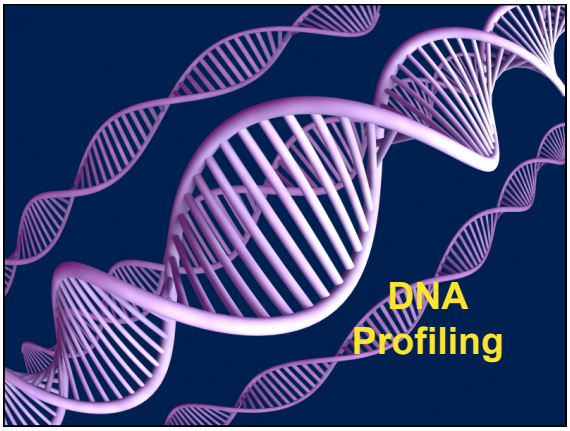
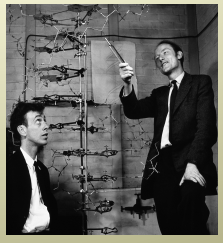


A Bit of History: Structure

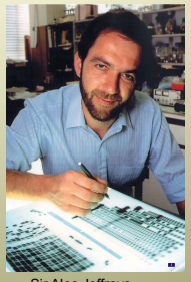
- The double helix structure of DNA was discovered in 1953 by Watson & Crick, two British scientists who based their work on Rosalind Franklin's data
- First described in a short article in *Nature*
- Kary Mullis (American) invented a method for duplicating targeted strands of DNA in 1983
- This method is called polymerase chain reaction (PCR)



Watson & Crick

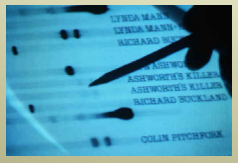
A Bit of History: DNA "fingerprinting"

- In the course of his research on variability in human DNA, Alec Jeffreys (British) developed a method of forensic DNA typing in 1985, originally called "DNA fingerprinting"
- "DNA fingerprinting" was first used in 1986 to catch a rapist/ murderer in England named Colin Pitchfork
- The case is chronicled in The Blooding, by Joseph Wambaugh



Sir Alec Jeffreys

A Bit of History: Pitchfork



DNA 101

What *is* it? *Where* is it?

- **DeoxyriboNucleic Acid**
  - The genetic material found in the nucleus of every every nucleated cell in the body
  - Has structure of a "double helix", like a twisted zipper or a twisted ladder
  - Made of long polymer strands of nucleotides (molecules made up of nitrogenous bases and sugar-phosphate groups)

DNA 101

- Blood (white blood cells)
- Roots of hair (epithelial cells)
- Saliva (epithelial cells)
- Semen (sperm cells)
- Skin, dandruff (epithelial cells)
- Sweat stains (epithelial cells)
- Vaginal fluids (epithelial cells)
- Nasal secretions (epithelial cells)
- Urine (epithelial cells)
- Feces (epithelial cells, rarely used)

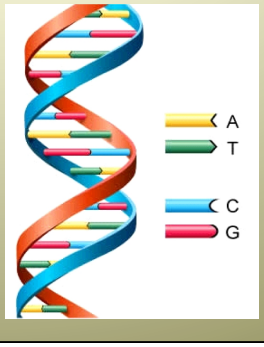
DNA is the same in every nucleated somatic cell, and is robust and stable

DNA 101

- Nucleotides contain either a purine or a pyrimidine base:

Pyrimidines
- Cytosine (C)
- Thymine (T)

Purines
- Guanine (G)
- Adenine (A)



DNA 101

- A "base pair" is like one wrung of the ladder (eg, one A with one T)
- There are approximately *3 billion* base pairs (C-G, A-T) of nucleotides in every nucleated cell.
- Some sequences of DNA code for certain proteins: genes.
- A gene is a hereditary unit that determines a particular characteristic in an organism.
- Genes are sequences of A,G,C,T *nucleotides.*
- The length and order of nucleotides determines the type of protein that is produced by that gene.
- Humans have ~20,000-25,000 genes

DNA 101

- DNA is organized into *chromosomes.*
- Every person gets 23 chromosomes from each parent, for a total of 46.
- Exactly half your DNA comes from each parent.
- The location of a gene on a chromosome is its *locus* (plural*: loci*).
- An *allele* is an alternate form of gene (for example, eye color)
- Each person inherits one allele from each parent at every locus.

DNA 101

- *Phenotype*: the physical characteristics (or exterior expression) of an organism's genetic makeup

- *Genotype:* 1. the genetic makeup of an organism;
- 2. the combination of alleles present at a particular locus, or at all loci present

Mother's Genotype: Bb

Mother's phenotype: Brown eyes

Father's Genotype: Bb

Father's phenotype: Brown eyes

	B	b
B	BB	Bb
b	Bb	bb

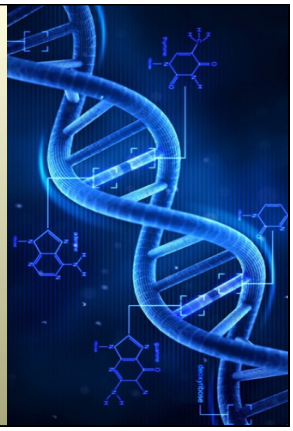
If they each have a blue recessive gene, two brown-eyed parents may have a blue eyed child.

In reality, inheritance is much more complicated than this Punnett Square diagram, but it offers a useful illustration of genotype and phenotype.

Junk DNA

- We share over 99% of our genome with other humans.
- However, that <1% of 3 billion nucleotides is still a significant and detectable level of variation.
- ***Most variation exists in the part of the genome that does not code for genes.***
- The function of this DNA, called "junk DNA", is mostly unknown.
- ENCODE (Encyclopedia of DNA Elements) is a SWG at UCSC that recently released several reports on the role of "junk DNA"
  - Biochemically active, and to have a regulatory function on genes.

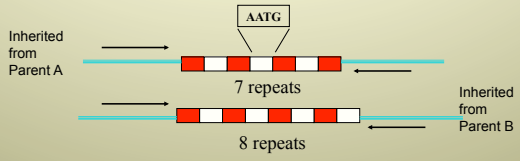
*Mutations in **junk dna** are tolerated and can accumulate with no affect on the organism… leading to increasing variation.*



Short Tandem Repeats

- **S**hort **T**andem **R**epeats (STRs)
  - **Short** because the sequences are short – usually 1-4 nucleotides in length
  - **Tandem** because they occur one after the other
  - **Repeats** because they are repeats of the same sequence
  - ATCGACCTTG-GCCG-GCCG-GCCG-GCCG-ATCGATTGACCTAAC
    = 4 **s**hort **t**andem **r**epeats of GCCG
- These are found in junk DNA
- The number of repeats of a particular sequence may vary between individuals.

Short Tandem Repeats



This person has 7 repeats of AATG from parent A, and 8 repeats of AATG from parent B, at this locus.

This person's genotype at this particular locus is 7,8 (or 8,7).

An Example of a STR in locus D7S280

- D7S280 is a locus on human chromosome 7. Its DNA sequence, as obtained from GenBank (a public DNA database) is gata.

```
aatttttgta ttttttttag agacggggtt tcaccatgtt ggtcaggctg
actatggagt tattttaagg ttaatatata taaagggtat gatagaacac
ttgtcatagt ttagaacgaa ctaacgatag atagatagat agatagatag
atagatagat agatagatag atagacagat tgatagtttt tttttatctc
actaaatagt ctatagtaaa catttaatta ccaatatttg gtgcaattct
gtcaatgagg ataaatgtgg aatcgttata attcttaaga atatatattc
```

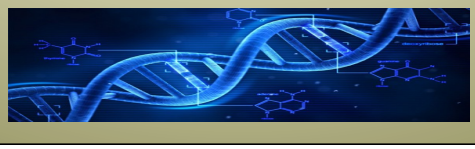
- Alleles at this locus have from 6 to 15 tandem repeats of the 'gata' sequence.
- In other words, every person has between 6 to 15 STRs of gata at this location (locus) in their DNA.

SO WHAT?

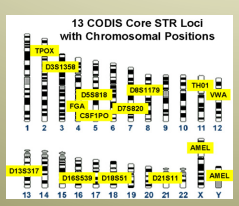
- If we look at only one locus (for example, D7S280), then the fact that every person has either 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 repeats of gata is not enough for us to tell them all apart, because too many people will have the SAME number of repeats at that locus. (For example, there may be *thousands* of people who have the genotype 7,8 at that locus.)
- HOWEVER, the more loci we look at, the fewer people will share numbers of repeats.
- AND, if we look at many loci, which we do in DNA profiling, then the number of people who share the numbers of repeats at all of the loci will be extremely close to ZERO.

And?

- This is where probability becomes relevant.
- Recall that probability means *the frequency of occurrence of an event.*
- The probability that you will have the exact same number of repeats at the same loci as anyone else is infinitesimally small (unless you have an identical twin).
- This is why DNA evidence is so powerful.



- Combined DNA Index System
- All forensic laboratories that use the CODIS system can contribute DNA profiles to the CODIS database.
  - The **Forensic Index** contains DNA profiles from crime scene evidence.
  - The **Offender Index** contains DNA profiles of individuals convicted of sex offenses (and other violent crimes) with many states now expanding legislation to include other felonies.
  - Looks at 13 loci



- A typical DNA case involves the comparison of two samples – an unknown or *evidence* sample, such as semen from a rape, and a known or *reference* sample, such as a buccal swab (inside cheek) sample from a suspect.
- If the DNA profiles obtained from the two samples are indistinguishable (they "match"), that is evidence for the court that the samples have a common source – in other words, that the suspect *is* the source of the semen.
- A logical and important legal question, then, is: how do we know that the suspect is the only person with this particular profile? How do we know that our suspect's DNA doesn't *just happen* to match the crime scene sample by chance?

A Sample Profile and Frequency Database

Locus	Alleles	#Times Observed	Dbase size	Frequency of Occurrence
CSF1PO	10 11	109 134	432	=.25 =.31
TPOX	8 8	229	432	=.53
THO1	6 7	102 64	428	=.24 =.15
vWA	16 16	91	428	=.21

How rare (or common) is this profile?
How do we figure it out?

How rare is the profile?

- The probability (frequency of occurrence) of a profile is determined using frequency databases (frequencies of alleles in a given population) and probability law
- The number of repeats found at each locus is an independent event -- in other words, the number of repeats at a locus is not related to the number of repeats found at another locus
- Probability Law: "The probability that two independent events may happen together is the *product* of their individual probabilities"… the PRODUCT RULE

A Sample Profile

Locus	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818
Genotype	15, 18	16, 16	19, 24	12, 13	29, 31	12, 13	11, 13
Frequency	8.2%	4.4%	1.7%	9.9%	2.3%	4.3%	13%

Locus	D13S317	D7S820	D16S539	THO1	TPOX	CSF1PO	AMEL
Genotype	11, 11	10, 10	11, 11	9, 9.3	8, 8	11, 11	X Y
Frequency	1.2%	6.3%	9.5%	9.6%	3.52%	7.2%	(Male)

- GENOTYPE ROW: tells you the number of repeats this person has at a particular locus.
- FREQUENCY ROW: tells you how often this particular combination of alleles appears in the general population. (Frequencies are determined simply by counting the number of alleles in a given population.

How rare is this profile?

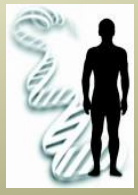
Locus	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818
Genotype	15, 18	16, 16	19, 24	12, 13	29, 31	12, 13	11, 13
Frequency	8.2%	4.4%	1.7%	9.9%	2.3%	4.3%	13%

Locus	D13S317	D7S820	D16S539	THO1	TPOX	CSF1PO	AMEL
Genotype	11, 11	10, 10	11, 11	9, 9.3	8, 8	11, 11	X Y
Frequency	1.2%	6.3%	9.5%	9.6%	3.52%	7.2%	(Male)

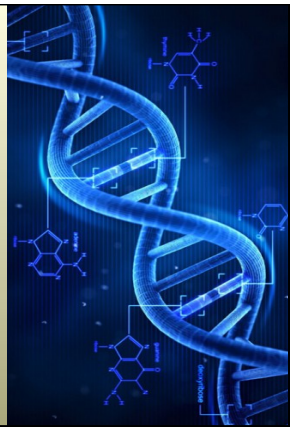
- .082x.044x.017x.099x.023x.043x.13x.012x.063x.095x.096x.0352x.072 = **.00000000000000000013642127!!!**
- So, the chance that this sample came from someone other than our suspect is
  LESS THAN 1 IN 100 QUADRILLION!

How do we get the profile? How do we find out what a person's alleles are?

1. Extract DNA from sample
2. Quantify the DNA
3. Amplify the DNA (PCR)
4. Separate & detect PCR products via Capillary Electrophoresis
9. Determine genotype
10. Compare to reference profiles
11. Consult population dbase & determine frequency of profile



- Remember, in DNA profiling, we are not concerned with genes, because that's not where the variation is.
- Rather, the DNA we look at is in the junk DNA regions.
- Every cell has only one copy of nuclear DNA, which is not enough for us to "see".
- So, we must first target the areas of DNA that we want to see more closely (the 13 loci) and amplify or copy it, so that there is enough for us to analyze.
- We do this using PCR…



PCR: Polymerase Chain Reaction

- PCR machines, or thermocyclers, use repeated cycles of heating and cooling to denature (unzip) and replicate (rezip) the DNA using many of the same enzymes found in cells which facilitate DNA replication naturally.

The PCR Song

- Believe it or not, you can learn a lot about PCR from this song….
- http://www.youtube.com/watch?v=x5yPkxCLads

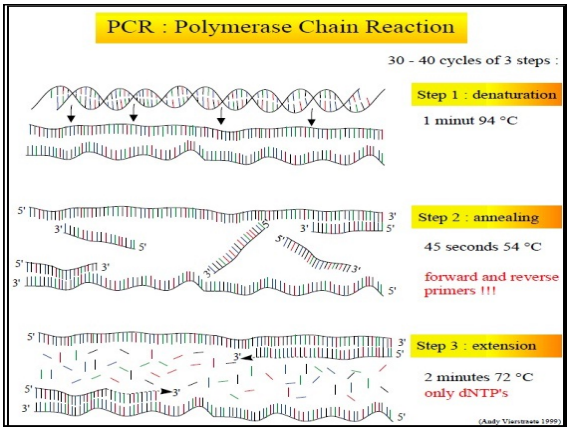
The PCR Song

"There was a time when to amplify DNA…You had to grow tons and tons of tiny cells.Then along came a guy named Dr. Kary Mullis, Said you can amplify in vitro just as well. Just mix your template with a buffer and some primers, nucleotides and polymerases, too. Denaturing, annealing, and extending. Well it's amazing what heating and cooling and heating will do.

PCR, when you need to detect mutations. PCR, when you need to recombine. PCR, when you need to find out who the daddy is (who's your daddy?). PCR, when you need to solve a crime."

PCR

- Using a thermocycler, the sample is denatured (double helix is unzipped) @ ~95°C
- Primers (oligonucleotides, typically 20 bases long) adhere to complimentary open strands (A-T, G-C) @ ~55°C
- The new DNA strand is synthesized with Taq polymerase @ 72 °C
- Process is repeated until there are enough copies of the DNA for detection using electrophoresis



Electrophoresis

Electrophoresis is the process of moving charged particles through a gel plate by applying an electric field (eg, negatively charged DNA will move toward a positive source).

- Following PCR, amplified DNA samples are separated by size (smaller particles move faster than larger ones) through the gel during electrophoresis.
- The process is automated using an instrument (a genetic analyzer) that reads the DNA by size -- a laser scans and detects the DNA samples as they electrophorese.

OHT 6.11

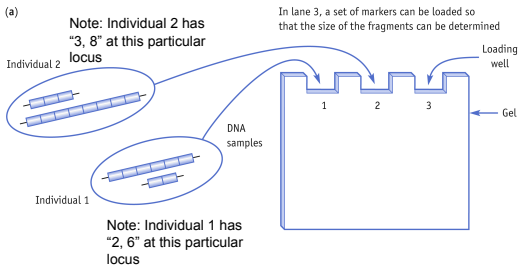
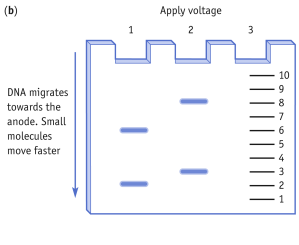


Figure 6.6 Separating DNA molecules according to their length: gel electrophoresis (a) loading the gel

OHT 6.12

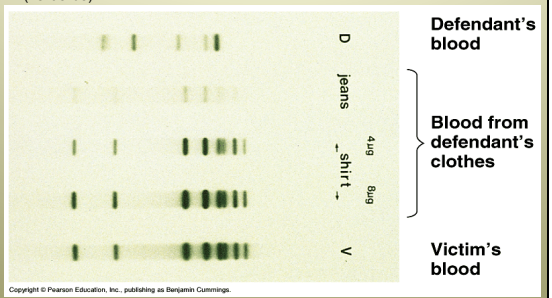


In the 3rd lane, a "ladder" sample is loaded, which has one fragment of every size. This acts as a control and allows you to determine the size of the DNA fragments in other wells.

Figure 6.6 Separating DNA molecules according to their length: gel electrophoresis (b) DNA migration

Electrophoresis Autoradiograph

We used to get "bands" of DNA that looked like this (below). Now we have automated DNA instruments, which show DNA not as bands but as peaks (next slide).



OHT 6.13

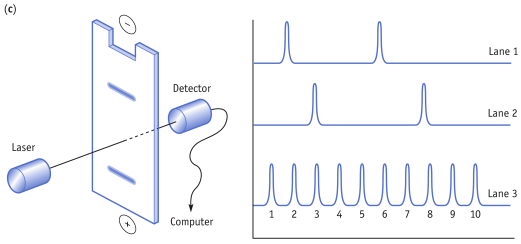


Figure 6.6 Separating DNA molecules according to their length: gel electrophoresis (c) detecting fluorescently tagged DNA by laser

OHT 6.2

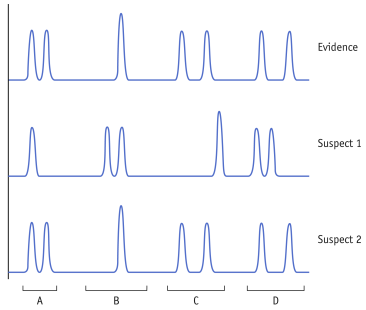


Figure 6.1 (continued) Simplified examples of DNA profiles

Jackson & Jackson: *Forensic Science*

OHT 6.16

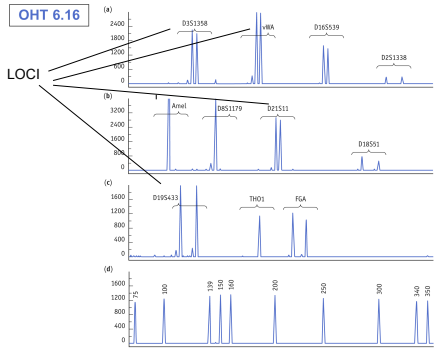


Figure 6.8 A modern DNA profile

Jackson & Jackson: *Forensic Science*

Other Types of DNA

- We've been talking about nuclear DNA (nDNA)
- There are other types of DNA that are useful for establishing genetic groups, but are otherwise not as discriminating
  - MtDNA (mitochondrial DNA) -- used to determine maternal relationships and maternal groups
    - EVERYONE has mtDNA. You inherited your mtDNA from your mother, without recombination with your father. Your mtDNA is an exact match with your mother's mtDNA, your mother's siblings, your mother's mother and all her siblings, and so on. If you are female, your daughters will continue this mtDNA.
  - Y-STR (short tandem repeats found on the Y chromosome -- used to determine paternal relationships and paternal groups in males
    - ONLY MALES HAVE YSTR. It is an exact match with your father, your brothers, your sons, your father's father and his male siblings….

mtDNA & Y-STR

Mitochondrial DNA

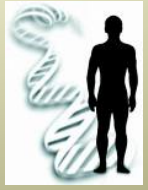
- 37 genes, 16569 base pairs
- 100-10,000 copies per cell (significantly more than nuclear DNA) make it useful for analyzing degraded samples
- Circular structure
- Non-coding D loop used for testing
- *Inherited maternally by all offspring*

Y-STR

- Short tandem repeats located on Y chromosome
- Useful for resolving mixtures between males & females
- *Inherited paternally by males only*

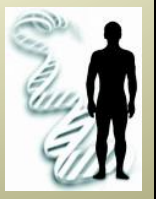
Some Considerations

- Although DNA is relatively stable, it does denature or get destroyed through enzyme action, from bacteria or through oxidation
- Therefore, samples should be collected quickly and preserved (usually by freezing if possible)
- Care should also be taken not to cross contaminate during collection -- including from collector!
- Blood is also a potential pathogen, so care must be taken to avoid exposing oneself to blood borne viruses like Hep B, tuberculosis or HIV



Some uses of DNA Profiling

- Identifying suspects in criminal cases
- Parentage testing
- Victim identification in mass disasters
- Identification of abducted children and reunification with families
  - UCB Human Rights & Forensic Science
  - http://www.law.berkeley.edu/12325.htm
- Animal identification- e.g. racehorse paternity, endangered species poaching
- Conservation biology and evolutionary studies



DNA Profiling can solve crimes

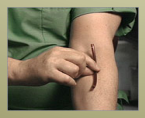
- The DNA profile is compared with those of the victim and the suspect.
- If the profile "matches" the suspect, it provides strong evidence that the suspect was present at the crime scene (**it does not necessarily prove he or she committed the crime).**
- If the profile doesn't match the suspect, then that suspect may be eliminated from the inquiry.

As the technology gets smarter, so too do the criminals

- A physician in Canada eluded authorities for years
- Accused of drugging and sexually assaulting patients, DNA profiles from semen samples from the assaulted women did not match Dr. Schneeberger
- Blood was drawn on 3 occasions in 1992, 1993 and 1996, but never came back as a match to the semen DNA
- Finally police obtained blood from a finger prick, swabbed the inside of his cheek and took hair samples
- The results matched the DNA from the semen found on the victims
- How did he get away with it?

As the technology gets smarter, so too do the criminals

- On the 3 occasions, blood was drawn from the same arm
- The last time the blood was drawn, the technician noticed that the blood looked brown and "old"
- Schneeberger had surgically implanted a piece of rubber tubing in his arm and filled it with stored blood from a patient



- DNA is also used in the identification of remains recovered in mass disasters

Post Mortem Identification Josef Menegle

- Josef Mengele was a Nazi war criminal notorious for grotesque human experiments that he carried out at the Auschwitz concentration camp.
- After the Second World War he fled from the Allies and escaped to South America. The fugitive succeeded in living out the rest of his days without being caught.
- In 1985 investigators went to the cemetery of Nossa Senhora do Rosario in the small Brazilian town of Embu to dig up the skeleton of a man who had been drowned in a swimming accident six years previously.
- Using DNA extracted from blood provided by Mengele's wife and son, it was concluded that it was more than 99.94% certain that the skeleton was Mengele's.

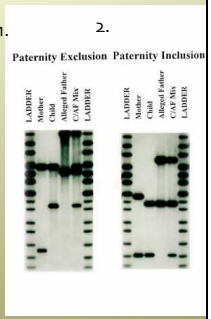
Paternity Cases

- Who's your daddy?

1.

2.

1. 2.



Paternity Cases

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

Exoneration

- Kirk Bloodsworth
  - Convicted in 1985 for the rape and strangulation of a 9-year old girl and sent to death row
  - In 1992, defense attorneys were successful in having a dime-sized semen stain on the girl's underpants tested against Bloodsworth's DNA
  - He was exonerated

Exoneration: The Innocence Project

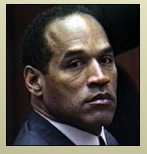
•312 post-conviction DNA exonerations in the US.
•Eighteen people had been sentenced to death before DNA proved their innocence and led to their release.
•Average served sentence: 13.6 years.
•70 percent of those exonerated by DNA testing are people of color.
•In almost 50 percent of DNA exoneration cases, the actual perpetrator has been identified by DNA testing.

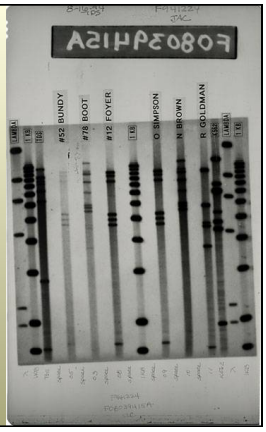
•The Innocence Project was involved in 172 of the 312 DNA exonerations.
•http://www.innocenceproject.org/

Causes of Wrongful Convictions

- Eyewitness misidentification
- Unvalidated or improper forensic science
- False confessions/admission
- Government misconduct
- Informants/snitches
- Bad lawyering

OJ Simpson





DNA Profiling

- **"I didn't understand the DNA stuff at all. To me, it was just a waste of time. It was way out there and carried absolutely no weight with me at all."**
- Post-trial commentary from a juror in the O.J. Simpson trial: V. Bugliosi, Outrage (New York: Dell Publishing, 1996).
- **"In a forensic setting, … an innocent suspect has little to fear from DNA evidence, unless he or she has an evil twin."**
- N. Risch & B. Devlin, "On the Probability of Matching DNA Fingerprints" (1992) 255 Science.