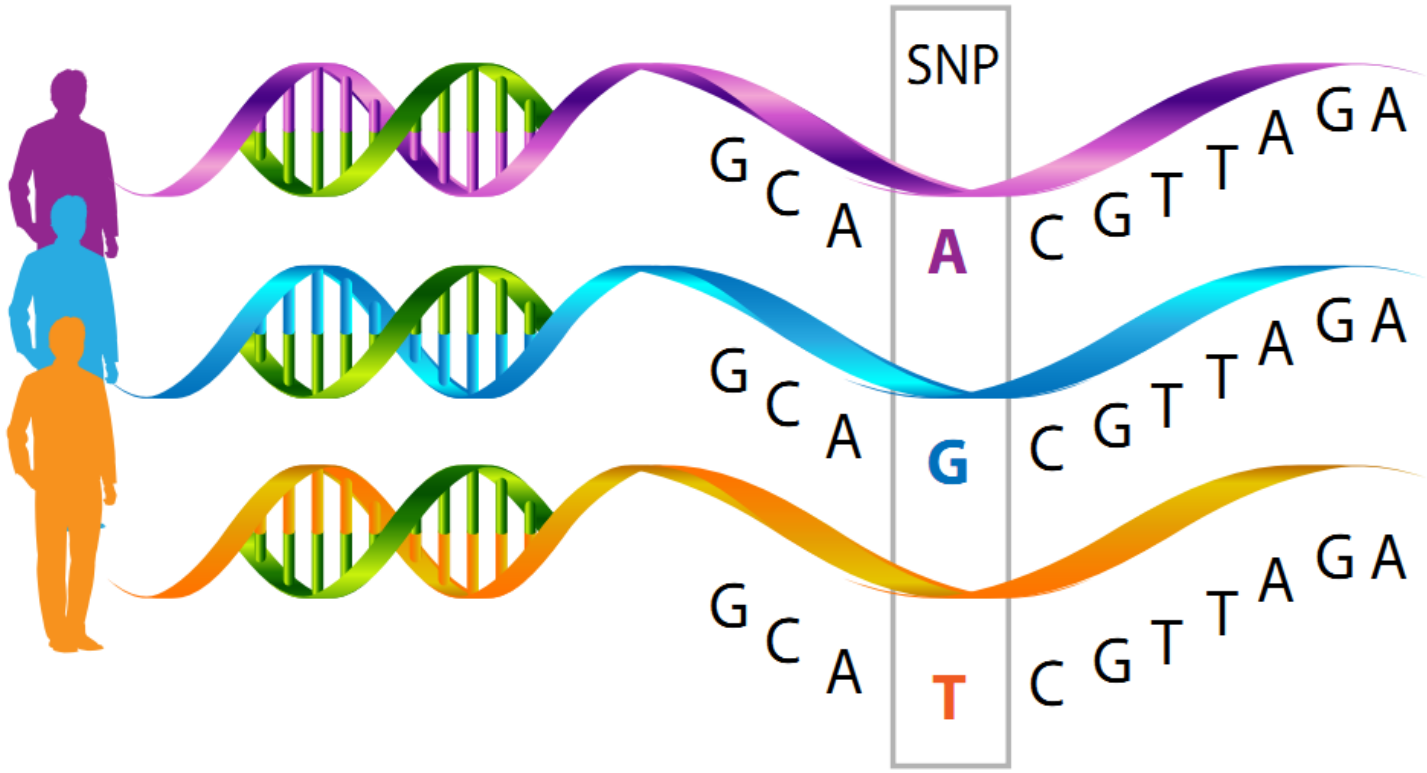
- **Polymorphisms** are inheritable differences in DNA sequences between individuals of a population
- Polymorphisms can take several forms incorporating one or more nucleotides or series of nucleotides
- Polymorphisms may or may not have a phenotypic effect

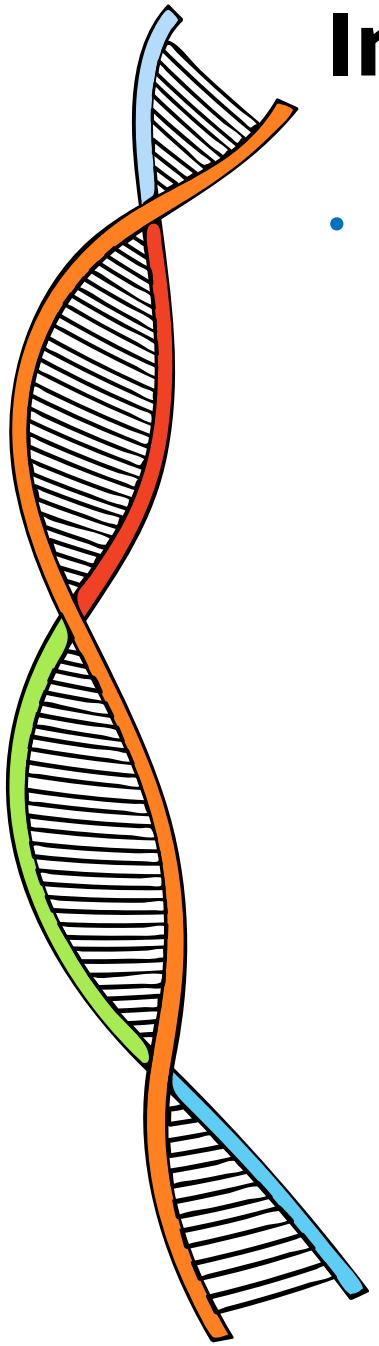




Single nucleotide polymorphisms (SNPs)

- SNPs are single nucleotide changes between individuals affecting either one or both alleles at the genomic location
- Inherited, therefore different populations have different proportions of SNPs
- Not really used in a forensic context until recently





Insertions and deletions

- **Indels** (insertions and deletions) are less common than SNPs but may have more of a biological effect as they disrupt the reading frame of the gene

wild-type sequence

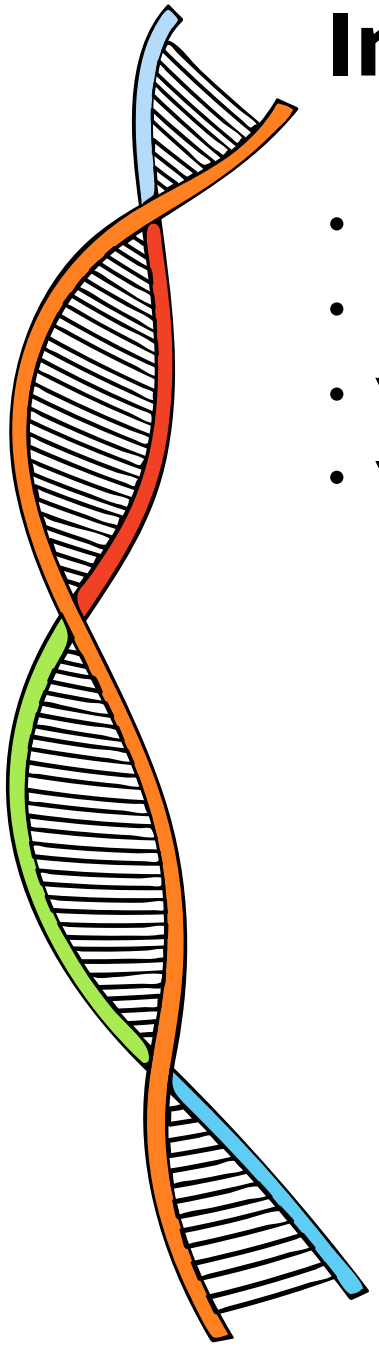
ATCTTCAGCCATAAAAGATGAAGTT

3 bp deletion

ATCTTCAGCCAAAGATGAAGTT

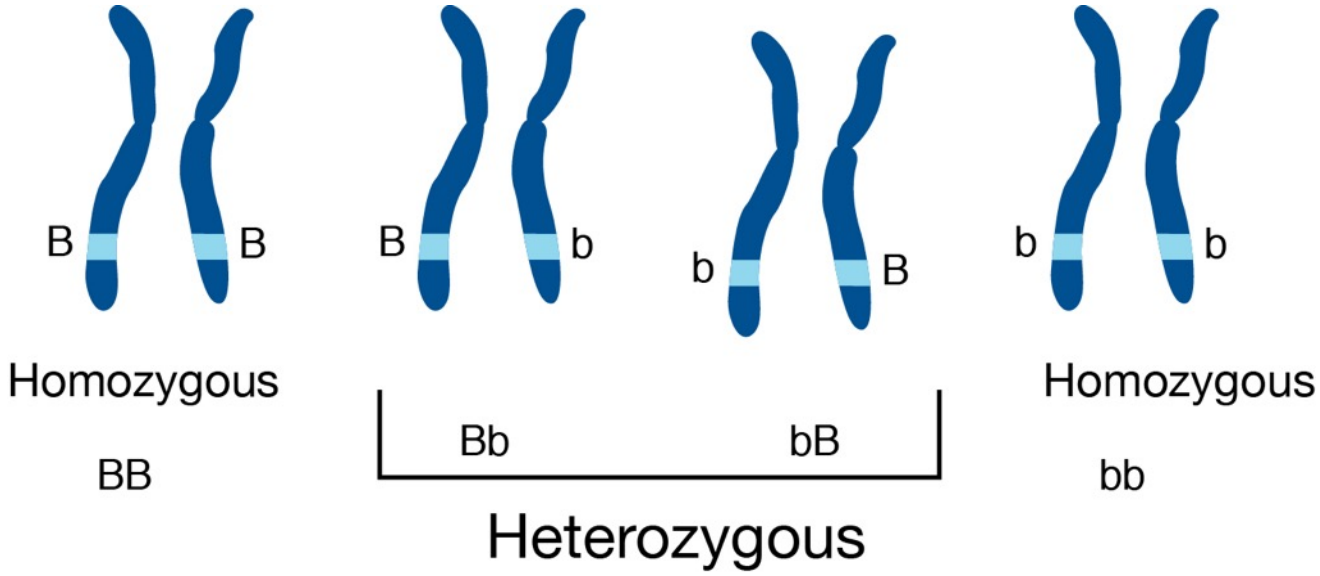
4 bp insertion (orange)

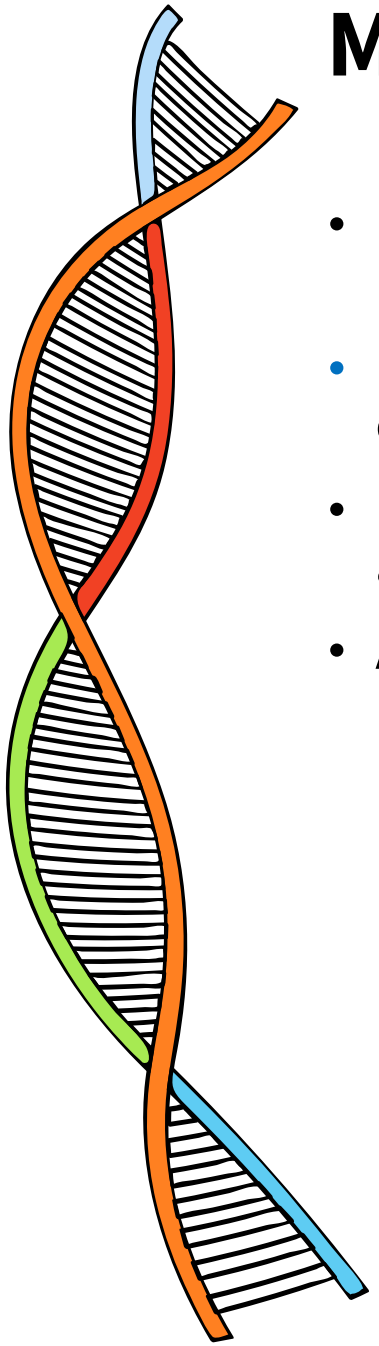
ATCTTCAGCCATATGTGAAAGATGAAGTT



Inheritance

- Models of genetic inheritance have been studied for over 150 years
- In humans, we inherit one of each chromosome from each of our parents
- Your genotype for each polymorphic location depends on the genotype of your parents
- You can be **homozygous** or **heterozygous** at each polymorphic position





Mendelian inheritance

- 1856-1866: Gregor Mendel conducted breeding experiments with pea plants
- **Blending theory** – offspring have a blend of the characteristics of their parents
- Mendel noticed that his pea plants did not adhere to blending theory
- A hybrid between a tall and a short pea plant did not yield a medium sized plant

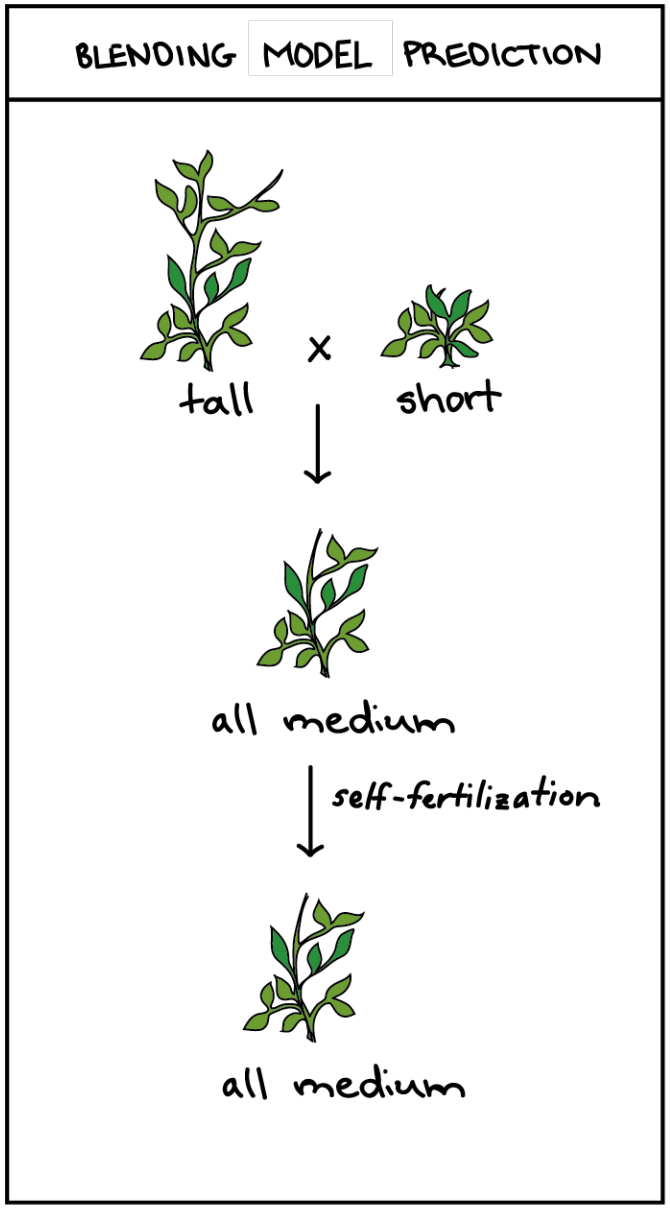
Gregor Mendel, the forefather of modern genetics

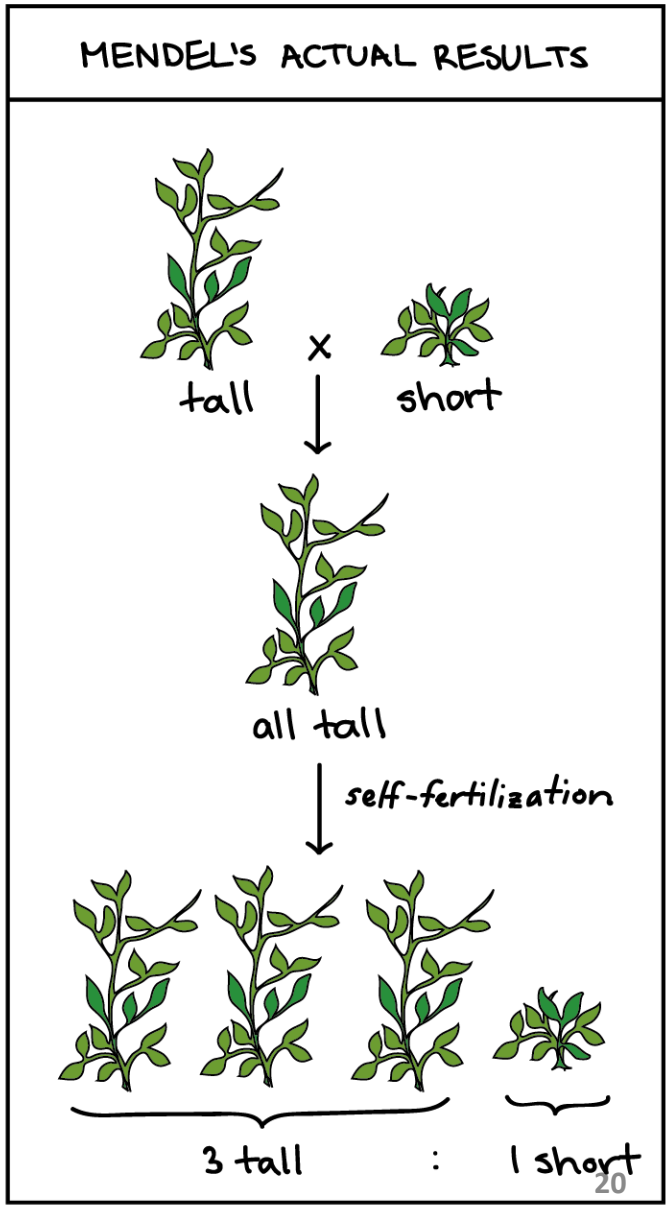
Mendelian inheritance

One feature (tall height) always concealed the other feature (short height) in the first generation.

The hidden form always presented in the second generation as a minority.

Mendel found that traits were controlled by pairs of heritable factors.





Mendelian inheritance

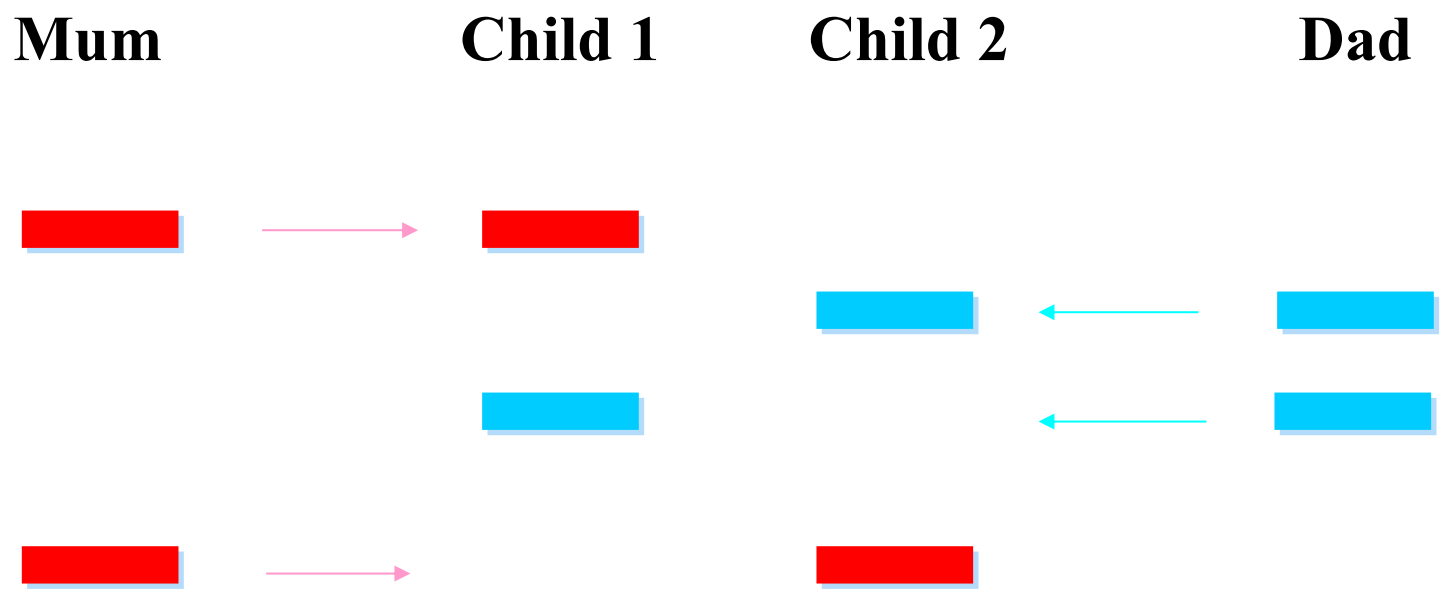
	T	t
T	 tall	 tall
t	 tall	 short

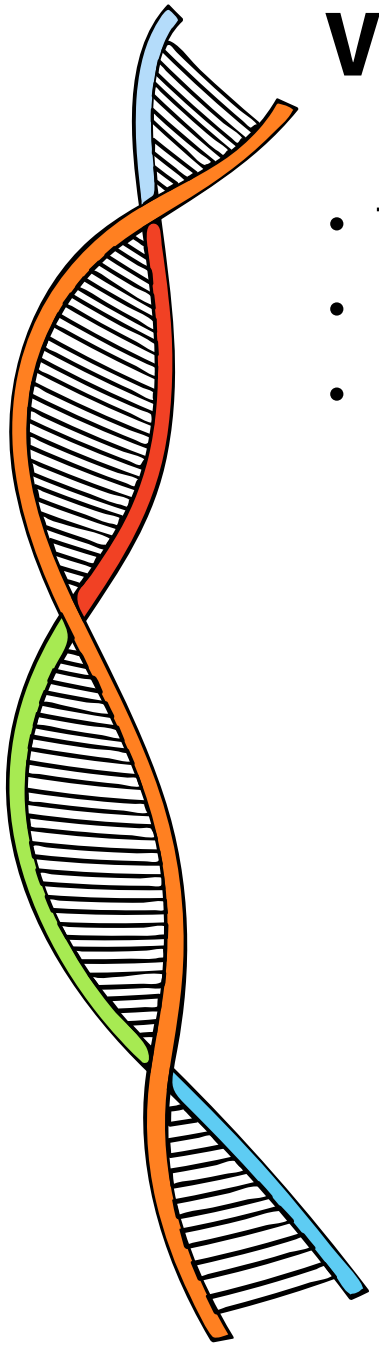
In this experiment, the phenotype is tall with a TT, Tt, and tT genotype.

The plant is only short with a tt genotype.

DNA fingerprinting is based on genetics

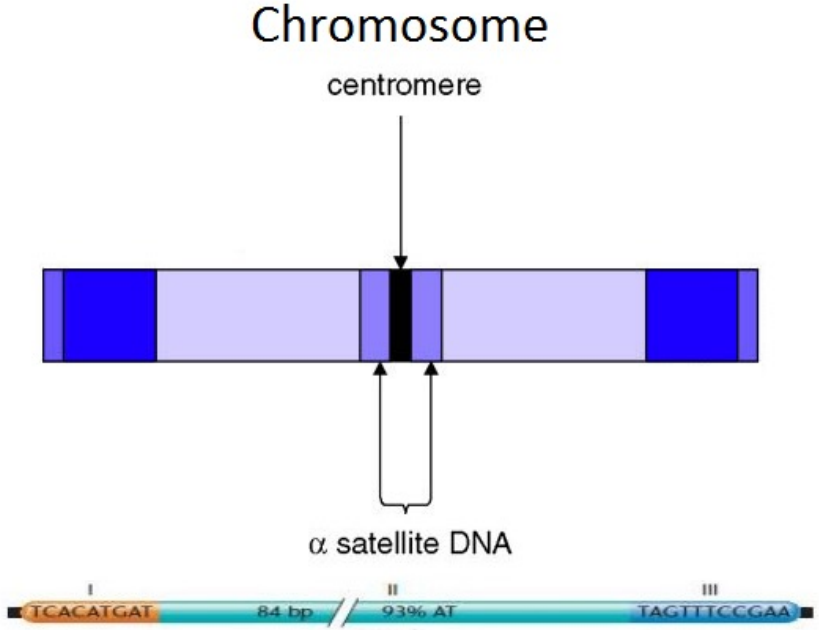
In the context of DNA fingerprinting, we inherit our DNA markers from each parent.

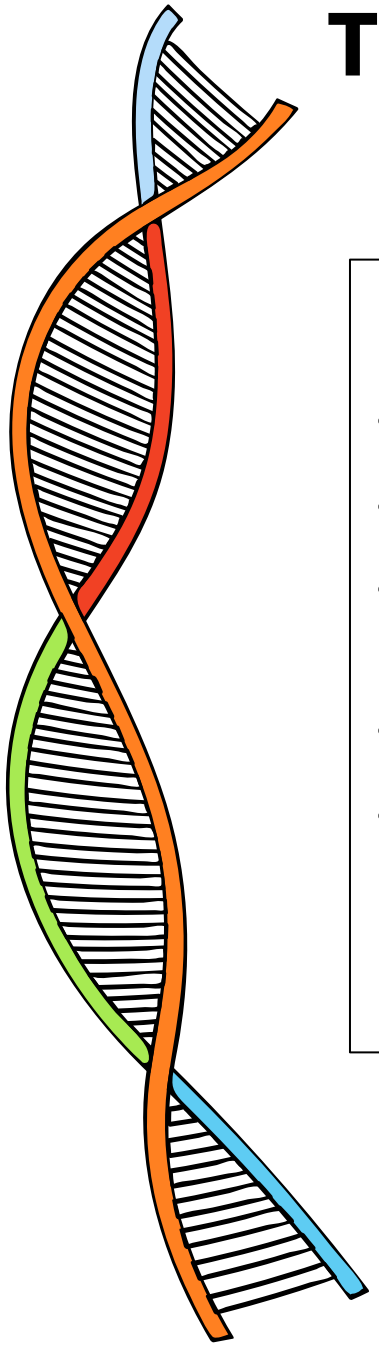




Variable regions of DNA

- The genome contains **satellites**: regions of DNA that are highly variable
- Highly conserved repeats of DNA motifs, but with a variable number of repeats
- For example: alphoid sequences
  - Located at the centromere of all chromosomes
  - Repeat units of 171 base pairs
  - Accounts for 3-5% of DNA in each chromosome





Types of satellites

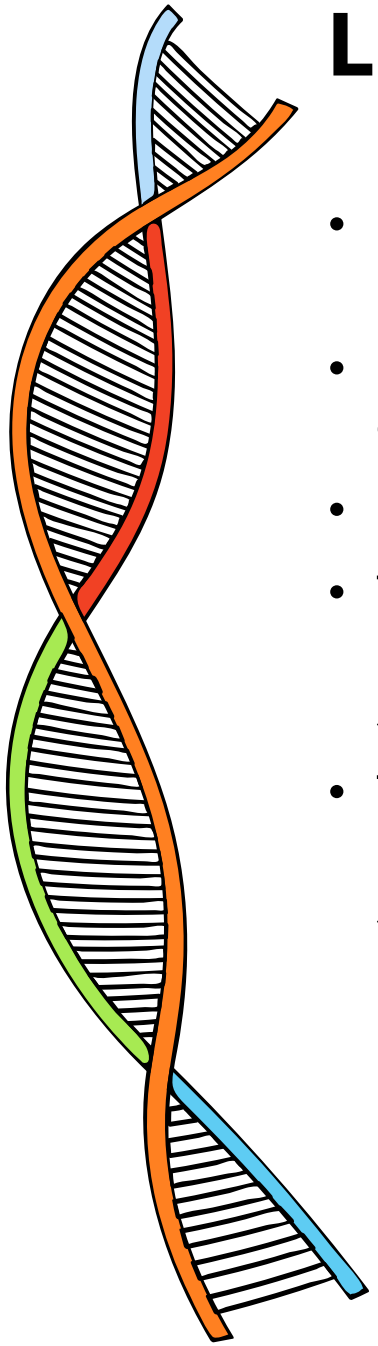
Minisatellites

- Motif length: 10-60 base pairs
- Repeated 5-50 times
- Occur at more than 1,000 locations across the genome
- High mutation rate
- High diversity

Microsatellites

- Motif length: 1-6+ base pairs
- Repeated 5-50 times
- Occur at thousands of locations across the genome
- High mutation rate
- High diversity
- Also called **short tandem repeats** (STRs)

Both types of satellites are called Variable Number Tandem Repeats



Loci

- Do we need to look at the entire genome to see differences between one person and another?
- By looking at one or more loci (genomic positions), we can use statistics to determine the odds of two people having the exact same number of repeated motifs in their VNTRs
- Remember that a person can be **homozygous** or **heterozygous** for an allele at each locus
- There are inheritable variations in DNA that can be used to determine the odds of guilt or to exclude a suspect from having been involved in a crime
- The frequency of the number of repeats for any locus is fixed within certain populations and form part of the statistical evaluation of the fingerprint result

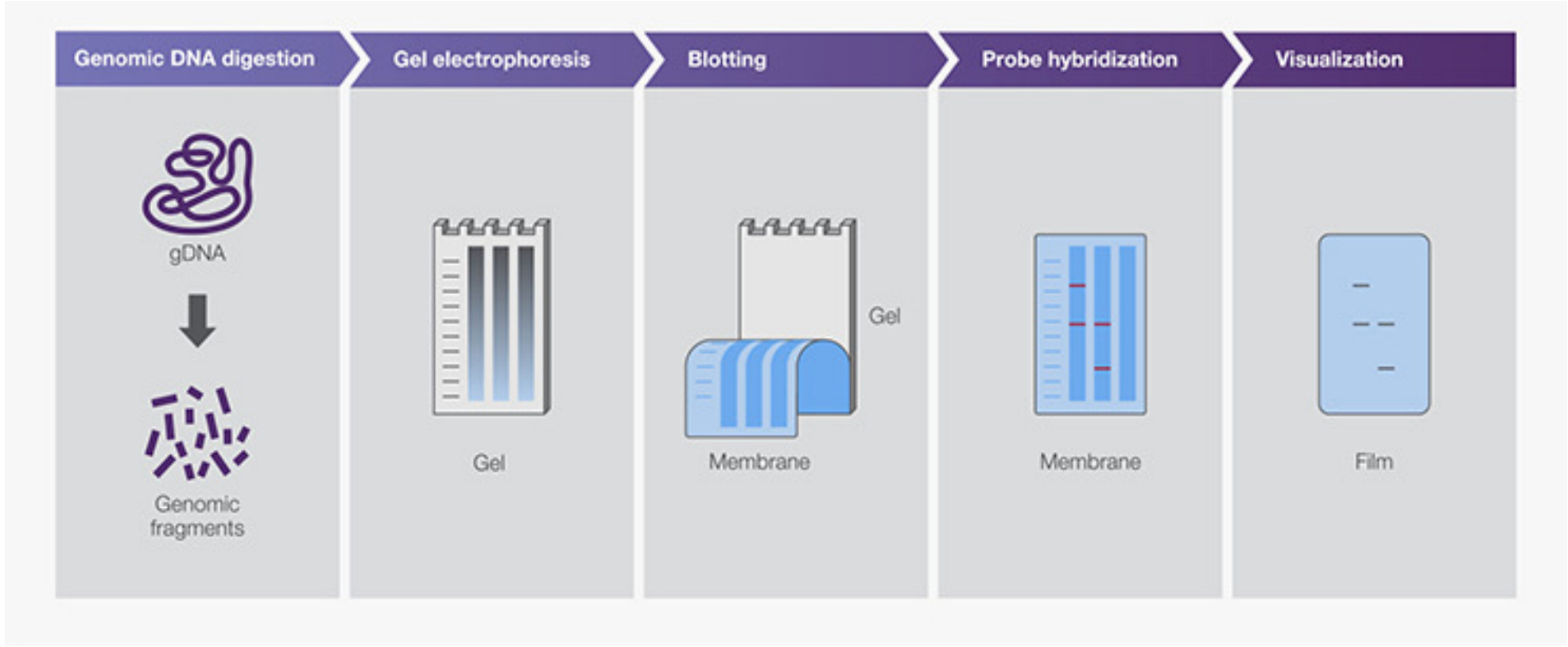
For example, this table shows the frequencies of different repeats (alleles) of three loci in the Sri Lankan population

Allele	Frequency		
	D3S1358	D5S818	D8S1179
8			1.1%
9		3.1%	1.1%
10		10.4%	17.0%
11		33.3%	9.1%
12		37.5%	8.0%
13		12.5%	9.1%
14	7.1%	3.1%	29.5%
15	31.6%		17.0%
16	30.6%		8.0%
17	22.4%		
18	8.2%		

https://pdfs.semanticscholar.org/02c1/1fc4222e2fd5c7657a59b7dd308ebe7aa688.pdf

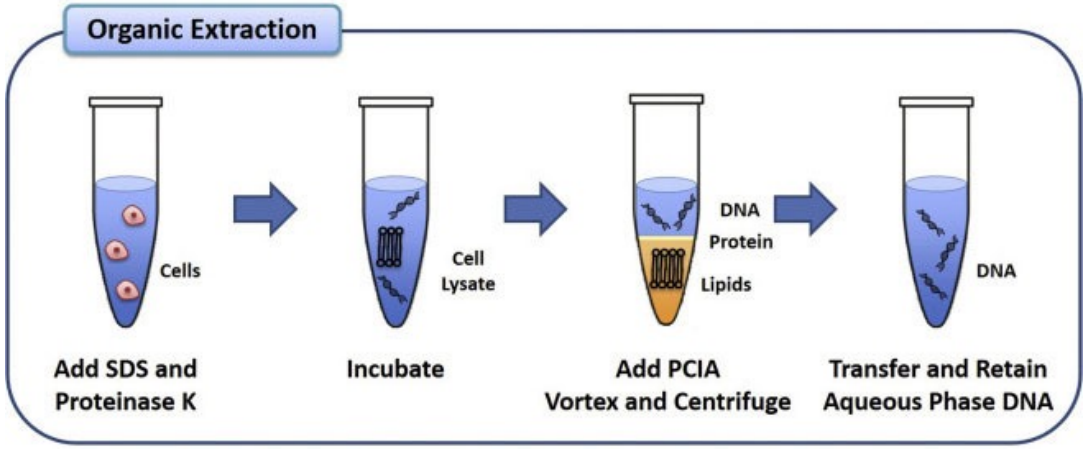
DNA fingerprinting method

DNA fingerprinting is based on a method called **restriction fragment length polymorphism** (RFLP)

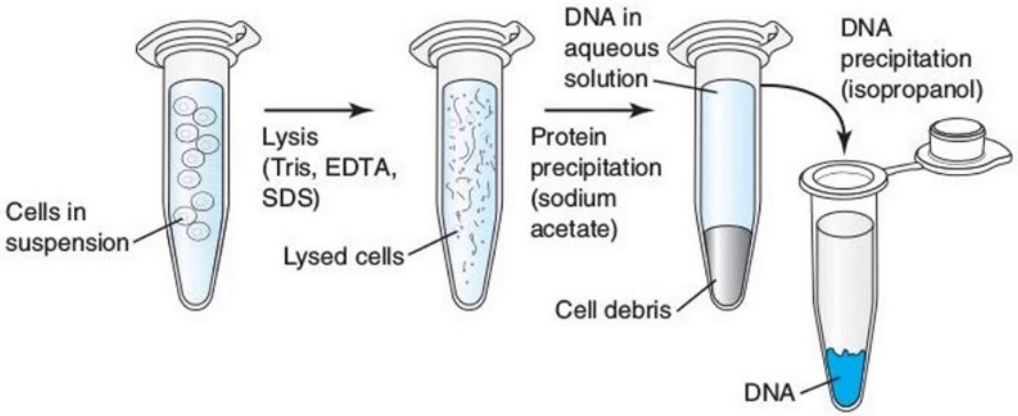


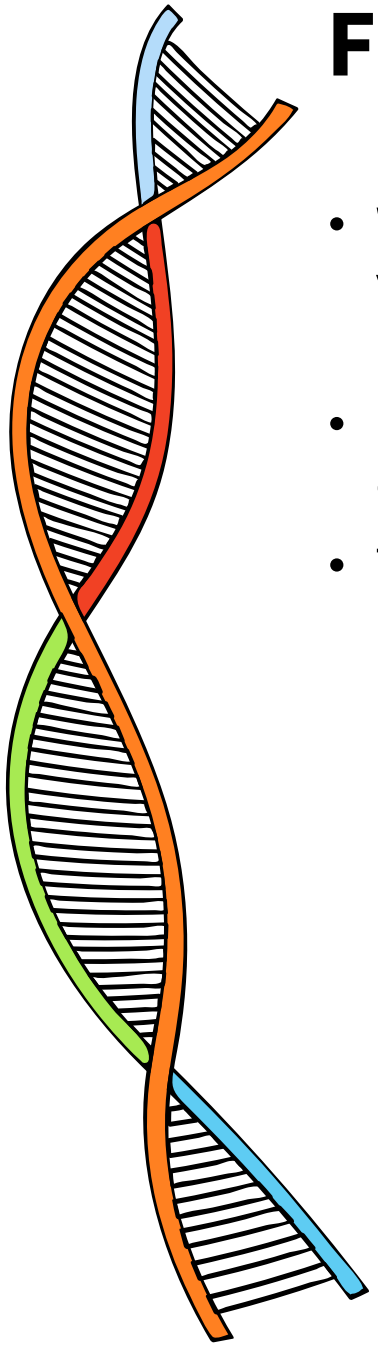
Isolation of DNA

- The first step of RFLP is to get access to the DNA
- There are many types of DNA extraction techniques that fall into 2 broad categories: **organic** and **inorganic** extraction



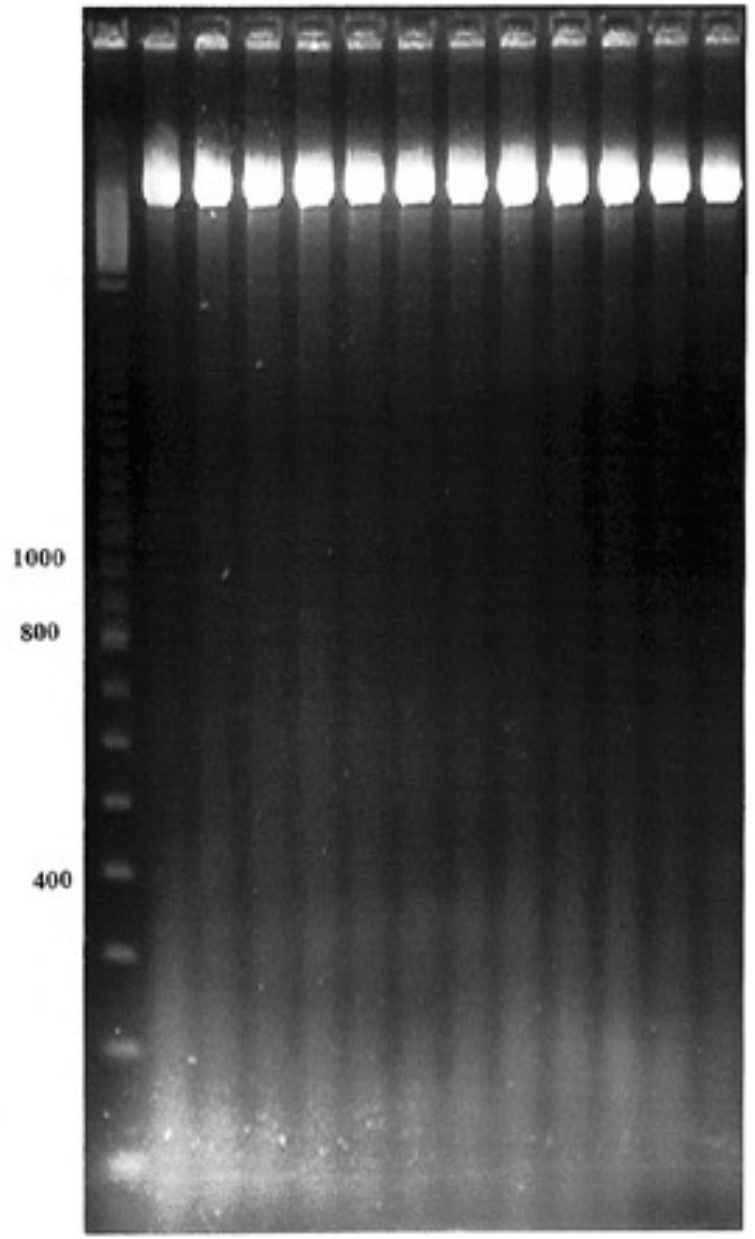
Inorganic extraction





Fragmentation of DNA

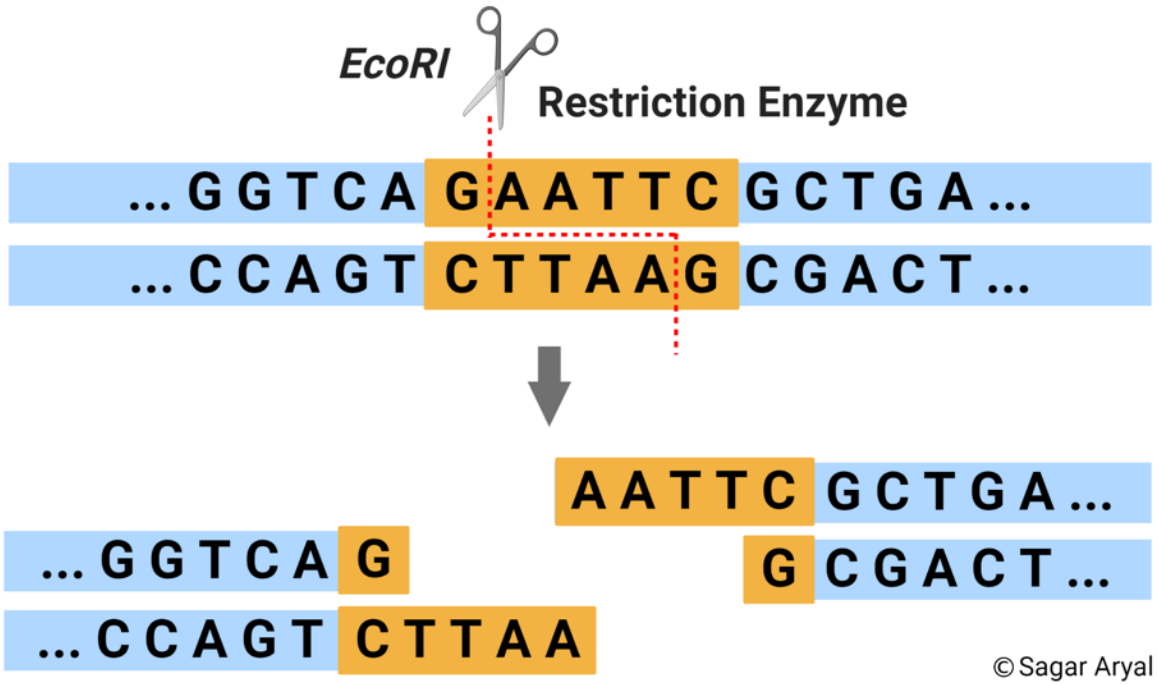
- We need to cut (**digest**) the DNA at these variable sites in order to see how many repeats are contained in them
- Even though our DNA is different to everyone else's, it still looks the same macroscopically
- To do this, we use specific enzymes called **restriction enzymes**



Undigested DNA

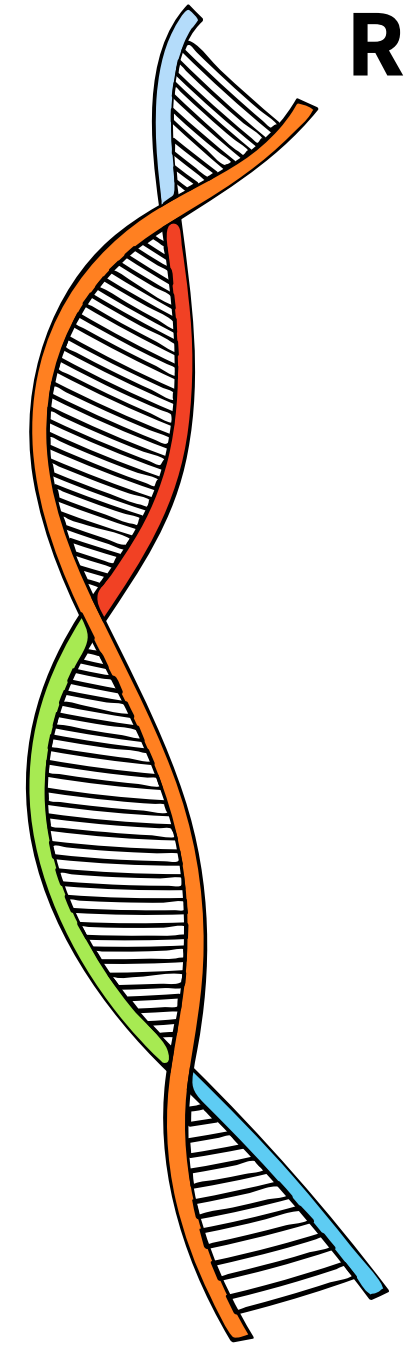
Restriction enzymes

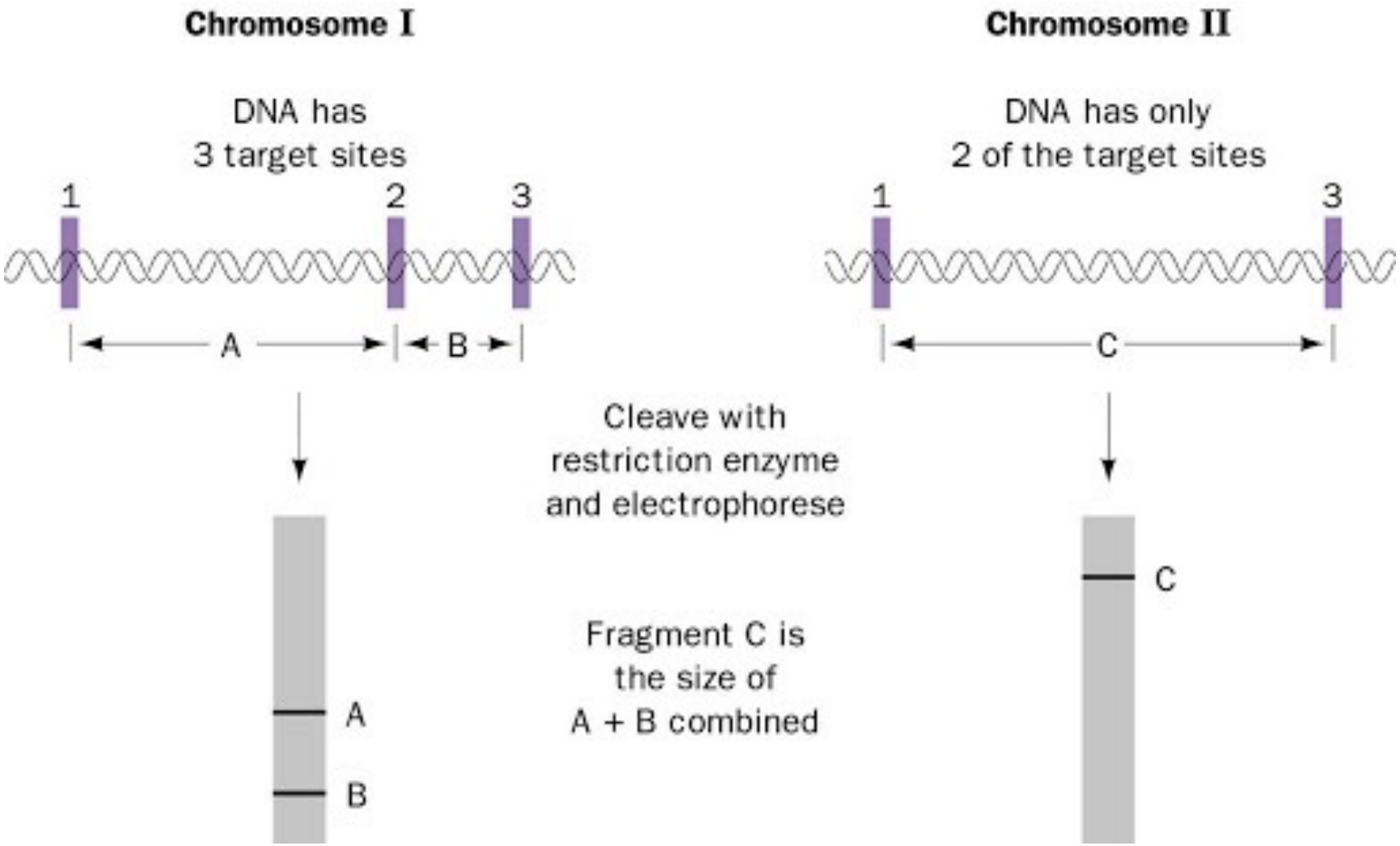
- Restriction endonucleases are found in bacteria and recognize, bind to and cut specific double stranded DNA sequences

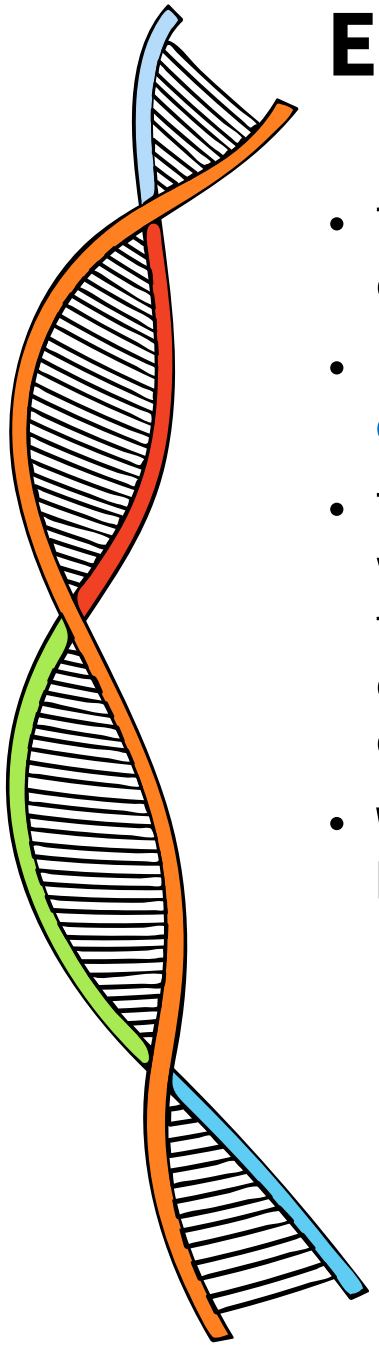


There are hundreds of restriction enzymes that recognise and cut specific sequences

Restriction enzymes

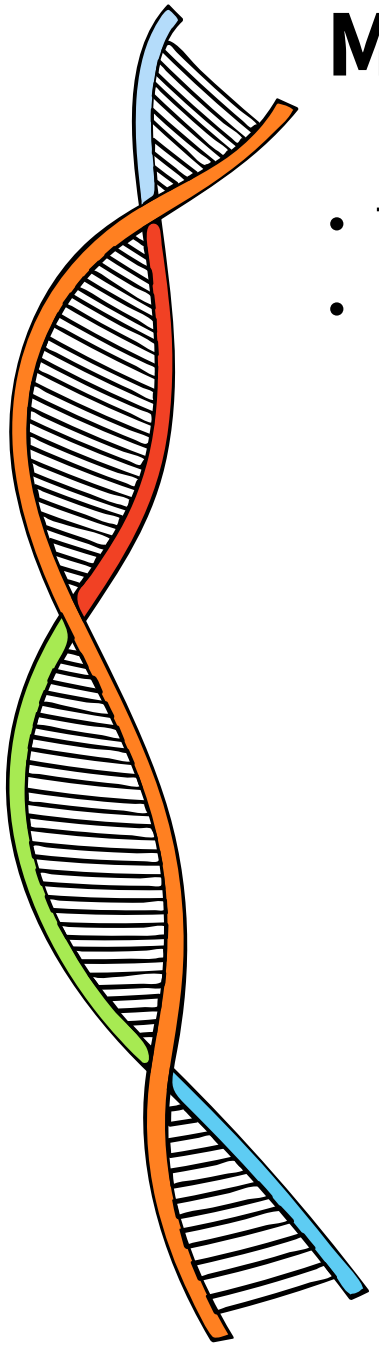






Electrophoresis

- The basis of fingerprinting is to measure the lengths of the fragments that the restriction enzymes produce
- In order to see the size, we need to separate the fragments in a process called **electrophoresis**
- This process relies on **agarose** which is allows DNA to move through it at a speed dependent on its size under an electrical current
- **What net charge does DNA have?**



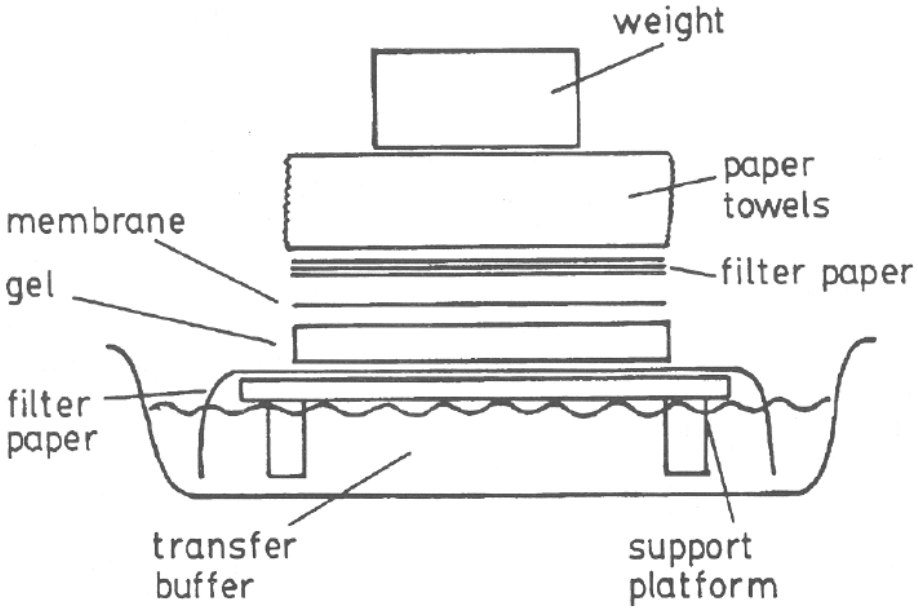
Membrane transfer

- The membrane used is nitrocellulose and positively charged,
- DNA moves from the gel to the membrane using capillary action – no electrical current is needed, just filter paper and a weight

Low water potential

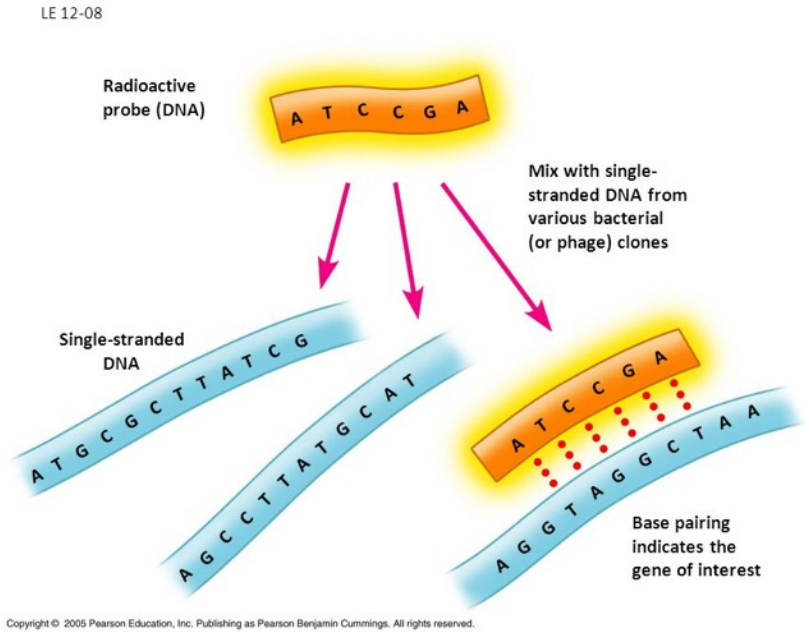
Direction of transfer

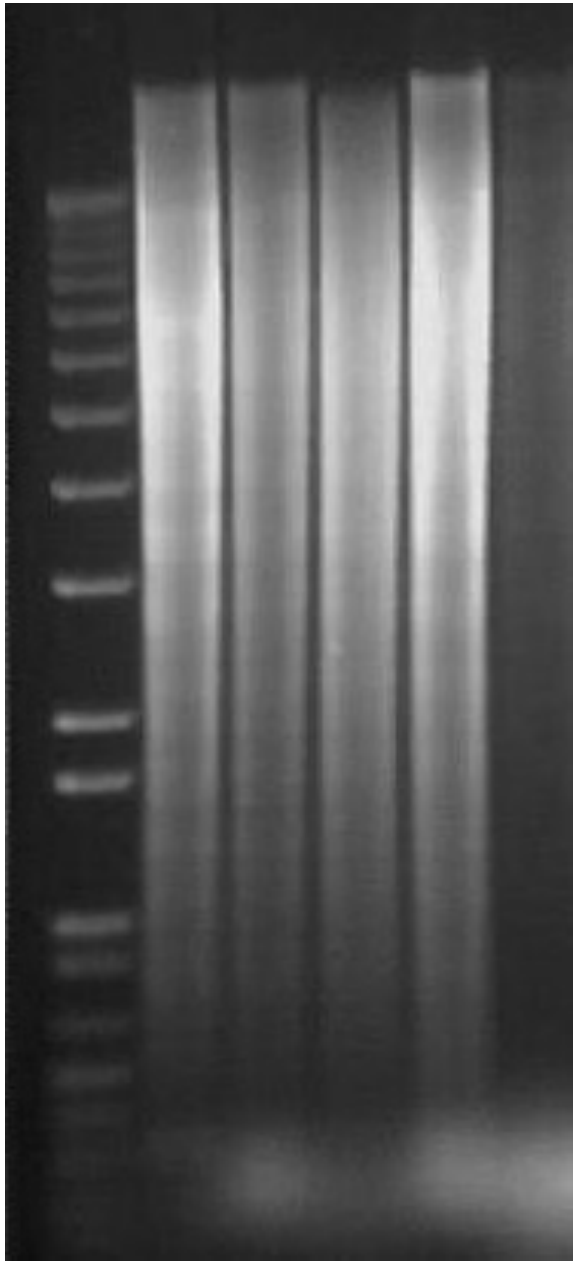
High water potential

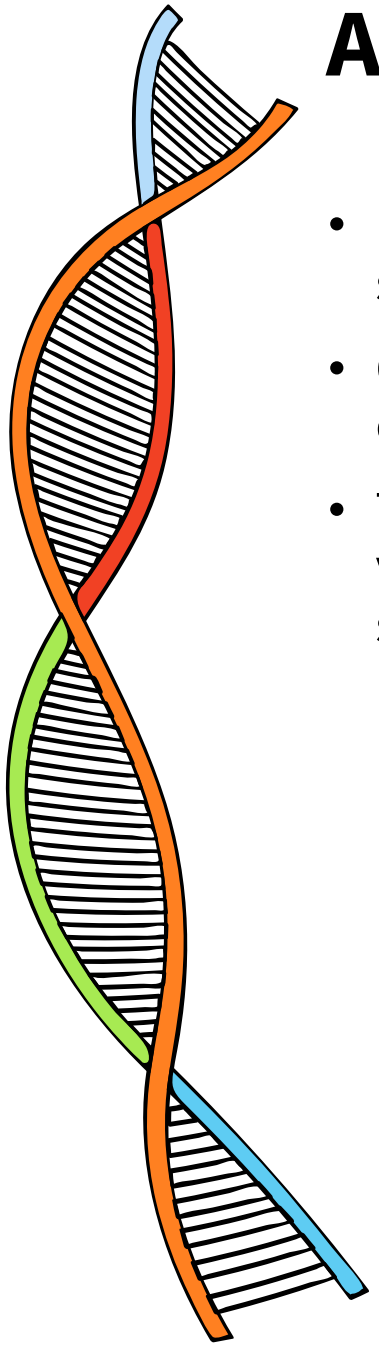


Probing and hybridization

- At this stage, each fragment in the smear of DNA has been transferred to the membrane
- In order to visualize only specific fragments, we need add probes with complimentary sequences to the fragments of interest
- Probes contain a radioactive label

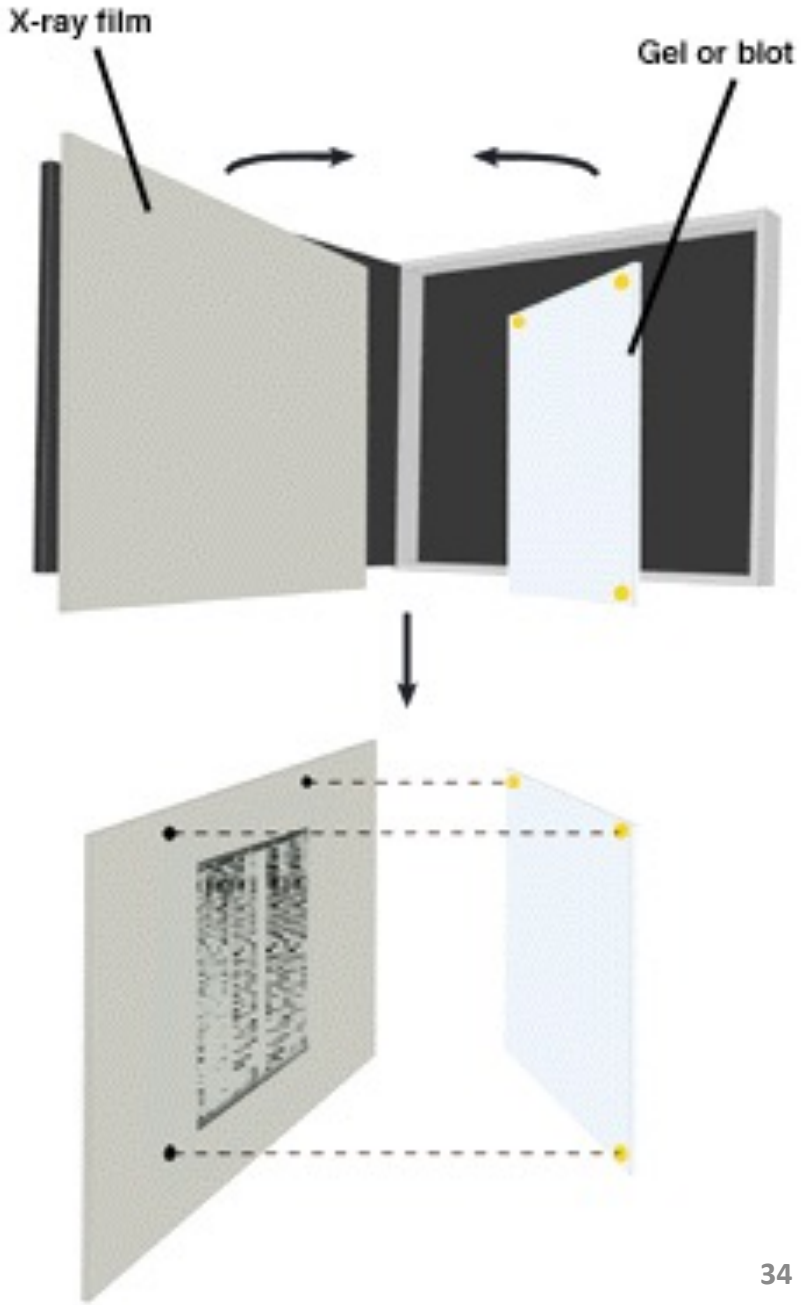






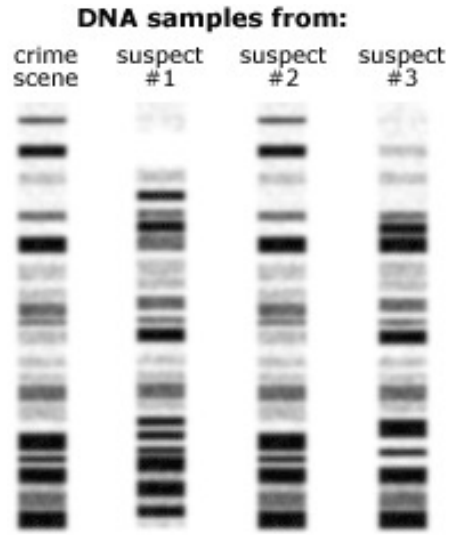
Autoradiography

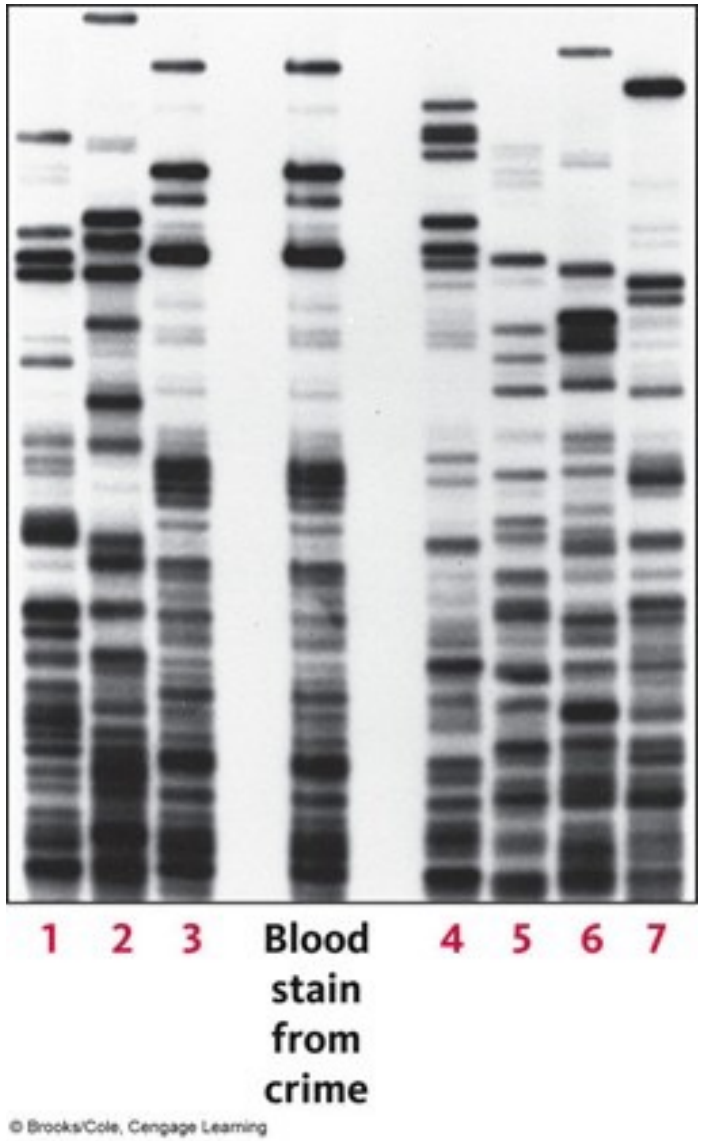
- Images of the blot can now be taken using standard X-ray
- Only the DNA with radioactive probes will be captured on the X-ray film
- These days, alternative methods for visualizing DNA fragments are in use that are safer and quicker

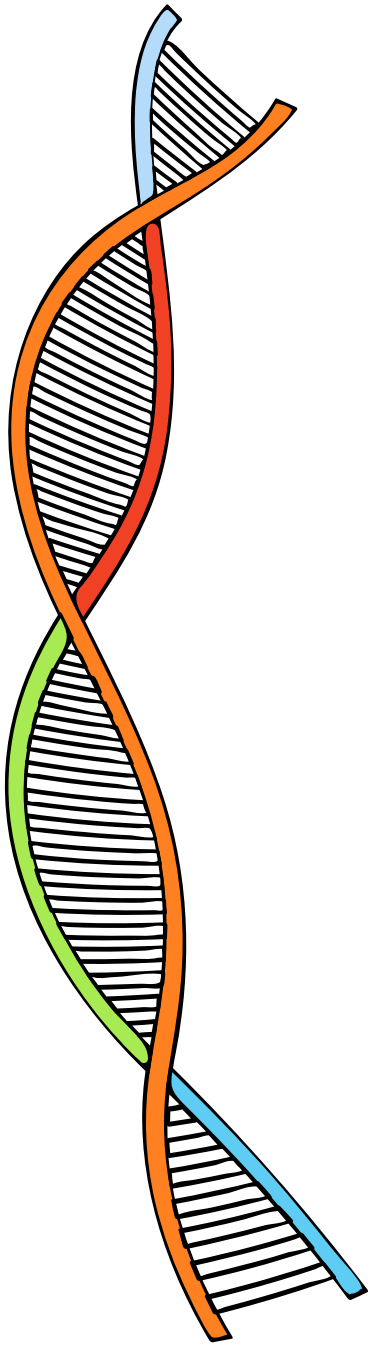


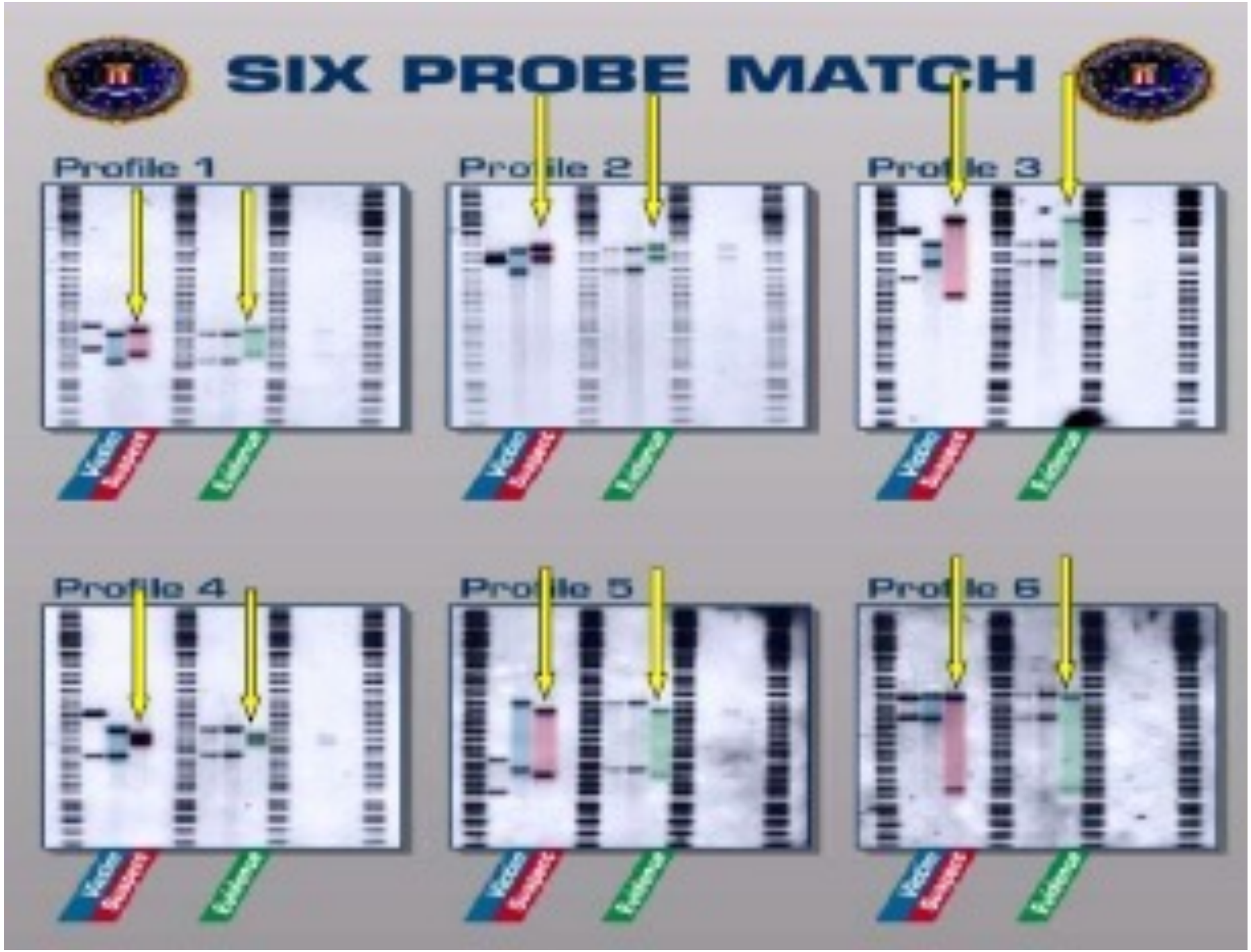
Analysis of variable DNA regions

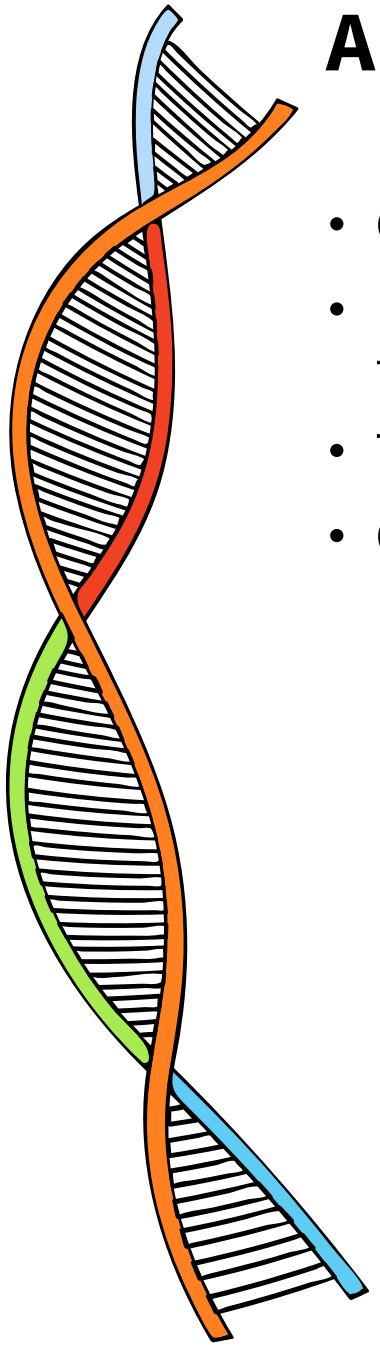
- What are we actually looking for?
- Is it enough to say one sample looks like another?
- What makes one genomic position more appropriate to look at than another?
- Are we using the scientific method by saying that the blood stain looks like the pattern in column 3?





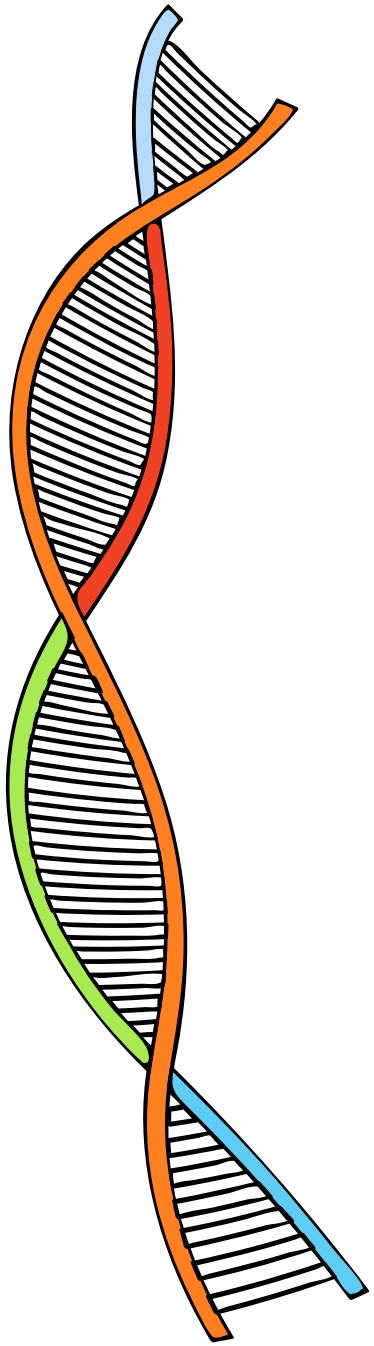


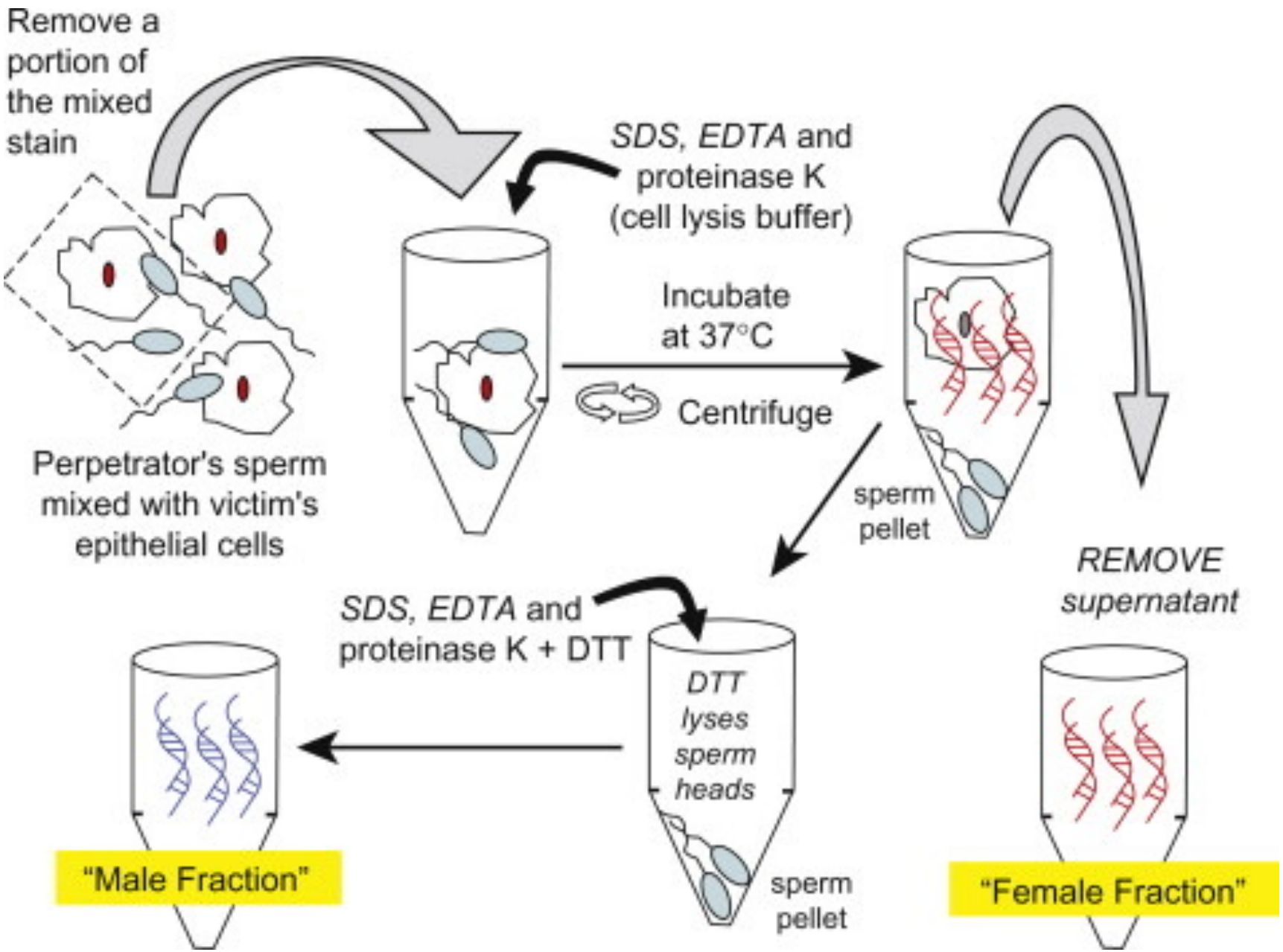

SIX PROBE MATCH
Profile 1
Profile 2
Profile 3
Profile 4
Profile 5
Profile 6

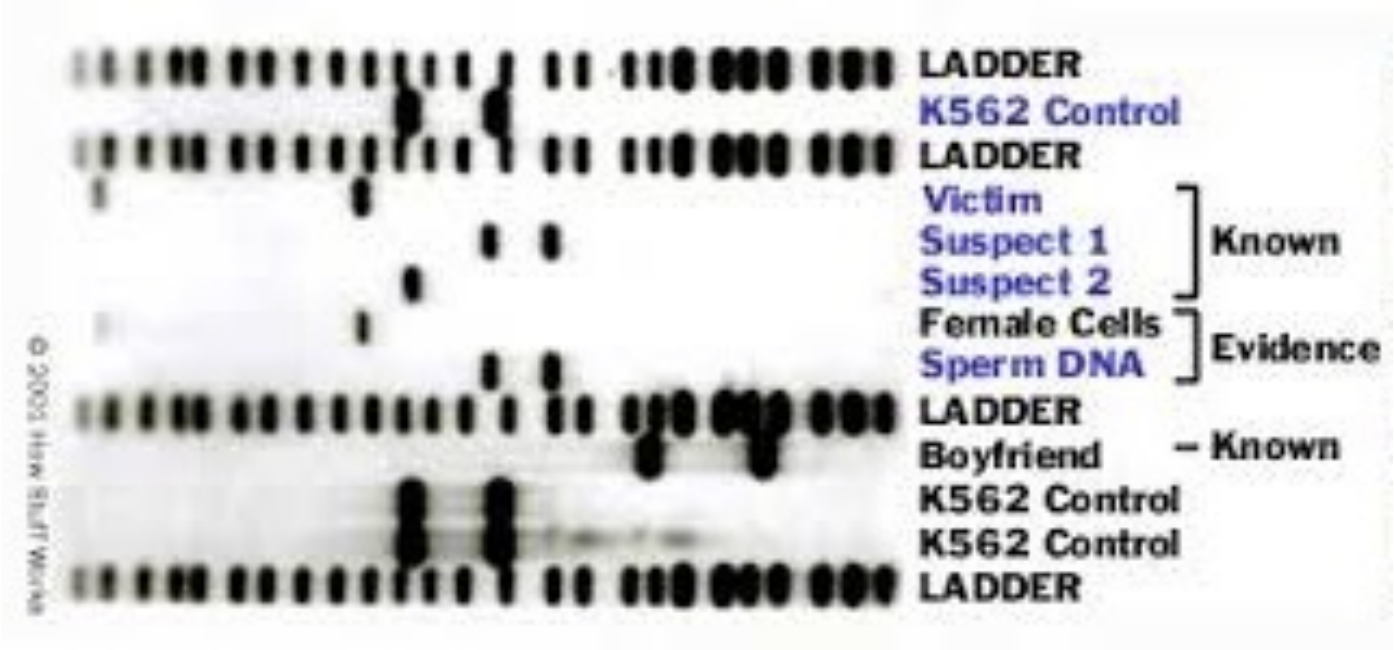


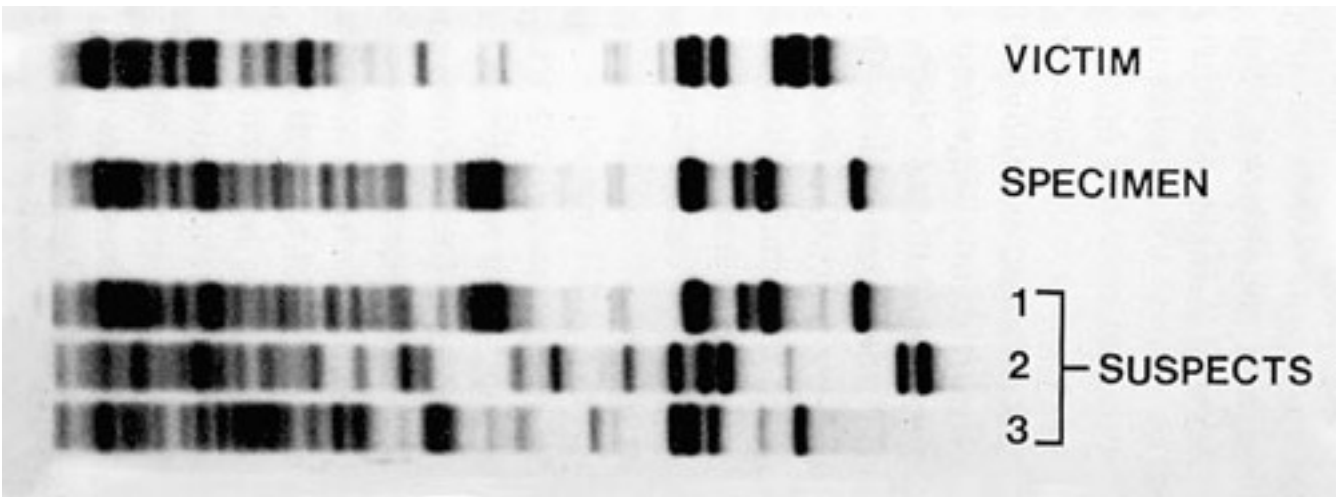
Analysis of male and female DNA in a mixed sample

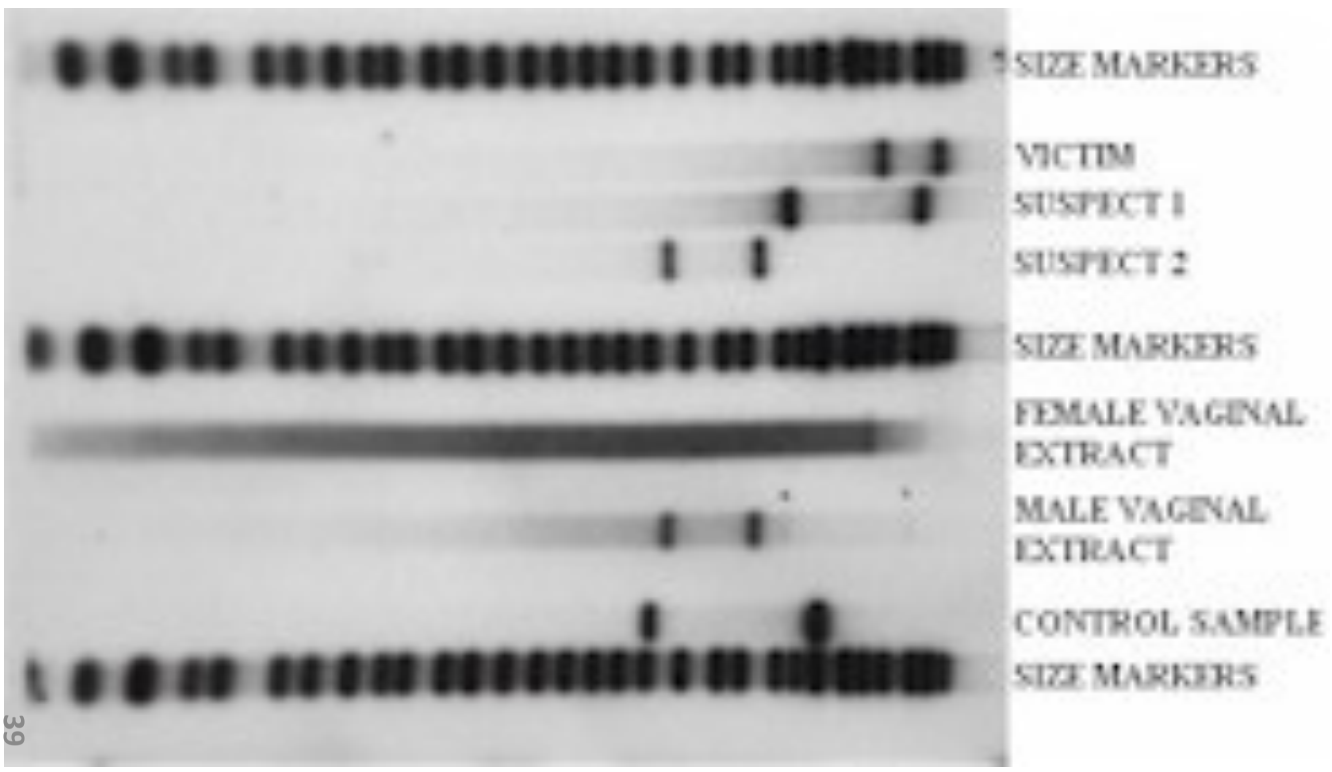
- Crimes involving sexual assault yield samples that are mixed
- It is essential to be able to separate the male and female fraction in order to perform DNA fingerprinting on the male fraction
- This is called **differential extraction**
- Otherwise, how can we potentially identify a suspect?




Remove a portion of the mixed stain
SDS, EDTA and proteinase K (cell lysis buffer)
Incubate at 37°C
Centrifuge
Perpetrator's sperm mixed with victim's epithelial cells
sperm pellet
REMOVE supernatant
SDS, EDTA and proteinase K + DTT
DTT lyses sperm heads
"Male Fraction"
sperm pellet
"Female Fraction"

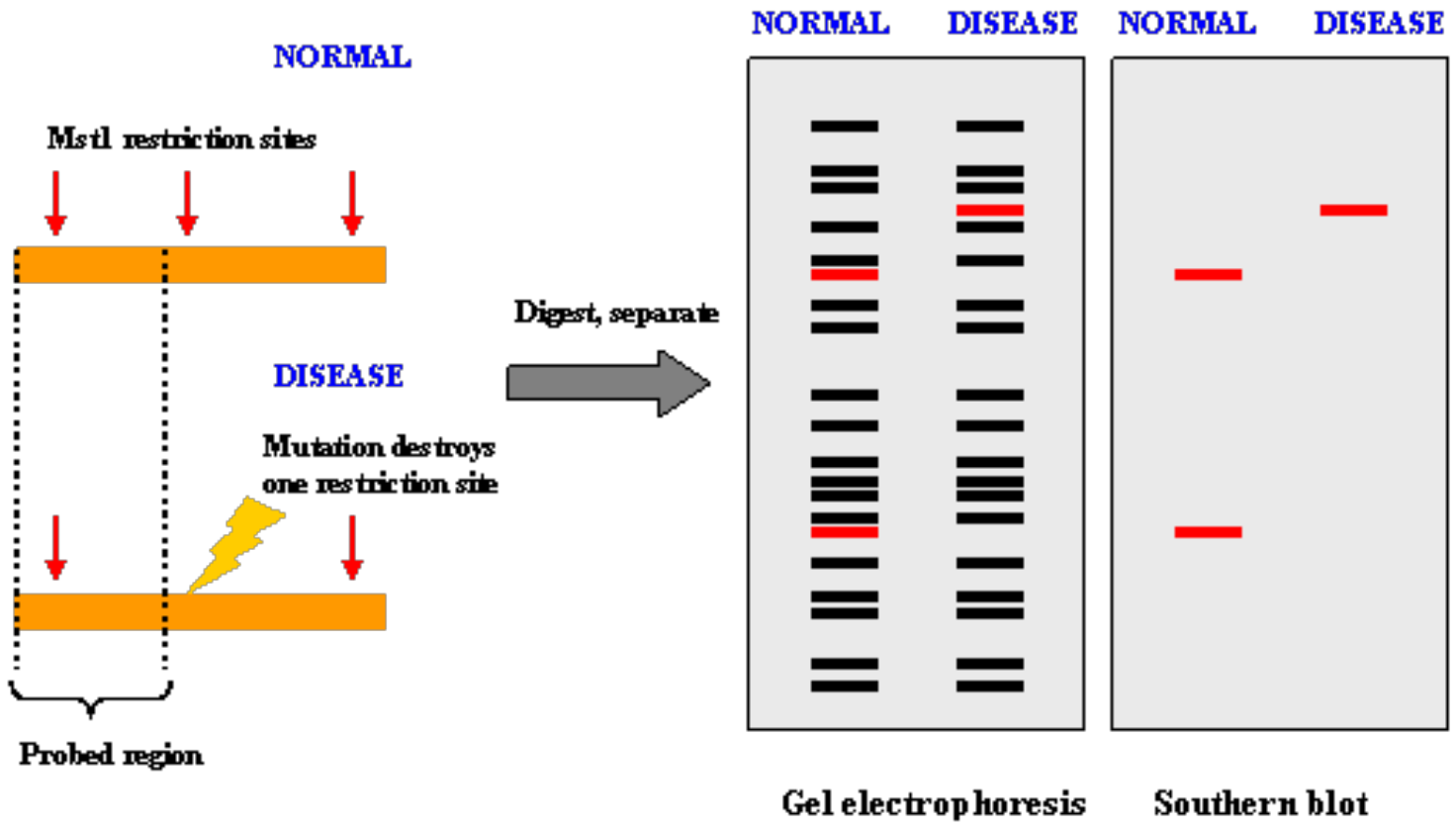

LADDER
K562 Control
LADDER
Victim
Suspect 1
Suspect 2
Known
Female Cells
Sperm DNA
Evidence
LADDER
Boyfriend
– Known
K562 Control
K562 Control
LADDER
© 2003 How Stuff Works


VICTIM
SPECIMEN
1
2
3
SUSPECTS


SIZE MARKERS
VICTIM
SUSPECT 1
SUSPECT 2
SIZE MARKERS
FEMALE VAGINAL EXTRACT
MALE VAGINAL EXTRACT
CONTROL SAMPLE
SIZE MARKERS

Variant analysis

- Certain mutations associated with disease can destroy the restriction site

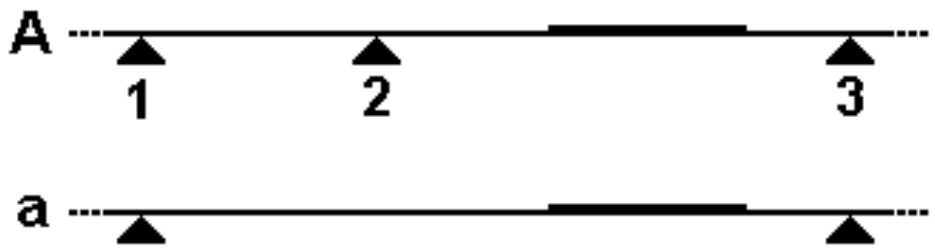


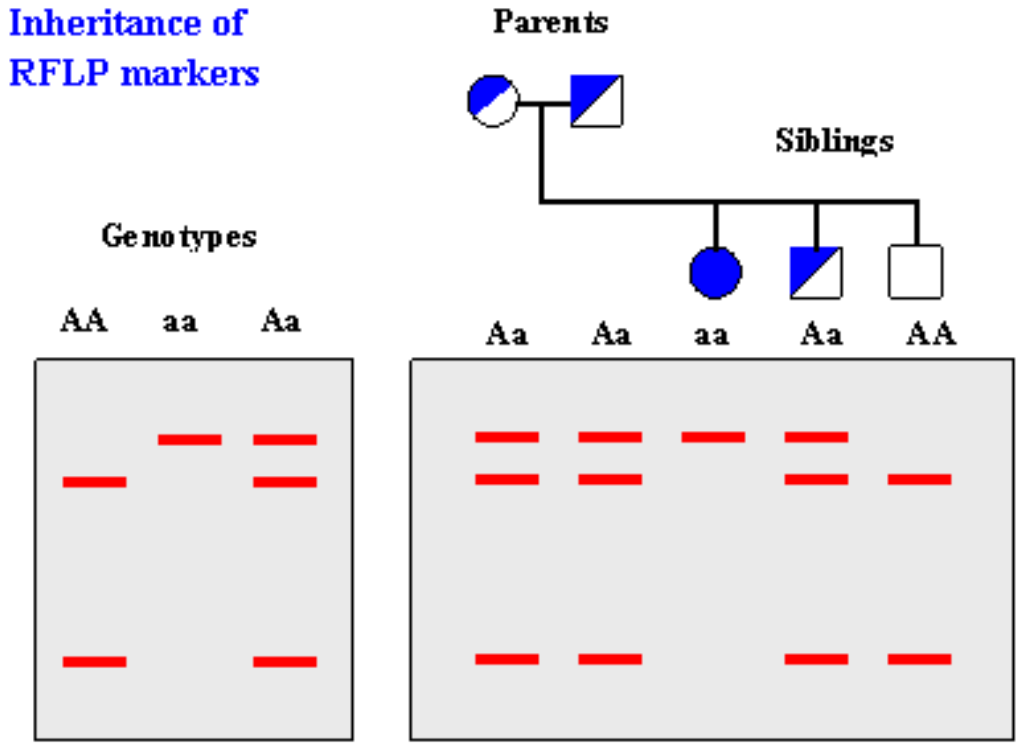
https://www.ncbi.nlm.nih.gov/probe/docs/techrflp/

Genotyping

- DNA fingerprinting can also be used for genotyping purposes

Schematic showing example of restriction enzyme site change, probably due to a single base substitution in site 2

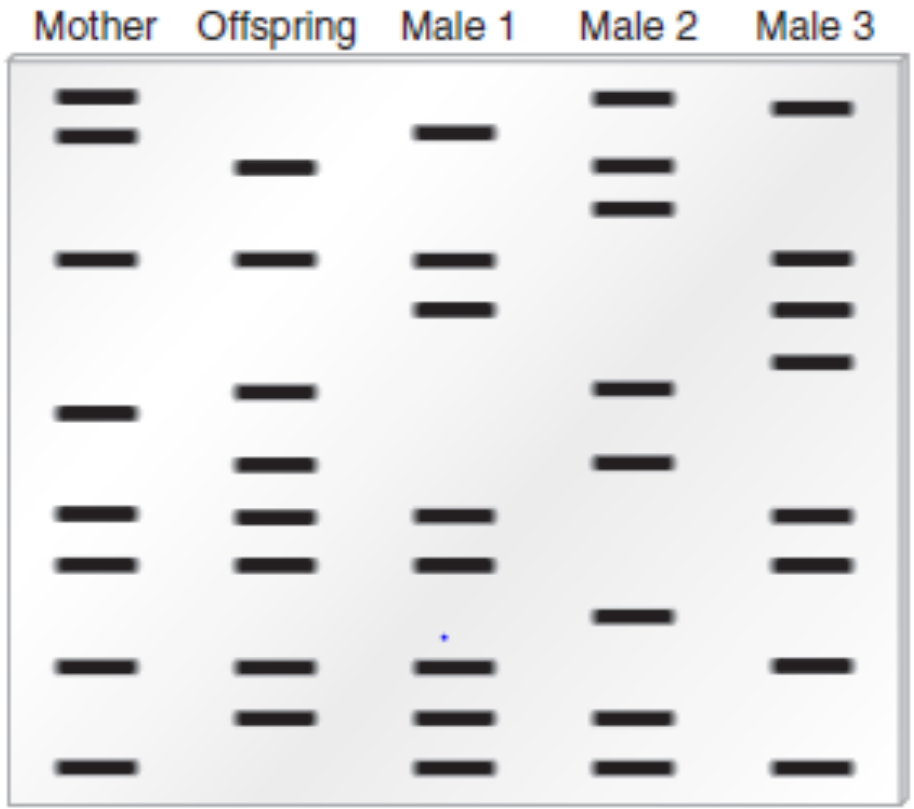




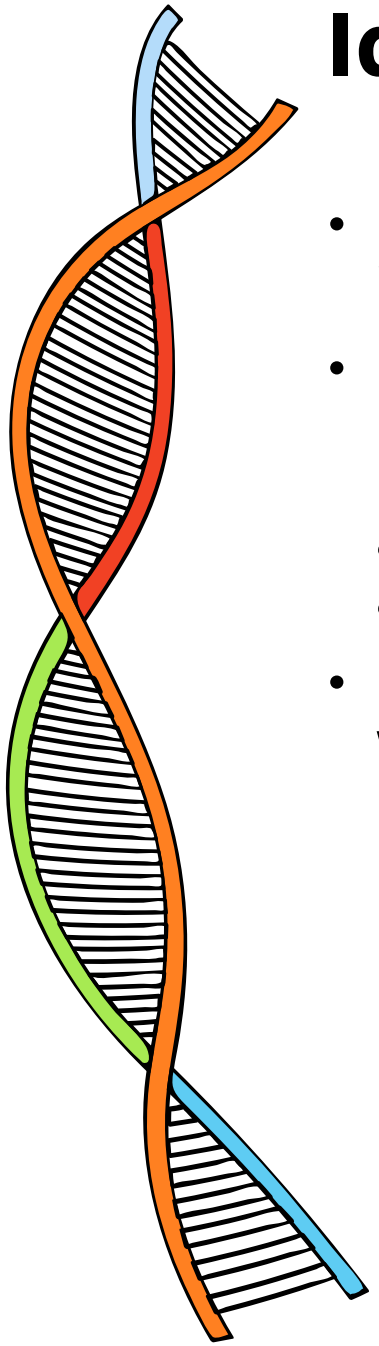
https://www.ncbi.nlm.nih.gov/probe/docs/techrflp/

Paternity testing

- Comparing alleles between the child, mother and potential father(s) can be used to determine paternity



- The child's alleles must be carried by either the father or the mother
- Who is not the father?
- Who is most likely to be the father?



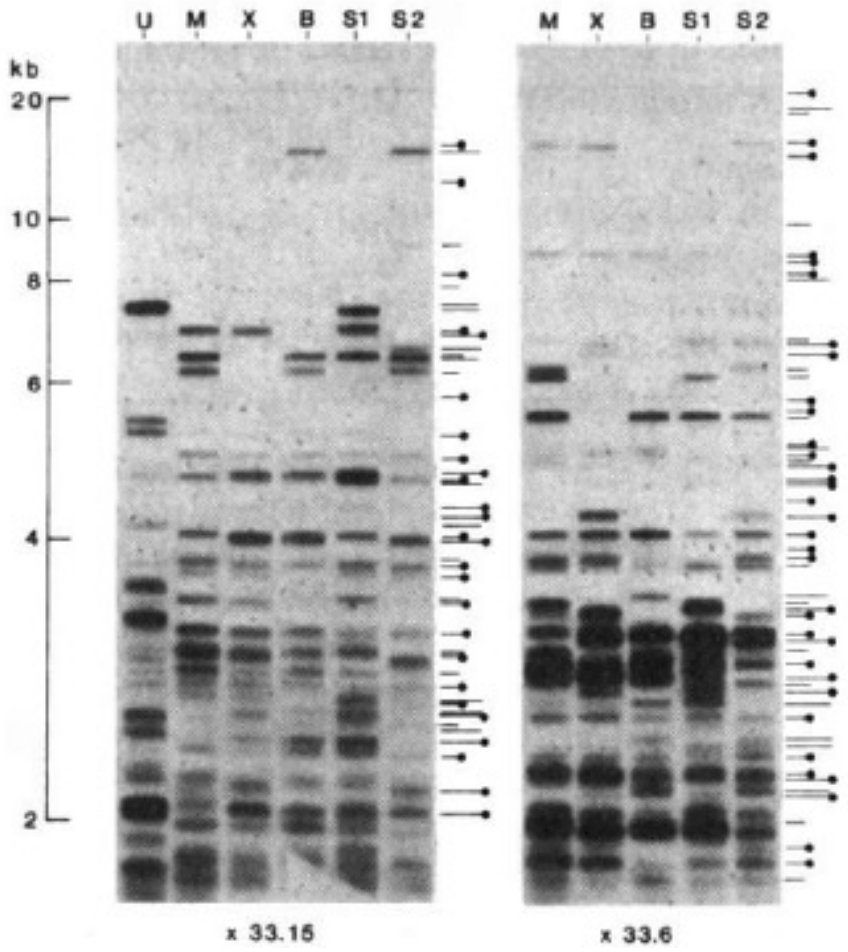
Identification

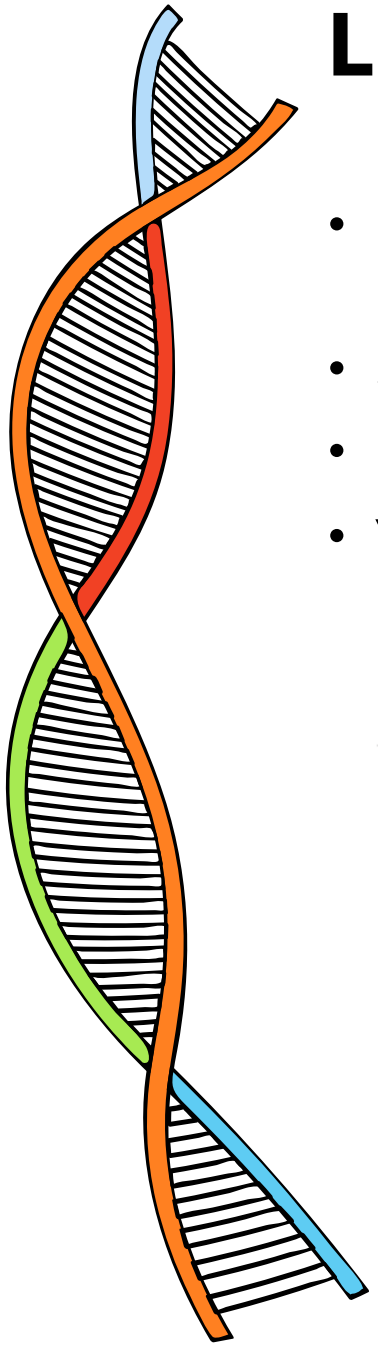
- DNA fingerprinting can be used for immigration purposes
- For example, a Ghanaian boy born in the UK returned to Ghana, but then came back to the UK. The government thought a substitution might have happened with a nephew.
- DNA fingerprinting showed that the boy was in fact the actual person who he said he was.

Positive identification of an immigration test-case using human DNA fingerprints

Alec J. Jeffreys, John F. Y. Brookfield & Robert Semeonoff

NATURE VOL. 317 31 OCTOBER 1985





Limitations

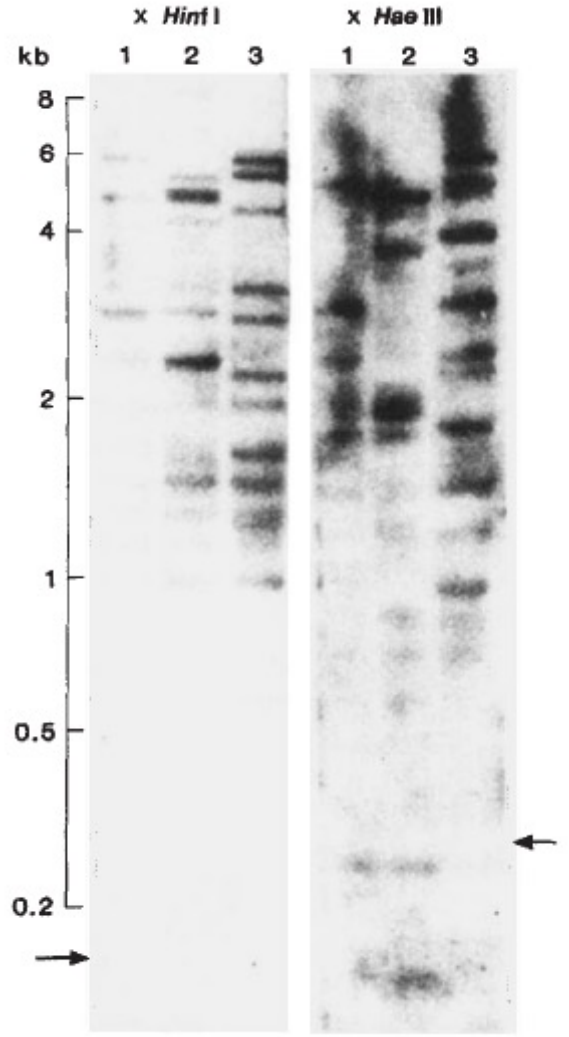
- Highly dependant on the quality and quantity of the DNA available
- Slow, tedious, and labour intensive
- Expensive and difficult to automate
- You need a LOT of DNA and time to do fingerprinting!

Hypervariable 'minisatellite' regions in human DNA

Alec J. Jeffreys*, Victoria Wilson* & Swee Lay Thein†

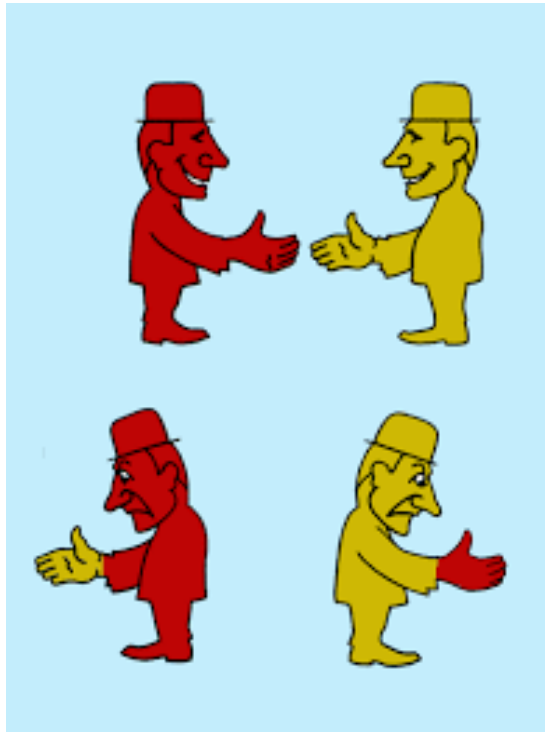
NATURE VOL. 314 7 MARCH 1985

Fig. 2 Detection of multiple 33-related sequences in human DNA. 10 μg samples of DNA from individual 1 (daughter), 2 (mother) and 3 (father) were digested with *Hin*fI or *Hae*III, electrophoresed through a 1% agarose gel and transferred by blotting[35] to a Pall Biodyne membrane to prevent the loss of small DNA fragments.

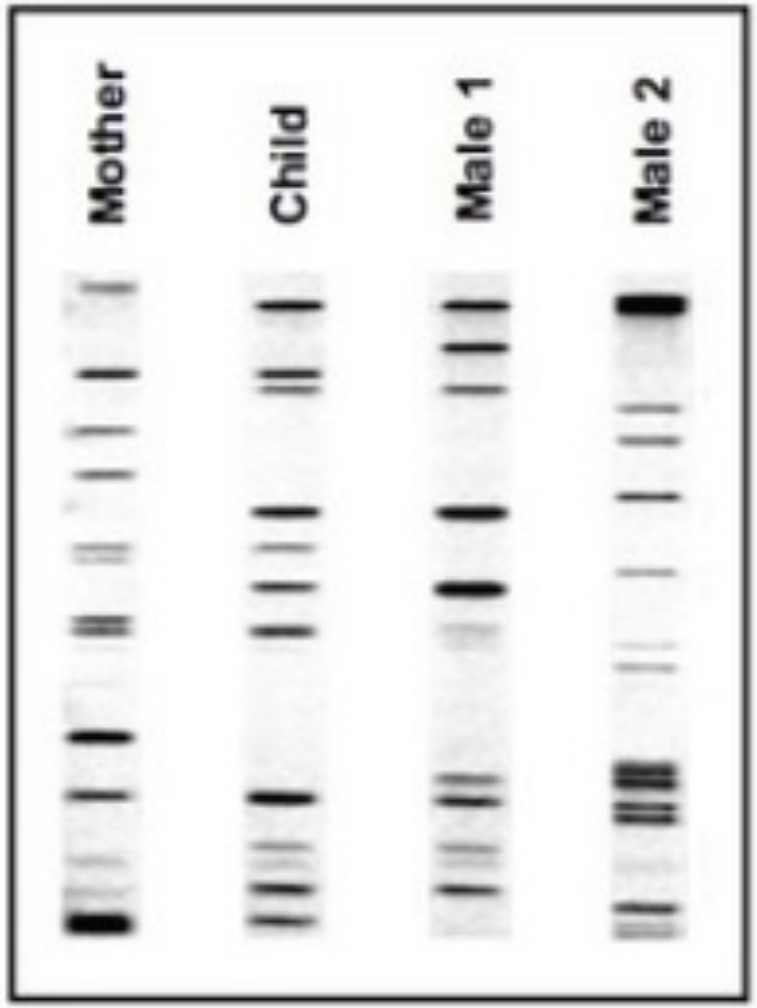


Limitations

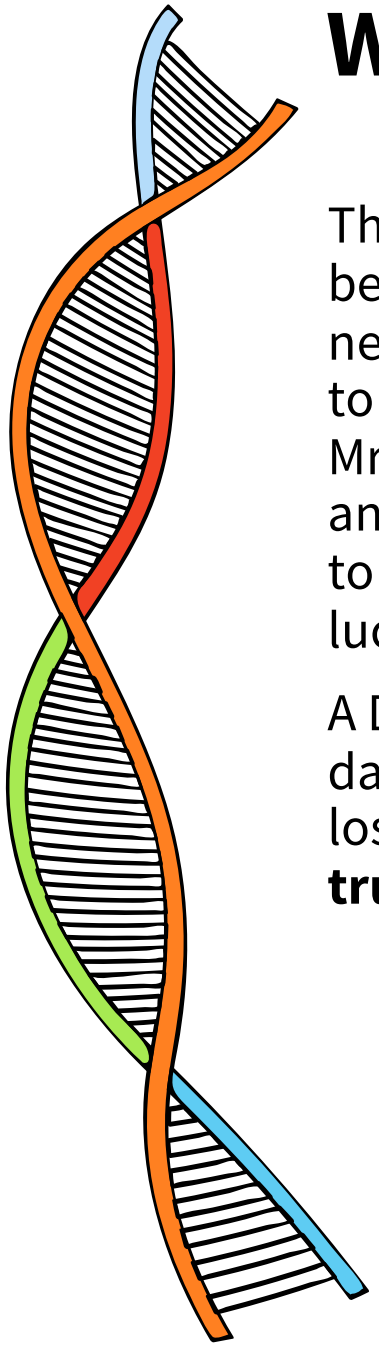
- DNA fingerprinting is destructive, so once you have performed the analysis you have no more sample left
- DNA fingerprinting is also as susceptible to contamination just like any other method



Worked examples



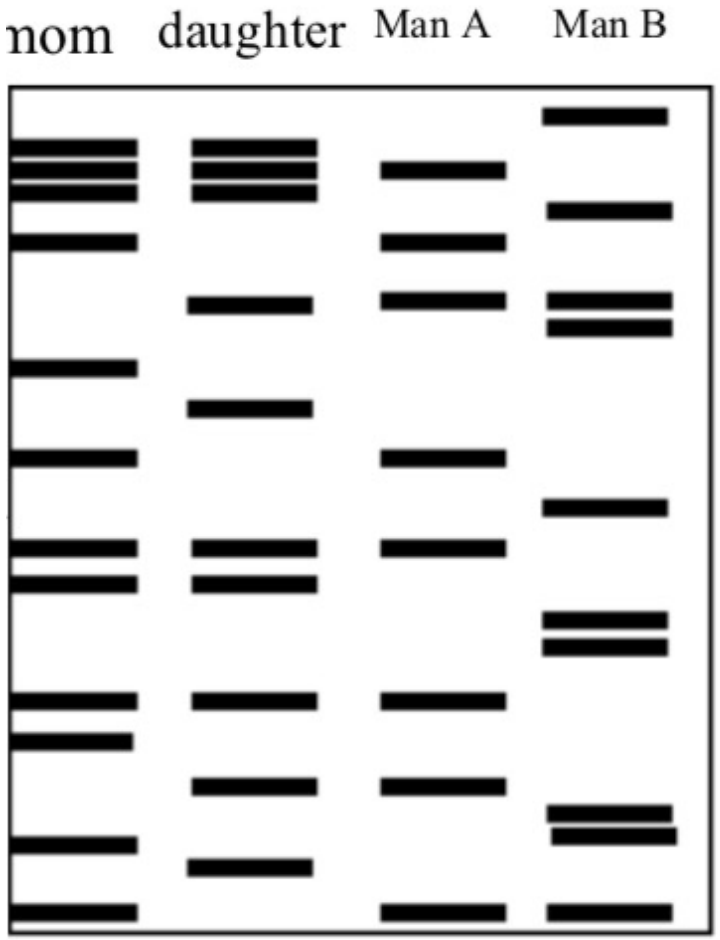
Who is the father?

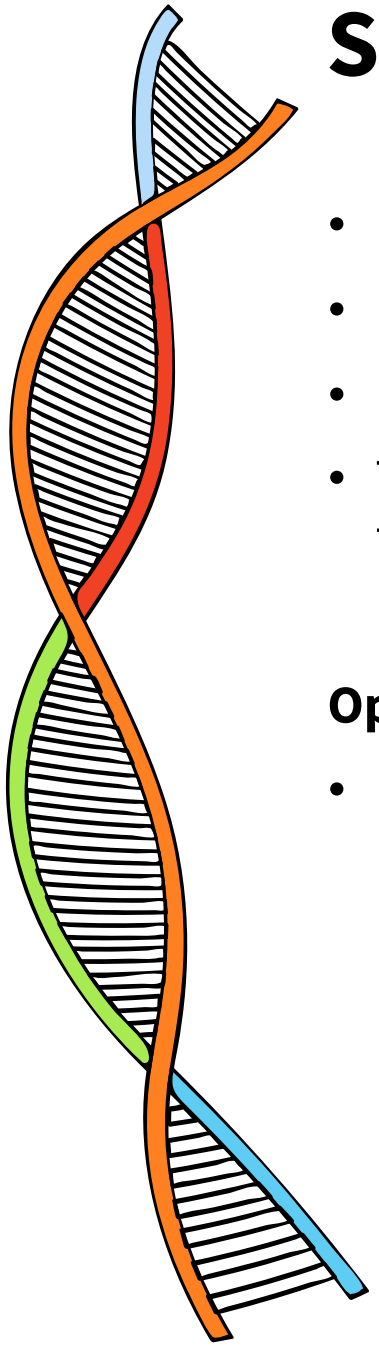


Worked examples

The millionaire, Mr. Big, has just died. He has left behind a wife, daughter and a large inheritance. The news of his death has brought forth 2 men who claim to be the long lost son of Mr. & Mrs. Big. Before Mr. & Mrs. Big were married they had an illegitimate child and had placed him up for adoption. They had tried to find him after they became wealthy but had no luck in locating him.

A DNA sample was taken from Mrs. Big, the Big daughter and the two men who claim to be the long lost son. **Which, if any, of the men are telling the truth?**





Summary

- DNA fingerprinting is the original method of DNA analysis
- Based on the genetic inheritance of VNTRs
- Multi-locus analysis is more powerful than single locus analysis
- Though it has been the industry standard for decades, it has particular limitations in terms of forensic analysis

Optional further reading:

- DNA Profiling and DNA Fingerprinting – **Multilocus DNA Fingerprinting** (available on Canvas)