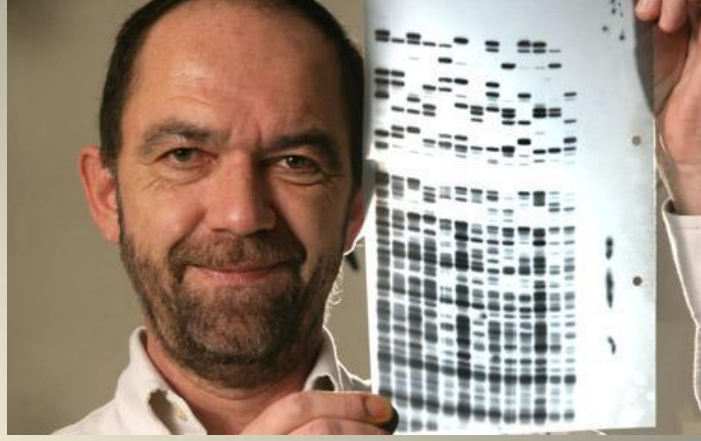
BIOTECHNOLOGY

DNA Fingerprinting

True or False?

- There is a lot of variability in our DNA when comparing ourselves to another person
- There are more noncoding regions than coding regions in our DNA
- The DNA regions that are the most different between individuals are the coding regions
- DNA fingerprinting looks at our genetic sequence to differentiate between individuals

DNA Fingerprinting



- Developed by Alec Jeffreys in 1984 (Leicester, England)
- A method that identifies an individual based on the patterns formed from the variations in the genetic code
- Also known as DNA profiling, genetic fingerprinting, DNA typing

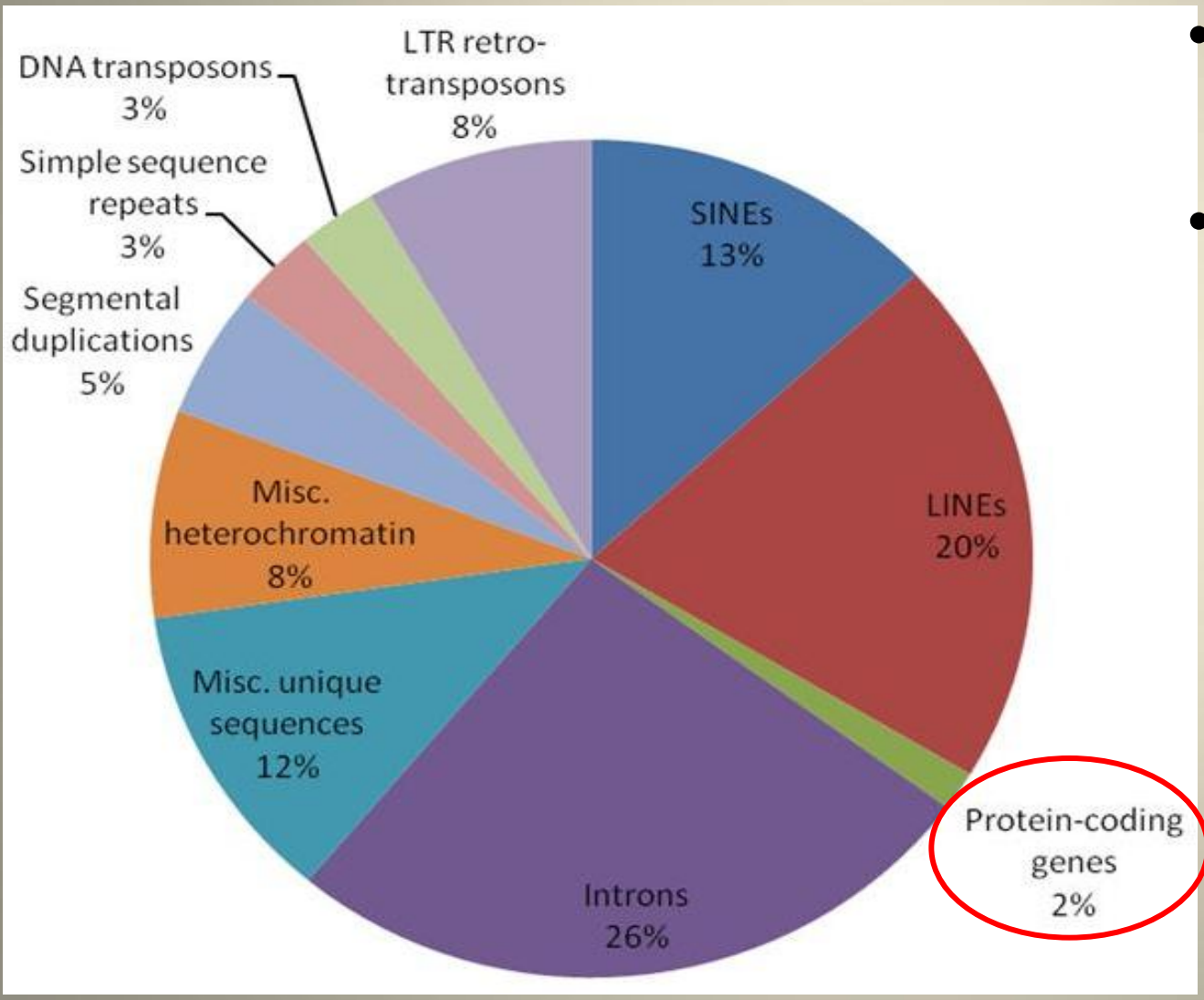
First Criminal Case Solved with DNA Fingerprinting Video: This Was the Birth of DNA Profiling (3:19)

https://www.youtube.com/watch?v=Fz6p8EgJZ3w

First criminal case to employ DNA fingerprinting

- Colin Pitchfork arrested (1987) and convicted on the rape and murder of 2 teenage girls (1983, 1986) based on DNA fingerprinting
  - First use of DNA fingerprinting to convict a criminal
  - First to be caught as a result of mass DNA screening
    - 5000 local men volunteered their DNA
    - Ian Kelley was paid by Pitchfork to masquerade as himself but was later found out
- Richard Buckland was the prime suspect
  - first to have innocence established with DNA fingerprinting

Human Genome



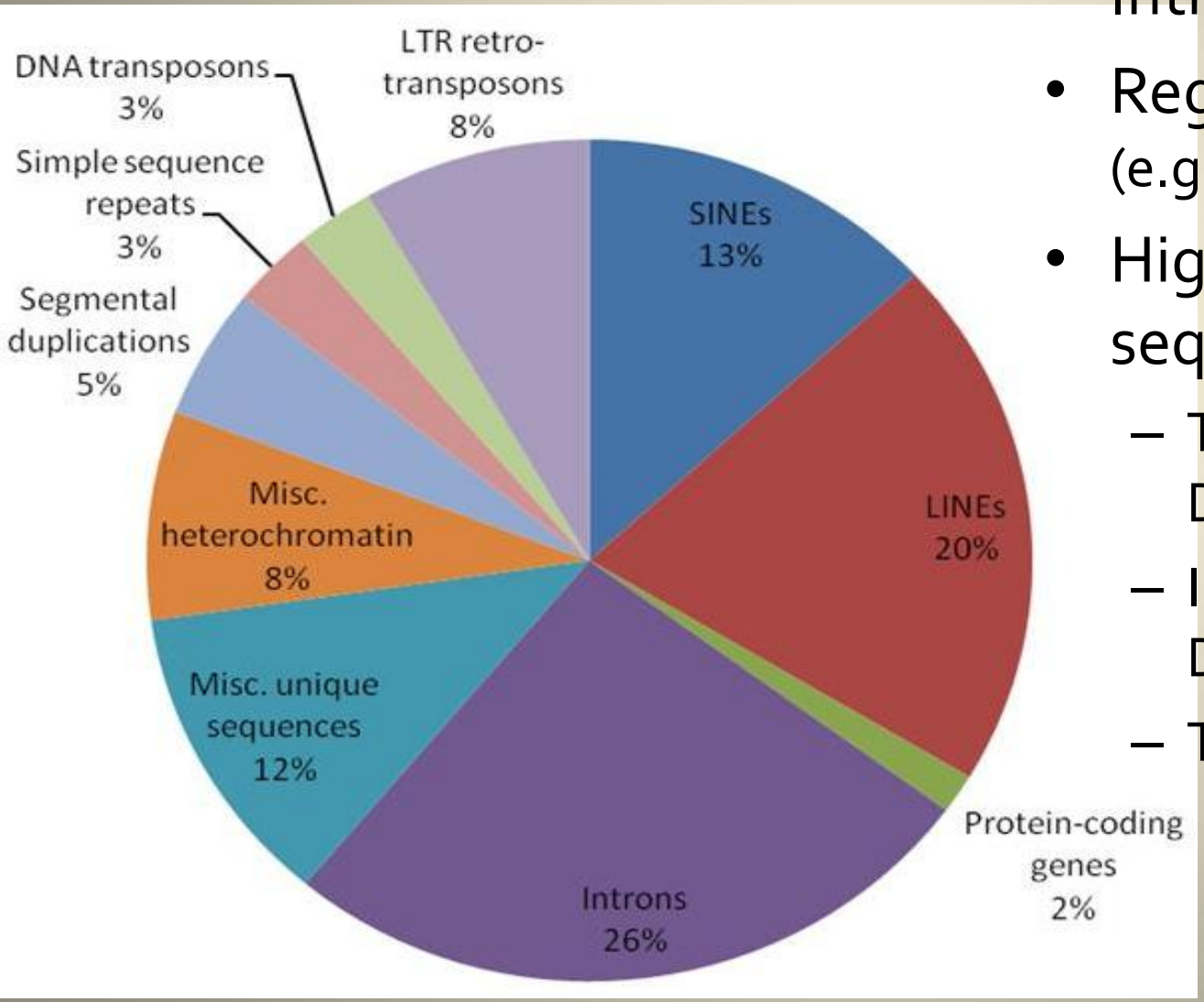
- 20,000-30,000 genes
- most of the DNA does NOT encode for a protein or RNA (is not a gene)

http://upload.wikimedia.org/wikipedia/commons/6/64/Components_of_the_Human_Genome.jpg

Coding & Noncoding Sequences

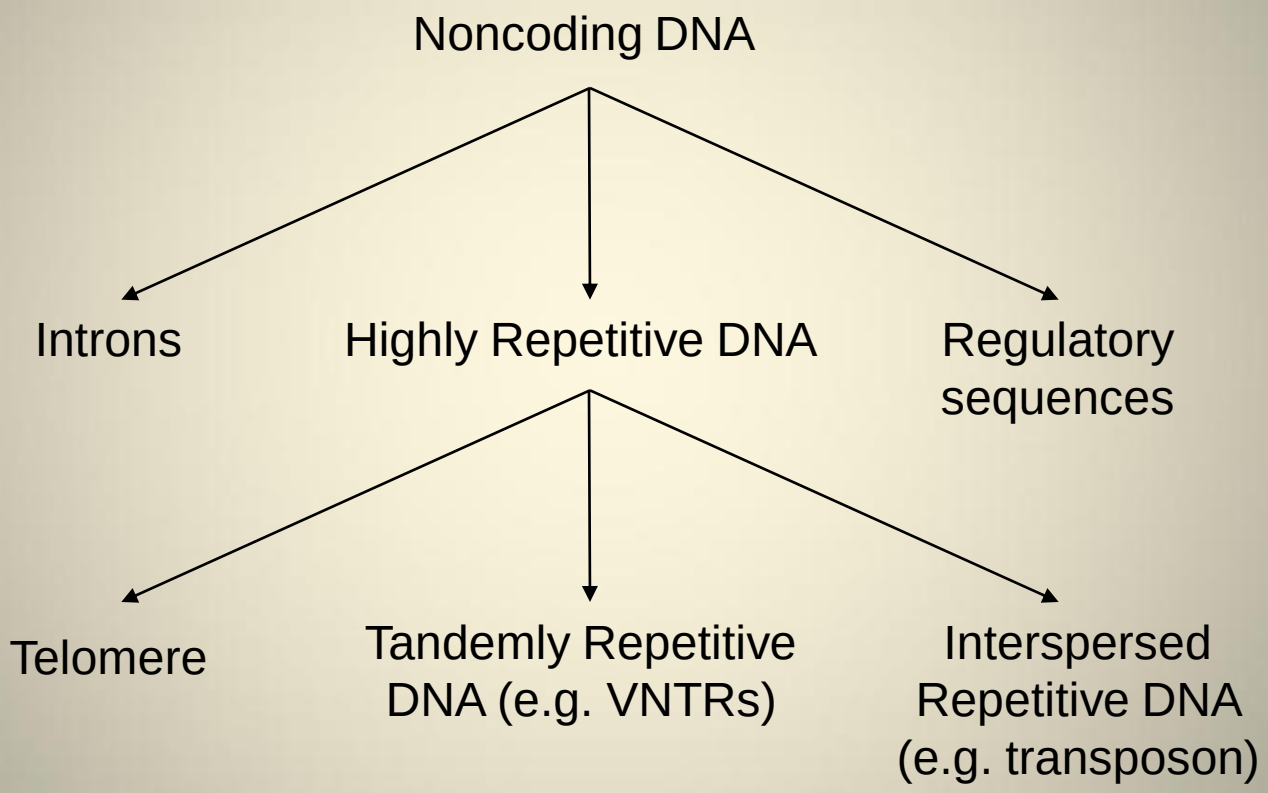
http://www.hhmi.org/biointeractive/dna/DNAi_coding_sequences.html

Classes of Noncoding Sequences



- Introns
- Regulatory sequences (e.g. promoter, UTR)
- Highly repetitive sequences:
  - Tandemly repetitive DNA (tandem repeats)
  - Interspersed repetitive DNA (transposons)
  - Telomeres

Noncoding DNA



Tandemly Repetitive DNA

- Also known as **Variable Number Tandem Repeats (VNTRs)**
  - Short identical DNA sequences repeated in series (tandem)
  - Example: OSC D1S80 locus, repeat length is 16bp
- Many types, often classified by length of the repeat
  - **Short tandem repeats (STR)** is a subcategory where the repeat length is short, most commonly 4 bases long
  - Example: D10S1248 locus repeat is GGAA (length is 4bp)

Characteristics of VNTRs

- Majority are **noncoding** thus the differences are harmless (no effect)
- But some examples of coding repetitive DNA
  - Fragile X and Huntington's
  - The number of repeats determine the phenotype

Example: Fragile X Syndrome

- First triplet repeat identified was the one in Fragile X syndrome
- Repeat sequence: **CGG** triplet on X chromosome
- Normal fragile X allele: repeated 30x in the 5' untranslated region of the first exon
- Fragile X syndrome: repeat is over 100x
- The most common single known cause of intellectual disability and autism

Example: Huntington's Disease

- Neurodegenerative disorder that affects muscle coordination and leads to cognitive decline and psychiatric problems
- Repeat sequence: **CAG**
- Individuals affected with HD typically have at least 36 repeats or greater.

Repeats	Disease
< 27	-
27 – 35	-
36 – 39	+ / -
> 39	+

Repeat Size	Median Age at Onset * (years)
39	66 (72-59)
40	59 (61-56)
41	54 (56-52)
42	49 (50-48)
43	44 (45-42)
44	42 (43-40)
45	37 (39-36)
46	36 (37-35)
47	33 (35-31)
48	32 (34-30)
49	28 (32-25)
50	27 (30-24)

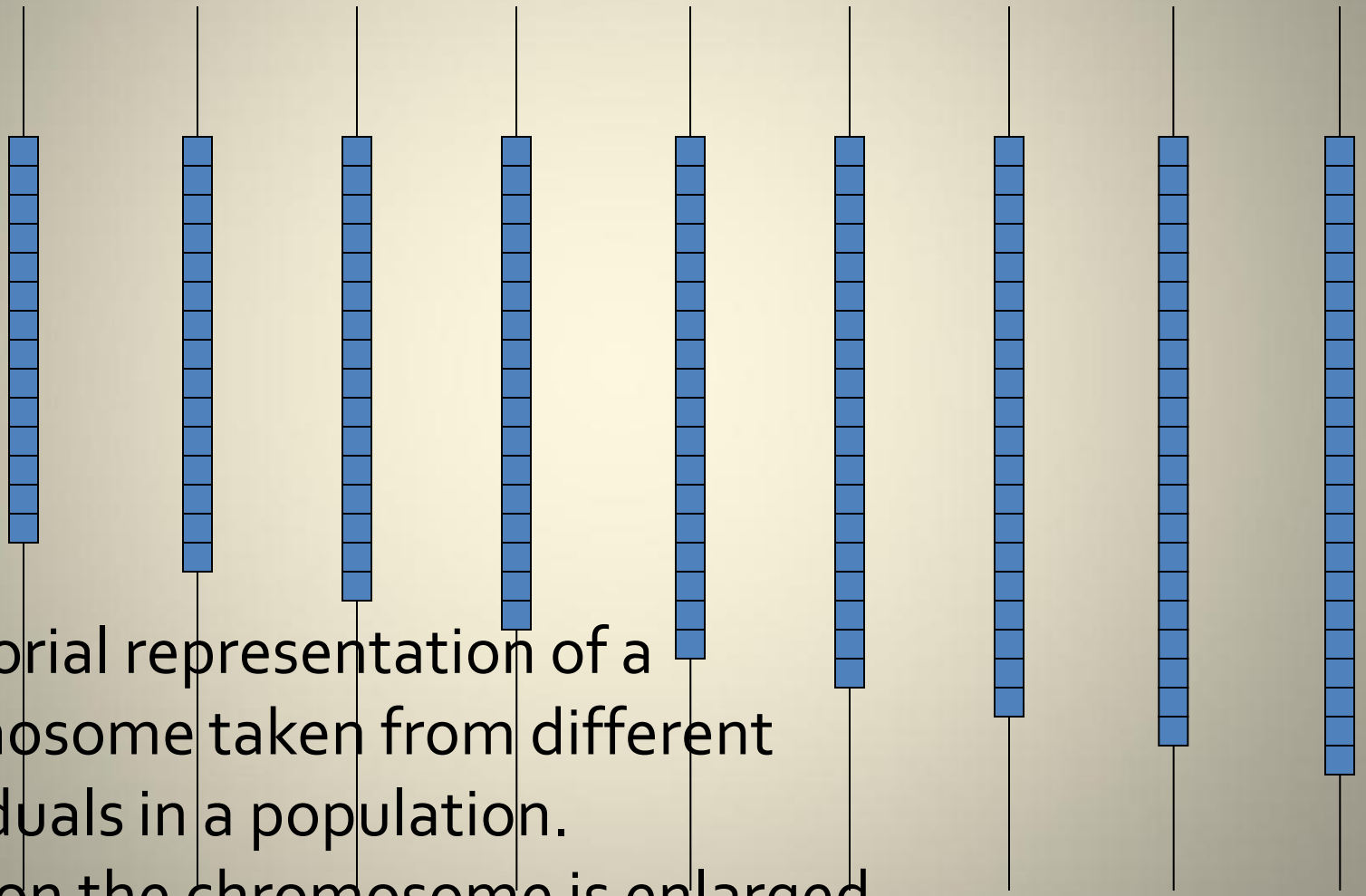
*Age by which 50% of individuals will be affected

Specific numbers of CAG repeat correlates to disease. Walker FO (2007). "Huntington's disease". Lancet 369 (9557): 218–28. Normal HD gene CAG repeats range from 10 - 27 repeats. A few normal individuals have intermediate HD gene CAG repeats of 27-35 repeats.
A current review of 1,049 persons (the majority of whom were symptomatic) has provided a determination of the likelihood of an age-of-onset for a given CAG repeat size for repeats between 39 50 repeats (Brinkman et al., 1997; Am. J. Hum. Genet. 60:1202-1210).

Characteristics of VNTRs

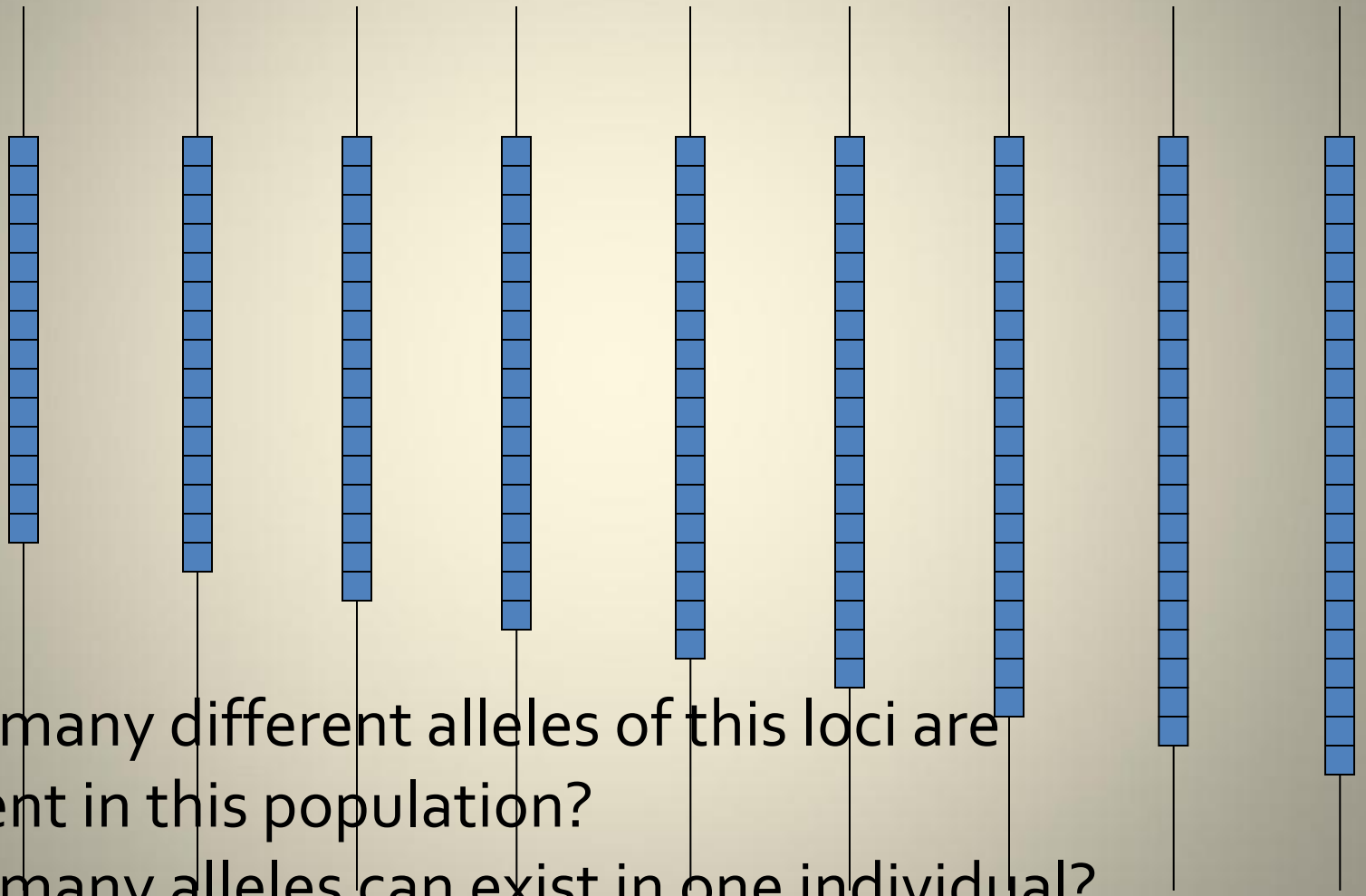
- **Polymorphic**
  - Word means: many forms
  - Meaning: many alleles
  - More than the standard 2 alleles (dominant and recessive)
- Cause of polymorphism
  - Allelic variation due to the number of repeats
  - Number of times the sequence (usually an STR) is repeated
  - The repeat sequence can be repeated many different number of times in the population

Example: Polymorphism



- A pictorial representation of a chromosome taken from different individuals in a population.
- A loci on the chromosome is enlarged to show the number of repeats.

Example: Polymorphism



- How many different alleles of this loci are present in this population?
- How many alleles can exist in one individual?

# repeats	14	15	16	17	18	19	20	21	22

Example of Polymorphism

Locus	D10S1248	D1S80
Repetitive sequence	GGAA	GTGGTCCTCCTTCCTG
Length of repeat		
Class of repeat		
Number of repeats	8-19	14-41
Number of variations		
PCR Fragment length	235-271 bp	200-700 bp

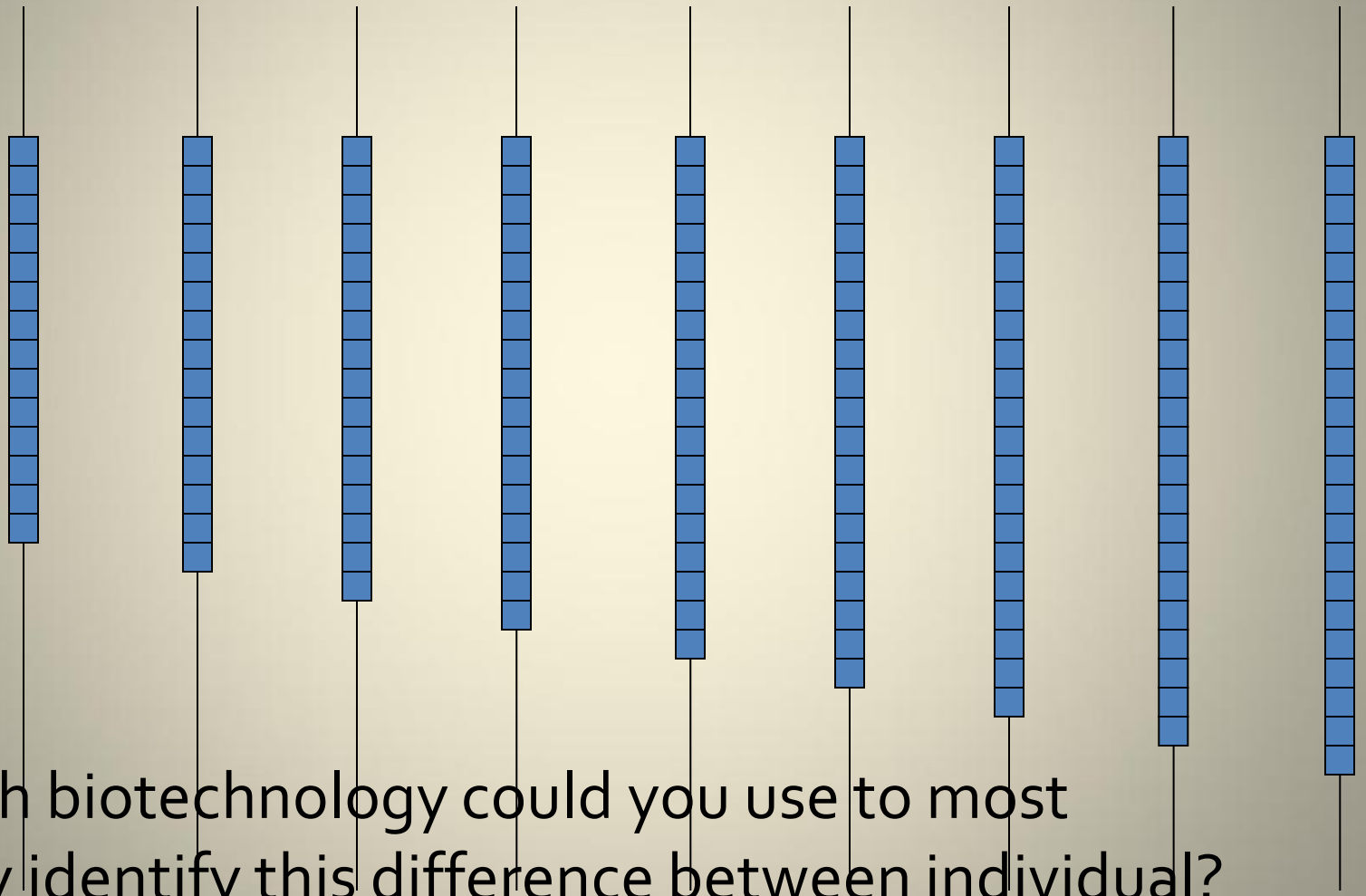
Example of Polymorphism

Locus	D10S1248	D1S80
Repetitive sequence	GGAA	GTGGTCCTCCTTCCTG
Length of repeat	4 bp	16 bp
Class of repeat	STR	VNTR
Number of repeats	8-19	14-41
Number of variations	12	28
PCR Fragment length	235-271 bp	200-700 bp

Allelic Variation Compared

- In a **population**, a polymorphic loci has many choices (alleles)
- In an **individual**, there are only a maximum of two alleles

Example: Polymorphism



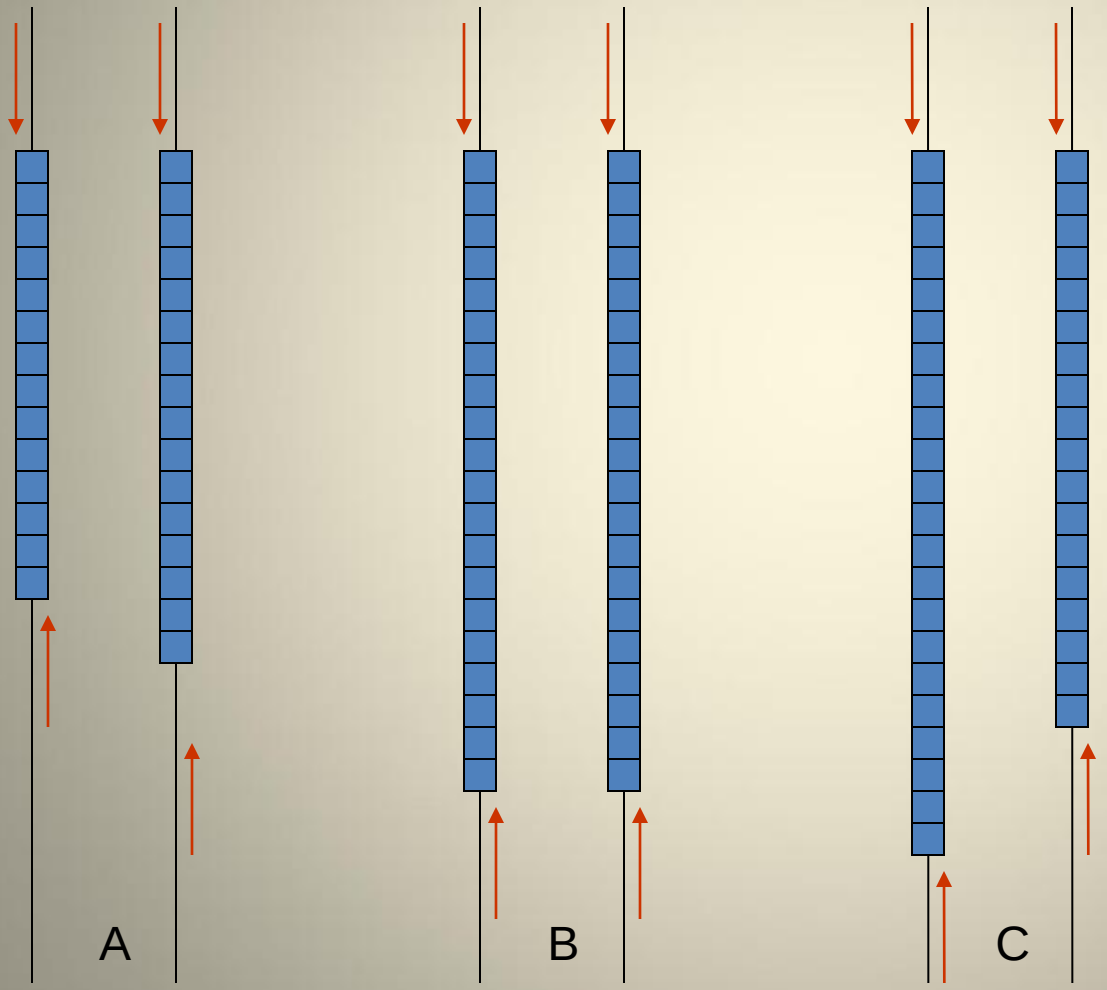
- Which biotechnology could you use to most easily identify this difference between individual?

# repeats	14	15	16	17	18	19	20	21	22

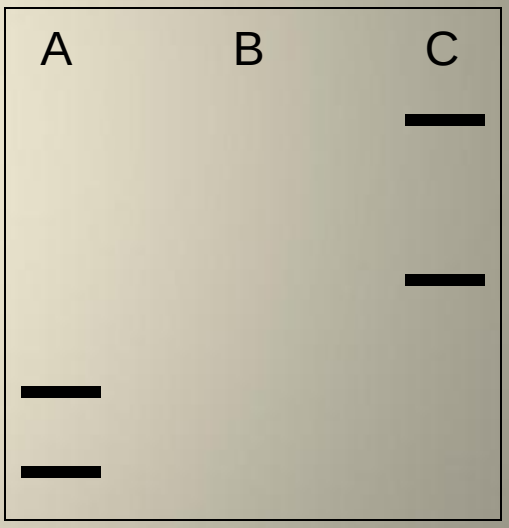
DNA Fingerprinting Method: STR Analysis

- PCR
  - Fragment of interest (an STR) is at a specific loci on the chromosome
  - Primers in PCR define/locate the fragment of interest
  - PCR amplifies the fragment
- Gel electrophoresis
  - Separates the PCR products by size
  - Visualizes the DNA fragments

OSC DNA Fingerprinting: D1S80



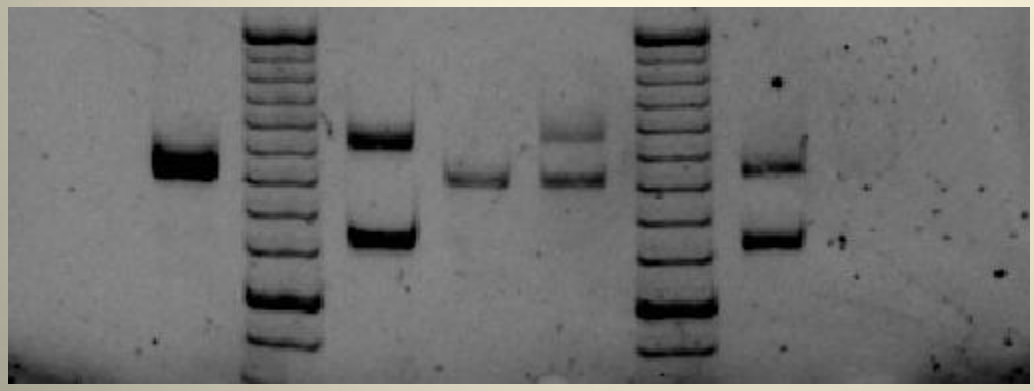
Legend
repeats
primers



How would person B's amplified fragments look on a gel electrophoresis?

Allelic Variation in an Individual

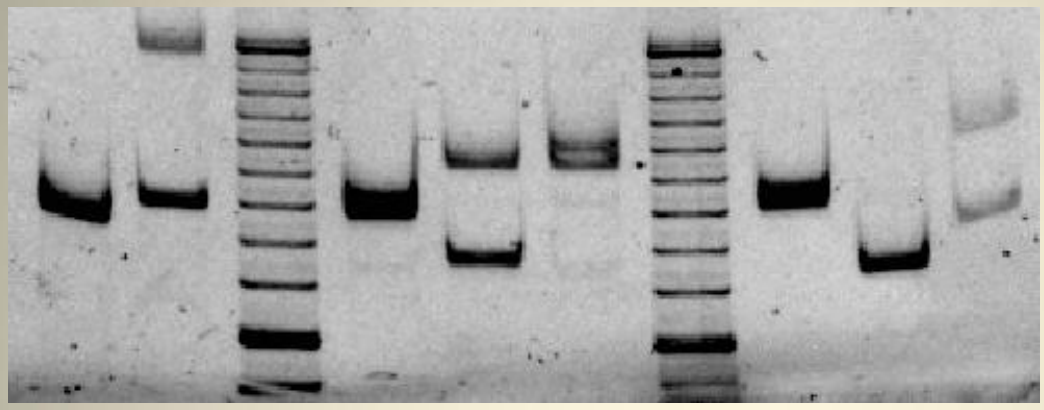
- Each person will have 2 copies but the copies can be different lengths (heterozygous) or the same length (homozygous)



Loci

- If all the loci present are homozygous, how many loci are represented?
- If all the loci present are heterozygous, how many loci are represented?

Allelic Variation

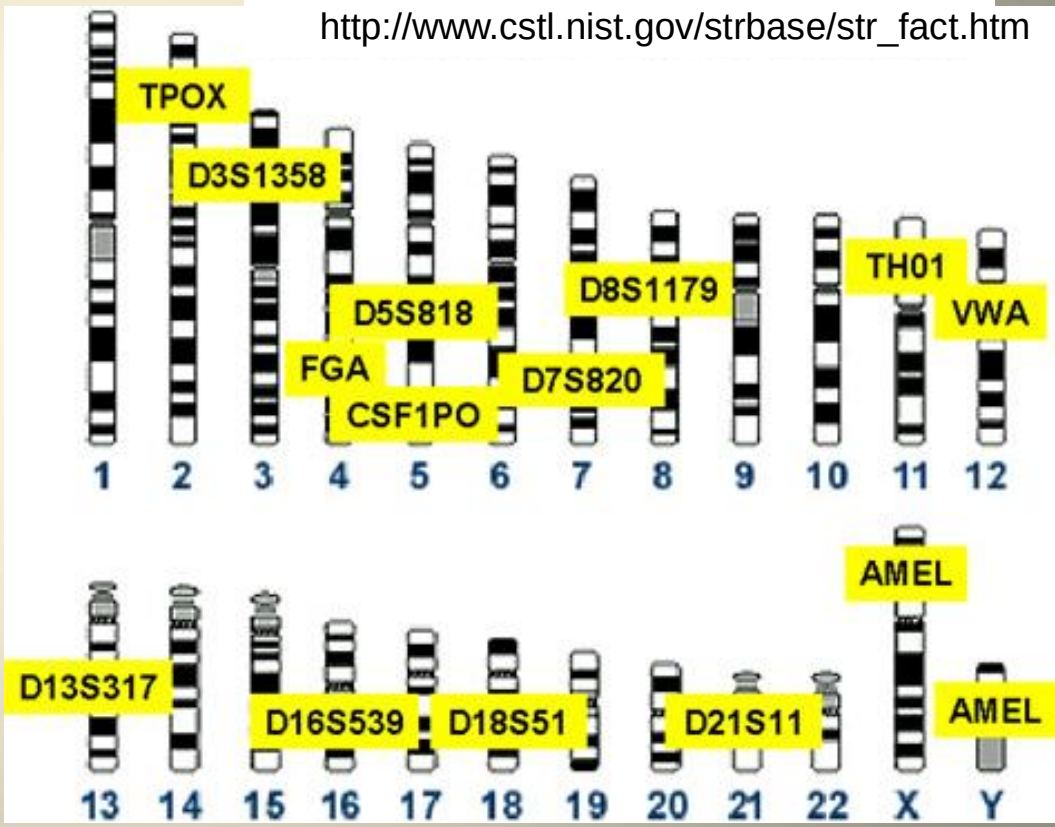


- The number of alleles at a polymorphic loci is small compared to the total world population
- An allele can be shared by 5 - 20% of all individuals
- What implications would this have?

Loci

- The power of STR analysis comes from looking at multiple STR loci simultaneously
- In North America, 13 loci are amplified plus one (AMEL) to determine sex

Info on each locus can be found at:
http://www.cstl.nist.gov/strbase/str_fact.htm



http://upload.wikimedia.org/wikipedia/commons/7/7a/Codis_profile.jpg

VNTR Application

- Polymorphism in VNTR useful for:
  - DNA Fingerprinting
  - studying evolution
- Both require looking at differences:
  - DNA Fingerprinting: differences between individuals
  - Evolution: differences between species

Why use noncoding regions?

- Coding regions are too similar between individuals and even species

Application of DNA Fingerprinting
Video: Alec Jeffreys and the first DNA Fingerprint (3:49)

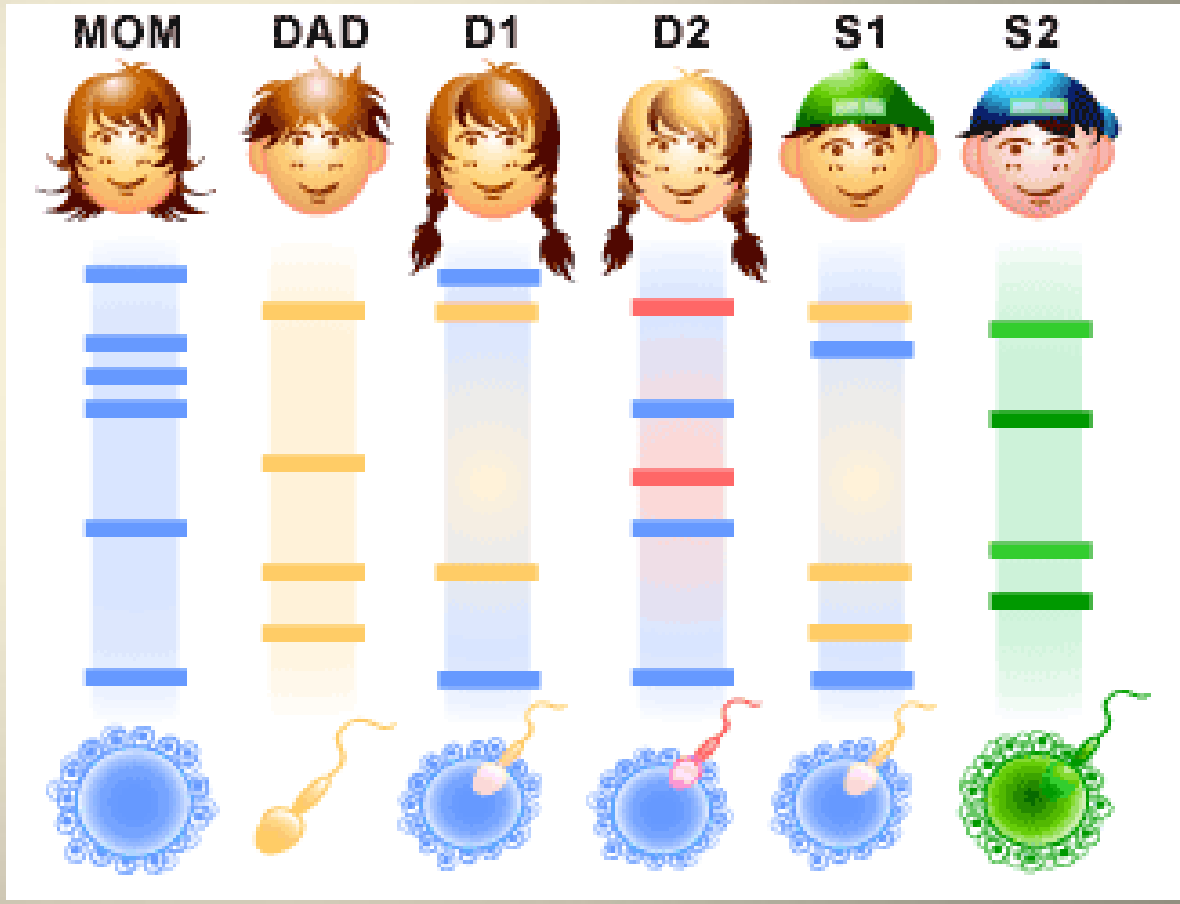
https://www.youtube.com/watch?v=Hzq7Tb10M1c

DNA Fingerprinting Application

- Paternity testing: identifying the father
  - Animation: http://www.sumanasinc.com/webcontent/animations/content/paternitytesting.html
- Criminal cases: eliminating suspects
- Identifying a corpse
- Immigration disputes
- Food testing (i.e. Champagne, horse meat)

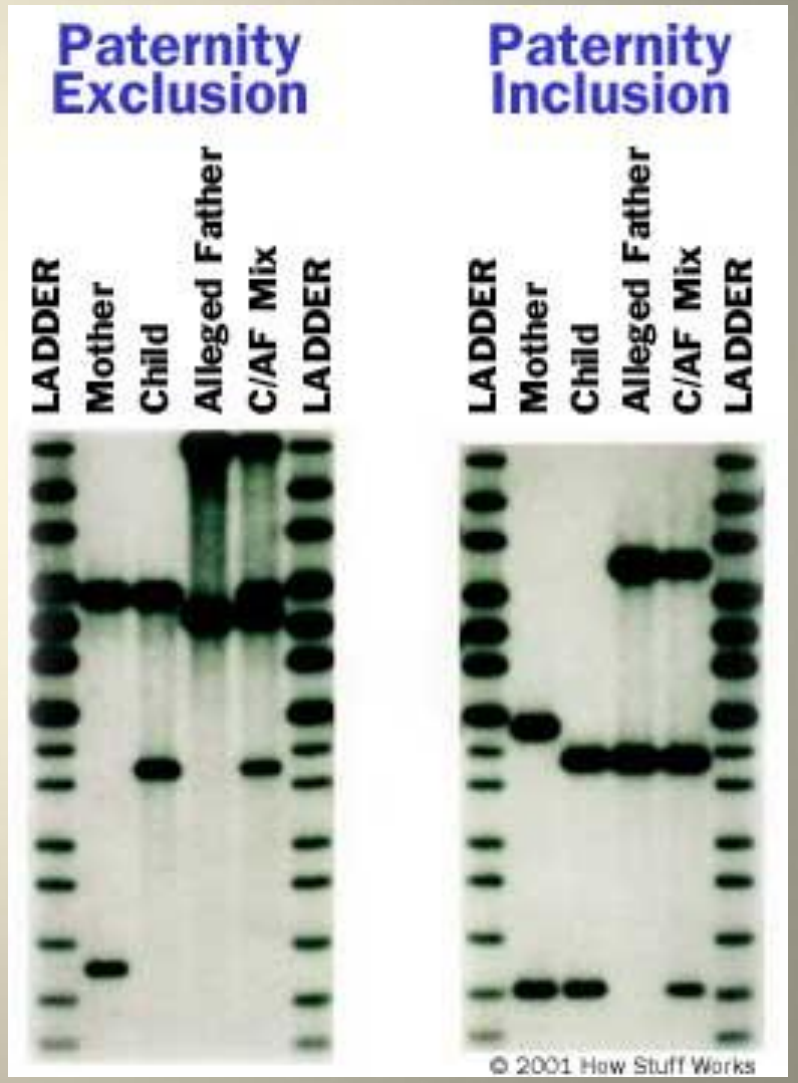
Paternity

- Used to identify a child's parents
- Each child inherits one set of chromosomes from each parent



Paternity

- What is the difference between paternity inclusion versus paternity exclusion?



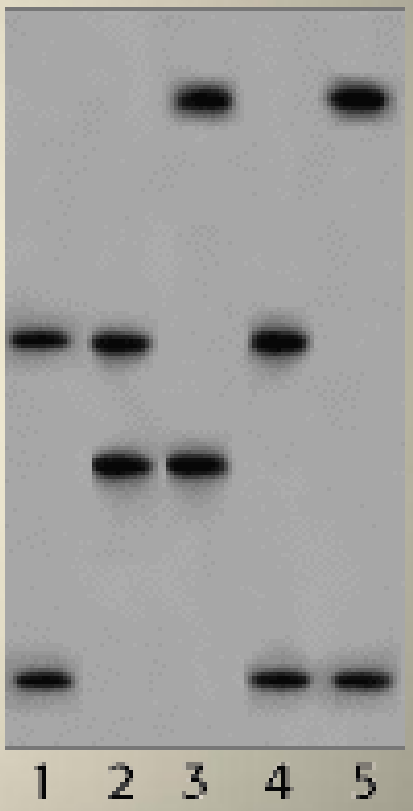
Paternity

- The chart shows 5 loci and the size of the fragments produced through DNA fingerprinting of each individual.
- Is it likely that the alleged is the father of the child? Explain.

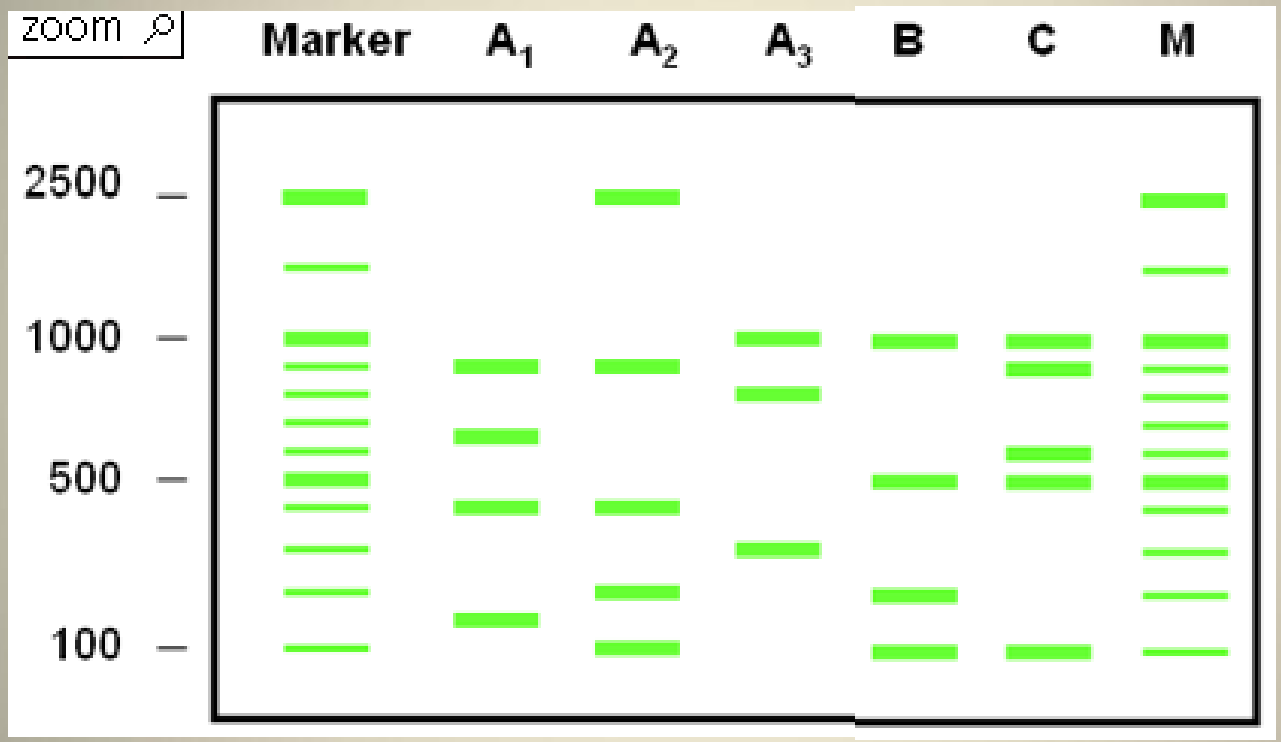
DNA Marker	Mother	Child	Alleged father
D21S11	28, 30	28, 31	29, 31
D7S820	9, 10	10, 11	11, 12
TH01	14, 15	14, 16	15, 16
D13S317	7, 8	7, 9	8, 9
D19S433	14, 16.2	14, 15	15, 17

Paternity

- The gel shows a family with 3 children, a biological mother and father.
- If lane 2 represents the biological mother determine which lane represents the father.
- Explain how you came to this conclusion.

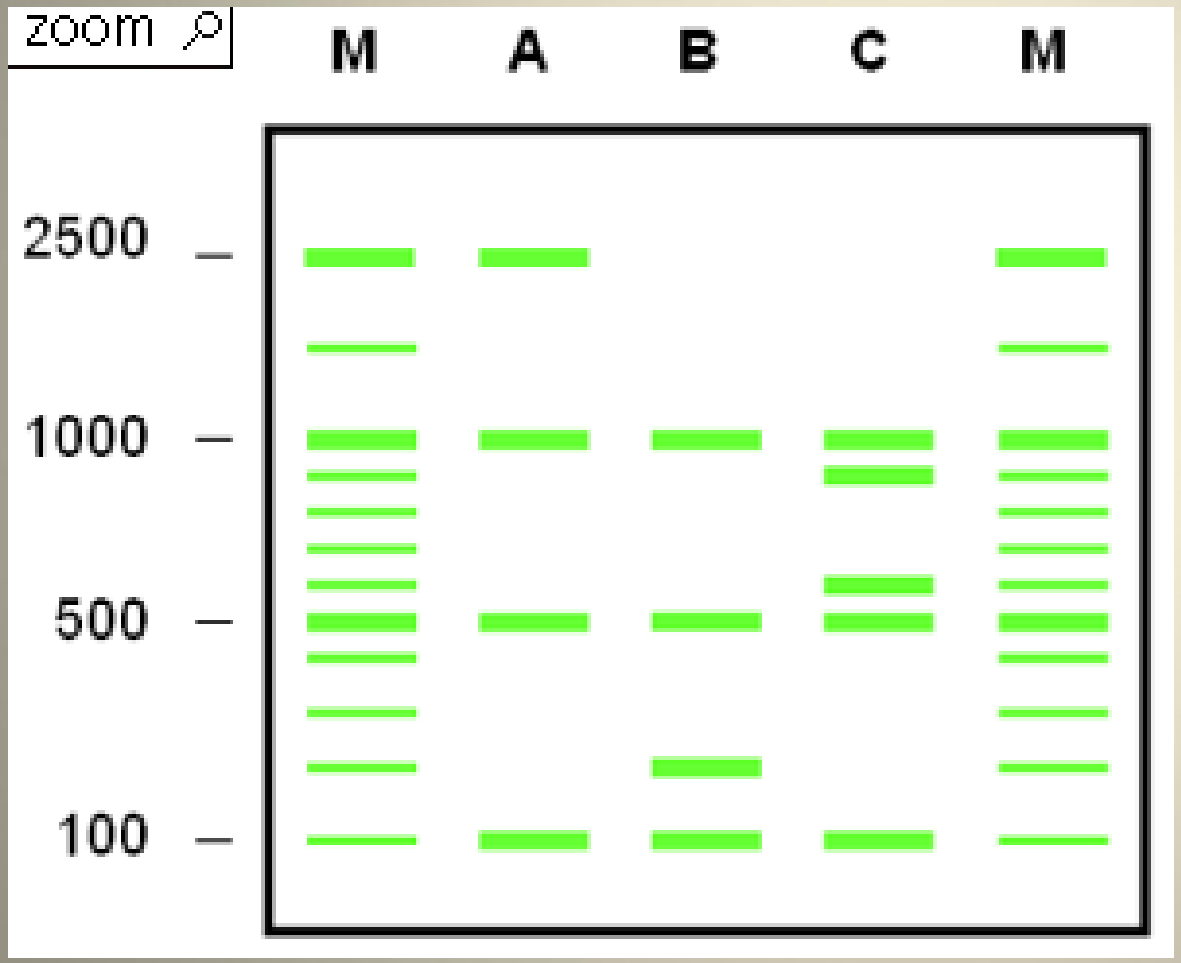


Paternity



- Assuming C is a parent of B, who is the other parent (A1, A2 or A3)?

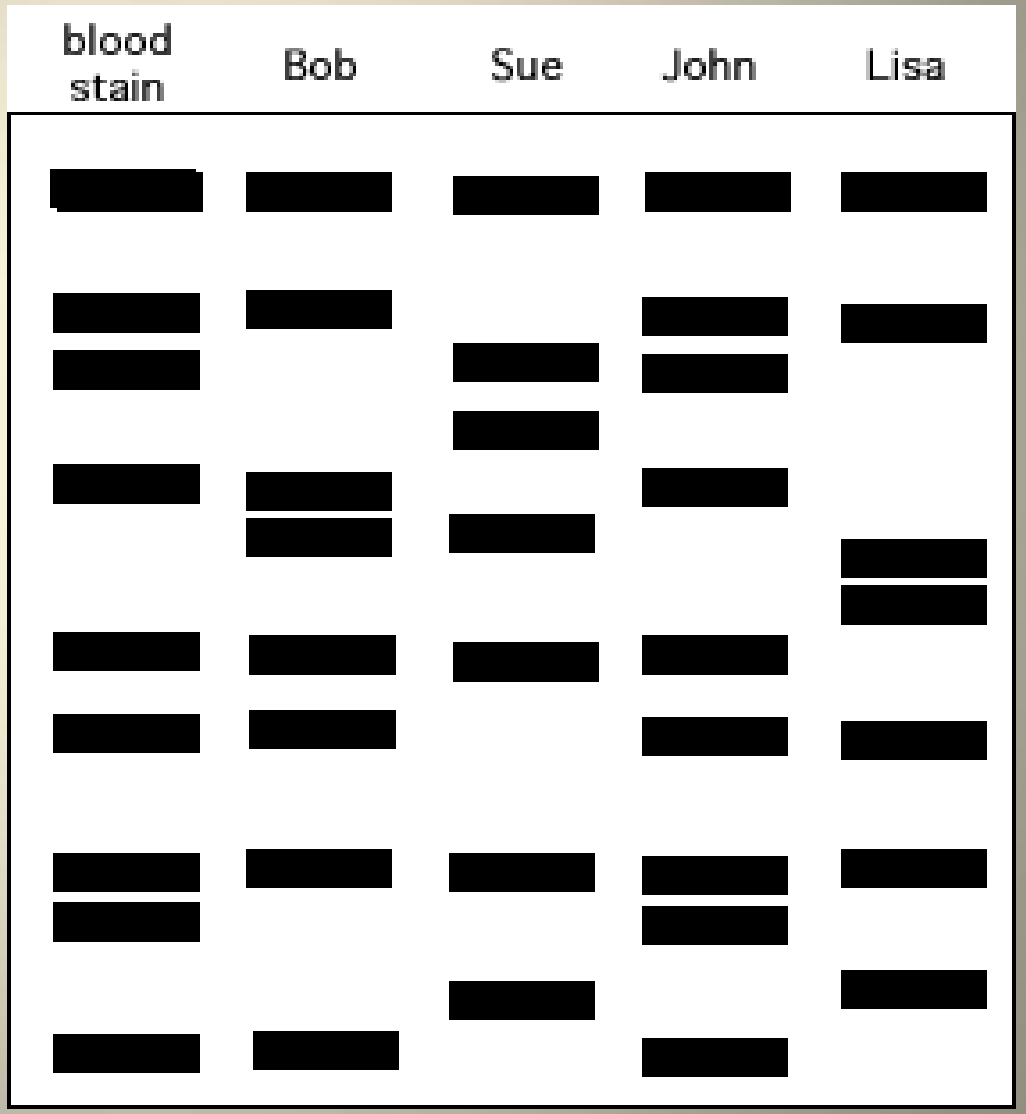
Paternity



- Can individual A, B and C be related (parent and child)?
- If so, who is who?

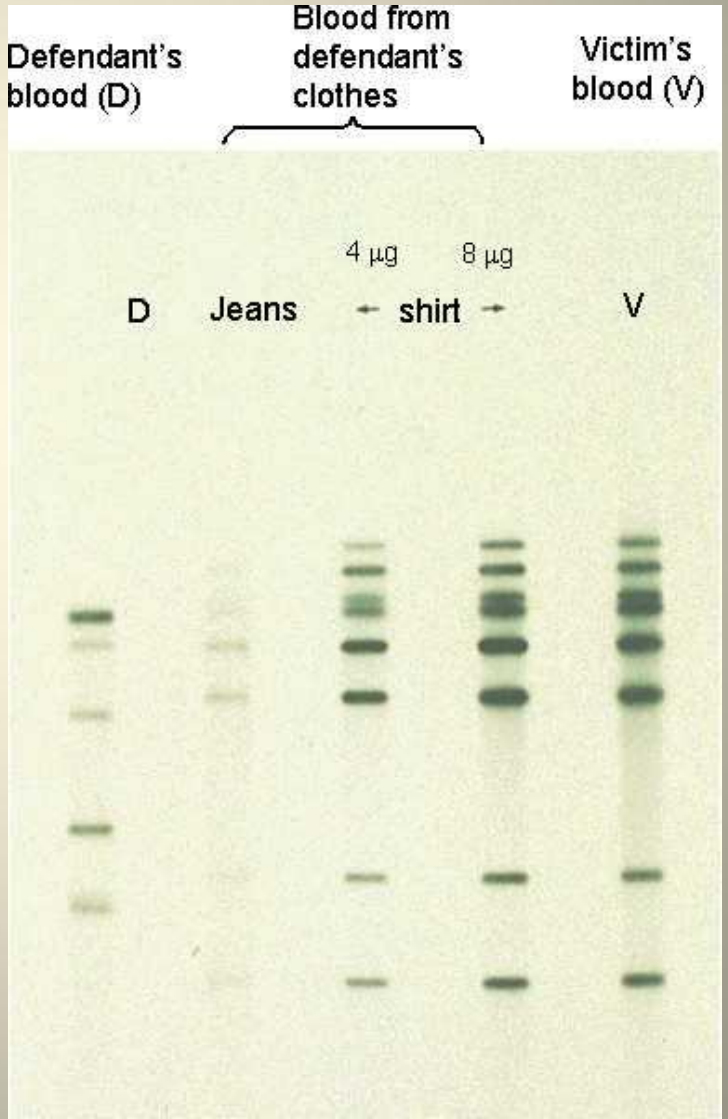
Criminal Case

- Which person is more likely to be suspected in the crime?



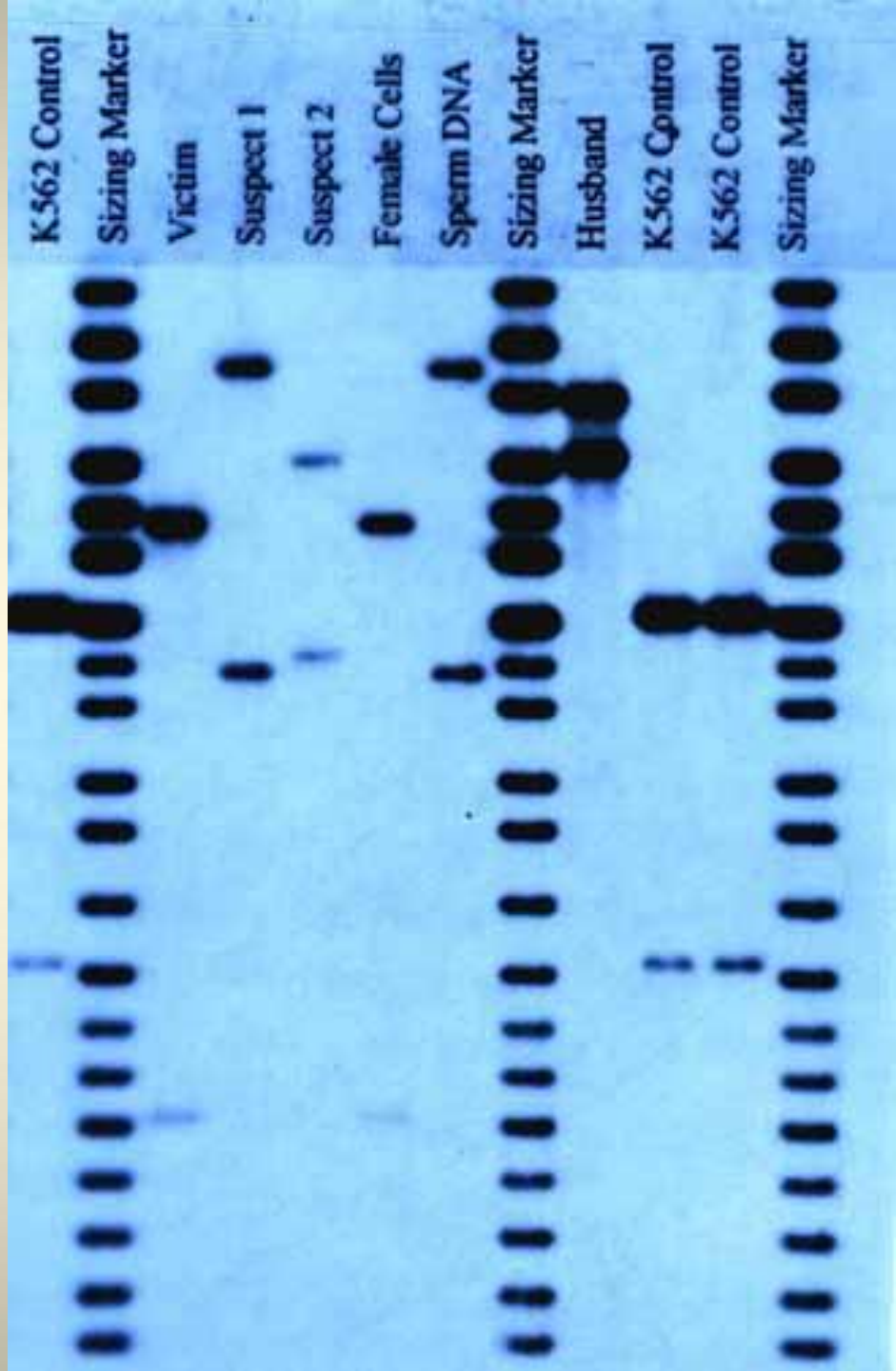
Criminal Case

- What does this DNA fingerprint tell you?



Sexual Assault Case

- Which DNA samples were collected at the crime scene?
- Which DNA samples were taken after the crime?
- Why was the husband's DNA also taken?
- What do you think the control was used for?



Activity: DNA Fingerprinting

The CASE

A married couple arranged with a surrogate to have a baby. The woman's egg was fertilized in vitro with her husband's sperm and the developing embryo was implanted into the surrogate mother. When the surrogate mother gave birth, she decided she wanted to keep the baby. She claimed that the developing embryo spontaneously aborted and the baby is the natural child of herself and her boyfriend. The case is taken to court to decide custody. A strong piece of evidence is DNA fingerprinting.